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ACTIVE AND PASSIVE GALACTOSE TRANSPORT IN YEAST

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SUMMARY

Galactose transport in *Saccharomyces cerevisiae*, strain Hansen C.B.S. 1172, was studied both in induced and in uninduced yeast.

1. It appeared that transport in uninduced cells is a passive, carrier-mediated, facilitated diffusion, whereas transport in induced cells is consistent with metabolically linked active transport, as characterized in a previous paper.

2. In addition to the differences between these two modes of transport discussed before, another distinction was found: active transport is highly pH-dependent, whereas passive transport is not.

3. Further it could be shown that transport in induced and in uninduced cells is accomplished *via* the same carrier. It is concluded that during adaptation to galactose a "permease" is induced, catalyzing the binding of galactose to the carrier and changing the carrier-mediated, facilitated diffusion into an active, permease carrier-mediated transport.

INTRODUCTION

From kinetic studies it has been concluded that hexose uptake in yeast is a carrier-mediated process[1,2], just as in a variety of other cells and tissues[3,4]. In previous papers[5–7] evidence has been presented indicating the existence of two different sugar transport mechanisms in yeast: a metabolically linked, active transport and a passive, carrier-mediated, facilitated diffusion. Some differences between these two transport systems were discussed.

Normally *Saccharomyces cerevisiae*, strain Hansen C.B.S. 1172, cannot metabolize galactose. The enzymes necessary for galactose metabolism can be induced, however. In another yeast strain it has been demonstrated that also a specific transport system for galactose can be induced[8,9]. The synthesis of the metabolic enzymes and of the transport system are under the control of different genes in this yeast strain.

Preliminary experiments with strain Hansen C.B.S. 1172 indicated that the transport system for galactose is also changed in this yeast, after adaptation[5]. In the present study these changes in the transport mechanism were examined in more detail in order to establish more firmly the differences between active and passive transport as characterized before[5,6]. Special attention was paid to the problem of whether both galactose transport mechanisms proceed *via* the same, or *via* different carriers.

METHODS

The yeast was grown on the liquid medium described previously[10]. The cultured yeast was starved aerobically overnight in distilled water and subsequently washed 3 times in about 30 vol. of distilled water. Adaptation to galactose was brought about as described before[5].

The experiments were performed in a buffered medium. In the pH range from 3.0 to 6.5 triethylamine–succinate–tartrate buffer was used[11]. In the pH range from 6.0 to 9.0 the suspensions were buffered with 0.025 M Tris–0.020 M succinic acid, adjusted to the desired pH with triethylamine.

Uptake of fermentable sugars was determined either directly by measuring the disappearance of the sugar from the medium, or indirectly by measuring fermentation rate, using standard Warburg technique. This indirect method may be used as a criterion for transmembrane transport, as discussed previously[6]. Experimental results were identical with both methods. Sorbose transport and uptake of galactose in uninduced or iodoacetate-poisoned cells was measured according to the procedure described by CIRILLO[12]. Intracellular sugar concentrations were calculated as indicated in that paper.

In a few experiments ^{14}C-labelled galactose was used and measured in a liquid scintillation counter, with the liquid scintillator described by BRAY[13].

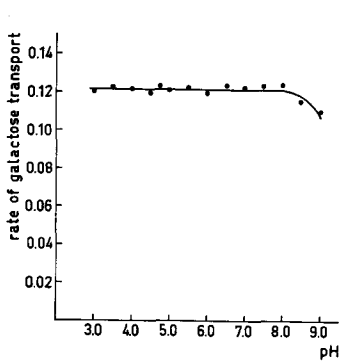
The analytical methods utilized were: for glucose, the glucose oxidase method, as modified by WASHKO AND RICE[14]; for galactose, the galactostat reagent (Worthington Biochemical Corporation) and the method of NELSON[15]; for sorbose, the method of DISCHE AND DEVI[16].

RESULTS

Effect of pH on the rate of galactose transport

The velocity of galactose transport in uninduced cells appears to be pH-independent, over a large pH range (Fig. 1). Only at extreme pH values does the rate of transport decrease slightly. The pH–activity curve of galactose uptake in induced cells is shown in Fig. 2. The same type of biphasic curve was found for the fermentation of glucose, as published before by ROTHSTEIN[17,18]. Further experiments revealed that a biphasic pH–activity curve is found for the uptake of all fermentable sugars tested so far. The location of the peaks is somewhat different for different substrates and, moreover, depends on the yeast strain. For instance, ROTHSTEIN found two maxima for glucose fermentation at pH 4.5 and 8.0; in the yeast strain used in the present experiments these glucose peaks were found at pH 4.3 and 5.5 and in still another yeast strain (Hansen, NCYC 240) at 4.5 and 6.5. Maltose fermentation in strain Hansen, CBS 1172, shows pH optima at 4.7 and 6.3. Transport of sorbose on the other hand shows the same pH-independence as galactose transport in uninduced cells (see Fig. 1).

ROTHSTEIN has shown that if K^+ is added to the yeast suspension, the peaks and valleys of the pH–activity curve are obliterated and fermentation proceeds with the same velocity over the pH range from 3.0 to 8.0 (refs. 11, 17). The same phenomenon was found for galactose transport in induced cells (Fig. 2), whereas galactose transport in uninduced cells was not influenced at all by K^+.



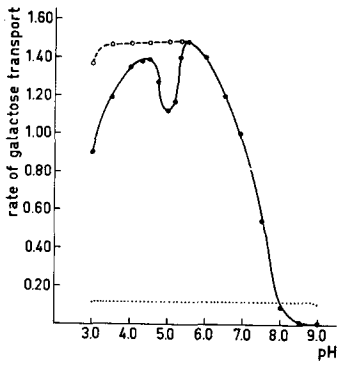
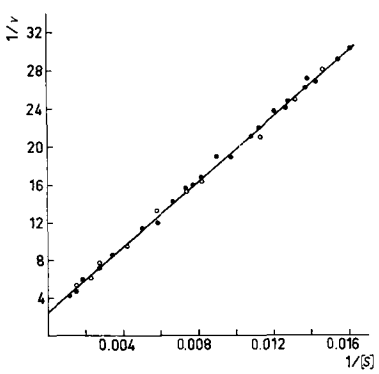


Fig. 1. The influence of pH on galactose transport in uninduced cells, at 25°. The yeast concentration was 2% wet weight and the galactose concentration 300 mM. The velocity of galactose transport is expressed in mmoles/g yeast per h, as calculated from the galactose influx during the first 10 min.

Fig. 2. The influence of pH on galactose transport in induced cells, at 25°, in the absence (●—●) and in the presence (○---○) of 0.1 M KCl. Galactose concentration: 300 mM; yeast concentration: 0.25%. The velocity of galactose transport is expressed in mmoles/g yeast per h. The dotted line indicates the velocity of galactose transport in uninduced cells, under the same experimental conditions.

Kinetics of galactose transport

Kinetic data of sugar transport by yeast fit the Michaelis–Menten equation for enzyme kinetics[1,5,6,19]. Plots of $1/v$ against $1/[S]$ reveal major differences between transmembrane galactose transport into induced and uninduced cells (Figs. 3 and 4). As expected from the experiments on pH influence on galactose transport discussed above, the kinetics of galactose transport in induced cells were affected by the pH of the medium, whereas the transport parameters of uninduced transport were not. In induced cells V appears to be pH-dependent, whereas K_m is not. Mean values (± standard error) for the four series of experiments (each carried out in 5-fold) were: for



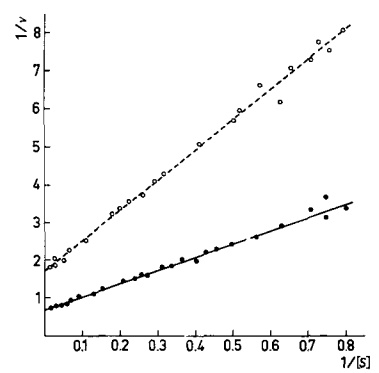


Fig. 3. Kinetics of galactose transport in uninduced yeast cells, at 25°, pH 4.5 (●—●) and pH 7.5 (○—○). The substrate concentration is expressed in mM, the rate of uptake in mmoles/g yeast per h.

Fig. 4. Kinetics of galactose transport in induced cells at 25°, pH 4.5 (●—●) and pH 7.5 (○---○). The substrate concentration is expressed in mM, the rate of transport in mmoles/g yeast per h.

uninduced transport at pH 4.5 and 7.5: $V = 0.383$ ($\pm$ 0.045) mmole/g yeast per h; $K_m = 653$ ($\pm$ 59) mM. For induced transport at pH 4.5: $V = 1.41$ ($\pm$ 0.10) mmoles/g yeast per h; $K_m = 4.9$ ($\pm$ 0.5) mM. For induced transport at pH 7.5: $V = 0.58$ ($\pm$ 0.06) mmole/g yeast per h; $K_m = 4.7$ ($\pm$ 0.4) mM.

Influence of temperature

Calculation of the energy of activation of transmembrane galactose transport according to the Arrhenius equation:

$$\ln v = K - \frac{E}{RT}$$

where v = rate of galactose transport, K = a constant, E = the energy of activation, T = the absolute temperature and R = the gas law constant, revealed that E varies slightly with temperature. To compare transport in induced and in uninduced cells, E was calculated in each case from transport rate data at 20° and 25°. It appeared that the energy of activation is not influenced by pH. A value of about 15000 cal/mole was found for both induced and uninduced transport.

Effects of Ni^{2+} and iodoacetate

The influx of galactose in uninduced cells is not inhibited by nickelous ions, nor by iodoacetate at a concentration of 1 mM. The influx in induced cells however is inhibited 76 % by $5 \cdot 10^{-5}$ M $NiSO_4$, at 25°, pH 4.5. Kinetic analysis demonstrates a decrease in V with no concomitant change in K_m. The influence of 1 mM iodoacetate on galactose transport in induced cells is shown in Fig. 5. V is reduced from 1.40 mmoles/g per h in normal induced cells to 0.47 mmole/g per h in iodoacetate-poisoned cells, whereas K_m increases from 4.8 mM to 290 mM.

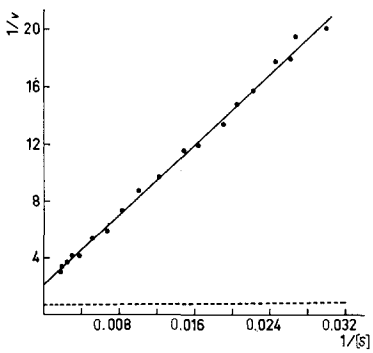


Fig. 5. Kinetics of galactose transport in induced, iodoacetate-poisoned yeast, at 25°, pH 4.5. The dotted line represents the kinetics of galactose transport in unpoisoned induced cells, under the same experimental conditions.

Transport at pH 8.5

Some additional experiments were performed to evaluate the transport characteristics in induced cells at pH 8.5. The extremely low rate of fermentation may be caused either by a very low rate of transmembrane transport or by inhibition of intracellular metabolic breakdown of the sugar at this rather high pH value. In the case of inhibition of metabolism, transmembrane sugar transport would no longer be

the rate-limiting factor of fermentation[18,19] and an intracellular accumulation of free sugar or metabolites should be expected.

Only traces of free sugar could be found inside the cells under these circumstances, with intracellular concentrations considerably lower than the medium concentrations (Table I), and reaching a maximum value within 10 min. Actually the intracellular concentrations of free sugar reached a much higher value both during galactose transport in uninduced cells at the same pH value, and during galactose transport in induced, iodoacetate-poisoned cells at pH 4.5 (Table I). With ^{14}C-labelled galactose no intracellular ^{14}C accumulation could be detected exceeding the values found in

TABLE I

TRANSPORT RATES OF GLUCOSE AND GALACTOSE IN YEAST, UNDER VARYING CONDITIONS

Sugar concentration in the medium: 300 mM; temperature: 25°. Total transport rates were calculated from initial transport rates, as deduced from fermentation velocities and/or intracellular sugar accumulation.

Substrate	*Conditions*	*Fermentation (mmoles/g yeast per h)*	*Intracellular concentration (mM) after 10 min incubation*	*Total transport rate (mmoles/g yeast per h)*
Galactose	Uninduced, pH 4.5	0.00	46.10	0.13
	Uninduced, pH 8.5	0.00	39.03	0.11
	Induced, pH 4.5	1.40	0.00	1.40
	Induced, pH 4.5, iodoacetate-poisoning	0.00	84.72	0.24
	Induced, pH 8.5	0.02	1.20	0.02
Glucose	pH 4.5	1.83	0.00	1.83
	pH 4.5, iodoacetate-poisoning	0.00	28.41	0.08
	pH 8.5	0.02	0.39	0.02

TABLE II

COMPETITION BETWEEN PAIRS OF SUGARS FOR THE HEXOSE TRANSPORT SYSTEM

The experiments were performed at 25°, pH 4.5. At pH 8.5 identical results were obtained. The substrate concentration was 400 mM. The inhibition data are mean values of at least 4 determinations.

Substrate	*Yeast, galactose induced:*	*Inhibitor*	*Concentration of inhibitor (mM)*	*% Inhibition of substrate transport*
Sorbose	—	Glucose	20	73.2
	—	Glucose	80	91.2
Galactose	—	Glucose	20	74.2
	—	Glucose	80	93.8
Sorbose	—	Galactose	20	< 1.0
	—	Galactose	80	< 1.0
	+	Glucose	20	73.1
	+	Glucose	80	93.2
	+	Galactose	20	42.6
	+	Galactose	80	62.8

yeast fermenting at pH 4.5. This rules out the possibility of an appreciable accumulation of intermediate metabolites of fermentation.

Similar results were obtained with glucose, as shown in Table I, where the transport and fermentation rates and the intracellular glucose concentrations are given for normal yeast at pH 4.5 and pH 8.5 and for iodoacetate-poisoned yeast at pH 4.5.

Competition and countertransport studies

In uninduced cells sorbose and galactose uptake are strongly inhibited by glucose, whereas galactose has little or no influence on sorbose transport. In induced cells both glucose and galactose strongly inhibit sorbose transport (Table II). This inhibition is not counteracted by extreme pH values: both glucose and galactose inhibit sorbose transport in galactose-induced cells at pH 8.5 to the same extent as at pH 4.5.

In uninduced cells glucose effects a rapid and extensive countertransport of sorbose and galactose, against a large concentration gradient (Fig. 6). Galactose does not evoke any appreciable countertransport of sorbose under these circumstances. In induced cells on the other hand, both glucose and galactose cause sorbose countertransport (Fig. 7).

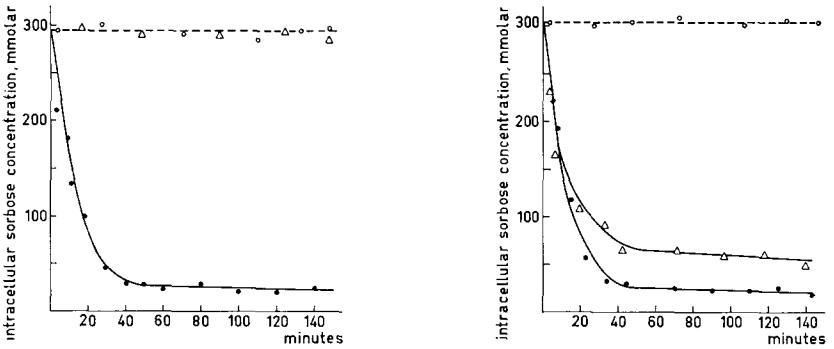


Fig. 6. Sorbose countertransport in uninduced cells; effect of glucose and galactose. The yeast was equilibrated with 300 mM sorbose by incubating 2 h at 25°. At zero time 300 mM glucose or galactose was added. At regular intervals the intracellular sorbose concentration was determined. ○– – –○, control; ●—●, glucose added; △– – –△, galactose added.

Fig. 7. Sorbose countertransport in induced cells, caused by glucose and galactose. For experimental conditions and symbols see the legend to Fig. 6.

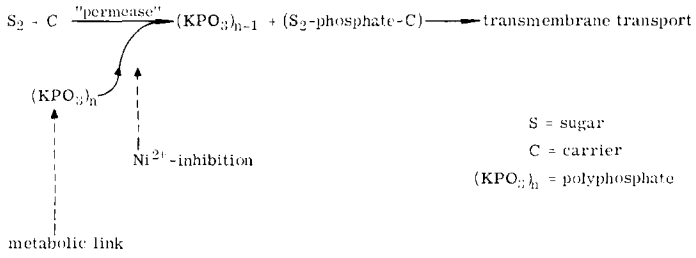
DISCUSSION

Experimental evidence for the existence of two hexose transport systems in yeast has been presented previously[5–7]. The fundamental differences between these two carrier-mediated systems were discussed and shown to be consistent with a transport model that can be summarized as follows:

1. Facilitated diffusion:

$$S_1 + C \rightleftharpoons (S_1C) \longrightarrow \text{transmembrane transport}$$

2. Active transport:



Sorbose is transported into the cell *via* the facilitated diffusion pathway, as is glucose in iodoacetate-poisoned yeast. In normal cells glucose, fructose and some other metabolizable sugars are taken up with the active transport system[5,6,10].

Galactose transport in CBS 1172 appeared to be an interesting topic in this respect. Normally galactose is a non-metabolizable sugar for this yeast strain. After appropriate induction however, this yeast can ferment galactose. As shown in this paper, uninduced galactose transport is not inhibited by nickelous ions nor by iodoacetate. This is in accordance with transport *via* facilitated diffusion[5]. After induction galactose transport is highly sensitive both to Ni^{2+} and to iodoacetate, as is characteristic for active transport. Ni^{2+} decreases V without affecting K_m (similar to the Ni^{2+} influence on active glucose transport[6]), whereas iodoacetate-poisoned yeast cells show a decreased V together with an increased K_m. Theoretically it could be expected that the K_m value in iodoacetate-poisoned yeast would equal the K_m of uninduced transport. As pointed out previously[5] however, some active transport is still observed in iodoacetate-poisoned cells, during the first seconds. Correction for this rapid transport component cannot be exact and may cause the observed discrepancy between the K_m values in uninduced and in iodoacetate-poisoned induced cells. The shift of K_m from 4.8 to 290 mM in induced cells, together with the absence of any iodoacetate influence on uninduced transport is, in any case, amply sufficient to demonstrate the difference between active and passive transport.

The major shift of transport parameters after induction evokes the question of whether the transport system in induced yeast is completely different from the carrier-mediated, facilitated diffusion in uninduced cells, referring to the more general discussion of whether separate carriers are involved in active and non-active transport[20]. The competition and countertransport studies described in this paper reveal that, both in induced and in uninduced yeast, glucose, sorbose and galactose share a common carrier, according to the generally accepted criteria[4,20–22]. Therefore it must be concluded that the galactose carrier in induced cells is identical with the galactose carrier in uninduced cells. The most obvious interpretation of the experimental results is that, during adaptation, the synthesis of an enzyme ("permease") is induced, catalyzing the binding of galactose to the carrier, according to the transport model suggested before[5,6], and resembling the KEPES permease–carrier model of transmembrane transport[23,24].

The influence of pH on sugar uptake can presumably be considered as an additional feature which differentiates active and passive transport in yeast. Sorbose and galactose transport in uninduced cells are pH-independent. Transport of galactose

in induced cells, as well as uptake of other actively transported sugars, show a typical biphasic activity curve. This pH influence is abolished by K^+ in the acid pH range, as was demonstrated before in the case of glucose transport[11,17]. This K^+ influence makes it doubtful whether the pH curve should be interpreted as a normal enzyme (permease) pH–activity curve. A possible explanation of at least part of the pH influence is that H^+ inhibits active transport in the same way as *e.g.* Ni^{2+} and UO_2^{2+} (refs. 6, 10), by changing the conformation of polyphosphate chains involved in active transport. In this way competitive expulsion of H^+ by K^+ would abolish the pH effects on transport, just as Co^{2+} counteracts the Ni^{2+} inhibition. In accordance with this hypothesis, many other cations (*e.g.* Co^{2+}, Mg^{2+} and Mn^{2+}) can abolish the pH effect in the acid range, as was found in preliminary experiments.

The experiments at pH 8.5 reveal that active transport of both glucose and galactose is very slow in the alkaline region. An additional inhibition of intracellular metabolism could not be ruled out, in view of the small accumulation of free sugar in the cells. A considerable inhibition of metabolism should not be expected however, as the intracellular pH change caused by varying the pH of the medium shows a pattern distinctly different from the observed transport inhibition[25].

The actual velocity of active glucose and galactose transport at pH 8.5 is much lower than the passive transport rate *via* the same carrier. Apparently the passive transport (under these circumstances potentially faster) is inhibited strongly by the slow, active transport. This reflects the unchanged ratio of K_m values for active and passive transport at this pH.

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