8 Control of metabolic dynamics: how the frequency of glycolytic oscillations in *Saccharomyces cerevisiae* is controlled by glucose transport

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Introduction

Saccharomyces cerevisiae engages in limit cycle oscillations after harvest at the diauxic shift, starvation of the cells and addition of glucose and cyanide [1]. Modelling studies have shown that control on both amplitude and frequency can be distributed [2]. We determined the control of the frequency by the glucose transporter by titrating maltose, a competitive inhibitor of glucose transport [3], and by measuring the transport kinetics. The glucose transport showed at least two kinetic components. The presence of both a high affinity and a low affinity component after starvation of diauxic shift cells may reflect the pre-growth conditions of the cells. In order to calculate the control coefficient, we modified the analysis by Bakker *et al.* [4], including the two kinetic components. Glucose transport only partly controlled the frequency of the oscillations.

Materials and methods

The yeast *Saccharomyces cerevisiae* (X2180, diploid strain) was grown under semi-aerobic conditions at 30°C on a rotary shaker in medium containing glucose (10 $g.l^{-1}$), YNB (6.7 $g.l^{-1}$) and potassium phthalate (100 mM) at pH 5.0. The cells were harvested at the diauxic shift, when glucose was exhausted (Glukotest, Boehringer

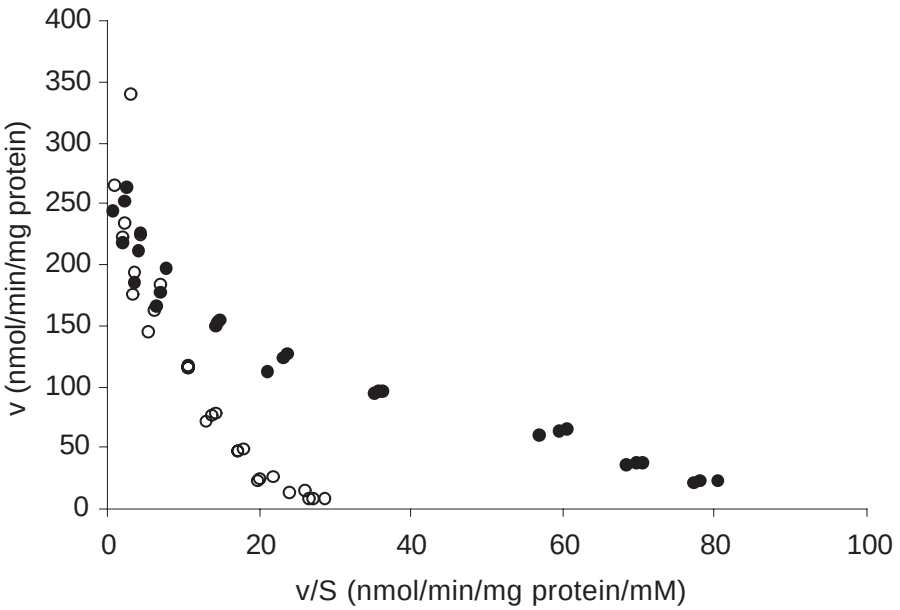

Fig. 8.1 Eadie-Hofstee plot of the zero-*trans* influx kinetics of glucose transport in the absence (•) and presence (o) of 100 mM of maltose.

Mannheim), washed twice with 100 mM potassium phosphate, pH 6.8, and resuspended in the same buffer. After starvation of the suspension for 2 hours at 30°C on a rotary shaker, the cells were again pelleted and resuspended in the same buffer to a protein concentration of approximately 5 $g.l^{-1}$.

Oscillations were induced in a thermostatically controlled cuvette at 25°C. Yeast cells were incubated and glucose was added to the suspension to a final concentration of 20 mM. Four minutes after the addition of glucose cyanide was added to a final concentration of 4 mM. In the case of maltose inhibition, maltose was added 2 min after the addition of glucose. The final maltose concentration ranged from 20 mM to 100 mM. The oscillations were monitored by measuring NADH fluorescence. The frequency was determined as the reciprocal of the time interval between subsequent maxima.

Glucose transport was measured in the absence and presence of 100 mM of maltose at 25°C in 100 mM phosphate buffer (pH 6.8) [5]. In the case of maltose inhibition, glucose and maltose were added simultaneously.

Results and discussion

The frequency of the glycolytic oscillations was determined at different maltose concentrations (Table 8.1).

The frequency decreased with an increasing maltose concentration. In order

Table 8.1 Frequency of glycolytic oscillations in the presence of different maltose concentrations.

[Maltose] (mM)	Frequency (min^{-1})
0	1.55
20	1.52
40	1.51
60	1.48
80	1.46
100	1.45

to quantify the sensitivity of the glucose uptake step for maltose we measured glucose transport in the presence and absence of maltose (Fig. 8.1). From these data the K_m and the V_{max} of the transporter as well as the K_i for maltose were obtained. The results showed that more than one transporter was present. Two symmetrical facilitated diffusion systems were fitted onto the data set using the following equation:

$$v = \frac{V_1^+ \cdot \frac{S_0}{K_{m,11}} - V_1^- \cdot \frac{S_i}{K_{m,12}}}{1 + \frac{S_0}{K_{m,11}} + \frac{S_i}{K_{m,12}}} + \frac{V_2^+ \cdot \frac{S_0}{K_{m,21}} - V_2^- \cdot \frac{S_i}{K_{m,22}}}{1 + \frac{S_0}{K_{m,21}} + \frac{S_i}{K_{m,22}}} \tag{8.1}$$

For the zero-*trans* influx measurements we assumed intracellular glucose to be zero, therefore equation 1 can be simplified to a two component irreversible equation. Fitting resulted in a high affinity ($K_{m,11}$ = 1.7 mM, V_1^+ = 152 nmol.min^{-1}.mg protein^{-1}) and a low affinity ($K_{m,21}$ = 99 mM, V_2^+ = 181 nmol.min^{-1}.mg protein^{-1}) component. Assuming each transporter to be symmetrical, the K_m for intracellular and extracellular glucose are equal for each, but differ between the two. Also the V_{max}^+ and V_{max}^- are assumed to be equal.

Using the kinetic constants derived via eq. 8.1, the K_i value for maltose can be estimated by fitting the following equation to the data from the uptake experiments in the presence of 100 mM maltose:

$$v = \frac{V_1^+ \cdot \frac{S_0}{K_{m,11}}}{\left(1 + \frac{I}{K_{i,11}}\right) + \frac{S_0}{K_{m,11}}} + \frac{V_2^+ \cdot \frac{S_0}{K_{m,21}}}{\left(1 + \frac{I}{K_{i,21}}\right) + \frac{S_0}{K_{m,21}}} \tag{8.2}$$

This equation holds for competitive inhibition and gives the following values: $K_{i,11}$ = 37.8 mM and $K_{i,21} = 9.9 \times 10^7$ mM. Apparently only the high affinity transporter is inhibited by maltose.

In order to be able to calculate the sensitivity of the transport step for maltose it is important to know the relevant conditions at which point this sensitivity needs to be calculated. Relevant are substrate, product and inhibitor concentrations. Intracellular glucose concentrations were calculated from the measured zero-*trans* influx rate and the measured glucose consumption flux, in the absence

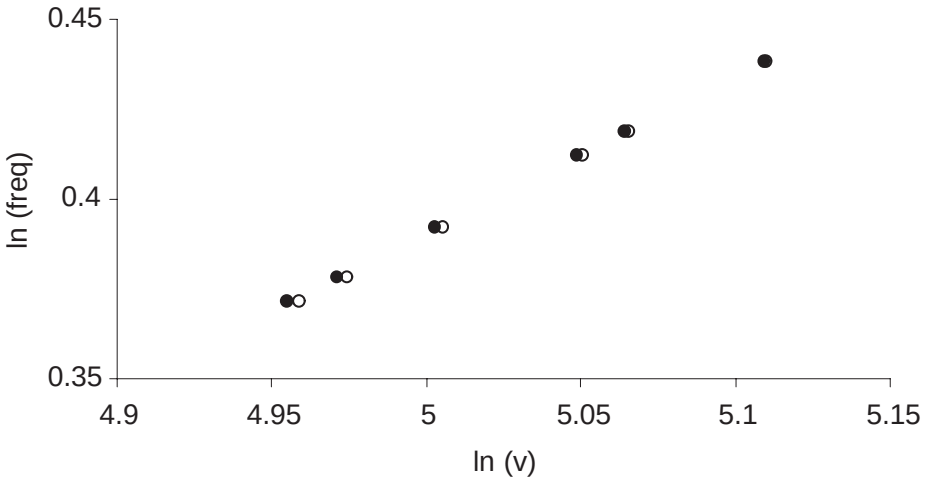

Fig. 8.2 Frequency of glycolytic oscillations plotted against the activity of the glucose transporter in ln-ln space. The slope of the curves represents the control coefficient for $K_{i,2}/K_{i,1}$ = 0.5 (•) and for $K_{i,2}/K_{i,1}$ = infinity (o).

of maltose (results not shown). The difference between this rate and the flux was attributed to inhibition of the latter by intracellular glucose. Using eq. 8.3, the internal glucose concentration was calculated to be 0.02 mM.

$$v = \frac{V_1^+ \cdot \frac{S_0 - S_i}{K_{m,1}}}{1 + \frac{S_0}{K_{m,1}} + \frac{S_i}{K_{m,1}} + \alpha_1 \cdot \frac{S_0 \cdot S_i}{K_{m,1}^2}} + \frac{V_2^+ \cdot \frac{S_0 - S_i}{K_{m,2}}}{1 + \frac{S_0}{K_{m,2}} + \frac{S_i}{K_{m,2}} + \alpha_2 \cdot \frac{S_0 \cdot S_i}{K_{m,2}^2}} \tag{8.3}$$

Maltose is not transported into the cell and therefore it inhibits only on the outside of the carrier. It thereby breaks the symmetry of the carrier. The binding however does affect the inside of the carrier and thus the rate of the backward reaction. This is reflected in two K_i values per transporter ($K_{i,11}$, $K_{i,12}$, $K_{i,21}$, $K_{i,22}$). From the experiments only one of the K_i values could be determined (i.e., in the absence of internal glucose). The effect of the other K_i values was calculated using the elementary rate equations. The ratio $K_{i,2}/K_{i,1}$ was varied between 0.5 and infinity. For the different maltose concentrations the rate of the uptake step can be calculated using the equation:

$$v = \sum_{j=1}^{2} \frac{\frac{S_0 \cdot V_j^+}{K_{m,j1}} - \frac{S_i \cdot V_j^-}{K_{m,j2}}}{\left(1 + \frac{I}{K_{i,j1}}\right) + \frac{\left(1 + \frac{[I]}{K_{i,j2}}\right) \cdot S_i}{K_{m,j2}} + \frac{S_0}{K_{m,j1}} + \frac{S_i \cdot S_0 \cdot \alpha_j}{K_{m,j2} \cdot K_{m,j1}}} \tag{8.4}$$

with the parameter values as determined in the transport and flux experiments.

The immediate effect of a change in parameter p is described by the elasticity

coefficient of enzyme i to parameter p. The elasticity coefficient is defined as

$$\varepsilon_p^i = \left(\frac{\partial \ln v_i}{\partial \ln p} \right)_{X_i} \tag{8.5}$$

where v_i is the activity of enzyme i. The derivatives are partial, i.e., the derivative is taken keeping all metabolite concentrations X_i that may affect the rate of enzyme i constant at their steady-state levels. The control coefficient of a step i on a variable X can be defined as

$$C_i^{X_j} = \frac{R_p^{X_j}}{\varepsilon_p^i} \tag{8.6}$$

where $R_p^{X_j}$ is the response coefficient, i.e., the response of a variable X_j to a change in parameter p.

The activities of glucose transport were calculated for the different maltose concentrations using eq. 8.4. The measured frequencies were plotted against these calculated activities of glucose transport in ln-ln space (Fig. 8.2). The control coefficients were calculated for the different values of the $K_{i,2}/K_{i,1}$ ratio as the slopes of the curves. From the effect of maltose on the frequency of glycolytic oscillations and from the activity of glucose transport the control coefficient was calculated to be 0.4 for the two values of the $K_{i,2}/K_{i,1}$ ratio.

These results show that the control of the frequency of glycolytic oscillations does not reside in one single enzyme but is distributed over other enzymes. Furthermore is should be noted that although we were not able to measure $K_{i,2}$ the control coefficients for the different ratios were sufficiently similar, for that inability to become irrelevant.

References

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