SUBSTRATE SPECIFICITY OF A GLUCOSE PERMEASE OF *ESCHERICHIA COLI*¹

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ABSTRACT

ROGERS, DEXTER (Utah State University, Logan) AND SHON-HUA YU. Substrate specificity of a glucose permease of *Escherichia coli*. J. Bacteriol. **84**:877-881. 1962.—A study was made of D-galactose uptake by galactose-negative *Escherichia coli* strain A (Weigle). Uptake probably occurred through a glucose-permease system, because D-glucose and a variety of nonmetabolizable glucose derivatives inhibited the accumulation of galactose and were themselves accumulated. D-Fructose did not inhibit galactose uptake. 6-Deoxy-D-galactose (D-fucose) was taken up by a different permease system. The glucose permease apparently favored pyranoses, and it required the 6-hydroxyl group of the substrate to a greater extent than any of the other hydroxyl groups. Although much of the absorbed glucose-permease substrate was recovered in the free form, a significant amount was recovered as the 6-phosphate ester. Depending on the conditions employed to study uptake, the 6-phosphate ester could amount to as much as 60% of the absorbed galactose.

A preliminary report on the uptake of galactose by a galactose-negative strain of *Escherichia coli*, strain A (Weigle), was given by Glass and Novick (1959). This strain lacks galactokinase and phosphogalactose uridyl transferase. Strain A must also lack the galactose permease (Horecker, Thomas, and Monod, 1960), because galactose uptake exhibits a higher K_m value than is recorded for the galactose permease (1.3×10^{-2} M vs. 7×10^{-6} M). The induction of the

galactoside permease, which can also transport galactose, is prevented with chloramphenicol (Rickenberg et al., 1956; Pardee, 1957). Galactose uptake by strain A is inhibited by α -methyl glucoside, a fact which has been interpreted to indicate that galactose is being taken up by a glucose permease (Glass and Novick, 1959; Monod, Halvorson, and Jacob, cited by Cohen and Monod, 1957).

MATERIALS AND METHODS

The methods employed to study permeation were essentially those used by Glass and Novick (1959). *E. coli* strain A (obtained from A. Novick) was grown aerobically at 37 C, to approximately 4×10^8 cells per ml, on a synthetic medium of the following composition (per liter): K_2HPO_4 , 7 g; KH_2PO_4 , 3 g; $(NH_4)_2SO_4$, 1 g; Na_3 citrate $\cdot$ $2H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; and lactic acid (85%), 2 ml. After appropriate growth (usually 0.34 to 0.42 g of dry cells per liter of culture), chloramphenicol was added (40 μ g per ml), and aeration was continued for 15 min. At that time, the test supplements were added as indicated, and aeration was continued for the indicated periods of time. After incubation, the cell suspensions were chilled and centrifuged without delay.

In practice, the cultures were diluted between 15 and 35% with the solutions of supplements, but, within each experiment, the extent of dilution was the same. Similar results were obtained if the cells were harvested and resuspended in fresh medium before measuring galactose uptake.

The harvested galactose-treated cells were resuspended in 0.1-culture volume of cold water and centrifuged without delay. The washed cells were extracted with 0.1-culture volume of water in a boiling-water bath for 15 min. The extracted residue was washed with 0.1-culture volume of water. The extract and washing were combined and analyzed for galactose as radioactivity in a

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lead-shielded, gas-flow, thin-window counter, assuming a sample layer of "infinite thinness." Uptake was sometimes measured as reducing sugar (Mateles, 1960) or by the anthrone method (Morris, 1948). These methods gave results that were similar to radioactivity measurements; however, their results were frequently lower than the latter values. For critical measurements, radioactivity was used.

The materials used were: D-galactose ("substantially glucose-free"), β -glucosidase ($3 \times$ crystallized), type V glucose-6-phosphate dehydrogenase (all Sigma Chemical Co., St. Louis, Mo.); D-galactose-1- C^{14} ("greater than 99% radiopurity"), 2-deoxy-D-glucose ("glucose-free"), 3-O-methyl-D-glucopyranose, α -methyl-D-glucoside, β -methyl-D-glucoside (all California Corp. for Biochemical Research); D-fucose (Mann Research Laboratories and Nutritional Biochemicals Corp.); barium galactose-6-phosphate (The British Drug Houses, Ltd.).

RESULTS

Identification of the glucose-permease system.

As stated previously, the uptake of D-galactose by strain A apparently proceeds by way of a glucose-permease system, because it is inhibited by α -methyl-D-glucoside (Glass and Novick, 1959). This relation was shown more directly by inhibiting galactose uptake with D-glucose (Table 1). Since D-fructose was not inhibitory under similar conditions, a pyranose rather than a furanose may be utilized by the glucose-permease system. This identification was confirmed by inhibiting galactose uptake with 2-deoxy-D-glucose (Table 1). The extent of inhibition was reduced by increasing the galactose concentration in the medium. Galactose uptake was also inhibited by 3-O-methyl-D-glucose. Thus, glucose and a variety of nonmetabolizable glucose derivatives inhibited galactose uptake. These results indicated that galactose was being taken up by a glucose-permease system, and probably in the pyranose form.

In the presence of an equimolar amount of glucose or 2-deoxyglucose, galactose uptake was inhibited 33 or 37%, respectively, instead of 50% as might be expected for compounds with comparable activities. An equimolar amount of 3-O-methylglucose inhibited galactose uptake by about 50%. It is conceivable, as Rotman (1959) suggested, that permeation involves a

TABLE 1. *Effect of glucose analogues on galactose uptake by strain A*

Galactose-1- C^{14} concn	+0.02 M Analogue	Galactose uptake*		Percentage inhibition of uptake
		Control	+ Analogue	
M				
0.02	D-Glucose	105	70	33
0.02	D-Fructose	105	121	(15)†
0.005	2-Deoxy-D-glucose	83	19	77
0.01	2-Deoxy-D-glucose	126	40	68
0.02	2-Deoxy-D-glucose	180	113	37
0.02	3-O-Methyl-D-glucose	2,868	1,412	51
0.005	D-Fucose	84	90	(7)†
0.01	D-Fucose	131	142	(8)†
0.02	D-Fucose	205	216	(5)†

* Count/min; as radioactivity in extracts of cells from 50-ml cultures treated for 12 min as indicated.

† Percentage stimulation of uptake.

sequence of reactions, and that each competitor may not be an effective inhibitor for every reaction. However, these inhibitors were taken up by the cells, and in the case of α -methyl glucoside (another competitor), it was both absorbed and phosphorylated, as was galactose. Thus, these inhibitors are also substrates, and, consequently, they should have relatively similar activities for each reaction. An additional explanation may be that the measurement of galactose uptake in the absence of a second sugar was sometimes low, for still unknown reasons. In support of this idea, noninhibitors, such as fructose and D-fucose, appeared to stimulate galactose uptake by as much as 15% (Table 1). Similar results were reported by Horecker et al. (1960), who could not attribute this effect to the metabolism of the noninhibitor sugars. Perhaps, as Mager (1959) noted, an increase in the osmotic pressure of the medium or the presence of certain compounds contributes to the functional stability of the cells.

The preceding evidence, together with previous work (Glass and Novick, 1959), established that the hydroxyl groups at carbon atoms 1, 2, 3, and 4 of the glucose-permease substrates do not exert primary effects on permeation. Since the 5-hydroxyl group is bound in a hemiacetal or

acetal linkage, its contribution is included in the pyranose structure. By a process of elimination, the 6-hydroxyl group must be the primary functional group for the glucose-permease system. This was substantiated by studies with D-fucose (6-deoxy-D-galactose). Fucose was absorbed by strain A, and its K_m value was 0.26×10^{-2} M. When added simultaneously, fucose did not inhibit galactose uptake (Table 1), and, consequently, these compounds were entering by different permease systems. The only structural difference between these compounds, the 6-hydroxyl group, controlled the entry by way of the glucose-permease system. The substrate specificity can, therefore, be defined as a 5-hydroxy-methylpyranose. Presumably, a D-pyranose is involved in the glucose-permease system.

Possible permeation intermediates. The well-known biochemical activity of the 6-hydroxyl group of the glucose-permease substrates suggested that permeation might be accompanied by reaction at this group. To investigate this possibility, cell extracts were analyzed for derivatives of galactose, and α - and β -methyl glucosides. A portion of each of these substrates was recovered as the 6-phosphate ester. This confirmed the significance of the 6-hydroxyl group, revealed by the kinetic studies, and suggested a possible phosphorylative mechanism of permeation.

Isolation of galactose-6-phosphate. An extract of galactose-treated cells was analyzed by paper chromatography, with 80% phenol as the solvent (Berry et al., 1951). Most of the absorbed material was recovered as free galactose (Fig. 1). The identification of the material, recovered from the galactose segment of the phenol chromatogram as galactose, was confirmed by chromatography with 2,4-lutidine (R_F 0.43; Berry et al., 1951). In addition to free galactose, a substantial amount of a galactose derivative was recovered at the origin of the phenol chromatogram. This experiment indicated that about 25% of the absorbed galactose was this non-mobile galactose derivative, although the results of other experiments revealed only about 8 to 10% as this material. [During 1960 and 1961, maximal uptake of galactose occurred consistently after about 15 min at 37 C, and the extent of phosphorylation of the absorbed substrate was observed to be about 8 to 10%. In

FIG. 1. Paper chromatography of an extract of galactose-treated cells, with 80% phenol as the solvent. The radioactivity that migrated at the solvent front probably represented "browning" products formed when the sample was dried on the paper.

1962, some change occurred in the test organism. Maximal uptake now occurs consistently between 1 and 3 min at 37 C, and the extent of phosphorylation has been found to be between 40 and 60%. These isolated observations suggest that the rate of galactose uptake and the extent of phosphorylation of the absorbed substrate may be related.]

Chromatography of another extract of galactose-treated cells on a Dowex-1-formate column revealed that most of the galactose passed through unadsorbed (Fig. 2). About 8% of the galactose was adsorbed to the Dowex-1-formate column and was eluted with formate buffer. This galactose derivative contained organic phosphorus, which was rapidly hydrolyzed with 0.1 M NaOH but not with 0.1 M HCl. Inorganic phosphate was released by treatment with a purified hexose phosphatase at a rate characteristic of hexose phosphates (Rogers and Reithel, 1960). The isolated material gave the blue color with anthrone that is characteristic of hexose derivatives, and it proved to be reducing by a modified Folin-Malmros test. Free galactose was identified in the phosphatase digest by chromatography with 80% phenol. This galactose derivative reacted with NaIO_4 under mild conditions (Morrison, Rouser, and Stotz, 1955), and yielded a substance that gave with diphenylamine the grass-green color characteristic of glycolaldehyde derivatives (Dische and Borenfreund, 1949). This treatment revealed the

FIG. 2. Chromatography of an extract of galactose-treated cells on a 0.6×6.0 cm column of Dowex-1 formate.

location of the phosphate group at the 6-carbon atom, because such a grouping prevents the fission of the bond between carbon atoms 5 and 6 by periodate treatment, with the result that these carbon atoms are recovered as a glycol-aldehyde derivative. The mole ratio of anthrone color-organic phosphorus-glycolaldehyde color after periodate treatment was 1.00:0.97:0.98. These reactions were consistent with the reactions of an authentic sample of galactose-6-phosphate.

Isolation of α -methyl glucoside-6-phosphate. An extract of cells treated with α -methyl glucoside was chromatographed on a Dowex-1-formate column. Although most of the extracted glucoside passed through the column unadsorbed, approximately 10% was adsorbed and eluted with 0.2 to 0.6 M formate buffer (pH 3.7). This glucoside derivative was eluted as a single peak, and it was found to be a nonreducing, anthrone-positive organic phosphate. After hydrolysis in 1 M HCl for 2 hr in a boiling-water bath, the mole ratio of reducing sugar-organic phosphorus was 1.00:0.99. The fact that a portion of the α -methyl glucoside was recovered as a phosphorylated derivative indicated that a 6-kinase or a phosphotransferase was probably involved, rather than the coupled reaction of a 1-kinase and mutase.

Isolation of β -methyl glucoside-6-phosphate. An extract of cells treated with β -methyl glucoside was chromatographed on a Dowex-1-formate column. Approximately 10% of the

FIG. 3. Reduction of triphosphopyridine nucleotide (TPN) by β -methyl glucoside-6-phosphate in the presence of glucose-6-phosphate dehydrogenase and β -glucosidase. The reaction mixture contained 1 μ mole of TPN (Na), 125 μ moles of glycylglycine buffer (pH 7.4), 20 μ moles of $MgCl_2$, 0.110 μ mole of β -methyl glucoside-6-phosphate (measured as reducing sugar after acid hydrolysis), and 1 Kornberg unit of glucose-6-phosphate dehydrogenase, in a volume of 2.80 ml. After no evidence of TPN reduction was observed, 2 mg of β -glucosidase were added in 0.20 ml of 0.25 M glycylglycine buffer (pH 7.4). The control was the mixture without the enzymes.

extracted glucoside was adsorbed on the column, and eluted with 0.2 to 0.6 M formate buffer (pH 3.7). This material was a nonreducing, anthrone-positive organic phosphate. It did not reduce triphosphopyridine nucleotide in the presence of glucose-6-phosphate dehydrogenase unless β -glucosidase was also present (Fig. 3). The mole ratio of reducing sugar (after hydrolysis)-organic phosphorus-glucose-6-phosphate (after β -glucosidase treatment) was 1.00:0.96:1.01. The specificities of these enzymes confirmed the deduction, made from periodate-diphenylamine treatment of the recovered galactose derivative, that phosphorylation occurred at the 6-hydroxyl group of the glucose-permease substrates.

DISCUSSION

Competitive inhibition of uptake appears to be a more reliable means of studying the structural specificity of a transport system than uptake measurements alone. This is especially true where there is evidence for different transport systems

handling similar structures, such as galactose and fucose. There is the further advantage that a metabolizable substrate can be employed as the competitor. The reservations expressed by Rotman (1959), when sequential reactions may be involved in permeation, appear to have been met because those sugars that are competitors are also absorbed and phosphorylated.

The substrate specificity of the glucose permease of strain A differs from the specificity of the glucoside permease described by Monod, Halvorson, and Jacob (cited by Cohen and Monod, 1957) because the glucose permease can absorb galactose. Otherwise, both permease systems resemble each other by being able to absorb glucose and α -methyl glucoside, but not fructose. Since the cells of strain A were not induced to form the galactoside-permease system, and since the K_m value for galactose uptake was about 2,000-fold greater than the K_m value recorded for the galactose-permease system, the other known means of transport for galactose were apparently ruled out. The absorbed material was clearly identified as galactose by paper chromatography. The validity of galactose uptake by way of a glucose-permease system was based not only on the competition for entry shown by glucose and glucose derivatives, but also on the fact that galactose, like α - and β -methyl glucosides, was absorbed and phosphorylated. Perhaps the glucoside permease is altered in some way so that galactose is hindered from approaching the permease site.

The preferred structures for the sugar transport systems of the mammalian erythrocyte and intestinal epithelium are summarized as a D-pyranose (LeFevre and Marshall, 1958) and a 2-hydroxy-5-methyl or substituted methyl-D-pyranose (Crane, 1960), respectively. Neither of these requirements offers a compelling argument for substrate phosphorylation during transport by these systems as that shown by the glucose permease of strain A. Therefore, a consideration of a phosphorylative transport mechanism is not intended for any other system than strain A at the present time. However, the general involvement of phosphorylation reactions has been suggested for several systems, including mammalian systems (Wilbrandt, 1954). It is this possibility that is the subject for a continuing study with strain A.

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