JOURNAL OF BACTERIOLOGY, Nov. 1979, p. 395-399
0021-9193/79/11-0395/05$02.00/0

Vol. 140, No. 2

Sucrose Transport by the *Escherichia coli* Lactose Carrier

KLAUS B. HELLER* AND T. HASTINGS WILSON

Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

Received for publication 15 May 1979

Several lines of evidence suggest that sucrose is transported by the lactose carrier of *Escherichia coli*. Entry of sucrose was monitored by an osmotic method which involves exposure of cells to a hyperosmotic solution of disaccharide (250 mM). Such cells shrink (optical density rises), and if the solute enters the cell, there is a return toward initial values (optical density falls). By this technique sucrose was found to enter cells at a rate approximately one third that of lactose. In addition, the entry of [^{14}C]sucrose was followed by direct analysis of cell contents after separation of cells from the medium by centrifugation. Sucrose accumulated within the cell to a concentration 160% of that in the external medium. The addition of sucrose to an anaerobic suspension of cells resulted in a small alkalinization of the external medium. These data are consistent with the view that the lactose carrier can accumulate sucrose by a proton cotransport system. The carrier exhibits a very low affinity for the disaccharide (150 mM) but a moderately rapid V_{max}.

Whereas several microorganisms transport and metabolize sucrose, *Escherichia coli* is completely sucrose negative. The impermeability of the plasma membrane of *E. coli* to this disaccharide is well known. This failure of sucrose to enter the cell has been used to advantage for osmotic stabilization of membrane vesicles (7), and Stock et al. (14) showed it to be an excellent marker for measurement of the water space external to the cytoplasmic membrane. It has therefore come as a surprise to discover that sucrose is a substrate for the lactose transport system.

MATERIALS AND METHODS

Strains. *E. coli* K-12 strain RE16 (*melB lacI*$^+\Delta ZY$ Str) was kindly provided by Jane Lopilato. The lactose-positive derivative RE16/F'*lac* (*melB lacI*$^+\Delta ZY$ Str/F'*lacIZ*$^+$ Y$^+$) was constructed. The proton symport experiments were conducted with strain GN2 [*melB*(Ts) *lacIZ*$^+$Y$^+$ *ptsI glk*] (5), which was kindly provided by D. Fraenkel.

Growth conditions. Cells were grown in medium 63 (2) supplemented with 1% tryptone (Difco) as the carbon source and 1 mM isopropyl-1-thio-β-D-galactopyranoside at 37°C. Cells were harvested after approximately three doublings. Cells were washed twice with 50 mM morpholinopropanesulfonic acid buffer (pH 7.0) to give a final optical density at 600 nm of about 3.0 (approximately 3.0 mg [dry weight] per ml).

Sucrose, inulin, and water spaces. The intracellular concentration of [^{14}C]sucrose was determined by a centrifugation technique (4) in which extracellular, periplasmic, and intracellular spaces were estimated with the use of appropriate chemical probes. This procedure is superior to the filtration method, for it requires no washing and the space of the remaining contaminating water can be estimated quantitatively. Cells of RE16 or RE16/F'*lac* (10 mg [dry weight] per ml) were exposed either to [^{14}C]inulin plus [^{3}H]H_2O or to [^{14}C]sucrose plus [^{3}H]H_2O. Samples (1 ml) were taken in duplicate at various intervals and layered on the top of 0.5 ml of silicone oil mixture (75% 550 fluid plus 25% 510 fluid; Dow-Corning, Lansing, Mich.) in 1.5-ml plastic microfuge tubes. After centrifugation for 1 min, the supernatant aqueous medium and part of the silicone oil were carefully removed, and the tip of the tube containing the cell pellet was cut off with a razor blade, placed in a scintillation vial containing 1 ml of 1 N NaOH, and incubated at room temperature with occasional shaking for about 30 min to suspend the cells. After neutralization with 2 N HCl, 9 ml of scintillation fluid (7.2 g of 2,5-diphenyloxazole [PPO], 1,200 ml of toluene, and 600 ml of Triton X-100 [10]) was added. We assume that the distribution of inulin and water in the pellet reflects the distribution in the test suspension and is not due to unspecific binding. The sucrose distribution was used to measure the space external to the plasma membrane in the transport-negative cell (RE16), since the disaccharide does not penetrate the membrane of *E. coli* (14). Inulin cannot cross the outer cell wall of *E. coli*, and therefore its distribution was taken as a measure of extracellular space. The difference between the sucrose space in the Y cell and the inulin space in this cell was taken as a measure of the periplasmic space. In these experiments it was assumed that the relative size of the periplasmic space of Y$^+$ and the Y cells was the same.

Permeation measured by the optical technique. Permeation of the carbohydrates was monitored by a photometric method which is based upon changes in light scattering resulting from plasmolysis (shrinkage) and deplasmolysis (reswelling) (8, 12). One milliliter of a 250 mM carbohydrate solution in 50 mM

morpholinopropanesulfonic acid (pH 7) was placed in a small cuvette and read in a Gilford spectrophotometer. A 0.10-ml sample of a concentrated cell suspension was added to the cuvette to give a final optical density at 600 nm between 0.8 and 1.0 and was mixed with a Pasteur pipette. The optical density was continuously monitored with a recorder attached to the spectrophotometer. Full scale of the recorder was set at 0.5, and an "off set" of 0.6 was routinely used after addition of cells so that the base line and the cell reading could be displayed on the same record. The difference between the reading after shrinkage in 125 mM NaCl (which does not enter cells readily) and the reading after dilution in 250 mM glycerol (which equilibrates rapidly [12]) was used for standardization ("total shrinkage"). The time span between addition of cells and reswelling to 50% of the total shrinkage due to substrate permeation is expressed as $T_{1/2}$ (12). The initial velocity of permeation is given as the change of optical density at 600 nm expressed as percent of total shrinkage per minute. Inhibitors and substrate analogs were added to the substrate solution before addition of the cells.

pH measurement. Proton movement was measured as described by West (16). Logarithmically grown cells were washed three times with 90 mM NaCl + 30 mM NaSCN and resuspended in this solution to give an optical density at 600 nm of about 3.0. This cell suspension was bubbled for at least 30 min with oxygen-free nitrogen in a narrow glass vessel containing a combined pH electrode. Small volumes of oxygen-free sugar solutions were then added, and the change of pH was recorded.

Chemicals. Methyl-β-D-thiogalactoside (TMG), thiodigalactoside (TDG), *p*-nitrophenyl-α-D-galactopyranoside, isopropyl-1-thio-β-D-galactopyranoside, and *p*-chloromercuriphenyl sulfonic acid were obtained from Sigma Chemical Co. *N*-Ethylmaleimide was obtained from Schwarz-Mann. Radioactive compounds and PPO were purchased from New England Nuclear Corp. [^{14}C]sucrose and [^{14}C]inulin were purified by means of paper chromatography before use. All other chemicals were of the highest purity commercially available.

RESULTS

Sugar entry measured with an optical method. The first series of permeability experiments was carried out with an osmotic technique in which cells were exposed to a hypertonic solution of carbohydrate. Initial rapid shrinkage (plasmolysis) was due to osmotic water movement and resulted in an increase in light scattering with a resulting increase in optical density. If entry of the carbohydrate occurred, the initial shrinkage was followed by a reswelling back to its initial volume, the rigid cell wall preventing further water entry. This swelling produced a decrease in light scattering and a fall in optical density. Since the rate-limiting step in the swelling process was sugar entry, the decrease in optical density was taken as an indirect measure of sugar permeability.

The results of representative experiments are shown in Fig. 1. Cells induced for the lactose transport system were quickly diluted into 250 mM lactose. The optical density observed 10 s later represented shrinkage of the cells, since it was similar to that obtained with cells exposed to hyperosmotic solutions of slowly penetrating substances such as NaCl. After this shrinkage, the optical density declined as sugar and water entered the cells. As a control, cells possessing no lactose transport system (*lacY*) were treated in a similar manner (upper curve in Fig. 1). After the initial shrinkage of such cells, there was only a slow reswelling, which is presumed to be due to some unrelated event (perhaps ionic) since it is seen with the other slowly penetrating substances such as NaCl. Lactose entry by this technique was also observed in transport-positive, β-galactosidase-negative (ZY^+) strains (data not shown).

When the experiment with the Y^+ cell was repeated with sucrose, a record similar to that of lactose, although slower in rate, was obtained (Fig. 1). The *Y* gene product was implicated in sucrose uptake, since the *Y* cell showed very slow swelling when exposed to this disaccharide.

The effects of several inhibitors on lactose and sucrose transport are shown in Table 1. Three lactose analogs known to possess affinity for the lactose carrier were found to inhibit sucrose entry. Sucrose transport was far more sensitive to these sugars than was lactose transport, suggesting a poor affinity of the carrier for sucrose. In addition, reagents which react with sulfhydryl groups blocked the transport of both lactose and sucrose.

Several other sugars were tested with this

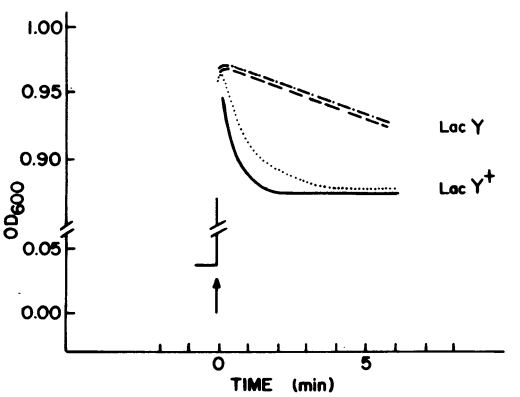

FIG. 1. *Optical changes associated with shrinkage and swelling of E. coli cells exposed to lactose and sucrose. Addition of cells (at the arrow) to a solution of 250 mM disaccharide resulted in a sudden increase in optical density.* (——, - - -) *lactose;* (····, ·-·-) *sucrose.*

technique (Table 2). Rapid entry of TMG, galactose, D-fucose, and cellobiose was observed. TDG, which is known to be a substrate but to be transported slowly, did not show rapid enough movement to be detected by this method. Likewise melibiose, a known substrate, showed only very slow entry in this assay.

Identification of intracellular sucrose. It was important to confirm the results of the original experiments by identifying sucrose within the cell. Since sucrose is non-metabolizable in *E. coli*, it was possible to follow radioactive sucrose and measure its distribution between intracellular and extracellular compartments. Cells were incubated in the presence of [^{14}C]sucrose plus [^{3}H]H_2O and, in parallel experiments, [^{14}C]inulin plus [^{3}H]H_2O. The cells were separated from the medium by centrifugation through silicone oil, and the pellet was analyzed for ^{3}H and ^{14}C. Inulin does not penetrate the outer membrane of *E. coli*, and sucrose does not penetrate the cytoplasmic membrane of a *lacY*-deleted strain. The difference between total water space and the sucrose space of the *Y* cell represented intracellular water space. The difference between the inulin space and the sucrose space in the *Y* cell was taken as the periplasmic space (14). Assuming that the periplasmic space was the same in both strains, the intracellular space of the Y^+ strain was calculated, and the intracellular sucrose concentration was determined. Figure 2 shows the mean value of five experiments in which cells were exposed to 50 mM [^{14}C]sucrose. During a 90-min incubation the disaccharide accumulated in the intracellular water to a concentration 1.6 times that in the external medium. The addition of 1 mM TDG, a galactoside with a high affinity for the lactose carrier, strongly inhibited sucrose entry into the cell. The sucrose space in the *Y* cell did not change with time. These experiments support the view that sucrose is transported by the lactose carrier.

Sucrose as an inhibitor of TMG uptake. Sucrose was found to inhibit the transport of the lactose analog TMG. At a concentration of 100

TABLE 1. *Inhibition of lactose and sucrose transport into RE16/F'lac by lactose analogs and SH-group blocking agents*[a]

Inhibitor[b]	$T_{1/2}$ (min)	
	Lactose	Sucrose
Control (no inhibitor)	0.50	1.3
TMG		
100 mM	0.60	2.3
TDG		
1 mM	—[c]	2.9
5 mM	0.55	∞
α-pNPG		
0.01 mM	0.55	2.9
0.5 mM	1.00	∞
NEM		
0.2 mM	0.90	1.8
0.5 mM	1.20	5.1
pCMP-SA		
0.04 mM	0.55	6.0
0.2 mM	0.90	∞

[a] The inhibitors were added to the 250 mM substrate solution prior to addition of cells. The $T_{1/2}$ was the half-time for equilibration (see Materials and Methods).

[b] α-pNPG, *p*-Nitrophenyl-α-D-galactopyranoside; NEM, *N*-ethylmaleimide; pCMP-SA, *p*-chloromercuriphenyl sulfonic acid.

[c] —, Not determined.

TABLE 2. *Rate of sugar entry into RE16/F'lac*[a]

Substrate	$T_{1/2}$ (min)	Initial velocity (Δ% total shrinkage per min)
L-Arabinose	∞	0
L-Ribose	>30	5
L-Xylose	3.0	5
Fructose	>30	1
D-Fucose	1.4	16
L-Fucose	3.6	8
Galactose	1.7	12
Glucose	>30	3
Mannose	>30	3
Rhamnose	2.0	9
Sorbose	∞	0
Cellobiose	0.7	12
Gentiobiose	>30	3
Lactose	0.5	61
TMG	0.8	20
TDG	>30	8
Maltose	∞	0
Melibiose	11.0	7
Sucrose	1.3	22
Trehalose	∞	0
Maltotriose	∞	0
Melizitose	∞	0
Raffinose	5.5	5
Stachyose	∞	0

[a] $T_{1/2}$ and initial velocity were calculated from the change of the optical density after addition of cells to 250 mM substrate solutions (see Materials and Methods). Values given represent the mean of at least three independent experiments.

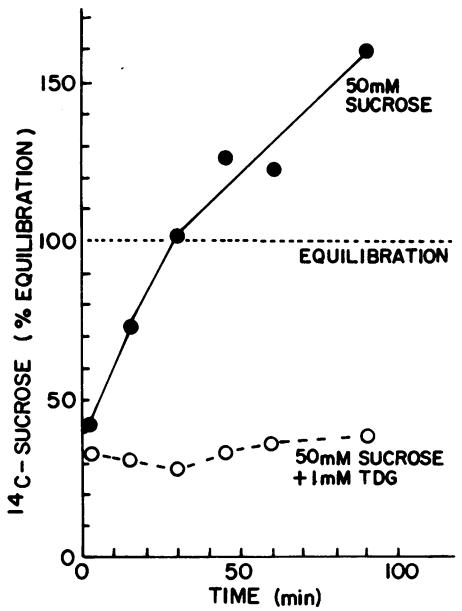

FIG. 2. *Accumulation of [^{14}C]sucrose by strain RE16/F'lac and the inhibition by TDG. [^{14}C]sucrose was purified by chromatography shortly before use. Equilibration of 100% indicates equal concentrations of sucrose in the intracellular and extracellular compartments. The results represent the mean values of five independent experiments. The mean value for the water space was 6.9 μl, that for the inulin space was 1.8 μl, and that for the sucrose space (in RE16) was 3.9 μl.*

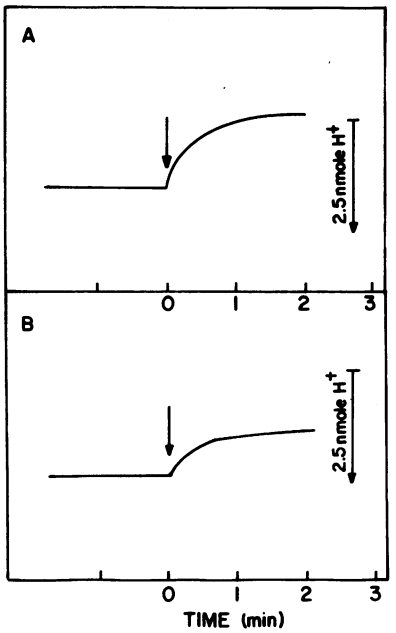

FIG. 3. *Effect of TMG and sucrose on the pH of a suspension of energy-depleted cells. The pH was measured in anaerobic suspension of E. coli strain GN2. Anaerobic samples of TMG (A) (final concentration, 5 mM) and sucrose (B) (final concentration, 150 mM) were added at the arrow.*

mM, sucrose showed a 30% inhibition of [^{14}C]-TMG (0.1 mM) transport; 250 mM disaccharide inhibited 80%. In parallel tubes, 50 mM and 125 mM NaCl were added to control for the osmotic effect of the disaccharide. Assuming simple competitive inhibition between sucrose and TMG, one can estimate that the K_m of the sucrose is of the order of 150 mM.

Stimulation of proton uptake by addition of sucrose. Lactose is transported into *E. coli* with concomitant proton uptake (16). If sucrose is taken up by the same transport system (as suggested by the previous experiments), it might be possible to detect a similar proton movement. *E. coli* K-12 strain GN2, which is unable to metabolize glucose, was used throughout these experiments, to prevent fermentation of glucose (a possible impurity in sucrose). When anaerobic cells of strain GN2 were exposed to 150 mM sucrose, a slow transient alkalinization was observed. Addition of 5 mM TMG led to a rapid and more pronounced proton uptake (Fig. 3). This result suggests that sucrose is taken up in *E. coli* cells by the lactose transport system via proton symport.

DISCUSSION

The widespread use of sucrose for osmotic stabilization of membrane vesicles and for gradient separation gives special significance to the present observations. Sucrose was not previously suspected as a substrate for the lactose carrier, since this disaccharide shows such a low affinity and its accumulation is extremely weak. However, Stock et al. (14) found that sucrose penetrated an *E. coli* strain possessing a lactose transport system to a greater extent than a cell uninduced for this transport system. They inferred that the periplasmic space was larger in the former cell than the latter. That sucrose is a substrate for the lactose carrier was an accidental observation in the course of our survey of sugar entry studied with an osmotic method.

A comment on the osmotic technique seems appropriate, since this method was central to the present studies. The plasmolysis-deplasmolysis technique of von Nägeli and Cramer (15) was applied to bacteria in 1903 by Fischer (3), who showed that when bacteria were suspended in concentrated solutions of nonpenetrating substances such as sucrose, water was withdrawn from the interior of the cell and the cytoplasm with its surrounding plasma membrane pulled

away from the rigid cell wall. Plasmolysis became less striking when low concentrations of salts were present in the plasmolyzing medium (13). If cells were suspended in concentrated solutions of penetrating nonelectrolytes such as glycerol, plasmolysis was quickly followed by a return to the original volume due to entry of glycerol and water. This technique for studying bacterial permeability has been used in several studies over the intervening years (6, 9). Recently the glycerol facilitator has been studied by this technique (1, 11, 12). West first used the method to study lactose entry via the *lacY* gene product (16).

The present study indicates that the osmotic method is ideal for study of sugars transported with a high maximal velocity irrespective of their affinity. It is interesting that a sugar such as TDG, with a well-known affinity for the lactose carrier, enters the cell sufficiently slowly that its movement cannot be detected with certainty by this method. On the other hand, lactose and sucrose enter the cell very rapidly. The inhibiting effect of several galactosides on the two disaccharides indicates that sucrose has far less affinity for the carrier than does lactose.

Chemical measurements of sucrose uptake confirm the transport of this disaccharide by the lactose carrier. The lactose analog TDG strongly inhibits entry. In these experiments it was demonstrated that sucrose could be accumulated within the cell to concentrations higher than in the suspending medium. Sucrose-proton cotransport provides the final support for the view that sucrose is a substrate for the lactose transport system of *E. coli*.

ACKNOWLEDGMENTS

K.B.H. was supported by grant He 959/3 from the Deutsche Forschungsgemeinschaft. This research was supported by Public Health Service grant 5-RO1-AM-05736-18 from the National Institutes of Health and by a grant from the National Science Foundation (PCM-78-00859).

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