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CARRIER-MEDIATED TRANSPORT OF D-RIBOSE BY *RHODOTORULA GLUTINIS*

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The kinetics of D-ribose transport by *Rhodotorula glutinis* were investigated over a 1 000-fold range of sugar concentrations. Analysis of the saturation isotherm revealed the presence of two carrier systems for D-ribose in the *Rhodotorula* plasma membrane. These two carriers exhibited K_m values of 1.3 and 30 mM. At saturating concentrations of D-ribose, the low K_m carrier contributes less than 10% to the total rate of transport. Although D-ribose is metabolized rapidly by *Rhodotorula*, intracellular free sugar concentrations exceed those in the medium. In addition, the transport of this pentose is inhibited by the proton-conductors, 2,4-dinitrophenol and carbonylcyanide *m*-chlorophenylhydrazone. These data suggest that *Rhodotorula* cells are capable of an energy-dependent, concentrative transport of D-ribose.

Introduction

The red yeast *Rhodotorula glutinis* is capable of transporting a wide variety of metabolizable and non-metabolizable monosaccharides, often against considerable concentration gradients [1]. A 1969 report by Horak and Kotyk [2] suggested that D-ribose was unique among the monosaccharides taken up by *Rhodotorula* in that the transport of this pentose did not appear to be energy-dependent or even carrier-mediated. These workers [2] observed that the rate of transport of D-ribose was directly proportional to the concentration of sugar from 5 μM to 5 mM and concluded that D-ribose entered the cells of *Rhodotorula* by a process resembling simple diffusion. As Barnett [3] has pointed out, however, the observed linearity between transport rate and D-ribose concentration could correspond to the lower part of a rectangular hyperbola representing the activity of a carrier-mediated transport system having a low affinity for D-ribose.

Methods

Rhodotorula glutinis (*Rhodosporidium toruloides*, ATCC 26194) was grown at 30°C in liquid medium [4] and harvested at mid-log phase. The harvested cells were washed three times with 0.1 M KH_2PO_4, suspended in 0.1 M KH_2PO_4 (5%, wet weight/volume), vigorously aerated on a magnetic stirrer for 1.5 h at room temperature, then packed in ice and maintained with gentle stirring at 0°C.

D-Ribose transport was measured by incubating cell suspensions with D-[1-^{14}C]ribose in a water-bath shaker at 30°C. Cells and sugar solutions were preincubated, and uptake was initiated by the addition of the cell suspension to a solution of the sugar in 0.1 M KH_2PO_4. At appropriate time intervals (usually 1 and 2.5 min), aliquots were withdrawn, filtered through a Millipore depth filter (AP2502500), and washed with 0.2 M KH_2PO_4 at room temperature. The cells plus the filter were transferred to scintillation vials along with 2 ml of 60% (v/v) ethanol and 10 ml of Triton X-114-based scintillation fluid [5]. Samples were counted in a Beckman Liquid Scintillation Spectrometer (LS-8000).

Initial velocity data for the saturation isotherm

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were analyzed by non-linear regression techniques as described previously [4].

Cell extracts for sugar analysis were prepared by extracting filtered cells with 60% (v/v) ethanol at room temperature for 2–3 h. The resulting suspensions were filtered and washed, and the filtrates were concentrated on a rotary evaporator (bath temperature approx. 30°C). Aliquots of these extracts were applied to thin layers of silica gel G (E. Merck 5539-9H) and the chromatograms were developed in acetone/water (90 : 10, v/v). Authentic D-ribose migrated with an R_F of 0.45 in this system while pentose phosphates remained near the origin. Radioactive spots were located on the chromatogram with a radiochromatogram scanner (Berthold, Model LB 276), cut out, and measured quantitatively by liquid scintillation spectrometry.

Studies on the liberation of $^{14}CO_2$ from D-[1-^{14}C]ribose by *Rhodotorula* were conducted in serum-capped flasks equipped with center wells containing filter paper and aqueous NaOH. Incubations were terminated by injections of 4% (w/v) perchloric acid, and the radioactivity of the $^{14}CO_2$ trapped in the center well was measured by liquid scintillation spectrometry.

D-[1-^{14}C]Ribose (50–60 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, IL and from New England Nuclear, Boston, MA. Unlabelled D-ribose and carbonylcyanide *m*-chlorophenylhydrazone were products of Sigma Chemical Co., St. Louis, MO. All other chemicals were of the highest purity commercially available.

Results and Discussion

In an earlier study [4], we reported a stimulation of the initial velocity of D-xylose transport by aeration of freshly harvested glucose-grown cells of *Rh. glutinis*. A similar effect of aeration was observed with D-ribose transport. The initial velocity of D-ribose transport was essentially constant in cells aerated from 60 to 120 min and more than 30 times the transport rate of cells which had been freshly harvested and maintained at 0°C. All the data presented in this report were obtained with cells which had been 'activated' by 90-min aeration at room temperature and then maintained at 0°C during the experimental period (1–2 h).

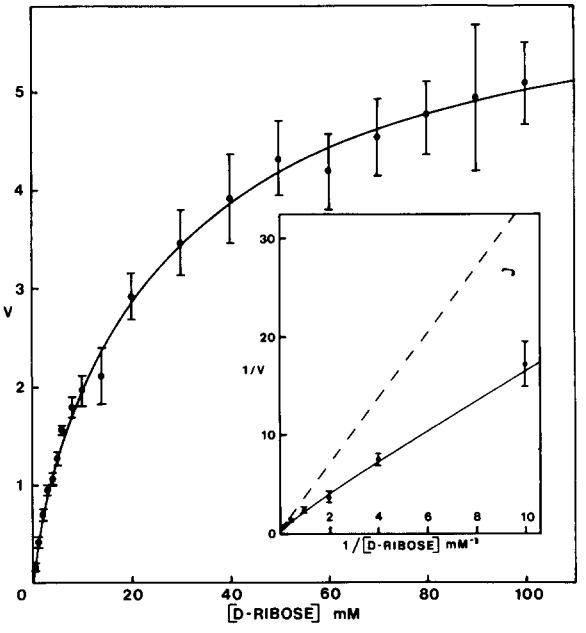

Fig. 1. Saturation isotherm for D-ribose transport by *Rhodotorula*. The insert is a Lineweaver-Burk plot of the portion of the data from 0.1 to 10 mM D-ribose. The solid lines are drawn from unweighted, non-linear regression fits to a two-carrier model for the transport process. The dashed line in the insert is from a similar fit to a one-carrier model. Velocities are expressed in nmol/min per mg (wet weight). Initial velocities were estimated from the differences in intracellular radioactivity between samples taken after 1 and 2.5 min of incubation.

The saturation isotherm for D-ribose uptake by *Rh. glutinis* is shown in Fig. 1. The figure contains 164 data points covering a 1 000-fold range of D-ribose concentrations. These data were tested for consistency with one-carrier (Model I) and two-carrier (Model II) models for the transport by weighted and unweighted, non-linear regressions:

Model I

$$v = \frac{V_1 \cdot [S]}{K_{m_1} + [S]}$$

Model II

$$v = \frac{V_1 \cdot [S]}{K_{m_1} + [S]} + \frac{V_2 \cdot [S]}{K_{m_2} + [S]}$$

The converged values of the constants and the variances for the overall fits are presented in Table I. With an unweighted fit, the difference in variance between the two models is slight and the K_{m_1} from the fit to Model II is rather ill-defined (Table I). The

TABLE I

COMPUTED PARAMETERS FROM THE SATURATION ISOTHERM FOR D-RIBOSE

The parameters were obtained from non-linear regression analyses of 164 points. The models considered were: I, a Michaelis-Menten function (one-carrier); II, a sum of two Michaelis-Menten equations (two-carrier). V is expressed in nmol/min per mg (wet weight) and K_m in mM. Parameters are reported ± S.E. The variance is $\Sigma W(v_{obs} - v_{calc})^2/(164$ minus the number of parameters evaluated). The weighting factor, W, was 1 for unweighted analysis and $1/v_{obs}^2$ for the weighted fit.

Parameter	Unweighted		Weighted	
	Model I	Model II	Model I	Model II
V_1	5.9 ± 0.1	0.56 ± 0.32	4.9 ± 0.1	0.56 ± 0.11
V_2	–	5.8 ± 0.2	–	5.7 ± 0.2
K_{m_1}	20 ± 1	1.3 ± 1.3	12 ± 1	1.3 ± 0.3
K_{m_2}	–	30 ± 5	–	30 ± 3
Variance	0.083	0.076	0.026	0.009

two-carrier model was not discarded on this basis, however, since the fit suggests that the second carrier, if present, is a low-K_m component with a small maximum velocity. Indeed, when the low concentration data from the saturation isotherm are graphically expanded by presentation in Lineweaver-Burk form (insert, Fig. 1), it is apparent that, in this region, the fit to the one-carrier model is inadequate (dashed line, Fig. 1) while the fit to the two-carrier model (smooth curves, Fig. 1) is quite acceptable over the entire range of concentrations. The data set used for this analysis contains a preponderance of points at high concentrations/high velocities and these points dominate an unweighted regression. An unweighted regression assumes that the variance of the velocities is constant [6]. In our experiments, however, the standard errors of the velocities generally increased with increasing velocity. This result can be seen in Fig. 1 and arises from the fact that the specific radioactivities of the D-ribose solutions employed decreased as the total concentration of D-ribose increased. Therefore, a weighted fit of the data was carried out using squared reciprocal velocities as weighting factors, assuming standard errors proportional to velocities [6]. The results of this analysis are presented in Table I. With the weighted fit, the difference in variance between the models is large and all parameters have acceptable standard errors. Significantly, the values of the converged parameters for the two-carrier model were essentially the same whether the fit was weighted or unweighted. In contrast, the parameters for the one-carrier model were altered markedly by the weighted analysis. Thus, the experimental data for the saturation isotherm appear to be more consistent with the two-carrier model system.

An earlier report from our laboratories [4] described the presence of two carriers in the *Rhodotorula* plasma membrane involved in the transport of D-xylose. These carriers, like those for D-ribose transport, exhibited markedly different Michaelis constants and maximum velocities [4]. In spite of these similarities, our current view is that the D-ribose carriers are not identical with the D-xylose carriers described earlier. This belief is based on the following observations: (a) Aeration of *Rhodotorula* cells for 1.5 h at room temperature results in a greater than 30-fold increase in the rate of D-ribose transport but only a 5–6-fold increase in D-xylose transport [4]. (b) 2-Deoxy-D-glucose is a potent inhibitor of D-xylose transport [4]. At 1 mM D-xylose, addition of 0.6 mM 2-deoxy-D-glucose resulted in greater than 70% inhibition of D-xylose transport [4]. In contrast, at 1 mM D-ribose, 0.6 mM 2-deoxy-D-glucose was only slightly inhibitory (approx. 20%) and the inhibition was of the 'partial' type with less than 70% inhibition even at 60 mM 2-deoxy-D-glucose (unpublished results).

The results described above clearly demonstrate the saturable nature of D-ribose transport by *Rh. glutinis*. It appears possible that the lack of curvature in the rate/concentration data reported previously

TABLE II

CHARACTERISTICS OF D-RIBOSE TRANSPORT AND METABOLISM

Cells were incubated at 30°C with an initial D-[1-^{14}C]ribose concentration of 1 mM. Intracellular concentrations were calculated with an estimated intracellular volume of 0.4 ml per g (wet weight) of cells [9]. The intracellular [D-ribose] was determined by thin-layer radiochromatography of cell extracts.

Incubation time (min)	$\frac{[\text{Ribose}]_{in}}{[\text{Ribose}]_{out}}$	$^{14}CO_2$ liberated (nmol)	Intracellular ^{14}C-labelled compounds (nmol)	Intracellular D-ribose (nmol)
1	0.9	–	14	9
5	3.3	3.2	50	31
10	5.9	6.8	90	53

by Horak and Kotyk [2] was due to differences in the strain or in the physiological state of the cells employed.

The transport of many monosaccharides by *Rh. glutinis* results in accumulation of sugar against considerable concentration gradients. In order to determine whether *Rhodotorula* cells are capable of concentrative transport of D-ribose, we examined concentration ratios, $[\text{D-ribose}]_{in}/[\text{D-ribose}]_{out}$, during 10 min incubations of the cells with radioactive sugar. Calculations based on total radioactivity taken up by the cells yielded concentration ratios approaching 10 by 10 min of incubation. That approach, however, neglects possible metabolic transformations of the sugar. Therefore, it was necessary to demonstrate that the intracellular radioactivity did in fact correspond to the free pentose. The results in Table II demonstrate that, under the conditions of our experiments, D-ribose metabolism begins in the first minute of incubation. At that time, only 64% of the intracellular radioactivity can be accounted for as D-ribose. The remainder is associated with other compounds which we did not identify specifically but which migrate, in our chromatographic system, with authentic pentose and pentulose phosphates. We also observed (Table II) a significant amount of $^{14}CO_2$ liberated from D-[1-^{14}C]ribose during 10 min of incubation. Previous studies [7,8] have demonstrated that *Rh. glutinis* metabolizes carbohydrates primarily by the hexose monophosphate pathway and our observations on the fate of intracellular D-ribose are consistent with its metabolism via this pathway. In spite of this early metabolism of D-ribose, concentration ratios well above 1 are observed during 10 min incubations (Table II). Thus, it appears that *Rh. glutinis* is capable of transporting this pentose against a concentration gradient.

Studies with proton-conducting uncouplers provide additional support for an energy-requiring, concentrative transport of D-ribose by *Rhodotorula*. Both 2,4-dinitrophenol and carbonylcyanide *m*-chlorophenylhydrazone inhibited the transport of D-ribose (Table III). The fact that these two, structurally dissimilar compounds produce similar effects on the transport suggests that their inhibitory action is not due to specific binding interactions with the D-ribose carriers but rather to their proton-conducting/energy-depleting effects on the *Rhodotorula* cell.

In summary, the results presented here demonstrate that the transport of D-ribose by *Rh. glutinis*

TABLE III

EFFECT OF PROTONOPHORES ON D-RIBOSE TRANSPORT

Cell suspensions (10%, w/v) were preincubated with the indicated concentrations of 2,4-dinitrophenol (DNP) or carbonylcyanide *m*-chlorophenylhydrazone (CCCP) for 10 min at 30°C. Uptake was initiated by addition of an aliquot of the preincubated cell suspension to an equal volume of radioactive sugar solution.

[DNP] (μM)	Transport of 0.5 mM D-ribose (% of control)	[CCCP] (μM)	Transport of 1.0 mM D-ribose (% of control)
0	(100)	0	(100)
25	89	5	58
50	67	10	24
100	21	50	8
200	2	100	2

is a carrier-mediated, energy-dependent process similar in these respects to the uptake of other monosaccharides by this organism.

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