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Regulation of glucose and maltose transport in strains of *Saccharomyces*

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SUMMARY

Growth of yeast cells on glucose resulted in complete inactivation of maltose transport and repression of the high affinity glucose transport system. When the cells were grown on maltose or subjected to substrate starvation, an increase in glucose and maltose transport was observed in both brewing and non-brewing yeast strains. The concentration of glucose employed in the growth medium was also observed to affect sugar transport activity. The higher the glucose concentration, the more pronounced the repressive effect. In addition, the time of growth of yeast on glucose or maltose also played a role in determining the rate of sugar transport. These results are consistent with the repressive effect of glucose on the high affinity glucose and maltose transport systems.

INTRODUCTION

Glucose or catabolite repression is one of the major metabolic regulatory mechanisms in yeast. In the presence of glucose a number of substrate utilizing systems are repressed [5,9]. For example, the repressing influence of glucose on maltose uptake and fermentation in yeast is well documented [1,4,8,10]. Kinetic analysis of glucose transport in *Saccharomyces cerevisiae* has demonstrated that glucose is transported by both high and low affinity transport systems [2,11]. The high affinity glucose transport system is under general glucose repression control [3,11]. That is, the high affinity transport system is repressed when cells are grown in the presence of glucose. Cells which are fully repressed in the high affinity transport system exhibit only low affinity glucose transport. Likewise, cells which are derepressed in the high affinity transport system do not exhibit low affinity glucose transport [2, 3]. Kinetic analysis has also demonstrated that maltose is transported in *Saccharomyces cerevisiae* by both high and low affinity transport systems [4,11]. Furthermore, both forms of the maltose transport system are regulated by catabolite inactivation [4].

Despite the considerable volume of published data on catabolite repression, its regulatory mechanism is still poorly understood. In this manuscript, the effect of yeast growth under derepression/repression conditions on sugar transport is reported.

MATERIALS AND METHODS

Chemicals

D-[U-^{14}C]Glucose (270 mCi/mmol) and D-[U-^{14}C]-maltose (420 mCi/mmol) were obtained from ICN Biomedicals (Irvine, CA). All other chemicals were obtained from commercial sources and were of the highest available purity.

Yeast strains and growth medium

The yeast strains employed in this study were, with their Labatt Culture Collection numbers, *Saccharomyces cerevisiae* haploid strain 1190 (*MAL2*, *MEL1*, *his4*, *leu2*, *suc*) and *Saccharomyces uvarum* (*carlsbergensis*) brewing lager polyploid strain 3021.

The yeast cells were subcultured in PYN medium which consisted of: peptone, 3.5 g; yeast extract, 3.0 g; KH_2PO_4, 2.0 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; glucose or maltose, 100.0 g; all dissolved in 1 l of distilled water and adjusted to pH 5.6.

Culture conditions

Fermentation and yeast growth studies were conducted in PYN media at 21 °C in 300 ml Erlenmeyer

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shake flasks containing various concentrations of glucose or maltose in 100 ml of media. Alternatively, fermentations were conducted in a Bio-Flo II fermentor containing 3 l of media. The yeast inoculum employed was 3.5 g wet weight of cells/l.

Sugar uptake studies

At specified times during fermentation, 10 ml of cell suspension was centrifuged at 4000 × *g* for 10 min at 4 °C. The supernatants were subjected to HPLC analysis to determine sugar concentrations as described elsewhere [8]. In the sugar transport studies, cells grown under various conditions were harvested by centrifuging at 4000 × *g* for 10 min at 4 °C. For glucose transport, cells were washed twice with ice-cold 100 mM potassium phosphate buffer, pH 6.6, and suspended in the same buffer at room temperature to a cell density of 5 mg dry weight/ml [8]. For maltose transport, cells were washed twice with ice-cold distilled water and suspended in 100 mM tartaric acid-Tris buffer, pH 4.2, at room temperature [8,13].

Transport was initiated by addition of 0.1 ml of radioactive substrate to 1.9 ml of cell suspension to give the desired final concentration. At 15-s intervals, 0.2 ml of cell suspension was withdrawn, filtered through 0.45-μm nitrocellulose filters and washed twice with ice-cold phosphate buffer. The filters were solubilized in scintillation fluid and radioactivity determined.

Biomass

At specified times during growth, 5 ml of cell suspension was withdrawn and centrifuged at 4000 × *g* for 10 min at 4 °C. The cell pellets were washed twice with distilled water and dried in an aluminum dish at 100 °C for 4 h.

RESULTS AND DISCUSSION

Transport of glucose and maltose

The results of 0.2 mM glucose and 0.2 mM maltose uptake studies in *Saccharomyces uvarum* (*carlsbergensis*) brewing strain 3021 and *Saccharomyces cerevisiae* haploid strain 1190 are presented in Fig. 1. The concentration of substrates employed in this study would reflect transport by the high affinity transport systems [11,12]. It can be seen in both the brewing and non-brewing yeast strains that pregrowth of yeast on 10% glucose results in complete inactivation of maltose transport. Maltose has been shown to be taken up in yeast by two kinetically different systems identified as high and low affinity transport systems. Both transport systems have been shown to be regulated by glucose-induced inactivation [4,6].

On the other hand, when the cells are pregrown on 10% maltose, an increase in glucose and maltose trans-

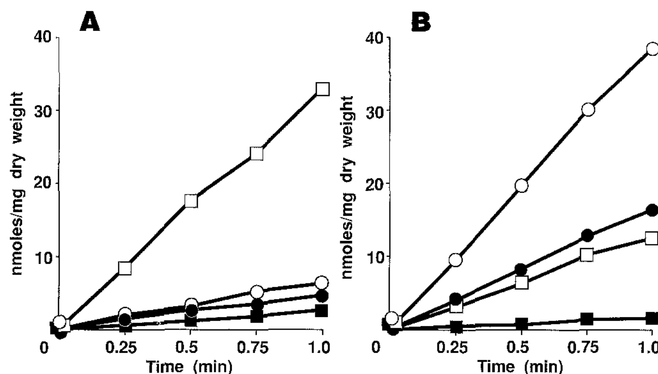

Fig. 1. Transport of 0.2 mM glucose and 0.2 mM maltose in *Saccharomyces uvarum* (*carlsbergensis*) brewing strain 3021 (A) and *Saccharomyces cerevisiae* strain 1190 (B). Glucose transport in glucose pregrown (●) and maltose pregrown (○) cells. Maltose transport in glucose pregrown (■) and maltose pregrown (□) cells. Cells were pregrown on 10% glucose and 10% maltose for 18 h.

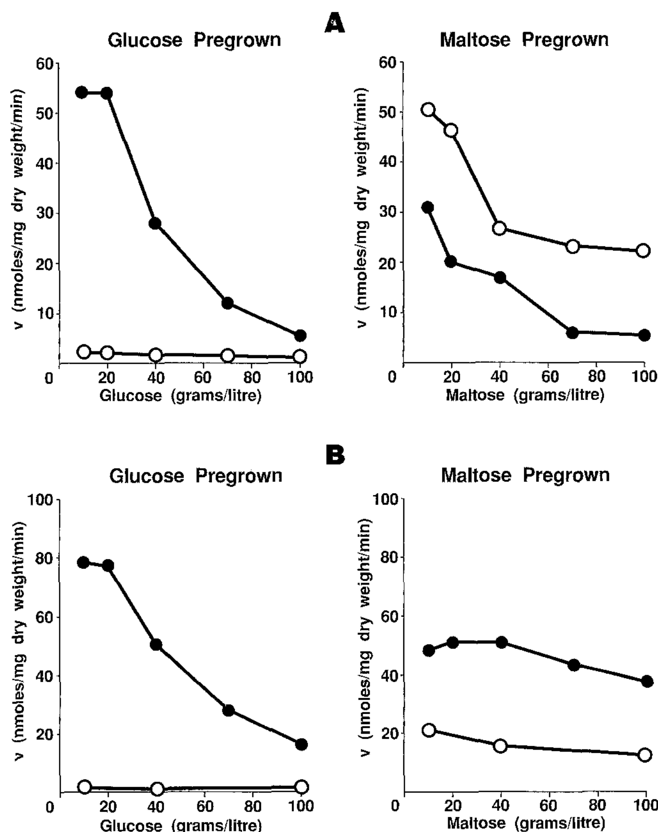

Fig. 2. Transport of 0.2 mM glucose and 0.2 mM maltose in yeast pregrown on various substrate concentrations. 0.2 mM glucose (●) and 0.2 mM maltose (○) transport was performed in *Saccharomyces uvarum* (*carlsbergensis*) brewing strain 3021 (A) and *Saccharomyces cerevisiae* strain 1190 (B) pregrown on glucose or maltose for 18 h.

TABLE 1

Concentration of glucose and maltose remaining in the media after 18 h of fermentation.

Initial substrate concentration (g/l)	Concentration after 18 h (g/l)			
	Strain 3021		Strain 1190	
	Glucose	Maltose	Glucose	Maltose
10	nd	nd	nd	nd
20	nd	nd	nd	nd
40	21.0	19.1	16.9	15.7
70	45.0	51.2	46.4	46.6
100	64.0	71.8	68.0	65.0

nd, not detected. The limit of detection is 0.5 g/l.

port rates are observed. These results are consistent with the repressive effect of glucose on maltose transport and utilization [1,4,8,10].

Effect of substrate concentration

The concentration of sugar employed in the growth medium was observed to significantly affect the rate of sugar transport (Fig. 2). Cells were pregrown on various concentrations of glucose (1–10%) for 18 h. With complete utilization of glucose, as in the case of growth on 1 and 2% glucose, maximum transport activity of 0.2 mM glucose was observed. As the concentration of glucose in the growth medium was increased, the concentration of glucose remaining in the growth medium after 18 h of growth also increased (Table 1). Associated with the increase in glucose concentration in the medium is a decrease in glucose transport activity (Fig. 2). Thus, the higher the glucose concentration in the medium, the more pronounced the repressive effect on glucose transport. It has been previously demonstrated that increasing the glucose concentration in the growth medium results in decreases in glucose utilization rates and yeast growth [7]. This would suggest that the glucose transport system is repressed by the high glucose concentrations. The high affinity glucose transport system has been shown to be regulated by glucose repression [3,11]. It can also be observed that maltose transport was inactivated in both yeast strains by growth on glucose. Even at the low glucose concentrations, in which maximum glucose transport activity was observed, maltose transport was still inactivated.

The concentration of maltose in the growth medium was also observed to have an effect on glucose and maltose transport rates in the lager brewing strain (Fig. 2A). As the concentration of maltose is increased in the growth medium, the concentration of maltose remaining in the medium after 18 h also increased (Table 1). An increase in maltose concentration resulted in a decrease in glucose and maltose transport rates. However, the concentration of maltose employed in the growth medium had only a slight effect on glucose and maltose transport activity in the laboratory haploid strain (Fig. 2B). In both cases, pregrowth of cells on maltose resulted in increased maltose transport activity. Growth of yeast on maltose would place the cells in a derepressed state exhibiting maximum sugar transport activity [11]. Furthermore, growth of the laboratory haploid strain on maltose resulted in derepression of glucose uptake, which is not observed in the industrial brewing strain. This may be related to a gene dosage effect since the laboratory strain possesses only the *MAL4* gene whereas the brewing strain possesses multiple copies of the six *MAL* genes. As such, the brewing strain ferments maltose at a faster rate than the laboratory strain [10]. The high copy of *MAL* genes in the brewing strain may lead to glucose uptake repression when grown on maltose. The reason for the effect of maltose on sugar transport in the brewing strain remains unclear and is currently under investigation.

Effect of growth time

The fermentation time was also observed to significantly affect the rate of glucose and maltose transport. Glucose transport rates in the lager brewing strain were

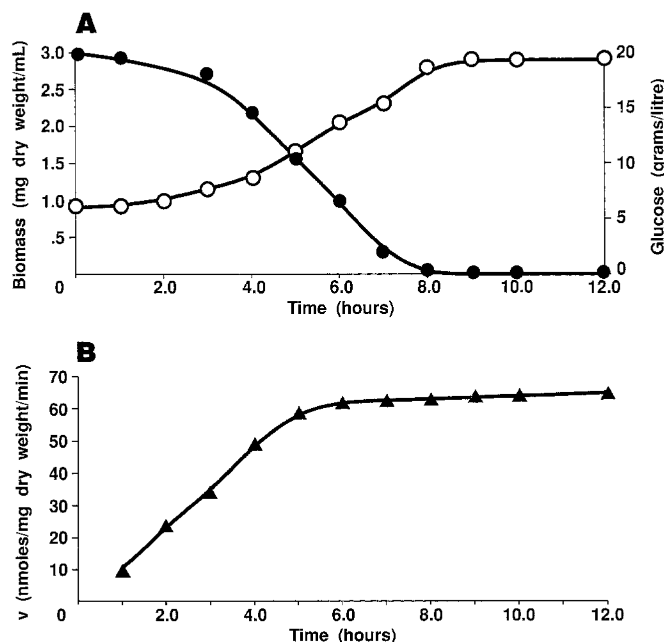

Fig. 3. Effect of growth time on 0.2 mM glucose transport in *Saccharomyces uvarum* (*carlsbergensis*) brewing strain 3021. (A) Cell biomass (○) and glucose remaining in the media (●) and (B) 0.2 mM glucose transport in cells pregrown on 2% glucose for various times.

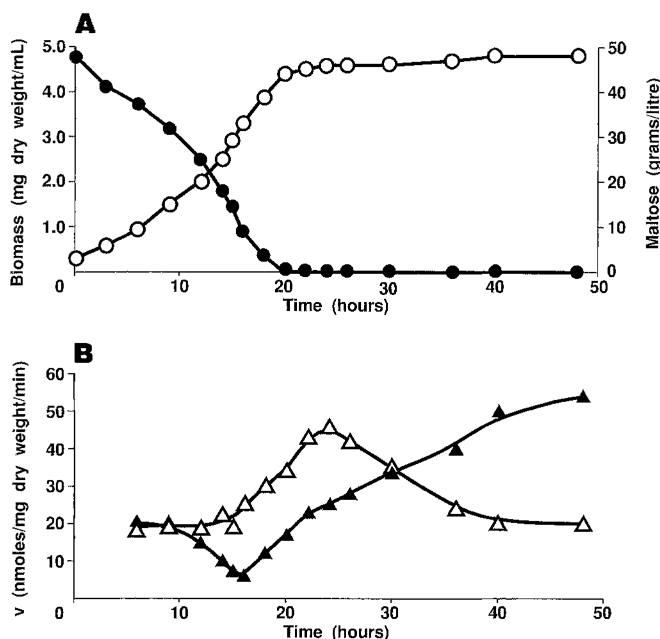

Fig. 4. Effect of growth time on 0.2 mM glucose and 0.2 mM maltose transport in *Saccharomyces uvarum* (*carlsbergensis*) brewing strain 3021. (A) Cell biomass (○) and maltose remaining in the media (●) and (B) 0.2 mM glucose (▲) and 0.2 mM maltose (△) transport in cells pregrown on 5% maltose for various times.

observed to increase as the growth time on glucose increased (Fig. 3). The increase in glucose transport activity coincided with a decrease in the glucose concentration in the medium, such that maximum glucose transport rates were achieved with complete utilization of glucose from the medium and maximum biomass. These results are in agreement with the repressive effects of glucose on the high affinity glucose transport system [3,11]. When glucose is depleted from the medium, maximum glucose transport activity is observed. As in previous cases, maltose transport was inactivated by growth of yeast on glucose (data not shown).

The transport of glucose and maltose was also found to be dependent on the growth stage of the brewing strain on maltose (Fig. 4). Glucose transport activity was observed to increase with increasing fermentation time. Maximum glucose transport rates were achieved in cells obtained after complete utilization of maltose from the medium. On the other hand, maltose transport activity increased to a point corresponding to complete utilization of maltose from the medium but subsequently decreased. As observed with growth of cells on various concentrations of maltose, it is not clear why maximum glucose transport rates are achieved only after complete utilization of maltose. We are currently investigating the role of maltose on glucose utilization in the brewing strain.

In summary, growth of yeast on glucose results in complete inactivation of maltose transport and repression of high affinity glucose transport. When the cells are grown on maltose or subjected to substrate starvation, an increase in glucose and maltose transport rates was observed in both brewing and non-brewing yeast strains. These results are consistent with the repressive effect of glucose on the high affinity glucose and maltose transport systems.

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