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D-Arabinose Countertransport in Bakers' Yeast

PETER O. WILKINS

Department of Microbiology, New Jersey College of Medicine and Dentistry, Jersey City, New Jersey 07304

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The initial rate of the glucose-induced countertransport of D-arabinose was measured at several concentrations of extracellular glucose. These data permit the calculation of the intracellular concentration of free glucose, and, if the rate of glucose metabolism is known, the maximal rate of glucose transport can be estimated. Since the maximal transport rate remained essentially constant when the extracellular glucose concentration was increased from 2 to 100 mM, the results are consistent with the hypothesis that, during glucose metabolism, glucose is transported across the yeast cell membrane by a symmetrical carrier system which functions independently of metabolism.

In a previous paper (10), some observations were presented on the glucose-induced countertransport of sorbose in bakers' yeast. These data provided indirect estimates of the intracellular glucose concentration under two sets of conditions: (i) when there were various concentrations of extracellular glucose, and (ii) when the rate of glucose metabolism was changed, independently of the extracellular glucose concentration, by altering the oxygen tension of the medium. The results suggested the existence of interactions between glucose transport and metabolism which are not revealed by direct glucose transport experiments involving metabolic inhibitors or nonmetabolizable glucose analogues. However, the nature of the apparent interactions was unexpected and the possibility that they represented artifacts produced by peculiarities in the transport of sorbose required further investigation.

Earlier experiments, e.g., those of Cirillo (1), had shown that sorbose transport includes a very rapid initial uptake equivalent to approximately 15% of the total amount accumulated. This was thought to represent an essentially instantaneous adsorption which did not affect countertransport or contribute to the intracellular sorbose concentration. The kinetic constants for sorbose transport reported by Wilkins and Cirillo (10) were calculated on this basis. Subsequent work in this laboratory indicated that, although most of the initial uptake occurs within 30 sec, it continues at a decreasing velocity for several minutes. Cirillo (*in press*) has shown that the initial uptake of sorbose, and several other nonmetabolizable sugars, is a complex process involving inducible components. He has also measured the time course of the initial uptake at 6-sec intervals with a continuous-flow apparatus (*personal communication*). These observations indicate that sorbose uptake rates derived from measurements at 1-min intervals represent the net effect of two processes and should not be used to calculate constants for substitution in countertransport rate equations.

In the experiments reported here, D-arabinose was the nonmetabolizable sugar. The rapid initial phase was not entirely absent from D-arabinose transport, but, as will be shown, it did not appreciably obscure the initial rates of the principal transport process. Intracellular glucose concentrations, calculated from initial rates of D-arabinose countertransport, increased from 0.9 to 6.9 mM when the extracellular concentration was increased from 5 to 100 mM. These results are compatible with the hypothesis that, in metabolizing yeast, glucose transport is mediated by a symmetrical mobile carrier system.

During glucose metabolism, the intracellular concentration of glucose can be expressed by the following equation (2, 8, 10):

$$\frac{dG_i}{dt} = \frac{T_{go}(G_o)}{G_o + K_{go}} - \frac{T_{gi}(G_i)}{G_i + K_{gi}} - q \qquad (1)$$

where q is the rate of glucose metabolism, G is the glucose concentration, T_g is the maximal glucose transport velocity, K_g is the apparent Michaelis constant for glucose transport, and the subscripts i and o refer, respectively, to inside and outside the cell membrane.

If it is assumed that intracellular glucose reaches a steady-state concentration where $dG_i/dt = 0$, and that glucose entry and exit are symmetrical processes in which $T_{go} = T_{gi}$ and $K_{go} = K_{gi}$, equation 1 can be reduced to

$$T_g = \frac{q}{\frac{G_o}{G_o + K_{go}} - \frac{G_i}{G_i + K_{gi}}} \quad (2)$$

If these assumptions are valid, experimental values for G_i and q should be such that T_g remains constant when G_o is varied. Thus, the determination of T_g can serve as a test for the operation of a symmetrical glucose transport mechanism during glucose metabolism.

The relationship between the intracellular glucose concentration and the countertransport of D-arabinose is shown in equation 3 (6):

$$\frac{dA_i}{dt} = \frac{T_a(A_o)}{A_o + K_a + \frac{K_a(G_o)}{K_{go}}} - \frac{T_a(A_i)}{A_i + K_a + \frac{K_a(G_i)}{K_{gi}}} \quad (3)$$

where A is the concentration of D-arabinose, T_a is the maximal velocity of D-arabinose transport, K_a is the apparent Michaelis constant for D-arabinose transport, and the subscripts o and i refer to outside and inside the cell. If $K_{gi} = K_{go}$, the determination of G_i from equation 3 is experimentally feasible.

MATERIALS AND METHODS

Preparation of yeast cells. The strain of *Saccharomyces cerevisiae* used was isolated from a cake of bakers' yeast (Anheuser-Busch, Inc., St. Louis, Mo.) and maintained on Sabouraud Dextrose Agar (Difco). For transport experiments, growth from a 24-hr slant culture was transferred to 250 ml of a liquid medium containing 1.0% Tryptone (Difco), 0.3% yeast extract (Difco), 2.0% glucose, and 0.4% K_2HPO_4 adjusted to *p*H 7.0. The cells were incubated overnight on a rotary shaker at room temperature. They were harvested and washed by centrifugation in distilled water.

D-Arabinose transport procedures. The reaction mixtures were contained in 50-ml Erlenmeyer flasks which were shaken in a water bath (15 C) throughout the experiment. Each flask contained: cells, 4% (v/v); ^{14}C-labeled D-arabinose (Nuclear-Chicago Corp., Des Plaines, Ill.), 4 μc; and unlabeled D-arabinose (Mann Research Laboratories, Inc., New York, N.Y.) to give the desired final concentration. The total volume was 10 ml. At appropriate intervals, 1-ml samples were transferred by pipette to membrane filters (Millipore Corp., Bedford, Mass.) containing 10 ml of ice-cold water, and the cells were washed on the filter in excess cold water. When partially dry, the membranes with adhering cells were transferred to centrifuge tubes containing 1.5 ml of 70% ethyl alcohol to extract the intracellular arabinose. The samples were then centrifuged and 0.9-ml portions were transferred to concentric planchets and dried under a lamp. The radioactivity of the samples was measured with a gas-flow counter (Baird-Atomic, Inc., Cambridge, Mass.).

In countertransport experiments, the cells were incubated in 300 mM D-arabinose for 2 hr, by which time the intracellular concentration was essentially equal to the extracellular concentration. Glucose was then added to the medium, and the resulting efflux, or countertransport, of arabinose was measured by the method described for determining the rate of arabinose uptake. Glucose solutions used for the induction of countertransport contained the same concentrations of labeled and unlabeled D-arabinose as the medium. Since glucose is metabolized during the countertransport of a nonmetabolizable sugar, the extracellular glucose concentration was kept constant by feeding glucose to the medium from a mechanically driven syringe. This increased the volume of the reaction system by 0.007 ml/min, and a correction for the resulting dilution of the cell suspension was applied to each sample. The rate of glucose feed was estimated from curve 1 in Fig. 1, which is a plot of the reciprocal of the rate of glucose metabolism (measured by the disappearance of glucose from the medium) versus the reciprocal of the glucose concentration. Glucose was determined by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.). Immediately after the removal of each sample, the glucose feed syringe was replaced by one containing 10% less glucose, so that the amount of glucose added remained proportional to the volume of cells in the flask. Ordinarily, five samples were taken to determine the countertransport rate. The feed system was then disconnected and three samples were taken to confirm the extracellular glucose concentration and the metabolic rate. The residue in each flask was centrifuged and the counts per minute per micromole of D-arabinose was determined from the supernatant fluids.

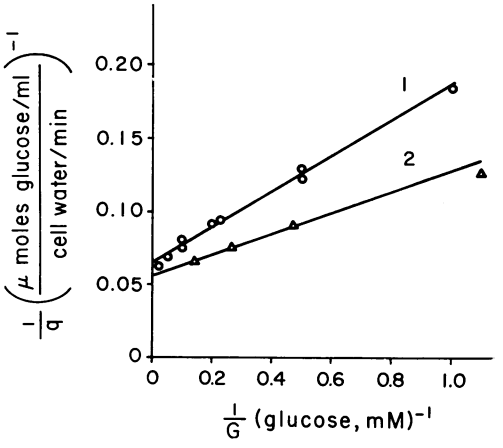

FIG. 1. *Double reciprocal plot of the rate of glucose metabolism (q) versus the glucose concentration (G). Curve 1: G = extracellular glucose. The maximal rate is 15.5 μmoles per ml of cell water per min. The apparent Michaelis constant is 1.8 mM. Curve 2: G = intracellular glucose. The maximal rate is 18 μmoles per ml of cell water per min. The apparent Michaelis constant is 1.3 mM. Temperature is 15 C.*

RESULTS

Calculation of intracellular arabinose. The calculation of molar concentrations of D-arabinose from radioactivity data requires an accurate estimation of the volume of cell water that is available as a solvent for sugars. Kotyk and Kleinzeller (4) examined several conditions which alter the sugar space in bakers' yeast and suggested that the intracellular water volume be set equal to the potentially free space for sugars. A sugar is said to occupy 100% space when it distributes throughout the intracellular water and reaches diffusion equilibrium with the external medium. This procedure was followed in the experiments reported here. At 15 C, the sugar space was usually 67% of the space at 30 C. This is similar to the D-xylose space at 15 C in a production strain of bakers' yeast described by Kotyk and Kleinzeller (4). Since some variation was observed, an extended time course of arabinose uptake was included in most experiments and the plateau, in a plot of intracellular arabinose versus time, was taken as representing the equilibration of internal and external sugar.

Figure 2 shows the first 5 min of uptake of D-arabinose and L-sorbose when the extracellular concentrations were 100, 300, and 1,000 mM. Straight lines drawn through the experimental points were extrapolated to the ordinate to indicate, approximately, the contribution of the initial rapid phase. For D-arabinose, this is no greater than 1.5% of the total accumulation and does not appreciably affect the calculation of the intracellular concentration.

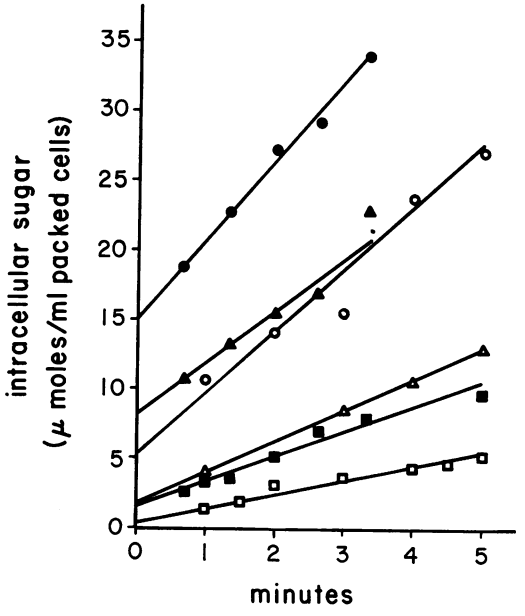

FIG. 2. *Initial uptake of D-arabinose and L-sorbose. Open symbols, D-arabinose. Closed symbols, L-sorbose. Extracellular concentrations: ○ and ●, 1,000 mM; △ and ▲, 300 mM; □ and ■, 100 mM. Temperature is 15 C.*

Determination of kinetic constants. Figure 3 is a Lineweaver-Burk plot of the reciprocal of the initial rate of D-arabinose uptake versus the reciprocal of the extracellular concentration. The rates were calculated from linear plots and extrapolated to the ordinate, similar to those shown in Fig. 2. The apparent Michaelis constant for D-arabinose transport is 800 mM, and the apparent maximal velocity (assuming the D-arabinose space to be 67%) is 26 μmoles per ml of cell water per min. As a test for their validity, these values were used to construct theoretical time course curves which were compared to experimental determinations of arabinose uptake (Fig. 4). The theoretical curves were constructed from the integrated form of the transport equation given by Sen and Widdas (7):

$$\ln \frac{A_o}{A_o - A_i} - \frac{A_i}{A_i + K_a} = \frac{K_a(T_a)}{(A_o + K_a)^2} \quad (t) \quad (4)$$

where t is the uptake time in minutes, and the other symbols are as given for equation 3. The value of t was calculated for arbitrary values of A_i when A_o was 100 mM (curve 1), 200 mM (curve 2), and 300 mM (curve 3). The close agreement between the experimental points and the theoretical curves in Fig. 4 indicates that values

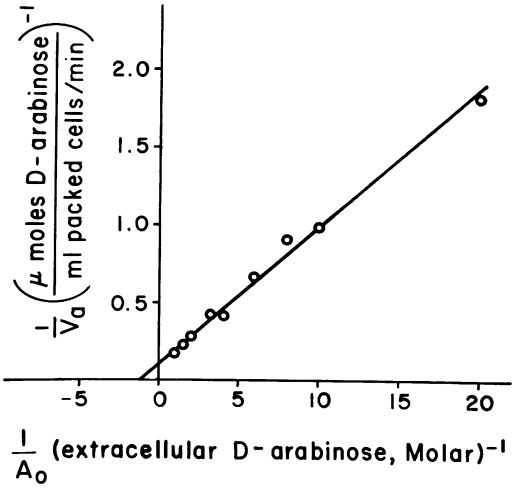

FIG. 3. *Double reciprocal plot of the rate of D-arabinose uptake (v_a) versus the extracellular concentration (A_o). Temperature is 15 C. The apparent maximal transport rate (T_a) is 26 μmoles per ml of cell water per min. The apparent Michaelis constant is 800 mM.*

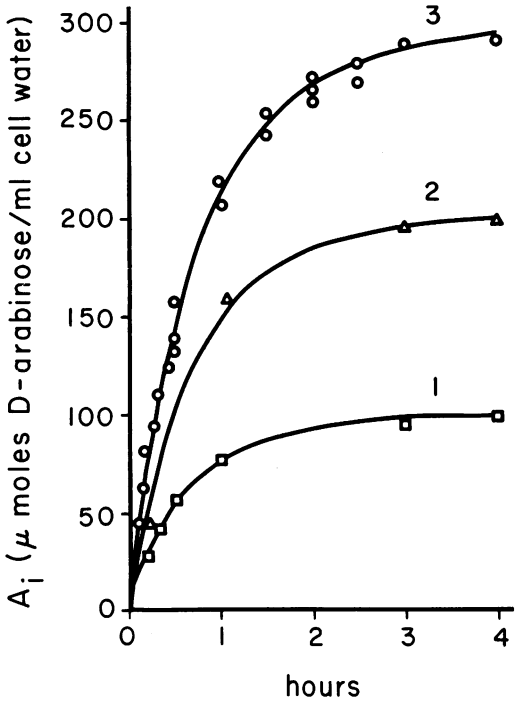

FIG. 4. *Time course of D-arabinose accumulation. The curves were constructed by substituting the values obtained from Fig. 3 for T_a and K_a in equation 4 when A_o was 100 mM (curve 2), 200 mM (curve 2), and 300 mM (curve 3). The points represent experimental values of intracellular D-arabinose expressed as micromoles per milliliter of cell water on the basis that the sugar space at 15 C is 67%.*

for K_a and T_a derived from Fig. 3 are characteristic of D-arabinose transport not only at the beginning of uptake but also in the plateau region where the efflux process is of major significance.

The apparent Michaelis constant for glucose transport (K_{go}) was determined graphically from the inhibition of arabinose transport by glucose (equation 3). Initial rates of arabinose uptake from an extracellular concentration of 300 mM were measured in the presence of several concentrations of glucose during short intervals when the efflux of arabinose and the loss of glucose from the medium were negligible. Under these conditions, a plot of the reciprocal of the rate of arabinose uptake ($1/v_a$) versus the extracellular glucose concentration (G_o) yields a straight line (Fig. 5). When $1/v_a = 0$, equation 3 reduces to

$$K_{go} = -\frac{K_a}{K_a + A_o}(G_o) \qquad (5)$$

where G_o is the glucose concentration at the intercept on the abscissa. Substituting $K_a = 800$ mM, $A_o = 300$ mM, and $G_o = -4.1$ mM in equation 5 yields $K_{go} = 3.0$ mM.

D-arabinose countertransport. Figure 6 shows the time course for the first 45 min of countertransport when A_o was 300 mM and G_o was 2, 5, 10, and 100 mM. Glucose was added to the medium at zero-time on Fig. 6, but the cells had been accumulating arabinose for 2 hr preceding the addition of glucose. According to Fig. 4, the intracellular arabinose concentration (A_i) is close to 270 mM after 2 hr of uptake. Therefore, in converting counts per minute per unit volume of packed cells to micromoles per milliliter of cell water, the original data were multiplied by factors which set A_i at 270 mM at the beginning of countertransport. The average deviation from the mean of the four factors used in the preparation of Fig. 6 is 3%; the greatest deviation is 7%. The rates of countertransport indicated in Fig. 6 are given in Table 1.

Calculation of G_i and T_g. Table 1 shows the concentrations of intracellular glucose (G_i) obtained by substituting in equation 3 the countertransport rates obtained from Fig. 6. The values for the remaining terms in equation 3 were those previously described: $T_a = 26$ μmoles per ml of

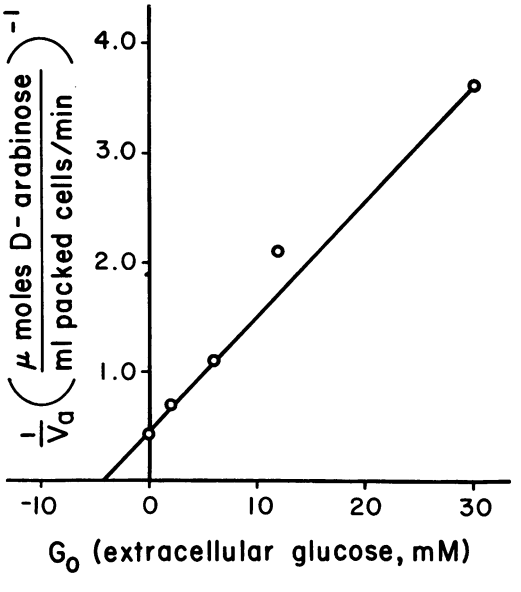

FIG. 5. *Competitive inhibition of D-arabinose transport by glucose. The reciprocal of the initial rate of D-arabinose uptake (v_a) is plotted against the extracellular glucose concentration (G_o). Extracellular D-arabinose, 300 mM. Temperature is 15 C. The apparent Michaelis constant for glucose influx (K_{go}), calculated according to equation 4, is 3.0 mM.*

cell water per min, K_a = 800 mM, K_g = 3 mM, A_o = 300 mM, and A_i = 270 mM.

The values for the apparent maximal rate of glucose transport (T_g) given in Table 1 were determined according to equation 2. The metabolic rates (q) required in this calculation were obtained from Fig. 1. The degree of success in maintaining a constant extracellular glucose concentration during the countertransport experiment is indicated by the difference between the initial and final glucose concentrations shown in Table 1. The discrepancies are not large enough to have a significant effect on the initial rate of countertransport or on the rate of glucose metabolism. When the average of the values found for T_g (53 μmoles per ml of cell water per min) is used in equation 2 to calculate G_i, the calculated values of G_i differ from the experimental values shown in Table 1 by no more than 10%. Since differences of this magnitude represent differences in the rate of countertransport which are within experimental error, the data indicate that T_g remains constant when G_o is increased from 2 to 100 mM.

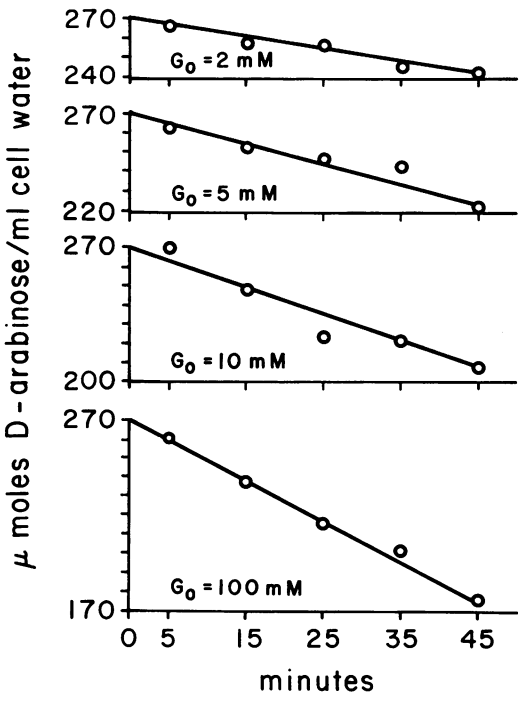

FIG. 6. *D-Arabinose countertransport. The counter transport of previously accumulated D-arabinose was induced by the addition of glucose to the medium at 0 min. The extracellular concentrations of glucose were maintained during the experiment by the addition of glucose from a mechanically driven syringe. Extracellular D-arabinose, 300 mM. Temperature, 15 C.*

TABLE 1. *Intracellular glucose concentration during D-arabinose countertransport*

Extracellular glucose (G_o)[a]		Rate of glucose metabolism (q)[b]	Rate of D-arabinose countertransport (dA_i/dt)[b]	Intracellular glucose (G_i)[c]	Apparent maximal rate of glucose transport (T_g)[b]
Initial	Final				
2.0	1.8	8	−0.6	0.9	47
5.0	4.6	11	−1.1	2.1	52
10.0	8.2	13	−1.4	3.7	59
100	97	15	−2.1	6.9	55

[a] Expressed as millimolar concentration.
[b] Expressed as micromoles per milliliter of cell water per minute.
[c] Expressed as micromoles per milliliter of cell water.

DISCUSSION

These results are consistent with the hypothesis that glucose transport in bakers' yeast is mediated by a symmetrical carrier system which functions independently of metabolism. The rate of influx is greater than the rate of metabolism, but, since efflux maintains the intracellular glucose concentration at a relatively low level, the net effect of transport is to limit the rate of metabolism. Curve 2 in Fig. 1 is a double reciprocal plot of the rate of metabolism (q) versus the intracellular glucose concentration (G_i). This curve indicates that, within the cell, the apparent Michaelis constant for glucose metabolism is 1.3 mM and the maximal rate is 18 μmoles per ml of cell water per min. However, since G_i does not increase above 7 mM, the rate of metabolism does not exceed 85% of its theoretical maximum. At lower concentrations of G_o, the rate-limiting effect of transport on metabolism is greater. When G_o is 2 mM, the rate of metabolism is 70% of the rate which would occur if G_i equilibrated with G_o.

The assumptions used in the derivation of equation 3 predict that the maximal transport rate should be the same for glucose and arabinose. This prediction is not borne out since T_g is approximately twice as rapid as T_a. The data do not provide an explanation for this difference. It can be shown, by setting $T_g = T_a$ and combining equations 2 and 3, that the rates of countertransport given in Table 1 are not compatible with a single value for T_g and T_a (if the other parameters are accepted as given) since the values of G_i thus obtained are impossible. If the countertransport rates were approximately twice as rapid, K_g would have the value found for K_a—26 μmoles per ml of cell water per min. Wilbrandt (9) noted differences in the maximal transport rate among some

of the sugars which use the glucose carrier system in red cells. Variations in the kinetic parameters of sugar uptake were also reported by Kotyk and Höfer (3) for *Rhodotorula gracilis* which transports sugars against a concentration difference. The theoretical implications of variations of this nature have been discussed by Wilbrandt (9) and by Regen and Morgan (5).

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