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GLUCOSE TRANSPORT IN NONGROWING YEAST

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The inducible, nonenergy-requiring glucose transport system of the yeast *Kluyveromyces lactis* is inactivated upon starving cells of glucose by (1) transferring logarithmic phase glucose-grown cells to synthetic medium containing a nonglycolytic carbon source, and (2) upon transition of logarithmic phase glucose-grown cells to stationary phase. The steady-state accumulation of nonmetabolizeable 6-deoxyglucose and the apparent K_m of transport of 6-deoxyglucose is the same in stationary phase cells and in logarithmic phase cells. The rate of transport is lower in the nongrowing cells. Restoration of activity requires energy and protein synthesis as well as inducer.

Introduction

Kluyveromyces lactis is an oxidative yeast that transports glucose via an inducible, nonenergy-requiring transport system [1,2]. Having observed the genetic regulation of the transport system, it became of interest to determine the mechanism of loss of carrier activity upon removal of glucose. It was found that when glucose-grown cells are starved of glucose in the stationary phase of growth or upon transfer to ethanol-containing medium, the glucose transport system is irreversibly inactivated.

Materials and Methods

All experiments were done using *Kluyveromyces lactis* Y123 (NRRL strain Y1118) grown in glucose synthetic medium, harvested, and washed as previously described [1]. The ethanol synthetic medium used had the same composition as did the glucose synthetic medium, but contained 0.2% ethanol instead of glucose. Cell viability was determined by plating cells on yeast maintenance agar which consisted of yeast extract, 3 g/l; malt extract, 3 g/l; peptone, 5 g/l; glucose, 10 g/l; and agar, 20 g/l (pH 5.4). Results are expressed as colony-forming units per ml.

Hexokinase (EC 2.7.1.1) and pyruvate kinase (EC 2.7.1.40) were assayed spectrophotometrically as before [2]. Cell free extracts were obtained by grinding cells with three times their weight of alumina for 5 min at 4°C. The concentration of glucose in the stationary phase medium was determined enzymatically via hexokinase activity.

In transfer experiments, cells were washed two times in 67 mM potassium phosphate buffer (pH 5.4), and resuspended in the indicated medium prewarmed to 30°C. Concentrations of additions to the media were as follows: 0.32 mM sodium azide; 1% ethanol; 2 mM phenylmethylsulfonyl fluoride (PMSF) in crystalline form; and 200 μg/ml anisomycin.

Transport experiments were done as before [1,3] using 0.125 Ci/mol 6-deoxy-D-[G-^{3}H]glucose (New England Nuclear; unlabeled, Sigma). Unless otherwise noted, cells were exposed to 40 mM substrate.

Abbreviation: PMSF; phenylmethylsulfonyl fluoride.

Results and Discussion

Cells growing in glucose-containing medium are starved of glucose during the stationary phase of growth (Fig. 1). After the cells have been in stationary phase for approx. 8 h, the glucose transport capacity of the cells decreased to 25% of the original activity. Cell viability did not decrease during this time period, nor did the specific activity of hexokinase, a constitutive enzyme [2]. Other experiments revealed that the activity of pyruvate kinase, also a constitutive enzyme [2], did not change during this time period. It is also noted that the pH of the medium, originally 5.4, decreased to approx. 4.4 during stationary phase. Other experiments revealed that the glucose transport system was inactivated when mid-logarithmic phase cells ($A = 0.5$, Fig. 2), were transferred to synthetic medium containing ethanol. After 4 h, during which time there was no change in the number of colony-forming units per ml, nor change in pH of the medium, 42% of the original transport activity remained. Aliquots of the same exponential-phase cells transferred to 67 mM potas-

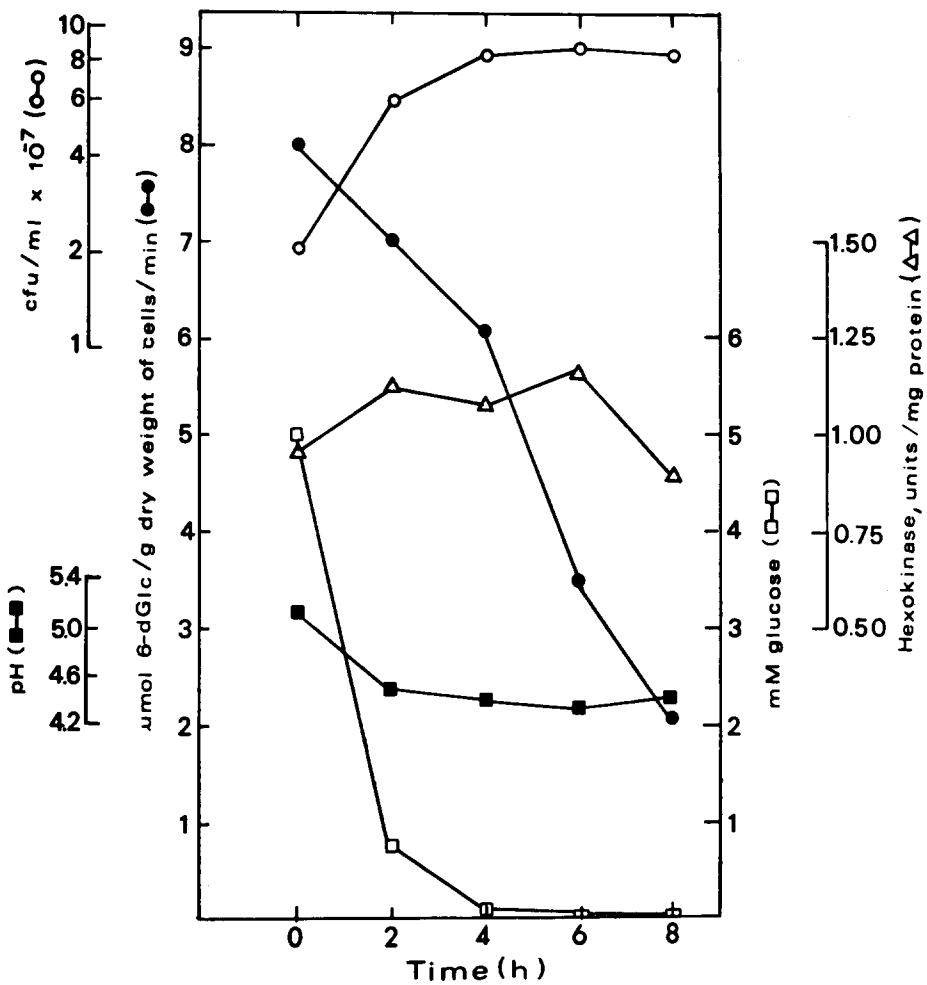

Fig. 1. Metabolic and cultural events associated with the transition of glucose-grown *Kluyveromyces lactis* from the late-logarithmic phase of growth to stationary phase. Cell viability was determined in the late-logarithmic phase of growth ($A = 2.0$) and at 2-h intervals during stationary phase. The rate of transport of 6-deoxy[^{3}H]glucose (6-dGlc) was determined every 2 h, as was the specific activity of hexokinase in crude cell free extracts. The pH of the growth medium and the concentration of glucose in the growth medium were also measured every 2 h. cfu, colony-forming units.

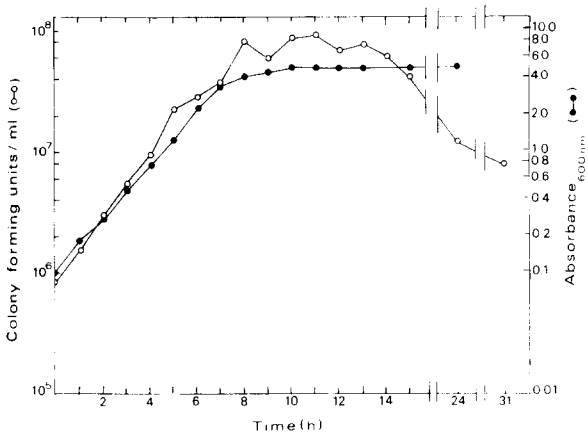

Fig. 2. Growth curve of *Kluyveromyces lactis* growing in glucose synthetic medium with shaking at 30°C. Viability was determined by plating cells in triplicate on yeast maintenance agar. Results are expressed as colony-forming units per ml.

TABLE I

THE EFFECT OF GLUCOSE STARVATION AND pH ON GLUCOSE TRANSPORT ACTIVITY

Cells were harvested, and washed two times with 67 mM potassium phosphate buffer. The rate of transport of 40 mM 6-deoxy[^{3}H]glucose, was determined in one aliquot of cells.

Cell age	Transport activity [a]		
	pH 5.4	pH 4.4	Stationary phase
Mid-logarithmic phase ($A = 0.5$)	80	84	–
Late-logarithmic phase ($A = 2.0$)	81	59	32

[a] Percent of zero-time transport activity after shaking at 30°C for 6 h in synthetic medium containing no carbon source at the pH indicated, or after being in stationary phase for 6 h.

sium phosphate buffer (pH 5.4) for the same time period retained 88% of transport activity. The lack of cell growth during stationary phase and immediately following transfer of cells to ethanol-containing medium indicates that the loss in glucose transport activity in the absence of inducer is not due to the dilution of existing carrier molecules after cessation of synthesis.

Attempts were made to reproduce the inactivation of carrier activity by starving mid-logarithmic phase cells ($A = 0.5$), and late-logarithmic phase cells ($A = 2.0$) in synthetic medium without a carbon source at pH of 5.4 and at pH of 4.4. The decrease in activity seen at both pH values with the two populations of cells was not as great as that seen in cells allowed to enter the stationary phase of growth (Table I). These results immediately suggested that factors in addition to glucose concentration and pH are involved in regulating the activity of the glucose transport system during the nongrowing state.

To determine any involvement of energy in the inactivation, sodium azide was added to late-logarithmic phase medium at a concentration that inhibited respiration but permitted a low level of fermentation [2]. Under these conditions, cells retained 85% of transport activity after 6 h, whereas cells incubated for the same time period without inhibitor retained 32% of original transport activity. A requirement for metabolic energy in carrier inactivation is suggested by these results. However, addition of ethanol, an energy source, to the stationary phase medium of these cells resulted in partial retention of carrier activity, for after 6 h, 63% of the transport activity of the late-logarithmic phase cells remained. It is noted, however, that when an aliquot of the same late-logarithmic phase cells instead were washed and incubated in ethanol synthetic medium (pH 5.4), 44% of initial activity remained after 5 h.

PMSF, a serine protease inhibitor, was also added to cells entering stationary phase. After 6 h incubation with PMSF, 69% of original transport activity remained as compared to 43% of original activity in untreated cells, suggesting a possible role of proteolysis in the inactivation process. Addition of anisomycin, an effective protein synthesis inhibitor in this yeast, to the late-logarithmic phase cells, had no effect on the rate of carrier inactivation. Therefore, if proteolysis is involved in carrier inactivation, the protease is already synthesized by late-logarithmic phase of growth. Failure of anisomycin to affect the rate of carrier inactivation during the stationary phase of growth suggests that PMSF is not interfering with the synthesis of proteins under these conditions as the inhibitor is known to do in *Escherichia coli* [4]. PMSF also interferes with energy production in *E. coli* [5], but

not in *Saccharomyces cerevisiae*, at least during 50 min incubation in the presence of inhibitor [6]. If such interference occurs in *K. lactis* the effect of PMSF on carrier activity may be related to the energy state of the cell rather than to the inactivation of protease activity. The retention of carrier activity seen with sodium azide supports this idea. It is noted, though, that cell death did not occur during 6 h incubation in the presence of PMSF, indicating that any reduction in energy level is not great enough to decrease cell viability.

Evidence that a reduction in number of existing carrier molecules is occurring in the nongrowing cells is presented in Fig. 3, where it is seen that the V of transport decreased upon transition of cells from logarithmic phase of growth to stationary phase, but the apparent K_m remained unchanged.

The steady-state accumulation of 6-deoxyglucose in late-logarithmic phase cells was compared with that in stationary phase cells. The total accumulation of labeled 6-deoxyglucose in late-logarithmic phase cells ($A = 2.0$) was 13.5 μmol/g dry weight of cells. Stationary phase cells harvested 3 h and 6 h later accumulated 13.6 and 13.8 μmol 6-deoxyglucose per g dry weight of cells, respectively, albeit at slower rates than exhibited by the growing cells.

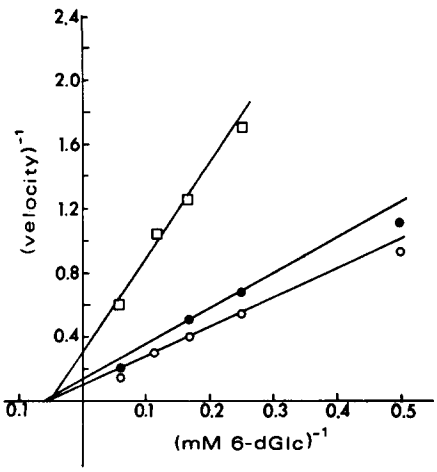

Fig. 3. Double reciprocal plots of accumulation of 6-deoxy-[^{3}H]glucose by growing and nongrowing *K. lactis*. The rate of accumulation was measured in late-logarithmic phase cells ($A = 3.0$) (○) and in cells harvested 3 h (●) and 8 h (□) later. The velocity is expressed as μmol 6-deoxyglucose (6-dGlc)/g dry weight of cells per min.

Following carrier inactivation, protein synthesis and energy are needed for restoration of transport activity as is the inducer (Fig. 4). The requirement for protein synthesis suggests again that proteolysis may be involved in the carrier inactivation. The further reduction in transport capacity seen when stationary phase cells are transferred to synthetic medium containing only sodium azide or anisomycin may reflect a requirement for glucose or a metabolite of it for maintenance of carrier integrity. Protein synthesis and energy were also needed to restore glucose transport activity in mid-logarithmic phase cells which had incubated for 4 h in ethanol synthetic medium.

As pointed out by Switzer [7], the requirement for protein synthesis for restoration of enzyme activity following inactivation may be to syn-

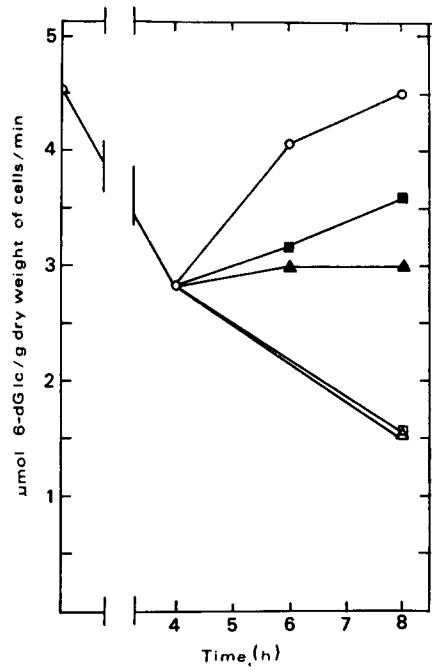

Fig. 4. Restoration of the glucose transport activity. Transport of 6-deoxy[^{3}H]glucose (6-d[^{3}H]Glc) was measured in early stationary phase cells ($A = 3.6$) and 4 h later. At 4 h, the remaining cells were transferred to fresh glucose synthetic medium containing no additions (○), 0.32 mM sodium azide (■), or 200 μg/ml anisomycin (▲). Other cells were transferred to synthetic medium (without carbon source) containing 0.32 mM sodium azide (□), or 200 μg/ml anisomycin (△). At the indicated time intervals, the rate of 6-deoxy[^{3}H]glucose transport was determined as before.

thesize not the enzyme in question, but rather a modifying one. The possibility that the glucose carrier of this yeast is subject to activation by a modifying enzyme and that it is the modifying protein that must be synthesized prior to restoration of transport activity cannot be ruled out at this time. As well, the activity of the glucose transport system may be regulated by a modifying protein via protein-protein interaction, a suggested means of regulation of the galactose transport system in *S. cerevisiae* [8]. Perhaps proteolysis of such a modifying protein is taking place.

Entry of *S. cerevisiae* into the stationary phase of growth results in an increase in activity of three major proteinases: proteinase A, proteinase B, and carboxypeptidase Y [9]. Any similar increase in proteolytic activity of *K. lactis* may contribute to the selective loss of activity of the glucose transport system seen in stationary phase cells. If the loss in transport activity in logarithmic phase cells transferred to nonglycolytic medium occurs via the same mechanism is not known at this time. The inactivation in stationary phase cells does appear to be more sensitive to pH than is the inactivation in logarithmic phase cells. Any involvement of energy in carrier inactivation in either population of nongrowing cells is unclear.

The nonenergy-requiring glucose transport system of *K. lactis* mediates transport of free glucose across the membrane in two directions, as shown by exchange diffusion of labeled 6-deoxyglucose [3]. Upon transition of glucose-grown cells to either stationary phase or nonglycolytic conditions, the rapid inactivation of the induced glucose transport system may serve to prevent loss of glucose formed intracellularly.

Acknowledgement

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