A MINIMAL TREATMENT OF AMOEBIASIS BY DRUG COCKTAIL AGAINST HEXOKINASE AND GLUCOSE TRANSPORTER IS PREDICTED BY STATISTICAL MODELLING

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Introduction

Amoebiasis is a major health concern in southern countries with yearly 50M infections and 70K death cases. [1] Entamoeba hystolytica is one causative parasite, responsible for the more severe infections. Entamoeba produces ATP only by glycolysis, therefore its enzymes are optimal targets for medical knock-down. Earlier modeling work concludes hexokinase as a rate limiting step. [4] However a new, extended and elaborated model presented here suggests glucose transporter as Nr.1 drug candidate being the single rate limiting step in the pathway. This result is reinforced by an early biochemical finding in 1974 by Serrano and Reeves. [2,3] Nevertheless, global sensitivity analysis reveals that the control of glycolytic flux easily swifts between glucose transporter and hexokinase. This switch-like behavior occurs at physiological parameters, pointing out that a cocktail of at least two drugs against these enzymes could be more effective for elimination of the parasite. To decide if the waggling flux control is indeed present *in vivo*, or just a possibility can be decided on further data on Entamoeba.

Materials and Methods

- A simple **ODE model** was set up, with most **parameters** of glycolytic enzymes **measured *in vitro***. [4,5]
- Expected result (from experimental work) is recovered, **glucose transport is the single rate limiting step,** but contradicts earlier modeling work, stating hexokinase is the rate limiting**.** [4]
- Global Sensitivity Analysis (tells about the effect of parameter variance) shows that **flux control easily shifts to hexokinase,** in a peculiar manner shown in Fig 2.

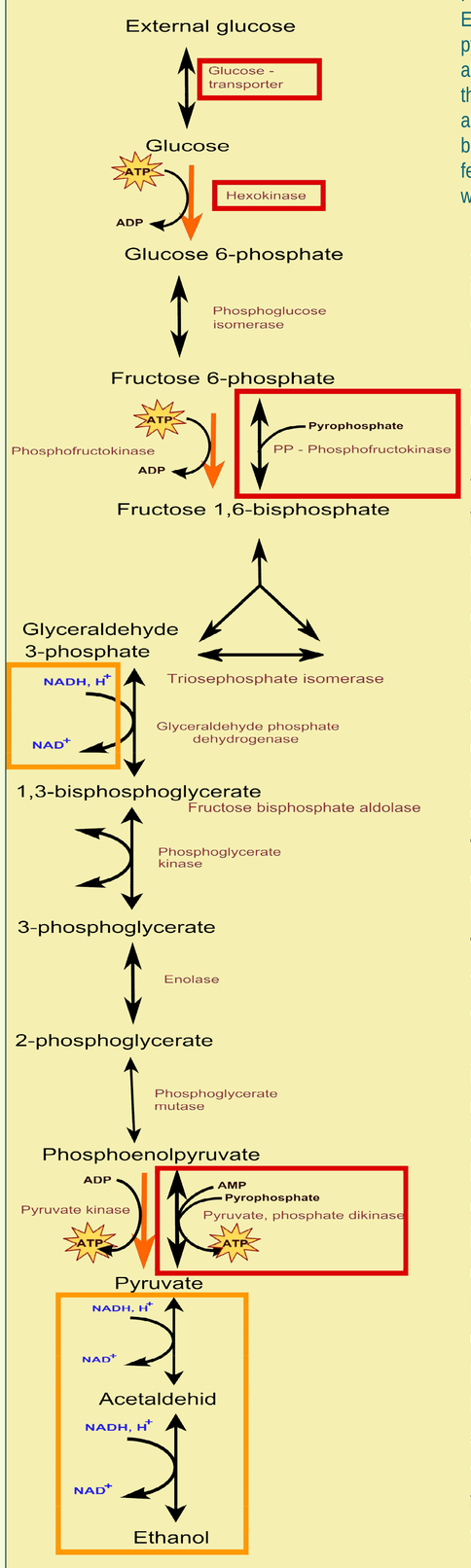

Figure 1: The glycolysis in Entamoeba features two reversible, pyrophosphate using enzymes, that allow much wider product feedback in the glycolytic chain. Some enzymes are also differently regulated. Red boxes mark the Entamoeba specific features, orange boxes, the parts that were included in the extended model.

- Model was **extended** to incorporate NADH-balance and downstream reactions. Fig 1, red squares.
- Model was refined, **more data was incorporated**. Km data for enzymes were taken as fixed, reverse and forward Vmax values were used to establish boundaries for each enzymes back- and forward rates.
- **Multiple parameter estimations** were carried out, and the extended model showed the „**flux control switch" in part of the results**, whereas **others showed hexokinase or glucose transporter control.**
- **Parameters showed** around 30% error among 100 independent fits, indicating **limited unidentifyability.**
- To decide whether this bimodality is indeed the case in patients, or at least in culture, more accurate data is required. Nevertheless, under a given circumstances **only a part of the cells may respond to a drug against a certain enzyme.**
- Further work was focused on the flux control switch.

Results and Discussion

- 38 Parameters (only Vmax values and balancing reactions) were fitted to 13 measured metabolite concentrations.
- **A fitted model with the bimodality** in flux control coefficients (FCC) was chosen, and **global sensitivity analysis** was applied. In this, mimicking dispersion of a natural cell population,

Parameter sets were sampled from a random uniform, or normal distribution, and in each case where a steady state was found, the flux control coefficients were calculated. They show the bimodal distribution shown in Fig 2.

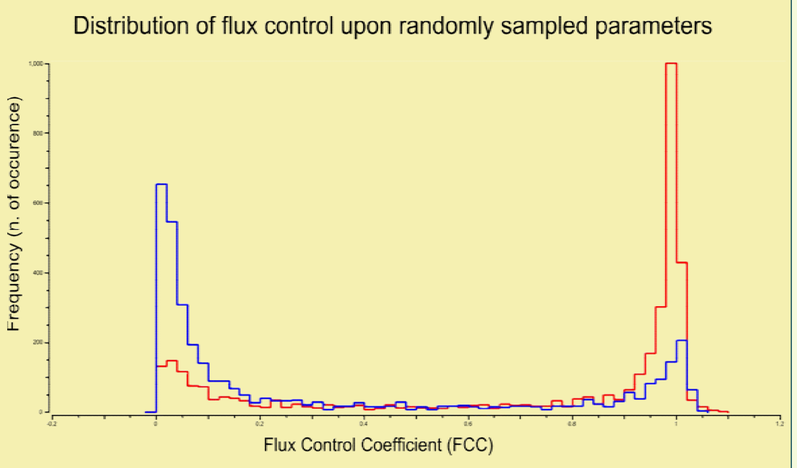

Figure 2: glucose transporter (red) and hexokinase (blue) takes either no, or rate limiting role in glycolysis. They take leading role complementary to each other. The flux control is otherwise not distributed, so other enzymes do not govern the flux.

Constraining random sampling of the parameters, it turned out that only parameters of these two enzymes account for the shift of the FCC, Vmax values in a more pronounced way, but also Km values had an effect. However, no single parameter alone showed remarkable correlation with the FCCs. Instead internal glucose [Gi] showed a clear correlation: Fig 3.

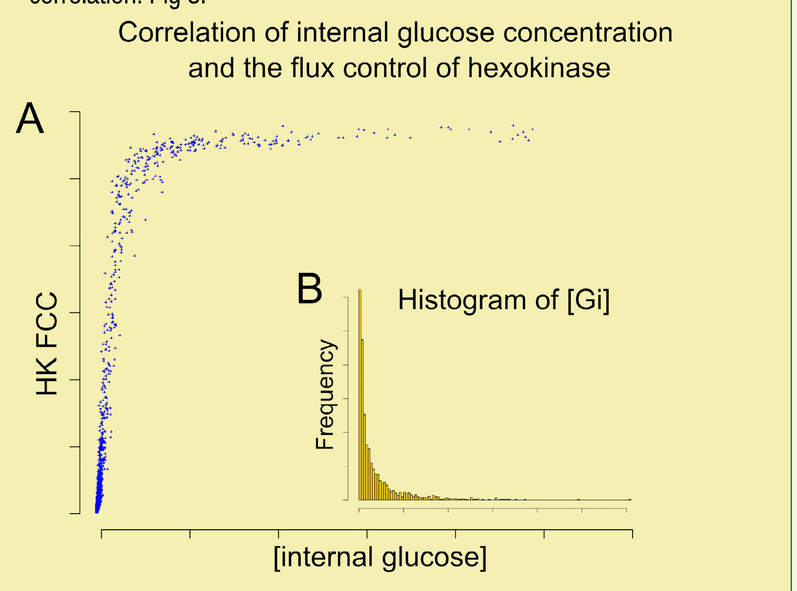

Figure 3: A: Positive correlation of internal glucose concentration with hexokinase FCC. One can see the bimodal histogram in Fig 2 summing up the occurrences towards the Y-axis (FCC). There is an opposite correlation of the internal glucose concentration to glucose transporter flux control., not shown for clarity. B: distribution of internal glucose concentration in the sampled parameter sets. The peak at very low concentration is represented as cluster at the bottom left corner of panel A.

Although no single reaction parameter correlated with the FCCs when all parameters were randomly sampled, we found that the quotient hexokinase Vmax / glucose transporter Vmax-fwd does correlate with flux control. Fig 4. Scanning single parameters showed, that GLUT Vmax (fwd and backward), HK Vmax and HK KATP influence FCCs.

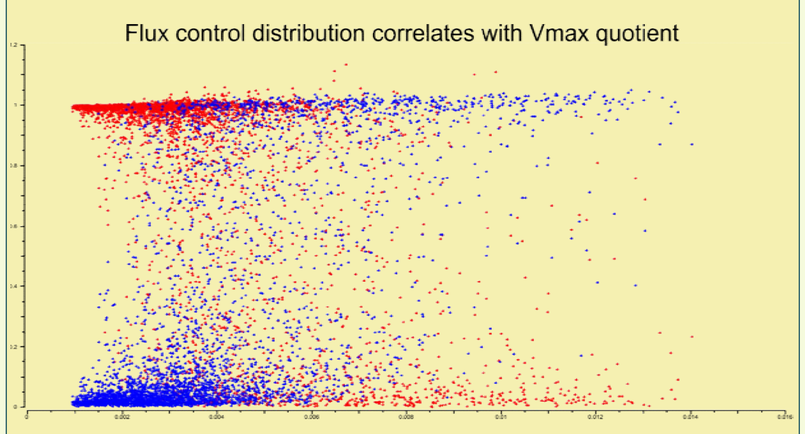

Figure 4: The glycolytic flux is controlled by glucose uptake if the Vmax quotient is low, if high it is controlled by hexokinase. The transition is though quite dispersed, as many other randomly sampled parameters affect the flux control distribution

Results and Discussion

Beside sampling all parameters at random, diagrams of reaction parameter dependence showed that three different parameters can account for the bimodal distribution of flux control coefficients. Fig 5.

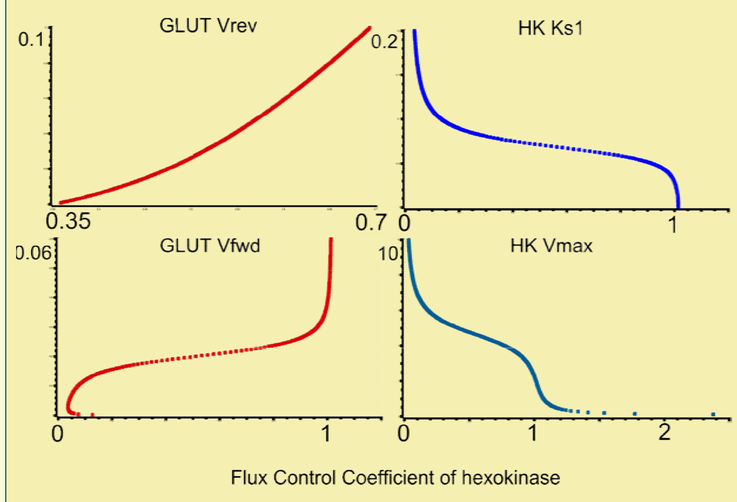

Figure 5: The dependence of flux control on enzyme kinetic parameters: Most binding constants as well glucose transporter reverse Vmax (A) does affect the Flux Control, but cannot account for the bimodal distribution. On the other hand glucose transporter fwd Vmax (C), hexokinase ATP binding constant (B) and Vmax (D) all can alone account for the flux control coefficients.

Similar switching phenomenon is also known from other models, as the yeast glycolysis model by Hvmne [6], or by Kell [7] or the trypanosome glycolysis by Bakker. [8]

Conclusion

- A model of Entamoeba hystolytica's central metabolism indicated that there is a bimodality in flux control within the range of in vitro measured parameter values, that may influence treatment efficacy. glucose transporter and hexokinase were identified as single and complementary rate limiting enzymes, since in a population of cells both may appear rate limiting, minimally a 2-component drug cocktail is suggested for treatment.
- It was found that 3 reaction parameters influence the rapid transition between a hexokinase and a Glucose uptake controlled regimes and that the quotient of Glucose and hexokinase forward Vmax-s correlate with the Flux control coefficients upon random sampling of all parameters, whereas single parameters show no correlation.

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