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Distribution of the Phosphoenolpyruvate:Glucose Phosphotransferase System in Fermentative Bacteria

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A number of selected fermentative bacteria were surveyed for the presence of the phosphoenolpyruvate:glucose phosphotransferase system, with particular attention to those organisms which ferment glucose by pathways other than the Embden-Meyerhof-Parnas pathway. The phosphoenolpyruvate:glucose phosphotransferase system was found in all homofermentative lactic acid bacteria tested that ferment glucose via the Embden-Meyerhof-Parnas pathway, but in none of a group of heterofermentative species of *Lactobacillus* or *Leuconostoc*, which ferment glucose via the phosphoketolase pathway. A phosphoenolpyruvate:glucose phosphotransferase system was also absent in *Zymomonas mobilis*, which ferments glucose via an anaerobic Entner-Doudoroff pathway. It thus appears that the phosphotransferase mode of glucose transport is limited to bacteria with the Embden-Meyerhof-Parnas mode of glucose fermentation.

The phosphoenolpyruvate (PEP):sugar phosphotransferase system first described by Kundig et al. (14) has been found in a broad range of procaryotic organisms, where it plays a significant physiological role not only in the uptake of sugars by group translocation, but also in the regulation of the carbon and energy metabolism of the cell (for reviews, see references 17 and 21). An early survey of the distribution of PEP:glucose phosphotransferase system (19) showed that it was present in bacteria with the capability of fermenting sugars, but was absent in members of genera that are characteristically strictly aerobic. This generalization has held up without significant exception in the case of the glucose-specific PEP:phosphotransferase system, but a fructose-specific PEP:phosphotransferase system has been reported in a number of strict aerobes (1, 23, 24, 26, 30) and in photosynthetic bacteria (22). We now report that the PEP:glucose phosphotransferase system is also not universally distributed among fermentative bacteria, but apparently is limited to those organisms which ferment glucose via the Embden-Meyerhof-Parnas (EMP) pathway.

The PEP:phosphotransferase system offers important advantages to organisms carrying out anaerobic glycolysis, as pointed out by Roseman (20). First, the system provides a tight linkage between the transport of a sugar and its subsequent metabolism; second, under conditions where energy supply is limited, the system allows for conservation of ATP, since the product of the transport event is a phosphorylated sugar which can enter catabolic and anabolic pathways directly. However, Kornberg and Smith (13) were the first to emphasize the importance of the PEP yield from glycolysis, when they suggested that an *Escherichia coli* mutant that was defective in the EMP pathway, and was thus forced to metabolize glucose via the hexose monophosphate shunt, grew sparsely on glucose because of a deficiency in PEP required both for transport and for biosynthesis. If this line of reasoning is pursued further, one would predict that the PEP:glucose phosphotransferase system would be operative in those organisms fermenting glucose via the EMP pathway, where there is a yield of two molecules of PEP per molecule of glucose fermented, but would be absent in those organisms fermenting glucose by other pathways, such as the phosphoketolase pathway (4, 5, 31) or the anaerobic Entner-Doudoroff pathway (7, 31), where the PEP yield is only one molecule of PEP per molecule of glucose fermented. This report shows that this prediction is realized. A preliminary report of this work has appeared (A. H. Romano and J. D. Trifone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K103, p. 153).

MATERIALS AND METHODS

Organisms. The following organisms, representing the indicated physiological type, were used. Homofermentative lactic acid bacteria: *Lactobacillus casei* ATCC 393, *Lactobacillus plantarum* ATCC 8014, *Streptococcus agalactiae* ATCC 13813, *Streptococcus faecalis* ATCC 19433, *Streptococcus mutans* FA1,

Streptococcus salivarius ATCC 13419, *Pediococcus cerevisiae* ATCC 25249; heterofermentative lactic acid bacteria (via pentose phosphoketolase pathway): *Lactobacillus brevis* ATCC 367, *Lactobacillus buchneri* ATCC 4005, *Leuconostoc dextranicum* ATCC 19255, *Leuconostoc mesenteroides* ATCC 10830; heterofermentative lactic acid bacteria (via hexose phosphoketolase pathway): *Bifidobacterium breve* ATCC 15700; alcoholic fermenter, via Entner-Doudoroff pathway: *Zymomonas mobilis* 10988.

Assay methods. All the lactic acid bacteria except *B. breve* were grown in tomato juice broth, to which had been added 1% Casamino Acids (both Difco); *B. breve* was grown anaerobically on thiol medium (Difco). *Z. mobilis* was grown in a medium containing 2% glucose, 1% yeast extract, and 1% peptone in distilled water, with pH adjusted to 6.8. Cells were harvested during logarithmic phase by centrifugation, washed twice with 0.15 M KCl at 5°C, resuspended at a density of approximately 200 mg (wet weight) per ml in a 0.1 M phosphate buffer (pH 7.0), and placed in an ice bath. The cells were decryptified by adding 0.01 ml of toluene per ml of cold cell suspension and agitating vigorously on a Vortex mixer, according to the method of Kornberg and Reeves (12). Protein content of 0.05 ml of cell suspension was determined by the method of Lowry et al. (15), after extraction with 1 ml of 1 N NaOH at 100°C for 15 min and neutralizing with 1 N HCl.

PEP:glucose phosphotransferase activity was measured as the PEP-dependent phosphorylation of 2-deoxy-D-[^{14}C]glucose, and hexokinase or glucokinase activity was measured as the ATP-dependent phosphorylation of D-[^{14}C]glucose, by a modification of the method of Richey and Lin (18) for the assay of glycerol kinase, whereby the anionic phosphorylated sugar product is adsorbed to DEAE paper. The utility of 2-deoxy-D-glucose as a substrate for the PEP:glucose phosphotransferase assay is based upon the observation that bacterial hexokinases do not catalyze the ATP-dependent phosphorylation of this analog (6, 19). The reaction mixture for the PEP phosphotransferase assay contained: 0.1 M Tris-hydrochloride containing 16 mM $MgCl_2$ (pH 7.5), 0.3 ml; 0.125 M PEP, 0.04 ml; 10 mM 2-deoxy-D-[*U*-^{14}C]glucose (0.5 μCi/μmol), 0.04 ml; toluenized cell suspension, 0.1 ml; distilled water, to 0.50 ml. For the glucokinase or hexokinase assay, 0.125 M ATP and 5 mM D-[*U*-^{14}C]glucose (0.5 μCi/μmol) were substituted for PEP and 2-deoxy-D-glucose, respectively. Control mixtures without PEP or ATP were run to determine the amount of endogenous phosphorylation of both D-glucose and 2-deoxy-D-glucose. Reactions were carried out at 30°C. Samples of 0.05 ml were removed at appropriate time intervals, placed on Whatman DE81 filter disks (2.3 cm) held on a vacuum funnel, and allowed to soak into the paper. The reaction was stopped by addition of 10 ml of 80% ethanol, and the vacuum pump was turned on. The filters were then washed three times with distilled water and placed into 10 ml of Bray scintillation fluid (2) for counting in a Packard Liquid Scintillation Spectrometer.

Uptake of 2-deoxy-D-glucose. Cells were grown to late logarithmic phase in tomato juice broth containing 1% Casamino Acids (Difco), harvested by centrifugation, washed twice with 0.9% NaCl, and resuspended in 0.05 M phosphate buffer (pH 6.5) containing 0.01 M $MgCl_2$ at a cell density of 0.2 to 0.5 mg (dry weight) per ml. To 5 ml of cell suspension incubated at 30°C were added uniformly labeled 2-deoxy-D-[^{14}C] glucose (0.2 μCi/μmol) and other additions as indicated. Samples of 0.5 ml were taken at appropriate time intervals, filtered through membrane filters (0.45-μm porosity), and washed with the suspension buffer held at ambient temperature. Filtration and washing were complete in 10 to 15 s. Control determinations of exit rate from *L. casei* and *L. brevis* showed less than 5% loss of incorporated radioactivity during the washing procedure. Filters with cells thereon were transferred to vials containing Bray scintillation fluid for counting as above.

Reagents. Uniformly labeled 2-deoxy-D-[^{14}C]glucose and D-[^{14}C]glucose were obtained from ICN and New England Nuclear Corp., respectively. PEP, ATP, L-arginine, and carbonylcyanide-*m*-chlorophenylhydrazone were obtained from Sigma Chemical Co.

RESULTS

PEP:glucose phosphotransferase activity. The time courses of the PEP-dependent phosphorylation of 2-deoxy-D-glucose (PEP:glucose phosphotransferase activity) and the ATP-dependent phosphorylation of D-glucose (glucokinase activity) catalyzed by toluenized cell suspensions of representative homofermentative and heterofermentative lactic acid bacteria are shown in Fig. 1. Table 1 shows the results of such assays carried out with a more extensive list of representative obligately fermentative bacteria. These data show that all the homofermentative lactic acid bacteria tested (*L. casei, L. plantarum, S. salivarius, S. faecalis, S. agalactiae, S. mutans,* and *P. cerevisiae*) exhibited PEP:glucose phosphotransferase activity, whereas none of the heterofermentative lactic acid bacteria that utilize the pentose phosphoketolase pathway of fermentation possessed this activity (*L. brevis, L. buchneri, L. dextranicum,* and *L. mesenteroides*). Similarly, *B. breve,* which carries out a hexose phosphoketolase heterolactic fermentation (5, 31), and *Z. mobilis,* which carries out an alcoholic fermentation via the Entner-Doudoroff pathway (7, 31), both lacked PEP:glucose phosphotransferase activity. All organisms tested showed ATP-dependent phosphorylation of D-glucose (glucokinase) activity, however, showing that the toluene treatment did decryptify the cells.

Uptake of 2-deoxy-D-glucose. Resting cells of *L. casei* accumulated 2-deoxy-D-[^{14}C]glucose in the absence of an exogenous energy source, whereas the heterofermentative species *L. brevis* and *L. dextranicum* did not (Fig. 2). This is consistent with the findings of Thompson and Thomas (29) that *Streptococcus lactis* ML3, a

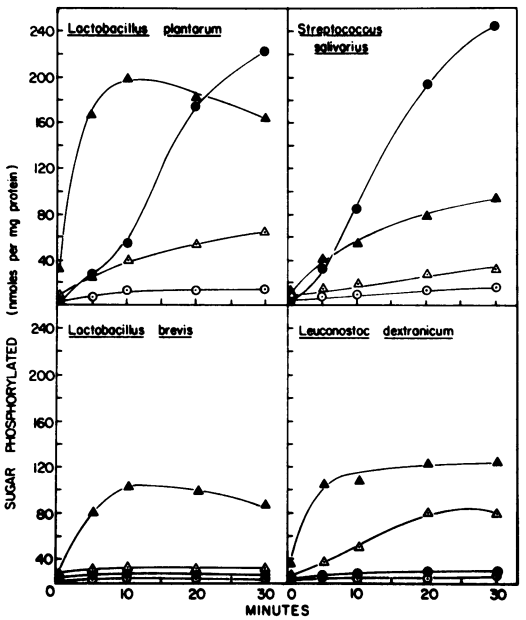

FIG. 1. *PEP-dependent phosphorylation of 2-deoxy-D-glucose and ATP-dependent phosphorylation of D-glucose by representative lactic acid bacteria. Toluenized cells were incubated with:* (○) *2-deoxy-D-glucose;* (●) *2-deoxy-D-glucose + PEP;* (△) *D-glucose;* (▲) *D-glucose + ATP. Details of reaction mixtures are in the text.*

homofermentative strain, accumulated 2-deoxy-D-glucose and thiomethylgalactoside at the expense of intracellular stores of PEP, whereas *S. lactis* 7962, which carries out a heterofermentative fermentation of lactose, did not accumulate thiomethylgalactoside unless a source of ATP was added. *L. brevis* accumulated 2-deoxy-D-[^{14}C]glucose when incubated in the presence of L-arginine (Fig. 3), a source of ATP via the arginine dehydrolase reaction (9, 25). That such ATP does not act directly in the accumulation of 2-deoxy-D-glucose, but rather acts through the generation of a proton motive force, as was clearly shown to be the case for lactose uptake in *S. lactis* 7962 by Kashket and Wilson (10, 11), is indicated by the inhibitory effect of the proton-conducting ionophore carbonylcyanide-*m*-chlorophenylhydrazone on the stimulatory action of L-arginine (Fig. 3).

We were not able to demonstrate accumulation of 2-deoxy-D-glucose in *Z. mobilis*, either without exogenous energy source or in the presence of L-arginine or D-fructose.

DISCUSSION

A survey of representative facultative anaerobic and strictly aerobic bacteria carried out in this laboratory a number of years ago showed that the PEP:glucose phosphotransferase system was characteristic of those genera possessing a fermentative capacity, but was absent in representative genera that had a strictly oxidative physiology (19). We now show that this group translocation system for the uptake of glucose is not universally distributed among fermentative bacteria, but rather apparently is limited to those organisms that ferment glucose via the EMP pathway. Thus, in addition to members of the *Enterobacteriaceae* and a number of

TABLE 1. *PEP phosphotransferase and glucokinase activities of toluene-treated cells*

Organism	PEP-dependent phosphorylation of 2-DOG[a]	ATP-dependent phosphorylation of D-glucose[a]
Lactobacillus casei	128	80
Lactobacillus plantarum	209	140
Streptococcus salivarius	228	62
Streptococcus faecalis	60	66
Streptococcus agalactiae	153	45
Streptococcus mutans	100	69
Pediococcus cerevisiae	120	68
Lactobacillus brevis	<1	81
Lactobacillus buchneri	<1	82
Leuconostoc dextranicum	4	38
Leuconostoc mesenteroides	4	102
Bifidobacterium breve	5	72
Zymomonas mobilis	<1	32

[a] Nanomoles per milligram of protein per 30 min; corrected for phosphorylation in the absence of added PEP or ATP. 2-DOG, 2-Deoxy-D-glucose.

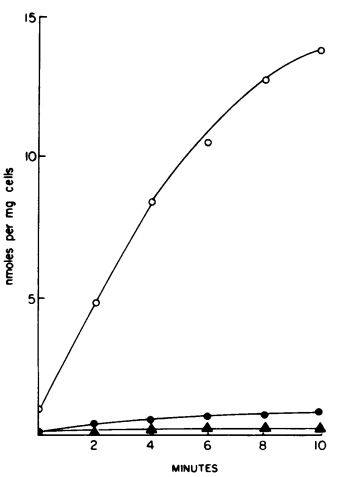

FIG. 2. *Uptake of 2-deoxy-D-glucose by representative lactic acid bacteria. Cells of L. casei* (○), *L. dextranicum* (●), *or L. brevis* (▲) *were incubated with 0.2 mM 2-deoxy-D-[^{14}C]glucose (0.2 μCi/μmol).*

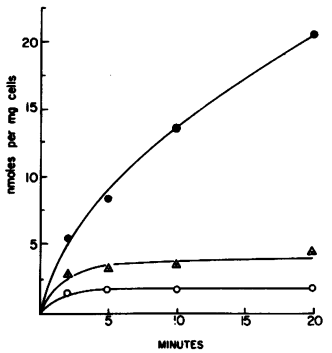

FIG. 3. *Effect of energy source and energy uncoupling ionophore on the uptake of 2-deoxy-D-glucose by L. brevis. Cells were incubated with 2 mM 2-deoxy-D-[^{14}C]glucose with no further addition* (○), *or with the addition of 40 mM L-arginine* (●) *or 40 mM L-arginine and 0.05 mM carbonylcyanide-m-chlorophenylhydrazone* (△).

other genera that are facultative or obligate anaerobes (for a review, see reference 21), this system is present in homofermentative lactic acid bacteria. It is absent, however, in heterofermentative lactic acid bacteria which ferment glucose via the pentose phosphoketolase pathway (4, 31), or via the hexose phosphoketolase pathway (5, 31). It is also absent in *Z. mobilis*, an anerobic pseudomonad that ferments glucose via the Entner-Doudoroff pathway (7, 31).

These results are teleonomically reasonable when one considers that the EMP pathway yields two molecules of PEP per molecule of glucose. Thus, if one molecule of PEP is used for transport, there is a net yield of one molecule that is available for energy or biosynthetic purposes, such as the synthesis of aromatic amino acids, or muramic acid that is required for cell wall polymer synthesis. On the other hand, the pentose and hexose phosphoketolase pathways, as well as the Entner-Doudoroff pathway, yield only one molecule of PEP per molecule of glucose metabolized. Thus, if this sole PEP were used for glucose transport, there would be none left for biosynthesis. This stoichiometric argument was first emphasized by Kornberg and Smith (13) as an explanation for the growth characteristics of *E. coli* mutants impaired in phosphofructokinase, and therefore impaired in generation of PEP via the EMP pathway, but capable of metabolizing glucose via the pentose phosphate cycle (where the PEP yield is but one molecule per molecule of glucose). Such mutants grew only sparsely on glucose, but relatively well on glucose-6-phosphate, a substrate that is transported independently of the PEP:glucose phosphotransferase system.

The results described here on the nature of the glucose transport system in lactic acid bacteria, as determined by use of the analog 2-deoxy-D-glucose, closely parallel the results obtained by others on the nature of the lactose transport system. Homofermentative strains of *S. lactis* such as C_2F and ML_3 transport lactose via the PEP:phosphotransferase system, whereas strain 7962, which carries out a heterofermentative fermentation of lactose (28), lacks a PEP:lactose phosphotransferase system (16, 29). This latter strain carries out an active transport of lactose, which is energized by a proton motive force (10, 11).

We were not able to measure a concentrative uptake of 2-deoxy-D-glucose by *Z. mobilis* either in the absence or in the presence of added energy sources. Thus, not only does this organism lack the PEP:glucose phosphotransferase system, but it apparently lacks a means of active transport of glucose. This may account for the distribution of this organism in nature: it is found only in environments with high sugar concentration, such as plant saps, fermenting ciders, honey, beer, etc. (for a review, see reference 25), where non-concentrative facilitated diffusion or simple diffusion may allow sufficient sugar uptake to support metabolism.

The generalization made in 1970 (19) that members of strictly aerobic bacterial genera lack the PEP:glucose phosphotransferase system appears to be still valid. However, a PEP:fructose phosphotransferase system that results in the formation of fructose-1-phosphate has been found in photosynthetic bacteria (22) and in a number of strictly aerobic bacteria, such as *Arthrobacter pyridinolis* (26) and a number of *Pseudomonas* species (2, 23, 24, 30). It is significant, however, that these organisms, which generally metabolize glucose via the Entner-Doudoroff pathway, metabolize fructose either exclusively via the EMP pathway, in the case of *Rhodopseudomonas capsulata* (3), or partially, in the case of the *Pseudomonas* species (2, 23, 24, 30), probably to the extent that the PEP requirement for transport of the sugar by the phosphotransferase system is satisfied. In any case, even these organisms appear to conform to the broader generalization, that the PEP:sugar phosphotransferase system is linked to the EMP mode of glycolysis.

The PEP:glucose phosphotransferase system has been found in a number of species of *Bacillus* (19), and its presence in *Bacillus subtilis*, which is generally considered to be a strict aerobe, has been cited as an exception to the generalization that the PEP:glucose phosphotransferase system is absent in strict aerobes

(21). However, most strains of this species, as well as other species of the genus *Bacillus*, are able to achieve limited growth anaerobically in the presence of a fermentable sugar, carrying out a 2,3-butanediol fermentation (31). Glucose is metabolized chiefly by the EMP pathway, both aerobically and anaerobically (8). Thus, this group of organisms does not present an exception to the generalization made here.

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