Sorbose Counterflow as a Measure of Intracellular Glucose in Baker’s Yeast

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ABSTRACT

Wilkins, Peter O. (New Jersey College of Medicine and Dentistry, Jersey City), and Vincent P. Cirillo. Sorbose counterflow as a measure of intracellular glucose in baker’s yeast. J. Bacteriol. 90:1605–1610. 1965.—The intracellular concentration of glucose in metabolizing baker’s yeast was determined indirectly from the glucose-induced counterflow of previously accumulated sorbose. The method is based on the concept that sugar transport in yeast is a symmetrical facilitated diffusion. The intracellular glucose concentration increased with an increase in the extracellular concentration and was higher in aerobiosis than in anaerobiosis. The concentrations were considerably greater than those obtained by direct analysis of intracellular glucose. Calculation of the apparent maximal velocity of glucose transport yielded values which varied with the rate of metabolism and the extracellular concentration. This suggests that during glucose metabolism the transport of hexoses includes elements that are not revealed by experiments involving metabolic inhibitors or nonmetabolizable sugars.

The transport of glucose across the cell membrane of baker’s yeast has been characterized by Cirillo (1962) as a carrier-mediated facilitated diffusion in which net transport is the result of independent influx and efflux processes each of which conforms to the rate equation for membrane transport given by Wilbrandt (1954):

\[ v = T \left( \frac{G}{G + K_s} \right) \]  \hspace{1cm} \text{(1)}

where \( v \) is the velocity of glucose transport, \( T \) is the maximal transport velocity, \( K_s \) is the apparent Michaelis constant for transport, and \( G \) is the concentration of glucose either outside the cell (for influx) or inside the cell (for efflux).

Thus, during glucose metabolism, the change in the intracellular concentration of glucose can be expressed as the rate of influx minus the rate of efflux minus the rate of metabolism as shown in

\[ \frac{dG_i}{dt} = T \left( \frac{G_0}{G_0 + K_{so}} \right) - T \left( \frac{G_i}{G_i + K_{si}} \right) - q \] \hspace{1cm} \text{(2)}

where \( q \) is the rate of glucose metabolism, and the subscripts \( i \) and \( o \) refer, respectively, to inside and outside the cell membrane.

Cirillo (1962) showed that, when glucose metabolism in yeast is blocked by exposing the cells to iodoacetate in a nitrogen atmosphere, the intracellular concentration of free glucose increases until it approaches the extracellular concentration. Under these conditions, influx and efflux are symmetrical processes in which \( K_{si} = K_{so} \) and \( T_{si} = T_{so} \). In the absence of metabolic inhibitors, intracellular glucose does not equilibrate with the extracellular concentration. Kotyk (1961) found that, when the external concentration was 5%, the internal concentration, after a brief initial peak, was 0.05%. Other investigators have failed to demonstrate any significant concentration of glucose in metabolizing cells (Burger and Hejtmova, 1961a; Cirillo, 1962). These results indicate that, during metabolism, the steady-state concentration of intracellular glucose may approach zero, and membrane transport, therefore, is the rate-limiting step in glucose utilization. However, Solis (1961) pointed out that intracellular glucose at a concentration sufficient to saturate hexokinase in yeast would be metabolized within a few seconds at room temperature. This suggests that measurements of intracellular glucose by direct analytical procedures are likely to yield erroneously low values.

An indirect method which circumvents this problem is presented by the glucose-induced counterflow of intracellular sorbose. Since sorbose
enters the yeast cell by facilitated diffusion but is not phosphorylated, the intracellular concentration of free sorbose increases until it equals the extracellular concentration. If glucose is added to yeast suspended in a solution of sorbose, previously accumulated sorbose will flow out of the cells at a rate which is a function of the intracellular glucose concentration. This countercflow of sorbose is a consequence of the fact that glucose and sorbose compete for the same transport system. Since glucose is metabolized, the intracellular concentration of free glucose is always lower than the extracellular concentration. The inhibition of sorbose transport by glucose is therefore less at the inner surface of the membrane than at the outer surface, and a net efflux of sorbose results.

The relationship of the intracellular glucose concentration to the rate of sorbose countercflow is shown in equation 3, which conforms to the equation for competitive inhibition of transport given by Rosenberg and Wilbrandt (1937): 

\[
\frac{dS_i}{dt} = \frac{T_e(S_e)}{S_e + K_e + \frac{K_s}{K_g}G_o} - \frac{T_i(S_i)}{S_i + K_i + \frac{K_s}{K_g}G_i}
\]  

(3)

where \(S_e\) is the extracellular concentration of sorbose, \(S_i\) is the intracellular concentration, \(T_e\) is the maximal rate of sorbose transport, and \(K_s\) is the apparent Michaelis constant for sorbose transport.

All of the terms in equation 3, with the exception of \(G_i\) and \(K_{ei}\), can be measured independently. Thus, if one assumes, as a working hypothesis consistent with the definition of facilitated diffusion, that \(K_{ei} = K_{go}\), and if \(G_i\), \(G_o\), and \(S_e\) remain constant, the intracellular glucose concentration can be determined. A simpler relationship, and one which obviates certain technical problems, can be obtained by setting \(\frac{dS_i}{dt} = 0\) in equation 3. This represents the counterflow steady state when net sorbose efflux has stopped and \(S_i\) is constant. Under these conditions,

\[
G_i = \frac{S_i}{S_o}(G_o + K_{go}) - K_{go}
\]  

(4)

Under the same steady-state conditions, and with the further assumption that \(T_{ei} = T_{go}\) during glucose metabolism, the intracellular glucose concentration can also be obtained from equation 2 by setting \(\frac{dG_i}{dt} = 0\). Thus

\[
G_i = \frac{K_{go}}{G_o + K_{go} - q} T_{go}
\]  

(5)

It is not possible to determine \(T_{go}\) independently, but it can be evaluated by substituting in equation 5 values for \(G_i\) obtained from either the initial rate or the steady-state level of sorbose countercflow. In the experiments reported here, this was done under two sets of conditions: (i) at various extracellular concentrations of glucose, and (ii) when the rate of metabolism was changed by altering the oxygen tension of the medium. If glucose transport in metabolizing yeast is completely described by equation 5, \(T_{go}\) should have the same value for all conditions tested. The fact that this did not occur suggests either that glucose transport involves interactions with metabolism which are not revealed when metabolic inhibitors or nonmetabolizable glucose analogues are used, or that, because of peculiarities in the uptake of sorbose, the counterflow steady-state technique for determining intracellular glucose is inaccurate.

**MATERIALS AND METHODS**

**Preparation of yeast cells.** Commercial baker's yeast (Anheuser-Busch Inc., St. Louis, Mo.) was washed by repeated centrifugation in distilled water until the supernatant fluid was clear. The packed cell volume was determined after centrifugation for 5 min at 3,000 \(\times \ g\), and a suspension was prepared that would give a concentration of 0.5% (v/v) in a 100-ml reaction system.

**Sugar transport procedures.** The reaction mixture was contained in a 500-ml Erlenmeyer flask fitted with a rubber stopper through which syringe needles were passed to serve as ports for the addition of sugar solutions and the removal of samples. The stopper also supported a stirring mechanism. Vigorous stirring, with free access to air, maintained an aerobic environment. Anaerobic conditions were provided by bubbling nitrogen through the system. The flask was immersed in a 15 C water bath since, at higher temperatures, the initial rates of sorbose transport were too rapid for satisfactory measurement.

In counterflow experiments, the cells accumulated sorbose for 1 hr from an extracellular concentration of 300 mm. Sorbose countercflow was then induced by adding glucose to the medium. The initial extracellular concentration of glucose (\(G_o\)) was maintained during the counterflow period by feeding glucose to the reaction mixture from a mechanically driven syringe. The rate of feed required to maintain a desired \(G_o\) was estimated...
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of sorbose in the cell extracts was expressed as micromoles per milliliter of cell water. The calculations required the consideration of (i) the decrease in cell density caused by feeding glucose to the medium, (ii) the fraction of accumulated sorbose that is osmotically free, and (iii) the volume of intracellular water. In most experiments, the glucose feed rate was 1.68 ml/hr. Thus, a 5-ml sample taken at 5 hr contains 8.4% fewer cells than a zero-time sample. In the experiments reported here, from 10 to 15% of the sorbose measured in the cell extracts was taken up by a rapid, temperature-independent process. Since this fraction is not displaced when glucose is added to the medium, it was not included in the calculation of the intracellular concentration of free sorbose. The intracellular volume of water that is available as a solvent for sugars represents 47% of the packed-cell volume at 30°C (Cirillo, 1962). At 15°C, however, the plateau in the time-course curve for sorbose transport, which represents the equilibration of internal and external sorbose, indicates that the intracellular volume is 33% of the packed-cell volume.

RESULTS

Determination of kinetic constants. The apparent Michaelis constant for glucose transport ($K_{m0}$)

from Fig. 1, which is a Lineweaver-Burk plot of the reciprocal of the rate of glucose metabolism (measured by the disappearance of glucose from the medium) versus $1/G_0$. Experiments in which the actual rate of metabolism differed by more than 10% from the estimated rate were discarded. Thus, the rate of change in $G_s$ during the counterclockwise period was no greater than 3% per hour when $G_s = 5\text{mM}$, and 0.2% per hour when $G_s = 100\text{mM}$. The counterclockwise steady state was assumed to exist when there was no significant difference between two determinations of $S_1$ spaced 1 hr apart.

At the end of the experimental period, several 5-ml samples were removed by syringe and ejected into centrifuge tubes containing 0.5 ml of 20 mM uranyl nitrate to stop the transport processes. After centrifugation, the supernatant fractions were analyzed to determine $G_u$. The packed cells were suspended and washed in 1 mM uranyl nitrate as described by Cirillo and Wilkins (1964). The washed cells were suspended in 5 ml of distilled water and boiled for 20 min to extract the intracellular sorbose.

Chemical analyses. Glucose was analyzed by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.). Sorbose was determined by the method of Diachte and Devi (1960).

Calculation of intracellular sorbose. The amount
was determined from the inhibition of sorbose transport by glucose as described by Cirillo (1962). Sorbose uptake in the presence of several concentrations of glucose was measured over short time intervals during which the uptake rates were linear and the loss of glucose from the medium through metabolism was negligible. Figure 2 shows the reciprocal of the rate of sorbose uptake, under aerobic and anaerobic conditions, plotted against the glucose concentration. The curves were drawn by the method of least squares. Their relationship to \( K_{go} \) is given by

\[
K_{go} = -\frac{K_s}{K_s + S_o} \quad (G_o)
\]

where \( G_o \) is the glucose concentration at the intercept on the abscissa, \( S_o \) is the extracellular sorbose concentration, and \( K_s \) is the apparent Michaelis constant for sorbose transport.

The aerobic and anaerobic values of \( G_o \) in Fig. 2 are \(-6.5\) and \(-5.9\) mM, respectively. Since the difference between these values is within experimental error, both curves were assumed, in the calculation of \( K_{go} \), to intercept the abscissa at \(-6.1\) mM. Substitution of \( G_o = -6.1\) mM, \( S_o = 300\) mM, and \( K_s = 244\) mM in equation 6 yields a value of 2.8 mM for \( K_{go} \).

\( K_w \) was determined from the Lineweaver-Burk plot of the reciprocal of the rate of sorbose uptake versus \( 1/S_o \) shown in Fig. 3. The circles in Fig. 3 represent aerobic conditions; the triangles represent anaerobic conditions. Since curves drawn through both sets of points by the method of least squares are superimposable, sorbose transport is independent of the oxygen tension of the medium.

**Determination of intracellular glucose.** According to equation 5, the intracellular concentration of glucose \( G_1 \) is a function of the rate of glucose metabolism \( q \) and the extracellular glucose concentration \( G_o \). \( G_1 \) was determined under two sets of conditions: in one series of experiments, \( q \) was changed independently of \( G_o \); in the other series, \( q \) changed as a result of changing \( G_o \).

In the experiments shown in Table 1, a twofold change in \( q \) was obtained by manipulating the oxygen tension of the medium. \( G_o \) was maintained at 4 mM, since this was the optimal concentration in a narrow range where the Pasteur effect could be demonstrated. Under anaerobic conditions, the counterflow steady-state concentration of intracellular sorbose \( (S_i) \) was 10% lower than in the corresponding aerobic determination. The values given for \( S_i \) are averages obtained from the analysis of four samples. Since the average

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<th>Table 1. Intracellular glucose concentration during aerobic and anaerobic metabolism*</th>
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* The reaction mixture was stirred vigorously to provide aerobic conditions and was saturated with \( N_a \) for anaerobic conditions. Intracellular sorbose was measured during the counterflow steady state. The intracellular glucose concentration and the apparent maximal velocity of glucose transport were calculated according to equations 4 and 5.

† Expressed as micromoles per milliliter of cell water per minute.

‡ Expressed as micromoles per milliliter of cell water.
deviation among the replicates was less than 3%.

A change in $G_i$ is compatible with the assumptions pertaining to equation 5, but a change in $T_g$ is not, since by definition $T_g$ is a constant, independent of $q$. The difference between the aerobic and anaerobic values of $T_g$ is greater than would be expected to result from experimental error. In order for the aerobic values of $T_g$ to be the same as the anaerobic values shown in Table 1, the aerobic values of $S_i$ would have to be 10% higher than those observed.

Figure 4 shows $S_i$ at various concentrations of $G_o$. In these experiments, the reaction mixtures were stirred at a constant rate in the presence of air so that $q$ changed only as a function of $G_o$ as shown in Fig. 1. The concentration of glucose which half saturates metabolism, according to Fig. 1, is 6.4 mM. Thus, in Fig. 4, $G_o$ ranges from approximately 0.5 to 20 times the Michaelis constant for glucose utilization. Determinations of $S_i$, when $G_o$ was increased to 200 mM, were not reproducible. At high glucose concentrations, significant deviations from the predicted rate of metabolism are difficult to detect, and, since $S_i$ is approaching its minimal value, errors introduced by the initial, irreversible adsorption of sorbose become increasingly important.

Values of $S_i$ taken from the curve in Fig. 4 were used to construct the corresponding curves for $G_i$ and $T_g$ shown in Fig. 5. Extrapolation of the $G_i$ curve indicates that it reaches a maximum of approximately 27 $\mu$moles/ml of cell water when $G_o$ is increased to infinity. $T_g$ increases similarly from 25 to 250 $\mu$moles per ml of cell water per min.

**DISCUSSION**

Taken at face value, these results indicate that, while $G_i$ does not equilibrate with $G_o$ during metabolism, its concentration is much higher than has been previously thought. This, and the observation that the apparent Michaelis constant for transport is smaller than the Michaelis constant for metabolism (Fig. 1 and 2), would argue against the conclusion of Burger and Hejmova (1961b) that transport is the rate-limiting step in glucose utilization. However, since the present method is indirect and dependent on several assumptions, the results are not conclusive.

The increase in $T_g$ which occurs when $q$ is increased, either independently of $G_o$, or as a function of $G_o$, suggests that changes in metabolism are linked to changes in the effective number of carriers that are available for glucose transport.
Other explanations, however, are equally probable. An apparent change in \( T_g \) might actually represent changes in the ratios of \( T_{go} \) to \( T_{gi} \), or of \( K_{go} \) to \( K_{gi} \), in equations 2 and 3. These ratios cannot be calculated from the data given here. Asymmetries of this kind in sugar transport have been studied with muscle preparations by Narahara and Özand (1963), but yeast cells have not yet proved amenable to similar treatment.

It is also possible that sugar transport in yeast is regulated, in part, by factors not included in equations 2 and 3. Sols et al. (1963) found that the level of glucose-6-phosphate in yeast was higher in aerobicosis than in anaerobiosis, but free intracellular glucose was always below saturation of hexokinase. They suggested that the end product of the pathway from extracellular glucose to glucose-6-phosphate controls the rate of glucose transport. Kipnis and Cort (1969) showed that 2-deoxy-6-phosphoglucone inhibited the transport of glucose, 2-deoxyglucose, and mannose in rat diaphragm. The inhibition was non-competitive and functioned at the inner surface of the membrane only. It is not known whether 2-deoxy-6-phosphoglucone inhibits sugar transport in yeast. Scharf (1961) found that 2-deoxyglucose inhibited glucose metabolism in yeast, but that 2-deoxy-6-phosphoglucone in the external medium did not. He concluded that the inhibitory effects of 2-deoxyglucose were best explained as effects on transport, but the possibility that 2-deoxy-6-phosphoglucone may function as an intracellular inhibitor remains to be investigated.

The inhibition of sugar transport by the feedback of hexose phosphate does not readily explain the results reported here, because the predicted changes in \( G_1 \) resulting from such inhibition are in the opposite direction to the changes observed. One might speculate, however, that although intracellular hexose phosphates may have a negligible effect on glucose transport, they may have sufficient affinity for the carrier to compete effectively at the inner surface of the membrane with a low-affinity substrate such as sorbose. Thus, if intracellular glucose-6-phosphate increases with an increase in \( G_o \), as suggested by the experiments on glucosamine transport of Burger and Hejmoa (1961b), a plot of \( S_1 \) versus \( G_o \) (Fig. 4) would plateau at a higher level than would be predicted from the initial part of the curve. If this is true, the minimal value of \( T_g \) shown in Fig. 5 (25 \( \mu \)moles per ml of cell water per min) may represent the true maximal velocity of glucose transport. Substitution of 25 for \( T_g \) in equation 5 yields values for \( G_1 \) that remain close to 0.5 \( \mu \)moles/ml of cell water when \( G_o \) is increased from 5 to 100 mM.

Because of peculiarities in the transport of sorbose, it is conceivable that the sorbose counterflow technique might yield erroneous values for the intracellular glucose concentration. This possibility is now being investigated with the use of other nonmetabolizable sugars which have different transport characteristics.

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**Literature Cited**


