BBA 75019

MOBILITY OF THE FREE AND OF THE LOADED MONOSACCHARIDE CARRIER IN *SACCHAROMYCES CEREVISIAE*

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(Received July 11th, 1966)

SUMMARY

An expression relating the mobilities of the free (D_C) and the loaded (D_{CS}) carrier was derived and two kinetic tests based on it were applied to monosaccharide transport in baker's yeast: (1) comparison of the Michaelis constant of transport (K_T) with the dissociation constant of the sugar–carrier complex (K_{CS}); (2) comparison of the initial rate of exit of a labelled sugar into a sugar-free medium with that into an equilibrium concentration of non-labelled sugar. Although the movement of the carrier across the membrane appears to be the limiting step of the transport process, the mobilities of the free and the loaded carrier were found to differ. For monosaccharides transported into the entire cell-water volume (represented by D-glucose, D-fructose, D-xylose and D-arabinose) $D_{CS}/D_C = 1.9–3.5$, whereas for sugars transported into only a part of the cell water (represented by D-galactose and D-ribose) $D_{CS}/D_C = 1$. The results (1) serve as further evidence for the existence of two carrier types in baker's yeast and (2) indicate that the simplified theory of carrier transport is not applicable to baker's yeast.

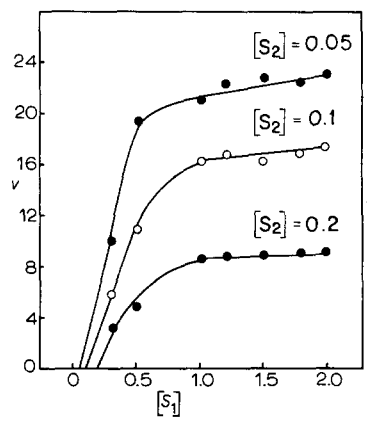
INTRODUCTION

Monosaccharide transport in baker's yeast is known to proceed only up to a diffusion equilibrium (*cf.* ref. 1). This property it shares with cells on which the generally accepted theories of carrier transport have been modelled, in particular mammalian erythrocytes (*e.g.* LEFEVRE[2], ROSENBERG AND WILBRANDT[3], MILLER[4]), but as distinct from the latter, the kinetics of this transport in yeast have not been studied very extensively (*cf.* refs. 5, 6) and a number of experimental observations on monosaccharide uptake await their kinetic interpretation. It was noted in an earlier paper[7] that the apparent Michaelis constant of monosaccharide uptake by yeast is not in agreement with the dissociation constant of the sugar–carrier complex as one would expect on the basis of the simplified theory where (1) the carrier moves with equal velocity whether free or loaded and (2) this movement is the limiting step of the transport process. This discrepancy stimulated an examination of the two assump-

tions. It is believed that the results might have a bearing on the mechanism of sugar transport in this cell type.

THEORETICAL

It has been shown that in human[8] and in rabbit[9] erythrocytes the rate of sugar transport through the cell membrane is limited by the actual movement of the substrate (in carrier-bound form) across the membrane and that an equilibrium can be assumed to exist on both sides of the membrane between the carrier C and the sub-



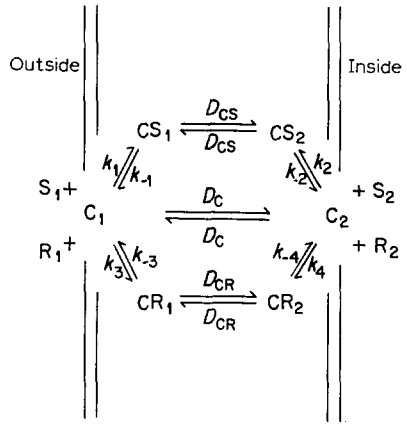


Fig. 1. Demonstration of E-kinetics of monosaccharide exit from baker's yeast. The theory of mobile carrier transport[10] predicts for saturation concentrations of substrate (very high $[S_1]$) that, if the movement across the membrane is much slower than the equilibration of the carrier with substrate at either side of the membrane, the *trans* concentration of substrate ($[S_2]$) has a pronounced effect on the rate of transport (E-kinetics) as is borne out in the figure. If the movement of the carrier–substrate complex were not limiting the curves would all merge at high S_1 concentrations (Z-kinetics). $[S_1]$, intracellular concentration of D-arabinose (%); $[S_2]$, external concentration of D-arabinose (%); v, the actual rate of exit derived from the tangent of the exit curves (in arbitrary units).

Fig. 2. A model for membrane carrier transport. The symbols are defined in the text.

strate S or R. A relevant test[10] was applied to the yeast cells and it was found that there, too, the equilibrium treatment is applicable (Fig. 1). The limitation by the movement across the membrane is also suggested by the low temperature coefficient of the overall process in the range from 15° to 35° (ref. 11).

In the presence of two substrates S and R the following equations should describe the movement of the carrier forms C, CS and CR:

$$v_C = D_C \left(C_{t,1} \frac{1}{S_1' + R_1' + 1} - C_{t,2} \frac{1}{S_2' + R_2' + 1} \right) \quad (1a)$$

$$v_{CS} = D_{CS} \left(C_{t,1} \frac{S_1'}{S_1' + R_1' + 1} - C_{t,2} \frac{S_2'}{S_2' + R_2' + 1} \right) \quad (1b)$$

$$v_{CR} = D_{CR} \left(C_{t,1} \frac{R_1'}{S_1' + R_1' + 1} - C_{t,2} \frac{R_2'}{S_2' + R_2' + 1} \right) \quad (1c)$$

where D_C, D_{CS} and D_{CR} are the mobilities of the free and of the loaded carrier with substrates S and R, respectively; $C_{t,1}$ and $C_{t,2}$ are the total concentrations of the carrier at the two sides of the membrane; S_1' and S_2' are the reduced concentrations of S at the two sides of the membrane ($S' = [S]/K_{CS}$ where $K_{CS} = k_{-1}/k_1 = k_{-2}/k_2 = [C][S]/[CS]$) and R_1' and R_2' those of R where $K_{CR} = k_{-3}/k_3 = k_{-4}/k_4 = [C][R]/[CR]$. The meaning of the symbols is made clearer by the scheme (Fig. 2).

Since the steady-state concentration of substrate intracellularly is equal to that in the medium it is assumed that $k_1 = k_2$ and $k_{-1} = k_{-2}$ and, similarly, $k_3 = k_4$ and $k_{-3} = k_{-4}$. Also the mobility of the carrier is taken to be equal in both directions.

As the carrier cannot escape from the membrane, the sum of Eqns. 1a–c must be equal to zero and hence, if $D_C \neq D_{CS} \neq D_{CR}$, necessarily $C_{t,1} \neq C_{t,2}$. (If $D_C = D_{CS} = D_{CR}$ then also $C_{t,1} = C_{t,2}$ as in the simplified theory.)

On adding the equations we obtain

$$\frac{D_C + D_{CS}S_1' + D_{CR}R_1'}{S_1' + R_1' + 1} = (Q - 1)\frac{D_C + D_{CS}S_2' + D_{CR}R_2'}{S_2' + R_2' + 1} \tag{2}$$

where $Q = 2C_t/C_{t,1} = 1 + C_{t,2}/C_{t,1}$. ($2C_t$ is the total concentration of the carrier in the membrane.)

Making the substitutions $x_1 = D_C + D_{CS}S_1' + D_{CR}R_1'$, $x_2 = D_C + D_{CS}S_2' + D_{CR}R_2'$, $y_1 = 1 + S_1' + R_1'$ and $y_2 = 1 + S_2' + R_2'$, we obtain $(Q-1) = x_1y_2/x_2y_1$.

Then

$$C_{t,1} = \frac{2C_tx_2y_1}{x_1y_2 + x_2y_1} \tag{3a}$$

and

$$C_{t,2} = \frac{2C_tx_1y_2}{x_1y_2 + x_2y_1}. \tag{3b}$$

Then the rate of transport of S in the presence of R is given by Eqn. 1b which, after suitable substitutions from Eqns. 3a and 3b, yields

$$v_S = 2D_{CS}C_t\left(\frac{x_2S_1' - x_1S_2'}{x_1y_2 + x_2y_1}\right) \tag{4}$$

If no R is present x_1 simplifies to $D_C + D_{CS}S_1'$, x_2 to $D_C + D_{CS}S_2'$, y_1 to $1 + S_1'$ and y_2 to $1 + S_2'$, so that

$$v_S = 2D_CD_{CS}C_t\frac{S_1' - S_2'}{2(D_C + D_{CS}S_1'S_2') + (D_C + D_{CS})(S_1' + S_2')} \tag{5}$$

For the initial velocity, when $[S_2] = 0$,

$$v_S = 2D_CD_{CS}C_t\frac{S_1'}{2D_C + (D_C + D_{CS})S_1'} \tag{6}$$

Comparing this equation with the common simplified form $v_S = V([S_1])/([S_1] + K_T)$ where $V = C_tD$ and K_T is the Michaelis constant of transport we observe that in the non-simplified case

$$V = 2D_CD_{CS}C_t/(D_C + D_{CS}) \tag{7a}$$

and

$$K_T = 2D_CK_{CS}/(D_C + D_{CS}) \tag{7b}$$

Hence

$$D_{CS}/D_C = 2K_{CS}/K_T - 1 \tag{8}$$

Relationship 8 shows the peculiar limitation on the ratio of the two constants which cannot become less than 0.5 if positive values for the mobilities are to be obtained.

To find the ratio of mobilities one can use two approaches: (1) to determine K_{CS} as well as K_T separately; (2) to compare the exit of labelled substrate from cells into a substrate-free medium with that into a medium containing non-labelled substrate at an equilibrium concentration.

With respect to (1): K_T can be determined from the initial velocity of uptake by any of the conventional techniques (Lineweaver–Burk plot and the like) although the inaccuracy of this procedure is very high (transient peaks and irregularities, see ref. 12). K_{CS} can be derived by the following procedure: We let the cells equilibrate with sugar S so that $[S_1] = [S_2] = [S]$, then add a negligible amount of radioactive substrate R (*cf.* WILBRANDT AND KOTYK[13]) and measure its rate of uptake. In this arrangement $[R] \ll [S]$ so that $x_1 = x_2 = D_C + D_{CS}S'$ and $y_1 = y_2 = 1 + S'$. Then, in analogy with Eqn. 4,

$$v_R = D_{CR}C_t \frac{[R_1] - [R_2]}{[S] + K_{CS}} \tag{9}$$

(since here $K_{CS} = K_{CR}$). If we measure consistently the half-time of uptake of R and use an equal amount of R at all concentrations of S, Eqn. 9 can be written $v_R = A/(K_{CS} + [S])$ where A is a constant.

For

$$[S] \gg K_{CS},\ v_R = A/[S] \tag{10a}$$

whereas for

$$[S] \ll K_{CS},\ v_R = A/K_{CS} \tag{10b}$$

Plotting the logarithm of v_R against that of [S] we obtain a curve with two rectilinear parts which can be extrapolated to intersect at a point where $[S] = K_{CS}$ (Fig. 3).

With respect to (2): Let us load the cells with labelled substrate R and then transfer them to (a) distilled water, (b) an equal concentration of unlabelled substrate

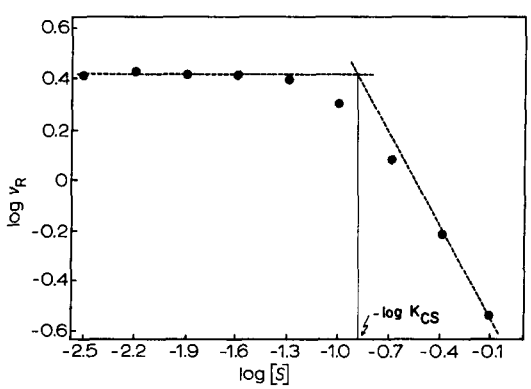


Fig. 3. Plot of the logarithm of the uptake rate of labelled substrate R added to an equilibrium of unlabelled substrate S. For details see the theoretical section. Figures for D-xylose from the paper of KOTYK[7] were replotted here to determine the dissociation constant of the D-xylose–carrier complex.

S. Then $D_{CS} = D_{CR}$, $K_{CS} = K_{CR}$ and $[R] \ll [S]$ since even in the labelled substrate there is a predominance of S. In case (a), $[R_1] = [R]$, $[R_2] = 0$, $[S_1] = [S]$, $[S_2] = 0$; in case (b), $[R_1] = [R]$, $[R_2] = 0$, $[S_1] = [S_2] = [S]$. Recalling Eqn. 4 we have for the initial rate of exit of R in case (a) $x_1 = D_C + D_{CS}S'$, $x_2 = D_C$, $y_1 = 1 + S'$ and $y_2 = 1$. In case (b) then $x_1 = x_2 = D_C + D_{CS}S'$ and $y_1 = y_2 = 1 + S'$.

Then for (a)

$$v_{R(a)} = 2D_{CS}C_t \frac{D_C R'}{D_C + D_{CS} S' + D_C(1 + S')} = 2C_t \frac{D_C D_{CS}[R]}{2D_C K_{CS} + [S](D_C + D_{CS})} \quad (11a)$$

and for (b)

$$v_{R(b)} = 2D_{CS}C_t \frac{(D_C + D_{CS} S') R'}{2(D_C + D_{CS} S')(1 + S')} = C_t \frac{D_{CS}[R]}{K_{CS} + [S]} \quad (11b)$$

Setting $v_{R(a)}/v_{R(b)} = a$ we can derive the expression for the ratio of mobilities:

$$\frac{D_{CS}}{D_C} = \frac{2K_{CS}(1 - a) + [S](2 - a)}{a[S]} \quad (12)$$

It may be seen that if very high [S] is chosen the expression reduces to $D_{CS}/D_C = 2/a - 1$, where $a = K_T/K_{CS}$ by analogy with Eqn. 8 (*cf.* ref. 14).

METHODS

Yeast. The baker's yeast strain *Saccharomyces cerevisiae* R XII from the Institute collection was maintained on malt–agar slopes and then grown in a synthetic medium with 2% glucose and yeast extract for 24 h at 30°. Before incubation it was washed with water, aerated for 3 h and again washed with distilled water.

Incubation took place in an atmosphere of argon (to prevent possible aerobic utilization of galactose after adaptation) at 30° in a water bath. In the exit experiments 18° was used to slow down the process. Samples of suspension (generally 0.6 ml containing 3–4 mg dry wt.) were removed at suitable time intervals after addition of sugar or after transfer to a new medium, filtered through a membrane filter (pore diameter 0.3–0.5 μ; HUFS Synthesia, Uhříněves, Czechoslovakia) placed on a special funnel attached to a water pump, washed twice with ice-cold water and the filter with the cell pellet was transferred for 10 min into a boiling 0.1% solution of Triton X. The time required for filtration of the sample, including the washing, never exceeded 12 sec. The suspension in Triton X was then transferred to aluminium planchets for radioactivity counting in a methane-flow Frieseke-Hoepfner apparatus.

When using glucose and fructose, incubation took place in the presence of $5 \cdot 10^{-4}$ M iodoacetamide which blocks sugar metabolism but permits the sugar to be transported[15].

Reagents. D-[1-^{14}C]Ribose, universally labelled D-[^{14}C]xylose, D-[1-^{14}C]arabinose, D-[1-^{14}C]galactose and universally labelled D-[^{14}C]fructose were obtained from the Radiochemical Centre, Amersham, England; universally labelled D-[^{14}C]glucose was from the Institute for Research, Production and Application of Radioactive Isotopes in Prague, Czechoslovakia. Non-labelled sugars were all from Hoffmann–La Roche, Basel, Switzerland. The selection of sugars was rather restricted by their availability in the labelled form.

Evaluation of uptake and exit curves. When the initial velocity was to be deter-

mined, the differentiated form of Newton's formula for equispaced arguments with associated decreasing differences was used (*cf.* Handbook of Chemistry and Physics, Chemical Rubber Publ. Co., p. 305) but it was always restricted to the first 20 sec of the process.

RESULTS AND DISCUSSION

Comparison of the K_{CS} and K_T. The K_{CS} values published previously[7] for D-xylose, D-arabinose and D-galactose were supplemented with those for D-ribose and related to the Michaelis constants of uptake of these sugars (Table I). Unfortunately,

TABLE I

COMPARISON OF K_{CS} AND K_T FOR VARIOUS SUGARS IN BAKER'S YEAST

Sugar	K_{CS} *(mM)*	K_T *(mM)*	D_{CS}/D_C*
D-Xylose	135	92–175	1.92–0.54
D-Arabinose	155	75–140	3.13–1.21
D-Galactose	21	20–41	1.10–0.02
D-Ribose	350	360–650	0.94–0.07

* Calculated from Eqn. 8.

the Michaelis constants obtained by this procedure are most unreliable and therefore only a semiquantitative difference between D-xylose and D-arabinose, on the one hand, and D-galactose and D-ribose, on the other, can be detected in the values. Much greater weight was attached to the exit procedure discussed below.

Comparison of the initial rate of exit into substrate-free and substrate-containing

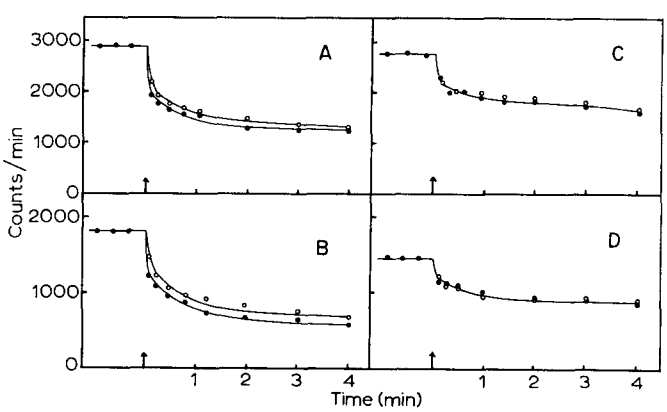


Fig. 4. Intracellular concentration of labelled D-arabinose (A), D-xylose (B), D-galactose (C) and D-ribose (D). Cells were preincubated for 2 h with the respective labelled sugar at a 0.3 M concentration. Then they were filtered, washed in ice-cold water (within 15 sec) and, at the point marked with the arrow, resuspended quickly in either distilled water (open circles) or 0.3 M solution of the corresponding non-labelled sugar (solid circles), all at 18°, in an atmosphere of argon.

medium. Fig. 4 shows a typical experiment of this type. All the results being summarized in Table II, it may be seen that D-xylose, D-arabinose, D-glucose and D-fructose show a clearly higher rate of exit into an equilibrium concentration of sugar whereas for D-galactose and D-ribose there is hardly any difference.

The difference between the two sugar types can serve as further evidence for a distinct carrier mechanism as discussed in detail elsewhere[11]. Assuming then, in accordance with the above paper, that the group of sugars comprising D-xylose, D-arabinose, D-lyxose, L-glucose, L-sorbose and all the metabolizable sugars, D-glucose, D-fructose and D-mannose, are all transported by the same carrier we may conclude that this carrier is affected in its mobility by a sugar being attached to it, this resulting in an asymmetry of distribution of the total carrier at the two membrane sides. Nevertheless, the movement of the carrier is the limiting step in monosaccharide transport.

The findings open up a field for conjectures as to the actual mechanism of the transport of sugars across the cell membrane.

(1) If the carrier moves across the membrane by translational diffusion it is difficult to visualize that the attachment of the probably relatively small monosaccharide molecule would alter its diffusion coefficient 2–3 times. It appears somewhat more likely that the transient binding of the sugar molecule might change the ease with which the carrier moves (perhaps by rotation) in the membrane (perhaps in an aqueous micropore).

TABLE II

INITIAL RATES OF EXIT OF LABELLED SUGARS INTO DIFFERENT MEDIA

Cells were preincubated with 0.3 M labelled sugar and after 120 min washed and resuspended. The values of v from several experiments have all been multiplied by a suitable factor to make the exit into water numerically equal.

Sugar added initially	*Resuspension medium*	v *(arbitrary units)*	a	D_{CS}/D_C*
D-[^{14}C]Xylose	Water	764	0.675	2.41
	0.3 M D-[^{12}C]Xylose	1132		
D-[^{14}C]Arabinose	Water	1118	0.703	2.28
	0.3 M D-[^{12}C]Arabinose	1582		
D-[^{14}C]Glucose**	Water	940	0.70	1.87
	0.3 M D-[^{12}C]Glucose	1341		
D-[^{14}C]Fructose**	Water	540	0.45	3.54
	0.3 M D-[^{12}C]Fructose	1201		
D-[^{14}C]Galactose	Water	631	1.01	0.99
	0.3 M D-[^{12}C]Galactose	624		
D-[^{14}C]Ribose	Water	238	0.97	1.11
	0.3 M D-[^{12}C]Ribose	246		

* Calculated from Eqn. 12.
** In the presence of $5 \cdot 10^{-4}$ M iodoacetamide.

The differences in the D_{CS} of individual sugars should then be reflected in different maximum velocities of uptake (*cf.* Eqn. 7a) but it follows, from a different aspect of the work[11], that the sugars in question possess very similar maximum rates. This might be due to the fact that the D_{CS}/D_C ratio for glucose and fructose was measured in the presence of iodoacetamide and if this is taken into account there remain the values for D-xylose and D-arabinose which are really almost identical.

(2) The identity of the D_{CS} and D_C for D-galactose and D-ribose tempts one to assume a distinctly different type of transport for these sugars although here, too, the mobile carrier seems to be involved[11]. Whether the difference between the two types of transport is merely quantitative must be elucidated by further experiments.

(3) A word should be said in this connection about a novel mechanism proposed by HECKMANN[16],[17] in which a S_N2 (substitution–nucleophilic–bimolecular) reaction between a fixed binding site with substrate and a free substrate molecule can account for all the phenomena previously observed (saturation kinetics, countertransport, competitive acceleration, exchange diffusion at high substrate concentrations). If such a model were to account for the present findings one would have to assume that the fixed binding site involved in the S_N2 reaction is more efficient if already coupled with a substrate molecule than without such coupling.

(4) The inequality of rates of movement of the free and the loaded carrier may be more common than would appear. MAWE AND HEMPLING[18] and LEVINE, OXENDER AND STEIN[14] showed much the same thing for glucose in human erythrocytes and it might be of interest to check the universality of this phenomenon as REGEN AND MORGAN[9] did not find it in rabbit erythrocytes.

The general conclusion that can be drawn from the present observations is, then, that the transport of monosaccharides in yeast is more complicated than permitted by the simplified carrier theory applied heretofore.

ACKNOWLEDGEMENTS

My thanks are due to Prof. A. KLEINZELLER for stimulating comments on the work described here and to Mrs. E. HOROVÁ for skilled technical cooperation.

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