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Characterization of low- and high-affinity glucose transports in the yeast *Kluyveromyces marxianus*

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Glucose transport in the yeast *Kluyveromyces marxianus* proceeds by two functionally and presumably structurally distinct transporters depending on the carbon source of the culture medium. In lactose-grown cells, glucose was taken up through a high-affinity H^+-sugar symporter ($K_m = 0.09$ mM), whereas a low-affinity transporter ($K_m = 3.5$ mM) was utilized in glucose-grown cells. The two transporters exhibited different substrate specificities. Galactose was demonstrated to be a selective substrate of the H^+-glucose symporter ($K_m = 0.14$ mM) and did not significantly enter glucose-grown cells. Fructose was a preferential substrate of the low-affinity carrier ($K_m = 3.5$ mM), but it entered lactose-grown cells through a high-affinity H^+-fructose symporter distinct from the H^+-glucose one. Other putative substrates of the two glucose transporters were identified by competition experiments. 2-Deoxyglucose recognized both carriers with a similar affinity, while the non-phosphorylatable analogues 6-deoxyglucose, 3-*O*-methylglucose and D-fucose exhibited a 10–30 fold preference for the high-affinity transporter.

Introduction

Feeding yeast cells with glucose induces the regulation of several enzymes. By contrast with the enzymes of the sugar metabolism, such as fructose 1,6-bisphosphatase and trehalase, for which a molecular mechanism has been proposed [1–3], the regulation of the sugar transport systems by the carbon source has received less attention [4–7].

An initial study of the effects of glucose on the lactose-assimilating yeast, *Kluyveromyces marxianus*, suggested the existence of two functionally distinct glucose transporters, the activity of which depends on the carbon source in the culture medium (Carvalho Silva, M. and Spencer-Martins, I., unpublished data). A characterization of these two glucose transports is presented in this paper.

Recently, evidence for the existence of two glucose transporters in *K. marxianus* has been published, based on the study of 2- and 6-deoxyglucose transport [8].

Materials and Methods

Preparation of cells

K. marxianus var. *marxianus* IGC 2587 was grown for 24 h at 25°C on a mineral medium containing vitamins [9] and glucose or lactose (0.5%, w/v), under mechanical shaking. Cells were harvested in the exponential phase of growth, unless otherwise indicated, centrifuged, washed

Abbreviation: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

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twice with ice-cold distilled water and resuspended in ice-cold distilled water at a final concentration of 40 mg (dry wt.) · ml^{-1}.

Sugar uptake

Cells (10 mg (dry wt.) · ml^{-1}) were preincubated for 10 min at 25 °C in 100 mM Tris-citrate (pH 5.0) (final volume, 0.2 ml). Uptake was initiated by the addition of the labelled substrate under stirring. At the end of incubation, cells were diluted with 5 ml of ice-cold distilled water and immediately filtered through GF/C glass-fiber filters (Whatman). Filters were washed twice with 5 ml ice-cold distilled water and counted in 10 ml 1,4-dioxane/10% (w/v) naphthalene/0.7% (w/v) 2,5-diphenyloxazol (PPO)/0.03% (w/v) 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP). Radioactivity was measured in a Beckman LS 8100 liquid-scintillator counter. Uptakes of [^{3}H]glucose, [^{3}H]galactose and [^{14}C]fructose were linear over the first 20–30 s (not shown). In most experiments, incubation was performed during 15 s. Results were corrected for nonspecific adsorption of the labelled sugar to the filters and/or the cells, determined by diluting the cells with 5 ml ice-cold distilled water before addition of the labelled sugar. This procedure gave the same results as the extrapolation to the time zero of the uptake values during the first 20 s. Inhibition by non-labelled sugars was assayed by adding simultaneously the labelled and non-labelled sugar. Inhibition by CCCP was assayed by preincubating the cells for 10 min at 25 °C with 100 μM CCCP added in ethanol (1%, v/v) or with ethanol alone (control).

The kinetic parameters of the sugar uptake were derived by linear regression of the data in the Eadie-Hofstee representation. In the case of a biphasic transport isotherm, an iterative procedure was applied.

Sugar-induced alkalinization

Proton signals associated with sugar transport were measured with a standard pH meter, PHM 82 (Radiometer, Copenhagen), connected to a Perkin-Elmer R 100 A Recorder. The pH electrode was immersed in a water-jacketed chamber kept at 25 °C and provided with magnetic stirring. To the chamber were added 4 ml of cells (10 mg (dry wt.) · ml^{-1}) in distilled water. The pH was adjusted to 4.8 and a baseline was recorded. When the pH reached 5.0, the sugar was added and the subsequent alkalinization was followed in the recorder. The slope of the initial part of the pH trace was used to calculate the initial H^+ uptake rate. Calibration was performed with known amounts of HCl or NaOH.

Materials

D-[1-^{3}H]Glucose, D-[1-^{3}H]galactose and D-[U-^{14}C]fructose were purchased from Amersham International and were used diluted with non-labelled sugar to a specific activity of 1000–2000 cpm · $nmol^{-1}$. D-Glucose, lactose, methyl α-D-glucoside, L-sorbose and D-xylose were from BDH Chemicals; D-galactose, 2-deoxy-D-galactose, 2-deoxy-D-glucose, 6-deoxy-D-glucose, D-fucose and 3-*O*-methyl-D-glucopyranose from Sigma Chemicals; D-fructose from Merck.

Results

Regulation of glucose transport

D-[^{3}H]Glucose transport in growing cells of *K. marxianus* is catalyzed by a low- or a high-affinity system, depending on the carbon source of the culture medium. With glucose as the carbon source (Fig. 1A), [^{3}H]glucose transport was characterized by a maximal velocity (V_{max}) of 32 nmol · min^{-1} · mg^{-1} (dry wt.) and a Michaelis constant (K_m) of 3.5 mM (Table I). If glucose was replaced by lactose in the culture medium (Fig. 1A, Table I), [^{3}H]glucose (up to 5 mM) was taken up with a much higher affinity (K_m = 0.090 mM) and a lower capacity (V_{max} = 15.5 nmol · min^{-1} · mg^{-1} (dry wt.)). Such a change in affinity was not observed when the cells were grown in a medium containing both glucose and lactose (K_m = 3.8 mM and V_{max} = 22 nmol · min^{-1} · mg^{-1} (dry wt.)).

Addition of 5 mM glucose to a suspension of lactose-grown cells induced a rapid alkalinization of the extracellular medium during the first 20 s, whereas no change of pH in that period time was observed with glucose-grown cells. As shown in Fig. 1B, this glucose-induced alkalinization followed Michaelis-Menten kinetics with respect to glucose concentration, with a K_m of 0.091 mM (Table I). This value is close to the K_m of [^{3}H]glucose transport in the same cells, suggesting that

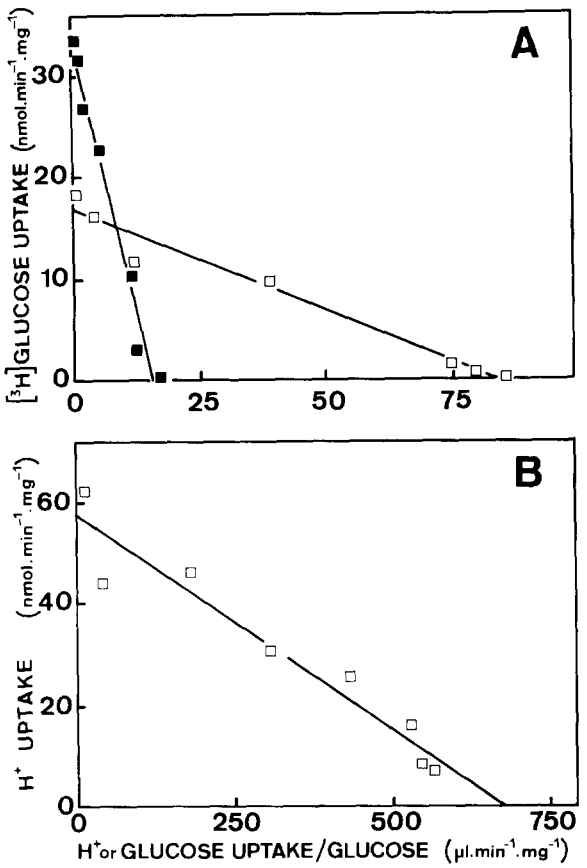

Fig. 1. Glucose transport in *K. marxianus*. This figure shows a typical transport experiment in the Eadie–Hofstee representation. (A) [^{3}H]Glucose transport. Glucose-grown cells [■] at 10 mg (dry wt.)·ml^{-1} in 100 mM Tris-citrate (pH 5.0) were incubated for 15 s with [^{3}H]glucose (final concn., 0.02–50 mM) and assayed for [^{3}H]-glucose transport as described under Materials and Methods. The same experiment was done with lactose-grown cells (□) and 0.002–12.5 mM [^{3}H]glucose. The straight lines were obtained by linear regression, yielding the following kinetic parameters: $K_m = 2.13$ mM and $V_{max} =$ 33.7 nmol·min^{-1}mg^{-1} (dry wt.) for glucose grown cells ($r =$ 0.984); $K_m = 0.178$ mM and $V_{max} = 16.4$ nmol·min^{-1}·mg^{-1} (dry wt.) for lactose-grown cells ($r = 0.939$). (B) Glucose-induced alkalinization. Lactose-grown cells were suspended in water at 10 mg (dry wt)·ml^{-1} and the pH was adjusted at pH 5.0. Addition of glucose (final concn., 0.015–5.0 mM) caused a transient alkalinization of the suspension which was followed by a pH electrode. Linear regression of the data provided and apparent $K_m = 0.0848$ mM and $V_{max} = 57.8$ nmol H$^+$·min^{-1}·mg^{-1} (dry wt.) ($r = 0.963$).

both processes are catalyzed by a H$^+$–glucose symporter. Consistent with this conclusion, the proton ionophore CCCP, at a 100 µM concentration, induced a 73% inhibition of [^{3}H]glucose transport in lactose-grown cells. As a control, only a 29% inhibition occurred with glucose-grown cells. In order to determine the symport stoichiometry, measurement of the V_{max} for glucose-induced alkalinization and [^{3}H]glucose uptake were done in same cell preparations. However, for unknown reasons, results ranged from 1.6 to 4.1 proton per glucose, reflecting a great variation in the V_{max} of the glucose-induced H$^+$ uptake (Table I).

It should be noted that, in several experiments, [^{3}H]glucose above 5–20 mM entered lactose-grown cells through a low-affinity component ($K_m > 5$ mM), in addition of the H$^+$-glucose symport. This low-affinity component was not inhibited by fructose (up to 100 mM), a property which distinguishes it from the low-affinity transport observed in glucose-grown cells (see below). This component, which may have little physiological significance, was not further investigated. Therefore, the term 'low-affinity transporter' in the text will refer only to the glucose transporter present in glucose-grown cells.

Another difference between low- and high-affinity glucose uptakes appears when testing the inhibitory capacity of various sugars (Table II). 6-Deoxyglucose, 3-*O*-methylglucose and particularly galactose and 2-deoxygalactose preferentially inhibited the high-affinity glucose transport compared to the low-affinity one, while the converse was true for fructose. Phlorizin (3 mM), an inhibitor of both Na$^+$-dependent and Na$^+$-independent glucose transport in animal cells [10,11], failed to inhibit the two glucose transports of *K. marxianus* (not shown). The inhibition by the sugars cited above was further characterized, in order to find selective substrates of the two transport systems.

Galactose is a substrate of the glucose high-affinity transporter

As shown in Fig. 2, the galactose inhibition of [^{3}H]glucose uptake in lactose-grown cells is competitive, with an inhibitory constant (K_i) of 0.13 mM ($n = 2$; Table IV). This result suggested that galactose might be a substrate of the glucose high-affinity transporter. Subsequently, D-[^{3}H]galactose uptake was characterized.

The [^{3}H]galactose uptake in lactose-grown cells followed Michaelis-Menten kinetics characterized

TABLE I

KINETIC PARAMETERS OF GLUCOSE, GALACTOSE AND FRUCTOSE TRANSPORT IN *K. MARXIANUS*

Sugar transport and sugar-induced alkalinization were determined as in Fig. 1. No sugar-induced alkalinization was detected in glucose-grown cells. Mean values ± S.E. are listed. *n*, number of experiments.

	Glucose-grown cells			Lactose-grown cells		
	K_m (mM)	V_{max} (nmol·min^{-1}·mg^{-1}(dry wt.))	*n*	K_m (mM)	V_{max} (nmol·min^{-1}·mg^{-1} (dry wt.))	*n*
[^{3}H]Glucose uptake	3.49 ± 0.81	32 ± 13	5	0.090 ± 0.045	15.5 ± 3.4	7
Glucose-induced alkalinization	–	–	–	0.091 ± 0.016	41 ± 19	4
[^{3}H]Galactose uptake	0.137	0.34	1	0.137 ± 0.011	18.6 ± 2.6	7
Galactose-induced alkalinization	–	–	–	0.077 ± 0.008	25.4 ± 9.7	3
[^{14}C]Fructose uptake	3.48 ± 0.50	60 ± 28	3	0.36 ± 0.07	38.1 ± 3.8	3
Fructose-induced alkalinization	–	–	–	0.62 ± 0.05	17.0 ± 1.5	3

by a V_{max} of 19 nmol · min^{-1} · mg^{-1} (dry wt.) and a K_m of 0.14 mM (Table I). As for glucose, galactose elicited a saturable transient alkalinization (Table I) and 100 μM CCCP produced a 74% inhibition of [^{3}H]galactose uptake. It was concluded that galactose enters lactose-grown cells through a H^+–sugar symporter. As presented in Table III, [^{3}H]galactose uptake was inhibited by glucose and 2- or 6-deoxyglucose in a similar way as the uptake of [^{3}H]glucose. The inhibition by glucose was competitive (not shown), with a K_i of 0.083 mM, a value close to the K_m of glucose

TABLE II

INHIBITION OF GLUCOSE TRANSPORT

Transport was measured at pH 5.0 after an incubation of 15 s with [^{3}H]glucose and the inhibitor added simultaneously. Inhibitor concentration was 10 mM. [^{3}H]Glucose was at 0.04 mM (lactose-grown cells) or 0.5 mM (glucose-grown cells). Uptake values in the absence of inhibitor were respectively 3.0 and 2.7 nmol·min^{-1}·mg^{-1} (dry wt.) for lactose- and glucose-grown cells. Results are means of two determinations.

Inhibitor	Glucose-grown cells (%)	Lactose-grown cells (%)
None	100	100
D-Glucose	27.2	0.75
2-Deoxy-D-glucose	25.4	18.9
6-Deoxy-D-glucose	82.4	23.9
3-*O*-Methyl-D-glucose	90.4	21.2
Methyl α-D-glucoside	100	84.0
D-Galactose	81.6	5.9
2-Deoxy-D-galactose	113	14.8
D-Fructose	44.7	78.2
L-Sorbose	117	83.8
D-Xylose	126	91.5

TABLE III

INHIBITION OF GALACTOSE AND FRUCTOSE TRANSPORT

Transport was measured as in Table II with 0.03 mM [^{3}H]galactose or with [^{14}C]fructose at 0.02 mM (lactose-grown cells) or 0.3 mM (glucose-grown cells). Inhibitor concentration was 10 mM for lactose-grown cells and 5 mM for glucose-grown cells. Results are means of two determinations.

Inhibitor	Glucose-grown cells	Lactose-grown cells	
	[^{14}C]-fructose transport (%)	[^{3}H]-galactose transport (%)	[^{14}C]-fructose transport (%)
None	100	100	100
D-Glucose	27.5	1.4	72.9
2-Deoxy-D-glucose	33.4	16.9	100
6-Deoxy-D-glucose	117	12.3	106
D-Galactose	112	2.3	100
D-Fructose	51.6	45.2	4.3

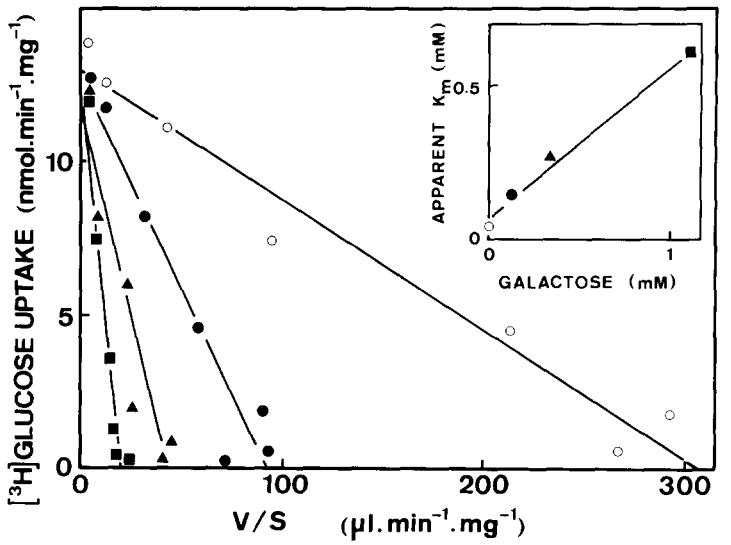

Fig. 2. Inhibition of [^{3}H]glucose transport in lactose-grown cells by galactose. This figure represents a typical inhibition experiment. [^{3}H]Glucose (0.002–4 mM) and galactose at a final concentration of 0 (○), 0.11 (●), 0.34 (▲) or 1.1 mM (■) were added simultaneously to lactose-grown cells (4.6 mg(dry wt.)·ml^{-1}). [^{3}H]Glucose uptake was assayed by filtration after 10 s. K_m and V_{max} in the absence of galactose were respectively 0.042 mM and 13.0 nmol·min^{-1}·mg^{-1} (dry wt.) ($r = 0.980$). Inset: the apparent K_m of [^{3}H]glucose uptake is plotted against the galactose concentration. The straight line obtained has a slope of 0.49 ($r = 0.995$), corresponding to a $K_i = 0.086$ mM for galactose.

uptake (Table I). These results demonstrated that glucose and galactose transport in lactose-grown cells are catalyzed by the same transporter.

Attempts to determine H^+/sugar stoichiometry of the symporter using galactose as substrate were made, but, similarly to glucose, a great variation in the V_{max} of the H^+ uptake was observed (Table I). The ratio of these V_{max} values for galactose and glucose, respectively, suffered a minor variation, but its value, surprisingly, differed from the unity: a mean value of 0.67 ± 0.12 ($n = 3$) was obtained, corresponding to an H^+/galactose stoichiometry ranging from 0.86 to 2.0.

Consistent with the change of galactose sensitivity of glucose transport (Table II), [^{3}H]galactose uptake almost completely disappeared when glucose was substituted for lactose in the culture medium: V_{max} decreased about 50-fold, while K_m remained constant (Table I). This slow [^{3}H]galactose uptake was inhibited 77% by 100 µM CCCP, showing that it represented a residue of the H^+–sugar symport described above. The activity of the symporter in glucose-grown cells was too low to allow the detection of any pH signal after addition of galactose.

Fructose is a substrate of the low-affinity glucose transporter

As mentioned above, glucose transport in glucose-grown cells was galactose-resistant and fructose-sensitive. Characterization of [^{3}H]glucose transport with increasing concentration of fructose showed that the inhibition by fructose was competitive (data not shown), with a mean K_i of 7.9 mM ($n = 2$, Table IV). D-[^{14}C]Fructose transport was further investigated.

[^{14}C]Fructose transport in lactose-grown cells was first examined. This transport was characterized by a V_{max} of 38 nmol · min^{-1} · mg^{-1} (dry wt.) and a K_m of 0.36 mM (Table I). As in the case of glucose and galactose, fructose uptake in these cells was catalyzed by a proton symport since (i) fructose induced a transient alkalinization with an apparent K_m of 0.62 mM (Table I) and (ii) 100 µM CCCP induced a 88% inhibition of [^{14}C]fructose transport. However, [^{14}C]fructose uptake was resistant to 10 mM concentrations of glucose, 2- and 6-deoxyglucose and galactose (Table III). H^+–fructose and H^+–glucose/galactose symports are thus catalyzed by separate carriers. The V_{max} of the fructose-induced H^+ uptake ex-

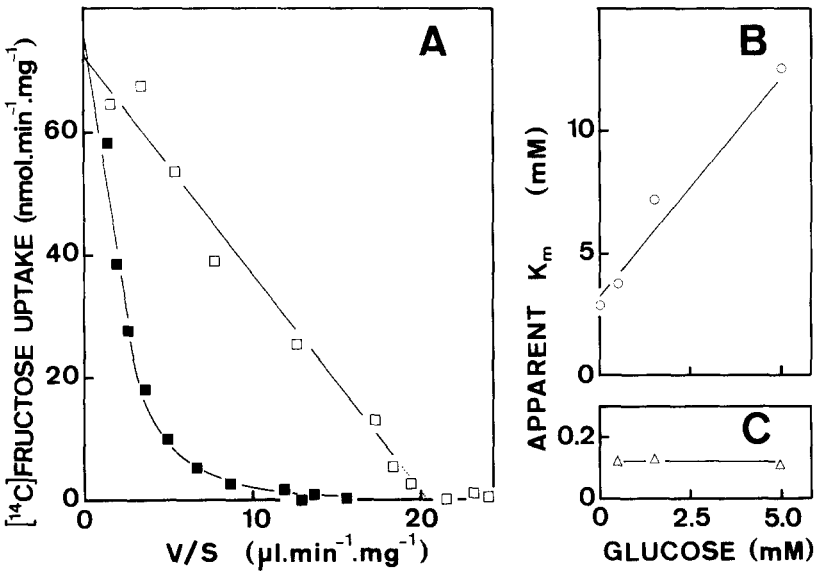

Fig. 3. Inhibition of the [^{14}C]fructose transport in glucose-grown cells by glucose. (A) Glucose-grown cells were harvested in the early stationary phase, preincubated in 100 mM Tris-citrate (pH 5.0) for 10 min at 25 °C and incubated for 15 s with [^{14}C]fructose (0.08–40 mM) in the absence (□) or presence (■) of 5 mM glucose. Linear regression of the data obtained in the absence of glucose provided $K_m = 3.62$ mM and $V_{max} = 72.6$ nmol·min^{-1}·mg^{-1} (dry wt.) ($r = 0.989$; $n = 8$). The curvilinear transport isotherm observed in the presence of 5 mM glucose, interpreted by the existence of two transport systems, was analyzed by an iterative procedure yielding the following kinetic parameters: $K_m = 0.209$ mM and $V_{max} = 2.34$ nmol^{-1}·min^{-1}·mg^{-1} (dry wt.) for the high-affinity component ($r = 0.963$; $n = 7$); $K_m = 17.4$ mM and $V_{max} = 73.0$ nmol·min^{-1}·mg^{-1} (dry wt.) for the low-affinity component ($r = 0.972$; $n = 7$). (B and C) A similar experiment was done with several glucose concentrations. The apparent K_m values of the low- (○) and high-affinity (△) components of the [^{14}C]fructose uptake are plotted against the glucose concentration. The straight line obtained for the low-affinity uptake has a slope of 1.94 ($r = 0.985$), corresponding to a $K_i = 1.65$ mM for glucose.

hibited a much lower variation than that for glucose or galactose (Table I), yielding an apparent stoichiometry of 0.46 ± 0.07 proton per fructose ($n = 3$).

Transport of [^{14}C]fructose in glucose-grown cells was characterized by a V_{max} of 60 ± 28 nmol · min^{-1} · mg^{-1} (dry wt.) ($n = 3$) and a K_m of 3.5 mM (Table I), a value similar to the K_i of fructose for the glucose low-affinity uptake (Table IV). As in the case of glucose, fructose did not elicit pH signals in these cells. The [^{14}C]fructose and [^{3}H]glucose low-affinity transports shared a similar substrate specificity (Tables II and III). The inhibition by glucose of the [^{14}C]fructose uptake was competitive (Fig. 3), with a K_i of 1.9 ± 0.6 mM ($n = 3$). It was concluded that fructose and glucose low-affinity uptake are catalyzed by the same transporter.

Interestingly, the inhibition by glucose revealed, in addition to this low-affinity uptake, a small high-affinity component ($K_m \approx 0.2$ mM), which became more significant when the glucose-grown cells were harvested in the early stationary phase (Fig. 3A). This high-affinity component, normally masked by the low-affinity one (Fig. 3A, control), was glucose-resistant (Fig. 3C) and may correspond to a residue of the H^+-fructose symport observed in lactose-grown cells.

Other sugars

Various other inhibitors of the two glucose transporters were characterized by competition experiments. Their K_i values are listed in Table IV. 6-Deoxyglucose, 3-*O*-methylglucose and D-fucose inhibited the high-affinity transporter more strongly than the low-affinity one, while 2-deoxyglucose had a similar affinity for both carriers. All these compounds were competitive inhibitors and may thus represent substrates of the glucose transporters. In the case of the high-affinity trans-

TABLE IV

INHIBITION CONSTANTS OF GLUCOSE TRANSPORT INHIBITORS

Inhibition constants of the [^{3}H]glucose transport inhibitors were determined as in Fig. 2, in glucose-grown cells (low-affinity transport) or in lactose-grown cells (high-affinity transport). For 2- and 6-deoxyglucose, the glucose high-affinity transporter was followed with [^{3}H]galactose as substrate. *n*, number of experiments; c; competitive; n.d., not determined.

Inhibitor	Glucose low-affinity transport			Glucose high-affinity transport		
	inhibition type	K_i (mM)	*n*	inhibition type	K_i (mM)	*n*
D-Glucose	c	3.49 ± 0.81 [a]	5	c	0.090 ± 0.045 [a]	7
2-Deoxy-D-glucose	c	0.80	1	c	1.5	1
6-Deoxy-D-glucose	c	27	1	c	2.6	1
3-*O*-Methyl-D-glucose	c	18	1	c	2.2	1
Methyl α-D-glucoside	n.d.	n.d.	–	c	40	1
D-Galactose	n.d.	>100	3	c	0.086–0.17	2
D-Fucose	n.d.	>100	1	c	3.9 ±1.6	3
D-Fructose	c	4.18–11.7	2	c	92	1

[a] K_m value from Table I.

porter, this conclusion is supported by the fact that these sugars, at approximately their K_i concentrations, elicited an extracellular alkalinization with lactose-grown cells (not shown).

Discussion

Characterization of two glucose transporters in K. marxianus

A first conclusion of the present work is that glucose can enter *K. marxianus* cells through two distinct transporters: a high-affinity glucose transporter ($K_m = 0.090$ mM) which is a H^+-sugar symporter and a low-affinity transporter ($K_m = 3.5$ mM) the activity of which is not associated with H^+ movement.

A paper reaching a similar conclusion has recently been published, based on the study of the 2- and 6-deoxyglucose transports in another strain of *K. marxianus* var. *marxianus* [8]. 6-Deoxyglucose was taken up through two distinct transport systems, a high-affinity H^+-sugar symporter ($K_m = 0.8$ mM) and a low-affinity transporter ($K_m = 90$ mM), both of which were utilized by 2-deoxyglucose with a same affinity ($K_m = 1.4$ mM). These K_m values agree reasonably with the K_i derived from the present competition experiments (Table IV).

The two glucose transporters exhibited distinct substrate specificities (Table IV; Ref. 8), a result which may reflect a difference in the structure of their active site. In increasing order, 3-*O*-methylglucose, 6-deoxyglucose, glucose, D-fucose and especially galactose exhibited a preference for the high-affinity transporter, while fructose was a better substrate for the low-affinity one. In the case of galactose, the affinities for the two transporters differed by more than 3 orders of magnitude. Therefore, [^{3}H]galactose transport may provide a simple and selective assay of the high-affinity transport in further studies of the adaptation of the transport systems to change in the carbon source. Note that evidence for the use of the galactose transport system by glucose and 6-deoxyglucose has also been presented for *Saccharomyces cerevisiae* [12].

Glucose high-affinity transport may correspond to a H^+-sugar symport, since (i) glucose and galactose elicited an alkalinization of the extracellular medium with an apparent K_m similar to their uptake K_m and (ii) the proton ionophore CCCP (100 μM) inhibited glucose and galactose high-affinity uptake. However, determinations of the stoichiometry of the symport, by comparison of the velocities of sugar uptake and sugar-induced alkalinization, exhibited little reproducibility from one cell preparation to another. The origin of this variation needs further research. By the same pro-

cedure, a H^+/glucose stoichiometry of 0.7 has been published for *K. marxianus* [13], but experiments were performed under conditions in which the two glucose transporters are present [8]. A study with the non-metabolizable substrate, D-fucose, which selectively recognizes the symporter (Table IV), has provided a H^+/fucose stoichiometry of 1 by comparison of the plateau values of D-fucose and H^+ transmembrane electrochemical gradients [14].

The low-affinity glucose transporter is not a H^+–sugar symporter, as indicated by extracellular pH measurement or treatment by 100 μM CCCP. It might proceed by facilitated diffusion or, as proposed by van Steveninck and co-workers, by a transport-associated phosphorylation mechanism [15,16]. The latter hypothesis would give a rationale to the observation that 6-deoxyglucose, a non-phosphorylatable glucose analogue, and 3-*O*-methylglucose, which is only slowly phosphorylated by glucokinase [17], have a lower affinity for this transporter than the phosphorylatable substrates, glucose and 2-deoxyglucose (Table IV and Ref. 8). Note that the lower affinity of 6-deoxyglucose compared to glucose has also been reported in *S. cerevisiae* [12,18] with the exception of one study [19].

Regulation of the glucose transport systems by glucose

Another main conclusion of this work is that the activities of the two glucose transporters are regulated by the carbon source of the culture medium. The H^+–glucose symport, assayed with its selective substrate, galactose, suffered a 50-fold inactivation when glucose was substituted for lactose in the medium (Table I). The low-affinity fructose-sensitive uptake of glucose present in glucose-grown cells was not observed in lactose-grown cells. In addition, the H^+-fructose symport observed in the latter cells was inactivated about 20-fold in glucose-grown cells (Table I and Fig. 3A). These regulations may be attributed to the presence of glucose, not lactose, since cells grown on either glucose and lactose or glucose alone possess a similar transport system.

In Ref. 8, the 6-deoxyglucose high-affinity transport in *K. marxianus* was shown to be subject to regulation, by comparing glucose-grown cells in the early and late stationary phases: this transport was present only in the latter, while the low-affinity one was observed in both cell preparations. The activation of the high-affinity transport during the stationary phase occurred 10 h after glucose was exhausted, an observation that led the authors to discard the disappearance of glucose as the cause of this process. As a matter of fact, the glucose consumption might necessitate a delay to exert its effect.

Glucose might regulate the transporters either at the gene level (glucose repression) or at the gene product level (catabolite inactivation, Ref. 20). The latter mechanism has been demonstrated for the glucose transport systems of *Candida wickerhamii* and *Pichia ohmeri*, where a similar regulation occurs [6,7,21], and for the galactose transporter of *S. cerevisiae* [4]. The mechanism of the regulation of the glucose transporters in *K. marxianus* is presently under investigation.

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