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Inducible Phosphoenolpyruvate-Dependent Hexose Phosphotransferase Activities in *Escherichia coli*

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1. A method is described for measuring the rate of phosphoenolpyruvate-dependent phosphotransferase activity for a variety of hexoses in toluene-treated suspensions of *Escherichia coli*. 2. The specific activities of the phosphotransferases that catalyse the phosphorylation of hexoses are greatly affected by the carbon source for growth. 3. In all strains of *E. coli* tested, fructose phosphotransferase activity is induced by growth on fructose. 4. Strains of *E. coli* differ greatly in the rate at which they phosphorylate glucose, but all strains possess at least a low glucose phosphotransferase activity under any tested condition of growth. Glucose phosphotransferase activity is further induced by growth on glucose; this does not occur in a mutant that lacks the ability to take up methyl α-D-[^{14}C]glucopyranoside and hence grows poorly on glucose. 5. When growing on fructose, two strains of *E. coli* synthesize the inducible glucose phosphotransferase system gratuitously, and to specific activities higher than observed during growth on glucose. A phosphotransferase catalysing the phosphorylation of mannose is similarly induced.

The utilization of a number of hexoses by enteric bacteria has been shown to involve the necessary activity of a phosphoenolpyruvate-dependent phosphotransferase system (Roseman, 1969; Kundig *et al.*, 1964). In this system, Enzyme I catalyses the transfer of the phosphate group from phosphoenolpyruvate to a nitrogen atom of a histidine moiety in a small protein (HPr) according to reaction (1) (Kundig & Roseman, 1971); in subsequent processes of the type represented in reaction (2) the appropriate Enzyme II catalyses the transfer of phosphate from the phosphorylated protein (phospho-HPr) to its specific sugar acceptor:

$$\text{Phosphoenolpyruvate} + \text{HPr} \xrightarrow{\text{Enzyme I}} \text{phospho-HPr} + \text{pyruvate} \quad (1)$$

$$\text{Phospho-HPr} + \text{hexose} \xrightarrow{\text{Enzyme II}} \text{hexose phosphate} + \text{HPr} \quad (2)$$

$$\text{Sum: Phosphoenolpyruvate} + \text{hexose} \rightarrow \text{hexose phosphate} + \text{pyruvate} \quad (3)$$

Evidence that this system plays the major role in the utilization of many hexoses by Enterobacteriaceae is derived mainly from a study of mutants affected in their ability to form HPr, Enzyme I or Enzyme II. This evidence was reviewed by Roseman (1969) and Lin (1970).

Kornberg & Reeves (1972) described briefly a method by which the phosphotransferase for methyl α-glucoside may be conveniently assayed. This method is now more fully explored and employed to assay the activities of phosphotransferases that catalyse the phosphoenolpyruvate-dependent phosphorylation of glucose, fructose and mannose in several strains of *Escherichia coli* when they are grown on a variety of carbon sources, and the induction of these phosphotransferase activities when cells are grown with glucose or fructose as the carbon source.

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Methods

Cell growth and decryptification with toluene

Cultures of *E. coli* are grown aerobically at 37°C on defined media containing salts (Ashworth & Kornberg, 1966) and a carbon source at 10 or 20mM; the media are supplemented with thymine, adenine and amino acids (40–100μg/ml) as required. The

absorbance of a cell suspension at 680nm multiplied by 0.68 is taken to equal mg dry wt. of cells/ml (Ashworth & Kornberg, 1966). Cells are harvested in the early phase of exponential growth (0.2–0.6mg dry wt. of cells/ml) by centrifugation at 20000*g* for 5min at 15°C. The harvested cells are suspended at 15°C in buffer containing 0.1M-sodium–potassium phosphate, pH7.2, and 5mM-$MgCl_2$; they are centrifuged as before and resuspended in this buffer. The washed cells are stored in ice until used on the same day. Alternatively, chloramphenicol to a final concentration of 100μg/ml is added to the samples withdrawn for harvest, which are then centrifuged and washed as described. No differences were observed when the two methods of harvest were applied to the same lot of cells. Cells were also grown in Oxoid Nutrient Broth (Oxoid Ltd., London S.E.1, U.K.) and harvested as described above.

Immediately before assay, a portion of the ice-cold cell suspension is placed in a 16mm×125mm test tube and vigorously agitated at top speed on a vortex-type mixer (Super-Mixer; Lab-Line Instruments Inc., Melrose Park, Ill., U.S.A.). While the agitation is continued, 0.01vol. of toluene–ethanol (1:9, v/v) is added by micro-pipette. Vigorous agitation of the suspension is continued for a further 60s, after which the tube is sealed with Parafilm and returned to the ice bath. When 10^5 toluene-treated cells were spread on a nutrient–agar plate, no colonies appeared after incubation for 48h at 37°C.

Bacterial strains

The three strains of *E. coli* K12 principally employed in this investigation are: B11 (Broda, 1967) *met*; K1 (Brice & Kornberg, 1967) *thy, met*; K2 (Brice & Kornberg, 1967) *arg, his, thr, leu, trp*. The preparation of the mutant K2.2w has been described by Kornberg & Smith (1972); it has the same amino acid requirements as strain K2, but lacks the ability to take up methyl α-D-[^{14}C]glucopyranoside. Strain K2.1.11^c (*his, thr, leu, pps, uhp*^{c}) was prepared from a genetic cross between the Hfr strain KL 16.11^c.21 (Ferenci *et al.*, 1971) and the F^- recipient K2.1t (Brice & Kornberg, 1967). Strain 0144, which was kindly provided by Professor A. H. Stouthamer (University of Nijmegen), requires only adenine for growth on media containing single carbon sources.

Standard phosphotransferase assay

Toluene-treated cells in buffer consisting of 0.1M-sodium–potassium phosphate, pH7.2, and 5mM-$MgCl_2$, equivalent to 10–100μg dry wt. of cells, are added to quartz cuvettes of 1cm light-path containing 1μmol of phosphoenolpyruvate, 0.1μmol of NADH, 0.3μg (2 units) of lactate dehydrogenase [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.] and sufficient buffer to give a final volume of 1.0ml after the addition of the carbohydrate substrate. A similar cuvette to which carbohydrate is not added serves as the control. The cuvettes are incubated at 30°C until the control, when monitored at this temperature in a recording spectrophotometer equipped with a thermostatically controlled cell compartment and automatic sample changer, exhibits a rate of decrease in extinction at 340nm that is linear with respect to time. In addition to achieving thermal equilibrium, this incubation period (which may last from 5 to 20min) ensures that any endogenous carbohydrate, which may be present in the cells, is consumed. Carbohydrate (5μmol) is then added to the experimental cuvettes and the decrease in absorbance followed at 340nm. Activity observed in the experimental cuvettes is corrected for any activity noted in the control. The molar extinction coefficient of NADH is taken to be 6.22×10^3 litre·mol^{-1}·cm^{-1}.

Other assays

L-Lactate oxidase is assayed in a cuvette containing toluene-treated cells, 0.1μmol of NADH, 0.3μg (2 units) of lactate dehydrogenase, and the usual buffer to a volume of 0.99ml. After thermal equilibrium is attained, the reaction is initiated by adding 0.01ml of 0.1M-lithium L-lactate. A similar cuvette to which the substrate is not added serves as the control. The progress of the reaction is measured as described above.

Sodium phosphoenolpyruvate was purchased from Sigma (London) Chemical Co. Ltd. It is assayed by observing the change in extinction at 340nm resulting from the addition of pyruvate kinase [Boehringer Corp. (London) Ltd., Ealing, London W.5, U.K.] to a cuvette containing buffer, lactate dehydrogenase, 1mM-ADP, 1mM-NADH and an appropriate sample of the stock phosphoenolpyruvate solution.

Results

Stoicheiometry of the pyruvate-formation assay

The phosphotransferase assay shows linear kinetics with respect to cell concentration in the range 10–100μg dry wt. of cells, and with respect to time for a period of at least 20min. It is dependent on added phosphoenolpyruvate, carbohydrate substrate and lactate dehydrogenase. On addition of one of the substrates in limiting amount, the stoicheiometry of reactions (1) and (2) is confirmed (Table 1).

Under the conditions of the standard assay the apparent K_m (glucose or fructose) is approx. 10^{-5}M. This low value facilitated the stoicheiometric observations on these carbohydrates, since limiting amounts were rapidly consumed and the reaction ceased

abruptly. On the other hand, the apparent K_m (phosphoenolpyruvate) is 3×10^{-4} M in the presence of 5 mM-fructose, and small amounts of added phosphoenolpyruvate are consumed at very slow rates in the reaction. To overcome this difficulty, limiting amounts of this substrate were added and the reaction was allowed to proceed for an extended period of time, after which the remainder of the substrate was assayed in the same cuvette by the addition of ADP and pyruvate kinase (Table 1).

Interfering enzyme activities present in toluene-treated cells

The control cuvette of the standard assay corrects for the small amount of NADH oxidase activity in the toluene-treated cells. That *E. coli* glucokinase does not interfere with the assay is shown by the result in Table 1; in this experiment a relatively large amount of ATP was added to the cuvette. A potentially serious interfering enzyme activity is that of L-lactate oxidase. Cells grown on nutrient broth and treated with toluene in the usual manner exhibited this enzyme in activity that amounted to over 200 nmol/min per mg dry wt. of cells. Under the conditions of the assay the apparent K_m (L-lactate) was 0.1 mM. When the pyruvate-formation assay is attempted with such cells, a spurious acceleration of the reaction rate with time is observed, which is due to the recycling of L-lactate to pyruvate, catalysed by this enzyme in the presence of air. Cells grown on a minimal medium with any of the carbon sources employed in this work reveal very little L-lactate oxidase activity (4–8 nmol/min per mg dry wt. of cells). Such small amounts of activity do not significantly affect the initial reaction rates observed in the standard phosphotransferase assay.

Phosphotransferase activities in cells grown on various carbon sources

The results listed in Table 2 were obtained with cells that had grown for many generations in the appropriate minimal medium in the presence of the indicated carbon source. These cells were assayed for the glucose and fructose phosphotransferase systems. With the three strains of *E. coli* investigated, fructose phosphotransferase activity is maximal in cells grown on fructose. Glucose phosphotransferase activity is highest when growth occurs on glucose, except in strain K2 grown on fructose, for which the value of 113 ± 6 nmol/min per mg dry wt. of cells is the highest listed in Table 2. It is almost double the value that this strain exhibits when grown on glucose. The phenomenon of growth on fructose inducing a higher activity of glucose phosphotransferase than of fructose phosphotransferase is designated the 'fructose kick' and has also been observed in the F^- strain 0144.

Induction of hexose phosphotransferase systems in the Hfr strain B11

Cells of the Hfr strain B11, grown on sodium gluconate as carbon source, exhibit low phosphotransferase activities for glucose, fructose and mannose. When such cells are washed and transferred to growth media containing glucose or fructose they show a significant time-lag before the onset of exponential growth; during and after this lag, the appropriate phosphotransferase activity is induced and maintained at a high value. Thus, after transfer of gluconate-grown cultures of *E. coli* B11 to media containing glucose as sole carbon source, the specific activity of the phosphotransferase for glucose rose from less than 5 to more than 60 nmol/min per mg dry wt. of cells, whereas that of the fructose phosphotransferase remained at less than 15 (Fig. 1*a*). In contrast, when such gluconate-grown cells were transferred to growth medium containing fructose as sole carbon source, the glucose phosphotransferase specific activity remained at less than 10 whereas that of the fructose system rose rapidly to more than 40 (Fig. 1*b*). These activities were maintained constant for several generations.

A third phosphotransferase activity, which catalyses the phosphoenolpyruvate-dependent phosphorylation of mannose, was also induced by growth on

Table 1. *Stoicheiometry of the pyruvate-formation assay*

The cuvettes contained 100 μg dry wt. of fructose-grown toluene-treated *E. coli* K2 cells. Apart from the concentration of the limiting substrate, the conditions were as described for the standard assay (see the Methods section). In the last experiment listed, the fructose concentration was 5 mM, and 37 nmol of phosphoenolpyruvate was added to the cuvette to initiate the reaction. After the reaction had proceeded for 28 min, 1 μmol of ADP and 2 units of pyruvate kinase were added, revealing 11 nmol of phosphoenolpyruvate that had not been consumed in the phosphotransferase reaction.

Limiting substrate and quantity consumed (nmol)	NADH oxidized (nmol)
D-Glucose (37)	36
D-Fructose (37)	36
D-Glucose* (19)	18
Phosphoenolpyruvate (26)	23

*ATP (1 μmol) was added to this cuvette.

Table 2. *Phosphotransferase activities in three strains of E. coli grown on various carbon sources*

Cells were grown in minimal media with the carbon source shown. The concentrations of glucose, fructose or sodium gluconate used for growth were 10mM, and of glycerol or sodium succinate 20mM. Harvesting, decryptification and assay were by the procedures described in the Methods section. The final concentrations in the cuvettes were: cells, 25–100μg dry wt.; sodium–potassium phosphate buffer, pH7.2, 95mM; $MgCl_2$, 4.9mM; phosphoenolpyruvate, 1mM; NADH, 0.1mM; lactate dehydrogenase, 0.3μg; D-glucose or D-fructose, 5mM. The S.E.M. is given in instances where five or more cell preparations were assayed (numbers of preparations are given in parentheses).

		Assayed phosphotransferase activity (nmol/min per mg dry wt. of cells)	
Strain	Carbon source	Glucose	Fructose
B11	Glucose	52±3 (5)	13
	Fructose	12	45
	Sodium gluconate	4	3
	Glycerol	6	3
	Sodium succinate	4	2
K1	Glucose	51	6
	Fructose	19	50
	Glycerol	12	3
K2	Glucose	62±3 (5)	6
	Fructose	113±6 (7)	85±7 (5)
	Sodium gluconate	31	5
	Glycerol	21±2 (6)	4.5±1 (6)
	Sodium succinate	13	1

glucose and, to a lesser extent, by growth on fructose. However, this mannose phosphotransferase activity that is induced during growth on glucose has a low affinity for mannose and requires a high (>1mM) concentration of mannose for half-maximal activity; its physiological role is uncertain.

Absence of induction of glucose phosphotransferase in a mutant that grows poorly on glucose

A mutant designated K2.2w, and found not to take up methyl α-[^{14}C]glucoside under standard conditions (Kornberg & Smith, 1972), differs in its properties from the other strains used in this investigation. The mutant grows well on fructose or glycerol, but very poorly on glucose. When grown on glycerol the cells exhibited glucose phosphotransferase activity of about 5nmol/min per mg dry wt. of cells, and this low value was not increased after transfer to glucose medium, although the cells grew slowly and the phosphotransferase activities for fructose and mannose increased slightly. These results are shown in Fig. 2(*a*), which also demonstrates that only about one generation of growth occurred on glucose medium during the 5h experiment. On transfer from glycerol to fructose medium, the phosphotransferase activity for fructose increased abruptly, that for mannose slightly and that for glucose not at all (Fig. 2*b*).

Gratuitous induction of phosphotransferases for glucose and mannose in fructose-grown cells: the 'fructose kick'

Fig. 3(*a*) shows that the induction of the glucose phosphotransferase system observed when glycerol-grown cells of the K2 strain are placed in glucose medium proceeded very much as has been described earlier for the Hfr strains, K1 or B11 (Table 2; Fig. 1). However, after transfer of the cells to fructose medium, hyperinduction of glucose phosphotransferase activity (the 'fructose kick') is observed. Fructose phosphotransferase activity is also induced under these conditions. Moreover, the cells form the phosphotransferase for mannose, which, though possessing only low affinity for the sugar, can nevertheless phosphorylate it at a rate even greater than that catalysed by the phosphotransferase for glucose. The results of a typical experiment in which K2 cells were transferred from glycerol to fructose medium are shown in Fig. 3(*b*). This experiment has been repeated on numerous occasions with and without chloramphenicol added to the cells at the time of harvest. When the results are plotted as the parameters (Δ total phosphotransferase activity) versus (Δ total cell mass), the curve representing fructose phosphotransferase shows the pronounced curvature expected when the induced enzyme is necessary for cell growth. On the other hand, in these co-ordinates the curves representing the mannose or glucose systems are

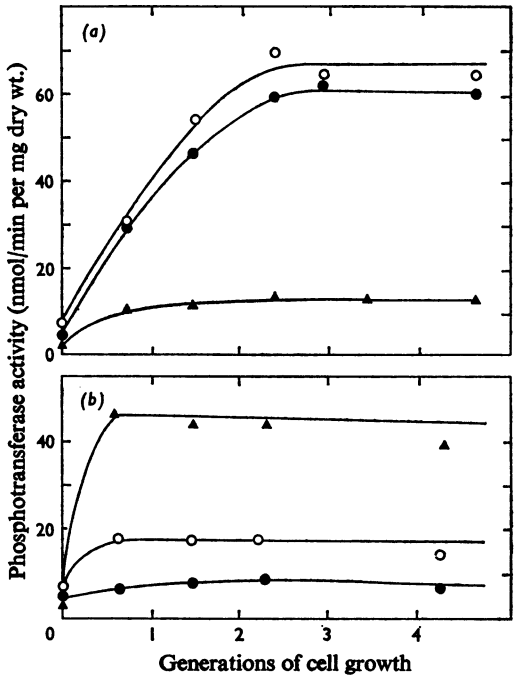

Fig. 1. *Induction of hexose phosphotransferase activity in E. coli strain B11*

Strain B11 (*met*) cells were grown on minimal medium containing methionine (50 μg/ml) and 10mM-sodium gluconate, washed with carbon-free medium, and transferred to media containing (*a*) 10mM-D-glucose or (*b*) 10mM-D-fructose as carbon source. Samples were withdrawn at appropriate intervals and the cultures were diluted with fresh medium as required to maintain exponential cell growth. Phosphotransferase assays of toluene-treated cells (see the Methods section) employed 5mM-D-glucose (●), 5mM-D-fructose (▲) or 25mM-D-mannose (○).

straight lines passing through the origin, a property that suggests that these systems are gratuitously induced.

To explore further the induction of the glucose and mannose phosphotransferases during growth on fructose, strain K2.1.11^c was employed. This organism carries the marker *uhp*^{c} and is therefore constitutive for the uptake of hexose phosphates (Ferenci *et al.*, 1971), and grows readily on fructose 1-phosphate. Like its K2 parent, it displays the 'fructose kick' when grown on fructose medium. It was grown on glycerol medium and transferred to minimal medium containing 5mM-fructose 1-phosphate as carbon source. After a 2.6-fold increase in cell mass, the total fructose phosphotransferase in the culture had increased by 18-fold, whereas total activity for glucose

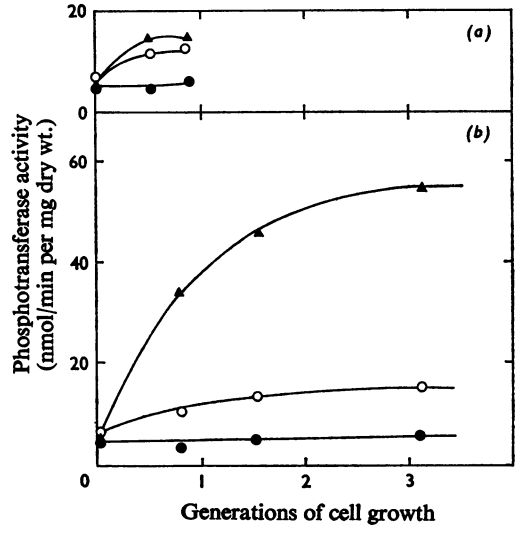

Fig. 2. *Induction of hexose phosphotransferase activity in E. coli strain K2.2w*

Strain K2.2w (*thr, his, arg, leu, trp*) cells were grown on minimal medium containing the required amino acids and 20mM-glycerol, washed with carbon-free medium, and transferred to media containing (*a*) 10mM-D-glucose or (*b*) 10mM-D-fructose as carbon source. Samples were withdrawn at appropriate intervals and the cultures were diluted with fresh medium as required to maintain exponential cell growth. Phosphotransferase assays of toluene-treated cells (see the Methods section) employed 5mM-D-glucose (●), 5mM-D-fructose (▲) or 25mM-D-mannose (○).

or mannose had increased only in direct proportion to the increase in cell mass. This shows that, whereas growth on fructose 1-phosphate induced component enzymes of fructose metabolism including the phosphotransferase (Kornberg, 1972), the 'fructose kick' is not elicited under these conditions.

Discussion

The pyruvate-formation method described briefly by Kornberg & Reeves (1972) and amplified in the present paper offers a convenient means of assaying the phosphoenolpyruvate-dependent phosphotransferase systems in *E. coli* cells that have been grown in media under conditions where the L-lactate oxidase activity of the organism is repressed. This repression occurs in minimal medium with a variety of carbon sources, but not in nutrient broth. The usefulness of the method depends on the rather unexpected finding that little of the potential NADH oxidase activity of the cell is revealed by decryptification with toluene. The recommended transferase assay employs, for

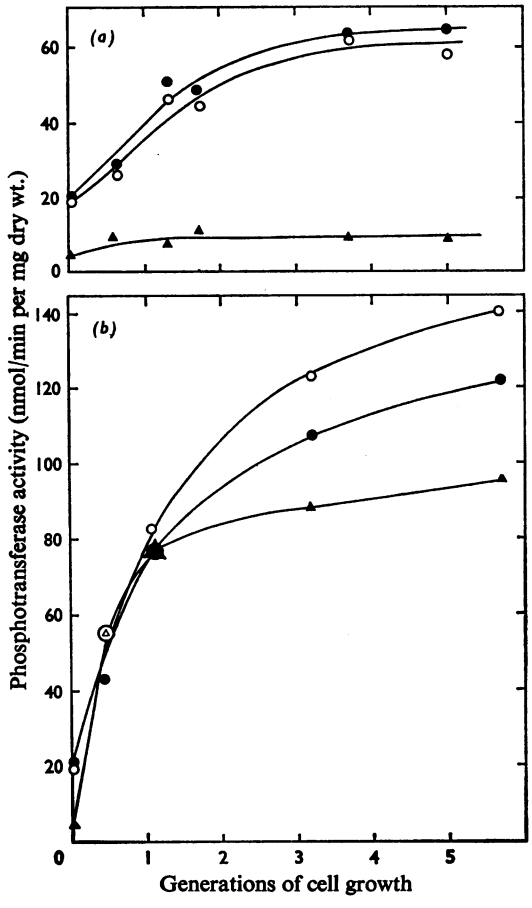

Fig. 3. *Induction of hexose phosphotransferase activities in E. coli strain K2*

Strain K2 (*thr, his, arg, leu, trp*) cells were grown on (*a*) glucose or (*b*) fructose and treated as described in Fig. 2, where the symbols ●, ▲ and ○ are identified.

reasons of economy, less than a saturating concentration of phosphoenolpyruvate. This demands that greater attention be paid to the concentration of this substrate than might otherwise be the case. The apparent K_m for phosphoenolpyruvate in the phosphorylation of 5 mM-fructose is in agreement with that found by Gachelin (1969) (with glucose as the second substrate). This relatively high K_m dictates that transferase activities measured by the standard method are about 75% of the maximum at the temperature of the assay (30°C).

The evidence presented in this paper supports the view (Ferenci & Kornberg, 1971*a*) that *E. coli* contains an inducible phosphotransferase system for the phosphorylation of fructose. Fraenkel (1968) showed that, at low concentrations of fructose, fructose 1-phosphate is formed; Ferenci & Kornberg (1971*b*) confirmed this observation, but also demonstrated that fructose 6-phosphate is formed at higher fructose concentrations.

That *E. coli* contains an inducible phosphotransferase system for glucose was suggested by Kornberg & Reeves (1972), who employed the glucose analogue, methyl α-D-glycopyranoside, as substrate. The existence of an inducible glucose phosphotransferase system is now confirmed in experiments with glucose as the substrate. A mutant organism that grows very poorly on glucose lacks the inducible glucose system, but contains a constitutive system at a very low activity. This constitutive system differs from the inducible one in at least one important, qualitative aspect: it is unable to react with methyl α-glucoside.

A novel finding is that in certain strains of *E. coli* the glucose phosphotransferase system is induced to high specific activities by growth on fructose. This is indeed an instance of gratuitous induction: fructose 1-phosphate does not induce the formation of the glucose phosphotransferase system in strain K2.1.11^c although fructose does.

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