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Short Communications

Correlation between Hexose Transport and Phosphotransferase Activity in *Escherichia coli*

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The utilization of a number of hexoses by enteric bacteria has been shown necessarily to involve the activity of a phosphoenolpyruvate-dependent phosphotransferase system (Kundig *et al.*, 1964). In this system an Enzyme I catalyses the transfer of the phosphate group of phosphoenolpyruvate to a nitrogen atom of a histidine moiety in a small protein (HPr); pyruvate is also formed in this step [reaction (1); Kundig & Roseman (1971)]. In subsequent processes, of the type represented in reaction (2), the appropriate Enzyme II catalyses the transfer of the phosphate group 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 study of mutants affected in their ability to form HPr or Enzyme I: such mutants are simultaneously affected in their ability to take up all sugars whose utilization involves the phosphotransferase system (Lin, 1970). Similarly, mutants of *Aerobacter aerogenes* that lack the Enzyme II for mannitol (Tanaka *et al.*, 1967), and mutants of *A. aerogenes* (Hanson & Anderson, 1968) and of *Escherichia coli* (Ferenci & Kornberg, 1971) that lack the Enzyme II for fructose, do not take up isotopically labelled mannitol and fructose respectively, although the utilization of other sugars is unimpaired. It is thus assumed that the phosphorylation of hexoses via the phosphotransferase system is an essential component of the process whereby these hexoses are transported into the cell (Roseman, 1969); this view is supported by the observation that, when either intact bacteria or 'membrane vesicles' derived from them take up methyl α-[^{14}C]glucoside, the radioactive material appears inside the cells or vesicles predominantly in a phosphorylated form (Rogers & Yu, 1962; Hagihira *et al.*, 1963; Kaback, 1968; Gachelin, 1970). However, to the best of our knowledge, no quantitative comparison between the rate at which a labelled hexose is taken up by intact cells and the rate at which the phosphotransferase system operates under similar conditions has so far been reported. It is the main purpose of this communication to describe procedures whereby such a comparison may be readily made, and to illustrate their utility by comparison of two strains of *E. coli* in which the extent and control of the uptake of methyl α-glucoside differs.

Cultures of *E. coli* are grown aerobically at 37°C on defined media containing salts (Ashworth & Kornberg, 1966) and a 10mM carbon source; the media are supplemented with amino acids (40–100μg/ml) as required. The bacteria are harvested by centrifugation in the early phase of exponential growth (at 0.2–0.4mg dry wt./ml), washed with the salts solution used for growth media and suspended in such a solution at 1.4mg dry wt./ml (method A). For assay by method B, the harvested cells are washed in a 'decryptification buffer', containing 0.1M-sodium–potassium phosphate buffer, pH7.2, and 5mM-$MgCl_2$, and are resuspended at 1.0mg dry wt./ml in this buffer.

Methods

Method A: uptake of methyl α-[^{14}C]glucoside. Into a conical flask (25ml) is placed 1ml of the bacterial suspension at 1.4mg dry wt./ml, and the flask is shaken at 37°C in a gyrotary water bath (Gallenkamp Ltd.) for 10min. Since this preincubation not only brings the well-aerated suspension to the desired temperature but also greatly lowers the intracellular stores of carbohydrate, it is necessary to add a readily

utilizable energy source before the addition of methyl α-[^{14}C]glucoside: if this is not done, the labelled analogue is taken up by, but is rapidly lost again from, the cells. Accordingly, 0.9ml of double-strength Oxoid nutrient broth (26g of powder/l) is added at 10min, and 5min later 0.1ml of 2mM-methyl α-[U-^{14}C]glucoside (3.8Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.) is added. Samples (0.1ml) are taken thereafter at 30, 75, 120 and 165s, and filtered, with suction, through Millipore filters (0.45μm pore size). The filters are rinsed with approx. 5ml of salts medium at 37°C, and transferred to glass vials containing 5ml of Bray's (1960) fluid for measurement of radioactivity in a Packard Tri-Carb model 4000 scintillation spectrometer. The quantities of radioactivity taken up are used to calculate the results as nmol of methyl α-glucoside incorporated/mg dry wt.

Method B: spectrophotometric assay of the phosphotransferase system. The suspension of cells in 'decryptification buffer' is thoroughly chilled in ice–water. A portion of the cold suspension is placed in a test tube and vigorously agitated on a Vortex mixer. By micro-pipette, and while the vigorous agitation is continued, is added 0.01vol. of toluene–ethanol (1:9, v/v); vigorous agitation of the suspension is continued for a further 1min. The suspension of toluene-treated cells is then kept in ice–water. The phosphotransferase activity of cells thus treated is stable and reproducible for many hours; the cells also manifest less NADH oxidase activity than when treated by the procedure of Gachelin (1969), on which this method is based.

The phosphotransferase activity is assayed as the phosphoenolpyruvate-dependent phosphorylation of appropriate hexoses, measured as the rate of decrease of extinction at 340 nm concomitant with the oxidation of NADH, as pyruvate [formed in reaction (3)] is reduced to lactate in the presence of added lactate dehydrogenase and NADH. The assay system contains, in a final volume of 1ml in a silica cell of 1cm light-path, 0.1μmol of NADH, approx. 3μg (2 units) of crystalline lactate dehydrogenase (Sigma Chemical Co. Ltd., London S.W.6, U.K.), 1μmol of phosphoenolpyruvate, 0.025–0.10ml of the toluene-treated cell suspension, and 'decryptification buffer' to 0.95–0.99ml. The mixture is equilibrated at 30°C and the reaction is started by the addition of 0.1–5.0μmol of the appropriate carbohydrate. Control cuvettes contain similar mixtures but with either phosphoenolpyruvate or the hexoses omitted.

Results and discussion

As shown in Fig. 1, the uptake of methyl α-[^{14}C]-glucoside is linear with time for only about the first minute, and its rate decreases thereafter as the intracellular concentrations of the analogue and its phos-

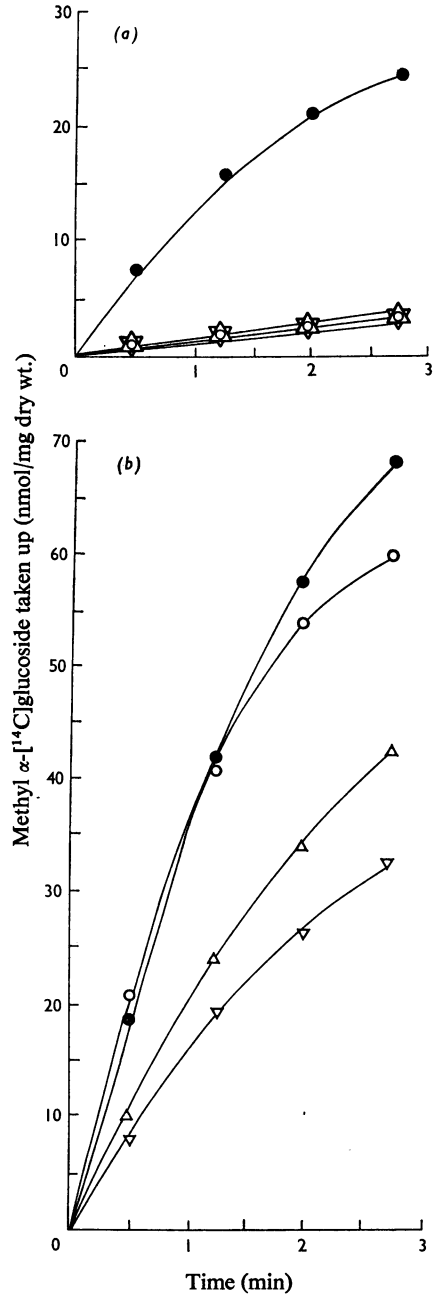

Fig. 1. *Uptake of* 0.1mM-*methyl* α-[^{14}*C*]*glucoside by washed suspensions of E. coli strains B*11 *and K*2

The cells (*a*, strain B11; *b*, strain K2) were grown on media containing glucose (●), glycerol (△), fructose (○) or gluconate (△) as sole carbon source. The rates of methyl α-glucoside uptake were measured as described in the text.

Table 1. *Rates of methyl α-glucoside uptake by intact cells and of phosphoenolpyruvate-dependent phosphorylation by toluene-treated cells*

Cultures of strain B11, an Hfr strain of *E. coli* K12 (*met*), and of strain K2, an F^- strain of *E. coli* K12 (*trp,his,arg,thr,leu*), were grown and assayed as described in the text. The rates of methyl α-glucoside uptake by intact cells (method A) were calculated from Fig. 1; the phosphotransferase activity of toluene-treated cells (method B) was measured in the presence of glucose-free 0.1 mM-methyl α-glucoside.

Strain	Carbon source for growth	Activity (nmol/min per mg dry wt.) Method A	Method B
B11	Glucose	15	17
	Fructose	3	3
	Gluconate	3	2
	Glycerol	3	3
K2	Glucose	38	56
	Fructose	44	99
	Gluconate	24	30
	Glycerol	19	23

phate approach the steady-state value (Gachelin, 1970). However, it is apparent that the Hfr strain B11 (Broda, 1967) takes up methyl α-glucoside from a 0.1 mM solution to a markedly smaller extent (Fig. 1*a*) than does the F^- strain K2 (Brice & Kornberg, 1967; Fig. 1*b*), and that the activity of this uptake system in the former strain is much more influenced by the nature of the carbon source on which the cells were grown than in the latter. Table 1 shows that the activity of the phosphotransferase system, measured with toluene-treated cells, is strikingly similar to the activity of the uptake systems as calculated from the initial rates shown in Fig. 1. Indeed, since methyl α-[^{14}C]glucoside taken up by strain K2 is known to be readily lost from the cells, the high rates obtained with method A are still minimal, and may be a measure of the retention of the labelled material in the cells rather than an accurate measure of the rates of uptake. It may well be that the rates obtained with method B are a more reliable index of uptake rates than are those measured directly. These considerations suggest that the activity of the phosphotransferase system may be equated with the rate of hexose uptake; they also show that the spectrophotometric method (B) here described is a convenient measure of the ability of *E. coli* both to take up and to phosphorylate methyl α-glucoside. Experiments (H. L. Kornberg & R. E. Reeves, unpublished work) show that this method can be equally readily used with any other sugar utilized via the phosphotransferase system.

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