Transport of maltose in *Saccharomyces cerevisiae*

Effect of pH and potassium ions

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The transport of maltose in *Saccharomyces cerevisiae* has been generally accepted as a H⁺–sugar symport, with a stoichiometrical ratio of 1:1. A simultaneous exit of K⁺ from the cells with the initial uptake of maltose has been reported previously. By using a K⁺-selective electrode and radioactive maltose, we were able to measure the exit of 1 mol of K⁺/mol of maltose taken up by the cells in the first 10–15 s. This stoichiometrical ratio is pH-independent. So, uptake of protons in a non-buffered cell suspension or exit of K⁺ in a buffered one can be used to measure initial rates of maltose uptake. We have used a K⁺ electrode and a pH electrode to study the effect of external pH and K⁺ respectively on the kinetic parameters of maltose transport. The following results were obtained: the apparent half-saturation constant for maltose (K_m) increased from 5.2 mM at pH 5.8 to 38.0 mM at pH 7.8; the same increase in pH halved the apparent maximum uptake rate (V_{max}); K⁺ had an inhibitory effect, decreasing V_{max} and increasing K_m at pH values above 5; K⁺ had a stimulating effect at pH values below or equal to 4. Under physiological conditions, i.e. lower pH outside, neutral pH inside and much higher [K⁺] inside the cell, and assuming symmetry of the system, a higher affinity for maltose is to be expected in the outer face of the plasma membrane. This behaviour of the system could explain, by itself, the maintenance of the high concentration of free maltose inside the cell (necessary because of the low affinity of the maltase), without significant back transport to the outside.

The maltose permease of *Saccharomyces cerevisiae* has been described as a proton symport. Seaston *et al.* (1973) showed that maltose was absorbed with protons by a mechanism that did not involve ATP directly, since it was functional in the presence of 2-deoxyglucose, antimycin and iodoacetamide.

The stoichiometrical ratio was established as about 1 mol of protons/mol of maltose (Eddy *et al.*, 1977; Serrano, 1977).

The inhibitory effect of protonophores studied by Serrano (1977) was interpreted as a proof for the electrogenic character of the transport system, which would use the electrochemical proton gradient across the plasma membrane as a source of energy.

The electrogenic character of the transport system was also studied by changes in fluorescence signals of carbocyanine dyes (Brocklehurst *et al.*,

1977); these results are not considered to be conclusive (Eddy, 1982), because of interference with mitochondrial events.

K⁺ is released by the yeast cells during the initial uptake of maltose. This was interpreted as a mechanism to maintain electrical balance, during the influx of protons, together with maltose, before the proton pump is activated (Serrano, 1977). The same efflux of K⁺ was observed in the presence of proton conductors (Seaston *et al.*, 1976), with a stoichiometrical ratio of about 1 mol of K⁺ released/mol of H⁺ taken up.

External pH has been shown to affect the specific rate of growth of a petite mutant of *S. cerevisiae* on maltose in the pH range 5–7, whereas it had no effect when the cells were growing on glucose or galactose (Olivero *et al.*, 1982). The specific growth rate (μ) depends on the growth yield (y) and the specific transfer rate of a nutrient

(k), according to the equation (van Uden, 1969):

$$\mu = k \cdot y$$

Data from continuous and batch culture suggested that the effect of pH on the specific growth rate might be related to the transport step, since no effect was detected on the growth yield.

In the present work we used measurements with a K⁺-selective electrode to study the effect of external pH and measurements with a pH electrode to study the effect of K⁺ on the kinetic parameters of maltose transport of *S. cerevisiae*.

Experimental

Organism, growth and harvesting conditions

Saccharomyces cerevisiae (I.G.C. 3507) was grown on a mineral medium (van Uden, 1967) containing 2% (w/v) maltose, in an orbital shaker, at 25°C. Exponential-phase cells (about 1 mg dry wt./ml of medium) were harvested by centrifugation, washed twice with water at 4°C and resuspended in water to a final concentration of about 100 mg dry wt./ml. These cells were kept in ice for 1 h before any experiment and were used during the next 4 h. The biomass in each batch of cells was determined by weighing the cells in 100 µl of suspension, after drying overnight at 80°C.

Measurement of maltose transport by the uptake of [¹⁴C]maltose

We used the method described by Serrano (1977). A 20 µl volume of buffer (0.1 M-Tris adjusted to the desired pH with tartaric acid) was mixed with 20 µl of cell suspension. After 5 min incubation at 26°C, 10 µl of [U-¹⁴C]maltose (about 100 c.p.m./nmol) was added; transport was stopped by the addition of 5 ml of ice-cold water after 5, 10, 15 and 20 s. The suspension was immediately filtered through Whatman 2.5 GF/C glass-microfibre filters, washed on the filter with 5 ml of ice-cold water and the radioactivity counted in a Beckman LS 8 100 liquid-scintillation system.

Controls were performed by adding 5 ml of cold water to the cells before the addition of radioactive solutions and proceeding as before.

[U-¹⁴C]Maltose was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Maltose was grade I from Sigma Chemical Co., St. Louis, MO, U.S.A.

Measurement of proton uptake

Proton uptake was measured with a combined pH electrode (G-202 B; Radiometer) attached to a standard pH-meter (PHM 62; Radiometer) connected to a flat-bed potentiometer recorder (6500; LKB). The pH electrode was immersed in a water-jacketed cell with magnetic stirring. First, 3.5 ml of

water and 0.5 ml of cell suspension were successively added and a base-line was obtained in the recorder, after adjustment to the required pH with HCl. The addition of maltose solutions switched on the uptake of protons, observed through the alkalization of the extracellular environment. From the slope of the initial part of the curve obtained in the recorder, initial rates of proton uptake were calculated. Calibrations were made with 10 mM-HCl. All assays were performed at 26°C.

Measurement of K⁺ efflux

K⁺ efflux was measured with a K⁺-selective electrode (F 2312 electrode; Radiometer), with as reference a double-bridge calomel electrode (R11; Philips), immersed in a conical water-jacketed cell with magnetic stirring. The electrodes were attached to the same equipment as the pH electrode in the proton-uptake measurements. To 3.5 ml of buffer (0.1 M-Tris adjusted to the desired pH with tartaric acid), 0.5 ml of cell suspension was added. A base-line was obtained, and the addition of maltose switched on the efflux of K⁺. From the slope of the initial part of the curve obtained in the recorder, initial rates of K⁺ efflux were calculated. Calibrations were made with 1 mM-KCl. All assays were performed at 26°C.

In the experiments for determining the maltose/K⁺ stoichiometrical ratio, the relative proportions of buffer and cell suspension were the same as in the [¹⁴C]maltose-uptake assays, for a final volume of 4 ml.

Results

Figs. 1(a) and 1(b) illustrate typical curves obtained with pH and K⁺ electrodes respectively. H⁺ influx and K⁺ efflux occurred simultaneously and immediately after the addition of the maltose pulse; we could not detect any time delay with the equipment with which we were working.

To study the effect of pH on the transport of maltose, the measurement of K⁺ efflux would be a useful tool, but first we need to calculate the maltose/K⁺ stoichiometrical ratio. We measured uptake of [¹⁴C]maltose and K⁺ efflux at different pH values and maltose concentrations to cover a range of uptake rates from the highest that we could get to the lowest that we could measure. Fig. 2 summarizes the results. The stoichiometrical ratio obtained from the slope of the regression line was 1.03 mol of K⁺ released/mol of maltose uptaken.

The measurements of K⁺ efflux could therefore be used as a measure of maltose transport, and we used the K⁺ electrode to study the effect of external pH.

Fig. 1. Alkalization (a) and increase in the extracellular K^+ concentration (b) of a cell suspension of *S. cerevisiae* grown on maltose, after the addition of a pulse of maltose.

The slope of the initial part of the curves was used to measure the initial rate of H^+ uptake (a) or K^+ efflux (b). To 4 ml of cell suspension, 100 μ l of 2M-maltose was added.

Fig. 2. Stoichiometry of initial maltose uptake and K^+ efflux in *S. cerevisiae*.

Initial maltose uptake was measured by the incorporation of [14 C]maltose, and K^+ efflux was measured with a selective electrode. Cells were suspended in 100mM-Tris adjusted with tartaric acid to pH 3.76 (●), pH 4.73 (○), pH 5.88 (■) and pH 7.16 (▽).

Effect of pH

We measured the effect of pH on the efflux of K^+ after the addition of a pulse of maltose (final concentration 50mM) in the pH range 2.7–8.5. There was a maximum in the range 4.5–6.0 with a strong decrease for pH below 4 and above 7. On varying the pH and the maltose concentration, the efflux of K^+ showed the kinetic pattern presented in Fig. 3, by Eadie–Hofstee plots. External pH affected both the apparent half-saturation constant (K_m) and the apparent maximum uptake rate

Fig. 3. Effect of external pH on the kinetic parameters of the maltose-transport system of *S. cerevisiae*.

The initial rates of maltose uptake were measured as K^+ efflux with a K^+ -selective electrode. The cells were suspended in 100mM-Tris adjusted with tartaric acid to pH 5.97 (□), pH 6.45 (○), pH 6.90 (●), pH 7.34 (△) and pH 7.75 (▲).

(V_{max}), and a summary of the values obtained is shown in Table 1.

The low rates obtained for low pH (confirmed with the uptake of [14 C]maltose; see Fig. 2) were not in agreement with the previous knowledge that the strain of *S. cerevisiae* used maltose at pH values below 3. We evaluated kinetic parameters in the range of low pH (below 4) and observed that K_m did not vary (about 5 mM) but that V_{max} was much lower (results not shown).

Effect of K^+

To study the effect of K^+ on the transport of maltose, we measured the uptake of protons as

Table 1. Effect of external pH on the kinetic parameters of the maltose-transport system of *S. cerevisiae*. Values were calculated from the Eadie-Hofstee plots of Fig. 3.

pH	$V_{\max}$ (mmol/h per g)	K_m (mM)
7.75	5.32	38.0
7.34	8.12	33.2
6.90	9.18	19.3
6.45	9.67	8.7
5.79	11.14	5.2

Fig. 4. Effect of K^+ on the maltose-transport system of *S. cerevisiae*.

The initial rates of maltose uptake were measured as H^+ uptake with a pH electrode. Maltose was added to a final concentration of 50 mM; Δ , pH 4.7; $\blacksquare$, pH 4.0; $\bullet$, pH 6.0.

Fig. 5. Effect of K^+ on the kinetic parameters of the maltose-transport system of *S. cerevisiae* at pH 4.5. The initial rates of maltose uptake were measured as H^+ uptake with a pH electrode. KCl was added to the cell suspension to a final concentration of 10 mM ($\square$), 50 mM (Δ) and 150 mM ($\circ$). Control is represented by $\bullet$.

Fig. 6. Effect of K^+ on the kinetic parameters of the maltose-transport system of *S. cerevisiae* at pH 6.25. The initial rates of maltose uptake were measured as H^+ uptake with a pH electrode. KCl was added to the cell suspension to final concentrations of 2.5 mM (Δ), 5 mM ($\square$), 7.5 mM ($\circ$) and 10 mM ($\diamond$). Control is represented by $\bullet$.

Table 2. Effect of some salts on the maltose-transport system in *S. cerevisiae*.

KCl, KSCN and NaCl were added to cell suspensions in the concentrations indicated. Initial rates of H^+ uptake were measured as indicated in the text, after the addition of a pulse of maltose to a final concentration of 50 mM.

pH	salt	Rate (mmol/h per g)
6.0	None	10.0
	KCl (10 mM)	3.1
	KSCN (10 mM)	3.0
	NaCl (10 mM)	6.1
	KCl (200 mM)	0
	KSCN (200 mM)	0
4.0	NaCl (200 mM)	2.4
	None	1.5
	KCl (10 mM)	7.5
	KSCN (10 mM)	7.2
	NaCl (10 mM)	8.7
	KCl (200 mM)	5.2
	KSCN (200 mM)	5.4
	NaCl (200 mM)	8.2

described in the Experimental section with the addition of KCl to the cell suspension. Fig. 4 summarizes the results obtained for a maltose concentration of 50 mM: a strong inhibitory effect at pH 6, a slight inhibitory effect at pH 4.7 and stimulating effect at pH 4.0. These aspects were studied in more detail at different pH values, and the Lineweaver-Burk plots of Figs. 5 and 6 illustrate the results. At pH 4.5 K^+ affected only $V_{\max}$ and transport could be measured for concentrations of KCl up to 150 mM. At pH 6.25 low concentrations

of KCl affected strongly the affinity of the system for maltose: no proton uptake could be detected for K^+ concentrations above 15 mM. For pH values in between, both V_{max} and K_m were affected (results not shown).

To elucidate how specific was the effect of K^+ , we measured the rates of uptake of protons in the presence of KCl, KSCN and NaCl. Table 2 summarizes the results.

Discussion

We can conclude from our data that pH values above 6 have an inhibitory effect on the physiological activity of the yeast by decreasing both V_{max} and the apparent affinity of the transport system for maltose (Table 1). This can be explained if we consider transport in terms of an enzymic reaction, catalysed by a permease, with two substrates, maltose and H^+ . Changes in the concentration of one of the substrates (H^+) will change the apparent kinetic parameters, even at saturating concentration of the other (Dixon & Webb, 1958).

The effect of K^+ in this range of pH goes in the same direction, as if K^+ were competing with H^+ , diminishing the degree of protonation of the carrier.

The stimulating effect of K^+ under acidic conditions (below pH 4) solves the apparent contradiction of growth and no transport under these conditions. In culture media, K^+ is usually present in concentrations at which maltose transport does occur, whereas in our experiments (Table 1 and Fig. 2) K^+ concentration was very low.

The nature of this K^+ -stimulating effect needs to be further elucidated. We can speculate that it may be simply a protective effect against excessively acidic extracellular conditions by increasing apparent pH.

The strain that we worked with, under the experimental conditions used, showed similar behaviour in the presence of KCl and NaCl, although Na^+ seemed to be less effective than K^+ both in the inhibitory effect at pH 6 and the stimulating effect at pH 4. On the other hand, the use of a permeant cation (SCN^-) or a non-permeant one (Cl^-) did not modify the K^+ effect (see Table 2). These results are in agreement with the mechanism that we postulated above, i.e. a direct effect of K^+ on the transport system, competing with H^+ .

Serrano (1977) reported that KCl inhibited, whereas NaCl or KSCN did not affect, maltose transport in *S. cerevisiae*. He used these data to explain the effect of K^+ on maltose transport as an inhibition of the K^+ efflux, this being necessary to restore the membrane potential of the cell during the initial H^+ -maltose uptake. However, he used

incubation times of 10–20 min, with the salts in 100 mM-Tris buffer; during this time possible changes induced in extra- and intra-cellular pH by SCN^- and/or Tris may have interfered with his results, in a range of pH in which the system is highly pH-dependent.

The intracellular H^+ and K^+ concentrations seem to be very important in maltose transport. Further experimental work should perhaps be done with plasma-membrane vesicles, as those described by Franzusoff & Cirillo (1983), in which both the extra- and intra-cellular conditions can be specifically defined, without interference from metabolism.

The results presented here have some important physiological implications. As Serrano (1977) pointed out, the fact that *S. cerevisiae* developed H^+ symport systems for maltose and other disaccharides may be related to the low affinity of intracellular maltase for maltose (K_m 10 mM). The cells must concentrate maltose from the culture medium, maintaining a high intracellular maltose concentration to keep a good rate of maltose hydrolysis. From our experimentally obtained data we can, just by kinetic considerations and assuming the symmetry of the system, explain the build-up of the maltose gradient without any back transport to the extracellular environment. Outside the cell, in a normal medium for growing yeasts, the pH is about 4 and the concentration of K^+ is low. Inside the cell, pH will be about 7 (Peña *et al.*, 1982) and the concentration of K^+ about 100 mM (Herbert *et al.*, 1971). Under these conditions K_m inside will be very high and no transport will occur.

These considerations imply that under normal physiological conditions the differences in pH and K^+ concentration between the inner face and the outer face of the plasma membrane determine that the H^+ -maltose symport is unidirectional.

Finally, we may remark that, with the system described above, the yeast can use the K^+ gradient as a useful energy source for the build-up of a sugar gradient through the plasma membrane, in addition to the protonmotive force.

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