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Reversible loss of affinity induced by glucose in the maltose-H^+ symport of *Saccharomyces cerevisiae*

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Glucose represses and inactivates maltose transport in *Saccharomyces cerevisiae*. The inactivation has been described as an irreversible process involving proteolysis. We have studied the inactivation of the maltose-H^+ symport in this yeast and have observed that the mechanism of inactivation depends on the physiological conditions. In resting cells there was a decrease in transport capacity. The rate of decrease was enhanced nonspecifically by the presence of a sugar, glucose being more effective than maltose. In growing cells, glucose induced a decrease in affinity of the H^+-symport which could be recovered by starvation, even in the presence of cycloheximide; there was no loss in capacity or, if present, this loss could be explained fully by the dilution due to repression during growth on glucose. We submit that in growing cells inactivation consists in a reversible modification of the permease not involving proteolysis.

Introduction

Glucose has two main effects on the maltose uptake of *Saccharomyces cerevisiae*. One is a rapid decrease of the rate of maltose uptake during incubation of maltose grown cells with glucose and has been considered as catabolic inactivation [1,2]. The other is the repression of the synthesis of maltose permease, i.e., catabolite repression.

The molecular bases of catabolite inactivation of the maltose permease are unknown. Görts [1] observed that its inactivation in the presence of glucose consisted largely in a decrease in affinity, and similar results were obtained for the galactose uptake system of the same yeast by Matern and Holzer [3]. As the recovery of the transport activity was suppressed in both cases by cycloheximide, these latter authors suggested that the mechanism of catabolite inactivation should involve a partial or total proteolysis of the permeases or even the degradation of a 'modifying protein' that, when present, would increase the affinity. More recently, Spencer-Martins and van Uden [4], studying the glucose transport systems of *Candida wickerhamii*, found that cells grown under conditions of derepression showed a high-affinity active transport system that suffered catabolite inactivation during incubation in 2% glucose, while a low-affinity passive system emerged concomitantly. They called this process catabolite interconversion and postulated a model after which the modifying protein that would increase affinity was sensitive to catabolite repression and catabolite inactivation, whereas the low-affinity part of the transport system was not.

In this work we have studied the effect of glucose on the maltose-H^+ symport, through the kinetic analysis of the initial uptake rates of the

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disaccharide by whole cells. We found that the effects of glucose depended on the incubation conditions and were partially reversed by starvation, even in the presence of cycloheximide. These results do not support the previous hypothesis that partial permease proteolysis would constitute the molecular basis of the effect. Instead, we suggest that a reversible modification of the permease, induced by glucose catabolism, takes place.

Materials and Methods

Growth, harvesting and preparation of cells

S. cerevisiae (IGC 3507) was grown in an orbital shaker at 25°C, on a mineral medium [5] containing 2% (w/v) of the appropriate sugar. Cells were harvested in the exponential phase of growth, when the culture was at about 1 mg dry weight/ml, by centrifugation, washed twice with water at 4°C, resuspended in water to a final dry weight of about 60 mg/ml and kept in ice. Starved cells were prepared by incubation in 67 mM KH_2PO_4 or, when indicated, in culture medium without any sugar (1–2 mg dry weight/ml) in an orbital shaker at 25°C. Growing cells were prepared from cells starved in medium by incubation in the same growth medium (1–2 mg dry weight/ml) with different sugars, as indicated in the text, in an orbital shaker at 25°C. Resting cells were prepared by incubation in 67 mM KH_2PO_4 with different sugars, as indicated in the text (1.2 mg dry weight/ml). Incubation was under the same conditions as for growing cells.

Measurement of proton uptake

Proton uptake was measured as we described earlier [6]. The pH electrode was attached to a pH meter connected to a recorder through a bucking-voltage device, which allowed measurements to 0.4 pH units full scale deflection. A baseline was obtained by the addition of 3.5 ml water and 0.5 ml cell suspension in a water-jacketed vessel with magnetic stirring. The addition of maltose solutions in appropriate amounts switched on the uptake of H^+, observed through the alkanization of the external environment. The slope of the initial part of the curve was taken as a measurement of proton uptake.

All experiments were performed at 25°C.

Measurement of maltose transport by the uptake of [U-^{14}C]maltose

We used the method previously described [6] measuring the incorporation of [U-^{14}C[maltose for periods of 10 s, in duplicate. 20 μl 100 mM citrate-Tris buffer (pH 5.0), 20 μl cell suspension and 10 μl [U-^{14}C]maltose (S.A. $\approx$ 1000 cpm/nmol) solution were incubated at 25°C for 10 s. Incorporation was stopped by the addition of 5 ml ice-cold water. Cells were immediately filtered, washed and counted in a liquid scintillation system.

[U-^{14}C]Maltose was purchased from Amersham International U.K. Maltose was from Sigma Chemical Co., St. Louis, MO, U.S.A.

Results

Effect of glucose on the maltose H^+-symport in growing cells

When maltose-grown cells, starved 1 h in growth medium without carbon source, were transferred into medium with 2% glucose, we observed the changes in kinetics illustrated in Fig. 1.

After 1 h incubation, the apparent affinity had decreased, whereas the capacity remained constant. The nature of the transport system was also maintained, since similar results were obtained measuring the initial transport rates either by the incorporation of [U-^{14}C]maltose or by H^+ uptake, showing the previously established stoichiometry of 1 proton per mol maltose [7,8]. The maltose H^+-symport lost affinity and no other system appeared to compensate for it.

Effect of glucose on the maltose-H^+ symport in resting cells

To study the effect of glucose in the absence of a nitrogen source, maltose grown cells, prepared as indicated in Materials and Methods, were incubated in 67 mM KH_2PO_4 (pH 4.5) plus 2% glucose. Maltose instead of glucose, and buffer alone, were also used to elucidate the specificity of this effect. The change in the kinetic parameters of maltose transport during incubation was studied in samples taken at different time intervals, measuring the H^+ uptake at several maltose concentration up to 20 mM.

In contrast with the results obtained with growing cells, the main effect in this case was on the

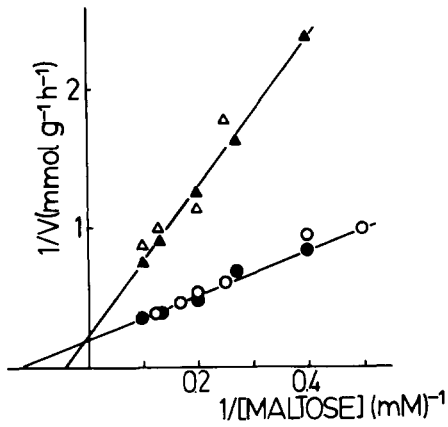

Fig. 1. Effect of glucose incubation on the maltose-H^+ symport of *S. cerevisiae* growing cells. Maltose grown, starved cells were assayed for their maltose-H^+ symport activity (circles). Then the cells were resuspended in growth medium with 2% glucose. After 1 h incubation the maltose-H^+ symport was assayed again (triangles). Two methods were employed to measure maltose transport (see Materials and Methods): H^+ uptake (open symbols) and [U ^{14}C]maltose uptake (closed symbols).

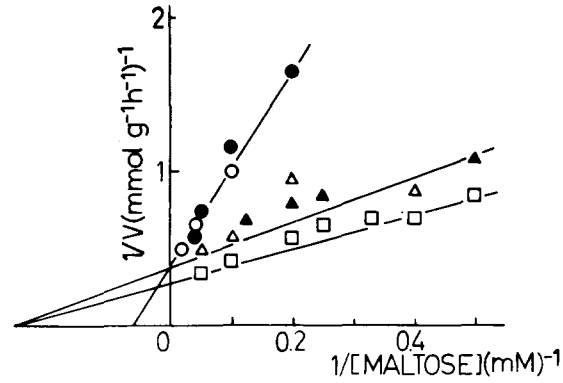

Fig. 3. Effect of glucose incubation on the maltose-H^+ symport of *S. cerevisiae* resting cells. Maltose-H^+ symport activity was measured by H^+ uptake in maltose-grown, starved cells (□); then the same cells were resuspended in 67 mM KH_2PO_4 (pH 4.5) plus 2% glucose for 1 h in the absence (○) and the presence (●) of cycloheximide. Finally they were starved for 1 h in the same buffer with (▲) or without (△) cycloheximide (6.25 μg/ml).

capacity and not on the affinity of the maltose transport system. Fig. 2 summarizes the results corresponding to these changes in V_{max}. After the first 30 min of incubation, during which there was an increase in V_{max}, specially significant in the cells with buffer alone or with glucose, the maximum transport velocity decreased. The rate of decrease was accelerated by the presence of a carbon source, glucose being more effective than maltose.

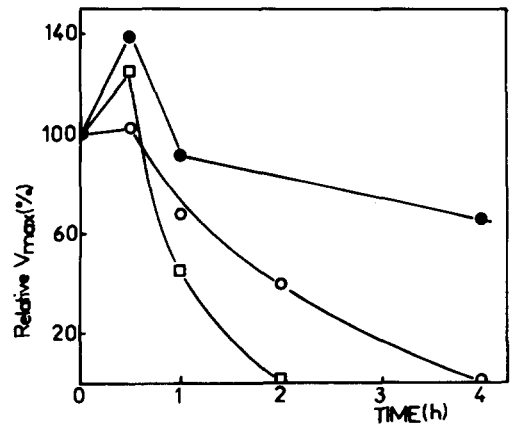

Fig. 2. Maltose-H^+ symport capacity of resting cells of *S. cerevisiae*. Maltose-grown cells were harvested and incubated with shaking at 25°C in 67 mM KH_2PO_4 (pH 4.5) alone (●), plus 2% maltose (○) or plus 2% glucose (□). At the time indicated the capacity was determined by H^+ uptake as described in Materials and Methods.

As starving the cells in buffer alone for a short time increased the maltose transport activity, we studied the effect of glucose incubation in resting but previously starved cells.

When starved cells were incubated for 1 h in buffer plus 2% glucose, the two effects already described for growing (decrease in affinity) and resting (decrease in capacity) cells occurred simultaneously, as is shown in Fig. 3. Moreover, the effect on affinity could be reversed by starving again the cells for 1 hr after glucose incubation. Cycloheximide at a concentration that inhibits completely protein synthesis in this strain (6.25 $\mu g \cdot ml^{-1}$) did not inhibit either the loss in affinity and capacity during glucose incubation or the recovery in affinity after starvation.

Discussion

All the previous studies on the effect of glucose on maltose transport in *S. cerevisiae* have been carried out using nonspecific methods, measuring net [^{14}C]maltose uptake or even maltose fermentation. In this work we report on a well-defined transport system: the H^+-symport.

The nature of the inactivation of this transport system depends on the incubation conditions. In the absence of nitrogen source, glucose leads mainly to a rapid disappearance of the system, presumably involving proteolysis. This effect is not specific, since maltose does the same, although at a lower rate (Fig. 2). Lagunas et al. [9] showed previously this effect of maltose in resting-cells. In any case, the catabolism of a carbon source in the absence of a source of nitrogen increased the inactivation rate, glucose being a better inactivator than maltose.

In growing cells there is no proteolysis of the system. The loss in capacity, when present, can be wholly explained by the dilution due to growth on glucose. Instead, a loss in affinity already described for maltose fermentation [1] was observed.

The molecular basis of this change in affinity remains unknown, and hypotheses suggested for other yeasts [3,4] do not seem to be applicable in this case: (1) it is not a case of catabolite interconversion [4], since a low-affinity system did not substitute for the high affinity H^+-symport (see Fig. 1); (2) partial proteolysis [3] does not seem to be involved, since the affinity loss was reversible: the results of Fig. 3 show that affinity could be recovered by starving the cells in the absence of a carbon source, even in the presence of cycloheximide.

It has been reported that the loss of affinity is irreversible [1,3]. This contradiction with our results may be explained by the method used by those authors to test the recovery of affinity. They incubated low-affinity cells with the sugar and cycloheximide: the presence of a fermentable carbon source in the absence of protein synthesis induces proteolysis (see Fig. 2 and Ref. 9). So the system did not recover affinity, but disappeared [3]. In our hands, when the cells were incubated without a carbon source, even in the presence of cycloheximide, affinity was recovered (Fig. 3). We submit that the loss of affinity is due to reversible modification of the permease, induced by glucose catabolism.

The nature of this modification on the permease is to be further elucidated. One of the effects of glucose addition to yeast cells is the immediate rise in cyclic-AMP intracellular concentration [10]. A cAMP-dependent phosphorylation of the permease could be a good candidate for the reversible chemical modification of the permease postulated above. In fact, such a phosphorylation of a transport system, decreasing the uptake rate, was described in rat adipocytes [11].

Based on our results, we think that the inactivation effect of glucose on the transport system of maltose in growing cells consists first in a loss in affinity due to a reversible chemical modification and then in a slow disappearance by dilution during growth, since glucose represses the synthesis of new permease.

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