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Localization of Phosphoglucose Isomerase in *Escherichia coli* and Its Relation to the Induction of the Hexose Phosphate Transport System

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The localization of phosphoglucose isomerase (PGI) was studied in relation to the induction of hexose phosphate uptake in *Escherichia coli*. The uptake system is induced only by extracellular glucose-6-phosphate (G6P); there is no induction by intracellular G6P. Fructose-6-phosphate (F6P) is an indirect inducer, and isomerization of F6P to G6P must occur before induction. PGI has been considered to be an internal enzyme; therefore, uptake of F6P by noninduced cells and leakage of the G6P formed would be required for induction. In this study, it was concluded that part of the PGI activity is located in the cell surface because: (i) uninduced, intact cells are able to convert F6P to G6P, whereas the activity of G6P dehydrogenase is not detectable; (ii) when cells are subjected to osmotic shock, about 10% of the PGI activity is found in the shock fluid; and (iii) sorbitol-6-phosphate (S6P) inhibits both PGI activity of whole cells and the induction of hexose phosphate transport system by F6P. S6P was not taken by intact cells. The data indicate that the isomerization of F6P to G6P can take place on the cell surface, and this explains the indirect induction of hexose phosphate transport by F6P.

The transport system for hexose phosphates in *Escherichia coli* is inducible (6, 13, 19, 24). Glucose-6-phosphate (G6P), when present in the medium in relatively low concentration (1×10^{-4} M), induces the uptake system, whereas no induction is observed by endogenously produced G6P, even when its internal concentration is as high as 6×10^{-2} M (4, 8, 25). The failure of internal G6P to induce has been ascribed to inhibition of induction by glucose or some product of glucose metabolism (25). However, externally added G6P is an effective inducer, without a lag period, even when a culture has remained for some hours with a high internal G6P content and with no induction having occurred (4, 25). (Accumulation of intracellular G6P was affected by use of a mutant lacking G6P dehydrogenase and isomerase [5].)

Winkler (25) made the interesting observation that fructose-6-phosphate (F6P) is also an inducer, but only indirectly, because it was ineffective in mutants lacking phosphoglucose isomerase (PGI). Thus, conversion of F6P to G6P is needed for the induction. According to Winkler (25, 26), the conversion occurs inside the *E. coli* cell, whereupon G6P leaks out and induces the hexose phosphate transport system from the outside. This explanation is not very satisfying. We do not find that internally generated G6P leaks out in sufficient amount to act as an external inducer, even in the mutant DF2000 (4) in which more than 0.06 M G6P accumulates internally. In the present paper, we demonstrate a more satisfying explanation for the mechanism of action of F6P. We show that part of the PGI of *E. coli* is a periplasmic enzyme, accessible to external sugar phosphate, as judged by the fact that it is released by osmotic shock and can be measured with intact cells. Also, a fraction of PGI can be inhibited in whole cells by a nonpenetrating compound, sorbitol-6-phosphate (S6P). Thus, F6P can be converted to G6P in the periplasmic space, providing an external inducer directly, without the need for transport of F6P and leakage outward of G6P from uninduced cells.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strain 7 (a derivative of K-12) was a gift from E. C. C. Lin (7).

Media and growth conditions. Cells were grown on a synthetic, phosphate-buffered medium (22) supplemented with 0.4% glycerol, 1.0% succinate or 0.5% glucose, at 37 C, in a rotary shaker. There were no

significant differences in the rate and extent of induction by cells grown on different carbon sources. The cells were harvested in mid-exponential phase of growth (approximately 10^9 cells per ml) by centrifugation, washed twice with cold 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 7.2) containing 0.03 M NaCl, and suspended in 0.033 M Tris-hydrochloride, *p*H 7.2 (1 g of packed cells per 80 ml).

Enzyme activities. A fraction of the cell suspension was subjected to osmotic shock (17), and another fraction was lysed by incubation for 15 min at 37 C with 10 μg of lysozyme per ml and 1×10^{-3} M ethylenediaminetetraacetic acid (EDTA), followed by freeze-thawing. Enzymatic activities were assayed in cell suspensions, cell lysates, shock fluids, and shocked cells. PGI activity was measured in a coupled assay by using G6P dehydrogenase (25). The reaction mixture contained 50 mM Tris-hydrochloride buffer (*p*H 7.6), 10 mM $MgCl_2$, 1 mM F6P, 0.2 mM nicotinamide adenine dinucleotide phosphate (NADP), 2.5 μg of G6P dehydrogenase per ml, and sample for assay. PGI activity was also assayed by direct measurement of F6P concentrations (20). G6P dehydrogenase activity was measured spectrophotometrically (5) under the same conditions as used for PGI activity, except that F6P was replaced by 0.4 mM G6P. Triose phosphate isomerase activity was measured in a coupled assay by using β-glycerophosphate dehydrogenase (1). Induction of β-galactosidase was obtained by growth in the presence of 5×10^{-5} M isopropyl-β-thiogalactoside, and the enzyme was assayed by following the rate of hydrolysis of *o*-nitrophenyl-β-galactopyranoside (15). Assays of inorganic pyrophosphatase (11) and 2',3'-cyclic phosphodiesterase (2) have been described. The activity of S6P dehydrogenase was measured spectrophotometrically (10). Protein was assayed according to Lowry et al. (14).

Uptake of hexose-6-phosphate. The uptake of G6P and S6P was measured as described by Dietz and Heppel (3). The cells were washed and suspended in the presence of chloramphenicol to 40 μg/ml. Hexose-6-phosphate was added to a final concentration of 8×10^{-5} M.

Preparation of membrane fractions. Cells were converted to spheroplasts by treatment with EDTA and lysozyme. The spheroplasts were lysed, and the membrane fraction was washed thoroughly, according to the procedure outlined by Kaback (12).

Chemicals and enzymes. The following reagents and enzymes were purchased from Sigma Chemical Co., St. Louis, Mo.: G6P, S6P, NADP, lysozyme, and ribonuclease; from C. F. Boehringer & Soehne, Mannheim, Germany: F6P, G6P dehydrogenase, PGI, glycerophosphate dehydrogenase, and triosephosphate isomerase; from Worthington Biochemical Corp., Freehold, N.J.: deoxyribonuclease; from Mann Research Laboratories, N.Y., N.Y.: IPTG; from New England Nuclear Corp.: G6P-*1*-^{14}C. S6P-*1*-^{14}C was prepared from G6P-*1*-^{14}C by reduction with $NaBH_4$ (27). All other chemicals were of analytical grade.

RESULTS

Enzymatic activities in whole cells and shock fluid. A number of enzymes have been assigned to a periplasmic region of gram-negative cells on the basis of: (i) histochemical evidence (16, 23), (ii) selective release by osmotic shock (9), (iii) the fact that they can be measured by using intact cells as a source of enzyme with nonpenetrating substrates (9), and (iv) the fact that the activity measured with intact cells can be inhibited by using an inhibitor which is unable to penetrate whole cells (L. A. Heppel et al., *in press;* 18). These criteria were applied to determine the localization of PGI in *E. coli*.

The activities of several enzymes were assayed by using intact control cells, whole shocked cells, cell lysates, and shock fluid (Table 1). Of the total PGI activity, 10 to 20% was expressed in whole cells, and 6 to 10% was found in the shock fluid. (The enzyme is not released from the cells during growth; only 0.3% of the total activity was found in the growth medium.) By comparison, less than 1% of G6P dehydrogenase, β-galactosidase, and inorganic pyrophosphatase and less than 2% of triosephosphate isomerase were found in the shock fluid. It is especially significant that G6P dehydrogenase, an enzyme that shares a common substrate with PGI, could not be detected in shock fluid or when intact control or shocked cells were tested; it could be measured only in lysates.

In the case of cyclic phosphodiesterase, which is entirely a periplasmic enzyme, 99% of the activity was released into the shock fluid. Less than 1% of the internal enzyme, inorganic pyrophosphatase, was released into the shock fluid, but 6 to 7% of the activity could be measured with whole cells. The assay is ordinarily carried out at *p*H 9, and conceivably *E. coli* cells are permeable to inorganic pyrophosphate under these conditions; however, similar results were obtained at *p*H 8. The possibility that a mediator exists for the entry of inorganic pyrophosphate is being investigated.

The results so far indicate that whole cells are capable of converting F6P to G6P, even when they have not been induced for the hexose phosphate transport system. A significant fraction of PGI appears to be a periplasmic enzyme. Incidentally, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (5×10^{-5} M) did not inhibit the activity of PGI in whole cells, though it completely inhibited hexose phosphate transport.

Induction of the hexose phosphate transport system: effect of S6P. Uptake of G6P was not detected in uninduced cells. Addition of G6P to the medium caused induction of the hexose phosphate transport system (Fig. 1). The induction began immediately after addition of

TABLE 1. *Enzymatic activities in E. coli strain 7 cells and shock fluid*

Determinations	Phosphoglucose isomerase		G6P-dehydrogenase		Triose-phosphate isomerase		β-Galactosidase		Pryophosphatase		2',3'-Cyclic phosphodiesterase	
	SA[a]	%[b]	SA	%	SA	%	SA	%	SA	%	SA	%
Cell lysate	630	100.0	109	100.0	1,001	100.0	2,050	100.0	1,673	100.0	120	100.0
Whole cells	63	10.0		<0.1	27	2.7	26	1.3	98	5.8	94	78.0
Shock fluid	1,205	8.3		<0.1	212	1.7	220	0.5	306	0.8	2,750	99.0
Shocked cells	21	3.2		<0.1	37	3.4	29	1.5	122	6.9	14	11.0

[a] SA, Specific activity, expressed as nanomoles per minute per milligram of protein.

[b] This column indicates the total activity measured on shocked cells, on shock fluid, or on suspension of intact cells. The total activity measured on an equivalent amount of completely lysed cells is taken as 100%.

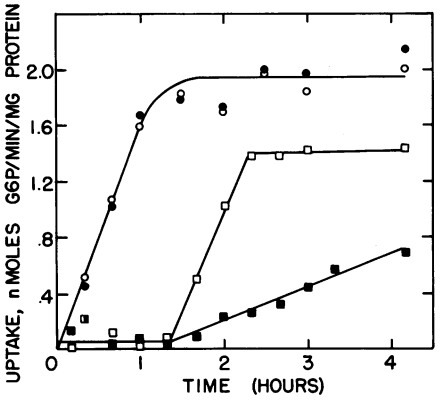

FIG. 1. *Induction of hexose phosphate transport system. Effect of sorbitol-6-phosphate. Hexose phosphates were added to cell suspension at zero time: G6P (5 × 10⁻⁵ M), ○; G6P (5 × 10⁻⁵ M) plus S6P (5 × 10⁻⁴ M), ●; F6P (10⁻⁴ M), □; F6P (10⁻⁴ M) plus S6P (5 × 10⁻⁴ M), ■.*

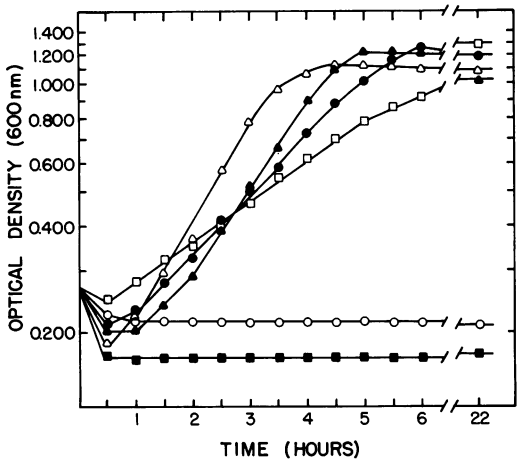

FIG. 2. *Growth of E. coli on different carbon sources. Carbon sources were added at zero time, to final concentration of 5 × 10⁻³ M. No carbon source, ○; glycerol, ●; G6P, △; F6P, ▲; sorbitol, □; S6P, ■.*

G6P, and its rate was linear, as described by Winkler (26). In the presence of F6P, the rate of induction was similar to that observed with G6P, but induction began only after 60 to 80 min. F6P is not an immediate inducer as is G6P, since conversion of F6P to G6P must take place before induction occurs as shown by Winkler (25).

S6P inhibits the induction of the hexose phosphate transport system when F6P is present in the medium, but not when G6P is used as the external inducer (Fig. 1). S6P is an inhibitor of PGI (21), and under the conditions used for the induction experiments the activity of PGI measured with whole cells was inhibited by 85%. Since S6P does not enter the cells (see below), the inhibition of PGI activity, and the resultant inhibition of induction of hexose phosphate transport by F6P, must occur external to the permeability barrier of the cells.

Several lines of evidence indicate that S6P does not enter the cell. Cells of strain 7 were unable to grow on S6P as a carbon source although they did grow on G6P, F6P, and sorbitol (Fig. 2). Growth on these carbon souces was not inhibited by S6P. A low, but significant, activity of S6P dehydrogenase activity was found in cell lysates (7.8 nmoles per min per mg of protein), but was not detected in whole cells. Uptake of labeled S6P was not detected in the cells even after 4 hr of growth in the presence of S6P, F6P, or a combination of F6P and S6P, under conditions used in the induction experiments (Fig. 1).

PGI activity in cell fractions. Since 10 to 20% of the total PGI activity was expressed in whole cells, the possibility that a fraction of the enzyme is membrane bound was checked. Membrane preparations were made by the method of Kaback (12), and PGI activity was measured at each stage of the procedure. As a control, the activities of other enzymes, both soluble and membrane bound, were assayed (Table 2). Only 2.2% of PGI activity was found

TABLE 2. *Activity of enzymes in cell fractions during preparation of membranes*

Fraction	Activity[a] of enzymes				
	Phospho-glucose isomerase	G6P dehy-drogenase	β-galacto-sidase	Pyrophos-phatase	Lactic dehy-drogenase
Cell lysate	100.0	100.0	100.0	100.0	100.0
Whole cells	18.8	<0.01	1.3	15.7	79.3
Supernatant of spheroplasts	16.0	4.7	9.1	16.5	0.4
Spheroplast lysate	84.3	95.1	91.0	83.6	70.5
Supernatant of spheroplast lysate	87.5	94.9	100.0	56.0	10.6
Washings	3.3	6.2	3.0	18.8	5.4
Membrane preparation	2.2	0.6	1.6	0.7	31.0

[a] Activity expressed as percentage of the total activity. The specific activities for cell lysates are indicated in Table 1. The specific activity of lactic dehydrogenase was found to be 892 nmoles per min per mg of protein in cell lysates.

in the membrane fraction, in contrast to 31% of D-lactic dehydrogenase, a known membrane-bound enzyme. We conclude that no significant fraction of PGI is membrane bound or else that the binding is very loose and is disrupted during isolation of the membranes.

DISCUSSION

Our observations, in conjunction with Winkler's findings, suggest that the indirect induction of the hexose-6-phosphate transport system by F6P is carried out largely outside of the cell membrane. F6P is converted to G6P in the periplasmic space by the PGI activity present in this region, and the G6P thereupon induces the transport system by an external route.

Mannose-6-phosphate (M6P) was found to be an inducer and a substrate of the hexose-6-phosphate transport system (24). Preliminary experiments indicated that the induction by M6P might be similar to the induction by F6P. M6P isomerase activity was found in the cells. About 15% of the total activity was expressed by whole, uninduced cells, and part of it was released by osmotic shock. Thus, M6P can be converted to G6P (via F6P) on the cell surface, and the G6P formed induces the transport system. The role of M6P and M6P isomerase in the hexose phosphate transport system is under current investigation.

The mechanism of the induction from outside is still obscure. We agree with Winkler (25), who considers that there are two major possibilities to explain why only exogenous G6P induces: (i) a small fraction of the G6P crossing the membrane would be converted to the "true inducer"; (ii) there is a membrane-associated induction-repression system. This system is oriented in such a manner that G6P can react with the repressor only if it first interacts with the external face of the cytoplasmic membrane.

Preliminary experiments were carried out to find whether a more efficient inducer (the true inducer) is produced during incubation of G6P with cells or cell fractions. These have provided no evidence for such a conversion.

It is not known whether the PGI activity expressed outside the cell is due to the same enzyme present inside the cell, or if there are two PGI isoenzymes, one external and the other internal.

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