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Modern Trends in System Biology. Virtual modeling and Regulation

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International Study Group for System Biology: Modern Trends in System Biology. Virtual modeling and Regulation

Edited by G.I. Podoprigora, Ya.R. Nartsissov

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Preface

Since the last year of XXth century, when Hiroaki Kitano firstly introduced term “system biology” this branch of natural science has been violently developing. It has successfully combined different fields of biology such as biophysics, biochemistry, molecular biology, molecular genetics, etc. The main reason for this trend was dual essence of scientific methodology for knowledge getting. On the one hand a researcher wants to work with a precisely pure system, which can have a small limited set of measurable values and on the other hand, his/her global aim is to augment the understanding of real biological object having complex multiply regulation. The first system is more or less simple and it has a propensity for application of physical approach. The second one is so complex that the tools of its investigation could be even philosophy or religion. Fine balance between such antipodal approaches finally forms intention to understand not only simple natural phenomena but also to comprehend how they shape a whole biological object. This way defines the set of mental instruments applying for researches. It includes mathematical modeling, statistics, computer programming and any kind of their combinations. Therefore, it is necessary for those who practise system biology to be experts not only in biology, but also in the computational/mathematical approaches needed.

History of International Study Group in Systems Biology (ISGSB) began in 1984 as a non-formal community of researchers specialized in mathematical modeling and experiments in the field of regulation and metabolic control. Initially it was identified under name The International Study Group of Biological Thermodynamics, Control and Kinetics (Biothermokinetics, BTK). The conference uniting this community had been held together with European Bioenergetics Conference (EBEC) but since 2000 it transformed
into an independent meeting. Friendly communication between interested scientific groups and involving of young scientists into research process helped to grow up the community. A major contribution to development of BTK-ISGSB has been made by Hans V. Westerhoff (Amsterdam), David A. Fell (Oxford), Reinhart Heinrich (Berlin), Triple J Group and many other colleagues who collaborated and discussed new results in a friendly atmosphere. In spite of the difficult period of the discipline re-defining and forming of novel interests ISGSB keeps the activity and looks forward with hope and optimism.

Indeed, papers in different area of system biology have been united in this volume and we suppose that this abundant variety helps to stimulate research interest of young scientists for further profound comprehension of Nature laws.

Yaroslav R. Nartsissov

Chairman of 14th Workshop of the International Study Group for Systems Biology 2010
Session 1

Fundamental problems of System Biology
Metabolic and Transcriptional Response to Cofactor Perturbations in Escherichia coli

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NADH and ATP play important roles as cofactors in a huge number of cellular reactions, and it is therefore of interest to dissect the role of these cofactors in different aspects of metabolism. We overexpressed an NADH oxidase and a soluble F1-ATPase in Escherichia coli to lower the level of either NADH or ATP, respectively. We used a global interaction network, comprising of protein interactions, transcriptional regulation, and metabolic networks, to integrate data from transcription profiles, metabolic fluxes, and the metabolite levels. We identified high-scoring networks for the two strains.

The results revealed a smaller, but denser network for perturbations of ATP level, compared with that of NADH level. The action of many global transcription factors such as ArcA, Fnr, CRP, and IHF commonly involved both NADH and ATP, whereas others responded to either ATP or NADH. Overexpressing NADH oxidase invokes response in widespread aspects of metabolism involving the redox cofactors (NADH and NADPH), whereas ATPase has a more focused response to restore ATP level by enhancing proton translocation mechanisms and repressing biosynthesis. Interestingly, NADPH played a key role in restoring redox homeostasis through the concerted activity of isocitrate dehydrogenase and UdhA transhydrogenase.

We present a reconciled network of regulation that illustrates the overlapping and distinct aspects of metabolism regulated by NADH and ATP. Our study contributes to the general understanding of redox and energy metabolism and should help in developing metabolic engineering strategies in E. coli.
To understand the biology at the system level, we must examine the structure and dynamics of cellular and organismal function rather than the characteristics of isolated parts of a cell or organism. The properties of systems, such as robustness, emerge as a central issue. An understanding of these properties may have and impact on the future of medicine. However, many breakthroughs in experimental devices, advanced and software are needed. The analytical methods are required before the achievements of systems biology, can live up to their much-touted potential.
Temperature compensation and entrainment of circadian rhythms under small temperature shifts

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The circadian clock helps an organism to anticipate daily variations in the environment and coordinate biological activities into a daily cycle. The rhythm persists autonomously in the absence of external time cues with a period of approximately 24h. However, single pulses of light, darkness, chemicals, or temperature can shift the clock phase. This allows entrainment of the clock to light and temperature cycles of exactly 24h. On the other hand, the period of the circadian rhythm is kept relatively constant within a physiological range of constant temperatures, i.e. the oscillator is temperature-compensated.

The mechanisms behind temperature compensation and temperature entrainment are not fully understood, both biochemically and mathematically. For some organisms temperature regulated processes within the circadian clock have been identified in recent years. However, how these processes contribute to temperature compensation has only partially been investigated and their influence on temperature entrainment is mostly unknown. Here, we theoretically investigate temperature compensation and entrainment in general oscillatory systems.

To obtain analytical results we restrict ourselves to small temperature shifts. This allows us to use the phase description method. We derive that every oscillator can be temperature compensated, if it has a reaction with a positive period control coefficient. Moreover, every temperature compensated oscillator is entrain-
able to external small-amplitude temperature cycles. The entrainment region of the oscillator gets larger with the variance of the external signal. Furthermore, we analyze different circadian clock models proposed in the literature with respect to these properties and estimate their potential for temperature compensation and entrainment.
Can a systems modeling approach be used to understand a complex pharmacokinetic/pharmacodynamic relationship? A case study of 5-lipoxygenase inhibition by Zileuton

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Systems modeling approaches are seen as the next step in the evolution of mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling. Although some recent publications have highlighted development of such models, few examples exist in literature on the successful application of this novel methodology within the drug development setting. We report the exploration of the hypothesis that complex literature-based systems models can be developed and applied during drug discovery and development of 5-lipoxygenase (5LO) inhibitors for asthma. Our initial interest focused on the human dose/time/effect (FEV1) relationship of a marketed 5LO inhibitor (Zileuton).

Objectives:

1. To develop a minimal systems model of 5LO inhibition and FEV1 regulation using literature data

2. To evaluate the possible mechanisms underlying the observed complex relationship between the PK and PD (FEV1) of Zileuton

3. To use the model to test alternate medical hypotheses
A systems model was developed integrating all known in *vitro*, in *vivo*, and clinical data on the relevant components of 5LO-mediated inflammatory path physiology and possible regulatory mechanisms involved in the response at the intracellular, cellular, and organism levels. This mathematical model contained the following components: (i) cell dynamics model of eosinophil (EO) maturation, migration, activation, and death, (ii) detailed biochemical model of 5LO operation, (iii) semimechanistic model of leukotriene (LT) biosynthesis in leukocytes, (iv) biophysical model of bronchoconstriction, and (v) PK model of Zileuton and its inhibition of the intracellular 5LO pathway. All model parameters were estimated on the basis of available literature data.

Multiple hypotheses were generated using the model to explain the observed delayed dose-response to Zileuton administration in asthmatic subjects. Simulations using the model indicated the following:

1. Acute bronchodilation after Zileuton administration was due to direct inhibition of LT synthesis. Doses of 400 and 600 mg maximally achieved this inhibition; hence, no dose-response is observed.

2. In the asthmatic state, high levels of activated inflammatory cells in the lung are driven by two positive feedback mechanisms.

   “Cellular LT production lead to activation and recruitment of further inflammatory cells.

   “Inflammatory cell derived cytokines, such as IL-5, lead to increased production and release of inflammatory cells from the bone marrow into blood.

3. Sustained high levels of inhibition of LT synthesis (> 85%) are required to interrupt these positive feedback mechanisms and so reduce the number of resident inflammatory cells, thus leading to reduced bronchodilatory stimuli (LT and non-LT such as hista-
mine) and subsequent chronic bronchodilation at doses greater than 400 mg.

4. The delay in the observation of dose-response is characteristic of EO cell lifespan in the airways.

5. As observed in literature, the model predicts that 5LO inhibition has inherently higher efficacy potential than LT receptor antagonism.
Session 2

Regulation and control in metabolic networks (part A)
Control of flux in the mammalian TCA cycle

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Abstract

As part of a collaborative project on mitochondrial metabolism, we have been developing a model of the TCA cycle and associated reactions in mammalian liver. The model involves simplified, saturable kinetics for the enzymes, with product inhibition, reversibility and effector actions included as appropriate. On testing the sensitivity of cycle flux to the values used for enzyme activity, we found that the flux control coefficients of the enzymes were highly variable, but there was a tendency for flux control to be distributed over a number of steps. If the activity of an enzyme with a significant amount of flux control was increased slightly, however, its degree of control fell away more abruptly than is normally the case, with control transferring to other enzymes. The consequence is that there is limited potential to increase the flux by activating any single enzyme. Although this behaviour is somewhat unusual, it can be reproduced in simplified models and there are precedents in other metabolic models. An explanation will be offered for why this behaviour can occur, and the implications discussed in relation to recent discoveries about the control of central carbon metabolism.

Introduction

As part of a collaborative project on multi-scale modelling of mitochondrial metabolism, we have been developing a
model of the tricarboxylic acid (TCA) cycle in mammalian liver. The aim is to combine this with a model of electron transport being developed in parallel by our collaborators. Here we report on some unusual behaviour of the flux control coefficients of enzymes in the model.

**Methods**

The processes currently incorporated in the model are: pyruvate dehydrogenase (PDH); reactions of the TCA cycle; electron-transport chain/ATP synthesis (as a single step process linking NADH generation via the cycle to ATP production); ATP hydrolysis; adenine-nucleotide exchange with the cytosol, and mitochondrial-cytosolic malate exchange. Concentrations of acetyl-CoA, TCA cycle intermediates, nicotinamide and adenine nucleotides are variables of the model. Most parameter values (enzyme kinetic and thermodynamic data and concentrations of external metabolites, e.g. pyruvate) have been derived from the literature for mammalian liver. Simplified Michaelis-Menten type equations [1,7] have been used throughout, except for cytosolic ATP hydrolysis which is a first order kinetic process. Only isocitrate dehydrogenase (NAD-dependent) and 2-oxoglutamate dehydrogenase were treated as irreversible, and product inhibition was included for all reactions except isocitrate dehydrogenase (NAD-dependent). The model has been simulated with COPASI [4] and ScrumPy [6].

**Results and Discussion**

The model reaches a steady state with fluxes and metabolite concentrations in the observed range for mammalian liver. The flux pattern shows features seen in $^{14}$C metabolic flux analysis experiments: approximately three-fold greater flux through pyruvate carboxylase than the cycle itself, and malate dehydrogenase running in reverse, feeding an outflow of malate to balance the input from pyruvate carboxylase.
The flux control coefficients for the TCA cycle flux show control can be distributed over a number of enzymes. However, a notable feature is that the control distribution varies abruptly if the activity of the enzyme with the greatest control is varied. This means that changing the activity of any single enzyme, even by a large amount, has relatively little impact on the cycle flux. We have previously observed an analogous exchange of control between rubisco and sedoheptulose bisphosphatase in a model of the Calvin cycle [3]. In this case, there is supporting experimental evidence that the in vivo activities of the enzymes are in the region where this exchange of control occurs.

Detailed examination of the TCA cycle model, and simplified variants showed that this behaviour was robust relative to many aspects of the representation. However, it did depend on an enzyme of the cycle becoming saturated with one of its substrates (in the strict sense of the enzyme rate becoming insensitive to change in the metabolite concentration, i.e. the elasticity starting to approach zero). When the activity of this enzyme is increased, material moves around the cycle and a different enzyme approaches saturation. It has generally been assumed that metabolic systems will avoid operating near saturation because of the danger that the steady state will break down with runaway metabolite concentrations. However, in cycles where there are additional constraints imposed by moiety conservation relationships, this danger can be mitigated. We propose therefore that the Calvin and TCA cycles may operate in this way to maximise the flux obtainable for a given investment in protein.

**Conclusion**

The dependence of the TCA cycle properties on the saturation characteristics of the enzymes demonstrates that it is important to represent enzyme saturability in kinetic models of metabolism, and that features may be lost by using approxima-
tions based on linear kinetics. The weak response of cycle flux to activation of a single enzyme underlines the need for multi-site modulation [2], also known as parallel activation [5] to generate significant increases in flux. A potential candidate for a new molecular mechanism to implement this will be proposed.

Acknowledgements

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References


Reconstruction and analysis of genome scale metabolic model of rice (*Oryza sativa*)

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Rice makes up 20% of total caloric intake for the human population as a whole. In order to assist the development of more efficient and nutritious rice cultivars, we are constructing a genome-scale metabolic model of *Oryza sativa*. The initial model has been built from the RiceCyc database. All reactions are checked for atomic balances and corrected where necessary and possible. The reactions unable to reach steady state owing to unbalanced production or consumption of reactants have been deleted. After addition of transport reactions for the input and output molecules we have obtained a working core model of more than 600 internal metabolites and 900 reactions.

The reconstructed network has been used to calculate some topological variables. Enzyme subset analysis shows that approximately half of the reactions exist in a subset with at least one other reaction. The metabolic capability (production of starch, cellulose, sucrose, amino acids, nucleotides, fatty acids, etc. from simple nutrients) of *O. sativa* is being analyzed using linear programming and the initial results show consistency with known biochemical knowledge. The reconstructed metabolic network is the first comprehensive network of a major crop.
How do nutrient resources shape the metabolic diversity within the Escherichia coli species?

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Abstract

In order to better understanding the role of ecological factors in the evolution of the species Escherichia coli, we studied the extent and structuration of diversity for 5 genetically distant strains grown in different environments for two sets of metabolic traits: the variation of protein abundances throughout the metabolic network and the variation of enzyme specific activity and catalytic constant. Our work reveals the huge metabolic diversity of the species and suggest new hypotheses about genotype-phenotype relationships. Available resources in the environment shape the general orientation of metabolism and drive preferential usage of a limited number of pathways. Genotypes determine the efficiency of the pathway through point changes in protein abundance in some environments only. However, variations of protein abundance do not fully translate into variation of enzyme activity because catalytic constants also exhibit both genetic and environmental
variation. The genetic variation of catalytic constants is negatively correlated to protein abundance and buffers the genetic variation of protein abundances.

**Introduction**

Microorganisms are remarkably adapted to a broad diversity of environments, including those which are very changing and unpredictable. Their ability to occupy these niches is in part due to their unsurpassed metabolic versatility and phenotypic plasticity. Among the medically important species, *Escherichia coli* species appears to be one of the most versatile one, being both a widespread commensal and a dangerous pathogen with large range of lifestyles. It has also huge genome plasticity with numerous gene loss and acquisition events occurring during the species evolution (Tenaillon et al., 2010). In this context, our objective was to gain a deeper understanding of the adaptation of the metabolism of *E. coli* species to different lifestyles. The quantitative description of metabolic diversity will pass through the integration of a broad range of heterogeneous experimental data into a model of cell functioning, which would ultimately be able to predict bacterial growth and survival given the environmental conditions.

Using whole genome sequences, whole genome metabolic networks have now been reconstructed for a large number of bacterial species, including *E. coli* strain *K12* [2]. Metabolites are linked to enzymes through the stoichiometry of the enzymatic reactions. The reactions and metabolites lists are built up through the integration of heterogeneous knowledge. Biomass composition rules can be used to constrain the system and were shown to correctly predict the metabolic fluxes leading to optimal growth (Ibarra et
al., 2005). However, those models only represent genetic diversity as the presence/absence of a reaction encoded by genes and do not take into account quantitative variation of enzyme activity. Metabolic Control Analysis explicitly address the question of the influence of enzyme activities on the steady state fluxes or metabolite concentrations [3,5] but experimental approaches are complex and can hardly be applied at the whole genome scale.

We chose here an intermediate approach to gain insight into the range of a variation for kinetic parameters like $V_{\text{max}}$ (maximum velocity) and $k_{\text{cat}}$ (catalytic constant), which are relatively easy to measure experimentally from quantitative proteomics and simple biochemical assays. $V_{\text{max}}$ is a composite parameter which depends on both $k_{\text{cat}}$ and enzyme concentration $[E]$ through the relationship $V_{\text{max}} = k_{\text{cat}}[E]$. While cellular enzyme concentrations are known to vary considerably within a species in a broad range of organisms [7], much less is known about the range of variation of kinetic parameters [1, 6, 8]. The aim of the work was to study the variation of protein abundances and enzyme activities throughout the metabolic network for different strains of the species $E. \text{coli}$ grown in different environments.

**Methods**

Quantitative proteomics was performed on 5 strains (IAI1, K-12, ED1a, Sakai, CFT073) grown in 4 different environments: two nutrient-complex media (LB and human urine), and two nutrient-simple media (minimal medium supplemented with glucose or gluconate). Proteins were extracted during the exponential growth stage and run on 2D gels. We identified 223 pro-
proteins distributed throughout the metabolic network and representative of the main biological processes. Network reactions were manually grouped into Pathway Categories using the Gene Ontology and our expert knowledge. Proteins were quantified using Progenesis. After normalization [9], the measure that we call protein abundance $E$ is proportional to the protein cellular concentration (moles/mg protein extract). Sources of variation for protein abundance $E_{ijkl}$ are the protein $\mu_i$, the nutrient medium $m_{ij}$, the strain $s_{ik}$ and the interaction between strains and nutrient medium $sxm_{ij}$.

The relative medium by protein effect $m_{ij}/\mu_i$ is a dimensionless measure of the relative effect of growing a strain in the nutrient medium $j$ on the abundance of protein $i$. The relative strain by medium by protein effect $[s_{ik} + (sxm_{ijkl})] / \mu_i$ is a dimensionless measure of the relative effect of growing the strain $k$ in the nutrient medium $j$ on the abundance of protein $i$. To see whether proteins that belong to the same pathway category behave the same when either the environment or the strain change, a Factorial Discriminant Analysis was performed separately on those two variables, with the Pathway Category as grouping factor. Whenever the Pathway Category effect is significant, the coordinates of the centroids of the group measure the average relative abundance of the proteins of the group. For a subset of 14 enzymes from central carbon metabolism, the enzyme specific activity $V_{ijkl}$ (moles/s/mg protein extract) was also assayed separately and used as a proxy for $V_{\text{max}}$. The catalytic constant was estimated by the ratio

$$kcat_{ijkl} = \frac{V_{ijkl}}{E_{ijkl}}$$
Standard errors for $k_{cat}$ were obtained by simulation of the residual variation, using the observed experimental errors for $V$ and $E$.

**Results & Discussion**

The 223 proteins (70% of which were enzymes) were well distributed throughout the 22 Pathway Category (between 2 and 39 proteins per category). Sources of variation for protein abundances were computed from ANOVA for each protein using (1). Quantification was highly repeatable (the mode of R2 was 0.70). We observed a large variation between strains and nutrient media for protein abundance (model coefficient of variation comprised between 0.04 and 2.29 with a mode at 0.43) and 212 proteins have at least one significant effect. Similar results were obtained by enzymatic assays for $V_{max}$, despite a lower repeatability (the mode of the R2 was 0.5). At least one effect was significant for all the enzymes, and the range of variation was much higher than for protein abundance (model coefficient of variation comprised between 1.43 and 1.81).

*Nutrient ressources determine the general orientation of metabolism*

Differences between media explain more than 50% of the total variation for protein abundance. All four nutrient media can be clearly distinguished by a specific distribution of protein abundances. Factorial Discriminant Analysis on the relative medium by protein effect with Pathway Category as grouping factor is highly significant: proteins from the same pathway category tend to be over- or under- abundant together. The first axis (40% of total variation)
separates the two nutrient simple media versus the two nutrient complex media. In simple media, proteins involved in amino-acid biosynthesis and energy production are over-abundant. The second axis (32% of total variation) is specific for gluconate medium, where the enzymes of the Entner-Doudoroff pathway are over-abundant. The third axis (12% of total variation) is specific for the human urine medium. In urine, acetogenic metabolism is important and the enzymes of acetate metabolism are over-abundant. Hence, the nutrient medium effect reflects the needs of the cell for the biosynthesis of metabolic precursors that are present in the culture medium. Available resources in the environment shape the general orientation of metabolism and drives preferential usage of a limited number of pathways which are those that are able to generate metabolic precursors from resources. Preferred pathways are characterized by the increase of protein abundance of all the pathway proteins.

**Differences between strains depend on the nutrient resource**

Differences between strains explain on average 26% of the total variation for protein abundance and translate in both strain and strain by medium interaction. Multivariate analyses on the relative strain by medium by protein effect show that all strain by medium combinations can be clearly separated by a specific distribution of protein abundances. However, those proteins do not cluster into Pathway Categories. Hence, each strain is characterized by point changes in protein abundance for some pathway proteins.
only. The set of proteins concerned by those point changes depend on the nutrient medium. These results suggest that genotypes may determine the efficiency of metabolic pathways by modulating the activity of enzymes with high control on the flux through changes in protein abundance.

But enzyme activities exhibit robustness to changes in enzyme abundance

The correlation between $V$ and $E$ depends on the catalytic constant $k_{cat}$ and it is expected to be positive if $k_{cat}$ is constant. Because both $V$ and $E$ vary between strains and between environments, we computed the correlation between $V$ and $E$ for each strain and each of the 14 enzymes over the four environments. We found a positive correlation in 60% of the cases. We also computed the correlation between $V$ and $E$ in each environment over the five strains and found a positive correlation in only 40% of the cases. Estimations of $k_{cat}$ confirm this result. Catalytic constants are variable between strains and between environments, but most of the variation is explained by differences between strains. Genetic variations for $k_{cat}$ tend to be negatively correlated with genetic variations for protein abundances. For this reason, variations of protein abundance do not fully translate into variation of enzyme activity.

The results will be further discussed in the light of Metabolic Control Analysis.
References

Avoiding presumptions - genetic analysis of metabolic networks

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As sequencing complete genomes remarkably sped up in recent years, the demand for reconstructed metabolic networks of various organisms is considerably rising. Nevertheless, combining those networks with experimental data remains particularly challenging. In a case study we reconstructed a metabolic network and analyzed its theoretical flux capabilities by applying Elementary Mode Analysis. One advantage of this approach is that all theoretical routes that can occur at steady state are calculated. In fact, it is still unclear whether organisms utilize only selected pathways, even if alternative routes are feasible to produce a target metabolite. So far, further methods that are designated to analyze a set of Elementary Modes, aim for strain optimization by preventing an organism to use less effective routes in order to potentially force it to maximize a yield of a particular target metabolite. However, this includes application of constraints that do not have to resemble the organisms true nature. We aimed for an analysis method of metabolic structure networks with minimal presumptions which is able to be applied for a diverse range of problems. Our method can be implemented to predict regulatory events of DNA/RNA binding factors and is applicable to analyze the effect of differentially expressed genes. We show that predictions of consequences from regulatory events are already valuable without including unproven presumptions, such as the evolution of maximizing metabolite yield.
Session 3

Regulation and control in metabolic networks (part B)
Modular analysis of metabolic control for large responses

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The cellular processes of organisms subject to changing environmental conditions or experimental manipulations show complex patterns of responses. The general laws governing these responses and the constraints limiting the patterns that could be achieved have been studied within the framework of metabolic control analysis (MCA).

Metabolic networks of intact systems show thousands of component reactions and variables, making full application of MCA being almost impossible. To solve this problem, modular strategies were introduced. Modular metabolic control analysis (MMCA) lumps large portions of the system into modules, leaving explicit the linking intermediates that are relevant to the questions that we aim to answer.

One important limitation of MCA and MMCA is that they strictly apply to infinitesimal changes in the variables. During the last years we have developed a MMCA that applies to large metabolic changes. The general theory for a system divided into two modules and one linking intermediate was developed. The component responses are described by elasticity coefficients for large changes. The systemic responses are quantified by control coefficients for large changes, with respect to parameters and with respect to rates. Conservation and summation constraints and relationships expressing the control coefficients in terms of the elasticity coefficients are derived. The definitions and relationships of the theory for large changes...
reduce to the expressions of traditional MCA when the changes are small. The elasticity coefficients for large responses may be calculated with data obtained from top-down experiments.

Examples are given to illustrate the application of the formalism. It is shown that, in some cases, extrapolation of results obtained for small (infinitesimal) changes to large responses leads to erroneous predictions.
How metabolic network design and constraints shape optimal adaptive responses

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Organisms continuously face dynamics in their environment and therefore adaptations are needed constantly. Adaptation restores vital network functions and improves fitness. Not everything is possible, network adaptation occurs within physicochemical and biological constraints. At present, adaptive responses of a network: why some enzymes go up in expression level whereas others go down, are poorly understood.

Proper functioning, in a fitness sense, or optimal functioning of a metabolic or signaling function depends on enzyme expression levels and their kinetic properties. This information suffices to predict responses of enzyme levels that restore optimal functioning upon changes in the environment. It is an open question whether gene networks can always realize those enzyme levels, given molecular information about the metabolic and signaling state. One can not rule out that constraints and trade offs are at play, at the level of the gene network, that prevent certain adaptive enzyme changes, as those would lead to a
reduction of fitness through some mechanism independent of the (sub-) network of interest.

Our results follow from the following insight: when the constraints acting on metabolism in terms of enzyme levels and other factors are known, optimal enzyme levels can be predicted from a kinetic model of metabolism alone. Then, the required constraint alterations in metabolic enzyme levels from one optimal metabolic steady state to the next upon a change in a metabolic parameter can be predicted. For differentially small changes in this parameter, optimal changes in enzyme levels under constraints can be understood in terms of metabolic network properties at the reference steady state to which the perturbation was applied.

We find that in order to understand adaptive responses constraints should be taken into account. For infinitely small perturbations changes in enzyme expression levels can be calculated using information of the reference state only. Based on our theory we predict that enzymes with high cost will have less adaptive changes in expression level. We also present theory, which relates the flux control coefficients at optimal networks in terms of constraints.
Entropic principles in carbohydrate metabolism

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Abstract

Most biopolymers, such as carbohydrates, chitins or peptides, are composed of a small number of monomeric building blocks which are linked by chemical bonds to form an enormous variety of unbranched or branched structures. In contrast to most enzymes in central metabolism, which catalyze one or a small number of precisely defined reactions, enzymes that are catalytically active on polymers usually catalyze a defined reaction pattern, but accept as substrates a wide variety of chemically similar structures.

The theoretical description of polymer active enzymes poses great challenges because every enzyme can in principle catalyze an infinite number of reactions. Obviously, a traditional approach in which differential equations are defined for every chemical species is inadequate.

Here, we particularly focus on carbohydrate active enzymes (CAZymes) and demonstrate that they are driven largely or exclusively by entropic gradients. We show that observed in vitro equilibrium distributions can be theoretically explained by a maximum entropy principle.
Introduction

Starch and glycogen are the most abundant energy storage carbohydrates in prokaryotic and eukaryotic cells. The monomeric units are exclusively glucose residues which are linked by α1,4- and α1,6-glucosidic bonds. Glucanotransferases are an important class of enzymes involved in both synthesis and breakdown of these carbohydrate storage compounds. They act on glucans, chains of glucose residues, and catalyze the transfer of maltosyl residues from one glucan to another. For example, disproportionating enzyme 1 (DPE1) is located in plant chloroplasts and is responsible for the removal of maltotrioses, chains of three glucose residues, which are inaccessible to the breakdown enzyme β-amylase, which cleaves two glucose residues from glucans with four or more glucose residues. If we focus on unbranched glucans, they can unambiguously be characterized by their degree of polymerization (DP), or chain length. If \( G_n \) denotes an α1,4 linked unbranched glucan, the removal of maltotrioses catalyzed by DPE1 is represented as \( G_3 + G_3 \rightarrow G_1 + G_5 \). However, in-vitro experiments show that the reaction pattern catalyzed by DPE1 is much more complex [2]. In equilibrium, glucans with a wide spectrum of DPs are observed and, moreover, the equilibrium DP distribution depends on the initial substrate. The experimental observations allow to conclude that DPE1 catalyzes a general reaction pattern \( G_n + G_m \rightarrow G_{n-q} + G_{m+q} \), in which the length of the transferred maltosyl residue, \( q \), is two or three.

A related enzyme, the cytosolic DPE2 involved in the metabolism of the soluble heteroglycan pool, catalyzes the transfer of single glucose residues between glucans and also displays similar equilibrium DP distributions. Interestingly, cytosolic phosphorylase (c-Pho), which catalyzes the transfer of a glucose residue onto inorganic phosphate, \( G_n + P_i \rightarrow G_{n-1} + G1P \), shows equilibrium DP distributions closely resembling those of DPE2.
Results

The reactions catalyzed by the above mentioned example enzymes are highly reversible. Calorimetric studies [3] have shown that bond enthalpies in linear glucans are independent on their position, which implies that the reactions catalyzed by DPE1 and DPE2 proceed without net enthalpy changes. We conjecture from this observation that increasing entropy must be the driving force for these reactions. If we define by $x_j$ the molar fraction of glucans with $j$ glucosidic bonds (and thus DP$=j+1$), a natural definition of the entropy arises from the Boltzmann-Gibbs formula

$$S = -\sum_j x_j \ln x_j$$

In an *in-vitro* experiment, the reaction mixture exchanges neither energy nor material with the environment and thus represents a thermodynamically closed system. In such a system, the equilibrium is characterized by maximum entropy. The reaction patterns of glucanotransferases impose two side constraints on the state variables $x_j$, namely the conservation of the total number of molecules, $\sum_j x_j = 1$, and the conservation of the total number of glucosidic bonds, $\sum_j j \cdot x_j = b$. Here, $b$ is a constant that is defined by the initial conditions ($b=\text{DP}_{\text{ini}}-1$) and that can thus be controlled experimentally. Maximizing the entropy (1) under the two constraints is easily performed by the method of Lagrangian multipliers and yields the equilibrium distributions

$$x_j = \frac{e^{-\beta_j}}{Z} \quad \text{with the partition sum } Z = \sum_j e^{-\beta_j} \quad \text{and } \beta = \ln \frac{b+1}{b}.$$
This predicted distribution agrees extremely well with experimentally observed equilibrium distributions for DPE1 and provides a consistent theoretical explanation of the dependence of the equilibrium distribution on the initial conditions.

For DPE2, it has additionally to be considered that this enzyme does not accept maltose as acceptor or maltotriose as donor [1]. This leads to the third constraint that the sum of glucose and maltose residues is conserved, \( x_0 + x_1 = m \), leading to an equilibrium distribution

\[
(3) \quad x_0 = \frac{m}{1 + e^{-\beta}}, \quad x_1 = \frac{m e^{-\beta}}{1 + e^{-\beta}}, \quad x_2 = (1 - m)(1 - e^{-\beta}), \quad x_{k+2} = x_2 e^{-\beta k},
\]

where \( \beta \) is determined from the implicit equation

\[
(4) \quad \beta - 2(1 - m)(1 - e^{-\beta}) = m e^{-\beta} + (1 - m) e^{-\beta}.
\]

Again, theoretically predicted distributions are in good agreement with experimentally observed equilibrium distributions.

The assumption of neutral enthalpy does not hold for c-Pho because bond enthalpies of phosphoester bonds and glucosidic linkages are considerably different. In this case, the driving force includes energetic and entropic contributions. The theoretical framework can still be applied by considering the respective contributions to the Gibbs free energy which, in equilibrium, assumes a minimum. Denoting the molar fraction of inorganic phosphate by \( v \), the molar fraction of glucose-1-phosphate by \( u \), and by \( \Delta g \) the difference in enthalpy between one mole glucosidic bonds and one mole phosphoester bonds, the Gibbs free energy can be written as

\[
(5) \quad G = \text{const.} + u \Delta g - TS,
\]

where now the entropy is defined as

\[
(6) \quad S = -R(u \ln u + v \ln v + \sum_j x_j \ln x_j).
\]
Minimization of $G$ has to be performed considering the conservation of total number of molecules, $u + v + \sum_j x_j = 1$, the conservation of the number of bonds, $v + \sum_j j \cdot x_j = b$, and the conservation of phosphate moieties, $u + v = p$, leading to

$$\frac{v}{u} = \frac{e^{-\beta RT}}{e^{-\Delta g RT}} \quad \text{end} \quad \frac{x_{k+1}}{x_k} = \frac{e^{-\beta RT}}{e^{-\Delta g RT}} \quad \text{for } k \geq s,$$

where $s$ is the minimal DP on which c-Pho is catalytically active. The exponent $\beta$ is determined by the implicit equation

$$\beta - s(1 - p) = p \frac{e^{-\beta RT} - \Delta g RT}{1 + e^{-\beta RT} - \Delta g RT} + (1 - p) \frac{e^{-\beta RT}}{1 - e^{-\beta RT}}$$

For $\Delta g = 0$, this equation is analogous to (4), explaining why the equilibrium DP distributions of c-Pho and DPE2 are highly similar.

**Conclusions**

We have provided a novel theoretical framework to describe the action of CAZymes, an important class on enzymes that catalyze a plethora of biochemical reactions which all follow a common pattern. In our theory, the parameter $\beta$ describes the equilibrium distribution of the reactants and can be determined from the initial conditions. This parameter thus provides a generalization of a simple equilibrium constant describing the equilibrium for a single reaction only.

We expect that our theory is widely applicable also to other classes of polymers, such as glycans, chitins and possibly even peptides. The theory in the present form allows only for the pre-
prediction of equilibrium distributions. However, this already allows predictions in which direction the concentration distribution will change if concentrations are known at a given time. A future challenge will be to expand this theory to non-equilibrium scenarios.

References


Genome scale reconstruction of *Salmonella typhimurium* LT2 metabolism

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Due to widespread bacterial resistance towards many classes of antibiotics there is an urgent need for novel agents effective against pathogenic bacteria. Metabolic enzymes are attractive targets for drug development due to their key role in physiology, conservation among pathogens, and the availability of high-throughput methods for their identification. Potential targets among single metabolic enzymes are however limited due to a high degree of metabolic redundancy in pathogens such as *Salmonella typhimurium* [1]. One approach for addressing this redundancy is to analyse the properties of an in silico representation of the complete known metabolic network of the organism, i.e. a genome scale stoichiometric model.

The metabolic model presented here was constructed based on metabolic data available from the MetaCyc database, including both experimentally verified enzyme reactions and reactions identified based on genome sequence. All reactions in the model were analysed for material imbalances, and reaction reversibility was assigned in accordance with experimental data when available. The model accounts for 948 reactions, including transport and intracellular reactions, 873 metabolites, and 1,003 genes. It is shown, using linear programming (LP), that the model can produce all biomass
precursors individually and in biologically relevant proportions, based on minimal glucose medium. Simulated growth on various carbon and nitrogen sources show good agreement with \textit{in vivo} data.

In order to identify reactions in the model that constitute an essential metabolic core the following analysis was conducted: The LP was set to produce fixed rates of biomass precursors, as well as a variable rate of a generic ATPase reaction, thus simulating an imposed energy demand on the network. Reactions that responded to the increasing energy demand by changing the rate over the range of fixed ATPase rates, were selected for further analysis. In contrast to simulation results obtained from an \textit{Arabiopsis} model [2] the \textit{Salmonella} model exhibited a more complex response, i.e. a larger fraction of reactions were responsive to the imposed energy demand.

The reactions identified by the above outlined analysis were highly variable in their correlation to the imposed rate of ATP hydrolysis. The cause of this variability and the uniqueness of the obtained metabolic responses is addressed.

\textbf{References:}

Session 4

System Biology and regulatory aspects of gene expression
Vertical genomics zooming into the gene-expression cascade

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Regulation Analysis, a quantitative methodology to dissect the regulation of metabolic fluxes in living cells, has revealed how regulation is distributed between metabolism and gene-expression. Depending on the conditions and on the genes of interest the relative importance of gene-expression and metabolic regulation vary dramatically. Moreover, also within the gene expression cascade, regulation is distributed. The old dogma of transcription being on the top of the regulation hierarchy has fallen apart. Various mechanisms of regulation at all levels from gene to functional protein have been identified. This complexity calls for an integrated, systems biology approach to understand the dynamics of the gene-expression cascade.

In the talk the question about how apparently simple mechanisms can lead to complex regulation at various levels of the gene-expression cascade will be discussed. Specifically, how dynamic modeling linked to experimental analysis of histon methylation led to new insights into the relation between histon methylation and the cell cycle will be shown. Furthermore, the functional implications of competition between macromolecules in the gene-expression cascade and how to assess these experimentally will be under discussion.
A general model for mRNA metabolism: from experiment to model and back

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Abstract

We present a general model for eukaryotic mRNA metabolism that considers multi-step enzymatic mechanisms of transcription initiation, mRNA elongation and degradation. We propose that measuring precursor and mature mRNA species accumulation and fitting it to the model allows determination of all the relevant transcription rate constants. We validate the approach experimentally on a nuclear-receptor regulated gene in a human cell line.

Introduction

In eukaryotes due to the repressive chromatin environment the regulation of gene transcriptional activity is linked to changes in chromatin structure at the gene promoter. Consequentially, regulated transcription initiation is an intricate process that requires coordinated action of multiple protein complex-
es involved in nucleosome modification and remodelling as well as pre-initiation complex formation. Recently, we have developed a detailed model of transcription initiation that reproduces transcriptional cycling of promoter states observed in cell population on different promoters and experimental cell systems [2,4,6]. The population-level cyclic changes in promoter chromatin state and protein occupancy result from the activity of single cells that is synchronous due irreversible sequential multi-step nature of transcription initiation mechanism. In agreement with existing experimental data, cyclic changes in chromatin accessibility for polymerase binding lead to bursts in mRNA production [5,7]. Under certain conditions synchronization of bursts results in cyclic changes of mRNA levels on population levels [3]. In current work we extend the model to include all stages of mRNA metabolism and test it experimentally.

Methods

For the mRNA time course measurement HepG2 cells were treated with 100nM of the PPARβ/δ agonist GW501516 or with vehicle (DMSO, 0.1% final concentration) and harvested at 15min intervals for 2.5h. Extracted mRNA was extracted and used for cDNA synthesis using oligo dT and random hexamer primers; fold induction of pre-mRNA (primer on intron1-exon1 border) and mature mRNA (primer on exon 1-exon3 border) relative to average DMSO-treated value was determined using real-time quantitative PCR. mRNA copy number per cell was determined using standard curve of Ct values versus amplified fragment copy number.

Results and discussions

We create a coarse-grained model of mRNA metabolism that can take into account multi-step enzymatic mechanisms of transcription initiation, elongation and degradation process-
es. We demonstrate that exact profile of mRNA accumulation after gene activation depends on the relative timescales of all the processes and propose that measurement of various mRNA species (precursor and mature) would allow determination of initiation, elongation, splicing and degradation times in a single experiment.

The approach is validated using adipose differentiation-related protein (ADRP) gene in human carcinoma cell line. ADRP expression is primarily regulated by peroxide proliferation activation receptor (PPAR), which can be activated by a specific synthetic ligand. We fit measured pre-mRNA and mature mRNA accumulation profiles using models with single-step and multi-step process description and validate fits using data from initiation inhibition experiments. The data is best described by the model with multi-step description of promoter cycle, mRNA elongation and degradation, rather than the single step one which is currently used to model transcription. The model also provides a better explanation for the mRNA degradation profiles found in the literature. We find that promoter cycling time is around 45 min and the mRNA burst size approximately 10 molecules which is consistent with previous observations [4,5], while the total elongation time is considerably longer (~50 min) than the available measurements [1].

The proposed model is liner and is shown to be identifiable for certain initial conditions, making it attractive for measuring transcription kinetic constants with minimal amount of perturbations. The approach can be extended to other genes and cellular systems.
References


Abstract

The signaling mechanisms of 48 nuclear receptors (NRs) have a universal design when the NR nucleo-cytoplasmic shuttling is concerned. We show how building a canonical NR signaling “blueprint” model could be followed by its parameterization for any particular NR under its relevant physiological conditions. We discuss perspectives of using this strategy whilst building the silicon human.

Introduction

NRs belong to a family of transcription factors involved in a diverse range of regulatory functions, such as the development, inflammation and metabolism [1-7]. NRs are synthesized in the cytoplasm and shuttle between nucleus and cytoplasm. When the NR binds its ligand (ligand for NR is a lipophilic molecule, usually with a steroid structure), the conformation of the NR changes. The change of the NR conformation changes its ability to bind with its response element (a
specific nucleotide sequence on the DNA). When the NR is bound to its response element, it participates in transcription initiation. In this way, the NR functions as a part of a signaling process where the ligand is the signaling molecule and the response is the change of the transcription of DNA into mRNA, which can then be translated to the corresponding protein. The amplitude of the transcriptional response depends on the concentration of liganded receptor near its response elements. Consequently, ligand-dependent changes of NR intracellular localization may be involved in the signaling process, to a different extent for each of the 48 members of the NR family. For example, a ligand-dependent shift of the receptor from cytoplasm to cell nucleus seems crucial in glucocorticoid receptor (GR) signaling. Although to a smaller extent, it can be also relevant for vitamin D receptor (VDR) signaling, only 10% of which is located in the nucleus in the absence of ligand, but 50% in its presence. Other receptors, e.g. the pregnane X receptor (PXR) may have nuclear localization both in the absence and presence of the ligand (see Table 1). Biologically, this looks very different and it could be perceived that the silicon human will require 48 entirely different NR models. But is this really true? Or could all NR models be considered as instantiations of the same network design?

**Methods**

The network diagram (Figure 1) presents a simplified canonical network structure of NR signaling.

The receptor can be present in two different pools: ligand-free NR (Nr) and ligand-bound NR (NrL). Both Nr and NrL can be transported through the nuclear membrane and compete for binding to a response element (Re) on the DNA. Based on this network structure we have built the “blueprint” kinetic model described in the Supplemental Information.
Several kinetic parameters (e.g. the \( K_d \) of GR-ligand binding) have been taken from literature (Supplemental Information, Table 2), others were fitted over a realistic physiological range towards reproducing the known systemic behavior. For example, the kinetics of ligand-free GR nuclear import was assumed to be equal to that of the nuclear import of Smad proteins [8].

The kinetic rate constant of ligand-free GR export and liganded GR import and export have been fitted to reproduce the GR intracellular localization as described in Table 1.

![Diagram](image)

**Figure 1. Canonical network structure of NR signaling**

Table 1. Intracellular localization of different NRs in the absence or presence of ligand (as fraction of molecules from the total number of molecules)

<table>
<thead>
<tr>
<th></th>
<th>Before ligand addition</th>
<th>After ligand addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nucleus</td>
<td>cytoplasm</td>
</tr>
<tr>
<td>GR</td>
<td>5 % (19 nM*)</td>
<td>95 % (102 nM*)</td>
</tr>
<tr>
<td>VDR</td>
<td>10 % (37 nM*)</td>
<td>90 % (97 nM*)</td>
</tr>
<tr>
<td>PXR</td>
<td>96 % (356 nM*)</td>
<td>4 % (4 nM*)</td>
</tr>
</tbody>
</table>

*taking into account that \( V_{nuc} = 0.45 \) pl and \( V_{cyt} = 1.55 \) pl and total number if NR is \( 10^5 \) molecules/cell

**Results and discussions**

The “blueprint” model presented on Figure 1 has been tuned for GR, VDR and PXR cases by changing parameters of ligand-free GR export and of liganded GR import and export. The results of this parameter tuning are presented on Figure 2. We could also establish that a sufficient transcriptional
response, taken as the ratio \((\text{Re} \text{NrL} + \text{Re} \text{NR})/\text{Retotal}\) can be observed in all 3 cases. Moreover, the timescale of the change in NR intracellular localization was similar to the one published in literature.

This demonstrates that different NR systems can be considered as instantiations of the same reaction network. Our NR model is still on its way to become a real silicon cell model that requires a future efforts. On the time being, that model is based on the hybrid strategy: some parameters are really measured, others are fitted, and others are guessed in a realistic physiological range. We used this model only as an example of a “blueprint” approach.

Another aspect of “blueprint” approach concerns the way of how online silicon cell models are being developed. For example, the network structure and the rate laws of every model at JWS site are fixed, but kinetic parameters can be changed locally by the user without affecting the default values stored in the curated database on the server [9]. In fact, what is stored is a “blueprint” model. For example, upon obtaining either better kinetic values or values for different physiological conditions, any experimentalist can easily re-parameterize the model and run simulations online. And in case of improving the model to a version to be published in any of a select number of journals, the investigator can send results to the model curating team, so that the default “blueprint” model can be continuously improved. The same approach could be promising for larger-scale models for the whole body human and will ultimately result in online silicon human.
Fig. 2. Time course of changes in intracellular localization and transcriptional response for GR, VDR and PXR models. The transcriptional response is taken to equal the ratio $(Re_{NrL} + Re_{Nr})/Re_{total}$, i.e. the fraction of REs attached to NR.
**Supplemental Information**

**Table 2. Model description**

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>v₁</strong> Nuclear response element binding liganded receptor:</td>
<td>$k_{1f} = 60 \text{ nM}^{-1}\text{min}^{-1}$; $k_{1b} = 24000 \text{ min}^{-1}$</td>
</tr>
<tr>
<td>$(k_{1f} \cdot \text{Re}_n(t) \cdot \text{Nr}<em>n(t) - k</em>{1b} \cdot \text{Re}<em>n(t)) \cdot V</em>{\text{nuc}}$ [nmoles/min]</td>
<td></td>
</tr>
<tr>
<td><strong>v₂</strong> Nuclear receptor binding nuclear ligand in the nucleus:</td>
<td>$k_{2f} = 60 \text{ nM}^{-1}\text{min}^{-1}$; $k_{2b} = 60 \text{ min}^{-1}$</td>
</tr>
<tr>
<td>$(k_{2f} \cdot \text{Nr}<em>n(t) \cdot L_n(t) - k</em>{2b} \cdot \text{Nr}<em>n(t)) \cdot V</em>{\text{nuc}}$ [nmoles/min]</td>
<td></td>
</tr>
<tr>
<td><strong>v₃</strong> Mass Action: Nuclear receptor that sits near plasma membrane binds ligand from the plasma membrane.</td>
<td>$k_{3f} = 60 \text{ nM}^{-1}\text{min}^{-1}$; $k_{3b} = 60 \text{ min}^{-1}$</td>
</tr>
<tr>
<td>$(k_{3f} \cdot \text{Nr}<em>c(t) \cdot L_n(t) - k</em>{3b} \cdot \text{Nr}<em>c(t)) \cdot V</em>{\text{cyt}}$ [nmoles/min]</td>
<td></td>
</tr>
<tr>
<td><strong>v₄</strong> Active transport of nuclear receptor between cytosol and nucleus:</td>
<td>For GR:</td>
</tr>
<tr>
<td>$Kappa_4f \cdot \text{Nr}_c(t) - Kappa_4b \cdot \text{Nr}_n(t)$ [nmoles/min]</td>
<td>$Kappa_4f = 0.1 \times 10^{-12} \text{ L/min}$; $Kappa_4b = 0.5 \times 10^{-12} \text{ L/min}$</td>
</tr>
<tr>
<td></td>
<td>For VDR:</td>
</tr>
<tr>
<td></td>
<td>$Kappa_4f = 0.1 \times 10^{-12} \text{ L/min}$; $Kappa_4b = 0.26 \times 10^{-12} \text{ L/min}$</td>
</tr>
<tr>
<td></td>
<td>For PXR:</td>
</tr>
<tr>
<td></td>
<td>$Kappa_4f = 0.1 \times 10^{-12} \text{ L/min}$; $Kappa_4b = 0.007 \times 10^{-12} \text{ L/min}$</td>
</tr>
<tr>
<td>Reactions</td>
<td>Parameters</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>v5</strong> Active transport of liganded nuclear receptor between cytosol and nucleus: $Kappa_{5f} \cdot N_r(t) - Kappa_{5b} \cdot N_r(t)$ [nmoles/min]</td>
<td>For GR:</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5f}=2 \times 10^{-12}$ L/min;</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5b}=0.025 \times 10^{-12}$ L/min</td>
</tr>
<tr>
<td></td>
<td>For VDR:</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5f}=0.3 \times 10^{-12}$ L/min;</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5b}=0.08 \times 10^{-12}$ L/min</td>
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<tr>
<td></td>
<td>For PXR:</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5f}=0.36 \times 10^{-12}$ L/min;</td>
</tr>
<tr>
<td></td>
<td>$Kappa_{5b}=0.002 \times 10^{-12}$ L/min</td>
</tr>
<tr>
<td><strong>v6</strong> Ligand diffusion between cytosol and nucleus: $Kappa_6 \cdot (L_c - L_n(t))$ [nmoles/min]</td>
<td>$Kappa_6=^{*}kappa_{Ligand diffusion}=32 \times 10^{-12}$ L/min; $L_c = 0.005$ nM</td>
</tr>
<tr>
<td><strong>v7</strong> Nuclear response element binding liganded receptor: $(k_7 \cdot Re_n(t) \cdot N_r(t) - k_{7b} \cdot Re N_r(t)) \cdot V_{nuc}$ [nmoles/min]</td>
<td>$k_{1f}=60$ nM$^{-1}$ min$^{-1}$; $k_{1b}=4800$ min$^{-1}$</td>
</tr>
<tr>
<td><strong>v8</strong> Nuclear receptor binding nuclear ligand in the nucleus: $(k_{8f} \cdot Re N_r(t) \cdot L_n(t) - k_{8b} \cdot Re N_r L_n(t)) \cdot V_{nuc}$ [nmoles/min]</td>
<td>$k_{2f}=60$ nM$^{-1}$ min$^{-1}$; $k_{2b}=60$ min$^{-1}$</td>
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### Balance equations

<table>
<thead>
<tr>
<th>Equation</th>
<th>Rate Expression</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{dL_{n}(t)}{dt} )</td>
<td>( \frac{(v_6-v_3)}{V_{\text{nuc}}} ) [nM/min]</td>
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</tr>
<tr>
<td>( \frac{dL_{c}(t)}{dt} )</td>
<td>0 (considered an infinite reservoir)</td>
<td></td>
</tr>
<tr>
<td>( \frac{dRe(t)}{dt} )</td>
<td>( \frac{(-v_1-v_7)}{V_{\text{nuc}}} ) [nM/min]</td>
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<tr>
<td>( \frac{dReN_{rn}(t)}{dt} )</td>
<td>( \frac{(+v_7-v_8)}{V_{\text{nuc}}} ) [nM/min]</td>
<td></td>
</tr>
<tr>
<td>( \frac{dReN_{rn}L_{n}(t)}{dt} )</td>
<td>( \frac{(+v_1+v_8)}{V_{\text{nuc}}} ) [nM/min]</td>
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</tr>
<tr>
<td>( \frac{dReN_{rn}L_{c}(t)}{dt} )</td>
<td>( \frac{(-v_1+v_5)}{V_{\text{cyt}}} ) [nM/min]</td>
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<tr>
<td>( \frac{dN_{rn}}{dt} )</td>
<td>( \frac{(+v_4-v_2-v_7)}{V_{\text{nuc}}} ) [nM/min]</td>
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<tr>
<td>( \frac{dN_{rn}L_{n}(t)}{dt} )</td>
<td>( \frac{(+v_2+v_5)}{V_{\text{cyt}}} ) [nM/min]</td>
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<tr>
<td>( \frac{dN_{rc}}{dt} )</td>
<td>( \frac{(-v_3-v_4)}{V_{\text{cyt}}} ) [nM/min]</td>
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<tr>
<td>( \frac{dN_{rc}L_{c}(t)}{dt} )</td>
<td>( \frac{(+v_3-v_5)}{V_{\text{cyt}}} ) [nM/min]</td>
<td></td>
</tr>
</tbody>
</table>

### Conserved Moieties

**DNA\text{\textsubscript{total}} (V_{\text{nuc}})**

\[
\text{Re}_n(t) \cdot V_{\text{nuc}} + \text{Re} \cdot N_r(t) \cdot V_{\text{nuc}} + \text{Re} \cdot N_rL_n(t) \cdot V_{\text{nuc}}
\]

[nmoles/cell]

\[
\text{DNA}_{\text{total}} \cdot V_{\text{nuc}} = 1000 \times 10^9 / N_A = 1.67 \times 10^{-12} \text{ nmoles (10}\text{\textsuperscript{5}} \text{molecules/cell)}
\]

**NR\text{\textsubscript{total}} (V_{\text{nuc}} + V_{\text{cyt}})**

\[
\text{Nr}_n(t) \cdot V_{\text{nuc}} + \text{Nr}_c(t) \cdot V_{\text{cyt}} + \text{Nr}_nL_n(t) \cdot V_{\text{nuc}} + \text{Re} \cdot N_rL_n(t) \cdot V_{\text{nuc}} + \text{Re} \cdot N_r(t) \cdot V_{\text{nuc}}
\]

[nmoles/cell]

\[
\text{NR}_{\text{total}} (V_{\text{nuc}} + V_{\text{cyt}}) = 10^4 \times 10^9 / N_A = 167 \times 10^{-12} \text{ nmoles (10}^5 \text{molecules/cell)}
\]
<table>
<thead>
<tr>
<th></th>
<th>( \text{Re}_a(0) )</th>
<th>( \text{Re} , \text{Nr}_a(0) )</th>
<th>( \text{Re} , \text{Nr}_L_a(0) )</th>
<th>( \text{Nr}_a(0) )</th>
<th>( \text{Nr}_L_a(0) )</th>
<th>( \text{Nr}_c(0) )</th>
<th>( \text{Nr}_L_c(0) )</th>
<th>( L_a(0) )</th>
<th>( L_m(0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{GR}: 3.52 \text{ [nM]} )</td>
<td>( \text{GR}: 0.17 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{GR}: 20 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{GR}: 101 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( 100 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
</tr>
<tr>
<td></td>
<td>( \text{VDR}: 3.37 \text{ [nM]} )</td>
<td>( \text{VDR}: 0.36 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{VDR}: 38 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{VDR}: 96 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \text{PXR}: 1.96 \text{ [nM]} )</td>
<td>( \text{PXR}: 1.7 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{PXR}: 355 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td>( \text{PXR}: 4 \text{ [nM]} )</td>
<td>( 0 \text{ [nM]} )</td>
<td></td>
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</tr>
</tbody>
</table>

**Volumes:** \( V_{\text{nucl}}=0.45 \times 10^{-12} \text{ L} \); \( V_{\text{cyt}}=1.55 \times 10^{-12} \text{ L} \); \( V_{\text{cell}}=2 \times 10^{-12} \text{ L} \);

\( N_A=\text{Avogadro’s number}=6.02 \times 10^{23} \); 1 L=1 litre=\( 10^{-3} \text{ m}^3 \); 1 nM=\( 10^{-9} \) moles/L.
References


Structure function relationships in HIV-I virus-host

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Human immunodeficiency virus-1 (HIV-I) has a minimal genome of only 9 genes, which encode 15 proteins. HIV-1 thus depends on the human host for virtually every aspect of its life cycle. The universal language of communication in biological systems, including between pathogen and host, is via signal transduction pathways. The fundamental units of these pathways are protein-protein interactions. Understanding the functional significance of HIV-I, human interactions require viewing them in the context of human signal transduction pathways. We focused our host pathogen study on the binding and competing interactions of HIV-I and human proteins. We showed that peptide motifs conserved across 70% of HIV-I subtype B and C samples occurred in similar positions on HIV-I proteins. We predicted which human proteins may be targeted by HIV-I by taking pairs of human proteins that may interact via motif conserved in HIV—I and the corresponding interacting protein domain. Our predictions were enriched with host proteins known to interact with HIV-I proteins ENV, NEF and TAT. Cellular pathways statistically enriched for our predictions include the T cell receptor signaling, natural killer cell mediated cytotoxicity, cell cycle, and apoptosis pathways. The current state of knowledge investigating Tat interactions with signal transduction pathways is still in its infancy but has made significant progress toward understanding HIV pathology. This area is of great interest because Tat is among a small group of newly discovered RNA–based regulators of transcription. What
is more important, however, are the implications of understanding these interactions concerning HIV-I infected individuals. With the failure to develop effective HIV vaccines after years of development, it is becoming more feasible to conjecture therapies that target Tat and signal transduction pathways promises to provide a wealth of information about transcriptional control as well as the regulation of immune cell activation.
Session 5

Virtual modeling of living systems
Use of linear programming and elementary modes analysis to investigate metabolic networks

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We have previously described a method by which a metabolic network can be hierarchically decomposed into separate modules [1]. This was based on an analysis of the null-space of the system, and, as such, identified correlations between individual reactions over all possible steady-flux distributions. A draw-back to this approach is that all reactions are assigned equal weighting in the analysis, regardless of likely differences in the magnitude of flux carried under realistic conditions. This in turn leads to a rather “unfocussed” modularisation.

More recently we have performed linear programming (LP) on a genome scale model of the Arabidopsis metabolic network, subject to variable constraints of energy demand [2]. This provides a great improvement in that now a much reduced set of reactions from the whole model can be identified.

In the present contribution we briefly review this work and then examine the results of a similar analysis of a medium-scale model of photo-synthetic metabolism, comprised of the core reactions of the chloroplast, cytosol and mitochondria. In this case the constraint is photon availability, and the response of LP solutions to variations in this constraint are examined and used to create a “metabolic tree”. The flux values obtained in the solutions are compared to the elementary modes of the system using a previously described algorithm for assigning fluxes to elementary modes [3]. In this way we identify how changes to flux in the system in the
face of changes of a realistic constraint can be described in terms of changes in utilization of elementary modes.

References


Multiple Environment Stoichiometric Analysis

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Constraint-based modeling has become an important tool in the analysis and understanding of genome-scale stoichiometric models of cellular systems. The development of these methods has been highlighted by rapid advances in ‘omics technologies that generate the data necessary for the creation of fully annotated “in silico” reconstructions of entire unicellular organisms[1].

One commonly used approach, Flux Balance Analysis (FBA), has been successfully employed in investigating the growth of microorganisms, under a single set of defined environmental conditions. However, in the wild, such an organism may experience changes in state that arise from sources either externally (e.g. a change in nutrient supply due to the microbe’s own activity or the activities of other species of microorganisms in its environment) or internally such as a mutation (deletion) in a particular gene which leads to a concomitant loss of (or large change in) cellular function. In this study we will be looking at the case where a microorganism is challenged to switch between multiple nutrient sources.

We use the genome scale metabolic reconstruction (GSR) of Escherichia coli K-12 [2] (iJR904) available from the BiGG Database [3] and analyze it with a newly developed tool for...
constraint based modeling: PyscesCBM. This software has been developed as a Constraint Based Modeling extension to the PySCeS simulation software [4] and allows for the interactive analysis and manipulation of genome scale models as well as the automatic construction of the linear programs needed for a multistate analysis.

The results of the multistate analysis show that when a changing nutrient source is considered, it may be advantageous for a microorganism to optimize its internal metabolism so that a minimum metabolic penalty is incurred when switching from one nutrient source to another.

References

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Spatial-time gradients and their modeling in virtual phantoms

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Modern trends in biomedical research are keenly focused on evident presentation of biological objects. They have to be described both in biochemical and structural properties in conditions which are very close to the native state of the considered species. In this case the system includes non-homogeneous non-steady state distributions of metabolites defined in the real complex geometrical pattern. In order to estimate these gradients and applied them to real biological objects the problem was divided into two parts in the present study. First of all the image of real biological object is converted into a virtual phantom and then the superposition of large set of small sphere sources for some kind of metabolites in consumed media is laid in the digital figure. Finally, the spatial areas of calculated concentrations can be matched to initial image and it, thereby, concerns practical aspects of virtual modeling because the result will describe particular case of real organ, tissue etc.

Key words: diffusion, virtual images, non-steady state gradients.

Introduction

Trying to create a mathematical model of some biological object, investigator is involuntarily going to simplify the analyzed system. The main reason for such action is a desire to contemplate an analytically representable pattern which still has more or less correct properties of the initial object. However, this way conceals
many reefs, which can appear as a great obstacle to discover the essence of living cell processes. Variety of life is inevitable substituted by limited range of regulatory properties of physical system and this approximation is not always satisfactory. Biophysicists solve this problem by using of sophisticated equation systems whereas following Denys N. Wheatley we can say that “…complex systems such as a living cell cannot be reduced to simple equations.” [1]. Nevertheless, before full stop this sentence at the present time should be extended by the words: “…without implication of potential computer simulation of considered objects”. It means that the lack of regulatory properties of living system could be partially (but essentially) substituted by a computer programming. Virtual objects are able to mimic precisely different dynamic parameters of the living prototypes. However, virtuality is not a life yet. On the other hand, there is no other chance to make a model besides simplify and fit initial system to some suitable physical problem.

Thus the most useful result will be that one which combines simplicity of physical system and complexity of life. In the present study an attempt has been made to represent possible application of diffusion to the real tissue patterns. This step can be considered as start of a new branch of system biology: metabolic imaging of living objects.

**Creation of virtual phantom of biological object**

As it is mentioned above, the first step to represent non-steady state distribution of metabolites in some object is the creation of digital space of modeling. In fact, one needs to determine the physical medium where the diffusion would occur. This process is implicitly fulfilled when a mathematician sets the problem for partial differential equations. The symmetry of the space, initial and the border conditions define the geometry and some properties of the considered medium. In general the geometry of the system supposes to be very simple. On the contrary, for the real object (tissue or or-
gan) geometry of the medium is extremely intricate and the creation of fitted and simplified phantom is a separate non trivial problem. In order to create a flat phantom of biological object GetData Graph Digitizer version 2.24 (© 2002-2008 S. Fedorov) has been used in the present study. The difference between 2D and flat phantom is in a finite thickness of analyzed layers. Flat phantom could be considered as a 3D phantom with a small dimension through one axe. The example of such phantom was shown in Figure 1.

First of all coordinate systems must be set up. It is reasonable to use Cartesian coordinates with initial point which is disposed in the one of image corners. For the considered example the initial point was placed in the left bottom corner.

Then digital scale of the image is marked on the graticule (Figure 1c). It is as high as 5.33 micm per pixel and the size of whole picture is 197(X) on 219(Y). Further essential procedure is a division of the object on the homogenous parts. Inside each of these parts the parameters which characterize the object assume to be fixed. The rules for the division depend on the prototype of the made phantom. It is easy to see that in the considered example there are several blood vessels which cross the area of mesentery tissue. Thus the vessels and the surrounding tissues are type I and type II of homogenous parts respectively. It should be stressed that vessel are not identical in their type. For example there is an arteriole among marked vessels (it is a vessel in the upper part of the image, pink line Figure 1d). That is why in type I of phantom space two subtypes could be also identified: i) arterioles ii) venules. This classification will cause the difference in given concentrations of oxygen and other metabolites in blood stream inside the vessels.

Since the considered area consists of only two generally different objects (tissue and vessels) so the geometry of phantom is initially made by fixing of coordinates (borders) for separated areas. It is reasonable to indicate vessels borders explicitly because
they are much less than surrounding tissues both in volume and in surface. Each vessel is approximate as a cylinder and it is described by the coordinates of the points laying on the central axis and by the diameter of outside border. Thus we obtain the set of $M$ vessels where each of them is divided on $N_i$ connecting cylinders:

$$\text{Vessels} := \left\{ \left( X_{\text{start}}^{ij}, Y_{\text{start}}^{ij} \right), \left( X_{\text{end}}^{ij}, Y_{\text{end}}^{ij} \right), d_{ij} \right\}^{N_i}_{j=1}^{M}_{i=1};$$

$$\forall j : j \leq N \Rightarrow \left( X_{\text{start}}^{ij}, Y_{\text{start}}^{ij} \right) \equiv \left( X_{\text{end}}^{i(j-1)}, Y_{\text{end}}^{i(j-1)} \right).$$

It is possible, that $j \neq k$, $d_{ij} \neq d_{ik}$, but the description of the vessels should be sensible made in such way when their diameters are fixed at least on some segments. The example of such phantom is shown in Figure 1d-e and it is also illustrated by the table of numbers.

Then remaining part of the considered area is divided on equal parts which have coordinates given by the rule:

$$\text{Tissue} = \left\{ \left( X^i, Y^i \right) \in \text{Vessels}, d = \max_{j \in \mathbb{N}} \left( d_{kj}, j = 1, ..., N_i, k = 1, ..., M \right) \right\}^{L}_{i=1};$$

Each pair mentioned above indicates the center of a small parallelepiped and thereby a mesh is formed with excluded lattice points on the image. All parameters of the model are considered as fixed ones inside the areas. Their sizes depend on the value of subinterval $L$. For acceptable accuracy of calculations it can be in the range from 100 to 200. The volume and the surface area of the homogenous parallelepipeds depend on the scale of initial image and they also define by the...
Figure 1 Creation of flat phantom for example of visual analysis of rat mesentery. For details see text.
aim of visual presentation of spatial-time distributions of concentration.

**Forming of spatial-time distribution of metabolite concentration in a virtual phantom**

Having formed a digital phantom of some biological object one needs to define the procedure how the gradients of metabolites have to be shaped in it. Let us consider a simple example of flat phantom. Without lost of generality it is supposed that the prototype consist of a short branch of blood vessel surrounding by nearly homogenous tissue. The phantom of this object can be created as it is described above (Figure 2a). It is assumed that the spheres with fixed diameters are placed along indicated line. Thus the chain of spherical sources has been explicitly represented. Diffusion of some metabolite occurs from sources into surrounding space if one considers the problem of tissue supply by bloodstream (Figure 2b). The phantom must be divided into elemental areas in order to represent the results of modeling. It could be made by placing of a grid with some defined scale. Grid pitch indicates the centers of mentioned areas (Figure 2c). A size of the grid depends on the quality of initial image and its definition but for usual case 40 000 locations (200x200) are quite enough. Then a value of metabolite concentration in each defined area and fixed time is the superposition of diffusion from all sources. The function for each source is the solution of a boundary problem where the coordinate corresponds to the distance from the source to considered area.

\[
\frac{\partial X(r,t)}{\partial t} = -k \cdot X(r,t) + D \cdot \left( \frac{\partial^2 X(r,t)}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial X(r,t)}{\partial r} \right)
\]

\[
\frac{\partial X(r,t)}{\partial r} \bigg|_{r=r_0} = -Q; \hspace{1em} X(r,0) = 0; \hspace{1em} r_0 \leq r < +\infty; \hspace{1em} 0 \leq t < +\infty
\]
The explicit form of the solution for border problem could be found elsewhere [2,3]. The solution depends on border flux $Q$ linearly and it raises $r_0^2$. The border condition for each source is given by researcher separately. In the represented example (Figure 2) the number of sources is equal to 22 therefore the concentration of some metabolite $X$ in $i^{th}$ area will be:

![Figure 2](image_url)

**Figure 2.** Step-by-step forming of sources in a phantom. For details see text.

In adjoining to sources areas (they are indicated in yellow in Figure 2d) it is defined the following rule: if the center of the area is overlapped with a source then the area is fully omitted and it is substituted on the source. If the area is extended on some (even small) distance from sources then it is consid-
ered on usual manner. In order to avoid complication of programming the places of sources are also included into the final gradient pattern and they are identified by the values on their borders.

The results of modeling are presented in Figure 3. The colored areas indicate the concentration of metabolite in some points of digital phantom. It is remarkable that there are several spherical centers in homogenous space of surrounding tissue where the metabolite can be produced or consumed (Figure 2d, Figure 3b, c). The flux through this centers disturbs a smooth gradients obtained for a long producing source imitating a blood vessel (Figure 2c). The final figure representing the pattern of gradient can be lay over the initial image by overlapping of the last points in the axes. This procedure is very useful for practical needs because the gradients are applied to the real example of biological system. The development of such methods could be considered as a good research tool for clinical investigations.

**Figure 3** Spatial gradients in phantom of biological object under different characteristics of the sources. The parameters of the solution are fixed and they are the following: $D = 5 \cdot 10^{-7} \text{cm}^2 / \text{s}$; $Q = 1 \text{mmole} / \text{cm}^2$; $k = 0.25 \text{s}^{-1}$; Diffusion occurs under different conditions. The first case corresponds to a single producer long source (A) and the next ones describe the gradients in the medium with several additional randomly placed sources (Figure 2d). One phantom includes producing sources (B, $Q_{\text{medium}} = 0.17 \text{mmole} / \text{cm}^2$) and another one yields a consumption of the metabolite in the same centers (C, $Q_{\text{medium}} = -0.17 \text{mmole} / \text{cm}^2$).
Discussion

It would be necessary to notice that in spite of a bounder problem with the first kind border conditions, the same one with the second kind border conditions allows to consider examples not only with sources which have different “power”, but also the cases where non-homogeneity is essentially different. It means that the medium with consuming centers can be modeled. These centers are the area in the phantom where the metabolite is absorbed at the border. The areas could be represented as simple sphere sources with fixed diameters where the border flux is negative (Q<0). It is remarkable that the medium is able to consume the metabolite itself and this property is explicitly localized in the constant $k$. However, the general sense of such areas is to represent evidently the structure of the biological object where metabolites can absorb. Prototypes for phantom centers could be a cluster of cells, elements of reticulum, mitochondria, etc. Whole distribution of the metabolite will be obtained by the superposition of its producing and consumer sources and the balance between these different fluxes depends on external condition and considered problem.

Thus, the result of presented study makes it possible to visualize metabolites gradients in a phantom of any kind of biological object and to retrace their further time dynamic.

Acknowledgements

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References


Vascular transport in plants: an advection-diffusion-reaction model of sucrose accumulation in sugarcane

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Introduction

Besides being of structural importance, the sugarcane stalk is also the primary storage organ for carbohydrates. Significant changes occur in sucrose metabolism when moving from immature to mature internodal tissue: sucrose accumulation increases, while growth slows down. Futile cycling of sucrose is an example of a process that negatively impacts sucrose accumulation. We have previously developed a kinetic model based on ordinary differential equations to simulate internodes 3–10 of a sugarcane stalk [5]; growth changes were modelled by substitution of internode-specific parameters. This approach has limitations, since phloem translocation is the result of advection due to an osmotically generated pressure gradient (the Munch hypothesis [3]).

To incorporate source/sink interactions, we here present a framework for solving a kinetic metabolic model when some of the species are subject to long-distance transport, allowing us to model all eight internodes at the same time.
Methods

The simplified phloem flow description of Thompson [4] is used to model advection. The model has compartmentalised species (the compartments are source, phloem, apoplast, symplast and vacuole), which, depending on the type of compartment, may undergo advection-diffusion, facilitated or active transport across a membrane or participate in chemical reactions. The model is cast as a set of Partial Differential Equations and is solved numerically with the FiPy software [2]. Sucrose translocation in the phloem is modelled explicitly, providing a bridge between internodes. Using known reactant concentrations as initial values, the model was solved numerically to produce an 11-h time course simulation. The model was also analysed using the Fourier Amplitude Sensitivity Test [1], a global sensitivity analysis method, to determine which parameters have the greatest influence on the model output.

Results and discussion

The model exhibited many of the traits observed experimentally in sugarcane, such as accumulation of carbohydrates and substantial futile cycling of sucrose. The most important factors affecting sucrose accumulation were predicted to be the synthesis and breakdown of sucrose in futile cycles and the rate of cross-membrane transport of sucrose. The model also showed that sucrose moves down a concentration gradient from the leaves to the symplast, where it is transported against a concentration gradient into the vacuole.

There was a net gain in carbohydrate accumulation in the realistic model, despite an increase in futile cycling with internode maturity. The model also predicts that the apoplastic space enters a steady-state relatively quickly and that many enzymes are almost saturated with their substrates, meaning that little kinetic regulation can take place.
We present a model of sucrose accumulation in sugarcane that spans eight internodes and four compartments. Reactions and translocation in these compartments were included. To our knowledge, this is the most comprehensive biochemical model of this nature to date. Given the scale and amount of detail, it could nevertheless still be solved in a reasonable time frame. Furthermore, a sensitivity analysis of the maximal activities was successfully carried out. This work has shown that integrating kinetic modelling (as done to date), to include physical processes like advection, is tractable.

While some of the results need to be confirmed experimentally, the present model provides a very comprehensive description of sucrose accumulation and is a rigorous, quantitative framework for future modelling and experimental design.

References


Applying whole cell modular modeling to eukaryotes

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We have developed modular models of a cell that can be used to explain common patterns in unicellular organisms. The shift to inefficient metabolism at higher growth rates is one such common pattern. Our modular models represent a self-replicating unit, consisting of modules that encode cell functions. To understand design principles of regulatory strategies we optimize fitness, which in this case is done by optimizing growth rate. With the simple modular model optimized for growth rate we see a switch to inefficient metabolism at increasing growth rates [1]. At the same time the model is simple enough so that we can have some understanding of why this is the optimal strategy.

The modular model of ref 1 represents a bacterial cell. To understand the properties of eukaryotes, we need realistic estimates of the costs and kinetic properties of the modules in the cell. Therefore, we will use data from yeast to give the modules more realistic properties. Also, we will expand the model with mitochondria and metabolic modules of eukaryotes. Once we are able to understand the optimal behaviour with respect to growth rate, we can try to understand what happens when the cell does not follow the optimal behaviour. Examples of non-optimal behaviour can be obtained by forcing cells to have a wrong or incomplete perception of the environment, as was recently observed experimentally [2].
References
Session 6

Modeling of glycolysis and its regulation
From wine to vinegar: control analysis of a mixed microbial population

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Abstract

We made a feasibility study on the experimental application of MCA on a simple eco-system consisting of the yeast Saccharomyces cerevisiae and the acetic acid bacterium Gluconobacter oxydans. Together these organisms catalyze the conversion of glucose to ethanol (yeast) and the subsequent conversion of ethanol to acetate (G. oxydans), which can be analyzed as a metabolic “pathway”. A kinetic model was constructed for the mixed population on the basis of kinetics for the pure cultures. A steady state was reached in the mixed population and by varying the relative concentrations of the two organisms we could quantify their flux control and concentration control coefficients. The yeast was shown to have full flux control, while the ethanol concentration control was shared and strongly dependent on the S. cerevisiae/G. oxydans ratio.

Introduction

The acidification of wine after its exposure to air, is mainly due to the activity of a group of organisms commonly referred to as the acetic acid bacteria. In this process the ethanol that is produced by the yeast Saccharomyces cerevisiae during the wine fermentation is oxidized to acetate by acetic acid bacteria. As such the two organisms form a small eco-system that catalyzes the conversion of glucose to acetate, which is very similar to an intracellular metabolic pathway. We here report on a feasibility study we made
towards the experimental application of metabolic control analysis (MCA), usually restricted to the analysis of metabolic pathways, to such a small eco-system.

Earlier work on the extension of MCA to ecosystems has been made in the 1990s by Giersch [2,4], and Schulze [3] and later by Westerhoff and Getz [1,5] who termed the new analysis, Ecological Control Analysis (ECA). Giersch was rather pessimistic on the experimental applicability, while Westerhoff and Getz were more optimistic, but thus far none of their theoretical analysis methods were applied experimentally. We set out to apply ECA experimentally to a very simple ecosystem, consisting of *S. cerevisiae* and an acetic acid bacterium, *Gluconobacter oxydans*.

**Methods**

We tried to construct the simplest possible eco-system, consisting of two microbial species. We decided to use *S. cerevisiae* and *G. oxydans*, which constitute a simple “pathway” from glucose to acetate with ethanol as an intermediate. We decided to incubate the microorganisms under non-growing conditions, again to simplify the system. *G. oxydans* was pre-grown under aerobic conditions on a yeast extract/ethanol medium, and *S. cerevisiae* was pre-grown on YPD medium under anaerobic conditions. Mixed cultures with varying concentrations of the two micro-organisms were incubated under aerobic conditions with glucose as substrate and changes in metabolite levels in the culture were followed with time using HPLC analysis. Flux and concentration control coefficients were determined for the steady state and a kinetic model was constructed to describe the time dynamics of the system.

**Results and discussion**

From initial mixed culture incubations it became quickly evident that the eco-system of *S. cerevisiae* and *G. oxydans* was
more complicated than the envisioned linear pathway from glucose to acetate via ethanol. Under the aerobic conditions that were necessary for the incubation of *G. oxydans*, yeast also showed a significant purely oxidative metabolism (converting glucose to CO2) and the *G. oxydans* oxidized significant amounts of glucose to gluconate. Despite these branches, the main carbon flux through the system still ran via ethanol to acetate, and we could incorporate the additional branches in our analysis.

We first characterized the pure cultures with respect to their sensitivity for the variables in the system, by incubating yeast and gluconobacter with varying concentrations of glucose, ethanol, acetate, and gluconate and quantifying the effect on their metabolic activities. Within the ranges that were observed in the mixed culture incubations none of the intermediates had a significant effect on the metabolic activities of *S. cerevisiae* or *G. oxydans*, with the exception of ethanol, for which *G. oxydans* showed a strong sensitivity at low concentrations. Kinetic rate equations were built for the metabolic activity of the two organisms and these equations were used for the description of the dynamics and the steady state of the mixed cultures.

In a typical mixed culture experiment, a quasi steady state for ethanol is reached after approximately 5 hours, at which point a constant glucose consumption and acetate production rate are observed. By varying the relative concentrations of the two organisms and measuring the effects on the steady state, we could determine the flux and concentrations coefficients.

The steady state acetate production rate was shown to be proportional to the *S. cerevisiae* concentration, and independent of the *G. oxydans* concentration, indicating that the yeast has full flux control. The control of the steady state ethanol concentration was shared over the two organisms and increased sharply with increasing *S. cerevisiae*/*G. oxydans* ratios. The experimental re-
Results could be simulated with the kinetic model based on the pure culture experiments but the parameter values needed to be adapted for a good description of the mixed culture experiments. Model analysis showed that the insensitivity of *S. cerevisiae* at the low ethanol concentrations as used in our incubations lead to the full flux control of the yeast, while the relative specific metabolic activities of the two organisms and the affinity for ethanol of *G. oxydans* were the main parameters affecting the steady state ethanol concentration.

In conclusion, we were able to experimentally analyze a very simple eco-system using metabolic control analysis. However, the intricateness of the experiments for even such a simple system could place serious limitations to the application of the theory to larger systems. The strength of ECA might lie more in the analysis of kinetic models for eco-systems than in a direct experimental application.

References

Heterosis: an emerging property of metabolic systems

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Abstract

From the generalization of Wright-Kacser-Burns biochemical model for dominance and epistasis to networks with several variable enzymes, we hypothesized that metabolic heterosis (hybrid vigor) could be observed because the response of the flux towards enzyme activities and/or concentrations follows a multi-dimensional hyperbolic-like relationship. To corroborate this, we reconstituted in vitro the upstream part of glycolysis, and simulated genetic variability by varying enzyme concentrations in the test tubes. Mixing 1:1 the content of “parental” tubes resulted in “hybrids” the flux of which was frequently heterotic. The level of heterosis could be quite well predicted from both the parental flux values and enzyme concentrations. Computer simulations based on a simplified model of flux-enzyme relationship fully confirmed this findings. In addition, they revealed that flux control coefficients of the parental enzymes provide the best predictor for heterosis.

Introduction

Plant and animal breeders have long known that crossing two lines has positive effects on hybrid fitness, and generally on quantitative, polygenic traits. This hybrid vigor, or heterosis, is now considered to be universal as it has been observed in a large range of animal, plant and micro-organism species. Until now, genetics, genomics and molecular approaches have failed to provide a convincing understanding of heterosis. For
that reason, it is repeatedly presented as a particular, and in some ways mysterious, phenomenon, and “it seems likely [...] that the complexity derives from its multigenic nature and that eventually a unifying principle will emerge” [1]. From in vitro experiments and in silico simulations, we show that heterosis is actually an emergent property of biological systems as soon as there is concavity of the genotype-phenotype relationship.

**Methods**

The in vitro system we reconstructed, based on the upstream part of glycolysis, is described in Fievet et al. [3]. It goes from hexokinase to glycerol 3-phosphate dehydrogenase. The flux was measured through the rate of NADH consumption at 390 nm. In order to mimic genetic variability in enzyme activities, we varied the concentrations of PGI (phosphoglucone isomerase), PFK (phosphofructokinase), FBA (fructose-1,6-bisphosphate aldolase) and TPI (triosephosphate isomerase) in the test tubes to create “parents”, each parent being defined by a particular vector of concentrations. The total enzyme concentration was constant. The “hybrids” were produced by mixing 1:1 the content of parental tubes, which corresponded to additivity of enzyme concentrations. We created 61 hybrids by crossing 36 parents.

For theoretical developments and computer simulations, we used the following hyperbolic relationship between the flux $J$ through a metabolic network and the enzyme concentrations $E_j$’s:

$$J = \frac{1}{\sum_{j=1}^{n} \frac{1}{X A_j E_j + X d_j E_{tot}}}$$

where $X$ is a constant, $A_j$ and $d_j$ are systemic parameters accounting for the kinetic behavior of enzyme $E_j$ in the net-
work and $E_{\text{tot}}$ is the total enzyme concentration [3,4,6]. In this relationship, increasing from 0 the concentration of one enzyme first results in a proportional increase in phenotypic value, then there is attenuation and finally saturation occurs. At the plateau of the curve, there is robustness of the flux towards enzyme variation. Optimum distribution of enzyme concentrations, i.e. the distribution which maximizes the flux for a given total enzyme concentration, as well as flux control coefficients were derived from equation (1). For simulations, the parental enzyme distributions were drawn at random with or without constraint on total enzyme concentration $E_{\text{tot}}$. In every situation, the fluxes of 2000 pairs of parents were computed. The hybrids were produced by virtual crossing the “parents”, and their flux was computed assuming additivity of enzyme concentrations. The relationships were analyzed between heterosis and various distances computed from parental fluxes, parental enzyme concentrations and parental control coefficients.

**Results & Discussion**

*Metabolic systems are heterotic*

Among the 61 *in vitro* hybrids, 17 exhibited mid-parent heterosis (hybrid flux higher than the mean of parental fluxes) and 21 best-parent heterosis (hybrid flux higher than the highest parental flux). Examining enzyme concentrations in the parents revealed that high values of best-parent heterosis occurred when the parental distributions were contrasted, and when one or both parents did not have enzyme concentrations close to the optimum distribution. This was confirmed by the positive correlation between the relative heterosis and a Euclidian distance between parents computed from enzymes whose concentrations were on both sides of the optimum value in the parents ($r = 0.31, p < 0.02$). A highly significant neg-
ative correlation was also found between relative heterosis and a “phenotypic” distance, the absolute difference between parental fluxes \((r = -0.33, p < 0.01)\). A multiple regression with both variables as predictors of heterosis resulted in a highly significant \(R^2\) values \((R^2 = 0.23, p < 0.0005)\).

**Heterosis results from the concavity of the flux-enzyme relationship**

The flux of the hybrid was computed using (1) and supposing additivity for enzyme concentrations. Because \(J=f(E_k)\) has a negative second derivative for all \(k\), we can use the standard concavity argument which states that

\[
J_{1*2} = f\left(\frac{E_{k1} + E_{k2}}{2}\right) \geq \frac{J_1 + J_2}{2} = \frac{f(E_{k1}) + f(E_{k2})}{2}
\]

Hence, heterosis for the flux is an emerging property of metabolic systems. Moreover, we showed that two enzymes at least need to be variable between the parents for best-parent heterosis to occur.

**Predicting heterosis**

In order to know if the cases of heterosis observed *in vitro* were consistent with the general model of flux response, we computed the theoretical heterosis values using the values of enzyme concentrations in the parental tubes and the values of parameters \(X_{A_j}\) and \(X_{d_j}\) estimated in an independent experiment [3]. The correlation between predicted and observed heterosis was very high \((r = 0.80; p < 10^{-14})\). This indicates that our approximation equation for the flux is reliable, which encouraged us to use it to analyse by simulation the relationships between heterosis and various predictor variables. For each level of constraint on total enzyme concentrations, either mid-parent or best-parent heterosis was observed for each of
the 2000 virtual crosses. The level of constraint altered the number of cases of best-parent heterosis, which varied from 529 (26 %) under strict constraint to 361 (18 %) under the loosest constraint. Best-parent heterosis was generally observed when the parents had close flux values, since there was a negative correlation between relative heterosis and the flux difference between parents ($r \approx -0.37$ to $r \approx -0.46$ [$p < 10^{-15}$]). Multiple regressions to predict heterosis from both the flux difference and the enzyme Euclidian distance between parents resulted in high $R^2$ values, from 0.40 (strict constraint) to 0.18 (loosest constraint).

The best variable to predict heterosis was by far the Euclidian distance computed from the parental control coefficients, with $R^2$ from 0.27 to 0.36. The reason could be that the control coefficients are integrative parameters which account for both kinetic parameters and concentrations of enzymes. The multiple regression with this variable and the flux difference between parents resulted in very highly significant $R^2$ values, from 0.65 (strict constraint) to 0.43 (loosest constraint). Adding any other variables in the multiple regression did not increase $R^2$ values. Those results show that it is possible to predict heterosis for the flux, provided some knowledge on parental metabolism.

In conclusion, heterosis appears as an emergent property of the concavity of the enzyme-flux curve. This concavity has been largely documented [2, 3], but could be valid more generally as it accounts for various fundamental and apparently unlinked observations, such as dominance of “high” alleles over “low” alleles [6], selective neutrality of molecular polymorphisms [5], “threshold effect” in genetic diseases [9], epistasis for gene expression and protein synthesis [7], or asymmetry of the effects of up- and down-regulated genes in metabolic engineering [8].
References


Studies of glycolytic flux regulation/control using permeabilised yeast cells

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Abstract

In this work the correlation between levels of adenosine nucleotides and glycolytic flux was studied using a permeabilised cell system by experiments and in silico simulations using a kinetic model of glycolysis. Experimentally, using a flow-through chamber, the ATP level was correlated to glycolytic flux by a parabolic relation with a maximum at 2-3 mM of ATP. The kinetic model did not describe this parabolic correlation; at high ATP levels above 4 mM the flux was further increased. Thus, other mechanisms than described by the kinetic equations used in the model are important at high ATP levels to reduce the glycolytic flux.

Introduction

The most important pathway in the central carbon metabolism, glycolysis has been known and studied for a long time. However, we still do not have the full knowledge of how the flux through the pathway is controlled and regulated but factors like glucose transport and activities of glycolytic enzymes have been found to participate to different extent [3, 4, 7, 9]. The enzymes indicated in these studies are mainly the ATP-coupled ones and thus the adenosine nucleotides themselves may play a role. ATP is the energy carrier of the cell and can be provided by glycolysis through substrate-level phosphorylation. Thus, the energy status of the cell is tightly linked to the glycolytic flux and the question is if individual adenosine nu-
cleotides and/or the ATP/ADP-ratio may impact the glycolytic flux. Some studies has indeed found correlations between ATP levels and glycolytic flux [5, 6]; at high levels of ATP the glycolytic flux was decreased. Larsson et al [6] used a permeabilised cell system, by which ATP levels are controlled within a wider range by external additions of the nucleotides. Additionally, such a system may be advantageous since one of the regulatory factors, the glucose transport, is circumvented. This study found that ATP was correlated to glycolytic flux by a parabolic relationship having a maximal flux at an ATP level of 1-1.5 mM.

In the present work, the effect of the adenosine nucleotides, ATP, ADP, and AMP, on the glycolytic flux has been studied using permeabilised yeast cells. Experiments have been performed in a special constructed flow-through chamber allowing constant conditions. Additionally, a kinetic model of glycolysis was constructed to represent the permeabilised yeast system and simulation results were compared to experimental data.

**Methods**

Spheroplasts were prepared [1] from Bakers’ yeast (*Saccharomyces cerevisiae*) cells (Jastbolaget AB, Rotebro, Sweden), and treated at a cell concentration of 1 g protein/l with nystatin (20 μg/ml, Sigma, Mycostatin) to obtain permeabilised cells as described [6]. The permeabilisation was done in a 7 ml flow-through chamber, sealed with glass fiber filters (Whatman GF/F) to retain cells inside, for 20 min before starting the flow of the ATP/ADP-containing buffer with MgSO₄ levels 2mM higher than actual ATP concentrations. The flow was constant and run for 20 min, after which samples were regularly taken from the cell free outlet during further 20 min and the buffer reservoir for metabolite analyses. The samples
(heat treated at 95°C, 2 min, supernatant stored frozen) were analysed with enzyme-coupled methods for adenosine nucleotides and sugar phosphates [2] and glucose (enzymatic kit from Roche). At steady state conditions, the metabolite levels and the glycolytic flux as glucose consumption rate were calculated.

A kinetic model of glycolysis (8) were adjusted to reflect the permeabilised cell system by omitting glucose transport and the ATPase and adenylate kinase reactions and setting the adenosine nucleotides as parameters. Further adjustments were done to include cell concentration as a parameter, the triosephosphate isomerase reaction with equilibrium kinetics \( V_{\text{max}} \) as 100 \( \mu \text{mol}/(\text{mg protein min}) \), equilibrium constant as 0.045), and glycogen and trehalose formation to follow first order kinetics of glucose-6-phosphate \( k_{\text{glycogen}} = 0.0215 \) and \( k_{\text{trehalose}} = 0.0086 \text{ min}^{-1} \), respectively). Other parameters were ethanol at 20 mM, fructose-2,6-bisphosphate at 0.02 mM, and glycerol at 0.15 mM. The differential equations of the model were encoded and solved using Mathematica both as time series and steady state results. To obtain a steady state solution the \( V_{\text{max}} \) of pyruvate decaboxylase was increased 1.4 times to 0.9 \( \mu \text{mol}/(\text{mg protein min}) \).

**Results & Discussion**

Experiments with permeabilised yeast cells were done with 5 mM glucose, 0.5 mM ADP, and ATP varying from 1-6 mM in the inflowing buffer. It was found that the relation of ATP to glycolytic flux followed a parabolic relation with the maximum at ATP levels between 2-3 mM (Figure 1A). Such a relation was described earlier [6] but with a maximum at slighter lower ATP levels of 1-1.5 mM. However, in that study all metabolites were added at start resulting in larger percentage changes at lower ATP levels which may explain the differenc-
es seen. Also, we have obtained rather few data points around the maximum and additional data are needed to point out the exact position of the maximum.

Figure 1. Glycolytic flux versus ATP concentration for A. two batches of spheroplasts (circles and triangles) and B. model simulations using the experimental values of the adenosine nucleotides as input (squares). The experimental data were normalized within each batch against the highest flux value.

In the kinetic model, the parameter values of glucose and cell levels were set to reflect the experimental conditions at 5 mM and 1 g/l, respectively, and the experimental values of ATP, ADP and AMP was used as input to the simulations. The correlation flux-ATP level showed a local maximum at 2 mM but at ATP levels higher than 4 mM there were large discrepancies between the experimental data and the model. The simulation data continuously increased whereas the experimental data decreased at higher ATP levels. Thus, at these higher ATP levels other factors than described in the rate equations used are determining the system. The sugar phosphates glucose-6-phosphate and fructose-6-phosphate were as well measured in the same samples and compared with the model outcome. It was found that experimental and modeling data of fructose-6-phosphate followed the same trends. On the other hand, the levels of glucose-6-phosphate were different at high ATP levels. The experimental data were lower than at intermediate ATP levels whereas the simulation data were markedly high-
er. This finding may be a part of the discrepancies found for the ATP-flux relation at high ATP levels.

Acknowledgments

I want to thank Anna Kaminski for performing measurements with permeabilised cells and Prof. Jacky Snoep for support during model construction. Financial support is acknowledged from the Knut and Alice Wallenberg and the Carl Trygger foundations, both in Stockholm, Sweden.

References


Integration of the metabolic and gene-expression regulation of yeast glycolysis into a kinetic computer model

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When Saccharomyces cerevisiae is confronted with nitrogen starvation, its glycolytic pathway responds predominantly by gene-expression regulation if the yeast has been growing under respiro-fermentative conditions, while cells from respiratory cultures exhibit a relatively strong metabolic response (van Eunen et al., IET Syst Biol 2010). The important role of metabolism that was observed under the latter conditions could not be explained directly from measured changes in intracellular metabolite concentrations. In the present work the data obtained from the experimental study were implemented in an existing kinetic model of glycolysis (Teusink et al., Eur J Biochem 2000) in order to get a better understanding of the regulation of glycolysis. At a first attempt, the original model did not reach a steady state under any of the conditions of this study. The following improvements were made to the model: i) using $V_{max}$ values measured under in vivo-like conditions (van Eunen et al., Febs J 2010), ii) adding the known inhibition of hexokinase by trehalose’ 6-phosphate, iii) redetermination of the kinetic parameters of glyceraldehyde-3-phosphate dehydrogenase
under *in vivo*-like conditions and *iv*) adding the known activation of pyruvate kinase by fructose 1,6-bisphosphate.

The improved model reached steady states at all experimental conditions studied. When the yeast was taken directly from a culture exhibiting respirofermentative metabolism, the predicted fluxes and metabolites concentrations corresponded well with the experimental data. Under the other conditions, however, extremely high concentrations of fructose 1,6-bisphosphate were found. In order to bring the concentration of fructose 1,6-bisphosphate down, whilst maintaining a good fit to the measured flux, it is necessary to regulate the influx of glucose via glucose transport and hexokinase strictly with respect to the downstream glycolytic reactions. Minor adaptations to the kinetics of hexokinase and glyceraldehyde-3-phosphate dehydrogenase simultaneously allowed a good fit under another two conditions, while the correspondence between model and experiment of the last condition improved substantially. The model was also tested for its dynamic response upon a glucose upshift. The results showed a surprisingly accurate correspondence between the model and corresponding experimental data.

The question how the lessons learnt from modeling of yeast glycolysis can be applied to other pathways and organisms will be discussed.
Experimental supply-demand analysis of yeast free-energy metabolism

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Abstract

Experimental supply-demand analysis of yeast anaerobic free energy metabolism shows that control of the glycolytic flux is shared between supply and demand. Metabolic supply of ATP (i.e. glycolysis), and its demand (cellular maintenance), were independently modulated in whole cells by means of respectively titrating with maltose (a competitive glucose uptake inhibitor) and benzoic acid (a metabolic uncoupler). Under the prevailing conditions (energy excess) the majority of glycolytic flux control was found to be situated in the supply block ($c_{supply}^f = 0.75, c_{demand}^f = 0.25$).

Introduction

Metabolism can be viewed as a molecular economy that is regulated by the laws of supply and demand [1]. All the reactions responsible for the synthesis of a common intermediate can be grouped together as the supply or producing block and all reactions that consume or metabolize to common intermediate make up the demand block [1]. As a modular version of MCA [2], supply demand analysis allows the control and regulation of metabolic processes to be expressed quantitatively [1]. Yeast anaerobic free energy metabolism can be viewed as a supply–demand system centered around the linking moiety
ATP-ADP. The supply block consists of all the reactions responsible for the production ATP, whilst the demand block refers to all cellular processes which require the net use of ATP [1, 3].

The degree of flux control assigned to the metabolic supply or demand may be experimentally determined through application of the double modulation method as originally described by Kacser and Burns [2], in which the result of independent perturbations in both the supply and demand blocks are measured as changes in the linking metabolite concentration and pathway flux [3]. By plotting rate characteristics (log-log space) that depict the relationship between the supply-demand flux and the linking metabolite or moiety, the sensitivities of supply and demand may be calculated [1 & 2]. These sensitivities can then be used to determine the flux and concentration control structure of the system in terms of supply and demand [1].

This thesis presents a direct experimental application of supply demand analysis to anaerobic free energy metabolism in yeast. The conserved ATP-ADP moiety expressed as the [ATP] / [ADP] ratio was used to quantify the free-energy state of these yeasts. As fermentations were assessed under oxygen limitation, glycolysis was viewed as the sole pathway responsible for the supply of ATP. By arresting cell growth through nitrogen deprivation, cell maintenance was regarded as the only source of free energy demand.

Methods

The yeast Saccharomyces cerevisiae (strain CEN.PK117-3D) was grown in batch culture on normal YPD medium. Fermentations were assessed under energy excess (non growing conditions and high [glucose]). Metabolic supply of ATP (i.e. glycolysis), and its demand for cellular maintenance, were in-
dependently modulated in whole cells by means of respectively titrating with maltose (a competitive glucose uptake inhibitor) and benzoic acid (a metabolic uncoupler). Glycolytic fluxes were measured \textit{in vivo} with $^{13}$C nuclear magnetic resonance (NMR) spectroscopy. The [ATP]/[ADP] ratio was quantified \textit{in vitro} (perchloric acid cell extracts) by means of $^{31}$P NMR spectroscopy.

\textbf{Results}

The ethanol production rate was used as the primary indicator of glycolytic flux. As $I_{suppy}$ must equal $I_{demand}$ at steady state, $I_{EtOH}$ was used to quantify both supply and demand. The respective addition of benzoic acid and maltose produced the desired effect concerning changes in the steady state [ATP]/[ADP] ratio and glycolytic flux (data not shown).

\begin{figure*}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Variations in the glycolytic supply and demand for ATP as a function of the steady state [ATP]/[ADP] ratio. ($\bullet$) depicts the supply rate characteristic when ATP demand is perturbed through benzoic acid addition. ($\circ$) depicts the demand rate characteristic when the glycolytic supply of ATP is modulated through maltose addition. The intercept of the supply and demand rate characteristic indicates the non perturbed steady state.}
\end{figure*}
In Fig. 1, the rate characteristics of supply and demand are presented. Data were analyzed via linear regression and elasticity coefficients were calculated as the gradient of the respective tangents of supply (-0.07) and demand (0.20). These elasticities were in turn then used to calculate the control coefficients presented in Table 1.

<table>
<thead>
<tr>
<th>Linking intermediate</th>
<th>$C^J_{\text{supply}}$</th>
<th>$C_{\text{[ATP]/[ADP]}^\text{supply}}$</th>
<th>$C^J_{\text{demand}}$</th>
<th>$C_{\text{[ATP]/[ADP]}^\text{demand}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ATP]/[ADP]</td>
<td>0.75</td>
<td>3.73</td>
<td>0.25</td>
<td>-3.73</td>
</tr>
</tbody>
</table>

**Discussion**

Our results report that under energy excess (high [glucose], non-growing conditions) the majority of flux control is situated in the supply. In addition, the large concentration control coefficients presented here point to the weakened homeostatic maintenance of the [ATP]/[ADP] ratio when energy is plentiful. Our data support the findings of Kroukamp et al. [3], who similarly found glycolytic flux control to reside in the supply.

In a previous report by Schaaff et al. [4], it was shown that the independent and simultaneous overexpression of key glycolytic enzymes in yeast did not lead to significant increases in pathway flux. Hofmeyr and Cornish-Bowden [1] interpreted these results as pointing to extrinsic flux control residing in the demand for ATP. Though these findings are in disagreement with the results presented here, the failure of Hofmeyr and Cornish-Bowden (2000) to incorporate the importance of the glucose transport step during their analysis may explain these discrepancies as originally proposed by Kroukamp et al. (2002) [3]. The inclusion of such a key control point [5,6,7] within the confines of glycolysis itself may indeed transfer the majority of flux control to the supply block, even when energy...
is freely available. In spite of this, yeast glycolytic flux control by the demand block would be better suited under conditions of energy excess, specifically concerning the homeostatic control of the \([ATP] / [ADP]\) ratio. It might be that there are other hierarchal systems or factors to consider, which may predominate metabolic regulation solely by the laws of supply and demand.

The work presented in this study may serve as an example for analyzing other metabolic networks with respect to supply and demand. Presently within our research group, this analysis is being extended to the microbes *Zymomonas mobilis* and *Lactococcus lactis*.

**References**

Session 7

Receptors and transporters of neurotransmitters. Modeling and application
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Glycine neurotransmitter transporters (GLYTs) catalyze Na+/Cl-/glycine cotransport and control the availability of glycine at the glycinergic synapses. GLYT1 is the main regulator of the glycine levels in the synaptic cleft whereas the neuronal GLYT2 supplies glycine unidirectionally to the presynaptic terminal by means of a glycine transport with higher Na+ coupling than GLYT1. We performed molecular dynamics simulations in the presence of Na+ on three-dimensional GLYT1 and GLYT2 homology models, and identified a conserved region in GLYT2 vestibule with high probability of Na+ interactions. The replacement of D471, located in transmembrane domain 6 (TM6) of GLYT2, altered Na+ dependence of glycine transport by reducing Na+ apparent affinity and cooperativity. The substitution of the equivalent position in GLYT1 (D295) had much lower impact. Electrophysiological recordings confirmed that mutation reduced the sodium affinity, and induced sodium leakage in GLYT2 but not in GLYT1. We have found that wild type GLYTs have different responses to lithium ion, which
inhibited glycine transport in GLYT1 but stimulated it in GLYT2. The mutation of Na2 site residues in both wild types abolished lithium-GLYTs interaction. Neutralization of D471, but not D295, altered the differential wild type lithium responses. Cysteine substitution of the homologous aspartates yielded GLYTs differentially sensitive to methanethiosulfonate (MTS) reagents in a way oppositely modulated by Na+ and Li+, thus, outlining the molecular basis of the GLYTs differential lithium phenotype. Our observations sustain that D471 of GLYT2 has cation selectivity properties.
Probability simulator of enzyme activity and its application to description of transmembrane currents through glycine receptor

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Questions of ion homeostasis are the major part of cell biophysics. In most cases researchers are interested how the ion accumulation in biological compartments has made. Other points of interest are the kinetic mechanisms and ways of regulation by external factors. Ion transport has major importance in case of forming of electrical potential at excitable membranes, especially at neuronal membranes. Thus transmembrane transport is essential not only for regulation, but for whole cell activity. Significant moment of these processes description is a need to estimate current-time dependences taking into account spatial location of neurons. In present work the computational method describing ion motion near membrane surface based on Langevine dynamics was applied to obtain current-time dependences in case of stochastic nature of open-shut processes in ion channels.

Introduction

The offered model consists of two self-sufficient parts that describe transmembrane ion currents occurrence and receptor-agonist interaction. First unit allows to evaluate total ion current through the ion channel on the assumption of receptor open-state. Thus time between conformational changes due to agonist binding is divided on short periods. During these peri-
ods ions behavior is described by Langevin equations for motion of charged particles in viscous medium as affected by electrostatic field. Second unit of the model formulizes a stochastic scheme for ligand binding. Probability values for open-shut change in receptor depend on binding constants and concentration of special agonist and the time of these processes is limited by conformational changes in channel protein domains. Whereas action of these two units of model is at different time scales it is able to combine them. To obtain evolution of single-channel ion currents in time two units were integrated into computational algorithm.

**Materials and methods**

It is the most convenient to describe the ligand-gated ion channels as this case will be anchored to conditions of alternate conduction states depending on agonist binding. Glycine Receptor is a member of ligand-gated ion channel family (LGIC). It has pentameric structure similar with major members of LGIC such as nicotinic acetylcholine receptor where five transmembrane subunits form pore via the α-helical domains TM2. After agonists binding GlyR opens its channel and it allows passive transport of Cl⁻ ions toward equilibrium potential. Such glycine mediated increase of Cl⁻ conduction leads to hyperpolarization [1].

As far as the main object of this investigation is a description of ion channels functioning, it is sequaciously to observe 3-compartment model [3] (Fig.1). Two of these compartments are the part of hydrophilic phase, whereas third compartment is a hydrophobic phase of membrane with protein incorporated. Geometry of ion channel and is obtained from receptors structure. For its precise description it is enough to construct the system of two conical vestibules connected by short nar-
row region, where inner and outer diameters are equal, \( d_{\text{in}} = d_{\text{out}} = 25\text{A} \). Cylindrical part has diameter \( d_{\text{ch}} = 6\text{A} \) [7].

![Diagram of modeling system](image)

Figure 1. Scheme of modeling system: I – external hydrophilic phase; II – part of membrane with incorporated receptor; III – internal hydrophilic phase.

Charged particles are randomly distributed in both hydrophilic compartments and motion is caused by interaction with each other and with fixed charges from hydrophobic phase. Aminoacids residues from protein or polar lipids from membrane can appear as these fixed charges, where residues positions are received from primary structure of transmembrane domains. It is important point in this case that interaction between particles has major influence to motions “randomness” than collision with solvent [2].

For the particle with mass \( m_i \) and charge \( q_i \) which is in motion in medium characterized by friction coefficient \( \gamma_i \) under the external electric field \( \vec{E}_i \) the motion equation is look like:
There \( F_i^R \) is a stochastic random force due to particle-solvent interaction. It is isotropic and its mean value is rapidly fluctuating near zero. Summary electric field is formed by both mobile and fixed charges and some macroscopic source. Here it is necessary to make some important remarks. As the initial spatial distribution is random, electric field function makes charged particles motion quasistochastic. It allows to estimate the system behavior, submitting Brownian Dynamics [6]. There is not enough to simulate transmembrane ion current for the whole channel description. It is necessary to give the chance for the researcher to obtain time-dependent currents taking into account activation/deactivation of channel. This question is uneasy as within the limits of the offered algorithm there is no possibility to build the system description of the given process. For precise description of experimental data it is necessary to take into account behavior of channel at close-state and time periods of open-shut processes. Thus it is essential to define rules for transition between concerned states so as it will be able to incorporate these rules into the computational algorithm.

Let’s consider one of possible approaches for the description of channel open-close processes under the ligand binding. The essence of the offered approach consists in sharing single channels structure analysis results, modeling of the processes occurring in catalytic cycle of one molecule of enzyme and integrated representation of total measured parameter by the set rules. In this case it is supposed that ligand-gated ion channel is somewhat identified with hypothetical “enzyme” which function is to associate “substrate” (ligand) and to release “product” (transmembrane current). The main role in possibility of such representation is formed by creation of the computer program simulating behavior of single
protein. Process is formalized not as form of single mathematical problem but as combination of several elementary computational modules. Each one of these units is represented as a theoretical model of single protein at microenvironment and show output characteristics interesting for researchers, for example quantity of molecules transformed per time. In this approach catalytic cycle of enzyme or receptors sequence of conformation changes always breaks into some elementary processes, i.e. such events which do not make all cycle up to the end but cycle is impossible without them. Substrate binding with proteins active center is the example of such event. Act of binding itself is not sufficient condition of reactions passing, but is apparently necessary for its realization. Moreover it is necessary to carry inhibitor and activator binding with receptor to processes mentioned above. There are 2 key parameters in description of such processes: probability of event and time of events pass. In a case considered in work the yielded process is a binding of a ligand (mediator) with a receptor, leading to ion channel opening. Thus it is supposed that the event is reversible and, hence, the elementary scheme will be fair:

\[
E + A \xleftrightarrow{\k_{\text{on}}} \xrightarrow{\k_{\text{off}}} EA
\]

The dissociation constant, obviously, looks like:

\[
K_d = \frac{[A] \cdot [E]}{[EA]} = \frac{k_{\text{off}}}{k_{\text{on}}}
\]
If now to assume that in considered system N protein molecules exists and the mean probability to find protein in free state or in complex will be equal to:

\[
\langle P_B \rangle = \frac{n_B}{N} = \frac{1}{1 + \frac{[A]}{K_d}}
\]

\[
\langle P_{BA} \rangle = \frac{n_{BA}}{N} = \frac{1}{1 + \frac{K_d}{[A]}}
\]

This expression characterizes mean probabilities for degradation and formation of enzyme-substrate complex. It is essential that the dissociation constant which is ratio of elementary reaction rates in a steady state can be obtained from thermodynamic characteristics. Thus combination of Brownian Dynamics methods for single-channel behavior description at open state and stochastic approach for time-dependent receptor functioning make possible investigation of receptor activity in whole.

**Results**

On an example of Glycine Receptor it is possible to simulate ion currents depending on time. According to the offered method, computational experiments for receptors opening caused by Glycine binding were made. Ion currents dependencies on time were received for various agonist concentrations. Opening and closing of the channel occurs at each step of receptor simulation and the opened state corresponds to the certain value of ion current (Fig.2). Obtained dependencies consist with experimental data for glycine receptor current [4,5]. Thus submitted addition for the computational algorithm allows not only to obtain mean values of single-channel trans-
membrane current but the time dependencies at whole. Such combination of “the single-channel current” and “agonist binding” approaches can play significant role in understanding of both receptor functioning and ion transport in whole cell.

![Figure 2](image)

**Figure 2.** Dependence of transmembrane ion current through glycine receptors single channel on time. Concentration of Glycine in system: $C_{\text{Gly}} = 2$ mM.

**References**

Computer simulation of glutamate transport via EAAT 4 neuron transporter

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Introduction

Amino acid L-glutamate is one of the major excitatory mediators in mammalian nervous system and plays a significant role in cognition, memory, learning and the other brain functions. Glutamate is also essential in the development of central nervous system, most neuron and glial cells have glutamate receptors [1]. Adequate glutamate uptake from the synaptic cleft is crucial for effective neurotransmission. Family of glutamate transporters consist of five members (EAAT 1-5—excitatory amino acid transporters 1-5) [2], and examination of EAAT 4 represented in postsynaptic membrane can provide additional data for System Biology. The purpose of the study is to estimate the glutamate concentration variation occurring after a single transporter EAAT 4 activation during synaptic neurotransmission.

Methods

According to literary data [3] a combined scheme for sequence of elemental events in glutamate transporter was developed. Algorithms proposed in the mentioned scheme were realized in virtual simulator of its activity. This simulator is independent software consisting of several logical blocks which described the probabilities of elemental steps during the neurotransmitter transport cycle. After the program execution the researcher can receive the amount of transported glutamate molecules per
time unit. In order to obtain statistically significant data due to huge fluctuations of the moved molecules number through a membrane via single transporter, the simulations were performed for a certain number of enzymes. The developed software enables carrying out virtual experiments in different sodium, potassium, chloride and glutamate concentrations.

**Results**

The time dependences of glutamate concentration were obtained under various environmental conditions. Distinct possibilities of chloride ion interactions with the glutamate transporter were shown to modify net trans-membrane flow of amino acid heavily and this effect is substantially determined by the system equilibrium. The occurrence of a few assumed probability schemes allowing combination of ligand ordered binding can lead to the variation in glutamate transport capacity. The results of the modeling disclose a feasible molecular mechanism of concentration dependent up/down regulation of EAAT 4 activity by transporting ions.

**Conclusion**

The developed software can be used for a virtual simulation of EAAT 4 transport cycle and the model allows to predict some features of the carrier which should be experimentally proved.

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Computer simulation of glycine transport in mammalian neurons

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Background

Neurotransmitters uptake is quite essential in various metabolic and functional processes in neural tissue. A simple tool making possible a quick and appropriate description of this process could be quite useful in the therapy of different neuronal diseases such as epilepsy, depression, psychosis, anxiety and neurodegenerative disorders [1]. Glycine is one of inhibitory neurotransmitters which play a significant role in the central nervous system functioning activating special receptors [2]. In physiological conditions the pool of this amino acid is regulated by a cascade of metabolic reactions and by membrane transport via specific transporters as well. The re-uptake of glycine into presynaptic terminals and surrounding glia is obligatory for the maintenance of low synaptic levels of the transmitter in the synaptic cleft. Glycine transporter type 2 (GLYT2) presented in the presynaptic membrane is a member of Na⁺-Cl⁻-dependent transport proteins family, which share a common structure with 12 transmembrane domains. In the process of its activity the translocation of the amino acid accompanies with binding of both one chloride and three sodium ions consequently.

Aim

The main goal of the present study was to develop a computer simulator of GLYT2 activity based on known experi-
mental data for quantitative estimation of membrane glycine transport.

**Methods**

A sequence of elemental events (Fig. 1) happening during the cycle of GLYT2 activity were summarized as a single scheme, which became a basis of an original software.

Fig. 1. Scheme of the elemental events during GLYT2 transporter cycle.

The algorithm of transporter simulator was developed using the probability approach describing the behavior of a single protein. As a result of such computations the number of translocated glycine molecules per time period has been evaluated. The computer experiments were carried out under different environmental conditions such as ion and glycine concentrations.

**Results**

As the major equilibrium constants of the transport steps are still unmeasured the reversibility degree of the glycine transport is also considered as a variable parameter. The val-
ues of equilibrium constant in the range 0.2 to 10 have the considerable effect on the pattern of glycine uptake (Fig. 2).

Fig. 2 Velocity of glycine uptake by GLYT2 as a function of equilibrium constant. Each value represents the mean ± s.e. for 10 experiments.

Using described software the time dependences of glycine, sodium and chloride ions amounts were obtained. Ligand cooperativity was observed not only for sodium ions (Hill coefficient is 3.6) but also for glycine (Hill coefficient is 1.8 for $K_{eq} = 2$) (Fig. 3). This unusual phenomenon can be eliminated by shifting of equilibrium to the influx transport of the amino acid.

Fig. 3. Velocity of glycine uptake by GLYT2 as a function of glycine concentration in the synaptic cleft. Used concentrations: $Na^+$ and $Cl^-$ in synaptic cleft 120 mM; $Na^+$ and $Cl^-$ in presynaptic terminal 5 mM; Gly-
cine in presynaptic terminal 30 mM. 3 equilibrium constants ($K_{eq}$) of 2, 10, 100 were used. Each value represents the mean ± s.e. for 10 experiments.

**Conclusion**

The developed software based on proposed probability algorithm can be used for a virtual experiments in GLYT2 activity simulation. The described model allows to predict some characteristics of the transporter functioning which can be experimentally proven. This software combined with glycine receptor model can be also used in research laboratories for evaluating of concentrations of chloride, sodium and glycine in synaptic cleft and presynaptic terminal in different time points during inhibitory signaling.

**References**


Session 8

Experimental model as a tool for research in system biology
Modular kinetic analysis of the effects of febrile-range hyperthermia on respiration in rat heart and liver mitochondria

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The elucidation of the molecular mechanism of the cell response to moderate heating is of importance for understanding the events that occur in the cell upon use of heating for therapeutic purpose or during illnesses that are associated with fever. The death or survival of different cells upon hyperthermia is determined by the molecular mechanisms that are not yet well established. The events following the exposure of cell to heat involve complex interplay of multiple pathways and factors operating at different regulatory levels (inducing changes in metabolic activities, signal transduction, and gene expression). Many reports have demonstrated mitochondria to be major targets of hyperthermic stress inside eukaryotic cells. Mitochondria are highly dynamic organelles exhibiting morphological as well as biochemical changes during physiological cell metabolism and stress responses [1]. These organelles may capture a central directory in the cells fate when affected by stress factors. That implies the need to determine the kinetic response of mitochondria from different healthy tissues (heart and liver) to moderate heating. In this study we evaluated the response of the oxidative phosphorylation system to febrile-range hyperthermia (40°C) by using modular kinetic analysis (MKA). We have compared the effect on the kinetic dependencies on the membrane potential $\Delta \psi$ of the proton leak, respiratory chain and the phosphorylation subsystems in isolated rat heart and liver mitochondria.
respiring with pyruvate plus malate. Our results indicate the absence of uncoupling and slight activation of mitochondrial oxidative phosphorylation by the febrile temperature in heart mitochondria and slightly shifted membrane leak kinetics towards higher rates and activated proton leak flux in liver mitochondria with no effect on the kinetics of the respiratory and phosphorylation modules.

MATERIALS AND METHODS

Mitochondria were isolated from the heart and liver of male Wistar rats and the protein in their suspension was estimated as described previously [2]. Mitochondrial respiration was measured at 37 and 40°C in the closed, stirred and thermostated vessel fitted both with a Clark type oxygen electrode and tetraphenylphosphonium (TPP⁺) selective electrode (A. Zimkus, Vilnius University, Lithuania) using 267 nM final concentration of TPP⁺. The incubation medium of heart mitochondria contained 30 mM Tris, 5 mM KH₂PO₄, 110 mM KCl, 10 mM NaCl, 1 mM EGTA, 5 mM NTA, 50 mM creatine, 0.875 mM CaCl₂ (1 μM free Ca²⁺), 5.17 mM MgCl₂ and mitochondria (0.3 mg protein), pH 7.2 [2]. The incubation medium of liver mitochondria contained 20 mM Tris, 5 mM KH₂PO₄, 110 mM KCl, 50 mM creatine, 2.3 mM MgCl₂ and mitochondria (0.5 mg protein), pH 7.2. The [ATP]/[ADP] ratio was clamped by adding excess creatine kinase (0.1 mg/ml) and 1 mM ATP. The experiments were performed using pyruvate + malate as oxidizable substrate (1 mM + 1 mM – for heart mitochondria, 5 mM + 5 mM – for liver mitochondria, Mitochondria were incubated with the respiratory substrate for 3 min (state 2) before the state 3 respiration was induced by addition of 1 mM ATP. Dissolved molecular oxygen at different temperatures (37 and 40°C) was determined using glucose oxidase catalyzed reaction between D-glucose and O₂ in
the incubation medium described above while the pH of the medium was strictly controlled at each temperature (pH 7.2).

Modular kinetic analysis was performed as described earlier [2]: the dependence of the flux through the respiratory module \((J_O)\) on \(\Delta \psi\) was determined by gradually inhibiting the flux through the phosphorylation module with the inhibitor of adenine nucleotide translocator carboxyatractyloside (0-1.75 nmol/mg protein) and this way modulating the value of \(\Delta \psi\) and concomitantly measuring the respiration flux \(J_O\) and \(\Delta \psi\) corresponding to each new steady state. The dependence of the flux through the phosphorylation module \((J_P)\) on \(\Delta \psi\) was determined by titrating the flux through the respiratory module with the respiratory chain inhibitor rotenone (0-0.06 nmol/mg protein), and concomitantly measuring the respiration flux \((J_O)\) and \(\Delta \psi\) corresponding to each new steady state. The phosphorylation flux \(J_P\) was calculated by subtracting proton leak flux \(J_L\) from the respiration flux \(J_O\) at the same value of \(\Delta \psi\) (i.e. \(J_P = J_O - J_L\)). The dependence of the flux through the proton leak module \((J_L)\) on \(\Delta \psi\) was determined by titrating the flux through the respiratory module with rotenone (0-0.08 nmol/mg protein) when the flux through the phosphorylation module was fully blocked by addition of excess oligomycin (2 \(\mu\)g/mg mitochondrial protein). The experiments of modular kinetic analysis were paired experiments: the measurements were performed at 37 and 40\(^\circ\)C using the same mitochondrial preparation.

**RESULTS AND DISCUSSION**

The aim of our experiments was to evaluate and compare the shift in the kinetic dependencies of fluxes through the proton leak, the respiratory and the phosphorylation subsystems on \(\Delta \psi\) induced by increase of temperature from 37\(^\circ\)C to 40\(^\circ\)C in mitochondria, isolated from rat heart and liver tissues.
The results of MKA in heart mitochondria show that increasing temperature from 37 °C to 40°C has no effect on the kinetics of phosphorylation and proton leak, but leads to slight activation of the reactions of the respiratory module. Although the respiration \( (J_O) \) and phosphorylation \( (J_P) \) fluxes through the modules of system in state 3 did not change significantly, increase in \( \Delta y \) value by 4 mV (Table 1) is consistent with the activation of the \( \Delta \psi \) producing respiratory module. Increase in \( \Delta \psi \) may be responsible for the higher value of the flux through the membrane leak module in state 3 \( (J_L) \). These results indicate the absence of uncoupling and slight activation of mitochondrial oxidative phosphorylation by the febrile temperature.

**Table 1.** Effect of increasing the temperature from 37 °C to 40 °C on the fluxes through the modules of oxidative phosphorylation and the membrane potential in heart mitochondria. Averages from \( n = 3 \) independent experiments; ± SEM. *, statistically significant difference, p<0.05.

<table>
<thead>
<tr>
<th>Parameters of oxidative phosphorylation</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>( J_O ), (nmol O·min(^{-1})·mg protein(^{-1}))</td>
<td>37 °C</td>
</tr>
<tr>
<td>( J_P ), (nmol O·min(^{-1})·mg protein(^{-1}))</td>
<td>320 ±14</td>
</tr>
<tr>
<td>( J_L ), (nmol O·min(^{-1})·mg protein(^{-1}))</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>( \Delta \psi ), (mV)</td>
<td>142 ± 1</td>
</tr>
</tbody>
</table>

MKA results for liver mitochondria show that increase of temperature from 37 °C to 40 °C slightly shifted membrane leak kinetics towards higher rates and activated proton leak flux at state 3. The effects of fever temperature on the kinetics of the respiratory and phosphorylation modules were not apparent. Considering significant decrease of \( V_3 \) (data not shown) we can assume that respiratory module is inhibited since the result can not be explained only by increased proton leak. The values of fluxes and the membrane potential in state 3 are presented in Table 2.
Table 2. Effect of increasing the temperature from 37 °C to 40 °C on the fluxes through the modules of oxidative phosphorylation and the membrane potential in liver mitochondria. Averages from n = 3 independent experiments; ± SEM. *, statistically significant difference, p<0.05.

<table>
<thead>
<tr>
<th>Parameters of oxidative phosphorylation</th>
<th>37 °C</th>
<th>40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{os}$ (nmol O.min$^{-1}$.mg protein$^{-1}$)</td>
<td>178 ± 4</td>
<td>134 ± 2*</td>
</tr>
<tr>
<td>$J_{p}$ (nmol O.min$^{-1}$.mg protein$^{-1}$)</td>
<td>160 ± 4</td>
<td>108 ± 2*</td>
</tr>
<tr>
<td>$J_{L}$ (nmol O.min$^{-1}$.mg protein$^{-1}$)</td>
<td>17± 1</td>
<td>25 ± 1*</td>
</tr>
<tr>
<td>$\Delta \psi$, mV</td>
<td>146 ± 1</td>
<td>145 ± 1*</td>
</tr>
</tbody>
</table>

Increase of temperature from 37 to 40 °C increased flux through the proton leak and inhibited the respiratory and phosphorylation flux. These three effects result in small decrease of the membrane potential (by 1 mV).

MKA revealed different effect of febrile-range hyperthermia on the respiration of mitochondria isolated from heart and liver tissues and the impact of this diverse kinetic response of mitochondria on heat sensitivity of different organs and tissues is not yet elucidated.

ACKNOWLEDGMENT

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Understanding the origin of mitochondrial (dys)function;
A new systems biology based research strategy

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Introduction
Mitochondrial dysfunction has been associated with the development of multiple diseases (e.g. type 2 diabetes) and the aging process. Currently, mitochondrial (dys)function is investigated using different strategies. For instance, PCr recovery measurements provide a non-invasive readout of in vivo mitochondrial function. However, a difference observed in vivo can originate from different sources at molecular scale, obscuring any straightforward interpretation of this type of data. Therefore also an alternative approach is often used: i.e. analyses muscle biopsy samples. Although these analyses can provide a wealth of information at a molecular scale, often many differences at this level are observed, and interpretation / prediction of their individual contribution to the in vivo (dys)function remains very challenging. To overcome these complicated problems we aim on: 1.) developing a detailed computational model capturing our current biochemical and thermodynamic knowledge of cell energy metabolism, and 2.) apply this model to relate in vitro characterization of skeletal
muscle at molecular scale to *in vivo* function read-out of the system.

Previous studies have been focused on aim 1: i.e. development and validation of a computational model. Here, we present an investigation of the feasibility and usefulness of the current model with respect to aim 2.

**Methods**

The research strategy was tested by investigating mitochondrial function in TA muscle of 8 and 25 week old male Wistar rats. Each group contained 10 animals.

*In vivo* mitochondrial function was determined from post exercise phosphocreatine recovery kinetics recorded by $^{31}$P MRS [1].

Molecular characterization of muscle phenotype was performed by applying biochemical (citrate synthase), Immunohistochemical (SDH activity and muscle fiber type composition) and western blotting (complex I-V, and ANT) analyses, as well as isolated mitochondria studies.

The computational model of cell energy metabolism included a detailed kinetic/thermodynamic model of mitochondria [2], glycolysis [3] and cellular ATP buffering [3].

**Results**

*In vivo* mitochondrial function decreased in the 25 week old rats compared to the 8 week old rats (time constant of PCr recovery 81+/-11s vs. 119+/-12s (mean+/-SD), respectively).

Characterization of the TA muscle at molecular scale revealed multiple differences between the two age groups: Isolated mitochondria studies showed a decrease in state 3 and state U respiration of 17%, indicating a decreased intrinsic mitochondrial function at the level of the dehydrogenases and
respiratory chain complexes. In addition, *in vitro* biomarkers of mitochondrial capacity (SDH staining, CS activity, western blot data) indicated a 20% decrease in mitochondrial capacity in the aged rats.

Next, model simulations were run to predict the impact of the changes at molecular scale to the *in vivo* observations. According to these predictions, the majority of the change in *in vivo* mitochondrial function originated from the decrease in mitochondrial capacity. In addition, the decrease in intrinsic mitochondrial function also contributed, albeit to a lesser extent.

**Discussions**

In this study a decreased *in vivo* mitochondrial function, as well as several differences at the molecular scale were observed. Although the data itself is interesting, its interpretation is very complex. The computational model was used to predict the contribution of each individual difference observed at molecular level to the *in vivo* function, thereby providing key information for guiding the interpretation of the data. We therefore feel that this work nicely illustrates the added value of computational modeling and a systems biology approach in studying mitochondrial (dys)function.

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Effect of glycine on pial brain vessels vasodilatation

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Glycine is widely known inhibitory neuromediator in central neural system. This amino acid is also very useful in clinical therapy of ischemic insult. However, the molecular mechanism of such essential property is not still clear. At the present study we has proved that application of glycine solution on uncovered pial brain tissue yields essential dilatation of blood vessels and this effect confirms previous results in mesentery and covered pial brain tissues [1,2]. This effect could explain enhance of glucose consumption in rat brain after glycine treatment [3,4]. Thus, the result of our investigation gives an evident proof of known clinical application of considered amino acid.

Acknowledgements

Authors very appreciate to Aleksey A. Selin for the assistance in the study of surgery technique. We would like to thank Prof. Gennady I. Podoprigora for his critical comments and discussion.

References


Mitochondria as therapeutic target of glycine in the model of ischemic stroke in vitro

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Abstract

Target and molecular mechanisms of therapeutic action of glycine are not sufficiently studied. This work is addressed to this problem. Study revealed that glycine prevents respiratory control index (RCI) decrease of isolated brain mitochondria after brief exposure to hypoxia. RCI after incubation in hypoxia was 67±5% of control values (without hypoxia), but RCI of mitochondria after hypoxia in the presence of glycine was 85±5% of control values. Incubation under hypoxia of mitochondrial suspension decreases calcium capacity (42±10% of control values). After incubation in hypoxic conditions for 10 minutes the generation of hydrogen peroxide significantly increases during oxidation of succinate. However if the incubation was conducted in the presence of glycine the rate of hydrogen peroxide formation was significantly lower. Thus in the model of ischemic stroke _in vitro_ occurs dramatic impairment of mitochondrial functions such as RCI and calcium capacity depletion and enhancement of hydrogen peroxide generation. But glycine effectively prevents those bioenergetic impairments.
Introduction

Glycine has been successfully used for the treatment of stroke [1]. Recently we have shown that glycine prevent respiratory control index depletion of mitochondria in the homogenate of the cerebral cortex after 24 hours common carotid artery occlusion in rats, along with preventing neuronal death [2]. But the target and molecular mechanisms of therapeutic action of glycine are not sufficiently studied. This work is addressed to this problem.

Results

For a detailed study of the mechanism of glycine action a model of isolated brain mitochondria hypoxia was chosen. The suspension of mitochondria was placed in a vial, from which oxygen was removed by blowing with nitrogen, and incubated for 10 minutes. It was found that in this model of hypoxia in vitro occurs a decrease of respiratory control ratio (RCI) of mitochondria after brief exposure to hypoxia. RCI after incubation in hypoxia was 67 ± 5% of control values (without hypoxia). Glycine (5 mM) prevented RCI decrease, being added to the incubation medium, RCI of mitochondria after hypoxia with glycine was 85 ± 5% of control values (Table 1). Then was performed study of molecular mechanisms of glycine action, namely Ca2 + capacity of mitochondria and the rate of hydrogen peroxide formation. It was found that as a result of incubation of mitochondria in hypoxia for 10 minutes Ca2 + capacity decreased to 42 ± 10% of the control value (Figure 1B). Glycine completely prevented this reduction in Ca2 + capacity (Figure 1A). There was a concentration dependence of this effect and it could be observed under not less than 2 mM of...
glycine. It was also found that the rate of hydrogen peroxide generation by mitochondria oxidizing succinate occurs with significant rate (Fig. 2C). After incubation in hypoxic conditions for 10 minutes the generation of hydrogen peroxide significantly increases (Fig. 2A). However if the incubation was conducted in the presence of glycine the rate of hydrogen peroxide formation was significantly lower (Fig. 2B).

**Table 1.** Glycine protects functional activity of isolated brain mitochondria during hypoxia. Values of respiratory control ratio (RCI) correspond to reduction of RCI (in %) relative to the RCI of intact mitochondria (without hypoxia).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>RCI (in %) (V3/V2 by Chance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without hypoxia (control)</td>
<td>100%</td>
</tr>
<tr>
<td>Hypoxia 10 min</td>
<td>67±5%</td>
</tr>
<tr>
<td>Hypoxia 10 min + glycine 5 mM</td>
<td>85±5%*</td>
</tr>
</tbody>
</table>

*p < 0.01 compared with control; * - p < 0.05 compared with Hypoxia 10 min. V3,V2 – rate of respiration in state 3 and 2 by Chance, respectively.

Fig. 1. Calcium capacity of isolated brain mitochondria at different conditions. Glycine protects mitochondria from reduction of Ca$^{2+}$ capacity induced by hypoxia. A, hypoxia 10 min; B, hypoxia 10 min + glycine 5 mM; C, intact mitochondria (without hypoxia). mito – mitochondria, Suc – succinate 5mM.
Conclusions

In isolated brain mitochondria after brief period of hypoxia (10 min) caused impairment of oxidative phosphorylation function of mitochondria. RCI depletion was effectively prevented by glycine. Study revealed that incubation under hypoxia of mitochondrial suspension decreases calcium capacity and activates generation of hydrogen peroxide. In the presence of glycine 5 mM that processes is almost completely inhibited.

Based on these findings next conclusions were made: in described model of hypoxia in vitro glycine works as protector, which attenuates ROS generation and prevents calcium capacity depletion. Thus glycine prevents bioenergetic impairment of mitochondria from brain tissue during hypoxia in the model of ischemic stroke.

References

Session 9

Application of system biology approach to description of ATP synthesis and anabolic processes
SysMO-MOSES - The effect of ATP, ADP and AMP on glycolytic enzymatic rates – standardisation of enzyme activity assays revisited

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Introduction

Systems biology comes in two flavours (bottom-up and top-down) and at present these two hardly meet. The aim of the SysMO project MOSES (MicroOrganism Systems biology: Energy and Saccharomyces cerevisiae) is to connect the two by developing an approach that starts both from the edges and the nodes of the network. This project is a joint effort of six European universities. A domino strategy is followed by i) extending systems biology along pathways that regulate cell function, ii) beginning with regulation through the most connective small molecules, and iii) proceeding with regulation of major pathways synthesizing or degrading those molecules at high flux rates. Once the network of regulation through such a molecule has been mapped and quantitatively understood (modelled), regulation through the second best connected molecule is addressed.

To demonstrate the principles of the approach, we have started with ATP (and ADP, AMP) as the most connected molecule and focus on the main pathways involved in catabolism, anabolism, and ATP consumption without driving growth.
(maintenance). The system is analysed step by step and dimension (e.g. metabolism, transcription etc.) by dimension. The Amsterdam group contributes to several aspects of the MOSES project, one of which will be discussed below.

To be able to exchange and integrate data of several research groups, it is essential to standardise the experimental procedures used [1]. In addition, care should be taken that the standardisation leads to data that is relevant, i.e. representative of the in vivo situation.

A clear example of this need for standardisation is the measurement of the kinetic parameters of enzymatic reactions. Typically, these parameters have been determined in vitro and under –allegedly- optimal conditions for each enzyme, resulting in widely varying conditions, even for enzymes in the same metabolic pathway. The Vertical Genomics Consortium, Yeast Systems Biology Network (YSBN) and the Standards for Reporting Enzymology Data (STRENDA) Commission have recently presented a standardised in vivo-like assay medium for kinetic studies on cytosolic yeast enzymes [2]. We have used this medium and other assay conditions described in this paper as a foundation for the enzyme kinetics measurements carried out within the framework of the MOSES project.

We set out to analyse the elasticities of the enzymes of the glycolytic and fermentation pathways in S. cerevisiae towards ATP, ADP and AMP and the regulation of the concentrations of these cofactors. This will allow us to quantify the contribution of the adenine nucleotides to the metabolic regulation. In this short presentation we will not discuss the results of this work in progress in detail, but rather focus on the process of implementing the proposed standardisation and extending it to be able to measure the effect of the adenine nucleotides on the enzyme rates.
Methods

The *S. cerevisiae* strain CEN.PK113-7D (MATa, MAL2-8Δ, SUC2, obtained from P. Kötter, Frankfurt, Germany) was grown in a one litre anaerobic glucose-limited chemostat culture at 30 °C and a dilution rate of 0.1 h⁻¹. The mineral medium and culture conditions used were essentially according to [3,4], with a glucose concentration of 125 mM in the feed. This growth condition represents the standard condition agreed on with the MOSES consortium.

The cell free extracts used for the enzyme activity measurements were prepared by disruption the cells with a bead-beating method as described in [2]. No phosphatase inhibitors were used.

The enzyme activity measurements were carried out under the *in vivo*-like conditions given in [2], with the alterations given below. When (additional) nucleotides were added, the same amount of MgSO₄ was added as well. Measurements were carried out in a Spectramax Plus plate reader (Molecular Devices). The activity of phosphofructokinase was not measured, due to lack of availability of the activator fructose 2,6-bisphosphate.

Results and discussion

We set out to collect a comprehensive set of data on the effect of physiologically relevant concentrations of ATP, ADP and AMP on the activity of the enzymes of the glycolytic and fermentative pathways in *S. cerevisiae*, under standardised conditions. We used the proposed *in vivo*-like assay medium as described in [2]. Apart from the base assay medium, assay conditions were kept the same as the assays under previously used optimal conditions. However, in certain cases we found that varying the substrate and/or activator concentrations re-
sulted in higher measured activities. Since we were among other things interested in determining the maximum enzyme activity, we decided to alter these concentrations whenever necessary (see Table 1). The base composition of the assay medium was left intact, hence still allowing standardised results.

Table 1 – Altered concentrations of substrates, activators, etc. in the activity assays of hexokinase (HXK), phosphoglucone isomerase (PGI), fructose 1,6-bisphosphate aldolase (ALD), triose phosphate isomerase (TPI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (ENO), pyruvate kinase (PYK), pyruvate decarboxylase (PDC), and alcohol dehydrogenase (ADH).

<table>
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<th>enzyme component</th>
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<tr>
<td>HXK ATP</td>
<td>1 mM</td>
<td>3 mM</td>
</tr>
<tr>
<td>NADP⁺ glucose</td>
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<td>1 mM</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>10 mM</td>
</tr>
<tr>
<td>PGI ATP</td>
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<td>0.15 mM</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>25 mM</td>
</tr>
<tr>
<td>ALD NADH</td>
<td>0.15 mM</td>
<td>0.15 mM</td>
</tr>
<tr>
<td>fructose 1,6-bisphosphate</td>
<td>2 mM</td>
<td>20 mM</td>
</tr>
<tr>
<td>TPI NADH</td>
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<td>0.15 mM</td>
</tr>
<tr>
<td>glyceraldehyde 3-phosphate</td>
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<td>24 mM</td>
</tr>
<tr>
<td>GAPDH NAD⁺ ADP</td>
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<td>5 mM</td>
</tr>
<tr>
<td>glyceraldehyde 3-phosphate</td>
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<tr>
<td></td>
<td>6 mM</td>
<td>4 mM</td>
</tr>
<tr>
<td>PGK ATP</td>
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<tr>
<td>NADH 3-phospho glycerate</td>
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<tr>
<td>PGM 2,3-diphospho glycerate</td>
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</tr>
<tr>
<td>3-phospho glycerate</td>
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</tr>
<tr>
<td>ENO 2-phospho glycerate</td>
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</tr>
<tr>
<td>PYK ADP</td>
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<tr>
<td>NADH fructose 1,6-bisphosphate phosphoenol pyruvate</td>
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<tr>
<td>PDC NADH cocarboxylase</td>
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Quite a few of the standard assays for the glycolytic enzymes contain so-called coupling enzymes to facilitate the coupling of the reaction under investigation to one where NAD(P)H or NAD(P)⁺ is oxidised or reduced, respectively, to monitor the reaction. Unfortunately, some of these coupling reactions require ATP or ADP, and the nucleotides needed to be excluded from the assay mixture since the effect of their
presence is under investigation here. Hence, the assays of phosphoglycerate mutase & enolase were altered by removing coupling enzymes from the reaction mixture. These reactions were monitored by the measuring the synthesis of PEP at 240 nm. Although ATP and ADP have a high extinction at 240 nm as well, we managed to carry out the measurements in the linear range of the spectrophotometer.

We have determined the activity of the enzymes of the glycolytic and fermentation pathway in cell extracts of the standard culture conditions, as given in Figure 1. The determined maximal activities are well in line with the glucose and ethanol fluxes through the system under these conditions (respectively 5.8 and 7.5 mmol (g DW)$^{-1}$ h$^{-1}$). The effect of ATP, ADP, and AMP on the enzymes activity will be discussed in our presentation.

Figure 1 – Determined maximum activities of enzymes of the glycolytic and fermentation pathway of the S. cerevisiae strain CEN.PK113-7D under the defined anaerobic chemostat conditions. Assay conditions as described above were used.
References


Not all downstream metabolites are the same: Modelling of the Mevalonate pathway

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Abstract

The mevalonate pathway is an essential metabolic pathway in eukaryotic organisms since it is responsible for sterol synthesis and the production of isoprenoid lipids needed for the generation of isoprenoid-containing molecules and for the prenylation of proteins. In this work we investigate the effects on downstream and upstream metabolites upon treatment with bisphosphonates, a class of drugs commonly-used to treat bone diseases. With this example we show that inhibiting an enzyme does not necessarily lead to a lower level of downstream metabolites.

Introduction

The mevalonate pathway generates several important metabolites including isoprenoids, important for the posttranslational prenylation of small GTPase proteins. The latter are required for normal cell function, including cell proliferation, differentiation, migration, and adhesion. In the liver the pathway’s main purpose is the production of cholesterol, an essential sterol but also a key player in human cardiovascular disease. The pathway is therefore a target of two important drug classes. On the one hand, statins are used to lower the production of cholesterol by inhibiting HMG CoA reductase [1], the
rate-limiting step in the pathway. The other class of drugs are bisphosphonates, used to treat common bone diseases such as post-menopausal osteoporosis, Paget's disease and cancer-induced skeletal disease [3]. Bisphosphonates have a high affinity for calcium and are thus quickly localized to bone surfaces from which they are mainly ingested by bone-destroying osteoclasts. Inhibition of the distal enzyme farnesyl pyrophosphate synthetase in osteoclasts causes loss of osteoclast function by interfering with the prenylation of small GTPases [5].

**Results**

We developed a kinetic model of the mevalonate pathway restricting ourselves to farnesyl pyrophosphate synthetase and its proximal and distal enzymes to study the effects of treatment with the bisphosphonate zoledronate (ZOL). The model is comprised of five main reactions that convert mevalonate diphosphate into...
Figure 2: Simulation results for various metabolite concentration and fraction of the outfluxes

isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMAPP), farnesyl pyrophosphate (FPP) and geranygeranyl pyrophosphate (GGPP), which primarily differ by their carbon chain length. Another three consuming reactions are added to prevent metabolite accumulation. This minimal model is depicted in Figure 1 in the shaded area. The reactions are modelled mainly with Michaelis-Menten kinetics with parameters from the literature where available [2,4]. The central reaction FPP synthetase is uncompetitively inhibited by ZOL. Figure 2 shows the concentrations of various metabolites upon treatment with different levels of ZOL. Interestingly, with increasing dosage of ZOL, the FPP level decreases and the IPP level increases as expected, but the level of the downstream product GGPP increases. The reason for this is that, at these metabolite concentrations, GGPP synthetase is not limited by IPP but by FPP. Around a ZOL concentration of 0.2, the enzymes are saturated with IPP and production of GGPP de-
creases due to the decreasing FPP. On the lower panel of Figure 2 the fraction of the outfluxes are shown. The ratio of GGPP and FPP outflux clearly changes with increasing ZOL concentration.

**Discussions**

Our model leads to the counterintuitive prediction of increasing levels of the downstream product GGPP upon inhibition of FPP synthetase. This could explain why very low concentrations of ZOL stimulate, rather than inhibit, the proliferation or differentiation of some cultured cell types. At higher concentrations of ZOL, the level of GGPP decreases, thus preventing normal protein prenylation. This effect is dependent on metabolite concentrations and enzymatic properties, and it is likely to be present in other pathways where reactions draw substrates from both upstream and downstream of a regulated reaction. This may be a way for nature to control the composition of metabolic precursors needed for further regulation. Moreover, it should be noted that such effects are difficult, if not impossible, to predict from a pathway diagram without the help of mathematical modelling.

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Mechano-probability Model of FoF1-ATPsynthase Catalytic Cycle: A Novel Approach to ATP Synthesis and Hydrolysis Description

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FoF1-ATPsynthase is a key enzyme for bioenergetics of the living cells. However precise mechanism of its functioning is not yet fully described. Moreover, up-to-date experimental approaches do not provide enough information for the thorough understanding of a complex enzyme mechanism [1]. Meanwhile computational methods are a simple and precise way to obtain various numerical data quickly and cheaply. In this work a new approach to FoF1-ATPsynthase description was applied to construct a single enzyme computer simulator, based on the main proved principles of its functioning.

The above model consists of three units, calculated simultaneously during the simulation. One of them represents description of step-by-step c-subunit core rotation. Another one evaluates the time of proton movement through the half-channels. The third part of the model formulizes a probability scheme for beta-subunits catalysis. The description of rotor movement with Langevine equation based on proven structural data allows us to obtain kinetically independent values of angular rotation velocity under various conditions [2]. To simulate the catalysis in beta-subunits, the process on each subunit was divided into several essential steps and then the probability of each event and the time of its occurrence were
evaluated. The flow graph of the elementary events on each subunit includes fixation of its state, occurrence and the type of bound nucleotide, evaluation of catalysis probability and possible nucleotide release. The overall probability scheme describes states of all catalytic subunits and conditions of their interconversion. To obtain ATP and proton fluxes the data about c-subunit cylinder rotation, proton transfer and the catalysis were integrated in the computer algorithm.

According to the introduced model ATP and ADP fluxes under different conditions for a single enzyme and mean values for a number of FoF1-ATPsynthases were obtained. The values of estimated fluxes agreed with known experimental data [3], and a catalytic equilibrium observed under physiological concentrations of ATP and ADP confirmed the reversibility of the process. The calculated time of proton transfer for a single half-channel based on diffusion coefficients and protonation/derprotonation constants was evaluated to be much less than the rotation and catalysis time respectively. Nevertheless, the proton flux was not negligible and it also agreed with known experimentally measured values. Thus, the simulator introduced in the present study can be used as a research tool for overall estimation of ATP/ADP yield in catalytic cycle of ATP synthase. The application of the above program could be spread on the modeling of ATP/ADP flux in various tissues and organs under physiological and pathological conditions.

References
A detailed kinetic model for mammalian skeletal muscle glycogen synthase and its regulation

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Abstract

Glycogen synthase and glycogen phosphorylase are generally recognized as key enzymes in the regulation of glycogen metabolism. However, the complex kinetics exhibited by glycogen synthase, in particular, makes it very hard to describe mathematically. Interest has been renewed in glycogen synthase kinetics by several studies that suggest it is not the flux-controlling enzyme of glycogen synthesis—as is traditionally believed. We constructed a detailed kinetic model of glycogen synthase that describes both its regulation by phosphorylation and allosteric modification. Glycogen synthase is modeled by an adapted Monod-Wyman-Changeux equation. Using rate characteristics, we show how both allosteric and covalent regulation of glycogen synthase serves to maintain metabolite homeostasis despite large changes in the glucose uptake rate.

Introduction

Glycogen synthase and glycogen phosphorylase are generally recognized as key enzymes in the regulation of glycogen metabolism. Yet, more than fifty years after their discovery, there exists no satisfactory kinetic description for either. This is due in part to the complex kinetics exhibited by these
enzymes: both are modified covalently and regulated by allostERIC effectors. Glycogen synthase, in particular, is extensively regulated, both allosterically by glucose-6-phosphate (G6P) activation and by ATP inhibition, and covalently by phosphorylation at nine serine sites.

Due to incomplete understanding of the extent of phosphorylation of glycogen synthase, earlier kinetic treatments of glycogen synthase employed a simple two-state phosphorylation model in which the enzyme exists as either the dephosphorylated, G6P-independent I form or the phosphorylated, G6P-dependent D form. More recent treatments that do take multisite phosphorylation into account often use the so-called fractional activity—the ratio of activity in the absence of G6P to the activity in the presence of saturating G6P—as a measure of inhibition by phosphorylation or activation by G6P. The fractional activity, although useful for assessing the relative importance of phosphorylation sites, provides little kinetic insight regarding the effects of phosphorylation.

Interest has recently been renewed in glycogen synthase kinetics as a result of the findings, as reviewed by Shulman et al. [3], that—contrary to the traditional understanding—glycogen synthase is not the rate-limiting enzyme of glycogen synthesis and that its extensive regulation serves to control metabolite homeostasis rather than the synthetic flux. This is in agreement with the conclusions drawn by Hofmeyr and Cornish-Bowden [1] in a theoretical exploration of the role of allosteric feedback and feedforward mechanisms.

Methods

In order to gain better insight in the regulatory design of glycogen metabolism, we have constructed a detailed kinetic model that includes both glycogen synthase multisite phosphorylation and allosteric modification.
The nine phosphorylation sites of glycogen synthase could theoretically result in 512 phosphorylation states. If one however takes into account that these phosphorylation sites are grouped together in two clusters that are phosphorylated hierarchically and ignores the sites that have negligible effects on glycogen synthase activity, it is possible to describe glycogen synthase as existing in only 18 states. We modeled the interconversion between these states by kinase and phosphatase reactions, which are themselves influenced by the hormonal regime.

The glycogen synthase rate equation is thus the sum of the rate equations of the 18 glycogen synthase states. It is conceivable that each glycogen synthase phosphorylation state could exhibit very different kinetics, requiring an enormous number of kinetic parameters to describe such a system mathematically. However, crystal structures of bacterial and archaeal glycogen synthase, and a comprehensive kinetic study by Piras et al. [2] suggest that the enzyme can be described by a Monod-Wyman-Changeux (MWC) model in which G6P, ATP and phosphorylation affect the equilibrium between an active (R) and inactive (T) conformation. We therefore modelled each glycogen synthase state with the MWC equation, using the same parameter values for the different states; these parameters were fitted to the experimental results of Piras et al. [2]. The R/T equilibrium constant, which has a different value for each of the 18 states, was calculated from results in the literature.

We included this description of glycogen synthase in a core model of glucose uptake and glycogen synthesis and analysed its regulatory design by means of supply-demand rate characteristics in which glucose uptake is a G6P supply subsystem and glycogen synthesis is a G6P demand subsystem.
Results and Discussions

We adapted the MWC equation to describe the effects of multiple phosphorylation and allosteric effectors on the kinetics of glycogen synthase (Figure 1). The rate equation describes the 18 phosphorylation states of glycogen synthase with only 27 parameters. It extends the application of the MWC model—a classic model of cooperativity and allosterism—to enzymes that undergo covalent modification.

\[ v = \sum_j \frac{k_{cat}[GS_j]\sigma_r}{(1 + \omega_t)L_j}\left(\frac{1 + \mu_t + \xi_t}{1 + \mu_r + \xi_r}\right)^n + (1 + \omega_r + \sigma_r) \]

\[ \sigma = \frac{[UDPG]}{K_m}, \omega = \frac{[ATP]}{K_{ic}}, \mu = \frac{[G6P]}{K_a}, \text{ and } \xi = \frac{[ATP]}{K_i} \]

Fig.1. Glycogen synthase rate equation. The phosphorylation state is denoted by \( j \). Each phosphorylation state is described by an adapted MWC equation in which G6P, ATP and phosphorylation alter the R/T equilibrium.

Supply-demand analysis around G6P reveals that phosphorylation and ATP increase the demand elasticity. This allows the G6P concentration to be maintained in a narrow range despite significant increases in the supply flux. Insulin-stimulation increases both the supply and demand fluxes, which further limits G6P concentration fluctuations.

It has been shown previously [1] that the role of allosteric regulation is to maintain metabolite homeostasis rather than the control of flux. We here demonstrate that phosphorylation could fulfil a similar role. The purpose of the extensive regulation of glycogen synthase is, therefore, not the control of glycogen synthetic flux, but rather the homeostatic maintenance of G6P concentration.
References


Kinetic modeling of deoxythymidine kinase in the pyrimidines biosynthesis pathway of *Escherichia coli*

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Deoxythymidine kinase (TDK) (EC 2.7.1.21) is one of the key enzymes in Pyrimidine Nucleotides Biosynthesis Pathway of *Escherichia coli* (Figure 1). TDK catalyzes the phosphorylation of deoxythymidine, or thymidine (TdR), as well as deoxyuridine (UdR) using different phosphate donors (ATP, dATP, GTP, dGTP, dCTP, ITP): 

\[
\begin{align*}
\text{ATP} + \text{thymidine} &= \text{ADP} + \text{thymidine 5'-phosphate}; \\
\text{dATP} + \text{thymidine} &= \text{dADP} + \text{thymidine 5'-phosphate}; \\
\text{dGTP} + \text{thymidine} &= \text{dGDP} + \text{thymidine 5'-phosphate}; \\
\text{dCTP} + \text{thymidine} &= \text{dCDP} + \text{thymidine 5'-phosphate}; \\
\text{GTP} + \text{thymidine} &= \text{GDP} + \text{thymidine 5'-phosphate}; \\
\text{CTP} + \text{thymidine} &= \text{CDP} + \text{thymidine 5'-phosphate}; \\
\text{ATP} + \text{deoxyuridine} &= \text{ADP} + \text{deoxyuridine 5'-phosphate}.
\end{align*}
\]

The catalytic action of TDK is controlled by the activators: ATP, dATP, dCDP and other deoxydiphosphates, and by the inhibitory effect of various compounds: dTMP, dTDP, dTTP, dGTP, dCTP, dUTP, as well as ADP, CDP, UDP, CTP, UTP.

Subsequently, the enzyme named deoxythymidylate kinase catalyses the conversion of dTMP to dTTP. In turn, dTTP serves...
as one of the substrates (additionally to dATP, dCTP, and dGTP) for DNA polymerase in DNA synthesis.

TDK attracts many investigators attention. Nevertheless, we have not found developed kinetic models of TDK in literature. The TDK action mechanism is of high complexity, therefore the choice of its specificity is crucial for modelling. We applied the steady-state and quasi-equilibrium approaches, elaborating the model.

Kinetic model of TDK catalytic cycle is elaborated based on the experimental data for Escherichia coli.

Literature available experimental data relevant to the kinetic properties of this enzyme (catalytic constants, Michaelis constants, and others) are not sufficient for reliable kinetic model development; therefore additional parameters had been obtained by fitting model curves against experimental data. Values of about 70 parameters were obtained using DB-Solve7.01 software. Parameters were fitted consequently, in groups corresponding to the data for different experiments, described in [1, 2]. The expression for reaction rate dependence on pH was also obtained.

Using the elaborated model it is possible to extrapolate the action of TDK to wide range of pH, as well as to predict the action of the enzyme in presence of different phosphate donors, activators and effectors in various combinations.

Using the elaborated kinetic model of TDK we can solve next practical tasks, which are important for microbiology and biomedicine:

1. Prediction of TDK behavior in the cell under different conditions.

2. Prediction of switching activities of TDK regarding to different phosphate donors in vivo and in situ.
TDK in the Pyrimidines biosynthesis pathway of Escherichia coli

Different activities of TDK with substrates TdR, UdR and different phosphate donors are shown
References


Concluding remarks

Systems biology represents a new rapidly developing interdisciplinary area of modern research. The interest displayed in it now is due to the necessity of gaining a deeper insight into the essence of biological processes occurring in a living organism taking into consideration an influence and interaction with diverse environmental factors both biotic and abiotic ones. On the other hand the new computer possibilities open practically unlimited possibilities in accumulation, processing, analysis, and integration of factual material coming from most successfully developing lines of investigation in medicine, biology and other natural sciences.

Discoveries and achievements in concrete areas of knowledge nourish Systems biology with new objective information, which allows the researchers in the field to advance, by way of feedback, new verifiable hypotheses and predictive concepts.

Todays trends in Systems biology with an emphasis on problems of virtual modeling and on those arising in studying regulatory processes were discussed at the regular 14th Workshop of the International Study Group for Systems Biology, hold on the 6-10th of September, 2010, attended by the representatives of the leading research teams engaged in this field from different countries.

The meeting has revealed a wide range of objects of research interests, from microbial to somatic cells, and has given particular attention to the analysis of molecular receptor structures, metabolic networks and regulatory mechanisms in interactions with environmental elements. By way of examples, various models in vitro, in vivo, and in silico are described including computational virtual phantoms with the outlook for developing silicon models of separate biological systems and even a human. Considered from the position of Systems biology, along with general theoretical questions, were genetically controllable and regulatory mechanisms operating in a metabolic network including the interaction of various
biostructures, prokaryotes and eukaryotes among them, as well as with environment. Also described were the kinetic models of analyzing the processes of metabolism and those of the genetic mechanisms of its regulation, as well as the prospects of creating the virtual phantoms of these processes. Special attention was given to questions of modeling the receptors and transporters for neurotransmitters, such as, the neuronal glycine transporter GLYT2. A number of communications were devoted to experimental models and to those of kinetic analysis for studying the mitochondrial response and understanding the origin of mitochondrial disfunction, particularly in the case of ischemic pathology, as well as for finding out the potentialities of natural metabolites, such as glycine, for their therapy-oriented correction. A number of communications were also devoted to the problems of bioenergetics and to the application of the modeling approach in Systems biology for describing ATP synthesis and anabolic processes.

The Institute of Cytochemistry and Molecular Pharmacology (ICMP) headed by Yaroslav Nartsissov arranged and hosted the said meeting. This institute focuses its main research interest on a comprehensive investigation of the mechanisms of action and on the estimation of medical products developed on the basis of natural metabolites, such as glycine, applying actively the modeling approach. A number of investigations carried out at the ICMP are presented in the proceedings of this meeting. It is worthy of note that in modeling preference is given to a complex approach involving both experimental biomodels (e.g., in studying microcirculation) as well as mathematical and virtual computerized phantom models.

It is necessary to separately stress the importance of international cooperation, as repeatedly exemplified at the Workshop. The development of collaborative investigations under agreed-upon protocols, which will, along with information interchange and databank accumulation, contribute to the optimization and standardization of methodological approaches as
well as to the accomplishment of large-scale projects. The distribution of the approaches developed by Systems biology and keeping the broad scientific community well-informed about the achievements in this area will, among other things, will promote more rational use of the available resources including biological ones. All the contributions made to the meeting are of undoubted interest for the experts engaged in the field of Systems biology as well as for a large circle of researchers.

Gennady I. Podoprigora

Editor of Modern Trends in System Biology
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