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EFFECT OF GLUCOSE ON THE ACTIVITY AND THE KINETICS OF THE MALTOSE-UPTAKE SYSTEM AND OF α-GLUCOSIDASE IN *SACCHAROMYCES CEREVISIAE*

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SUMMARY

1. During incubation of maltose-grown *Saccharomyces cerevisiae* cells in nutrient medium with glucose, the maltose-uptake system was almost completely inactivated within 90 min. During this deadaptation, the K_m of the maltose-uptake system increased from 4 to 50 mM.

2. The activity of α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) remained rather constant during incubation of maltose-grown cells in a nutrient medium with glucose.

3. The presence of maltose in the nutrient medium with glucose did not protect the maltose-uptake system against inactivation. The inactivation was scarcely affected by partial inhibition of glycolysis or by anaerobiosis during deadaptation. Incubation with ethanol did not inactivate the uptake system. Cycloheximide in the medium with glucose led to a two-fold slower inactivation either by inhibiting the protein synthesis or by interfering with the glucose metabolism.

4. A rapid recovery of the maltose-uptake system occurred when inactivated cells were incubated in a maltose nutrient medium; protein synthesis appeared to be a prerequisite for the recovery process.

INTRODUCTION

There is ample evidence that the uptake of maltose and of other α-glucosides in several strains of baker's yeast is an active transport process[1–4]. During deadaptation of maltose-grown cells in a medium with glucose, the maltose permease is inactivated, resulting in the development of crypticity of the cells to maltose metabolism[1].

For several other enzymes in yeast, a rapid inactivation *in vivo* during glucose metabolism has been described, *e.g.* for galactozymase[5] and for malate dehydrogenase[6,7]. The mechanism of this inactivation process is not fully understood.

As part of our investigations on the influence of different carbon sources on the carbohydrate metabolism of yeast[8], we studied the effect of glucose on the activity of α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) and of the maltose-

uptake system during deadaptation of maltose-grown cells. Whereas α-glucosidase is rather stable during deadaptation, the maltose-uptake system decreases in activity with a rapid increase in the K_m. Conditions that affect this decrease in activity were investigated.

MATERIALS AND METHODS

Saccharomyces cerevisiae, CBS 1171, was used in these experiments. The yeast was grown with 2% maltose as described previously[8]. Cells in the logarithmic phase were harvested by either centrifugation or filtration on a membrane filter and were washed thoroughly. They were incubated in either 0.067 M KH_2PO_4 or a nutrient medium with 2% glucose. For anaerobic incubations, the flasks were continuously flushed with O_2-free N_2. The nutrient medium in this case contained 12.5 μg ergosterol and 12.5 μl Tween 80/ml dissolved according to ANDREASEN AND STIER[9]. The cells were periodically rapidly harvested by filtration and were washed.

The rate of sugar uptake was determined by measuring the anaerobic fermentation rate at 28° for 30 min with conventional Warburg techniques. Each vessel contained 2 mg dry weight of yeast suspended in 0.067 M KH_2PO_4 and 10 μmoles glucose or 5 μmoles maltose, unless otherwise stated. Since transmembrane transport is the rate limiting step in sugar fermentation, this indirect method can be used as a criterion for sugar transport[10–12] (see RESULTS). In some experiments we used ^{14}C-uniformly labeled maltose and measured the radioactivity in the CO_2 with the liquid scintillator described by BRAY[13].

For the determinations of α-glucosidase activity, cell-free extracts were prepared as described elsewhere[8], and the enzyme was assayed by the rate of hydrolysis of *p*-nitrophenyl-α-D-glucoside at 28° (see ref. 14). Protein in the extracts was determined according to LOWRY *et al.*[15].

RESULTS

Changes in the activity of the maltose-uptake system and of α-glucosidase during deadaptation of maltose-grown cells

When cells from a maltose-grown culture are incubated in 0.067 M KH_2PO_4, the activity of maltose fermentation decreases gradually. The activity of glucose fermentation diminishes to a lesser extent (Table I). Cells from a maltose-grown culture that are incubated in a nutrient medium with 2% glucose lose the capacity to ferment maltose much more rapidly, while the capacity to ferment glucose increases (Table I). The rapid decrease in maltose fermentation is apparently not due to a loss of glycolytic activity.

The decrease in maltose fermentation is caused either by a loss of α-glucosidase activity or by a loss of the activity of the maltose-uptake system. During incubation in 0.067 M KH_2PO_4, the specific activity of α-glucosidase remains constant. During incubation in a nutrient medium with glucose, a gradual decrease in the specific activity occurs, but this is less than expected by dilution of the enzyme during growth without further synthesis of the enzyme.

The rate limiting step in the maltose consumption is therefore not the α-glucosidase or glycolytic activity; this is in agreement with the results of SOLS AND

DELAFUENTE[10]. It can be concluded that the decrease in maltose fermentation is due to a decrease in the activity of the maltose-uptake system. This is also shown by experiments in which the disappearance of maltose from the medium was directly determined with an anthron method and was compared with the anaerobic CO_2 production with maltose as substrate. With both methods the same decrease during deadaptation in a medium with glucose was found.

The decrease can partly be explained by dilution of the maltose-uptake system because of growth of the cells without further synthesis of this system. The percentage of the maltose-uptake system which is inactivated during growth with glucose is $[(a-b)/a] \cdot 100$ (a is the calculated activity expected when the system is merely diluted by growth, and b is the determined activity). From the results presented in Table I, Column 2, it appears that during growth with glucose a great inactivation occurs.

In order to follow this inactivation of the maltose-uptake system more closely,

TABLE I

CHANGES IN SUGAR UPTAKE BY MALTOSE-GROWN CELLS DURING INCUBATION WITHOUT MALTOSE

Maltose-grown cells were incubated in 0.067 M KH_2PO_4 or in nutrient medium with 2 % glucose. At the times indicated the cells were rapidly harvested, washed, and resting suspensions were prepared. Sugar uptake was indirectly determined as described in MATERIALS AND METHODS. The standard error of the mean followed by the number of experiments in brackets are given.

Incubation	*Substrate*		
	Maltose		*Glucose*
	Percentage of original activity	*Inactivation (%)*	*Percentage of original activity*
1.5 h in 0.067 M KH_2PO_4	78 ± 4 (6)	22 ± 4 (6)	84 ± 3 (6)
3 h in 0.067 M KH_2PO_4	53 ± 5 (4)	47 ± 5 (4)	81 ± 5 (5)
1.5 h in nutrient medium with 2 % glucose	12.1 ± 1.1 (4)	82 ± 2 (4)	145 ± 9 (4)
3 h in nutrient medium with 2 % glucose	2.9 ± 0.5 (4)	92 ± 2 (4)	143 ± 8 (4)

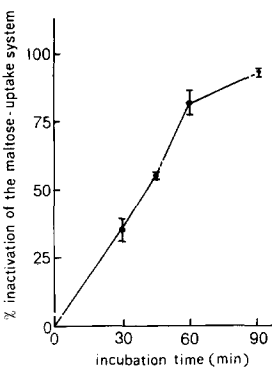


Fig. 1. Inactivation of the maltose-uptake system during incubation of maltose-grown cells in nutrient medium with 2 % glucose. For experimental details see the legend to Table I.

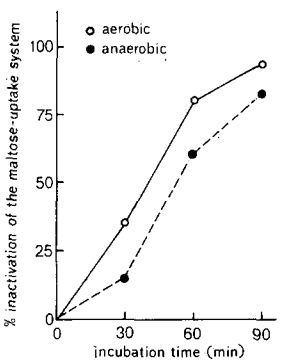


Fig. 2. Effect of anaerobic conditions on the inactivation of the maltose-uptake system during incubation in nutrient medium with 2 % glucose. For experimental details see under MATERIALS AND METHODS and the legend to Table I.

cells were harvested at short intervals. During the first 60 min after transfer of maltose-grown cells in a nutrient medium with glucose, a rapid inactivation occurred (Fig. 1); sometimes an increase in activity or a lag in the inactivation was found during the first 15 min of incubation. Incubation in a KH_2PO_4 solution with 2% glucose resulted in a slight decrease in the rate of inactivation.

Effect of different carbon sources and inhibition of metabolism and protein synthesis on the inactivation of the maltose-uptake system

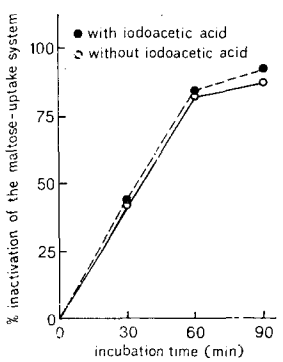
The maltose-uptake system is not inactivated when glucose is replaced by ethanol in the incubation medium; apparently glucose is an important factor in the inactivation process.

The decrease in the maltose-uptake system during a 45-min incubation in nutrient medium with 2% glucose is 34%; in nutrient medium with 2% glucose and 1% maltose it is 38%. Experiments on the effect of 2% glucose on the production of ^{14}C-labeled CO_2 from [^{14}C]maltose show that the consumption of maltose is inhibited by glucose by about 30%. Thus maltose does not protect the uptake system against inactivation by glucose, and this lack of protection is not due to inhibition of maltose uptake by glucose.

The inactivation of the maltose-uptake system under anaerobic conditions is after a lag of 0.5 h approximately as fast as under aerobic conditions (Fig. 2).

The presence of 0.2 mM iodoacetic acid in the incubation medium inhibits the growth by about 60%, the aerobic CO_2 production by about 50% and the respiration by about 10%. It does not affect the inactivation of the maltose-uptake system (Fig. 3). A higher concentration of iodoacetic acid (1 mM) which completely inhibits the aerobic CO_2 production cannot be used because of irreversible effects on the cells.

6.25 μg/ml cycloheximide in the incubation medium inhibit the growth by about 65%, the aerobic CO_2 production by about 60% and the respiration by about 30%. The inactivation of the maltose-uptake system is about 2 times slower with cycloheximide than without it (Fig. 4).



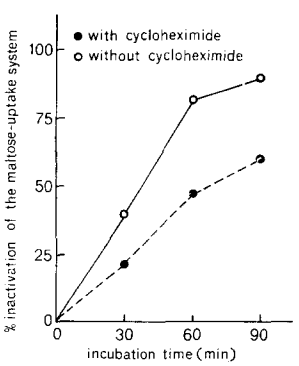


Fig. 3. Effect of iodoacetic acid on the inactivation of the maltose-uptake system during incubation in nutrient medium with 2% glucose. For experimental details see the legend to Table I. The concentration of iodoacetic acid was 0.2 mM.

Fig. 4. Effect of cycloheximide on the inactivation of the maltose-uptake system during incubation in nutrient medium with 2% glucose. For experimental details see the legend to Table I. The concentration of cycloheximide was 6.25 μg/ml.

Recovery of the maltose-uptake system after deadaptation

When maltose-grown cells after incubation in culture medium with 2 % glucose are incubated in culture medium with 2% maltose, a rapid recovery of the maltose-uptake system occurs (Fig. 5). Cycloheximide inhibits this recovery suggesting that protein synthesis is involved. This is supported by the fact that recovery does not occur in a medium with 0.067 M KH_2PO_4 and 2% maltose but without a nitrogen source.

Changes in the kinetics of the maltose-uptake system and of α-glucosidase during deadaptation

To acquire kinetic data we varied the concentration of substrate in the determinations of the activity of the maltose-uptake system and of α-glucosidase. Plots of $1/v$ against $1/[S]$ reveal that the K_m of the maltose-uptake system sharply increases

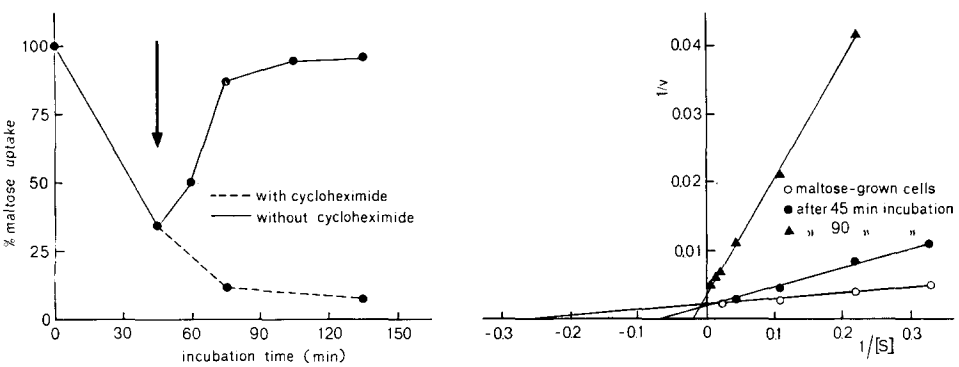


Fig. 5. Recovery of the maltose-uptake system by incubation of deadapted cells with maltose and with or without cycloheximide. Maltose-grown cells were incubated in nutrient medium with 2 % glucose for 45 min. After rapidly washing these cells were incubated in nutrient medium with 2 % maltose with or without cycloheximide (6.25 μg/ml). For further experimental details see the legend to Table I.

Fig. 6. Changes in kinetics of the maltose-uptake system during incubation of maltose-grown cells in nutrient medium with 2 % glucose. Substrate concentration is expressed in mM, the rate of maltose uptake in μl CO_2 produced/2 mg dry weight per 30 min.

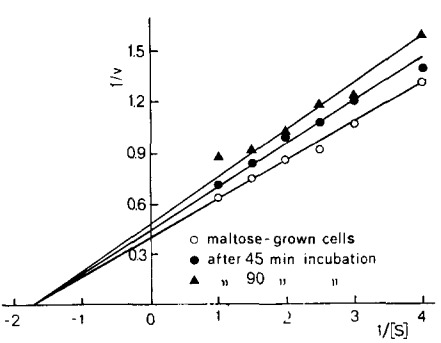


Fig. 7. Changes in kinetics of α-glucosidase during incubation of maltose-grown cells in nutrient medium with 2 % glucose. Substrate concentration is expressed in mM *p*-nitrophenyl-α-D-glucoside, the activity in μmoles *p*-nitrophenyl-α-D-glucoside split/mg protein per min. For experimental details see MATERIALS AND METHODS.

during deadaptation, whereas V remains rather constant (Fig. 6). The K_m of maltose-grown cells is about 4 mM, after 45 min of deadaptation it is about 13 mM and after 90 min about 50 mM. The decrease in the activity of the maltose-uptake system as described above is caused largely or completely by the decrease in the affinity of the uptake system for its substrate. During recovery of the maltose-uptake system the K_m decreases to its original value within 1 h. The K_m of α-glucosidase remains rather constant during deadaptation with a value of about 0.5 mM whereas V slightly decreases (Fig. 7).

DISCUSSION

The rapid increase in the K_m of the maltose-uptake system during deadaptation of maltose-grown cells in a glucose nutrient medium is surprising. To our knowledge there have been few investigations on K_m changes in regulatory phenomena. A 125-fold decrease in the K_m of the galactose-uptake system in yeast during induction has been reported[16].

The mechanism of the decrease in activity of the maltose-uptake system is not known. Since V is rather constant during deadaptation, it is improbable that proteolysis of components of the maltose-uptake system is involved. Conformational changes in the components (carriers or permease molecules) of the uptake system or conversion into inactive subunits, both resulting in a decrease in affinity, are possible. It is not known whether these changes during deadaptation are correlated with a shift from metabolically linked active transport into passive transport. During induction the transport of α-thioethyl-D-glucopyranoside changes from facilitated diffusion into active transport[3,4], as has also been shown for galactose transport[16]. HEREDIA *et al.*[17], however, do not agree with the claims for active sugar transport in yeast.

The presence of glucose during deadaptation appears to be necessary for the inactivation process. Incubation with ethanol which can be oxidized by these cells[8] does not result in inactivation. Blocking of respiration by anaerobiosis during deadaptation slightly affects the inactivation process. These findings can be explained by the assumption that glycolytic metabolism is a prerequisite for the inactivation. This has been suggested for the inactivation of malate dehydrogenase[7]. Apparently the 50% inhibition of aerobic CO_2 production by iodoacetic acid is not enough to decrease the inactivation process.

The presence of cycloheximide during deadaptation results in a two-fold slower inactivation; whether this is due to its strong interference with glucose metabolism or to its inhibition of protein synthesis cannot be concluded. It has been suggested that in the inactivation of malate dehydrogenase by glucose[6] and of ornithine transcarbamylase by arginine[18], an inactivating protein is involved. A catabolite of glucose might induce this inactivating protein[6].

The recovery of the maltose-uptake system needs protein synthesis as appears from the inhibiting effect of cycloheximide and the necessity of a nitrogen source. The components of the maltose-uptake system may be synthesized *de novo* from amino acids, or the recovery is due either to conformational changes or to the assembly of subunits in which peptide-bond formation on the ribosomes is involved.

The large decrease in the activity of the maltose-uptake system and the stability of α-glucosidase during deadaptation leads to the development of crypticity. The

occurrence of crypticity to maltose metabolism has been described in another strain of yeast, but the decrease in activity is much slower and is found only in the absence of a nitrogen source[1].

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