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The influence of ATP on sugar uptake mediated by the constitutive glucose carrier of *Saccharomyces cerevisiae*

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The glucose carrier of *Saccharomyces cerevisiae* transports the phosphorylatable sugars glucose, mannose, fructose and 2-deoxy-D-glucose (2-dGlc) and the non-phosphorylatable sugar 6-deoxy-D-glucose (6-dGlc). Reduction of the ATP concentration by, for example, incubating cells with antimycin A, results in a decrease in uptake of 2-dGlc and fructose. These uptake velocities can be increased again by raising the ATP level. These results establish a role of ATP in sugar transport. Transport of glucose and mannose is less affected by changes in the ATP concentration than 2-dGlc and fructose uptake, while the 6-dGlc transport is independent of the amount of ATP in the cells. Also, reduction of the kinase activity by incubation with xylose diminished transport of 2-dGlc and fructose, while the uptake of glucose and mannose remained unchanged. It is discussed that these results are due to transport-associated phosphorylation with ATP as substrate and the hexokinases and the glucokinase as phosphorylating enzymes.

Introduction

The constitutive glucose carrier of the yeast, *Saccharomyces cerevisiae,* appears to have the characteristics of a phosphotransferase system [1,2]. This can, for example, be concluded from experiments using the phosphorylatable glucose analog 2-dGlc, which reveal that this sugar enters the cell in the 2-dGlc-6-phosphate form [3,4].

The mechanism of this apparent transport-coupled phosphorylation is only partly solved. Recently, it became clear, that the hexokinases, PI and PII, and the glucokinase play an important role in this transport. Kinaseless mutants lack the apparent low K_m uptake component of the glucose and fructose transport [5–7] normally present in wild-type cells, while inactivation of the kinase with xylose diminished the 2-dGlc transport [8].

The two most important hypotheses explaining the coupled phosphorylation are:

(1) The phosphoryl donor is peripherally localized polyphosphate. The sugar and a phosphate group of polyphosphate are bound to the carrier and at the inside of the membrane a carrier-bound kinase forms the sugar phosphate [1,9].

(2) The sugar is transported, and at the inside of the cell sugar phosphate is formed by a kinase at the expense of ATP. This kinase can be bound to the carrier, so that the sugar enters the cell as a sugar phosphate [3], or the kinase occurs in sufficient excess in the cell, so that it phosphorylates the sugar as fast as it enters the cell [4].

In the present paper, the role of ATP on transport was studied by changing the cellular ATP concentration. It will be shown, that transport of 2-dGlc and fructose is diminished by lowering the ATP concentration, whereas transport of glucose and mannose is affected to a lesser extent. Transport of the non-phosphorylatable sugar, 6-dGlc, is independent of the ATP concentration.

Abbreviations: 2-dGlc, 2-deoxy-D-glucose; 6-dGlc, 6-deoxy-D-glucose.

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Materials and Methods

The yeast *Saccharomyces cerevisiae* strain Delft I (CBS 1172) was grown on a synthetic medium with glucose as carbon source, harvested, and washed as described before [10].

Inactivation of the kinases was performed by the xylose treatment of DelaFuente [11], with the modification as described in a previous paper [8].

Sugar transport was measured aerobically at 20 °C (unless indicated otherwise), using 0.1 ml of a 10% (wet weight/volume) yeast suspension buffered with 0.1 M Tris-maleate at pH 4.5 and a tracer amount of (labeled) sugar. After 5 s of incubation, 10 ml of ice-cold water was added and the cells were isolated by filtration on cellulose-nitrate filters (0.45 μm pore, Schleicher and Schuell). After washing the filters with 10 ml ice-cold water, radioactivity was determined by scintillation counting using Picofluor 30 (Packard) scintillation liquid. The blank in each experiment was determined by adding the labeled substrate together with the 0 °C water [5].

Antimycin treatment was done by incubating a 10% (wet weight/volume) yeast suspension in 0.1 M Tris-maleate (pH 4.5) with 200 μg antimycin A per g wet weight of cells for 1 h at 20 °C (unless indicated otherwise).

ATP was assayed by the method of Addanki et al. [12] using Sigma FLE-50 firefly lantern extract.

For determination of the hexokinase- and glucokinase activities, the cells were permeabilized with toluene as described by Serrano et al. [13]. The kinase activity in the permeable cells was measured according to Bergmeyer et al. [14].

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

Results

The constitutive hexose translocator of *S. cerevisiae* appears to catalyze transport of a wide variety of carbohydrates [15–17]. Table I shows that this also holds for the strain used in this study, i.e., *S. cerevisiae* CBS 1172. It can be seen that glucose, fructose, mannose, 2-dGlc and 6-dGlc inhibit each others transport. The K_i values obtained from these results are close to the ones published before [15–17] (K_i(glucose), 1.8 mM; K_i(fructose), 6.7 mM; K_i(mannose), 19 mM; K_i(2-dGlc), 1.4 mM; and K_i(6-dGlc), 50 mM) and, moreover, are similar to the K_m values of transport of the respective sugars [2,16,18].

Even though 2-dGlc and 6-dGlc are transported in these (glucose-grown) cells by the same carrier, a fact that also follows from the mutual competitive inhibition as shown in Fig. 1, the data of Table I indicate that 6-dGlc transport is less sensitive to inhibition by phosphorylatable sugars than 2-dGlc uptake. This is even more apparent when the inhibition is studied in cells reduced in respiratory capacity by the presence of antimycin. Table II reveals that uptake of 6-dGlc is hardly affected by the presence of 5 mM mannose, whereas 2-dGlc influx is strongly inhibited by mannose. Since the K_m of mannose uptake in this

TABLE I

MUTUAL INHIBITION OF GLUCOSE, MANNOSE, FRUCTOSE, 2-dGlc AND 6-dGlc TRANSPORT

The initial uptake rate was measured at 20 °C, using a tracer amount of sugar and 5 mM of the inhibitor except for 6-dGlc of which 200 mM is used for inhibition. Transport is given as a percentage of the uptake rate of the control (without inhibitor).

Inhibitor	Transport (% of control uptake)				
	glucose	fructose	mannose	2-dGlc	6-dGlc
–	100	100	100	100	100
Glucose	–	27	21	22	34
Fructose	56	–	55	53	65
Mannose	79	81	–	68	87
2-dGlc	18	21	19	–	29
6-dGlc	19	22	22	16	–

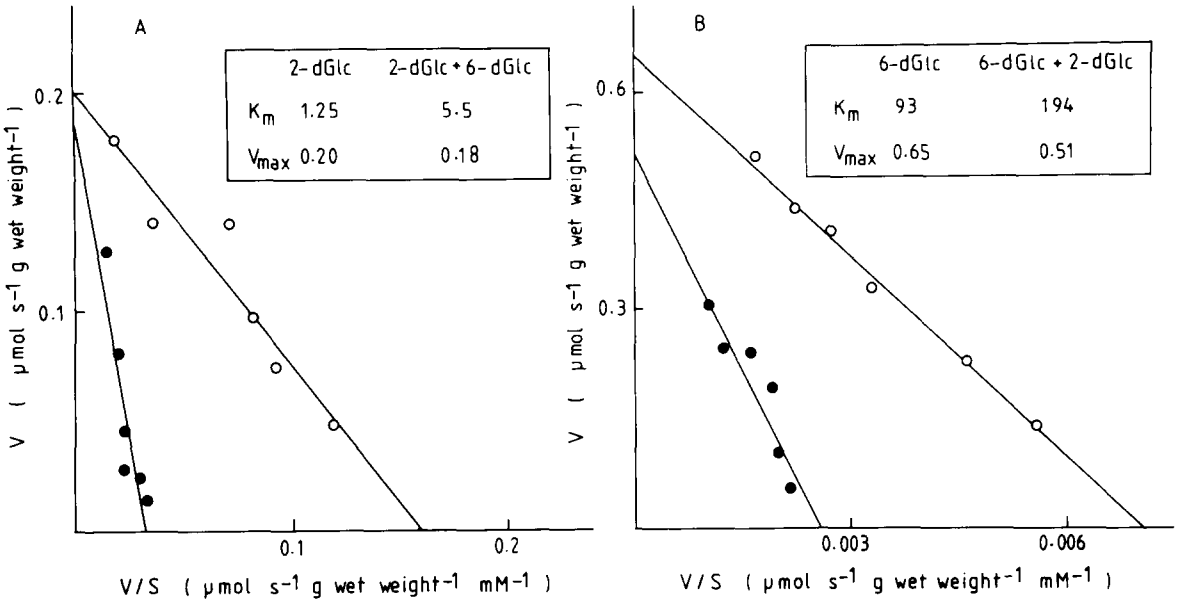

Fig. 1. Eadie-Hofstee plots of the mutual competition between 2-dGlc and 6-dGlc uptake. Influx was determined from the linear uptake measured at 5, 10, 15 and 20 s at 25 °C. (A) ○, 2-dGlc uptake; ●, 2-dGlc uptake in the presence of 200 mM 6-dGlc. (B) ○, 6-dGlc uptake; ●, 6-dGlc uptake in the presence of 4 mM 2-dGlc. The lines are calculated using linear regression.

yeast is 20 mM, competition for the carrier can maximally give a 20% reduction in uptake velocity. Therefore, it should be concluded that the strong inhibition of 2-dGlc transport is caused by a secondary effect of mannose. Since the main difference between 2-dGlc and 6-dGlc uptake is the lack of phosphorylation of the latter, the effect of mannose is expected to be a change in the amount of high-energy phosphate. Fig. 2 shows the cellular ATP concentrations during uptake of 5 mM mannose. The ATP level drops immediately after adding mannose to the cells, whereas after some time the reverse is observed. Table III gives data on the effect of antimycin and mannose

TABLE II

REDUCTION OF THE 2-dGlc AND 6-dGlc UPTAKE RATES IN THE PRESENCE OF 5 mM MANNOSE

The initial influx was measured at 15 °C, taking samples at 10, 20, 30 and 40 s. Uptake was measured under aerobic conditions or in antimycin-treated cells in the presence or absence of 5 mM mannose. The influx was given as percentage of the uninhibited transport.

Conditions	Influx as percentage of the control	
	2-dGlc	6-dGlc
Aerobic conditions	65	88
Aerobic conditions in antimycin-treated cells	20	100

TABLE III

THE EFFECT OF ANTIMYCIN TREATMENT AND MANNOSE INCUBATION ON 2-dGlc TRANSPORT

The uptake velocities were determined at 15 °C taking samples after 10, 20, 30 and 40 s. A tracer amount of 2-dGlc was used.

Conditions	Additions	2-dGlc influx as percentage of the aerobic uptake rate
Aerobic cell suspension	no	100
Aerobic conditions in antimycin-treated cells	no	39
	5 mM mannose together with 2-dGlc	8
	5 mM mannose, 5 min before 2-dGlc	87

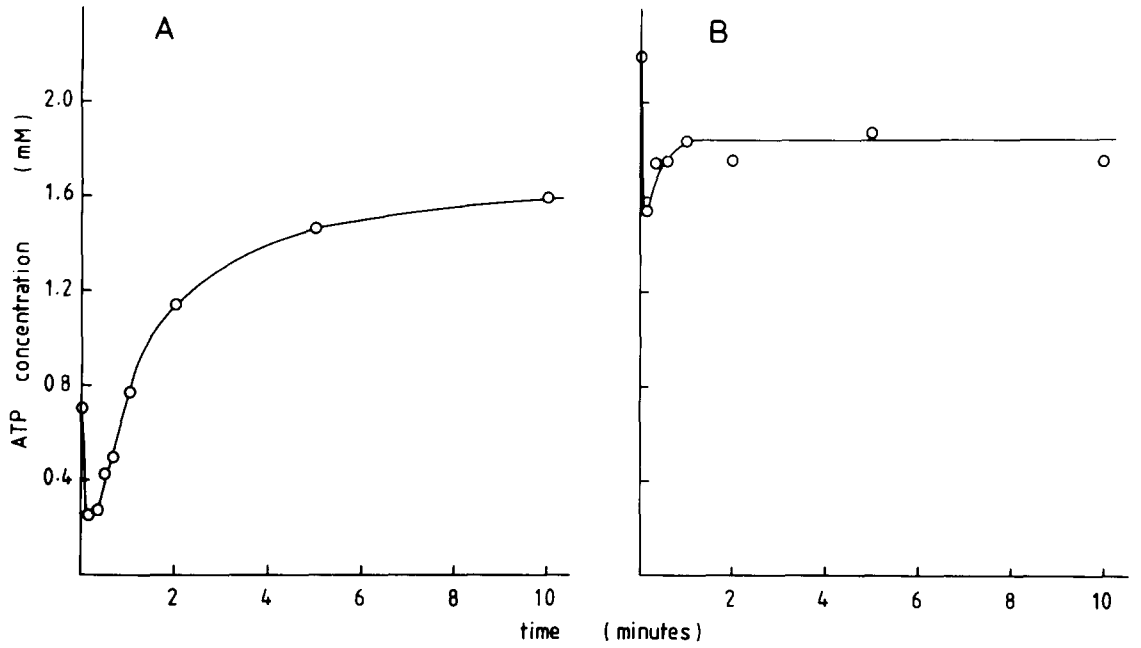

Fig. 2. Changes in the cellular ATP level by incubating a 10% (wet weight/volume) yeast suspension in 0.1 M Tris-maleate (pH 4.5) under aerobic conditions with 5 mM mannose. (A) The cells were treated with antimycin A, before mannose was added ($T = 15\,^{\circ}$C). (B) The mannose incubation was performed at 20 °C in the absence of antimycin A.

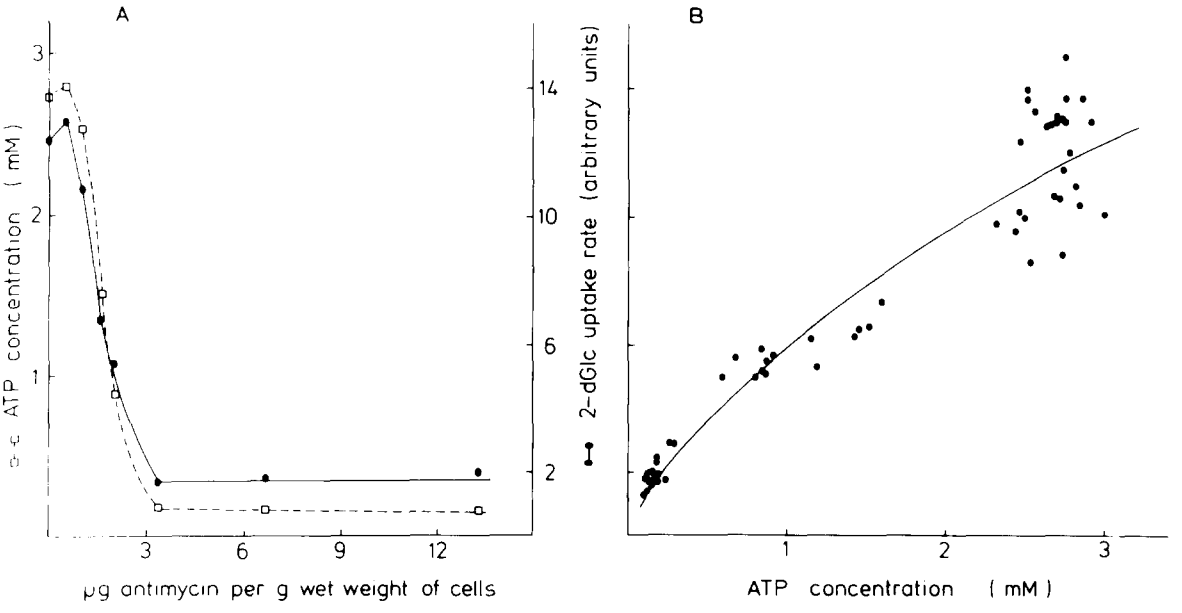

Fig. 3. (A) Relationship between the ATP concentration in the cells and the uptake rate of 2-dGlc. Cells were incubated with different concentrations antimycin A for 10 min at 25 °C, and, subsequently, uptake of a tracer amount of 2-dGlc and the ATP level was determined. (B) The uptake rate of 2-dGlc plotted against the ATP concentration.

TABLE IV

THE EFFECT OF ANTIMYCIN TREATMENT AND MANNOSE INCUBATION ON GLUCOSE, MANNOSE, FRUCTOSE AND 2-dGlc TRANSPORT

The uptake velocities were determined at 20 °C using tracer amount of sugar.

Conditions	Addition	Sugar influx as percentage of the aerobic uptake rate			
		glucose	mannose	fructose	2-dGlc
Aerobic cell suspension	no	100	100	100	100
Aerobic conditions in cells treated with antimycin A	no	81	97	54	16
	5 mM mannose together with the sugar	35	–	6	2
	5 mM mannose, 5 min before the sugar	64	–	52	59

incubation on 2-dGlc uptake. Comparison of Table III with Fig. 2 indicates that 2-dGlc influx varies in a similar way as the cellular ATP level. This suggests a relation between ATP and 2-dGlc transport. Data coming from cells treated with variable amounts of antimycin demonstrate that 2-dGlc influx changes in parallel with the ATP levels. Titrating the yeast cells with antimycin leads to a gradual reduction of the ATP concentration and the 2-dGlc uptake rate (Fig. 3A). The replot of the data as shown in Fig. 3B indicates a role of ATP in 2-dGlc transport.

Another method to vary the amount of ATP in the yeast is to incubate antimycin A-treated cells for 2 min with 12.5 mM tetramethyl-*p*-phenylenediamine and 25 mM ascorbate. This treatment restores the reduction of oxygen [19]. The ATP level increases from 0.17 mM to 2.2 mM, the uptake of a tracer amount of 2-dGlc increases from 0.85 to 7.0 (indicated in arbitrary units). These data confirm the conclusion that ATP is involved in the regulation of 2-dGlc uptake.

As the 6-dGlc transport velocity is not changed by the incubation with antimycin A, the regulation of transport by ATP is expected to be characteristic for phosphorylatable sugars. This was tested by measuring the effect of changing the ATP concentration on transport of phosphorylatable sugars. Table IV shows that fructose and 2-dGlc uptake are strongly influenced, whereas glucose and mannose uptake are less affected by manipulation of the ATP content through antimycin or mannose incubation. Even though transport of all these phosphorylatable sugars apparently depends on ATP, the sensitivity towards ATP can be different.

In a previous publication, it was shown that 2-dGlc uptake, in contrast to 6-dGlc transport, depends on active sugar kinases [8]. Incubating the yeast with xylose reduces the kinase activity [8,11] as well as the 2-dGlc uptake rate. Xylose pretreatment, however, does not lead to inactivation of the transport of all phosphorylatable sugars. Table V indicates that the glucose and mannose uptake velocities are not reduced, whereas fructose and 2-dGlc influx are diminished by a decrease of kinase activity. This shows that glucose and man-

TABLE V

EFFECT OF REDUCING THE KINASE ACTIVITY OF THE CELLS WITH XYLOSE ON SUGAR UPTAKE

The initial uptake rate is determined in antimycin-treated cells pretreated with xylose and ethanol or only ethanol (control cells). The sugars were used in tracer concentrations. The influx is given as the ratio between the initial transport rates in the xylose-treated cells (V_{xylose}) and the control cells ($V_{control}$).

Sugar	$V_{xylose}/V_{control}$
Glucose	1.04
Fructose	0.51
Mannose	1.12
2-dGlc	0.24

nose transport not only differ from fructose and 2-dGlc uptake by their sensitivity towards ATP, but also in their sensitivity towards kinase activities.

Discussion

Glucose, fructose, mannose, and the glucose analogues 2-dGlc and 6-dGlc are transported through the same carrier in *S. cerevisiae* CBS 1172 (see also Refs. 15–17). It has been suggested before that this constitutive transport system can catalyze transport associated phosphorylation with polyphosphates as primary phosphoryl donors [1,2]. The results presented in this paper, however, establish a role of ATP in sugar transport. This suggests that ATP either regulates transport by acting as an (allosteric) modulator of transport, or by being directly involved in phosphorylation of the sugar.

Regulatory properties of ATP on substrate affinities and catalytic reactivities of transport systems have been described for, e.g., the glucose carrier of erythrocytes [20]. The fact, however, that 2-dGlc and fructose transport are more sensitive to ATP than glucose or mannose uptake, makes the possibility of ATP as an allosteric modulator of sugar transport in *S. cerevisiae* less likely. Therefore, it is probable that the role of ATP in transport is caused by a direct involvement of ATP in transport-associated phosphorylation. In this model, the intracellular kinases are supposed to be the phosphorylating enzymes.

One of the main conclusions from this study is that glucose and mannose uptake are less ATP sensitive than 2-dGlc and fructose transport. This can be explained by the differences in the affinities of the hexokinases and the glucokinase for these sugars and for ATP [21–23]. Compared with the hexokinases, the glucokinase has the highest affinity for glucose and mannose, whereas the hexokinases have a higher affinity for 2-dGlc and fructose than the glucokinase [21–23]. Since transport was measured in this study at a very low sugar concentration, it can be expected that glucose and mannose are phosphorylated by the glucokinase and fructose and 2-dGlc by the hexokinases. The fact that the glucokinase has a higher affinity for ATP than the hexokinases [21,22] may explain the differences in ATP sensitivity.

Support for this model comes from the studies with xylose-treated cells. Xylose treatment causes inactivation of the kinases [23]. However, the inactivation of the glucokinase is prevented by high ATP levels in the cell. Thus, xylose treatment will mainly affect the hexokinases when cells are also supplemented with a metabolizable substrate (see also Ref. 24). Xylose incubation as described in this paper was carried out in the presence of ethanol. This resulted in a decrease of glucose phosphorylation in permeabilized cells of 82% and 51% when assayed with 2.7 mM and 27 μM ATP, respectively. This indicates that indeed the glucokinase, having a low K_m for ATP [21,22], was relatively unaffected by xylose preincubation. Concomitantly, glucose and mannose uptake (measured at low concentrations) were not influenced by xylose treatment, contrasting the effect on 2-dGlc and fructose transport.

The conclusion of differential use of sugar kinases in transport-associated phosphorylation is confirmed by data on 2-dGlc transport in a hexokinase-less mutant [21]. It was observed that this mutant did not have any significant uptake of 2-dGlc, again indicating that 2-dGlc uptake depends on the presence of active hexokinase(s) and not on the glucokinase. Finally, the fact that hexokinase-less mutants can grow on glucose as well as on mannose [25] suggests that transport of these sugars is not strictly dependent on the activity of the hexokinases, and is consistent with the view that uptake of these carbohydrates mainly depends on the glucokinase.

The data presented here suggest a model in which transport-associated phosphorylation utilizes the hexokinases as well as the glucokinase, whereas uptake of non-phosphorylatable sugars is not regulated by the kinases. The uptake of 2-dGlc and fructose is mainly controlled by the hexokinases. Glucose and mannose will preferentially be phosphorylated by the glucokinase. As both glucose and mannose have similar affinities for the hexokinases as 2-dGlc [21,22], it seems possible that glucose and mannose transport can also be controlled by the hexokinases, when the glucokinase is not sufficient (e.g., at high sugar concentrations). These results resemble those of

Bisson and Fraenkel [5], who showed that high-affinity fructose transport needs active hexokinases, whereas glucose uptake still proceeds with a high affinity when only the glucokinase is present. The mechanism by which the kinases are involved in transport-associated phosphorylation is, as yet, not clear. Even though a direct association between carrier and kinase(s) has been suggested [3,5], no direct evidence is present for the existence of such multi-enzyme complexes. Further experimentation is needed to clarify the relation between the carrier and the kinases.

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