Properties of the Sugar Carrier in Baker's Yeast

I. Kinetics of Transport

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Received May 7, 1964

ABSTRACT

A kinetic test evolved for distinguishing between mobile carrier transport in which one or two substrate molecules are transported at a time was applied to sugar transport in *Saccharomyces cerevisiae* and it was found that the mechanism here involves attachment of one sugar molecule to one molecule of carrier.

Incidental to the test, the dissociation constants of some sugar-carrier complexes were determined.

The diversity of sugar transport mechanisms in different cells is discussed.

It is now generally accepted that sugars are transported into the cell of baker's yeast by a mobile membrane carrier with high substrate specificity, as evidenced by selective uptake of stereoisomers, saturation kinetics of the process at high substrate concentrations and by the existence of countertransport (Burger, Hejmová & Kleinzeller, 1959; Cirillo, 1959, 1961). Views have been advanced both for (Burger & Hejmová, 1961) and against (Scharff, 1962) the identity of this carrier with the enzyme hexokinase which would thus fulfil a dual function: one of attaching and transporting the sugar, another of phosphorylating it with the aid of adenosine triphosphate. These views will be dealt with in subsequent communications.

Transport of sugars in *Saccharomyces cerevisiae*, whether metabolized or not, generally proceeds without requirement for metabolic energy up to a diffusion equilibrium (Kotyk & Kleinzeller, 1963; Cirillo, 1961; see, however, Okada & Halvorson, 1963, who observed active transport of α-thioethylglucopyranoside in some yeast strains) and the process is symmetrical for entry and exit of sugars (Kotyk & Kleinzeller, 1963). In this respect baker's yeast resembles another exhaustively studied type of cell, viz. the human erythrocyte (e.g. Wilbrandt & Rosenberg, 1961). There, too, no up-hill transport of sugars occurs save for the phenomenon of induced countertransport (Rosenberg & Wilbrandt, 1957) and it has appeared, further information lacking, that the sugar carrier in human erythrocytes and in baker's yeast is a similar entity differing in specificity for substrate but operating on the same principle.

It has been shown recently (Kotyk & Wilbrandt, 1963, 1964) that in human erythrocytes sugars appear to be transported in the form of a complex of the carrier with two sugar molecules attached, this arrangement being able to decrease the number of hydroxyl groups protruding from the complex by mutual bonding between the two sugar molecules. Stein (1961) who has conceived the idea of sugar dimer formation (although without an operative carrier) has proposed to attribute some previously observed anomalies of sugar transport in other cells to this dimer formation. Indeed, the overwhelming

uniformity of basic metabolic pathways throughout nature might justify such extrapolation and it has therefore been tested whether the kinetics of sugar transport in baker's yeast, hitherto considered identical with that in human erythrocytes, is actually such even in respect of the number of molecules attached to the carrier.

THEORETICAL

Transport of substances across cell membranes under conditions where the mobility of the carrier or carrier complex is limiting for the whole process (where therefore equilibrium exists on both sides of the membrane with respect to the carrier, C, substrate, S, and whatever C-S complexes may be formed) is generally expressed by

$$v_S = f(D_C,\ D_{CS},\ D_{CSS}\ldots,\ K_{CS},\ K_{CSS}\ldots) \\ /c_1 f(S_1) - c_2 f(S_2)/ \qquad (1)$$

where D_C, D_{CS}, and D_{CSS} are the mobilities of the free carrier and its substrate complexes, K_{CS}, K_{CSS} the dissociation constants of the complexes, c_1 and c_2 the carrier concentrations at the two sides of the membrane (under particular conditions, these can also be functions of the other variables) and S_1 and S_2 the corresponding substrate concentrations. When two sugars, S and R, are concerned the appropriate constants and variables must be introduced (D_{CR}, D_{CRR}, possibly D_{CRS} and D_{CSR}, K_{CR}, K_{CRR} etc., and R_1 and R_2).

1. In previous considerations (e.g. Wilbrandt, 1961) it has been assumed that only C, CS (and CR) exist, that their mobilities are equal ($= D$) and that therefore $(c_1) = (c_2) = C_t$. In conformity with Michaelis-Menten character of the active (= transporting) complex formation the rate of transport of a single sugar is expressed by

$$v_S = C_t D\left[\frac{S_1}{S_1 + K_s} - \frac{S_2}{S_2 + K_s}\right] = \\ = V\left(\frac{S'_1}{S'_1 + 1} - \frac{S'_2}{S'_2 + 1}\right) \qquad (2)$$

where $S' = S/K_S$ and C_t is the amount of carrier in the membrane.

When another sugar, R, is present

$$v_S = V\left[\frac{S'_1}{S'_1 + R'_1 + 1} - \right. \\ \left. - \frac{S'_2}{S'_2 + R'_2 + 1}\right] \qquad (3)$$

2. If, however, two substrate molecules can be attached to the carrier, a much more complex situation evolves. Then the carrier can occur in the forms C, CS and CSS (and CR, CRR, CSR and CRS if two substrates are present). For the carrier cycle to be operative the vectorial sum of movements of these carrier forms must be equal to zero and this can be achieved only when $(c_1) \neq (c_2)$ since the mobilities of the various carrier forms are not assumed to be equal. The values of c_1 and c_2 can be calculated with respect to C_t and substituted into the equation for the movement of S:

$$v_S = v_{CS} + 2v_{CSS}\ (+ v_{CSR} + v_{CRS})$$

whence

$$v_S = 2C_t\,\frac{x_2 y_1 - x_1 y_2}{x_2 w_1 + x_1 w_2} \qquad (4)$$

If a single substrate, S, is present, the symbols have the following meaning:

$$x = D_C + S'(D_{CS} + D_{CSS}S'')$$
$$y = S'(D_{CS} + 2D_{CSS}S'')$$
$$w = 1 + S'(1 + S'');$$

if S and R are present:

$$x = D_C + S'(D_{CS} + D_{CSR}R''' + \\ + D_{CS}S'') + R'(D_{CR} + \\ + D_{CRS}S''' + D_{CRR}R'')$$
$$y = S'(D_{CS} + 2D_{CSS}S'' + \\ + D_{CSR}R''') + D_{CRS}R'S'''$$
$$w = 1 + S'(1 + S'' + R''') + \\ + R'(1 + R'' + S''')$$

where $S' = S/K_{CS}$, $S'' = S/K_{CSS}$, $S''' = S/K_{CRS}$, $R' = R/K_{CR}$, $R'' = R/K_{CRR}$, $R''' = R'/K_{CSR}$, the constants K_{CS}, K_{CSS}, K_{CRS} etc. being the dissociation constants equal to (C)(S)(CS), (CS)(S)/(CSS), (CR)(S)/(CRS) etc.

Several specific features emerge from this type of kinetics as are described in detail elsewhere (Wilbrandt & Kotyk, 1964) and several tests based on them can be made use of for distinguishing between the transport of single (mono-complex) and paired (di-complex) substrate molecules. The most readily per formed and most reliable is the following.

If we permit a substrate S to equilibrate with the cells so that $(S_1) = (S_2)$ and then add a constant amount of the labelled form (R) of the same substrate such that $(R) \ll (S)$ the rate of uptake of R is defined as follows:

(1) on the basis of mono-complex theory:

$$v_R = V\left(\frac{R'_1}{S'+1} - \frac{R'_2}{S'+1)}\right) \qquad (5)$$

Since $K_{CS} = K_{CR}$

$$v_R = V\frac{R_1 - R_2}{S + K_{CS}} \qquad (5a)$$

If now the half-time of uptake of R is consistently measured $(R_1) - (R_2) =$ constant, and

$$v_R = \frac{K}{S + K_{CS}} \text{ where } K \text{ is a constant.} \qquad (6)$$

For low concentrations of S, $K_{CS} \gg (S)$ and $v_R =$ constant; for high concentrations of S, $K_{CS} \ll (S)$ and $v_R =$ constant/(S).

A plot of log v_R against log(S) is shown by curve 1 in Fig. 1.

(2) On the basis of di-complex theory: Here $D_{CS} = D_{CR} = D_1$, $D_{CSS} = D_{CSR} = D_{CRR} = D_{CRS} = D_2$ and $K_{CS} = K_{CR} = K_1$, $K_{CSS} = K_{CRR} = K_{CRS} = K_{CSR} = K_2$ (hence $S'' = S'''$, $R'' = R'''$); moreover, $(R) \ll (S)$ and $(S_1) = (S_2) = (S)$.

Then from eq. (4) after several algebraic steps

$$v_R = C_t\frac{(D_1 + D_2S'')(R_1 - R_2) + D_2S'(R_1'' - R_2'')}{1 + S' + S'S''} = C_t\frac{(D_1K_2 + 2D_2S)(R_1 - R_2)}{K_1K_2 + K_2S + S^2} \qquad (7)$$

(For the sake of simplicity, symbols S and R stand here for concentrations of the two substrates.)

The transport of di-complex nature can predominate over mono-complex transport by virtue of (*a*) greater mobility of the carrier di-complex so that $D_2 \gg D_1$, and K_1 is similar to K_2, or (*b*) greater affinity for the attachment of a second molecule over the first one so that $K_2 \ll K_1$ and D_1 is similar to D_2.

If either or both of the mechanisms are operative eq. (7) becomes

$$v_R = 2C_tD_2(R_1 - R_2)\frac{S}{S(S + K_2) + K_1K_2} \qquad (8)$$

Here, for $(S) \ll K_1, K_2$, $v_R =$ constant $\times$ (S); for $(S) \gg K_1, K_2$, $v_R =$ constant/(S).

and hence the logarithmic plot of v_R against (S) goes through a maximum. Curve 2 in Fig. 1 shows the extreme case when $D_1 = 0$ and $K_2 = 4K_1$ (based on the arbitrary assumption that the affinities of the carrier for the first and second molecule of substrate are equal). When either $D_1 = 0$ or K_2 is not completely negligible as compared with K_1 the left-hand part of the theoretical curve exhibits a slope of less than +1 when logarithmically plotted (cf. Wilbrandt & Kotyk, 1964).

METHODS

Yeast. Resting cells of commercial Swiss yeast and Czech yeast were used in the experiments.

Incubation was carried out at 30° C, anaerobically (passing oxygen-free nitrogen through the suspension to minimize the possibility of inducing enzyme systems for the utilization of the substrates used) in suspensions of approximately 10 mg. yeast dry weight/ml. 0.15M NaCl with appropriate sugar added. One-ml. samples were withdrawn at suitable time intervals, filtered through a Millipore filter and washed twice with ice-cold water. The pellet on filter was extracted with 2 ml. 5% $ZnSO_4$, followed by the addition of 2 ml. 0.3N $Ba(OH)_2$ and centrifugation. An aliquot of the supernatant was evaporated to dryness in a small glass beaker, 2 ml. 99% ethanol added to the residue and left overnight. On the next day, 12 ml. scintillation liquid was added and radioactivity measured in the beaker on a Tracerlab Liquid Scintillation counter.

RESULTS

Three sugars were selected for testing the prevalence of mono-complex or di-complex transport in baker's yeast, viz. D-galactose, D-arabinose and D-xylose, the choice being restricted by three factors: (1) the sugar must be taken up by the cell, (2) it must not be metabolized, (3) it must be available in the radioactive form.

The yeast suspension was always equilibrated with the unlabelled form for 2 hrs. (then the intracellular concentration was found to equal the extracellular one) and then a negligible amount of the radioactive species dissolved in the corresponding concentration of the unlabelled sugar added. Kinetics of uptake of the radioactive form was linear and it was therefore justified to take the reciprocal of the half-time of uptake as the measure of the rate. Galactose formed an exception in that its uptake curves were practically linear and no equilibrium level of radioactive galactose was reached even after 2 hrs. Here, the slopes of uptake curves were used as the measure of uptake velocity. Whether this indicates the beginning of utilization of galactose will not be taken up here.

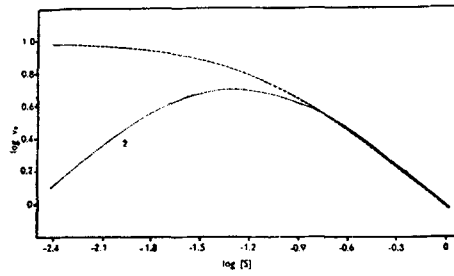

Fig. 1. Theoretical plots of the rate of uptake of a labelled sugar R added in equilibrium to the unlabelled sugar S. Curve 1 — mono-complex theory, K_{CS} = 0.1M; curve 2 — di-complex theory, K_{CS} = 0.025M, K_{CSS} = 0.1M, $D_1 = 0$, $D_2 = 1$.

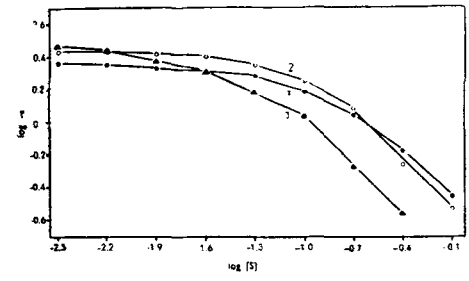

Fig. 2. Experimental values corresponding to Fig. 1 as found for three different sugars in baker's yeast. Curve 1 — D-arabinose; curve 2 — D-xylose; curve 3 — D-galactose.

Fig. 2 shows that for all the three sugars the uptake is mono-complex in character as represented by curve 1 in Fig. 1.

The plot of v_R against (S) makes it possible to determine the K_{CS} of the sugar,

Table 1. Apparent dissociation constants of the sugar-carrier complex in baker's yeast

D-Xylose	0.13—0.14M
D-Arabinose	0.15—0.16M
D-Galactose	0.05—0.07M

simply by extending the linear portions of the curve and reading the substrate concentration at the point of intersection. The K_{CS} values of the three sugars as found here are shown in table 1.

DISCUSSION

The typically mono-complex transport of sugars in baker's yeast demonstrated here is in conformity with views held heretofore and confirms the findings published previously (Kotyk & Kleinzeller, 1963). In the work underlying the earlier publication smooth convex curves for the plot of initial rate of D-xylose uptake against concentration were obtained while an S-shaped curve would be predicted on the basis of the di-complex treatment given here (cf. Wilbrandt & Kotyk, 1964).

The K_{CS} value of the D-xylose-carrier complex found here is lower than the apparent K_m of transport reported in the abovementioned paper for reasons associated most likely with inequal mobilities of the free carrier and the sugar-carrier complex as will be discussed in the following paper of this series.

The mono-complex type of sugar transport in yeast thus differs from that in human erythrocytes. The fact raises the question of the evolutionary significance of this diversity of transporting systems, particularly in view of the established fundamentally different mechanism transporting sugars up-hill in the intestine (Crane, 1960) and kidney (Krane & Crane, 1958; Kleinzeller & Kotyk, 1961), on the one hand, and in microbial species like *Escherichia coli* (e.g. Kepes, 1960) and *Rhodotorula gracilis* (Kotyk & Höfer, in press), on the other. It would appear that, unlike in the case of the basic metabolic pathways, there is no single and universally distributed sugar carrier in all living cells but rather that such molecular entity and mechanism have acquired the sugar-transporting function as were most readily adaptable to the purpose under given environmental (and physiological) conditions.

Before it is justified to venture further into a discussion of the peculiarities of the various carriers more knowledge about their molecular characteristics is necessary.

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СВОЙСТВА ПЕРЕНОСЧИКА САХАРА В ПЕКАРСКИХ ДРОЖЖАХ I. КИНЕТИКА ТРАНСПОРТА

А. Котык

Кинетический тест, разработанный для распознавания транспорта подвижным переносчиком, когда одна или две молекулы субстрата перемещаются одновременно, был использован при изучении транспорта сахара у дрожжей Saccharomyces cerevisiae. Было установлено, что этот механизм включает связывание 1 молекулы сахара с 1 молекулой переносчика. При тестировании были определены константы диссоциации некоторых комплексов сахар-переносчик. — Обсуждается различие механизмов транспорта сахара в различных клетках.