THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 246, No. 9, Issue of May 10, pp. 2891-2897, 1971
Printed in U.S.A.

Studies on the Uptake of Hexose Phosphates

III. MECHANISM OF UPTAKE OF GLUCOSE 1-PHOSPHATE IN *ESCHERICHIA COLI**

(Received for publication, October 19, 1970)

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SUMMARY

A wild strain of *Escherichia coli* utilizes glucose 1-phosphate by hydrolysis of the ester at the cell surface, after which the glucose moiety but not the phosphate group enters the cell. However, when these cells are induced by treatment with glucose 6-phosphate, they are able to take up both glucose 6-phosphate and glucose 1-phosphate without preliminary hydrolysis. Mutant strains of *E. coli* that lack Enzyme I of the phosphotransferase system are unable to grow in the presence of glucose or glucose 1-phosphate as a carbon source. These strains show uptake of ^{14}C-glucose 1-phosphate after being induced with glucose 6-phosphate.

Mutant strains have been isolated that lack Enzyme I and are constitutive for the hexose phosphate transport system. They can grow on glucose 6-phosphate or glucose 1-phosphate, but not on glucose as carbon source. In these strains the uptake of ^{14}C-glucose 1-phosphate involves entry of the entire molecule, and uptake is inhibited by glucose 6-phosphate.

It is concluded that glucose 1-phosphate may be used by *E. coli* after preliminary hydrolysis by a surface phosphatase which is effective at neutral pH. In addition, glucose 1-phosphate is a substrate, but not an inducer, for the hexose phosphate transport system that is active with glucose 6-phosphate and related compounds.

In recent years evidence has appeared that a number of phosphate esters can be transported into *Escherichia coli*. One such uptake system is specific for L-α-glycerophosphate (1). A second system is able to transport glucose 6-phosphate, fructose 6-phosphate, and mannose 6-phosphate (2–5). To date, no other phosphate esters are known to penetrate the protoplasmic membrane as the unsplit ester.

With respect to glucose 1-phosphate there is disagreement in the literature. According to one report (6) *E. coli* are not able to utilize this ester, but another communication states that a mutant *E. coli* was able to grow with glucose 1-phosphate as a carbon source even when it could not be induced to transport glucose 6-phosphate (5). We have reinvestigated the problem and present evidence that there are two mechanisms by which *E. coli* can grow on glucose 1-phosphate at a normal rate. In wild strains the ester is hydrolyzed, presumably by a surface phosphatase, after which the glucose moiety but not the phosphate group enters the cell. However, when these cells are induced by treatment with glucose 6-phosphate, they are able to take up both glucose 6-phosphate and glucose 1-phosphate without preliminary hydrolysis. Furthermore, mutants can be isolated in which the transport of both glucose 6-phosphate and glucose 1-phosphate has become constitutive.

* This work was supported by United States Public Health Service Research Grant AM-11789 and National Science Foundation Grant GB-7093X.

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EXPERIMENTAL PROCEDURE

Materials

Bacteria—Strain E15 (7) carries a deletion for alkaline phosphatase but is wild type with respect to transport. Strains MM6 (8) (obtained from Dr. D. Fraenkel, Harvard University), as well as MOX19 (9) and 1103 (9) (from Dr. C. Fox) are deficient in Enzyme I of the phosphotransferase system (10, 11); consequently they are unable to grow on glucose as a carbon source. Strain GN2 (8) lacks both Enzyme I and glucokinase.

Media—The synthetic medium (CRM) was similar to that of Cohen and Rickenberg (12) and contained (per liter, pH 7.3, adjusted with KOH) 13.6 g of KH_2PO_4, 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.5 mg of $FeSO_4 \cdot 7H_2O$, and 1 ml of a trace metal solution containing 480 mg of $FeCl_3 \cdot 6H_2O$, 280 mg of $MnCl_2 \cdot 4H_2O$, 270 mg of $CaCl_2$, 2000 mg of $ZnCl_2$, 290 mg of H_3BO_3, and 130 mg of $CoSO_4$ per liter. Tryptone broth consisted of 10 g of Difco Bactotryptone and 5 g of NaCl per liter.

Enzymes and Chemicals—Muscle phosphorylase *a* and glycogen were from Sigma Chemical Corporation. The Glucostat kit was purchased from Worthington Biochemical Corporation. Thin layer plates were Uniplate, precoated, 250 μ with Avicel, a product of Analtech, Inc., Newark, Delaware. ^{14}C-glucose 1-phosphate and ^{14}C-glucose 6-phosphate were from New England Nuclear Corporation and Nuclear-Chicago, respectively.

Methods

Growth of Bacteria

Bacteria were grown on a rotary shaker at 37°. Growth was measured by removal of samples whose optical density at 600 mμ was determined in the Gilford model 240 spectrophotometer. Alternatively, cell suspensions were shaken in 125-ml Erlen-

meyer flasks equipped with a side arm and growth was measured with a Klett colorimeter equipped with a number 42 filter. The approximate relationship between absorbance in the Gilford machine and number of bacteria per ml is: $0.12 = 0.9 \times 10^8$, $0.48 = 4.1 \times 10^8$, $0.7 = 5.5 \times 10^8$, $1.11 = 12 \times 10^8$.

Assay for Uptake of Glucose 1-Phosphate and Glucose 6-Phosphate

All transport assays were carried out at ambient temperatures. A cell suspension (approximately 20 mg per ml, wet weight) was treated with 40 μg per ml of chloramphenicol and incubated with gentle agitation for 4 min. The radioactive substrate was then added and at 1 min and 2 min a sample of 0.3 ml was filtered through a Millipore membrane filter (0.45 μ pore size), previously wetted with cold Buffer I (0.15 M NaCl-5×10^{-4} M $MgCl_2$-0.01 M Tris-HCl, pH 7.3). The cells were filtered dry and washed on the filter with 10 ml of the same cold buffer. The filters were glued to 3.0-cm aluminum planchets with rubber cement, dried under a heat lamp, and counted in a Nuclear-Chicago thin window counter. To determine the specific activity of the substrate, samples were placed in 0.1 ml of water on an aluminum planchet, dried down, and counted. In calculating specific activities no account was taken of self absorption by the cells on the Millipore filter. Various other wash conditions were tested with similar results.

We determined that the extent of uptake of radioactivity is linear with time for up to 3 min (points at 15, 30, 60, 120, 180, and 240 sec). This was true both for ^{14}C-glucose 1-phosphate and ^{14}C-glucose 6-phosphate. Furthermore, linearity was observed both for strain E15 and for the various hexose phosphate transport mutants described herein.

In all competition experiments on uptake described in this paper, the nonradioactive competitor was added simultaneously with the radioactive substrate at zero time.

Assay for Enzyme I Activity

Assay for Enzyme I activity was carried out essentially as described by Kundig and Roseman (10).

Assay for Hydrolysis of Hexose Phosphate Esters by Intact Cells and by Cell-free Extracts

Measurement of P_i Release—A cell suspension was prepared (1 g per 80 ml, wet weight) in the buffer indicated for each experiment. Chloramphenicol was added (40 μg per ml). After 4 min the sugar phosphate was added, and gentle agitation was begun. At intervals a sample of 0.8 ml was removed and pipetted into 0.3 ml of cold 0.5 M trichloracetic acid. After centrifugation in the cold, 1 ml of supernatant solution was assayed for P_i by adding 0.5 ml of cold reagent A (4 ml of 16% ammonium molybdate in 10 N H_2SO_4 mixed with 36 ml of H_2O and 2 g of $FeSO_4$). The mixture was transferred to a 37° water bath for 30 sec and returned to an ice bath until its optical density at 740 mμ could be measured. With cell extracts or shock fluid the treatment with chloramphenicol was omitted. This method (13) is suitable for use with labile phosphate esters.

Measurement of Glucose Release—This was necessary when the preparation contained relatively high concentrations of P_i. Samples (0.2 ml) of the reaction mixture were incubated and at various times placed in a boiling water bath for 2 min and stored in an ice bath, after which the Glucostat assay was performed.

Synthesis of Glucose 1-Phosphate Labeled with ^{32}P

The incubation mixture contained 330 μmoles of ^{32}P-inorganic phosphate with a specific activity of 1.76×10^7 cpm per μmole, 13.2 mg of glycogen, 230 units (0.2 mg) of phosphorylase *a*, and 5 μmoles of ethylenediaminetetraacetate in a total volume of 1.2 ml. After 24 hours at 30°, 1 ml of 95% ethanol was added to remove enzyme and excess glycogen. The mixture was centrifuged at 10,000 $\times$ *g* in the cold for 15 min. To precipitate excess ^{32}P-labeled inorganic phosphate, the supernatant was treated with 1 ml of 0.3 M magnesium acetate and 0.4 ml of 1.5 M NH_4OH. The mixture was centrifuged, the supernatant was treated with 0.05 ml of 1 M K_2HPO_4, 0.05 ml of 0.3 M $MgCl_2$, and 0.005 ml of 1.5 M NH_4OH, and the mixture was centrifuged once more. The supernatant solution was lyophilized almost to dryness and submitted to thin layer chromatography in methanol-formic acid-water (16:3:1, v/v/v) and 95% ethanol-1 M ammonium acetate-0.1 M EDTA (70:30:1).

When the chromatograms were scanned for ^{32}P all of the activity coincided with a marker of authentic glucose 1-phosphate. The preparation of ^{32}P-glucose 1-phosphate was stored at −90°.

RESULTS

Studies on Uninduced Cultures of Strain E15

Strain E15 carries a deletion of the alkaline phosphatase gene but is wild type with respect to transport activity. It is able to grow with glucose 6-phosphate as a carbon source but only after a lag period during which an active transport system for this ester is induced. The experiments in this section are concerned with uninduced cells.

Growth on Glucose 1-Phosphate as Carbon Source—Uninduced strain E15 was able to grow with glucose 1-phosphate as carbon source without a lag period and the rate of growth was comparable to that obtained with glucose. No growth was obtained with fructose 1,6-diphosphate or galactose 6-phosphate, although the free sugars were able to serve as a source of carbon.

The experiments just cited were carried out with Medium CRM which contains 0.1 M phosphate. When Medium E, containing only 4×10^{-3} M phosphorus was used the growth curves for glucose and glucose 1-phosphate were again comparable. Barely perceptible growth was noted with fructose 1,6-diphosphate, galactose 6-phosphate, and ribose 5-phosphate, and this was suppressed by increasing the concentration of phosphate to 0.05 M. These data suggested that the last three compounds were utilized after hydrolysis by a surface phosphatase that was sensitive to inorganic phosphate. But to our surprise, it appeared that glucose 1-phosphate also was utilized only after preliminary hydrolysis.

This was shown as follows. Uptake experiments on *E. coli* E15 were carried out with glucose 1-phosphate labeled with ^{32}P in the phosphate moiety or with ^{14}C in the glucose portion of the molecule. It is evident (Table I) that the bacteria took up ^{14}C but there was no significant entry of ^{32}P. This would indicate that the glucose moiety penetrated the cell after removal of phosphate by hydrolysis.

Competition Experiments—Consistent with these results is the fact that nonradioactive glucose inhibited the uptake of radioactivity when cells were incubated with ^{14}C-glucose 1-phosphate, but glucose 6-phosphate did not inhibit (Fig. 1). Deoxyglucose also inhibited the uptake of ^{14}C-glucose 1-phosphate (Fig. 2). These data all suggest that glucose 1-phosphate is hydrolyzed

TABLE I

Fate of glucose and phosphate moieties during uptake of glucose 1-phosphate

The bacterial cells of strain E15 were grown on Medium CRM plus 0.01 M glucose 1-phosphate to an optical density at 600 mμ of 0.703, harvested, and washed twice with Medium CRM (unsupplemented) at room temperature. The standard uptake assay was used with 0.01 M Tris, pH 7.3, 0.15 M NaCl, 5×10^{-5} M $MgCl_2$, and 50 μg of chloramphenicol per ml of reaction mixture. Strain MM7 was grown on Medium CRM plus 0.02 M pyruvate and treated similarly. Two separate incubations were run in an identical manner except that one was with 8.8×10^{-5} M ^{32}P-glucose 1-phosphate and the other with 8.4×10^{-5} M ^{14}C-glucose 1-phosphate.

Cells	Uptake of radioactive label	
	^{14}C	^{32}P
	mμmoles/mg protein/2 min	
E15	6.4	0.23
MM7[a]	14.3	17.0

[a] Strain MM7 was derived by spontaneous mutation from strain MM6. Strain MM6 lacks Enzyme I of the phosphotransferase system and cannot grow on glucose or glucose 1-phosphate as carbon source. Strain MM7 also lacks Enzyme I and cannot grow on glucose, but it is able to grow on glucose 1-phosphate.

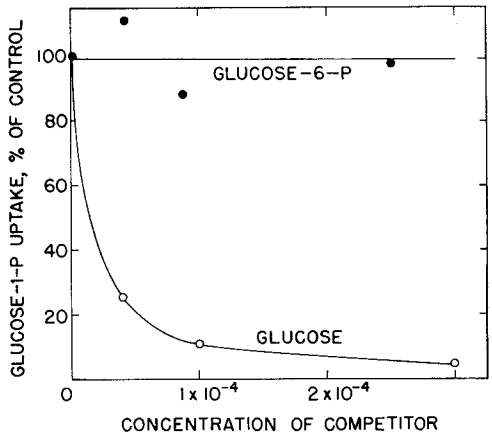

FIG. 1. Effect of glucose or glucose 6-phosphate on the uptake of ^{14}C-glucose 1-phosphate. Strain E15 (alkaline phosphatase deletion, wild type with respect to transport) was grown on Medium CRM supplemented with 0.04 M glycerol. The uptake of ^{14}C-glucose 1-phosphate in the presence of nonradioactive glucose or glucose 6-phosphate was measured as described under "Methods." One hundred per cent corresponds to 8-mμmole uptake per 2 min per mg of protein.

by a surface phosphatase active in the presence of 0.1 M phosphate, after which the glucose moiety is transported into the cell.

Studies on Strains Unable to Utilize Glucose

Strains MM6, MOX19, and 1103 lack Enzyme I of the phosphotransferase system and are unable to grow on a synthetic medium with glucose as a source of carbon (8, 9). Strain GN2 lacks glucokinase as well (8). All of these strains could be induced for transport of glucose 6-phosphate with this ester as the inducer, and good growth was obtained when glucose 6-phosphate was available as a source of carbon.

When Medium CRM was supplemented with glucose 1-phosphate none of these strains were able to grow, as would be expected if they could not utilize glucose. The earlier results (above) indicated that glucose 1-phosphate was split by *E. coli* prior to uptake of the glucose moiety. However, after long incubation periods of from 18 to 40 hours, the bacteria did begin to grow and the doubling time during exponential phase was nearly as short as for glucose 6-phosphate. A typical experiment is shown in Fig. 3. The cells which eventually grew on glucose 1-phosphate were carried through a number of transfers on nutrient agar slants, after which they were inoculated successively into tryptone broth and Medium CRM supplemented with 0.015 M pyruvate. The culture isolated in this manner had altered growth properties; a new strain appeared to have arisen by spontaneous mutation. This strain was able to grow on glucose 1-phosphate or glucose 6-phosphate without a lag period but could not grow on glucose (Fig. 4). The culture was assayed for Enzyme I and the results were still negative. It was clearly not a revertant. Secondary mutants of this type were obtained from MOX19, MM6, 1103, and GN2.[1]

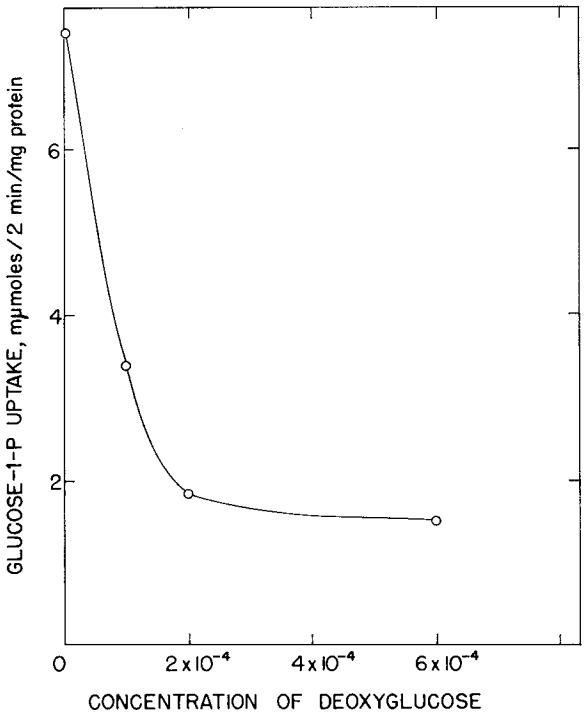

FIG. 2. Competition by deoxyglucose for the uptake of glucose 1-phosphate. Strain E15 were grown in Medium CRM plus 1×10^{-2} M glucose and harvested in midexponential phase of growth. Uptake of ^{14}C-glucose 1-phosphate, at a concentration of 8.3×10^{-5} M, was measured as described under "Methods" with addition of deoxyglucose as indicated.

Uptake measurements gave the following results. The transport of glucose 6-phosphate in the mutant strains was constitutive, as uninduced cells were able to take up this ester (Table I). Furthermore, in these strains, glucose 1-phosphate was taken up as such by a constitutive transport system. This was shown

[1] The mutants were purified through several single colony isolations on selective media and stored in stab cultures. When such stored cultures were later grown on nonselective media and colonies were streaked, most were stable mutants. However, spontaneous revertants were found which had the property of being still Enzyme I negative but which had lost the constitutive transport of both glucose 1-phosphate and glucose 6-phosphate.

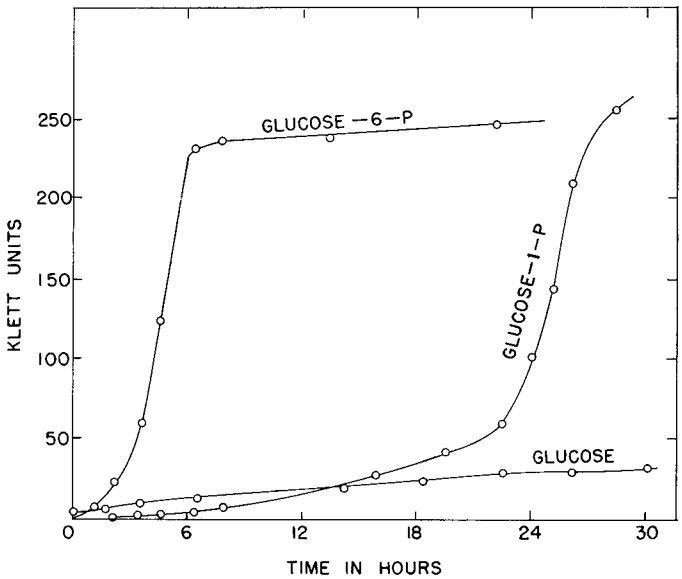

FIG. 3. Spontaneous mutation of strain MM6 resulting in ability to grow on glucose 1-phosphate. Strain MM6, which lacks enzyme I of the phosphotransferase system, was grown to mid-exponential phase in Medium CRM plus 0.01 M pyruvate. Subcultures were made to Medium CRM with carbon supplements as indicated. There was no significant growth on glucose after 48 hours of incubation.

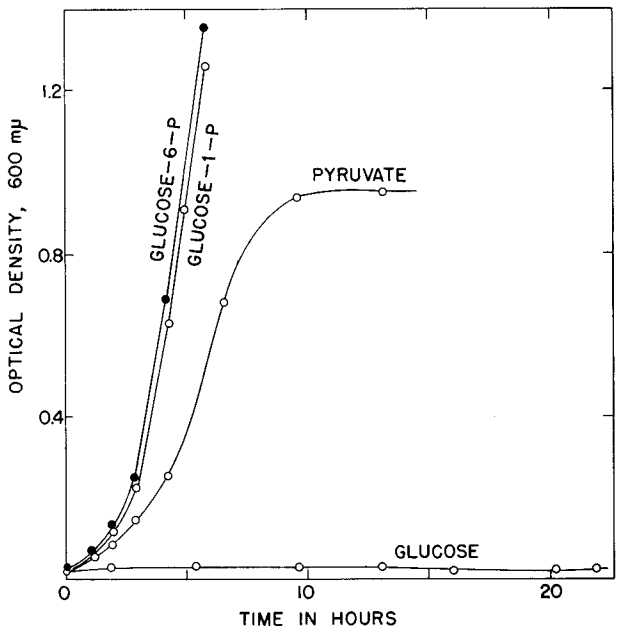

FIG. 4. Growth of strain MM7. This mutant strain of *Escherichia coli*, which is constitutive for the uptake of the two glucose phosphate esters, was carried on tryptone agar slants and transferred successively to tryptone broth and Medium CRM plus 0.02 M pyruvate. Cells were then inoculated in Medium CRM supplemented with 1×10^{-2} M glucose 6-phosphate, glucose 1-phosphate, pyruvate, or glucose.

in the following way. The uptakes of ^{14}C-labeled and ^{32}P-labeled glucose 1-phosphate were measured under identical conditions. The ^{14}C- and ^{32}P-labeled moieties were taken up in approximately equivalent amounts; thus, both the glucose and the phosphate portions of the molecule entered the bacterial cell (Table I). Further, in contrast to the results with E15, the transport of glucose 1-phosphate was inhibited by glucose 6-phosphate but not by glucose (Fig. 5). Thus, it appears that in our mutants, glucose 1-phosphate and glucose 6-phosphate share a constitutive transport system.

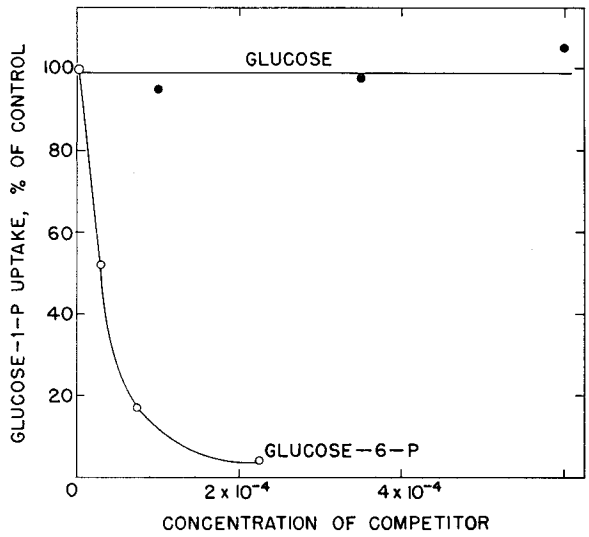

FIG. 5. Effect of glucose or glucose 6-phosphate on the uptake of ^{14}C-glucose 1-phosphate. Strain MM7 (lacks enzyme I, constitutive for glucose 6-phosphate transport) was grown on Medium CRM plus 0.01 M pyruvate. The uptake of ^{14}C-glucose 1-phosphate in the presence of nonradioactive glucose or glucose 6-phosphate was measured as described under "Methods." One hundred per cent corresponds to 15-mμmole uptake per 2 min per mg of protein.

Studies on Induced Cultures of E. coli

Studies with Strain MOX19—Strain MOX19 lacks Enzyme I of the phosphotransferase system. When cells were grown in the presence of pyruvate and transport activity was tested, the uptake of ^{14}C-glucose 1-phosphate was very low (Table II). On the other hand, cells induced for glucose 6-phosphate transport showed an excellent rate of uptake of ^{14}C-glucose 1-phosphate. Thus, the induced glucose 6-phosphate transport system, as well as the constitutive system, is able also to take up glucose 1-phosphate.

Studies with Strain E15—It is more difficult to demonstrate effects of induction with wild type E15 cells, because they are able to take up ^{14}C-glucose and grow in the presence of ^{14}C-glucose 1-phosphate, even if they have not been induced by treatment with glucose 6-phosphate. Nevertheless, the data do suggest that induction for glucose 6-phosphate transport produces a new system for taking up glucose 1-phosphate as well. Thus the rate of uptake of radioactivity from uninduced E15 corresponded to 6 to 7 mμmoles of ^{14}C-glucose per 2 min per mg of bacterial protein (Fig. 2). After induction with glucose 6-phosphate the uptake of ^{14}C-glucose 1-phosphate rose to 19 mμmoles per min per mg (Fig. 6). Uptake of ^{14}C-glucose 1-phosphate by uninduced cells is inhibited by glucose but not by glucose 6-phosphate (Fig. 1). However, with induced cells the uptake of ^{14}C-glucose 1-phosphate did show inhibition by glucose 6-phosphate (Fig. 6). Thus, an additional mechanism for uptake of glucose 1-phosphate was induced when cells were treated with glucose 6-phosphate.

Glucose 1-Phosphatase Activity of Strain E15

The experiments described in the first section of "Results" indicated that, in the case of E15, glucose 1-phosphate was

TABLE II

Uptake of glucose 1-phosphate by strain MOX19 grown in presence of pyruvate or glucose 6-phosphate

Strain MOX19 lacks Enzyme I of the phosphotransferase system; it cannot grow on glucose or glucose 1-phosphate as an energy source. Cells were grown on Medium CRM plus 0.0005% Bactotryptone, and this was supplemented with 0.01 M glucose 6-phosphate of 0.02 M pyruvate. They were harvested in mid-exponential phase, washed twice with Medium CRM, and suspended in this medium for the standard uptake assay (see "Methods").

Carbon source	Concentration of glucose 1-phosphate	Uptake of ^{14}C-glucose 1-phosphate
		mμmoles/mg protein/2 min
Pyruvate	4×10^{-4}	1.0
Glucose 6-phosphate	5.6×10^{-4}	11.5

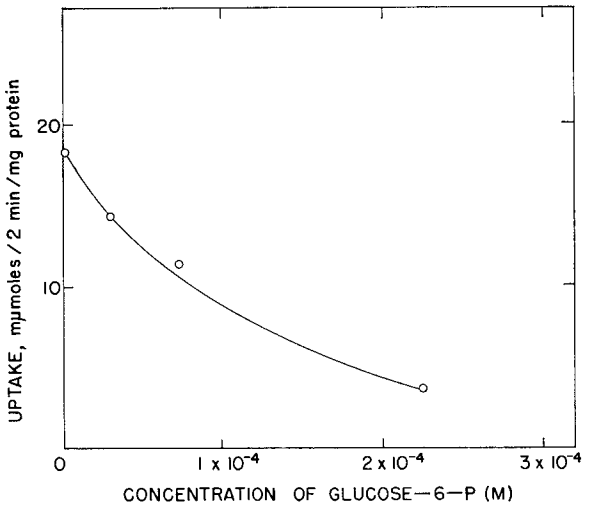

FIG. 6. Effect of glucose 6-phosphate on the uptake of ^{14}C glucose 1-phosphate by wild type *Escherichia coli* induced for transport of glucose 6-phosphate. Strain E15 were grown to midexponential phase on Medium CRM with 0.01 M glucose 6-phosphate as carbon source. The cells were harvested and washed, and the uptake of ^{14}C-glucose 1-phosphate (8×10^{-5} M) was measured as described under "Methods," in the presence of the indicated concentrations of nonradioactive glucose 6-phosphate.

hydrolyzed by intact cells after which the glucose moiety was taken up by the glucose permease system. Experiments with whole cells were then carried out which revealed that under the conditions of the glucose 1-phosphate uptake assay the rate of hydrolysis and the rate of uptake were equal (Table III). In some experiments the rate of hydrolysis was nearly double the rate of uptake of radioactivity. Further, the rates of hydrolysis of fructose 1,6-diphosphate, fructose 1-phosphate, galactose 6-phosphate, and glucose 6-phosphate were less than 10% of that of glucose 1-phosphate.

The phosphatases of *E. coli* have been the subject of several recent publications (15–17); the enzyme accounting for most of the hydrolytic activity against glucose 1-phosphate of *E. coli* is termed acid hexose phosphatase. It is released by osmotic shock (16, 18). If acid hexose phosphatase were responsible for the splitting of glucose 1-phosphate prior to uptake of the glucose moiety one would expect whole cells to hydrolyze other hexose phosphates and utilize the sugars. The enzyme is relatively

TABLE III

Comparison of rate of hydrolysis of glucose 1-phosphate by intact cells and rate of uptake of ^{14}C-glucose 1-phosphate

E. coli E15 were grown on Medium CRM plus 0.01 M glucose to an optical density at 600 mμ of 0.74, washed 2 times with 0.15 M NaCl-0.01 M Tris-HCl, pH 7.4-5 $\times 10^{-4}$ M $MgCl_2$, and suspended in the same medium. Other procedures are described under "Methods."

The concentration of glucose 1-phosphate was 1×10^{-3} M for the hydrolysis studies. In other experiments a series of concentrations were tested and K_m was 3×10^{-4} M. A level of 1×10^{-3} M is saturating and less than 10% of the substrate was utilized. For all uptake studies in this paper the concentration of hexose phosphate was 8.5×10^{-5} M, and less than 10% of radioactivity was taken up by the cells. The value for K_m of transport was 1×10^{-5} M for ^{14}C-glucose 1-phosphate in uninduced strain E15; however, only the uptake of radioactivity was measured, and it is not implied that the entire molecule was taken up. Both the uptake measurements and hydrolysis studies were carried out at 23°.

Substrate	Other additions	Rate of P_i release	Rate of uptake
		μmoles/mg protein/hr	
1×10^{-3} M glucose-1-P		0.38	0.37
1×10^{-3} M glucose-1-P	1.1×10^{-5} M FCCP[a]	0.41	0.06
1×10^{-3} M glucose-1-P	1×10^{-2} M glucose	0.34	0.01

[a] Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

TABLE IV

Activity of purified acid hexose phosphatase against various phosphate esters at pH 5.8 and pH 7.3

Compound	Relative activity[a]	
	pH 5.8	pH 7.3
Glucose 1-phosphate[b]	100[c]	79
Glucose 6-phosphate	75	27[d]
Galactose 6-phosphate	70	0
Fructose 1-phosphate	77	29
Fructose 1,6-diphosphate[b]	67	0
2-Deoxyglucose 6-phosphate	0	0
Ribose 5-phosphate	11	0

[a] The rate of hydrolysis of glucose 1-phosphate at pH 5.8 was set at 100. The reaction mixtures were buffered with 0.1 M acetate, pH 5.8, or 0.1 M Tris-HCl, pH 7.3. Acid hexose phosphatase was purified from shock fluid by DEAE-cellulose chromatography (16).

[b] For crude shock fluid the relative rates were (*a*) glucose 1-phosphate, 100 at pH 5.8 and 84 at pH 7.3; (*b*) fructose 1,6-diphosphate, 99 at pH 5.8 and 10 at pH 7.3.

[c] The activity of purified acid hexose phosphatase against glucose 1-phosphate was 9000 μmoles hydrolyzed per mg of protein per hour at 37°.

[d] At pH 7.3 in acetate buffer, it is evident that glucose 6-phosphate is hydrolyzed about one-third as fast as glucose 1-phosphate. However, when intact cells are tested in growth medium (buffered at pH 7.3 with 0.1 M phosphate) the rate for glucose 6-phosphate is approximately 5% of that observed for glucose 1-phosphate. This explains why glucose 6-phosphate does not compete with glucose 1-phosphate for entry in uninduced cells, where the ester must first be hydrolyzed before the sugar moiety is transported (Fig. 1).

TABLE V

Effect of cell concentration on inhibition of ^{14}C-glucose 1-phosphate uptake by glucose

The rationale for these experiments is as follows. If ^{14}C-glucose 1-phosphate forms ^{14}C-glucose near the cell surface which mixes with glucose in the medium, then the uptake of radioactivity will be reduced by ^{12}C-glucose in the medium. Further, this effect will be magnified in a dilute cell suspension.

E. coli E15 were grown in Medium CRM plus 0.01 M glucose, harvested at an optical density at 600 mμ of 0.700, and washed twice with 0.15 M NaCl-1 × 10^{-3} M $MgCl_2$ (23°). The uptake of radioactivity with ^{14}C-glucose 1-phosphate was studied in a medium containing 0.15 M NaCl, 5 × 10^{-4} M $MgCl_2$, 0.01 M Tris-HCl, pH 7.3, and trace metals as in Medium CRM. Approximately equal amounts of cells were filtered (see "Methods").

Cell concentration	^{14}C-Glucose-1-P	Glucose	Glucose-1-P uptake		Rate (+ glucose)/rate (− glucose)
			30 sec	2 min	
mg protein/ml	M		*mμmoles/mg protein*		
0.417	8.5 × 10^{-5}			4.56	0.65
0.417	8.5 × 10^{-5}	5 × 10^{-5}		3.00	
0.059	8.5 × 10^{-5}			2.40	0.24
0.059	8.5 × 10^{-5}	5 × 10^{-5}		0.57	
0.436	1.33 × 10^{-4}		4.86	20.8	0.50
0.436	1.33 × 10^{-4}	6.25 × 10^{-5}	1.60	9.58	
0.0436	1.33 × 10^{-4}		5.07	13.0	0.19
0.0436	1.33 × 10^{-4}	6.25 × 10^{-5}	0.81	2.29	

nonspecific for various hexose 6-phosphates and α-linked hexose 1-phosphates (16). However, neither fructose 1,6-diphosphate nor galactose 6-phosphate supported growth. This could be explained when it was found that the activity of crude shock fluid against these esters was quite low at pH 7.3 compared with pH 5.8 (Table IV). Similarly, a purified preparation of acid hexose phosphatase hydrolyzed fructose 1,6-diphosphate and galactose 6-phosphate at pH 5.8 but not at pH 7.3, whereas the same preparation hydrolyzed glucose 1-phosphate rapidly at both pH 5.8 and 7.3.

Uptake of ^{14}C-glucose 1-phosphate was usually measured in phosphate-buffered media. This did not inhibit the surface phosphatase, which showed equal activity in 0.1 M phosphate and in phosphate-free incubation mixtures. It is of interest that 1.1 × 10^{-5} M carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and 1 × 10^{-2} M glucose both greatly inhibited the uptake of radioactivity from ^{14}C-glucose 1-phosphate by *E. coli* E15, but did not affect the rate of hydrolysis of the ester by whole cells (Table III). Presumably these agents interfered with active transport of the glucose derived from glucose 1-phosphate.

Since free glucose did not accumulate in the medium when strain E15 took up ^{14}C-glucose 1-phosphate, the question arose whether the glucose moiety was formed in the periplasmic space and was transported into the cell without mixing with glucose that might be present in the medium. Experiments to check this point revealed that glucose derived from ^{14}C-glucose 1-phosphate did equilibrate with glucose in the medium (Table V). Thus, adding the same concentration of glucose to the medium inhibited the rate of uptake of ^{14}C-glucose 1-phosphate to a much greater extent with dilute cell suspensions than with more concentrated cell suspensions (Table V). With dilute suspensions any ^{14}C-glucose released from ^{14}C-glucose 1-phosphate would be more effectively diluted if it got into the medium.

DISCUSSION

The present data indicate that there exist in *E. coli* two separate pathways for the uptake of glucose 1-phosphate, both of which are active enough to permit good growth on this ester as a carbon source. In the first pathway, glucose 1-phosphate is hydrolyzed, after which the glucose moiety is absorbed by the cell while phosphate is excluded. We assume that hydrolysis is catalyzed by a phosphatase localized near the surface of the cell. An acid hexose phosphatase previously described (15–17) appears to have the properties required to perform this function. It is highly active even in the presence of 0.1 M phosphate and while its formation is repressed by growth in the presence of glucose or glucose 6-phosphate (15, 16), sufficient activity remains to account for the rate of utilization of glucose 1-phosphate. For some reason, at neutral pH this enzyme is much more active against glucose 1-phosphate than it is against fructose 1,6-diphosphate or galactose 6-phosphate. This could account for failure to grow on the two last named compounds.[2]

Since glucose 1-phosphate enters the cell after preliminary hydrolysis, it is understandable that free glucose should compete for entry while glucose 6-phosphate does not, and it is also clear why mutants unable to grow on glucose are also unable to grow on glucose 1-phosphate. The mutants used in this study lack Enzyme I of the phosphotransferase system (10, 11).

From the mutant strains lacking Enzyme I a second mutation arose with the ability to grow on glucose 1-phosphate but not glucose. This strain transported glucose 1-phosphate as such, and with labeled compounds it was shown that the glucose and phosphate moieties were taken up in approximately equal amount. In this new mutant organism the transport of glucose 1-phosphate was inhibited by glucose 6-phosphate but not by glucose. This is exactly the reverse of what happens with wild type *E. coli*. The same mutation was characterized by an alteration in the transport of glucose 6-phosphate, which now became constitutive where it had been inducible.

These data suggest that glucose 1-phosphate and glucose 6-phosphate share a common transport system in the mutant. It is likely that when wild strains are induced for transport of glucose 6-phosphate they also are able to take up glucose 1-phosphate by the same system. The situation is complicated in wild strains because they are able to hydrolyze glucose 1-phosphate rapidly and then take up the glucose moiety. However, when wild strains were induced with glucose 6-phosphate the uptake of radioactivity upon incubation with ^{14}C-glucose 1-phosphate was not only greatly increased compared with noninduced cells, but also showed substantial inhibition in the presence of nonradioactive glucose 6-phosphate.

Thus it appears that when wild strains of *E. coli* are induced for transport of glucose 6-phosphate they acquire an extra mechanism for uptake of glucose 1-phosphate; the glucose moiety may now enter as part of an intact glucose 1-phosphate

[2] Strain DF2000 (see preceding paper (19)) behaved like E15 in that it has an active surface hexose phosphatase that hydrolyzed glucose 1-phosphate. Entry of radioactivity when cells are incubated with ^{14}C-glucose 1-phosphate is competed for by glucose, as in the case of E15. Glucose 1-phosphate causes growth stasis in DF2000-fed glycerol in the same way as glucose itself (see Fraenkel (20)).

molecule or after preliminary hydrolysis. This is why the rate of uptake of ^{14}C-glucose 1-phosphate, measured by radioactivity, was increased when wild strains were induced. However, it is likely that the two systems interact in some way as strictly additive effects cannot be obtained. The effect of induction is much more easily observed in strain MOX19 which lacks Enzyme I. Here, the uptake of ^{14}C-glucose 1-phosphate rises nearly 12-fold when the cells are induced with glucose 6-phosphate.

Pogell *et al.* (4) made a thorough and careful study of glucose 6-phosphate uptake and their data are of considerable interest to us. Of the hexose phosphates that they tested, only nonradioactive glucose 6-phosphate, glucose 1-phosphate, fructose 1-phosphate, and fructose 6-phosphate markedly decreased the accumulation of labeled glucose 6-phosphate by cells induced with the last named compounds. Also, they observed that the accumulation of ^{14}C-glucose 1-phosphate was increased significantly in glucose 6-phosphate-grown cells. We confirm both of these observations. However, they go on to consider the suggestion that separate permeases for hexose 6-phosphates and glucose 1-phosphate may exist, both being derepressed by growth on either glucose 1-phosphate or glucose 6-phosphate. With this we disagree.

Since completion of this work a paper by Fukui and Miyairi (21) has appeared, describing a specific transport system for glucose 1-phosphate in *A. tumefaciens*. It is quite different from the *E. coli* system, because glucose 1-phosphate acts as an inducer and neither glucose nor glucose 6-phosphate inhibits the uptake of ^{14}C-glucose 1-phosphate. Mention should also be made of an interesting new technique for isolating mutants in the hexose 6-phosphate transport system (22).

After this paper was first submitted for publication we became aware of significant and interesting new developments from H. L. Kornberg's laboratory (23). Ferenci, Kornberg, and Smith observed that an *E. coli* mutant devoid of the ability to grow upon fructose can grow on fructose 1-phosphate. However, this ability to utilize fructose 1-phosphate is manifested only by cultures that have been exposed previously to substances that induce the uptake system for hexose 6-phosphates and persists for only about 1½ doublings on fructose 1-phosphate. This implies that fructose 1-phosphate is not an inducer of the UHP[3] system but can be transported by it. (This is similar to the claim that we make for glucose 1-phosphate.) The case for fructose 6-phosphate, as observed by Winkler (5) is different. Growth in the presence of fructose 6-phosphate induces the hexose phosphate uptake system, but only if the cells contained phosphoglucose isomerase.

Ferenci *et al.* (23) selected mutants that grew rapidly on fructose 1-phosphate. Such mutants were UHP constitutive; that is, they were derepressed for uptake of ^{14}C-glucose-6-phosphate and ^{14}C-fructose 6-phosphate. Quite recently, these same workers observed that mutants of Enzyme II of the fructose uptake system revert on fructose 1-phosphate to give UHP-constitutive cells.[4] Finally, Dr. Kornberg, with a phosphotransferase Enzyme I mutant made and mapped as described earlier (24), has independently confirmed our observation that UHP-constitutive cells can be isolated after spontaneous mutation in the presence of glucose 1-phosphate.[4] Genetic studies on the hexose 6-phosphate (UHP) transport system are being actively pursued in the laboratories of Kornberg and Winkler, and such studies should clarify our understanding of this system.

[3] The abbreviation used is: UHP, uptake system for hexose 6-phosphates.

[4] H. L. Kornberg, personal communication.

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J. Biol. Chem. 1971, 246:2891-2897.