Control and regulation of glycolysis in Trypanosoma brucei

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Summary

Trypanosoma brucei is a unicellular, eukaryotic parasite causing the African sleeping sickness. When living in the mammalian bloodstream this organism depends solely on glycolysis for its ATP supply. Trypanosome glycolysis differs from that in other organisms, because seven enzymes catalysing the conversion of glucose into 3-phosphoglycerate are confined to a specialized organelle: the glycosome. Furthermore, the glycosomal enzymes appear hardly sensitive to compounds that regulate the corresponding enzymes in other organisms.

The central question addressed in this thesis is which steps control the glycolytic flux in bloodstream form *T. brucei.* According to Metabolic Control Analysis the control exerted by an enzyme on the steady-state flux through a metabolic pathway is expressed quantitatively by a flux control coefficient, defined as the relative change of the flux divided by the relative change of the activity of this enzyme at constant activities of all other enzymes. If an enzyme has a flux control coefficient of 1, it is the rate-limiting step of the pathway. If its flux control coefficient is zero, it exerts no control. The definition also allows to analyse situations where the enzymes share control. The distribution of flux among pathway enzymes is determined by their kinetic properties. Most of the glycolytic enzymes from *T. brucei* have been characterized kinetically under fairly uniform conditions. This should enable one to calculate the distribution of control in *T. brucei.* With this aim the kinetic data were used to construct a detailed model of trypanosome glycolysis. The calculated flux and metabolite concentrations corresponded reasonably well to experimental values (Chapter 2). The model also reproduced the inhibition of anaerobic glycolysis by glycerol, although in the model the amount of glycerol required to inhibit glycolysis was lower than experimentally determined. This discrepancy diminished when the transport of glycerol 3-phosphate across the glycosomal membrane was coupled to that of dihydroxyacetone phosphate via an exchange mechanism (Chapter 3).

This improved model was then used to investigate which steps control the glycolytic flux under physiological conditions (Chapter 3). Most surprisingly there was no single answer: in the physiological range of glucose concentrations the control shifted between the glucose transporter on the one hand and aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK) and glycerol-3-phosphate dehydrogenase (GDH) on the other hand. The other kinases, which are often thought to

control glycolysis, exerted only little control. The utilization of ATP did not control the flux either, demonstrating that trypanosome glycolysis is supply-driven rather than demand-driven.

The precise value of the flux control coefficient of the glucose transporter was very sensitive to uncertainties of parameter values and could not be estimated reliably. Therefore, its control coefficient was determined experimentally by titrating with phloretin, an inhibitor of glucose transport. To measure the effect of the inhibitor on the activity of the transporter itself a rapid (5 s) assay of uptake of radiolabelled glucose was developed (Chapter 4). At high glucose concentrations the measured uptake rate was high enough to explain the steady-state glycolytic flux. Phloretin proved to be a competitive inhibitor. Its effect on the transporter, therefore, depended not only on the extracellular glucose concentration, but also on the intracellular glucose concentration. Since the inhibition of glucose efflux had not been measured, one of the K_i's was unknown and had to be estimated. This resulted in an uncertainty in the flux control coefficient: at 5 mM glucose it was between 0.3 and 0.5 (Chapter 5). At a very low glucose concentration (0.5 mM) the glucose transporter assumed all flux control, in agreement with model predictions. In contrast to previous speculations (Gruenberg, J., *et al.* (1978) *Eur. J. Biochem.* **89**, 461-469) these results prove that glucose transport is not *the* rate-limiting step of trypanosome glycolysis: under physiological conditions it is just one of the controlling steps, but it shares control with other enzymes. One of these steps was the transport of pyruvate across the plasmamembrane, which had a flux control coefficient of 0.1, as was measured by titrating with the inhibitor UK5099 (Chapter 5). Most probably, the other controlling steps are ALD, GAPDH, PGK and GDH, as the model predicted, or otherwise the transporters of glucose, 3-phosphoglycerate, glycerol 3-phosphate and dihydroxyacetone phosphate across the glycosomal membrane, which had not been included in the model.

One of the intended applications of this study is the optimization of selectivity of drugs against African sleeping sickness. If an enzyme has a high flux control coefficient in the parasite, while the corresponding enzyme in the mammalian host has a low flux control coefficient, an inhibitor of this enzyme should inhibit the steady-state flux more selectively than it inhibits the isolated enzymes (Chapter 1). From the above results is may be predicted that trypanosome glycolysis is most sensitive to inhibition of the glucose transporter, followed by inhibition of ALD, GAPDH, PGK and GDH. This was confirmed by simulations in which large activity changes were considered (Chapter 3). The enzymes catalysing irreversible reactions, hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PYK), seemed to be present in excess and are mediocre targets from this

perspective. The flux control exerted by the glucose transporter in various host tissues, has never been determined directly. Our results warrant further research into this control. Other authors calculated that the activities of ALD, GAPDH and PGK could be decreased substantially in erythrocytes before clinical symptoms should be expected (Schuster, R. and Holzhütter, H.-G. (1995) *Eur. J. Biochem.* **229**, 403-418). Consequently, a cocktail of inhibitors against these enzymes may prove to be effective against sleeping sickness.

It has been proposed that inhibition of the glycolytic flux is impossible, because most inhibitors are competitive and the cell can overcome the inhibition by increasing the concentrations of the competing substrates (Eisenthal, R. and Cornish-Bowden, A. (1998) *J. Biol. Chem.* **273**, 5500-5505). Indeed, the required $[I]/K_i$ ratios may be higher than expected, but the problem may be solved by designing inhibitors that compete with coenzymes, of which the concentrations cannot increase unrestrictedly. The flux should be inhibited most effectively if such inhibitors would be combined with inhibition of the biosynthesis of the corresponding coenzymes (Chapter 7).

Until now it is unknown why the first part of glycolysis of trypanosomes and other Kinetoplastida takes place in glycosomes, while in other organisms all glycolytic enzymes reside in the cytosol. The aforementioned model of trypanosome glycolysis was used to investigate the consequences and possible function of compartmentation of glycolysis. Two models were compared: one with the glycosome present and one in which the glycosomal membrane had been removed and the glycosomal enzymes had been diluted in the cytosol. The results did not support the prevailing hypothesis that the compartmentation of glycolysis causes the high glycolytic flux in trypanosomes. It was shown, however, that two risks of the design of glycolysis were overcome by compartmentation.

In glycolysis ATP is first invested before net production takes place. This makes the first enzymes, HK and PFK, virtually irreversible and a high activity of these enzymes will, if unregulated, result in accumulation of hexose phosphates. This indeed happened, if no glycosomal membrane was present. If glycolysis was compartmentalized, as it is in *T. brucei*, however, the activities of HK and PFK were limited by the low glycosomal [ATP]/[ADP] ratio, since net production of ATP by pyruvate kinase occurs only in the cytosol.

Due to the investment of ATP in the beginning there is also a risk that glycolysis cannot get started anymore, once the ATP concentration has become too low to activate HK and PFK. Since *T. brucei* does not store any carbohydrates this may occur when it is deprived of a carbon source. Within the glycosome the sum of the concentrations of phosphorylated compounds is conserved, as the phosphate group is only transferred from

one organic compound to another, but not converted into inorganic phosphate inside the glycosome. This pool of phosphorylated metabolites may serve as a short term free-energy storage, required to start up glycolysis after brief starvation. One may speculate that the glycosome has taken over the function of regulation of enzyme activities and, partly, of storage of carbohydrates. Once they had acquired the glycosome, Kinetoplastids may have lost the latter features and thereby the compartmentation of their glycolysis became irreversible.