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Intracellular Hexose-6-Phosphate:Phosphohydrolase from *Streptococcus lactis*: Purification, Properties, and Function

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An intracellular hexose 6-phosphate:phosphohydrolase (EC 3.1.3.2) has been purified from *Streptococcus lactis* K1. Polyacrylamide disc gel electrophoresis of the purified enzyme revealed one major activity staining protein and one minor inactive band. The M_r determined by gel permeation chromatography was 36,500, but sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a single polypeptide of apparent M_r 60,000. The enzyme exhibited a marked preference for hexose 6-phosphates, and the rate of substrate hydrolysis (at 5 mM concentration) decreased in the order, galactose 6-phosphate > 2-deoxy-D-glucose 6-phosphate > fructose 6-phosphate > mannose 6-phosphate > glucose 6-phosphate. Hexose 1-phosphates, *p*-nitrophenylphosphate, pyrophosphate, and nucleotides were not hydrolyzed at a significant rate. In addition, the glycolytic intermediates comprising the intracellular phosphoenolpyruvate potential in the starved cells (phosphoenolpyruvate and 2- and 3-phosphoglyceric acids) were not substrates for the phosphatase. Throughout the isolation, the hexose 6-phosphate:phosphohydrolase was stabilized by Mn^{2+} ion, and the purified enzyme was dependent upon Mn^{2+}, Mg^{2+}, Fe^{2+}, or Co^{2+} for activation. Other divalent metal ions including Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, and Ni^{2+} were unable to activate the enzyme, and the first four cations were potent inhibitors. Enzymatic hydrolysis of 2-deoxy-D-glucose 6-phosphate was inhibited by fluoride when Mg^{2+} was included in the assay, but only slight inhibition occurred in the presence of Mn^{2+}, Fe^{2+}, or Co^{2+}. The inhibitory effect of Mg^{2+} plus fluoride was specifically and completely reversed by Fe^{2+} ion. The hexose 6-phosphate:phosphohydrolase catalyzes the in vivo hydrolysis of 2-deoxy-D-glucose 6-phosphate in stage II of the phosphoenolpyruvate-dependent futile cycle in *S. lactis* (J. Thompson and B. M. Chassy, J. Bacteriol. **151**:1454–1465, 1982).

Starved cells of many species of lactic acid bacteria including *Streptococcus lactis* (27, 36, 42), *Streptococcus faecalis* (17), *Streptococcus pyogenes* (32), and *Lactobacillus casei* (5, 6, 34) maintain an endogenous reserve of phosphoenolpyruvate (PEP), 3-phosphoglyceric acid, and 2-phosphoglyceric acid. The presence of this intracellular PEP pool has permitted the study and characterization of sugar:phosphotransferase systems (PTS; 10, 16, 31) in physiologically intact cells. In *S. lactis*, non-metabolizable sugar analogs, e.g., 2-deoxy-D-glucose (2DG) and methyl-β-D-thiogalactopyranoside (TMG) (see references 20 and 42 for TMG transport by the atypical strain *S. lactis* 7962), are transported by mannose-PTS and lactose-PTS, respectively. The phosphorylated derivatives accumulate intracellularly to a concentration of 30 to 40 mM (36, 42). Similarly, when starved cells are treated with iodoacetate (to inhibit glycolysis via inactivation of glyceraldehyde 3-phosphate dehydrogenase [36]), glucose 6-phosphate and galactose 6-phosphate accumulate upon incubation of the cells with glucose or galactose (37, 38). High levels of intracellular sugar phosphates are not maintained by the cells, however, and a decline in the intracellular concentration of these derivatives occurs simultaneously with appearance of free sugar in the medium. The half-times for efflux of 2DG, TMG, and galactose from cells previously loaded with 2DG 6-phosphate (2DG-6P), TMG-6P, and galactose 6-phosphate are approximately 3, 11, and 3 min, respectively. Studies by Thompson and Saier (41) and Thompson and Chassy (39) indicated an intracellular locus for the dephosphorylation process, and additional evidence of intracellular sugar phosphate:phosphohydrolase activity in vivo was obtained from studies concerned with the bacteriostatic effect of 2DG upon *S. lactis* (39, 40). Inhibition of growth was attributed to the dissipation of PEP

(and indirectly, ATP) during recycling of the glucose analog via a three-stage futile cycle involving: stage I, translocation and phosphorylation of 2DG via the mannose-PTS; stage II, hydrolysis of accumulated 2DG-6P; and stage III, efflux of free 2DG.

This report describes the purification of an intracellular hexose 6-phosphate (H6P):phosphohydrolase (EC 3.1.3.2) from *S. lactis* K1. Some of the kinetic, biochemical, and physical characteristics of the phosphohydrolase are presented, and a role for the enzyme in the regulation of sugar metabolism is proposed.

MATERIALS AND METHODS

Organism and growth conditions. *S. lactis* K1 (a typical group N streptococcus) was obtained from the culture collection of the New Zealand Dairy Research Institute, Palmerston North, New Zealand. Cells were grown, and frozen cultures were maintained in the complex medium described previously (41).

Enzyme assays. H6P:phosphohydrolase activity was routinely determined by a modification of the procedure of Thompson and Chassy (39), using [^{14}C]2DG-6P as a substrate. One enzyme unit is defined as the amount of enzyme which hydrolyzes 1 μmol of [^{14}C]2DG-6P min^{-1} at 37°C. The standard reaction mixture (100 μl) contained 100 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES; pH 6), 10 mM $MgCl_2$, 5 mM [^{14}C]2DG-6P (0.2 μCi μmol^{-1}), and enzyme protein. After incubation at 37°C for 60 min, the reaction was terminated by transfer of 90 μl of reaction mixture to a polypropylene microcentrifuge tube containing 0.95 ml of distilled water and 0.25 ml of Dowex-1–acetate ion-exchange resin. The suspension was mixed thoroughly, and the resin was removed by sedimentation in a Beckman microfuge. Enzyme activity was calculated from the [^{14}C]2DG present in 0.9 ml of supernatant fluid. The sample was mixed with 10 ml of Hydrofluor liquid scintillation cocktail, and radioactivity was determined with a Packard Tri-Carb 2660 LS spectrometer. Enzyme activity (micromoles of [^{14}C]2DG-6P hydrolyzed or [^{14}C]2DG formed per milligram of protein per minute) was directly proportional to protein concentration, and the reaction proceeded at a linear rate for at least 60 min. In kinetic analysis, the concentration range of [^{14}C]2DG-6P was varied from 1 to 50 mM. In studies of substrate specificity, unlabeled phosphate compounds were present in the assay at a final concentration of 10 mM, and P_i was determined by the colorimetric micromethod of Hørder (19). The standard assay system (1 ml) for nonspecific phosphatase activity contained 100 mM MES buffer (pH 6.0), 5 mM *p*-nitrophenylphosphate, 5 mM $MgSO_4$, and enzyme protein. After 60 min of incubation at 37°C, the reaction was terminated by addition of 2 ml of 1 M Na_2CO_3 solution. The absorbance of the sample at 420 nm was recorded on a Gilford 300N spectrophotometer, and *p*-nitrophenol was determined by reference to a standard curve.

Purification of H6P:phosphohydrolase. (i) Growth of cells. *S. lactis* K1 was grown at 32°C for 16 h in two 10-ml volumes of complex medium containing 5 mM 2DG and 14 mM sucrose as the energy source. Samples (0.5 ml) of the culture were transferred to each of 15 bottles containing 800 ml of freshly prepared medium. The organisms were grown without aeration at 32°C until stationary phase (ca. 16 h). The cultures were then chilled in an ice bath, and the cells were harvested by centrifugation (13,000 × *g* for 15 min at 4°C) in a Sorvall GS-3 rotor. The supernatant fluids were discarded, and the cell pellets were washed twice by resuspension and centrifugation from 2× 200-ml volumes of 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.3) containing 1 mM EDTA, 1 mM dithiothreitol, and 5 mM $MnSO_4$ (HEDM buffer) at 0°C. A final yield of 49 g (wet weight) of cells was obtained from the 12 liters of culture.

(ii) Preparation of cell extract. All purification procedures were conducted at 0 to 4°C. The wet cell pellet was resuspended to a volume of 140 ml in HEDM buffer at 0°C and mixed with 375 g of 0.10- to 0.17-mm glass beads prewashed in HEDM buffer. The cells were disrupted by 2× 3-min periods of vigorous agitation in a Bead beater (Biospec Products, Bartlesville, Okla.) previously chilled to 0°C (38). A 3-min period of cooling was allowed between the periods of agitation. The beads were permitted to settle, and the supernatant fluid was decanted and retained. The beads were rinsed with 50 ml of HEDM buffer, and the wash fluid was added to the previous supernatant. Residual intact cells and cell debris were removed by low-speed centrifugation (27,000 × *g* for 30 min at 4°C). The supernatant fluid (ca. 190 ml) was collected and further clarified by high-speed centrifugation (220,000 × *g* for 2 h), using a type 60 Ti rotor in a Beckman L2-65B ultracentrifuge. The cell-free extract was transferred to membrane dialysis sacs (Spectrapor no. 1; 14.6-mm dry cylindrical diameter; molecular weight cutoff, 6,000 to 8,000). The samples were dialyzed overnight at 4°C against two changes of 4 liters of 20 mM HEPES buffer, pH 7.3, containing 1 mM dithiothreitol, 1 mM EDTA, and 5 mM $MnSO_4$. The retentate was clarified by centrifugation at 27,000 × *g* in a Sorvall SS34 rotor for 20 min at 4°C. The cell extract was concentrated under 50 lb/in^2 of N_2 gas by Diaflo ultrafiltration (Amicon YM-30 membrane) to a volume of 80 ml.

(iii) DEAE-Sephacel chromatography I. The 80 ml of cell extract was transferred (at a flow rate of 1.6 ml min^{-1}) onto a DEAE-Sephacel anion-exchange column (2.3 by 40 cm) previously equilibrated with HEDM buffer. After 27 fractions (10 ml per fraction) had been collected, the absorbed proteins were eluted with a complex gradient (formed by an LKB model 11300 Ultrograd mixer) of 0.1 to 0.40 M KCl in HEDM buffer. The absorbance of the eluate at 280 nm was recorded with an Isco model UA-5 absorbance-fluorescence detector. Samples (100 μl) of alternate fractions were assayed for H6P:phosphatase and PNPP phosphatase activities.

(iv) Ultrogel AcA54 gel filtration chromatography. Fractions 76 to 84 from the previous step which contained H6P:phosphatase activity were pooled and concentrated to 7 ml by pressure ultrafiltration through an Amicon PM-30 membrane. A 5-ml sample of concentrated extract was transferred to minidialysis sacs and further reduced to a volume of 3 ml by using Carbowax 20,000 (polyethylene glycol 20M). After addition of 0.5 ml of glycerol, the enzyme preparation was chromatographed on the Ultrogel AcA54 column

(2.5 by 92 cm) equilibrated with HEDM buffer. Fractions 94 to 102 contained H6P:phosphatase activity; these were pooled (46 ml) and concentrated to 3 ml by Diaflo ultrafiltration. Molecular weights of the two phosphatases were estimated from plots of peak elution volume versus log M_r prepared with a series of known standards (2).

(v) **DEAE-Sephacel chromatography II.** The concentrated enzyme from Ultrogel AcA54 chromatography (3 ml) was applied to a second DEAE-Sephacel column (1.4 by 30 cm) which previously had been equilibrated in HEDM buffer containing 0.1 M KCl. The absorbed materials were eluted with a gradient of KCl as described previously for DEAE-Sephacel step I. The flow rate was 45 ml^{-1} h and 9-ml fractions were collected. The H6P:phosphatase eluted shortly after the start of the KCl gradient (0.13 M KCl) in the second of three sharply defined protein peaks. Approximately 80% of the total enzyme activity applied to the column was recovered in fraction 15. This fraction was concentrated in a 10-ml Amicon concentration system to a final volume of 3 ml. This preparation (30 μg of protein ml^{-1}) was used in all subsequent experiments.

HPLC. Molecular exclusion by high-pressure liquid chromatography (HPLC) was performed with a Chromatronix Instruments model 3500 and a TSK gel 3000 sw type of 150,000 M_r exclusion, 7.5-mm inside diameter by 600 mm. (Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan). The sample applied (100 μl) was obtained from DEAE-Sephacel column II and contained 15 to 20 μg of protein. HPLC was carried out at 310 lb/in^2, using 0.05 M MES buffer (pH 6.0) containing 5 mM $MgSO_4$ at a flow rate of 0.5 ml min^{-1}. Fractions of 0.5 ml were collected (full-scale absorbance at 200 nm = 0.02 U). The major protein and enzyme activity peaks were coincident in fractions 46 to 51. A sample was dialyzed to remove salts, lyophilized, and then resuspended in Laemmli sample buffer for sodium dodecyl sulfate (SDS) electrophoresis.

Protein assay. Throughout the purification, protein was determined by the Coomassie brilliant blue dye-binding assay of Bradford (4), using bovine serum albumin as the standard.

PAGE. Proteins present in samples at various stages throughout the enzyme purification were determined by polyacrylamide disc gel electrophoresis (PAGE), using the method of Davis (8). Discontinuous SDS-PAGE in 1.5-mm-thick gels was performed by the modified Laemmli (25) procedure described in the operating manual for the LKB model 2001 vertical slab gel apparatus. Samples for SDS-PAGE were prepared by boiling for 3 min in Laemmli sample buffer, and electrophoresis was conducted at 14°C for 4.5 h at 20-mA constant current.

Visualization of protein and phosphohydrolase activities in gels. Neutral 7.5% polyacrylamide tube gels were prepared with 50 mM bis-(2-hydroxyethyl)iminotri(hydroxymethyl)methane (BIS-TRIS) buffer (pH 7.2) containing either 5 mM $MgSO_4$ or 1 mM EDTA. No stacking gel was used with the neutral gel system, and the gels were pre-electrophoresed overnight at 50 V. Before electrophoresis, samples were dialyzed against 5 mM HEPES buffer (pH 7.3) containing either 2.5 mM $MgSO_4$ or 1 mM EDTA and, after freeze-drying, were reconstituted in a minimum volume of distilled water. After application of the samples electrophoresis was performed (4 h, 80 V), using a neutral running buffer of 0.2 M BIS-TRIS (pH 7.2) containing 5 mM $MgSO_4$ or 1 mM EDTA. Protein bands were visualized by staining with Coomassie blue R-250 by the procedure of Diezel et al. (9). H6P:phosphohydrolase activity in gels was detected by a modification of the method of Allen and Hyncik (1). The gels were soaked for 15 min at 37°C in 50 mM MES buffer (pH 6) containing 5 mM $MgSO_4$ and 50 mM glucose 6-phosphate. The substrate solution was changed, and incubation was continued for another 15 min. P_i liberated by the enzyme was localized by the lead nitrate-sodium sulfide conversion method described previously (1, 21).

Reagents. Radiolabeled compounds, including 2-[1-^{14}C]DG-6P disodium salt (52.2 mCi $mmol^{-1}$) and β-D-[*methyl*-^{14}C]thiogalactopyranoside (54.7 mCi $mmol^{-1}$), were from New England Nuclear Corp., Boston, Mass. Hydrofluor liquid scintillation cocktail was obtained from National Diagnostics, Somerville, N.J. Analytical-grade anion-exchange resin AG1-x8 (100 to 200 mesh, acetate form), dye-binding protein assay (Bio-Rad protein assay), and materials for PAGE were purchased from Bio-Rad Laboratories, Richmond, Calif. Ultrogel AcA54 was from LKB (Rockville, Md.) and DEAE-Sephacel anion-exchange resin was obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Diaflo ultrafiltration membranes were from Amicon Corp., Lexington, Mass. Glucose 1-phosphate was purchased from Schwarz BioResearch, Mount Vernon, N.Y. Glucose 6-phosphate, dithiothreitol, and *p*-nitrophenylphosphate were from Calbiochem, La Jolla, Calif. PEP and galactose 1-phosphate were from Boehringer-Mannheim Corp., New York, N.Y. Buffers and other phosphorylated compounds were obtained from Sigma Chemical Co., St. Louis, Mo. β-D-[*Methyl*-^{14}C]thiogalactopyranoside 6-phosphate was prepared by using starved cells of *S. lactis* as described by Thompson and Saier (41).

RESULTS

Enzyme purification. We found previously (39) that cell extracts of *S. lactis* 133 catalyzed the hydrolysis of 2DG-6P. The extract prepared from strain K1 hydrolyzed both *p*-nitrophenylphosphate and 2DG-6P. To determine whether a hexose phosphate-specific enzyme was present, we attempted to purify the enzyme from nonspecific phosphatase(s). After chromatography of a high-speed supernatant on DEAE-Sephacel, two phosphatase activities were detected (Fig. 1). The enzyme present in the peak eluting at 0.16 M KCl (fractions 60 to 75) hydrolyzed *p*-nitrophenylphosphate, whereas H6P:phosphatase activity was found in the peak eluting at 0.18 M KCl (fractions 76 to 84). These two activities were pooled separately, and after concentration each was subjected to gel permeation chromatography on Ultrogel AcA54. The *p*-nitrophenylphosphate:phosphohydrolase emerged at an elution volume corresponding to a protein of M_r 53,000 (data not shown). The H6P:phosphatase activity eluted in fractions (94 to 102) corresponding to an M_r of 36,500 (Fig. 2A). The

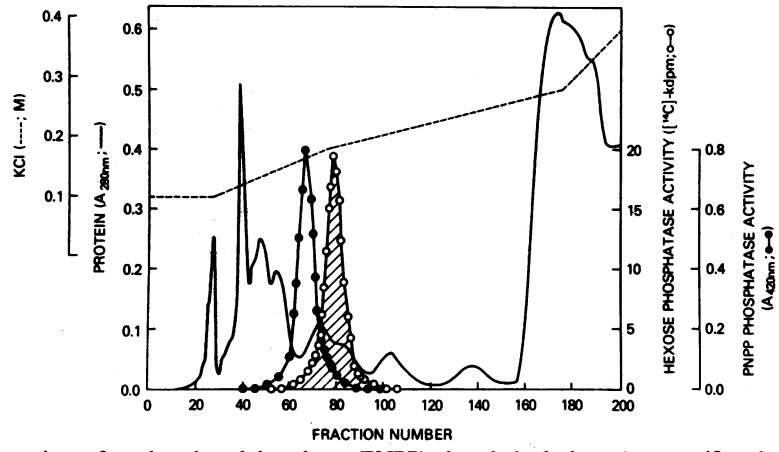

FIG. 1. Separation of *p*-nitrophenylphosphate (PNPP):phosphohydrolase (nonspecific phosphatase) and H6P:phosphohydrolase by DEAE-Sephacel ion-exchange chromatography. The dialyzed high-speed supernatant (80 ml) prepared from cell extracts of *S. lactis* K1 was loaded onto a column (2.3 by 40 cm) equilibrated with HEDM–0.1 M KCl at a flow rate of 1.6 ml min^{-1}. Proteins were eluted in 10-ml fractions with a complex gradient from 0.1 to 0.4 M KCl (------). The protein content of the effluent was monitored continuously (absorbance at 280 nm [$A_{280\ nm}$]; ——). Aliquots of fractions were assayed for both H6P:phosphohydrolase activity (O; hatched peak) and PNPP-phosphohydrolase activity (●).

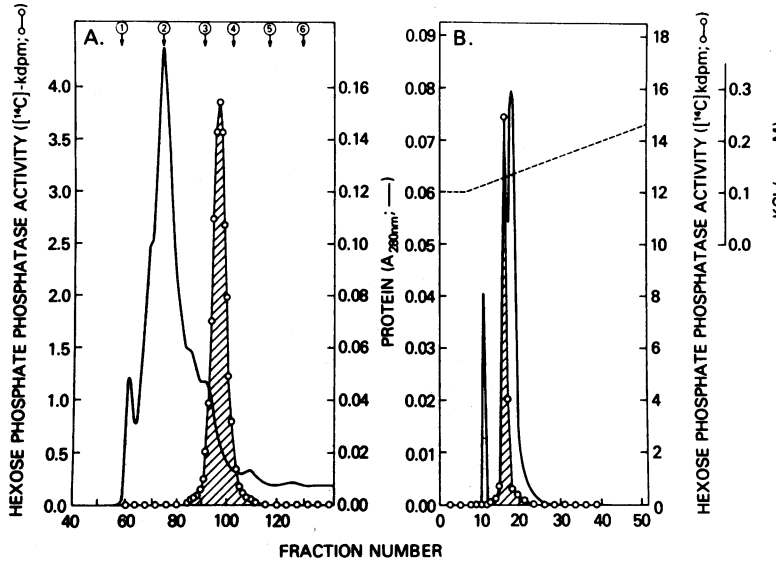

FIG. 2. Purification of H6P:phosphohydrolase activity by steric exclusion chromatography on Ultrogel AcA54 (A) and rechromatography on DEAE-Sephacel (B). (A) The pooled concentrated fractions (3 ml) of H6P:phosphohydrolase from DEAE-Sephacel (I) chromatography were applied to an Ultrogel AcA54 column (2.5 by 92 cm) pre-equilibrated with HEDM buffer (flow rate, 0.5 ml min^{-1}). Fractions (3 ml) were assayed for H6P:phosphohydrolase activity (O; hatched area), and protein was continuously monitored (absorbance at 280 nm [$A_{280\ nm}$]; ——) as described in the text. Arrows indicate elution points of standard proteins in decreasing order of size (M_r): 1, void volume determined by ferritin (540,000); 2, bovine serum albumin (68,000); 3, ovalbumin (43,000); 4, carbonic anhydrase (29,000); 5, chymotrypsinogen (25,700); and 6, RNase (13,800). (B) Fractions containing enzyme activity from Ultrogel AcA54 chromatography were concentrated to 3 ml and applied (45 ml h^{-1}) to a second DEAE-Sephacel column (1.4 by 30 cm) equilibrated with HEDM–0.1 M KCl. Fractions (9 ml) were collected over the course of elution with a linear gradient of KCl in HEDM (------). Protein and enzyme activity were determined as described in (A).

specific activity of the enzyme was increased 40-fold by this purification step (Table 1). The pooled fractions from Ultrogel chromatography were concentrated and rechromatographed on DEAE-Sephacel (Fig. 2B). The second of the three sharply defined protein peaks (fraction 15) contained 80% of the enzyme applied to the column. The final preparation represented a 6% overall recovery and an 860-fold purification of the H6P:phosphatase (Table 1). The purified enzyme remained fully active for at least 3 weeks at 4°C in HEDM buffer and retained ca. 90% activity after lyophilization or freezing (−20°C).

Protein analyses and activity staining by PAGE. To assess the degree of purity of the enzyme, samples from different stages of purification were analyzed by PAGE (Fig. 3, lanes A to D). Coomassie blue staining of the final preparation (Fig. 3, lane D) revealed one major and one minor protein band. To determine which (if any) of the components represented the enzyme, attempts were made to stain for H6P:phosphohydrolase activity in situ or after elution of the proteins from the basic gels. These experiments were unsuccessful. A neutral gel system was then used in an attempt to stabilize the enzyme during electrophoresis. It was possible to detect phosphohydrolase activity with enzyme purified through the first DEAE step, using the neutral system containing 5 mM $MnSO_4$, but no activity was detectable in gels loaded with preparations purified through the Ultrogel AcA54 step (data not shown). It was subsequently found that the stability of the enzyme was enhanced by omission of the stacking gel and by pre-electrophoresis of the resolving gel. When the final preparation (Table 1) was dialyzed against EDTA and electrophoresed in a neutral gel containing 1 mM EDTA (minus $MgSO_4$), two protein bands were detected (Fig. 3, lane E): the major component exhibited H6P:phosphohydrolase activity by activity stain (Fig. 3, lane F) but the minor lower band was inactive. When 5 mM $MgSO_4$ was included in the gel and in the electrophoresis buffer, diffuse but noncoincident bands for protein and activity were detected (data not shown). These results suggest that multiple forms of the enzyme may be generated during electrophoresis in buffers containing metal ions or that the presence of the metal simply alters the electrophoretic mobility of the protein.

TABLE 1. Summary of the purification procedure of H6P:phosphohydrolase from *S. lactis* K1

Purification step	Total activity (U)	Total protein (mg)	Sp act (U/mg)	Purification (fold)
Crude extract (225,000 × *g* supernatant)	21.15	1,392	0.02	
DEAE-Sephacel I	12.00	46	0.26	17.5
Ultrogel AcA54	3.25	0.30	10.83	722
DEAE-Sephacel II	1.17	0.09	13.00	867

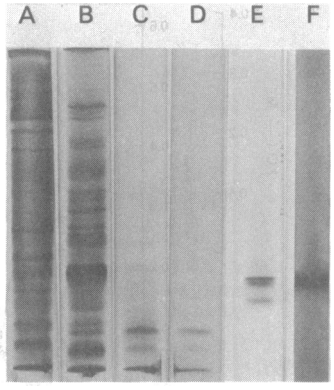

FIG. 3. PAGE and activity staining of H6P:phosphohydrolase preparations. Lanes A to D show the protein profiles (by basic gel electrophoresis) of samples obtained from different stages of enzyme purification: A, extract (250 μg of protein) from high-speed ultracentrifugation; B, from DEAE-Sephacel (I) (~130 μg); C, from Ultrogel AcA54 (5 μg); and D, after DEAE-Sephacel (II) chromatography (2 μg). The gels were stained with Coomassie brilliant blue R-250. In lanes E and F 5-μg samples of protein from DEAE-Sephacel (II) were electrophoresed in neutral polyacrylamide tube gels and subsequently stained for protein (lane E) and enzyme activity (lane F) in the presence of Mg^{2+} and with glucose 6-phosphate as a substrate.

Molecular weight determination by HPLC and SDS-PAGE. A lyophilized sample (ca. 15 μg of protein) of the enzyme obtained from Ultrogel AcA54 was chromatographed by HPLC, using a molecular sizing TSK column. One major and one minor (10% of total) protein peak was obtained, and only the major peak exhibited H6P:phosphohydrolase activity. The M_r of the enzyme by reference to elution points of protein standards was 37,000. When the protein comprising the activity peak was freeze-dried and subsequently electrophoresed on SDS-PAGE, a single polypeptide of M_r 60,000 was detected by Coomassie blue stain.

Temperature and pH optima. Optimum enzyme activity (with 2DG-6P as substrate) occurred at pH 6.0 in 0.1 M MES buffer containing 5 mM Mg^{2+} ion. The rate of sugar phosphate hydrolysis decreased rapidly below pH 6 and above pH 6.5. Of the buffers tested, MES was superior to HEPES or Tris and phosphate buffer was inhibitory. The optimum temperature for enzyme activity was 40°C.

Metal ion requirement. In initial attempts at

TABLE 2. Effect of divalent cations on activity of H6P:phosphohydrolase[a] from *S. lactis* K1

Divalent cation[b]	Sp act (μmol of 2DG-6P hydrolyzed mg of protein^{-1} min^{-1})	
	−NaF	+NaF[c]
None	ND[d]	ND
Mg^{2+}	1.11[e]	ND
Fe^{2+}	0.79	0.87
Co^{2+}	0.76	0.60
Mn^{2+}	0.63	0.56

[a] The enzyme preparation was dialyzed for 2 h against 0.1 M MES buffer (pH 6.0) containing 1 mM EDTA. The EDTA was then removed by dialysis (2 h) against buffer alone.

[b] Present in assay at 5 mM; 2DG-6P concentration, 5 mM.

[c] NaF final concentration, 10 mM.

[d] ND, No detectable activity.

[e] Under the same assay conditions the specific activity of the enzyme before dialysis was 2.28.

enzyme purification it was found that the H6P:phosphohydrolase was inactivated during passage through Ultrogel AcA54. However, active enzyme was recovered upon inclusion of a divalent metal (Mg^{2+} or Mn^{2+}) in the HEPES elution buffer. Subsequently, 5 mM Mn^{2+} was included in all preparation buffers together with 1 mM dithiothreitol. The essential requirement of a divalent cation for enzyme stability and activation was demonstrated after dialysis of the preparation against EDTA (Table 2). The enzyme specifically required a divalent cation for activation, and this requirement could be satisfied by any one of the following: Mg^{2+}, Fe^{2+}, Co^{2+}, or Mn^{2+}. Other metal ions, including Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Ni^{2+}, failed to activate the phosphatase, and the first four cations were potent inhibitors. In the standard assay a significant basal level of enzyme activity (Fig. 4A) occurred due to the

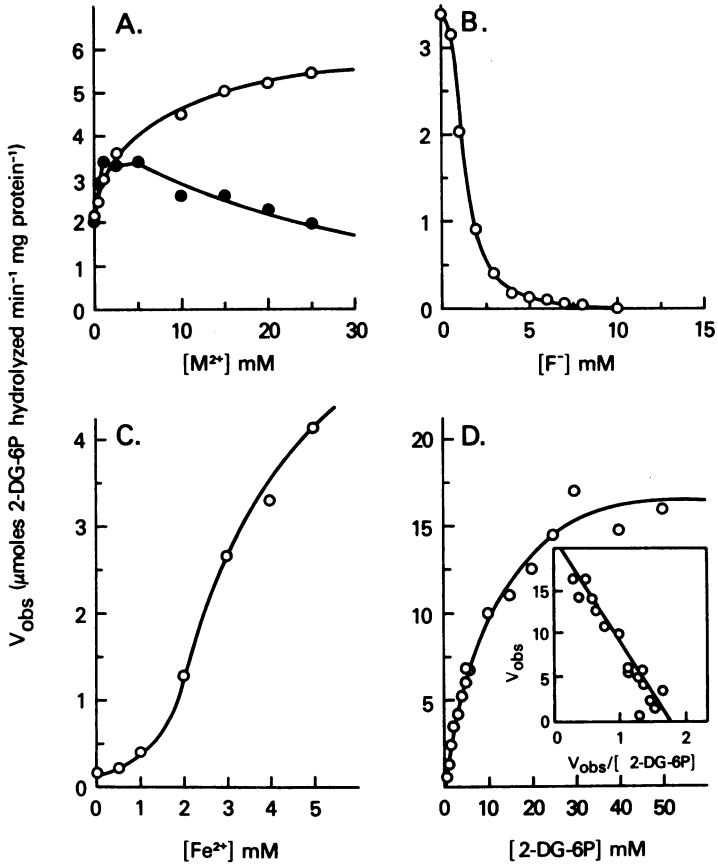

FIG. 4. Stimulation of H6P:phosphohydrolase activity by divalent metal ions (A). Assays containing various amounts of $MgSO_4$ (○) or $MnSO_4$ (●) were performed as described in the text. (B) Inhibition of enzyme activity by fluoride ion. The standard assay contained 5 mM $MgSO_4$ and various concentrations of NaF. (C) Reversal of fluoride inhibition by Fe^{2+} ion. Standard assays contained 5 mM NaF and 5 mM $MgSO_4$, and these were supplemented with increasing concentrations of $FeSO_4$. (D) Substrate saturation characteristics of the H6P:phosphohydrolase and the dependence of the rate of 2DG-6P hydrolysis (V_{obs}) on 2DG-6P concentration. The inset shows an Eadie-Hofstee transform of the same data.

presence of 0.12 mM Mn^{2+} ion introduced upon addition of the enzyme (in HEDM buffer). The rate of 2DG-6P hydrolysis was proportional to Mn^{2+} ion concentration up to 1 mM (Fig. 4A), but at levels > 5 mM Mn^{2+} was inhibitory. In contrast, Mg^{2+} was slightly less effective than Mn^{2+} in activating at low concentrations, but the former cation did not inhibit at higher concentrations.

Fluoride inhibition. Previous studies (39) showed that (i) efflux of 2DG from cells preloaded with 2DG-6P was inhibited by fluoride (F^-) ion and (ii) an F^--sensitive sugar phosphate phosphatase was present in crude cell extracts. Fluoride ion also inhibited the purified enzyme, but the degree of inhibition by the halide was dependent upon the nature of the divalent cation present in the assay (Table 2). When Mg^{2+} was included in the system, 1 and 5 mM F^- caused 50 and 98% inactivation, respectively. No detectable 2DG-6P hydrolysis occurred in the presence of 10 mM NaF (Fig. 4B; Table 2). However, when Mn^{2+}, Fe^{2+}, or Co^{2+} served as the activator, 10 mM F^- inhibited the enzyme activity by only 10 to 15%. Furthermore, inhibition by the combination of Mg^{2+} plus F^- was completely and specifically reversed by Fe^{2+} ion (Fig. 4C).

Inhibitor studies. The sulfhydryl (-SH) reagents *N*-ethylmaleimide, iodoacetate, and iodoacetamide caused no significant inhibition when present in the assay at a final concentration of 10 mM. A number of compounds were tested for their capacity to modify the activity of the H6P:phosphohydrolase. It was found that 25 mM PEP and 2,3-diphosphoglyceric acid reduced the rates of 2DG-6P, galactose 6-phosphate, glucose 6-phosphate, and fructose 6-phosphate hydrolysis by 50%, but equivalent concentrations of 2- and 3-phosphoglyceric acids were either without effect or were slightly stimulatory (data not shown). Both arsenate and P_i (25 mM) inhibited the enzyme by 50%. Free sugars and other glycolytic metabolites had no significant effect upon the activity of the phosphatase.

Substrate specificity and kinetic parameters. The dependence of the rate of 2DG-6P hydrolysis upon substrate concentration was studied in the presence of 25 mM Mg^{2+} (Fig. 4D), and the kinetic parameters determined were as follows: K_m = 11 mM and V_{max} = 21 μmol of 2DG-6P hydrolyzed mg of protein^{-1} min^{-1}. The specificity of the enzyme was determined from the rate of P_i liberation from a wide range of potential substrates, including sugar phosphates, phosphorylated glycolytic intermediates, and nucleotides (Table 3). With the exception of ribose and ribulose 5-phosphates, only hexose 6-phosphates were readily hydrolyzed by the enzyme. The hexose and hexulose 1-phosphates were cleaved at <10% the rate of the corresponding 6-phosphate derivatives, and fructose 1,6-diphosphate and glucose 1,6-diphosphate were hydrolyzed more slowly than their fructose and glucose 6-phosphate counterparts. The marked preference of the enzyme toward H6P substrates is clear from the data in Table 3. Neither *p*-nitrophenylphosphate, nucleotides, pyrophosphate, nor other glycolytic intermediates were readily hydrolyzed by the enzyme.

DISCUSSION

We have described the purification of an intracellular H6P:phosphohydrolase from *S. lactis* K1. Chromatography on DEAE-Sephacel resolved this enzyme from a *p*-nitrophenylphosphate:phosphohydrolase of approximate M_r 53,000, and the sugar phosphate phosphatase was further purified by gel permeation chromatography and a second DEAE-Sephacel step. PAGE and SDS-PAGE indicated that the final preparation was nearly homogeneous. The apparent M_r estimated from the two gel filtration methods (Ultrogel and HPLC) was 36,500, but SDS-PAGE revealed a single polypeptide of M_r 60,000. The latter technique appears to be a more reliable index of M_r (43) and may provide the best estimate for that of the phosphohydrolase. The anomalous behavior on gel filtration may indicate either (i) deviation in structural form of the enzyme from the more globular proteins used as size standards or (ii) an adsorptive interaction between the protein and gel matrix resulting in retardation and, hence, in an underestimation of molecular weight.

The purified enzyme showed a marked preference for sugar phosphates containing the phosphoryl group at C-6 of the hexose molecule. Glucose 1-phosphate and galactose 1-phosphate were hydrolyzed at <2% of the rate of the corresponding 6-phosphate isomers (Table 3). A free hydroxyl group at the C-1 (anomeric) center facilitates rapid cleavage of sugar phosphate substrates, since (i) addition of a second phosphate group (i.e., glucose 1,6-diphosphate, fructose 1,6-diphosphate) considerably reduced the rate of hydrolysis and (ii) P_i liberation from TMG-6P (−S · CH_3 substitution at C-1) was 90% slower than that observed from galactose 6-phosphate. Previously we had shown that starved cells of *S. lactis* contained high levels of 2- and 3-phosphoglyceric acids as well as PEP, and these glycolytic intermediates (total concentration, ca. 30 to 40 mM) were maintained by the cells for many hours (27, 42). The three intermediates were not hydrolyzed by the purified phosphohydrolase (Table 3). The results of this investigation and high-resolution ^{31}P-nuclear

TABLE 3. Substrate specificity of purified H6P:phosphohydrolase from *S. lactis* K1

Substrate[a]	Sp act (μmol of P_i produced mg of protein^{-1} min^{-1})	Relative rate (%)[b]
2DG-6P	5.67	100
D-Galactose 6-phosphate	6.87	121
D-Fructose 6-phosphate	5.27	93
D-Mannose 6-phosphate	3.53	62
D-Glucose 6-phosphate	3.33	59
D-Glucose-1,6-diphosphate	1.67	29
D-Glucosamine 6-phosphate	1.60	28
D-Ribose 5-phosphate	1.43	25
6-Phospho-D-gluconate	1.10	19
D-Ribulose 5-phosphate	0.78	14
TMG-6P	0.54	10
DL-α-Glycerol phosphate	0.47	8
D-Fructose 1-phosphate	0.40	7
D-Fructose 1,6-diphosphate	0.30	5
Mannitol 1-phosphate	0.27	5
Dihydroxyacetone phosphate	0.13	2
D-Galactose 1-phosphate	0.13	2
ATP	0.10	2
ADP	0.10	2
AMP	0.10	2

[a] Substrate concentration, 10 mM, except ATP, ADP, and AMP which were present at 5 mM. Compounds not hydrolyzed by the enzyme: *p*-nitrophenyl phosphate, pyrophosphate, 2-phosphoglycerate, 3-phosphoglycerate, 2,3-diphosphoglycerate, phosphoenolpyruvate, D-glucose 1-phosphate, and D-sorbitol 6-phosphate.

[b] Relative rate of hydrolysis with respect to 2DG-6P (100%).

magnetic resonance experiments with whole cells (J. Thompson and D. Torchia, unpublished data) indicate that the enzyme functions primarily as a phosphohydrolase, with water serving as acceptor for the phosphoryl (leaving) group. Free sugars could not replace water as phosphoryl acceptor, and we have been unable to demonstrate phosphotransferase activity with the bacterial enzyme. This behavior contrasts with that of the bifunctional glucose 6-phosphate: phosphohydrolase described by Nordlie (for review, see reference 29) which in mammalian cells exhibits both phosphohydrolase and phosphotransferase activities. The purified enzyme from *S. lactis* required a divalent metal (Mg^{2+}, Mn^{2+}, Co^{2+}, or Fe^{2+}) for activation, and our observation that activity was rapidly lost upon omission of divalent cation (Mg^{2+}, Mn^{2+}) from the buffer during purification appears unique among hexose phosphate:phosphohydrolases. In addition, the enzyme exhibits some unusual interactions with metal and fluoride (F^-) ions. For example, (i) significant inhibition by F^- occurred only in the presence of Mg^{2+} (Table 2), and (ii) inactivation by the combination of Mg^{2+} plus F^- was immediately and specifically reversed by addition of Fe^{2+} to the assay system. The role of divalent metals in the enzymatic mechanism and the mode of F^- inhibition are not known. However, the observation that F^- inhibits the hydrolysis of 2DG-6P and TMG-6P in preloaded cells (39, 41) suggests that Mg^{2+} ion may be the physiologically important metal activator in vivo.

Data in Table 4 show that the enzyme prepared from *S. lactis* exhibits properties quite different from hexose phosphate:phosphohydrolases purified from *Escherichia coli* (11), *Salmonella typhimurium* (21, 22, 33, 44), and *Neisseria meningitidis* (26). This is particularly evident with respect to substrate specificity, F^- sensitivity, and localization of enzyme activity. However, the studies of Koch (23), Gachelin (13), Kundig (24), Winkler (45), and Haguenauer and Kepes (14, 15) with *E. coli* and those of Hengstenberg et al. (18) with *Staphylococcus aureus* have demonstrated the presence of intracellular H6P:phosphohydrolase(s) in these bacteria (Table 4). The enzyme(s) has not been purified or extensively characterized, but Haguenauer and Kepes (14, 15) showed that α-methylglucoside 6-phosphate:phosphohydrolase activity was distinct from alkaline and other nonspecific phosphatases in *E. coli*. Furthermore, like *S. lactis*, the intracellular enzyme in *E. coli* was not saturated in vivo by the highest concentrations of α-methylglucoside 6-phosphate established by the $EII^{glc}/EIII^{glc}$ PTS (31). Kundig (24) suggested that the H6P:phosphohydrolase activity was a property of partially purified $EIII^{glc}$ of the glucose-PTS, but recent data of Postma and coworkers (35) and Meadow and Roseman (28) indicate that the phosphatase and $EIII^{glc}$ are separate entities which may have copurified during Kundig's isolation procedure.

Physiological functions. Recently we described a novel PEP-dependent 2DG futile cycle in *S. lactis* (39, 40). The operation of this 2DG:2DG-6P cycle, like the α-methylglucoside:α-methylglucoside 6-phosphate cycle described previously in *E. coli* (13, 14, 45), causes the dissipation of PEP due to an alternating sequence of phosphorylation and dephosphorylation of the analog (Fig. 5). In growing cells of *S. lactis* the energetic deficiency results in bacteriostasis (39, 40). It is our contention that the H6P:phosphohydrolase mediates the second stage of this futile cycle. Although recycling of a non-metabolizable glucose analog is depicted in Fig. 5, it seems probable that futile cycling of metabolizable sugars may also occur in some strains of *S. lactis* exhibiting the Lac^- Gal^d phenotype (7, 30). Such isolates, which have been obtained from *S. lactis* 133, H_1, and C_{10} (7), lack the plasmid which encodes the genes necessary for lactose

TABLE 4. Comparison of properties of H6P:phosphohydrolase from *S. lactis* K1 with hexose phosphatase activities in other microorganisms

Parameter	*S. lactis*[a]	*E. coli*[b]	*Salmonella typhimurium*[c]	*E. coli*[d]
Localization	Cytoplasmic	Periplasmic	Periplasmic	Cytoplasmic
Mol wt	36,500[e], 60,000[f]	NR[g]	Dimer (37,000-M_r subunits)	20,000
pH optimum	6.0	5.5–6.0	6.0	7.2
Effect of M^{2+} ions	Activated by Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}	No specific requirement; inhibited by Co^{2+}, Hg^{2+}, Zn^{2+}	No specific requirement; inhibited by Co^{2+}, Hg^{2+}, Zn^{2+}	Activated by Mg^{2+}, Mn^{2+}
Effect of EDTA	Inhibited by 1 mM	No inhibition at 10 mM	No inhibition at 5 mM	Inhibition
Effect of fluoride	Inhibition with Mg^{2+} (reversed by Fe^{2+})	NR	Half-maximum inhibition (0.08 mM)	Inhibition
Relative affinity (glucose 6-phosphate as substrate)	Low (K_m ~ 20 mM)	High (K_m ~ 0.3 mM)	High (K_m ~ 1.5–2 mM)	NR
Specificity	H6P	H1P and H6P	H1P and H6P	H6P
Regulation of activity	Modification by ATP, glycolytic intermediates and P_i?	NR	NR	Regulated by PEP, under metabolic control?
Regulation of enzyme synthesis	Constitutive, elevated by growth in presence of 2DG	Catabolite (glucose) repression	Regulation by cAMP, repression by glucose	Constitutive?
Function	Regulation of cellular H6P levels, detoxification	Hydrolysis of extracellular sugar-P for growth	Hydrolysis of sugar-P in medium	Detoxification

[a] Data from this study.
[b] Data from reference 11.
[c] Compiled from references 21, 22, 33, 44.
[d] Data compiled from 13–15, 24, 45.
[e] Determined by gel filtration.
[f] Determined by SDS-PAGE.
[g] NR, Not reported.

transport and catabolism. The organisms retain the capacity for growth on galactose (via the Leloir pathway) but exhibit a longer generation time and a more heterolactic fermentation pattern than the parental (Lac^+) strain grown on the same sugar. In addition, the Lac^- Gal^d organisms accumulate high levels of galactose 6-phosphate (ca. 20 to 40 mM [7]), which they are unable to metabolize due to absence of the Lac plasmid-encoded enzymes of the D-tagatose 6-phosphate pathway (3, 7). In this investigation we have found that galactose 6-phosphate is the best substrate for the H6P:phosphohydrolase (Table 3). Cells of *S. lactis* 133 Lac^- Gal^d also possess this enzyme (Thompson and Chassy, unpublished data), and the abnormally high levels of galactose 6-phosphate maintained during growth may represent a steady state in which the rate of formation of galactose 6-phosphate via the galactose-PTS (30, 37), is equal to the rate of hydrolysis of the sugar phosphate by the phosphohydrolase. The intracellular galactose

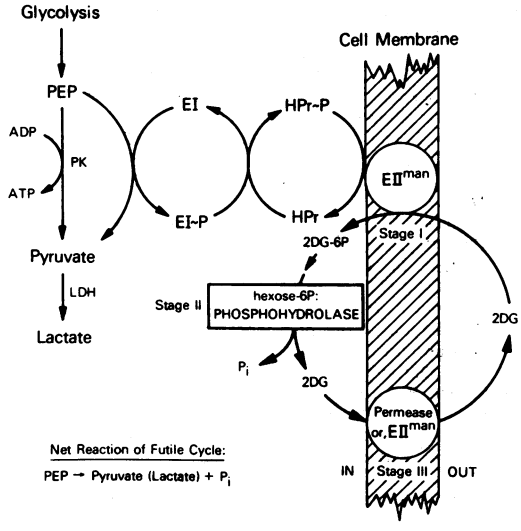

FIG. 5. Participation of H6P:phosphohydrolase in stage II of the PEP-dependent, 2DG futile cycle in *S. lactis*. Abbreviations: EI and EI~P, free and phosphorylated forms of enzyme I; HPr and HPr~P, free and phosphorylated forms of a histidine-containing phosphocarrier protein; EII^{man}, enzyme II (recognition) component of the mannose-PTS; PK, pyruvate kinase (EC 2.7.1.40; ATP:pyruvate 2-*O*-phosphotransferase); LDH, lactate dehydrogenase (EC 1.1.1.27; L-lactate:NAD^+ oxidoreductase).

formed may then (i) exit the cell via the galactose permease (20, 37), thereby completing a futile cycle, or (ii) enter the Leloir pathway after phosphorylation by the ATP-dependent galactokinase. The hydrolysis of each molecule of galactose 6-phosphate would result in a net loss of one molecule of PEP. In addition, the abnormally high galactose 6-phosphate concentration may impede the rate of galactose metabolism by the Leloir pathway. These two factors may, in part, account for the slower growth rate of Lac^- Gal^d isolates on galactose.

Although galactose 6-phosphate, glucose 6-phosphate, and fructose 6-phosphate are substrates for the H6P:phosphohydrolase (Table 3), it is unlikely that significant dephosphorylation of these glycolytic intermediates occurs during growth of wild-type strains because the intracellular concentrations of the compounds are considerably lower than the K_m for the enzyme. It should also be noted that fructose 1,6-diphosphate, which is present in growing cells at a concentration of 15 to 30 mM (27, 36, 42), was not readily hydrolyzed by the purified enzyme. In *S. lactis* the H6P:phosphohydrolase may play two important roles. First, the enzyme may serve as a detoxification mechanism for the hydrolysis (and eventual expulsion; 32, 39, 40) of potentially harmful and non-metabolizable sugar phosphates. Second, the phosphohydrolase may permit the cell to maintain the concentration of specific glycolytic intermediates (e.g., glucose 6-phosphate, fructose 6-phosphate) within acceptable limits during growth (see reference 12). Our studies with *S. lactis*, and those of Koch (23), Kundig (24), and Haguenauer and Kepes (14, 15) with *E. coli*, suggest an association of the H6P:phosphohydrolase with the bacterial PTS. It remains to be seen whether the enzyme is a regulatory component of the group translocation system or whether the phosphatase itself is subject to metabolic regulation.

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LITERATURE CITED

1. **Allen, J. M., and G. Hyncik.** 1963. Localization of alkaline phosphatases in gel matrices following electrophoresis. J. Histochem. Cytochem. **11**:169–175.
2. **Andrews, P.** 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. **91**:222–233.
3. **Bissett, D. L., and R. L. Anderson.** 1974. Lactose and D-galactose metabolism in group N streptococci: presence of enzymes for both the D-galactose 1-phosphate and D-tagatose 6-phosphate pathways. J. Bacteriol. **117**:318–320.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Chassy, B. M., and J. Thompson.** 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β-D-phosphogalactoside galactohydrolase activities in *Lactobacillus casei*. J. Bacteriol. **154**:1195–1203.
6. **Chassy, B. M., and J. Thompson.** 1983. Regulation and characterization of the galactose:phosphoenolpyruvate-dependent phosphotransferase system in *Lactobacillus casei*. J. Bacteriol. **154**:1204–1214.
7. **Crow, V. L., G. P. Davey, L. E. Pearce, and T. D. Thomas.** 1983. Plasmid linkage of the D-tagatose 6-phosphate pathway in *Streptococcus lactis*: effect on lactose and galactose metabolism. J. Bacteriol. **153**:76–83.
8. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum. Ann. N.Y. Acad. Sci. **121**:404–427.
9. **Diezel, W., G. Kopperschläger, and E. Hofmann.** 1972. An improved procedure for protein staining in polyacrylamide gels with a new type of Coomassie brilliant blue. Anal. Biochem. **48**:617–620.
10. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**:385–418.
11. **Dvorak, H. F., R. W. Brockman, and L. A. Heppel.** 1967. Purification and properties of two acid phosphatase fractions isolated from osmotic shock fluid of *Escherichia coli*. Biochemistry **6**:1743–1751.
12. **Fraenkel, D. G.** 1968. The accumulation of glucose 6-phosphate from glucose and its effect in an *Escherichia coli* mutant lacking phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. J. Biol. Chem. **243**:6451–6457.
13. **Gachelin, G.** 1970. Studies on the α-methyl glucoside permease of *Escherichia coli*. A two-step mechanism for the accumulation of α-methylglucoside 6-phosphate. Eur. J. Biochem. **16**:342–357.

14. **Haguenauer, R., and A. Kepes.** 1971. The cycle of renewal of intracellular α-methylglucoside accumulated by the glucose permease of *E. coli*. Biochimie **53**:99–107.
15. **Haguenauer, R., and A. Kepes.** 1972. NaF inhibition of phosphorylation and dephosphorylation involved in α-methyl-D-glucoside transport in *E. coli* K12. A pH dependent phenomenon sensitive to uncoupling agents. Biochimie **54**:505–512.
16. **Hays, J. B.** 1978. Group translocation transport systems, p. 43–102. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, New York.
17. **Heller, K., and R. Röschenthaler.** 1978. β-D-phosphogalactoside galactohydrolase of *Streptococcus faecalis* and the inhibition of its synthesis by glucose. Can. J. Microbiol. **24**:512–519.
18. **Hengstenberg, W., J. B. Egan, and M. L. Morse.** 1968. Carbohydrate transport in *Staphylococcus aureus*. VI. The nature of the derivatives accumulated. J. Biol. Chem. **243**:1881–1885.
19. **Hørder, M.** 1972. Colorimetric determination of orthophosphate in the assay of inorganic pyrophosphatase activity. Anal. Biochem. **49**:37–47.
20. **Kashket, E. R., and T. H. Wilson.** 1972. Role of metabolic energy in the transport of β-galactosides by *Streptococcus lactis*. J. Bacteriol. **109**:784–789.
21. **Kier, L. D., R. Weppelman, and B. N. Ames.** 1977. Resolution and purification of three periplasmic phosphatases of *Salmonella typhimurium*. J. Bacteriol. **130**:399–410.
22. **Kier, L. D., R. Weppelman, and B. N. Ames.** 1977. Regulation of two phosphatases and a cyclic phosphodiesterase of *Salmonella typhimurium*. J. Bacteriol. **130**:420–428.
23. **Koch, A. L.** 1971. Energy expenditure is obligatory for the downhill transport of galactosides. J. Mol. Biol. **59**:447–459.
24. **Kundig, W.** 1974. Molecular interactions in the bacterial phosphoenolpyruvate-phosphotransferase system (PTS). J. Supramol. Struct. **2**:695–714.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
26. **Lee, Y.-P., J. R. Sowokinos, and M. J. Erwin.** 1967. Sugar phosphate phosphohydrolase. I. Substrate specificity, intracellular localization, and purification from *Neisseria meningitidis*. J. Biol. Chem. **242**:2264–2271.
27. **Mason, P. W., D. P. Carbone, R. A. Cushman, and A. S. Waggoner.** 1981. The importance of inorganic phosphate in regulation of energy metabolism of *Streptococcus lactis*. J. Biol. Chem. **256**:1861–1866.
28. **Meadow, N. D., and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Isolation and characterization of a glucose-specific phosphocarrier protein (III^{glc}) from *Salmonella typhimurium*. J. Biol. Chem. **257**:14526–14537.
29. **Nordlie, R. C.** 1971. Glucose-6-phosphatase, hydrolytic and synthetic activities, p. 543–610. *In* P. D. Boyer (ed.), The enzymes, vol. 4. Academic Press, Inc., New York.
30. **Park, Y. H., and L. L. McKay.** 1982. Distinct galactose phosphoenolpyruvate-dependent phosphotransferase system in *Streptococcus lactis*. J. Bacteriol. **149**:420–425.
31. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate:sugar phosphotransferase system. Biochim. Biophys. Acta **457**:213–257.
32. **Reizer, J., and C. Panos.** 1980. Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. Proc. Natl. Acad. Sci. U.S.A. **77**:5497–5501.
33. **Rephaeli, A. W., I. R. Artenstein, and M. H. Saier, Jr.** 1980. Physiological function of periplasmic hexose phosphatase in *Salmonella typhimurium*. J. Bacteriol. **141**:1474–1477.
34. **Romano, A. H., J. D. Trifone, and M. Brustolon.** 1979. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in fermentative bacteria. J. Bacteriol. **139**:93–97.
35. **Scholte, B. J., A. R. Schuitema, and P. W. Postma.** 1981. Isolation of III^{Glc} of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Salmonella typhimurium*. J. Bacteriol. **148**:257–264.
36. **Thompson, J.** 1978. In vivo regulation of glycolysis and characterization of sugar:phosphotransferase systems in *Streptococcus lactis*. J. Bacteriol. **136**:465–476.
37. **Thompson, J.** 1980. Galactose transport systems in *Streptococcus lactis*. J. Bacteriol. **144**:683–691.
38. **Thompson, J., and B. M. Chassy.** 1981. Uptake and metabolism of sucrose by *Streptococcus lactis*. J. Bacteriol. **147**:543–551.
39. **Thompson, J., and B. M. Chassy.** 1982. Novel phosphoenolpyruvate-dependent futile cycle in *Streptococcus lactis*: 2-deoxy-D-glucose uncouples energy production from growth. J. Bacteriol. **151**:1454–1465.
40. **Thompson, J., and B. M. Chassy.** 1983. Regulation of glycolysis and sugar:phosphotransferase activities in *Streptococcus lactis*: growth in the presence of 2-deoxy-D-glucose. J. Bacteriol. **154**:819–830.
41. **Thompson, J., and M. H. Saier, Jr.** 1981. Regulation of methyl-β-D-thiogalactopyranoside-6-phosphate accumulation in *Streptococcus lactis* by exclusion and expulsion mechanisms. J. Bacteriol. **146**:885–894.
42. **Thompson, J., and T. D. Thomas.** 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. J. Bacteriol. **130**:583–595.
43. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.
44. **Weppelman, R., L. D. Kier, and B. N. Ames.** 1977. Properties of two phosphatases and a cyclic phosphodiesterase of *Salmonella typhimurium*. J. Bacteriol. **130**:411–419.
45. **Winkler, H. H.** 1971. Efflux and the steady state in α-methylglucoside transport in *Escherichia coli*. J. Bacteriol. **106**:362–368.