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Effect of xylose incubation on the glucose transport system in *Saccharomyces cerevisiae*

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Incubation of *Saccharomyces cerevisiae* with xylose and ethanol for 16 hours leads to a decrease of hexokinase (and glucokinase) activity in the cells. It does not alter the levels of polyphosphate, orthophosphate and ATP. The transport of the glucose derivative 2-deoxy-D-glucose, a sugar that can be phosphorylated, is inhibited after this treatment, whereas transport of 6-deoxy-D-glucose, which has a blocked phosphorylation site, is not inhibited. Even though, both deoxyglucoses use the same transport system. The decrease in initial velocity of 2-deoxy-D-glucose transport is most pronounced under anaerobic conditions. Incubation of the cells with antimycin A, a treatment which has a similar effect as anaerobiosis, shows, that the inhibition of the transport of 2-deoxy-D-glucose is presumably the result of an increase in the K_m of the carrier transport. Transport of glucose is probably regulated by kinase enzymes.

In some yeasts the transport of glucose and glucose derivatives seems to involve a phosphotransferase system [1–3]. Pulse-labeling experiments, designed to determine the temporal order of appearance of labeled sugar in the intracellular pools, revealed that 2-deoxy-D-glucose (2-dGlc) first appears in the cell in the phosphorylated form [1–3]. This apparent transport coupled phosphorylation can be explained by different models. Based on circumstantial evidence, a transport-associated phosphorylation has been proposed with peripherally localized polyphosphate as the phosphoryl donor [1,4,5]. In other investigations Bisson and Fraenkel demonstrated, that in wild type *Saccharomyces cerevisiae*, glucose uptake has a high-affinity and a low-affinity component. In mutants, lacking both hexokinases and glucokinase, only the low-affinity component was present. These observations suggest the involvement of kinases in transport of glucose in yeast [6–8].

The existence of a low- and high-affinity state of the glucose carrier has been shown also by Serrano and DelaFuente [9]. However, they suggested that the transition between these states might be regulated by an intermediate of sugar metabolism.

In this paper, the role of the kinases in hexose transport was studied by inactivation of the kinases with xylose. Xylose irreversibly inactivates the hexokinases PI and PII [10–13] and the glucokinase [13]. This inactivation occurs in vitro as well as in vivo [10,12–14]. It will be shown, that inactivation of the kinases in vivo results in a decrease in uptake of 2-dGlc, whereas 6-deoxy-D-glucose (6-dGlc) transport is not affected.

Saccharomyces cerevisiae strain Delft I (CBS 1172) was grown with glucose as carbon-source, harvested and washed as described before [15].

For inactivation of the kinases a 10% (wet wt./v) yeast suspension was incubated for 15–16 h in a medium containing 1% (v/v) ethanol and 0.3 M xylose. As a reference, cells were incubated

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with 1% (v/v) ethanol only. The suspensions were incubated on a GFL rotary shaker. Afterwards, the cells were collected by centrifugation and washed three times with ice-cold distilled water [10].

For determination of the hexokinase and glucokinase activity 2 g (wet weight) of yeast was suspended in 18 ml 100 mM Tris-HCl (pH 7.0). After adding 50 g glass beads with a diameter of 0.4–0.5 mm the yeast was broken by shaking for 1 min in a Braun disruptor, cooled with solid CO_2. The glass beads were removed by filtration on a G4 glass filter. After centrifugation for 10 min at $1200 \times g$ the kinase activity was measured in the supernatant according to Bergmeyer et al. [16], with the exception that the ATP concentration was 2.7 mM instead of the described 0.64 mM.

6-dGlc and 2-dGlc transport were measured at 25°C, unless indicated otherwise, using 10% (wet wt./v) yeast suspensions buffered with 0.1 M Tris-maleate at pH 4.5, as described before [5].

Fermentation was measured using the standard Warburg technique.

Polyphosphate and orthophosphate were determined as described previously [17].

ATP was assayed by the method of Addanki et al. [18] utilizing Sigma FLE-50 firefly lantern extract.

2-[2,6-^{3}H]dGlc and [U-^{14}C]xylose were purchased from Amersham International. 6-[^{3}H]dGlc was obtained from New England Nuclear.

As has been shown before [10,13], incubation of *S. cerevisiae* with D-xylose results in inhibition of hexokinases and glucokinase. In the strain used in the present study (CBS 1172) incubation of cells with 300 mM xylose for 15–16 h resulted in a 85% reduction in glucose phosphorylating activity. Also transport of 2-dGlc is reduced in xylose-treated cells. Table I shows the effect of incubating *S. cerevisiae* cells with xylose and ethanol on anaerobic and aerobic 2-dGlc uptake. As can be seen, the initial rate of the anaerobic 2-dGlc uptake is much lower in cells incubated with xylose than in control cells. Under aerobic conditions a much smaller effect is seen.

Since the fermentation rate of glucose in yeast cells becomes limited by the rate of transport [9], measurement of the fermentation rate gives an indication of the transport velocity. The rate of fermentation in xylose-treated cells is about 80% lower than in control cells, again indicating the inactivation of the glucose transport activity by xylose treatment (not shown).

Transport of glucose and 2-dGlc closely involves phosphorylation of the sugar. An explanation for the low uptake in cells with reduced kinase activity, could be a decrease in one of the phosphate pools. Therefore the levels of polyphosphate, orthophosphate and ATP were measured both in treated and in non-treated cells. As shown in Table II, there is no significant difference in phosphate pools of treated and non-treated cells.

To exclude the possibility, that the decreased 2-dGlc and glucose transport was caused by competition of residual xylose for the glucose carrier, control experiments were conducted. Utilizing

TABLE I

THE INFLUENCE OF XYLOSE TREATMENT ON AEROBIC AND ANAEROBIC DEOXYGLUCOSE TRANSPORT

The velocity of dGlc uptake is given in nmol dGlc per gram wet weight of cells per second. V_{xylose} and $V_{control}$ represent influx of dGlc in xylose-treated resp. control cells. For the anaerobic uptake experiments samples were taken at 15, 30, 45, 60 and 75 s, for the aerobic 2-dGlc influx samples were taken at 4, 8, 12, 16, 20 and 24 s, for the aerobic 6-dGlc experiments samples were taken at 15, 30, 45 and 60 s.

Anaerobic		Aerobic		Temp. (°C)	V_{xylose}	$V_{control}$	Inhibition of dGlc transport (%)
2-dGlc	6-dGlc	2-dGlc	6-dGlc				
0.1 mM				25	0.37	1.8	79.5
1 mM				25	1.76	4.4	60
1 mM				10.8	0.85	1.87	54.6
	1 mM			15	0.247	0.28	12
		1 mM		15	14	24	41.7
			1 mM	25	1.57	2.19	28.3

TABLE II

POLYPHOSPHATE-, ORTHOPHOSPHATE- AND ATP POOLS IN XYLOSE-TREATED AND CONTROL CELLS

The results are given in μmol orthophosphate units per gram wet weight of yeast for polyphosphate and orthophosphate. The amount of ATP is given in μmol ATP per gram wet weight of yeast.

Pool	Incubation	
	xylose treated	control
Polyphosphate	12.7 μmol/g	12.3 μmol/g
Orthophosphate	17.5 μmol/g	17.7 μmol/g
ATP	0.295 μmol/g	0.3 μmol/g

[^{14}C]xylose, it was established that after the 16 h incubation period with 300 mM xylose equilibration was reached. During the subsequent washing procedure, the intracellular xylose concentration decreased to about 150 mM. Assuming subsequent equilibration of the xylose during the preincubation before the 2-dGlc uptake measurements, a maximal extracellular concentration of 7.5 mM should be expected. In control experiments it appeared that 2-dGlc transport was not measurably affected by the presence of 15 mM xylose. Thus the decreased 2-dGlc transport in xylose-pretreated cells cannot be caused by xylose competition for the carrier. This is corroborated by experiments, in which the xylose-pretreated cells were washed as described in the methods section, subsequently incubated for 25 min at 25°C in water and washed again three times. Although this will result in much lower intra- and extracellular xylose concentrations during the 2-dGlc uptake measurements, 2-dGlc uptake was unaffected by the extra washing procedure. In fact these results were expected, as the K_m value of xylose for the glucose carrier is in the range of 160–300 mM [9,19].

These results exclude the possibility that transport reduction is caused by circumstantial factors. Therefore it is concluded, that the effect of xylose treatment on glucose and 2-dGlc uptake is linked to the effect of xylose treatment on hexo- and glucokinase activities, thus suggesting a close relation between transport and kinase activity.

6-dGlc can utilize both the glucose carrier and the inducible galactose transporter [7,20]. Under our culture conditions only the constitutive glucose carrier [7,20,21] is present, since 6-dGlc influx was unaffected by the presence of 10 mM galactose. However, 5 mM 2-dGlc inhibited 6-dGlc uptake strongly (> 80%). Therefore it can be concluded, that 2-dGlc and 6-dGlc use the same carrier in this yeast, viz. the glucose permease. Even though xylose treatment does not affect 6-dGlc influx significantly (Table I).

To determine whether xylose treatment affects the affinity or the maximal uptake velocity of 2-dGlc, the kinetic parameters of transport had to be determined. At high 2-dGlc concentrations, accurate measurements of initial transport velocities require a fast-sampling technique. For practical reasons this technique could not be used under anaerobic conditions, as sampling under these conditions has to be done utilizing (relatively slow operating) gass-tight syringes. Therefore anaerobic conditions were mimicked by aerobic conditions in the presence of antimycin A. This drug reduced the O_2 consumption rate about 99%, as judged from measurements of dissolved O_2 using a Clark type electrode system. 2-dGlc transport in cells incubated with 200 μg antimycin A per gram (wet weight) cells, did not differ significantly from transport under anaerobic conditions (Fig. 1). It should be noted, that the aerobic uptake in the same time interval results in 7 μmol 2-dGlc per gram wet weight for the control and 5 μmol 2-dGlc per gram wet weight for the cells incubated with ethanol and xylose.

The velocity of dGlc transport in cells which were incubated with antimycin A is shown in Table III. It should be noted that, again, 6-dGlc uptake is insensitive to xylose treatment. The inhibition of 2-dGlc transport became less at higher 2-dGlc concentrations, suggesting an altered K_m of the glucose transporter in xylose-treated cells. This can also be concluded from the corresponding Eadie-Hofstee plot (Fig. 2). The mean K_m and V_{max} values of different measurements were for control cells a K_m of 0.49 ± 0.009 mM and V_{max} of 19 ± 2 nmol $\cdot s^{-1} \cdot g^{-1}$ and for xylose-treated cells a K_m of 1.61 ± 0.15 mM with V_{max} of 13 ± 1.5 nmol $\cdot s^{-1} \cdot g^{-1}$.

These results show that phenotypic kinaseless cells behave in a similar way as the genetic kinaseless cells of Bisson and Fraenkel [6–8]. They found a low- and high-affinity glucose transport in strains containing at least one of the glucose kinases and

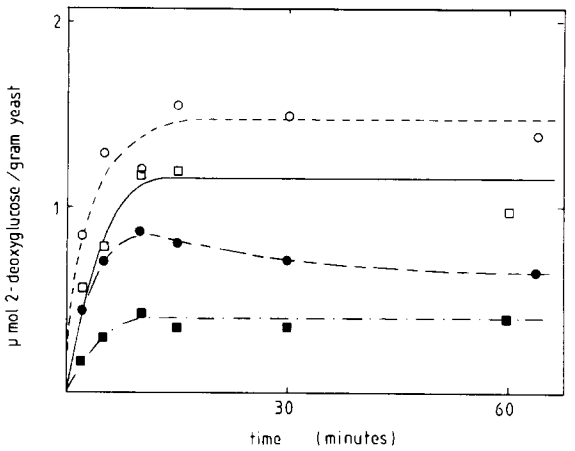

Fig. 1. Comparison between 2-dGlc uptake in cells incubated under anaerobic conditions and cells incubated with antimycin A. Uptake was measured in a 5% wet weight per volume of yeast suspension. Antimycin A (200 μg/g wet weight cells) was added to the cells 1 h before starting transport measurements. Initial 2-dGlc concentration: 1 mM. □, anaerobic 2-dGlc uptake in control cells; ○, 2-dGlc uptake in control cells, incubated with antimycin A; ■, anaerobic 2-dGlc uptake in xylose-treated cells; ●, 2-dGlc uptake in xylose-treated cells, incubated with antimycin A.

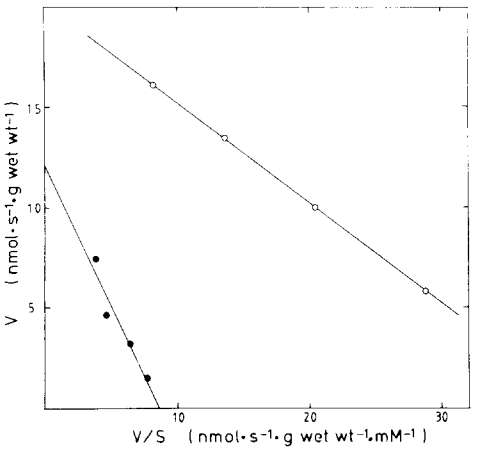

Fig. 2. Eadie-Hofstee plot of 2-dGlc uptake in cells incubated with antimycin A. Antimycin A (200 μg/g wet weight cells) was added to the cells 1 h before starting transport measurements. ○, control cells; ●, xylose-treated cells.

only a low-affinity uptake in yeast strains lacking these enzymes. This suggested that the kinase can alter the apparent K_m of the transporter a result similar to the one presented in Fig. 2. However, an important difference between our data and those of Bisson and Fraenkel is, that the transport of 6-dGlc is not affected by kinase inactivation, although both deoxysugars utilize the same carrier. This seems to indicate, that phenotypic kinase inactivation only influences transport of glucose analogues, that can be phosphorylated.

There is a second difference between the results of Bisson and Fraenkel and our observations. They found a high-affinity and a low-affinity uptake system for both glucose and 6-dGlc [6,7]. In our case only a high-affinity uptake system for 2-dGlc transport is found (Fig. 2), whereas 6-dGlc uptake only shows a low-affinity system. A clear explanation for this discrepancy can not be given as yet. However, it is possible, that differences in growth conditions can lead to the appearance or disappearance of a second transport system (see e.g. Ref. 20).

Serrano and DelaFuente [9] found, that the K_m value of sugars for the carrier is 2–10-fold lower in the presence of fermentation than in its absence. They proposed, that an intermediate of sugar metabolism regulates the carrier. However, it is unlikely that a similar mechanism can explain our results, since 6-dGlc transport is not influenced by xylose treatment. Therefore our results should be explained by another mechanism.

Xylose inactivates the hexokinases and the glucokinase without affecting the phosphate pools

TABLE III

INFLUENCE OF XYLOSE TREATMENT ON DEOXYGLUCOSE TRANSPORT IN CELLS TREATED WITH ANTIMYCIN A

Influx was determined from the linear uptake measured at 5, 10, 15 and 20 s. Antimycin A (200 μg/g wet weight of cells) was added to the cells 1 h before starting transport measurements. The velocity of dGlc uptake is given in nmol dGlc per gram wet weight of cells per second. V_{xylose} and $V_{control}$ represent influx of dGlc in xylose-treated resp. control cells.

Concentration		V_{xylose}	$V_{control}$	Inhibition of dGlc transport (%)
2-dGlc	6-dGlc			
0.2 mM		1.5	5.8	74
0.5 mM		3.2	10.2	69
1 mM		4.6	13.5	66
2 mM		7.5	16.2	54
	1 mM	3.52	3.48	0

(Table II) and reduces the transport of 2-dGlc (Table I). So it is tempting to postulate, that there is a direct coupling between (one of) the kinases and the glucose carrier. Inactivation of the kinases results in an altered transport, by changing the K_m for sugars that can be phosphorylated. So when the sugar phosphorylation capacity is reduced, the apparent affinity of the sugar for the carrier is altered, resulting in a higher K_m. 6-dGlc transport will not be affected by the xylose treatment. This sugar cannot be phosphorylated and already has a low-affinity for the carrier, and thus the K_m will not change on xylose treatment.

Finally, the fact that transport inhibition by xylose-induced kinase inactivation is more pronounced under anaerobic than under aerobic conditions, can be explained by assuming energization of the transport process via the kinase activity. In anaerobic cells the phosphoryl donor cannot be replenished [5], as under aerobic conditions. So, inactivation of the kinases will be very critical for transport activity. As shown in Table I, anaerobic transport is indeed stronger inhibited by xylose pretreatment than aerobic transport.

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