JOURNAL OF BACTERIOLOGY, June 1990, p. 2871–2876
0021-9193/90/062871-06$02.00/0

Vol. 172, No. 6

Continuous-Culture Study of the Regulation of Glucose and Fructose Transport in *Kluyveromyces marxianus* CBS 6556

ERIK POSTMA[1] AND PETER J. A. VAN DEN BROEK[2]*

Department of Microbiology and Enzymology, Delft University of Technology, 2628 BC Delft,[1] and Department of Medical Biochemistry, Sylvius Laboratory, University of Leiden, Wassenaarseweg 72, 2333 AL Leiden,[2] The Netherlands

Received 26 September 1989/Accepted 16 February 1990

Regulation of transport of D-glucose and D-fructose was studied in *Kluyveromyces marxianus* grown in continuous culture. Both substrates could be transported by at least two different transport systems, low-affinity transport and high-affinity proton-sugar symport. The low-affinity transporter, specific for both glucose and fructose, was constitutively present and was apparently not regulated by carbon catabolite repression. Regulation of the activity of the glucose- and fructose-specific proton symport systems appeared to proceed mainly through catabolite repression. Activation of symport did not need the presence of specific inductor molecules in the medium. Nevertheless, the capacities of the proton-sugar symporters varied in cells grown on a wide variety of carbon sources. The possibility that the control of proton symport activity is related to the presence of specific intracellular metabolites is discussed.

Sugar transport in yeasts can proceed by a number of different mechanisms. Facilitated diffusion and proton-sugar symport have been established for many different types of yeast (8, 16, 19, 20). Moreover, it is claimed that glucose transport in *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* can proceed through a phosphotransferase (10, 17).

The activity or the presence of sugar transport proteins in the plasma membrane is strongly dependent on the environmental conditions. In 1969, down regulation of carrier activity was described for the first time for maltose transport in *S. cerevisiae* (7) and seemed to be due to catabolite inactivation and catabolite repression (for definitions, see reference 9). Later, these effects were also described for other transport systems in *S. cerevisiae* (2, 11) and some *Candida* strains (12, 19). Up regulation, defined as the enhancement of transport activity, is assumed to be caused by reversal of the catabolite effects and is in some cases regulated by induction (4, 15).

In a previous paper, four possible transport systems in *K. marxianus* were described (3). One transport system seemed to be a glucose- and fructose-specific transporter with apparent phosphotransferase properties. The other three carriers appeared to be proton-sugar symporters, specific for, respectively, lactose, fructose, and glucose-galactose. Regulation of the activity of the sugar carriers has been described by Gasnier (5) and De Bruijne et al. (3). It was found that the proton-sugar symporters were strongly dependent on the environmental conditions, down regulation proceeding through catabolite effects. However, doubts have remained about some aspects of regulation of sugar carriers in *K. marxianus*; conflicting data were presented in these two papers on the question of whether the low-affinity glucose carrier is constitutively present. Moreover, suggestions have been made about the inducibility (by low concentrations of the substrates) of the fructose- and glucose-galactose-specific cotransporters (3).

Most studies on regulation of cellular functions have been carried out by using cells grown in batch. Even though this way of growing yeast cells is easy, it has as the important disadvantage that growth conditions are not constant and to some extent are uncontrollable. These problems can be overcome by using continuous cultivation, wherein the steady-state conditions are constant. The applicability of continuous cultures to studies of regulation of transport processes has been shown for several yeast species (13–15).

In this paper, the regulation of glucose and fructose transport was studied in *K. marxianus* grown on different carbon sources in continuous culture.

MATERIALS AND METHODS

Growth conditions. *K. marxianus* CBS 6556 was grown in a fermentor (Applikon Dependable Instruments, Schiedam, The Netherlands) with a 1-liter working volume at 40°C for the sugar substrates and at 33°C for ethanol, glycerol, and acetate. The medium composition was as described by Bruinenberg et al. (1) (with a 10-fold-greater amount of $Na_2MoO_4 \cdot 2H_2O$). The cultures were carbon limited except when nitrogen was limiting with sucrose as the carbon source (see Results). With nitrogen limitation, the sucrose/ammonium sulfate ratio was 25:1 (wt/wt). The concentration of the carbon sources in the medium was 10 g · liter^{-1} except for growth on xylose and galactose, for which 12.5 g · liter^{-1} was used.

Oxygen levels were measured by using a Clark-type oxygen electrode and were kept above 50% air saturation. The pH was kept at 4.5 by using 2 M KOH. To prevent disintegration of sugars, the sugar stock solutions were sterilized by steaming at 100°C for 15 min, followed by 10 min at 110°C and subsequent cooling. By this procedure, the normally indicated impurities were not considerably increased. The contaminations of the sugar carbon sources with glucose and fructose, before and after sterilization, are listed in Table 1.

Determination of dry weight. For dry weight measurements, nitrocellulose filters (pore size, 0.45 μm; Gelman Sciences, Inc., Ann Arbor, Mich.) were used. After removal of the medium by filtration, the filter was washed with

* Corresponding author.

TABLE 1. Contamination of sugar carbon sources with glucose and fructose before and after sterilization

Carbon source	Contamination (%)[a]			
	Glucose		Fructose	
	1	2	1	2
Glucose			ND	ND
Fructose	0.04[b]	0.04[b]		
Sucrose	0.00	0.14	0.00	0.12
Galactose	0.04	0.04	0.00	0.01
Xylose	0.09	0.10	0.01	0.01

[a] 1, Before sterilization; 2, after sterilization. ND, Could not be determined because of the high glucose level.
[b] The high fructose concentration interfered with the assay.

demineralized water and then dried in a R-7400 Magnetron oven (Sharp Inc., Osaka, Japan) for 15 min.

Determination of residual substrate concentrations. Residual substrate concentrations were measured by using the rapid freezing method in liquid nitrogen as described previously (14). In some cases, steady-state residual substrate concentrations were determined by a dialysis method. A pipe with a dialysis membrane (6) was inserted in the fermentor. On the dialysis side, the medium (total volume, 9 ml) was pumped through a sterile loop at a flow rate of 3 ml · min^{-1}. Samples from this loop could be withdrawn aseptically. Experiments showed that equilibration of substrates between the culture medium and the dialysis loop required 10 to 15 h. Glucose and fructose were determined with a Boehringer kit (no. 676543).

Transport assay. Transport was measured at 37°C as described previously (14). Briefly, cells were incubated aerobically at the same cell concentration as in the fermentor. ^{14}C-labeled sugar was added, and after 5 s the reaction was stopped by adding ice-cold 0.1 M LiCl. After the cells were filtered and washed with 0.1 M LiCl on 0.45-μm-pore-size cellulose nitrate filters, radioactivity was determined by liquid scintillation counting. Corrections for passive diffusion into the cells and for binding of the sugar to the filters and the cells were made from measurements with cells that were heat inactivated by incubation for 2 min at 100°C. Control experiments had revealed that these heat-inactivated cells had lost carrier-mediated transport, leaving cells structurally intact. Therefore, the amount of sugar taken up by specific transport systems was calculated, at each sugar concentration, from the difference between normal and heat-inactivated cells. This difference was in all cases 700 to 20,000 dpm (depending on the sugar concentration) higher than the aspecific background of 150–400 dpm. Kinetic constants were obtained by measuring transport (in triplicate) at 11 concentrations ranging from 25 μM to 15 mM for D-glucose and from 50 μM to 20 mM for fructose. The apparent kinetic constants were obtained by computer curve fitting of the data (20). Linear or biphasic kinetics were fitted with, respectively, one or two kinetic components.

Measurements of H^+-sugar symport. Measurements of sugar-dependent alkalinization were performed at 37°C essentially as described by Van Urk et al. (21). Briefly, cells were harvested from steady-state cultures by centrifugation, washed two times in potassium phthalate buffer (1.25 mM, pH 5.0), and resuspended in this buffer to a concentration of 10 g (dry weight) · $liter^{-1}$. Proton transport was measured aerobically, using a pH electrode (Ankersmit) connected to a Philips PW 9421 pH meter equipped with a Kipp BD 40 recorder. Calibration was done with a standard NaOH solution. Sugar-stimulated proton uptake was calculated from the amount of protons disappearing from the medium immediately after addition of the sugar and was corrected for the base-line drift. The kinetic constants were obtained from proton flux measurements at sugar concentrations in the range of 50 μM to 1 mM.

TABLE 2. Biomass yield and glucose and fructose concentrations in chemostat cultures of *K. marxianus* CBS 6556 at different dilution rates[a]

Carbon source	Dilution rate (h^{-1})	Yield ($g \cdot g^{-1}$)	Concn (μM)	
			Glucose	Fructose
Glucose	0.1	0.43	26	6
Glucose	0.2	0.43	75	8
Fructose	0.2	0.43	16	59
Sucrose	0.2	0.45	30	39
Xylose	0.1	0.43	6	0
Galactose	0.1	0.41	5	0
Glycerol	0.1	0.44	20	0
Ethanol	0.1	0.61	15	3
Acetic acid	0.1	0.33	6	0
Sucrose N limitation	0.2	0.34	5,500	9,500

[a] Variations in determinations of biomass yield and sugar concentration were 5 and 25%, respectively.

Materials. D-[U-^{14}C]glucose and D-[U-^{14}C]fructose were obtained at 10 GBq · $mmol^{-1}$ from Amersham International. All other chemicals were obtained in the best purity available from commercial sources.

RESULTS

Characterization of growth. *K. marxianus* CBS 6556 was grown in continuous culture on a number of different carbon sources. The growth yields and residual glucose and fructose concentrations as determined by the rapid freezing method in liquid nitrogen are shown in Table 2.

Glucose in the medium was observed under all growth conditions, even when the medium was not supplied with a sugar carbon source (ethanol, glycerol, and acetate media). This finding suggested that this rapid sampling technique could introduce artifacts in the measurement of residual substrate. Therefore, residual substrate in the fermentor was measured by an other method: a dialysis probe containing a compartment that is in equilibrium with the medium and free from cellular contamination was inserted in the fermentor. Residual glucose and fructose were determined in samples from this probe. With growth on glucose, fructose, and sucrose, almost identical data were obtained with the freez-

TABLE 3. Comparison of residual sugar concentrations in carbon-limited chemostat cultures of *K. marxianus* obtained with the rapid freezing and dialysis methods

Carbon source	Dilution rate (h^{-1})	Concn (μM)[a]			
		Freezing method		Dialysis method	
		Glucose	Fructose	Glucose	Fructose
Glucose	0.1	27	6	35	8
Ethanol	0.1	15	3	0	0
Fructose	0.2	16	59	5	80
Sucrose	0.2	30	39	21	37

[a] The variation in determinations was 25%.

TABLE 4. Kinetic constants of glucose uptake in aerobic chemostat cultures of *K. marxianus* grown on various carbon sources[a]

Carbon source	Dilution rate (h^{-1})	K_{m1} (mM)	V_{max1} (μmol · g [dry wt]$^{-1}$ · min^{-1})	K_{m2} (mM)	V_{max2} (μmol · g [dry wt]$^{-1}$ · min^{-1})
Glucose	0.2	0.030	52	3.0	145
Fructose	0.2	0.052	36	4.4	440
Sucrose	0.2	0.050	54	2.0	220
Xylose	0.1	0.134	78	7.4	220
Galactose	0.1	0.060	19	5.8	115
Glycerol	0.1	0.107	63	2.0	165
Ethanol	0.1	0.037	4	2.5	190
Acetic acid	0.1	—	—	1.6	150
Sucrose N limitation	0.2	—	—	4.0	135

[a] The variation in determinations was maximally 20% (14). —, Transport system not detected.

ing and dialysis methods (Table 3). However, in ethanol cultures, the extracellular glucose concentration determined with the dialysis probe was zero, indicating that in that case the rapid sampling techniques indeed had overestimated the extracellular sugar concentration. It should be noted that during growth on sucrose, all external substrate was quantitatively hydrolyzed to glucose and fructose as a result of the presence of extracellular inulinase or invertase (18).

Characterization of sugar transport. Transport of glucose and fructose into yeast cells grown on different carbon sources was estimated after 5 s of uptake (control experiments revealed that uptake was linear with time for 10 s). Table 4 shows the kinetic constants of glucose transport. It follows from these data that glucose, depending on the growth conditions, can be transported by two transport systems: a high-affinity carrier with a K_m of about 70 μM and a low-affinity carrier with a K_m of about 3.5 mM.

Table 5 shows the data for fructose transport. Also with fructose, two transport systems could be observed: one with a K_m of approximately 100 μM and one with a K_m of about 8 mM.

The activity of proton-sugar symporters was determined by measuring proton uptake in weakly buffered yeast suspensions. The apparent affinity constants of sugar-stimulated proton influx revealed that both glucose and fructose could

TABLE 5. Kinetic constants of fructose uptake in aerobic chemostat cultures of *K. marxianus* grown on various carbon sources[a]

Carbon source	Dilution rate (h^{-1})	K_{m1} (mM)	V_{max1} (μmol · g [dry wt]$^{-1}$ · min^{-1})	K_{m2} (mM)	V_{max2} (μmol · g [dry wt]$^{-1}$ · min^{-1})
Glucose	0.2	0.082	107	8.4	400
Fructose	0.2	0.073	102	7.4	500
Sucrose	0.2	0.104	116	6.5	420
Xylose	0.1	0.118	68	2.6	280
Galactose	0.1	0.140	3	10.4	125
Glycerol	0.1	0.066	128	4.1	310
Ethanol	0.1	0.150	38	9.0	140
Acetic acid	0.1	0.145	50	11.9	270
Sucrose N limitation	0.2	—	—	12.5	260

[a] The variation in determinations was maximally 20%. —, Transport system not detected.

TABLE 6. Affinity constants and apparent H^+-sugar stoichiometries of high-affinity glucose and fructose uptake determined by alkalinization of weakly buffered cell suspension of *K. marxianus*

Carbon source	Glucose		Fructose	
	K_m (μM)	H^+-glucose stoichiometry	K_m (μM)	H^+-fructose stoichiometry
Glucose	22	1.8	107	0.8
Fructose	55	0.6	350	0.8
Sucrose	75	1.5	180	0.8
Xylose	ND[a]	0.1	300	1.1
Galactose	60	2.2	—[b]	—
Glycerol	50	0.3	70	0.5
Ethanol	—	—	ND	0.4
Acetic acid	—	—	100	0.8
Sucrose N limitation	—	—	—	—

[a] ND, Affinity constant could not be accurately determined because of large variations in capacity obtained at low sugar concentrations.
[b] —, No alkalinization was observed upon addition of sugar.

cause, with high affinities, proton uptake (Table 6). The close similarity between the affinity constants obtained for glucose- and fructose-dependent alkalinization and the affinity constants for high-affinity glucose and fructose uptake (Tables 4 and 5) indicates that the high-affinity glucose and fructose transports are mediated by proton-sugar symporters. It should be noted, however, that the theoretical possibility of sugar-OH^- antiport can not be excluded.

As has been reported by Gasnier (5), proton flux measurements in *K. marxianus* can have enormous experimental scatter. In the present study the same was observed. Table 6 shows that indeed large variations in proton-sugar stoichiometries could be observed. Even though it is apparently difficult to determine exact stoichiometries in this yeast (see also Gasnier [5]), average proton-sugar stoichiometries of 1.3 for glucose and 1.0 for fructose could be observed, indicating that glucose and fructose are cotransported with one proton.

Galactose-limited growth. Glucose- and fructose-proton symporters were present in *K. marxianus* under all sugar-limited conditions. Galactose enters *K. marxianus* via the glucose-proton symporter. Although this carrier was present under galactose-limited conditions, its capacity was low (Table 4). To sustain growth, the residual galactose concentration in the fermentor should be relatively high. This was indeed observed: a residual galactose concentration of 300 μM was established. Fructose-proton symport in galactose-grown cells was extremely low (Table 5). It was rationalized that the relatively high galactose concentrations in the fermentor might cause a catabolite repression effect on the fructose-proton symporter. Therefore, *K. marxianus* was grown under dual limitation of fructose and galactose. If galactose (or an intermediate in galactose metabolism) has a repressive effect on the fructose-H^+ symporter, the residual substrate concentration of fructose should be increased. At a growth rate of 0.20 h^{-1}, the fructose concentration increased from 59 ± 15 μM to 110 ± 20 μM during, respectively, fructose- and fructose-galactose-limited growth (Tables 3 and 7). Moreover, transport measurements, performed at 50 μM fructose, revealed that fructose influx was reduced to 36% of the value of cells grown on fructose only.

When a galactose pulse (8 mM) was given to a mixed galactose-fructose-limited steady-state culture, a rapid increase in the residual fructose concentration was observed,

TABLE 7. Residual sugar concentrations in fructose-galactose-limited cultures of *K. marxianus* growing at different dilution rates[a]

Dilution rate (h^{-1})	Concn (μM)					
	Freezing method			Dialysis method		
	Glucose	Fructose	Galactose	Glucose	Fructose	Galactose
0.10	30	60	95	3	70	90
0.20	35	110	5,100	0	100	4,800

[a] Fructose and galactose were present in the cultures at concentrations of 10 and 3.3 g · liter^{-1}, respectively. The variation in determinations was maximally 25%.

indicating that rapid repression of this carrier occurred (Fig. 1). This result was confirmed by sugar uptake experiments, which showed that 1 h after the galactose pulse, fructose influx, measured at 50 μM fructose, was decreased to 47% compared with the level in cells before the galactose pulse.

Specific sugar consumption rate and sugar transport. In steady state, the specific rate of sugar consumption (q_{obs}) by chemostat grown cells can be calculated by the equation q_{obs} = dilution rate/cell yield. This sugar consumption in the fermentor should be balanced by the actual sugar transport (14), which can be calculated with Michaelis-Menten kinetics on the basis of residual sugar concentrations and measured kinetic constants. Table 8 shows the data for growth of the yeast cells on sugars. It follows that q_{obs} and transport flux are indeed closely similar.

DISCUSSION

Residual sugar in the fermentor. This report presents a study on the regulation of sugar transport in *K. marxianus* cultured in a chemostat. In the steady state, chemostat cultures have constant and well-described growth conditions. Surprisingly, it was found that in the case of growth on nonsugar substrates, glucose and, in some cases, fructose could be observed in the growth medium. It appeared, however, that this residual glucose and fructose was an artifact of the fixation method. When a dialysis probe was used to determine residual substrate concentration (Table 3), no glucose or fructose could be detected. The glucose observed with the fixation of cells in liquid nitrogen might be attributed to the release of sugar from the cell interior as a consequence of cell membrane permeabilization. If all of the cells suffered glucose loss, the internal glucose concentration in ethanol-, glycerol-, and acetate-grown cells can be calculated to be between 0.8 and 2.5 mM. It must be noted that part of the glucose determined under these growth conditions is in fact glucose-6-phosphate. This indicates that at least part of the glucose is derived from an internal pool.

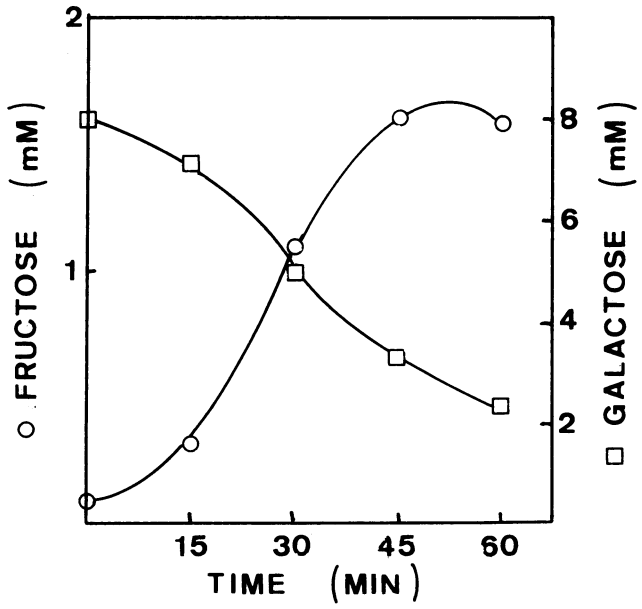

FIG. 1. Concentration of galactose and fructose in a dually (fructose-galactose) limited chemostat culture of *K. marxianus* CBS 6556 growing at a dilution rate of 0.10 h^{-1} after a galactose pulse of 8 mM.

TABLE 8. Comparison of the calculated in situ flux (q_{calc}), based on transport data and residual substrate concentrations, and the observed in vivo (q_{obs}) sugar flux in aerobic chemostat cultures of *K. marxianus* CBS 6556[a]

Carbon source	Flux (μmol · g [dry wt]$^{-1}$ · min^{-1})		q_{calc}/q_{obs}
	q_{calc}	q_{obs}	
Glucose	41	43	1.0
Fructose	46	43	1.1
Sucrose	56[b]	43[c]	1.3
Galactose	16[d]	23	0.7
Sucrose N limitation	175[b]	57[c]	3

[a] The in situ fluxes are expressed as the sum of the individual fluxes calculated from the residual substrate concentrations (Table 2) and the affinities of the individual carriers (Tables 4 and 5). Average q_{calc}/q_{obs} was 1.4.
[b] Sum of glucose and fructose fluxes via their individual carriers.
[c] Expressed as glucose-fructose equivalents.
[d] Based on the determined residual galactose concentration of 300 μM in the fermentor and the assumption that galactose transport characteristics are equal to glucose transport characteristics.

Although the method of cell fixation in liquid nitrogen may introduce artifacts for the solutes that are nonlimiting (Tables 2, 3, and 7), the fixation method in liquid nitrogen gives accurate data for the limiting substrate(s) when *K. marxianus* is grown on sugar substrates (Tables 3 and 7).

Low-affinity glucose and fructose transport. Regulation of glucose and fructose transport was studied by comparing yeasts grown on different carbon sources. It follows from the results given in Tables 4 and 5 that uptake of glucose and fructose can proceed by both low- and high-affinity transport, as was described before (3, 5). In these reports, it was shown that fructose and glucose share the same low-affinity transporter. In contrast, however, to the data of Gasnier (5), Tables 4 and 5 show that the low-affinity system was present under all growth conditions. In light of the wide variety of carbon sources used in this study, it therefore seems likely that, at least in this strain, the low-affinity transport system is constitutively present and is not sensitive to catabolite repression. However, examination of the V_{max} values revealed that the low-affinity system was expressed to different extents when grown in different carbon sources. This finding might indicate variations in the amount of the low-affinity transport protein, although variation of secondary transport effectors, such as membrane composition, or sugar kinases cannot be excluded.

Regulation of H^+-sugar symporters. Competition studies have previously shown (3, 5) that *K. marxianus* can contain separate proton symporters for high-affinity uptake of either

glucose or fructose. The data obtained with nitrogen-limited growth confirm the previous conclusion (3) that down regulation of cotransport can proceed through catabolite repression. This effect is already maximal at a relatively low sugar concentration (15 mM) in the medium. This concentration dependence is similar to the one found for *Candida utilis* (13).

In contrast to down regulation, up regulation seems to be a still rather obscure process. In a previous study, it was suggested that fructose-proton symport would be inducible (3). The inductor of this high-affinity fructose carrier would be fructose at low concentrations (high fructose concentrations result in catabolite repression). Therefore, cells grown in the absence of fructose should have a lower capacity for fructose-proton symport than cells taken from a medium with (low concentrations of) residual fructose. Since glycerol-grown cells have the highest V_{max} and zero residual fructose, it should be concluded that induction by its substrate does not play a role.

Similar reasoning can hold for regulation of the glucose-galactose-specific cotransporter. Again, the capacity of the high-affinity transporter in glycerol-grown cells was at least as high as in glucose-grown cells, even though the glycerol growth medium did not contain glucose. Apparently also the H^+-glucose symporter is not inducible by its natural substrates.

Since regulation of glucose- and fructose-proton symport activity in *K. marxianus* does not proceed through induction by its substrate, the simplest model would assume catabolite repression to be the main regulatory factor. However, the activation of the H^+-sugar symport does not proceed in a similar way for the different cotransport systems. Batch experiments showed that activation of the H^+-fructose symporter, after depletion of glucose in the growth medium, precedes that of the H^+-glucose symporter (3). At the time that this fructose-proton symporter becomes apparent, the culture is growing on its fermentation products, ethanol and acetate. The glucose-proton symporter, however, becomes apparent only when these fermentation products are almost completely consumed. These observations in batch cultures have now been confirmed by using ethanol- and acetate-limited chemostats. Under these conditions, only the fructose-proton symporter was observed (Tables 4 and 5). Furthermore, glycerol is a good activator of the H^+-glucose symporter, whereas ethanol is not (3), which clearly correlates with the observation in carbon-limited chemostats, where glycerol and xylose give the highest capacity of the H^+-glucose symporter.

An interesting aspect is the growth under dual limitation of fructose and galactose. Under these conditions, the residual fructose concentration is increased and correlates well with the observed decrease in transport capacity: $q_{calc}/q_{obs} = 1.2$. The repression effect was, however, rather limited (the fructose concentration increased from 59 to 110 μM [Tables 3 and 7]) and did not seem to be caused by the external galactose concentration, for increasing the dilution rate from 0.10 to 0.20 h^{-1} gave a 50-fold increase in the galactose concentration but only a 2-fold increase in the fructose concentration.

K. marxianus belongs to the group of yeasts capable of growing on polyfructosides. Therefore, this yeast contains the exoenzyme inulinase, which hydrolyzes these extracellular compounds to fructose. As was found in this study for the fructose-proton symporter, regulation of the activity of inulinase proceeds through catabolite repression, fructose does not seem to be an inductor of activity, and activity is high in cells grown on ethanol and glycerol (18). This may indicate that the fructose-proton symporter and inulinase share, to some extent, a common regulatory pathway.

This study confirmed that proton-sugar symport is regulated by carbon catabolite repression. However, the inductive role of low concentrations of their substrates, as was found for glucose-proton symport in *C. utilis* (13), could not be established. Therefore, up regulation should be explained by a different mechanism. Two main possibilities exist. (i) Relief of catabolite repression leads to full expression of proton symporters. Subsequently, an intracellular (allosteric) affector should control the activity of the transporters. (ii) An intracellular inducer is involved in regulating the level of expression of the symporters. At the moment, it is not possible to distinguish between these models. However, it is clear that internal metabolites are of importance in regulating the activities of the fructose- and glucose-proton symporters. Moreover, it appears likely that these substances are different for the two symporters. Further experimentation is needed, however, to determine the nature of these regulating molecules.

LITERATURE CITED

1. **Bruinenberg, P. M., J. P. Van Dijken, and W. A. Scheffers.** 1983. An enzymic analysis of NADPH production and consumption in *Candida utilis*. J. Gen. Microbiol. **129**:965–971.
2. **Busturia, A., and R. Lagunas.** 1986. Catabolite inactivation of the glucose transport system in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **132**:379–385.
3. **De Bruijne, A. W., J. Schuddemat, P. J. A. Van den Broek, and J. Van Steveninck.** 1988. Regulation of sugar transport systems of *Kluyveromyces marxianus*: the role of carbohydrates and their catabolism. Biochim. Biophys. Acta **259**:569–576.
4. **Dickson, R. C., and K. Barr.** 1983. Characterization of lactose transport in *Kluyveromyces lactis*. J. Bacteriol. **154**:1245–1251.
5. **Gasnier, B.** 1987. Characterization of low- and high-affinity glucose transport in the yeast *Kluyveromyces marxianus*. Biochim. Biophys. Acta **903**:425–433.
6. **Gibson, T. D., and J. R. Woodward.** 1988. Continuous, reliable on-line analysis of fermentation media by simple enzymatic/spectrophotometric assays. Anal. Chim. Acta **213**:61–68.
7. **Görts, C. P. M.** 1969. Effect of glucose on the activity and the kinetics of the maltose-uptake system and of α-glucosidase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **184**:299–305.
8. **Höfer, M., and P. C. Misra.** 1978. Evidence for a proton/sugar symport in the yeast *Rhodotorula gracilis* (*glutinis*). Biochem. J. **172**:15–22.
9. **Holzer, H.** 1976. Catabolite inactivation in yeast. Trends Biochem. Sci. **1**:178–181.
10. **Jaspers, H. T. A., and J. Van Steveninck.** 1975. Transport-associated phosphorylation of 2-deoxy-D-glucose in *Saccharomyces fragilis*. Biochim. Biophys. Acta **406**:370–385.
11. **Matern, H., and H. Holzer.** 1977. Catabolite inactivation of the galactose uptake system in yeast. J. Biol. Chem. **252**:6399–6402.
12. **Peinado, J. M., A. Barbero, and N. Van Uden.** 1987. Repression and inactivation by glucose of the maltose transport system of *Candida utilis*. Appl. Microbiol. Biotechnol. **26**:154–157.
13. **Peinado, J. M., P. J. Cameira-Dos-Santos, and M. C. Loureiro-Dias.** 1989. Regulation of glucose transport in *Candida utilis*. J. Gen. Microbiol. **135**:195–201.
14. **Postma, E., W. A. Scheffers, and J. P. Van Dijken.** 1988. Adaptation of the kinetics of glucose transport to environmental conditions in the yeast *Candida utilis* CBS 621: a continuous-culture study. J. Gen. Microbiol. **134**:1109–1116.
15. **Postma, E., W. A. Scheffers, and J. P. Van Dijken.** 1989. Kinetics of growth and glucose transport in glucose-limited chemostat cultures of *Saccharomyces cerevisiae* CBS 8066.

Yeast **5**:159–165.

16. **Romano, A. H.** 1982. Facilitated diffusion of 6-deoxy-D-glucose in bakers' yeast: evidence against phosphorylation-associated transport of glucose. J. Bacteriol. **152**:1295–1297.
17. **Rothstein, A., and J. Van Steveninck.** 1966. Phosphate and carboxyl ligands of the cell membrane in relation to uphill and downhill transport of sugars in the yeast cell. Ann. N.Y. Acad. Sci. **137**:606–623.
18. **Rouwenhorst, R. J., L. E. Visser, A. A. Van der Baan, W. A. Scheffers, and J. P. Van Dijken.** 1988. Production, distribution, and kinetic properties of inulinase in continuous cultures of *Kluyveromyces marxianus* CBS 6556. Appl. Environ. Microbiol. **54**:1131–1137.
19. **Spencer-Martins, I., and N. Van Uden.** 1985. Inactivation of active glucose transport in *Candida wickerhamii* is triggered by exocellular glucose. FEMS Microbiol. Lett. **28**:277–279.
20. **Van den Broek, P. J. A., and J. Van Steveninck.** 1980. Kinetic analysis of simultaneously occurring proton-sorbose symport and passive sorbose transport in *Saccharomyces fragilis*. Biochim. Biophys. Acta **602**:419–432.
21. **Van Urk, H., E. Postma, W. A. Scheffers, and J. P. Van Dijken.** 1989. Glucose transport in Crabtree-positive and Crabtree-negative yeasts. J. Gen. Microbiol. **135**:2399–2406.