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Compartmentation in the Induction of the Hexose-6-Phosphate Transport System of *Escherichia coli*[1]

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The induction of the hexose-6-phosphate transport system was investigated. Glucose-6-phosphate (G6P) at concentrations as low as 10^{-4} M was able to induce this system in wild-type cells, as well as in mutants lacking phosphoglucose isomerase or G6P dehydrogenase. Growth in the presence of fructose-6-phosphate (F6P) induced the system only if the cells contained phosphoglucose isomerase. Furthermore, glucose and F6P were found to induce the system only if the extracellular concentration of G6P became appreciable in the medium as a consequence of the leakage of intracellular G6P formed from the glucose or F6P. Intracellular G6P was not an inducer even at high concentrations. The metabolism of glucose inhibited the induction of the hexose-6-phosphate transport system. Hypotheses for this compartmentalization of inducer and membrane-associated induction are presented.

The transport system responsible for the uptake of hexose-6-phosphates in *Escherichia coli* has been previously described (3, 7, 9). This transport system is induced in wild-type cells by growth in the presence of glucose-6-phosphate (G6P) or fructose-6-phosphate (F6P), but not by growth on glucose or fructose. Evidence that separate systems are involved in the transport of hexose-6-phosphates and the corresponding free hexoses was obtained by the isolation of hexose-phosphate transport negative mutants which would grow on hexoses but not hexose-6-phosphates (9). In the present study, mutants blocked in phosphoglucose isomerase and G6P dehydrogenase (1, 2) were used to characterize the nature of the inducer. Mutants lacking both of these enzymes are unable to grow on glucose or G6P. However, these mutants accumulate large intracellular pools of G6P when grown in the presence of glucose because of the phosphoenolpyruvate-phosphotransferase of the glucose transport system (6). Heppel (5) reported that the double mutant was not induced for G6P transport when grown in the presence of glucose but was induced when grown with G6P. The present study demonstrates that extracellular G6P functions as an inducer of hexose-6-phosphate transport, but that neither intracellular G6P nor F6P (intra- or extracellular) will induce this system.

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MATERIALS AND METHODS

Bacterial strains. The wild-type parental strain was *E. coli* K-10, a prototrophic Hfr with normal sugar metabolism (Fig. 1). The mutants were DF1800, which lacks both phosphoglucose isomerase (Pgi^-) and G6P dehydrogenase or zwischenferment (Zwf^-), DF40 (Pgi^-, Zwf^+), and DF1801 (Pgi^+, Zwf^-). The DF family of mutants, kindly provided by D. Fraenkel, has been previously described (1, 2).

Media and growth conditions. Minimal salts medium 63 (9) supplemented with thiamine (1 μg/ml) and 1% Difco Casamino Acids as carbon source was the standard medium. Casamino Acids were preferable to glycerol because the pronounced stasis seen with the latter (2) was not observed with Casamino Acids. The standard starting inoculum was 0.18 mg (wet weight) per ml. Cells were grown for 3 to 4 hr at 37 C with shaking, and growth was monitored with a Klett photometer at 420 nm. A tris(hydroxymethyl)aminomethane (Tris)-based medium free from inorganic phosphate (4) at *p*H 8.0 with β-glycerol phosphate (0.05%) and gluconate (0.4%) was used for derepression of alkaline phosphatase.

Uptake assays. Cells were harvested and washed at room temperature and resuspended to a known turbidity in medium 63 plus chloramphenicol (50 μg/ml) without carbon source. Uptake of ^{14}C-labeled substrates was determined by membrane filtration as previously described (9), except that all operations were performed at room temperature with filters of 0.45 μm average pore size. G6P was present in the

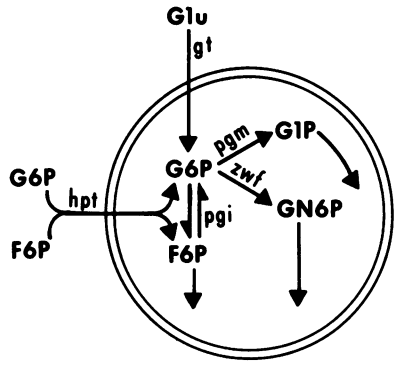

FIG. 1. *Scheme of carbohydrate metabolism pertinent for these studies. Abbreviations: hpt, hexose-6-phosphate transport system; gt, glucose transport system; zwf, glucose-6-phosphate dehydrogenase; pgi, phosphoglucose isomerase; pgm, phosphoglucomutase; Glu, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; G1P, glucose-1-phosphate; GN6P, gluconate-6-phosphate. The cell membrane is indicated by the double circle.*

assay mixture at a concentration of 0.5 mM and 0.1 μc/ml. The cells on filters were dried, and their radioactivity was counted by liquid scintillation spectrometry in toluene-Omnifluor, 0.4% (New England Nuclear Corp., Boston, Mass.).

Spectrophotometric assays. G6P was measured by use of G6P dehydrogenase as described by Fraenkel (2). Intracellular G6P was determined after hot-water extraction of filtered and washed cells. Fructose-6-phosphatase was measured in the same way as G6P after the addition of phosphoglucose isomerase. Alkaline phosphatase was measured by hydrolysis of *p*-nitrophenyl phosphate. The assay system contained, in addition to intact cells, Tris-hydrochloride (*p*H 8.0, 0.67 M) and *p*-nitrophenyl phosphate (16 mg) in a total volume of 3 ml.

Chemicals. Chloramphenicol was a gift of Parke, Davis & Co., Detroit, Mich. Glucose-^{14}C-6-phosphate was purchased from New England Nuclear Corp.; potassium G6P, from Calbiochem, Los Angeles, Calif.; and G6P dehydrogenase (type XV), phosphohexose isomerase (grade III), and the sodium salt of F6P, from Sigma Chemical Co., St. Louis, Mo.

RESULTS

F6P versus G6P as the inducer. The double mutant DF1800 (Pgi$^-$, Zwf$^-$) was used to determine whether both G6P and F6P were inducers of hexose-6-phosphate transport. As shown in Fig. 2, G6P at concentrations as low as 10^{-4} M was an effective inducer both in the wild type, K-10, and in DF1800. Once induced, the mutant, like the wild type (9), transported both F6P and G6P. Similar results were obtained with the single mutants DF40 and DF1801. On the other hand, F6P was not an inducer in the double mutant, although F6P was able to induce this transport system in the wild type and in the single mutant DF1801 (Pgi$^+$, Zwf$^-$; Fig. 3). F6P was also unable to induce DF40 (Pgi$^-$, Zwf$^+$). Thus, F6P functions as an inducer only when phosphoglucose isomerase is present in the cell to convert F6P to G6P.

Effect of dilute culture conditions. The large pool of intracellular G6P accumulated by the mutant DF1800 when grown with glucose made it feasible that induction from internal G6P might occur in this strain, even though the neutral hexoses were previously shown not to induce the hexose-phosphate transport system in the wild

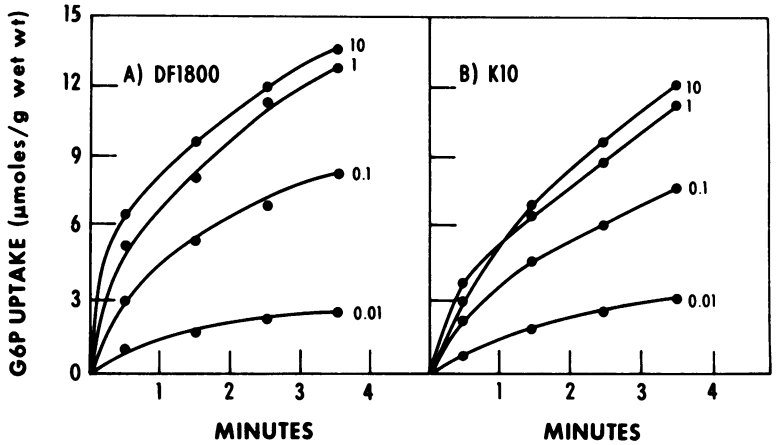

FIG. 2. *G6P uptake after growth with G6P: (A) DF1800, (B) K-10. Cells were grown under standard conditions as described in Materials and Methods. The Medium 63-thiamine-Casamino Acids medium was supplemented with the indicated concentrations (millimolar) of G6P. Cells were harvested, and the uptake of G6P was determined.*

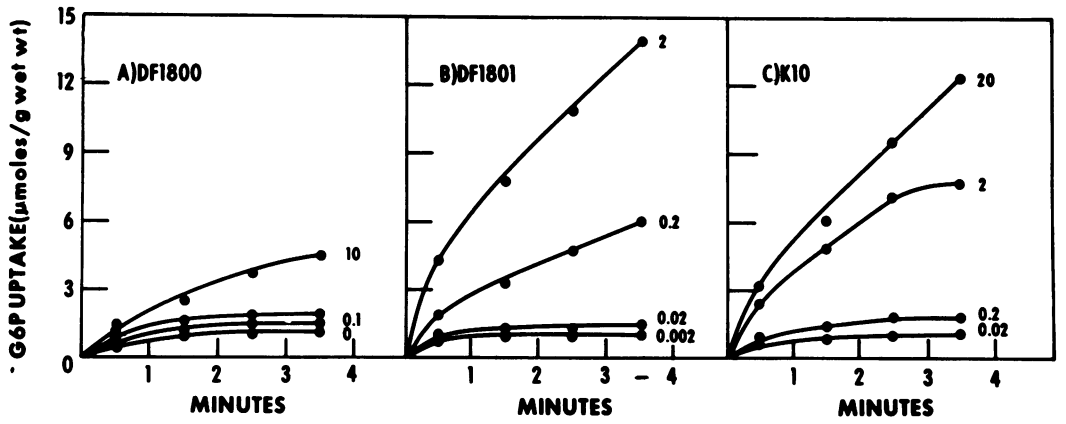

FIG. 3. *G6P uptake after growth with F6P: (A) DF1800, (B) DF1801, (C) K-10. Conditions as in Fig. 2 except that medium was supplemented with the indicated concentration (millimolar) of F6P.*

type (9). The induction observed when DF1800 was grown with glucose (Fig. 4) is, however, an artifact. As shown in Fig. 4, DF1800 grown with various concentrations of glucose under standard conditions appeared to be induced by the glucose; however, if the starting inoculum was decreased, but the glucose concentration was maintained at a high level, there was no induction (curve DIL-10). These results suggested that a high intracellular concentration of G6P will not induce, whereas a relatively low concentration of G6P in the medium will. Table 1 shows data obtained with DF1800 grown in the presence of 10 mM glucose with various starting inocula. As can be seen, the intracellular concentration remained high with inocula of any size and did not correlate with the level of induction. On the other hand, the concentration of G6P in the medium rose as the inoculum was increased, and the level of induction increased in parallel.

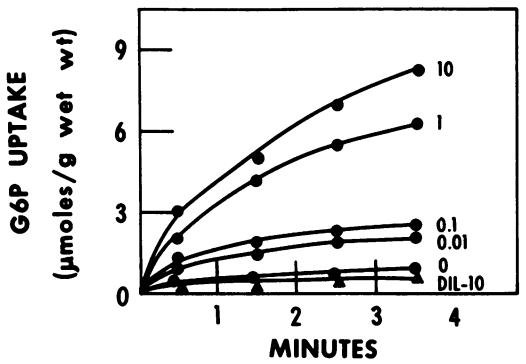

FIG. 4. *Effect of dilute culture conditions on G6P uptake in strain DF1800, after growth with glucose. Conditions as in Fig. 2 except that the dilute culture (curve DIL-10) had a starting inoculum 5% of the standard culture. Supplementation of the medium with glucose was at the concentrations indicated (millimolar).*

TABLE 1. *Correlation of the level of induction with the extracellular pool of G6P in DF1800 grown with glucose*

G6P concn (μM)		G6P uptake (μmoles/g, wet wt)	
Extracellular	Intracellular	0.5 min	3.5 min
4	12	0.08	0.18
12	—	0.18	1.0
22	—	0.22	1.1
31	—	0.61	1.4
35	12	0.90	2.9
94	—	3.0	10.0
150	10	5.2	12.0

To test the hypothesis that under the standard growth conditions cells grown with glucose were leaking G6P into the medium and this exogenous G6P was the inducer, the medium derived from a standard culture was tested for the presence of an inducing factor. As shown in Fig. 5, the medium from a standard culture, when reinoculated with a dilute inoculum, produced induction (curve SD). However, the medium from a dilute culture would not induce a dilute inoculum (curve DD), but would induce a standard inoculum (curve DS) with the concomitant leakage of more G6P. Treatment of the standard medium with nicotinamide adenine dinucleotide phosphate (NADP) plus G6P dehydrogenase to remove G6P rendered it unable to induce a dilute inoculum (curve SZD). Measurements of the G6P content of the media correlated with the level of induction as measured by uptake assays.

Another test of the hypothesis was to derepress alkaline phosphatase so that the G6P leaking from the cells would be hydrolyzed. In this case,

G6P would not build up in the medium and induction would not be expected to ensue. As shown in Fig. 6, under conditions of phosphate depression, G6P was still able to induce the transport of G6P, although an effect of the lowered concentration of G6P in the medium by the action of the phosphatase was observed. Glucose, however, while effective when alkaline phosphatase was repressed, caused no induction in the derepressed culture.

Similar experiments with dilute cultures of cells (Pgi^+) grown with F6P are shown in Fig. 7. Again, the induction was not observed when the cells were grown under dilute conditions but was observed under standard conditions. Figure 8 demonstrates that the medium from a standard culture of K-10 grown with F6P contained sufficient G6P to induce hexose-6-phosphate

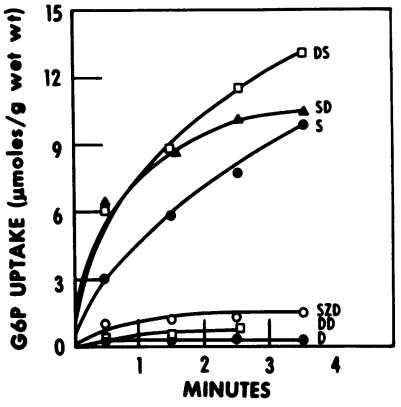

FIG. 5. *Induction of G6P uptake in a dilute culture of strain DF1800 by growth in the medium from a culture grown with glucose. Cells were grown with glucose (10 mM) under standard and dilute conditions as described in Fig. 4. Cells harvested from these cultures were assayed for G6P uptake: curve D, dilute culture; curve S, standard culture. The medium from both cultures was assayed for G6P. NADP (0.66 mg/ml) was added to the medium from the standard culture and G6P dehydrogenase was added to a portion of this culture to remove G6P. All three media, the dilute, the standard plus zwischenferment, were then sterilized by filtration. The next morning these media were reinoculated with dilute or standard inocula and grown as before; the cells were harvested and assayed for G6P uptake. Curve DS, dilute medium with standard inoculum; curve DD, dilute medium with dilute inoculum; curve SD, standard medium with dilute inoculum; curve SZD, standard medium plus zwischenferment with dilute inoculum.*

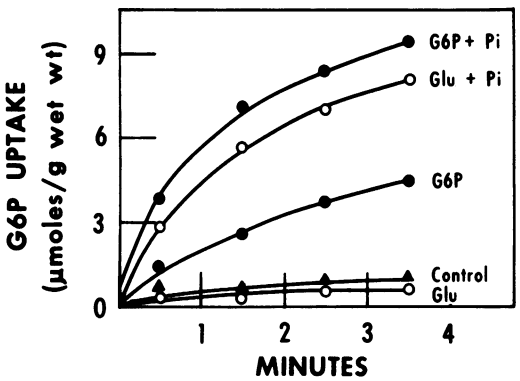

FIG. 6. *Effect of derepression of alkaline phosphatase on the induction of G6P uptake in cells of strain DF1800 grown with glucose. A standard inoculum of cells was added to the Tris-based medium described in Materials and Methods. G6P was added at 0.15 mM where indicated; glucose was added at 10 mM where indicated (Glu). In curves labeled Pi, the inorganic phosphate concentration was adjusted to 10 mM to repress and inhibit alkaline phosphatase.*

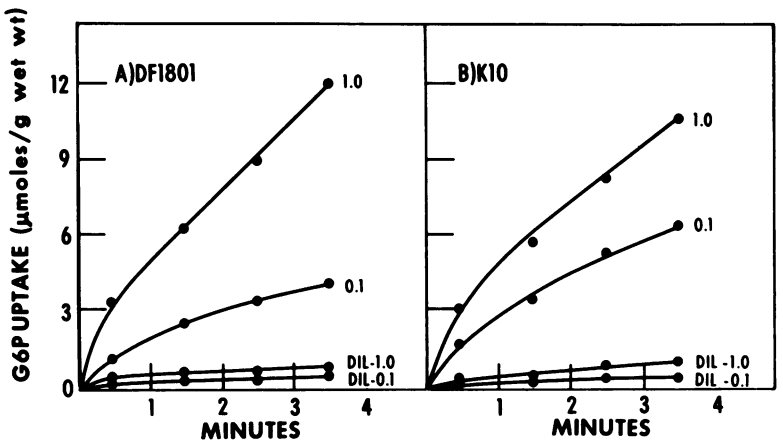

FIG. 7. *Effect of dilute culture conditions on G6P uptake after growth with F6P: (A) DF1801, (B) K-10. Conditions as in Fig. 4, except that supplementation was with F6P at the indicated concentrations (millimolar).*

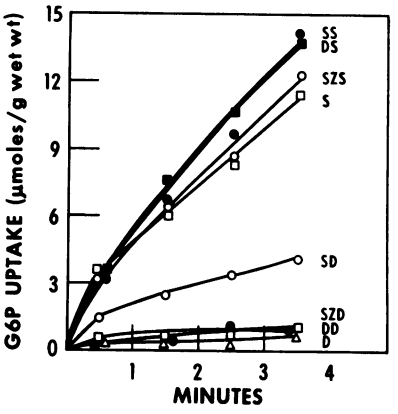

FIG. 8. *Induction of G6P uptake in a dilute culture of strain K-10 by growth in the medium from a culture grown with F6P. Conditions and experimental design as in Fig. 6 except that F6P (1 mM) was in the medium instead of glucose and F6P (1 mM) was added to the filtered medium before the second growth. As in Fig. 6, the last letter of the abbreviations represents the inoculum (dilute or standard) and the preceding letters represent the medium (dilute, standard, or standard plus zwischenferment).*

transport when a dilute inoculum was added (curve SD). Again, as in Fig. 5, the addition of NADP plus G6P dehydrogenase to the medium removed G6P so that a dilute culture could not be induced (curve SZD). However, after this treatment with zwischenferment, the addition of a standard inoculum to the medium replenished the G6P and the culture was induced (curve SZS).

Repression by glucose. The addition of glucose fully inhibited the induction which resulted from the leakage of G6P from a standard culture of K-10 grown with F6P (Fig. 9). No inhibition was observed when the G6P level in the medium was elevated to 1 mM. Similar results were observed in strains DF40 and DF1801. Although the mechanism of this inhibition (repression) is not known, it would account for the lack of induction from any leakage of G6P in K-10, DF40, or DF1801 grown with glucose.

DISCUSSION

The hexose-6-phosphate transport system is unusual in that its substrates, G6P and F6P, are more common as metabolic intermediates than as carbon sources occurring in the natural environment. To enjoy the economy of an inducible active transport system for hexose-6-phosphates, the cell must become induced when G6P is present in low concentration in the medium but remain repressed in the presence of intracellular G6P. Although the amount of intracellular G6P in wild-type organisms is too small to measure accurately (2), the large pool of G6P formed in the mutants lacking both phosphoglucose isomerase and G6P dehydrogenase made possible the demonstration of a difference in the ability of intracellular and extracellular G6P to induce the hexose-6-phosphate transport system.

Heppel (5) first pointed out this difference in a similar mutant, DF2000 (Pgi^-, Zwf^-). He found no induction of this transport system in the presence of glucose when the cells were grown either with glycerol as carbon source, in which stasis occurred, or with gluconate as carbon source, in which there was no stasis. The level of G6P which spilled into the medium in these experiments was similar to the levels in the dilute cultures used here, in which no induction occurred. This would explain why induction was never found with glucose. When some of the experiments done with DF1800 were repeated with DF2000, the two mutants were found to be identical in their behavior toward induction.

The ability of cells that contain phosphoglucose isomerase to be induced by F6P demonstrates a requirement for intracellular conversion of F6P to G6P and the subsequent leakage of this G6P to form exogenous G6P. No induction by F6P can occur in the absence of phosphoglucose isomerase. However, in cells which can metabolize glucose, the G6P formed from glucose does not induce. This observation seems paradoxical, because a larger intracellular G6P pool would be expected from the metabolism of glucose than from F6P. The explanation of the lack of induction from the G6P which leaked out of the cells

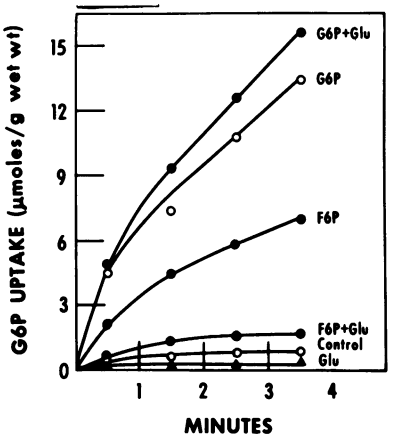

FIG. 9. *Inhibition by glucose of the induction of G6P uptake in K-10 grown with F6P. Cells were grown under the standard conditions. Medium was supplemented as indicated: G6P (1 mM), F6P (1 mM), glucose (10 mM).*

metabolizing glucose presumably involves inhibition of the induction by glucose, or, more likely, some product of glucose catabolism. An inhibitory effect of glucose could be observed in DF1801 or wild-type cells in the presence of F6P as the source of G6P.

The nature of the difference between G6P outside the cell and G6P inside the cell is an intriguing question but experimentally difficult to answer. Sercarz and Gorini described a similar situation where exogenous arginine serves as the co-repressor, but endogenous arginine does not (8). Two basic models are possible. In one there would be a unique pathway of G6P metabolism which is membrane-associated. In this case, a small fraction of the G6P crossing the membrane would be converted to the "true inducer," which could then exert its effect on cytoplasmic repressor. Since the great majority of the G6P crosses the membrane unaltered, the separation of the few molecules of true inducer would present a formidable problem. The second model would require a membrane-associated induction-repression system. It is possible that G6P can react with a cytoplasmic repressor molecule only when G6P is in the membrane phase. The alternative that the repressor is membrane-bound makes the association of G6P, repressor, and deoxyribonucleic acid difficult to visualize. Work is in progress with regulatory mutants of the hexose-6-phosphate transport system in an attempt to test these hypotheses.

ACKNOWLEDGMENTS

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