JOURNAL OF BACTERIOLOGY, Nov. 1984, p. 755–763
0021-9193/84/110755-09$02.00/0

Vol. 160, No. 2

Isolation of a Novel Protein Involved in the Transport of Fructose by an Inducible Phosphoenolpyruvate Fructose Phosphotransferase System in *Streptococcus mutans*

LUCIE GAUTHIER, DENIS MAYRAND, AND CHRISTIAN VADEBONCOEUR*

Groupe de Recherche en Ecologie Buccale, Ecole de Médecine Dentaire, Université Laval, Québec, Canada

Received 9 March 1984/Accepted 14 August 1984

Fructose transport in *Streptococcus mutans* LG-1 is mediated by at least two distinct phosphoenolpyruvate fructose phosphotransferase systems. One system is constitutive and consists of membrane components enzyme II as well as enzyme I and heat-stable protein. The other system is inducible and requires, in addition to enzyme I and heat-stable protein, a soluble substrate-specific protein for catalytic activity. This protein factor, designated III^{fru}, was purified by DEAE-cellulose chromatography, hydroxylapatite chromatography, molecular sieving on Sephadex G-75, and preparative electrophoresis. The purified preparation showed only one protein band, with a molecular weight of 12,600, on sodium dodecyl sulfate-urea-polyacrylamide gel electrophoresis, on gel electrophoresis with the discontinuous buffer Tris-glycine, and after electrofocusing in gel (pI ≃ 3.7). The molecular weight of the native protein determined by gel filtration at 4°C was 51,000. Immunodiffusion experiments performed with immunoglobulins prepared against the purified III^{fru} from *S. mutans* LG-1 suggested that other *S. mutans* strains possessed a III^{fru}. No precipitin bands, however, were detected with extracts from *S. salivarius*, *S. sanguis*, *S. lactis*, *S. faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus casei*, and *Escherichia coli*.

The transformation of carbohydrates to organic acids by oral streptococci is of prime importance in the formation of dental caries (3, 49) although the relative cariogenicity of various carbohydrates is still controversial. Sucrose has been identified as one of the most cariogenic sugars because it promotes plaque formation by *Streptococcus mutans* (19); in addition, many studies have demonstrated the enamel demineralization and cariogenic potential of fructose (4, 8, 24, 39). The amount of this carbohydrate in the diet has increased considerably during the last 12 years consequent to the use of high-fructose corn syrup in processed foods (W. H. Bowen, Spec. Suppl. Abstr. Proc. Sweeteners and Dent. Caries, 1978, p. 147–155). For these reasons, the metabolism of fructose by oral streptococci, particularly *S. mutans*, may be of significance in the development of dental caries.

Metabolism of carbohydrates in bacteria is initiated by the transport of these carbohydrates across the cellular membrane. Under specific conditions of growth (14, 21), this process is mediated in *S. mutans* mainly by the phosphoenolpyruvate (PEP) sugar phosphotransferase (PTS) system (6, 13, 28, 33, 38, 40, 41). Although fructose phosphotransferase activity has been reported in *S. mutans* (41), little is known about the fructose PTS in this cariogenic oral bacterium. Very recently, we have shown that *Streptococcus salivarius* and *S. mutans* possess a constitutive fructose PTS (44). We have also found that transport of xylitol in *S. mutans* is mediated by this constitutive fructose PTS (L. Trahan, M. Bareil, L. Gauthier, and C. Vadeboncoeur, Caries Res., in press). Spontaneous mutants defective in this constitutive fructose PTS could be easily obtained by repeated culturing of *S. mutans* cells in the presence of glucose and xylitol (18; Trahan, et al., in press). These fructose PTS-negative mutants grow on fructose although less rapidly than does the wild type (18). This suggested the presence of another fructose transport system in *S. mutans*. Indeed, in this communication we report the presence of a second fructose PTS in *S. mutans*. In addition, a new protein factor required for the PEP-dependent phosphorylation of fructose by this system has been purified from *S. mutans* LG-1 and partially characterized. This factor has been called III^{fru}.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. mutans* strain LG-1 was from our own collection. It was characterized by the methods of Facklam (15) and Thomson et al. (L. A. Thomson, W. A. Little, R. Facklam, and R. Gherma, Abstr. Annu. Meet. Am. Assoc. Dent. Res. 1983, no. 108, p. 181) and classified as *S. mutans* serotype *c*. Acid formation by this strain was observed in the presence of mannitol, sorbitol, inulin, lactose, melibiose, raffinose, sucrose, and salicin. Strain LG-1 X^r is a spontaneous mutant derived from LG-1 (18) and, as opposed to the wild type, was devoid of fructose PTS activity after growth of the cells on glucose when the activity was measured with purified enzyme I (EI) and heat-stable protein (HPr) (Trahan et al., in press). The source of the other strain used in this study was reported previously (45). Purity of the strains was confirmed by their Gram stain properties and colony characteristics on mitis salivarius agar (Difco Laboratories) and Trypticase (BBL Microbiology Systems)-yeast extract (Difco Laboratories) agar. Bacteria were grown in a medium containing (per liter) 17 g of Trypticase, 3 g of yeast extract, 5 g of NaCl, and 2.5 g of disodium phosphate. Sugars were sterilized by filtration with a Millex-GS membrane filter (0.22 μm; Millipore Corp.) and added aseptically to the medium. Growth was monitored by reading the optical density at 660 nm on a Bausch & Lomb Spectronic 20 spectrophotometer. To obtain large quantities of cells, we grew the cultures with gentle stirring in six 20-liter Pyrex (Corning Glass Works) bottles each containing 12 liters of medium. Each bottle was inoculated with one overnight culture of 300 ml. Cells were harvested at the end of logarithmic growth by continuous-flow centrifugation in a

* Corresponding author.

Beckman model J2-21 centrifuge with a model JCF-Z rotor. The culture (72 liters) yielded ca. 120 g of wet cell paste that was washed twice with 750 ml of 10 mM potassium phosphate (pH 7.0) containing 0.5 mM 2-mercaptoethanol (2-ME) and chloramphenicol (5 μg/ml) and was kept frozen at −20°C.

Preparation of enzyme II-containing membranes. Cells were ground with levigated alumina (3 g of alumina per g of wet cell paste) for 20 min in a refrigerated mortar, and 2 ml of 10 mM potassium phosphate (pH 7.5) containing 14 mM 2-ME and 1 mM EDTA was added for each gram of cells. Alumina was removed by centrifugation at 3,000 × *g* for 5 min, and cell debris and cells were sedimented at 16,000 × *g* for 20 min. The supernatant was collected, and membrane fragments were sedimented at 200,000 × *g* for 4 h. The supernatant from this high-speed centrifugation was used for the partial purification of EI and HPr and for the purification of III^{fru}. The pellet resulting from this centrifugation was washed with 10 mM potassium phosphate (pH 7.5) containing 14 mM 2-ME and 1 mM EDTA and then was stored at −20°C and used as enzyme II (EII) fraction.

Partial purification of EI and HPr from *S. salivarius*. A method for the complete purification of EI and HPr from *S. salivarius* has been reported (45). Studies reported in this paper were conducted with EI and HPr partially purified from *S. salivarius* by this previous method. Phosphotransferase proteins isolated from *S. salivarius* exhibit enzymatic cross-reactivity with those from *S. mutans* (unpublished data).

The membrane-free supernatant obtained from ca. 100 g of wet cells by centrifugation at 200,000 × *g* was loaded on a DEAE-cellulose column previously equilibrated with 10 mM potassium phosphate (pH 7.5)–14 mM 2-ME–1 mM EDTA. The column was first washed with buffer and then with 0.1 M KCl. This fraction contained HPr and was further purified by chromatography on a column of Sephadex G-75 (Pharmacia Fine Chemicals, Inc.). The column of DEAE-cellulose was then eluted by using a gradient of 0.1 to 0.5 M KCl. EI activity was recovered at 0.35 M KCl. Those fractions containing high enzyme activity were pooled and dialyzed against 10 mM potassium phosphate (pH 6.8)–14 mM 2-ME–0.1 mM EDTA. This sample was loaded on a column of hydroxylapatite (Bio-Rad HT) equilibrated against the aforementioned buffer. The column was washed successively with the equilibration buffer and with a linear gradient of 10 to 100 mM potassium phosphate. The active fractions were concentrated by ultrafiltration with an Amicon cell with an XM50 membrane and were dialyzed against 10 mM sodium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 6.8)–0.1 M KCl–14 mM 2-ME–0.1 M EDTA. The preparation at this stage of purification showed several protein bands when subjected to acrylamide gel electrophoresis, but it was free of EII and HPr activities.

Purification of HPr from *S. mutans*. The complete purification of HPr from *S. mutans* was achieved by a method slightly different from that reported for *S. salivarius* (45). The fractions eluted at 0.1 M KCl on DEAE-cellulose were pooled and concentrated by ultrafiltration with an Amicon ultrafiltration cell with a YM5 membrane. The concentrated sample was loaded onto a column of Sephadex G-75 (2.6 by 95 cm) that had been equilibrated with 10 mM potassium phosphate buffer (pH 7.5) containing 14 mM 2-ME, 0.1 mM EDTA, and 0.1 M KCl. After elution with the same buffer, those fractions containing HPr were pooled, concentrated by ultrafiltration, and dialyzed against the aforementioned buffer without KCl. HPr was subsequently purified by a preparative polyacrylamide gel electrophoresis with the discontinuous buffer system of Davis (10).

Purification of the fructose-specific protein factor III^{fru}. Purification was started from cells of strain LG-1 X^r obtained from six 12-liter cultures grown on 0.5% fructose. All steps were carried out at 4°C, and unless otherwise mentioned, all buffers contained 14 mM 2-ME and 0.1 mM EDTA. The presence of III^{fru} in column fractions was detected by measuring the rate of phosphorylation of [^{14}C]fructose in the presence of partially purified HPr and EI from *S. salivarius* and membrane fragments obtained from fructose-grown cells of strain LG-1 X^r. Care was taken to ensure that the activity increased linearly with the amount of III^{fru} added to the assay medium. A membrane-free cellular extract was obtained as described above. The extract was applied to a DEAE-cellulose column (5 by 25 cm) previously equilibrated with 10 mM potassium phosphate (pH 7.5) and 1 mM EDTA. The column was washed first with buffer and then with 1 liter of 0.1 M KCl. The column was then eluted with a 3-liter gradient of 0.1 to 0.5 M KCl, collecting 25-ml fractions. The fructose-specific factor was recovered at 0.35 M KCl, together with EI. Those fractions containing activity were pooled and dialyzed against 10 mM potassium phosphate (pH 6.8). The dialyzed sample was loaded on a column of hydroxylapatite (Bio-Rad HT; 2.6 by 15 cm) (mixed with 10% [vol/vol] of cellulose powder [Whatman CF11] to increase the flow rate) equilibrated against 10 mM potassium phosphate (pH 6.8). The column was washed successively with 150 ml of equilibration buffer and with a linear gradient (1 liter) of 10 to 150 mM potassium phosphate (pH 6.8) at a rate of 40 ml/h, collecting 5-ml fractions. The fructose-specific factor was eluted at the end of the gradient and was free of EI activity which was recovered at ca. 40 mM potassium phosphate. Those fractions containing III^{fru} were concentrated by ultrafiltration with an Amicon cell with a YM5 membrane and then were applied to a Sephadex G-75 column (2.6 by 95 cm) equilibrated with 10 mM potassium phosphate (pH 7.5) and 0.1 M KCl. After elution, the active fractions were pooled, concentrated by ultrafiltration, and dialyzed against 10 mM potassium phosphate (pH 7.5). A sample of this pooled fraction containing 4 to 5 mg of protein in 5 ml of 10 mM potassium phosphate (pH 7.5), 20% glycerol, and 0.0005% bromophenol blue was then layered on the top of a column of polyacrylamide gel (4 by 6.5 cm) in a Canalco Prep-Disc Electrophoresis apparatus. The gels (a 0.5-cm-long stacking gel [pH 6.7] and a 6-cm-long separating gel [pH 8.9]) and buffers were prepared by the method of Orr et al. (31) with the Tris-glycine discontinuous buffer system. Electrophoresis was performed under a constant voltage of 500 V. When the bromophenol blue reached the bottom of the separating gel (after ca. 5 h), elution was initiated (60 ml/h), and 3-ml fractions were collected. The active fractions were pooled, dialyzed against 10 mM potassium phosphate (pH 7.5), concentrated, and stored at −20°C in the presence of 20% glycerol.

Enzyme assays. 6-Phosphofructokinase (6-PFK) and 1-Phosphofructokinase (1-PFK) activities were measured as previously described (9) with cell extracts prepared by sonication. The PEP-dependent sugar phosphorylation was determined through isolation of the radioactively labeled sugar phosphates by precipitation with $BaBr_2$ (45). Specific activity is expressed as nanomoles of sugar phosphorylated per minute at 37°C either per milligram of cytoplasmic protein or of membrane protein, as indicated in the tables.

Electrophoresis. Electrophoresis of III^{fru} was performed by the methods of Davis (10) and Swank and Munkres (42).

The molecular weight of the protein was determined by the method of Swank and Munkres (42). Proteins were incubated 2 h at 37°C in the presence of 3% sodium dodecyl sulfate (SDS)–5% 2-ME–8 M urea in 0.01 M H_3PO_4 adjusted to pH 6.8 with Tris. Partial cyanogen bromide cleavage products of sperm whale myoglobin from Pharmacia Fine Chemicals, Inc., were used as molecular weight markers. The gels were stained with Coomassie blue R250. After destaining, the gels were scanned at 595 nm with a Beckman DU-8 spectrophotometer.

IEF in polyacrylamide gels. Isoelectric focusing (IEF) was carried out in gels by the method of Righetti and Drysdale (35). Polyacrylamide gels of 10 cm in length were prepared in glass tubes (5 by 12.5 cm). A mixture of LKB Ampholines (2%) in the pH range of 3 to 10 were polymerized in the gels together with 10 μg of the protein sample. IEF was run at 1 mA per gel until the voltage reached 200 V and then was run at a constant voltage of 200 V for 12 h. After IEF, the gels were gently extruded from the gel tubes with water. Some gels were used for determination of the pH gradient, whereas others were fixed immediately with 10% trichloroacetic acid (3 times at 12 h each) and stained with Coomassie blue G250 by the method of Reisner et al. (34). The pH gradient was determined by cutting the gels into 0.5-cm sections and placing each of these into 1 ml of distilled water. The pH of the gel-containing water was measured after 4 h with a pH electrode.

Gel filtration. The molecular weight of III^{fru} was estimated by gel filtration at 4°C on an Ultrogel AcA34 column (2.6 by 95 cm) equilibrated with 10 mM potassium phosphate (pH 7.5)–0.1 M KCl–14 mM 2-ME–0.1 mM EDTA. Calibration of the column was accomplished by running separately the following standard proteins: RNase A, chymotrypsinogen A, ovalbumin, bovine serum albumin, and alcohol dehydrogenase. The void volume was estimated with blue dextran. The molecular weight of III^{fru} was determined by the method of Laurent and Killander (25).

Identification of the reaction products. The experiments were conducted with membranes of lactose-grown cells of strain LG-1 incubated in the presence of an excess of EI and HPr and with membranes of fructose-grown cells of LG-1 X^r incubated in the presence of an excess of EI, HPr, and concentrated, dialyzed, membrane-free cellular extract (550 μg of proteins) of fructose-grown cells. Both incubations were carried out in 1.8 ml of the following reaction medium: 1 mM [*U*-^{14}C]fructose (0.5 Ci/mol)–4 mM $MgCl_2$–2 mM PEP–2 mM 2-ME–10 mM NaF in a 5 mM triethanolamine buffer (pH 7.0). After 120 min of incubation at 37°C, the mixtures were heated at 100°C for 2 min and then centrifuged for 25 min at 15,000 × *g*. The action of 6-PFK on the phosphorylated derivatives was studied by using 0.9 ml of the mixtures obtained after centrifugation at 15,000 × *g*. The pH of these samples was adjusted to 8.0, and 2 mM ATP–3 mM $MgCl_2$–20 U of 6-PFK (Sigma Chemical Co. no. F6877) was added. The solutions were incubated 150 min at 37°C, heated at 100°C for 2 min, and then centrifuged at 15,000 × *g* for 25 min. The mixtures were concentrated by evaporation at 37°C to ca. 100 μl. The reaction products were identified by paper chromatography. Samples (5 μl ≃ 38,000 dpm) of each extract were applied to Whatman no. 1 chromatography paper. Development was ascending, at 20°C for 15 h. The solvent was 1 M ammonium acetate (pH 5)–95% ethanol −0.1 mM disodium ethylenediamine-tetraacetic acid (30:70:1 [vol/vol/vol]) (17). Unlabeled standards of fructose 6-phosphate, fructose 1-phosphate, and fructose 1,6-diphosphate were run on the same chromatogram. Strips of 1 cm each were cut and counted as described for filter membranes. Unlabeled standards were located with the acid molybdate solution of Hanes and Isherwood (22).

Production of antibody. Antiserum against III^{fru} was obtained from two New Zealand white female rabbits after immunization by multisite subcutaneous injections. The primary inoculation consisted of 100 μg of protein emulsified in Freund complete adjuvant (Difco Laboratories). A second injection of protein in Freund incomplete adjuvant was given 6 weeks after the first injection. The rabbits were exsanguinated by cardiac puncture 15 days after the second injection, and the blood was left to clot for 1 h at room temperature. After storage at 4°C overnight, the serum was collected by centrifugation and immunoglobulins were purified by the method of Calendar and Berg (5). Saturated ammonium sulfate solution (1 volume) was added to the whole serum, and the precipitate was dissolved in 25 ml of 20 mM potassium phosphate, pH 6.5. After dialysis against this buffer, the globulin solution was loaded on a column of DEAE-cellulose (1.5 by 20 cm) equilibrated against the same buffer. The proteins not retained on the column included most of the IgG.

Immunochemical procedures. Double-diffusion immunoprecipitations were conducted with microscope slides (2.5 by 7.5 cm) coated with 1% agar (Difco Laboratories) in 10 mM sodium borate (pH 7.8)–0.15 M NaCl–1 mM NaN_3. Diffusion was allowed for 24 h at room temperature. In all experiments, the center well contained 10 μl of undiluted, purified immunoglobulins, and the antigen wells contained μl of cellular crude extracts. Preimmune serum was used as a control.

Protein determination. Proteins were usually measured by the method of Lowry et al. (27) with serum bovine albumin as the standard. Protein determination of membrane preparations was carried out by the method of Lowry et al. (27) after a preincubation of the samples with 1 N NaOH (10 min, 37°C). Proteins in column fractions were detected by the method of Bradford (2).

RESULTS

Evidence for the presence of two fructose PTS systems in *S. mutans* LG-1. To detect the presence of two fructose PTS systems in *S. mutans*, we have measured fructose phosphotransferase activity with EII-containing membranes isolated from glucose- and fructose-grown cells of strains LG-1 and LG-1 X^r. The activity was determined by using an excess of partially purified EI and HPr in the presence or absence of concentrated, dialyzed, crude membrane-free extracts. Results are summarized in Table 1. When the experiment was conducted in the absence of cellular extract, fructose PTS activity was detected with wild-type LG-1 membranes but only at the background level with membranes isolated from mutant strain LG-1 X^r. Addition of crude membrane-free extracts to the reaction medium restored fructose PTS activity in membranes of the mutant strain and enhanced the activity in the wild-type membrane. Growth of the mutant cells at the expense of fructose resulted in a four- to fivefold increase of fructose phosphotransferase activity. From these results, it could be hypothesized that *S. mutans* LG-1 possessed two fructose PTS systems; one was constitutive and did not require any other soluble components than EI and HPr to be active. This system was missing in mutant strain LG-1 X^r, but this strain possessed another fructose PTS with activity that could not be detected solely in the presence of EI and HPr.

TABLE 1. Phosphoenolpyruvate-dependent phosphorylation of fructose by isolated membranes

Source of membranes	Growth sugar	Additions[a]	Sp. act (nmol of phosphorylated product/min per mg of membrane protein)
S. mutans LG-1	Glucose	EI + HPr	27.5
	Fructose	EI + HPr	38.1
	Glucose	Extract + EI + HPr	56.4
	Fructose	Extract + EI + HPr	73.8
S. mutans LG-1 X^R	Glucose	EI + HPr	0.66
	Fructose	EI + HPr	0.57
	Glucose	Extract + EI + HPr	2.46
	Fructose	Extract + EI + HPr	10.73

[a] The reaction medium contained membranes (22.5 μg of protein), membrane-free extract (where indicated, ≈650 μg of protein), excess of EI and HPr, 1 mM [*U*-^{14}C]fructose (0.1 Ci/mol), 4 mM $MgCl_2$, 2 mM PEP, 2 mM 2-ME, and 10 mM NaF in a 50 mM sodium phosphate buffer (pH 7.0). In all of the assay, the reaction was dependent on the amount of membranes in the reaction medium.

Absence of a fructose-specific HPr in *S. mutans* LG-1 X^r. Mugharbil and Cirillo (30) have found that an α-methylglucoside-resistant mutant of *Mycoplasma capricolum* possessed a fructose-specific HPr (HPr^{fru}) that exhibited chromatographic characteristics similar to those of the nonspecific HPr found in the wild type. To determine if such an HPr occurred in strain LG-1 X^r, we purified the HPr from glucose- and fructose-grown cells of strains LG-1 and LG-1 X^r and tested the ability of the purified proteins to catalyze the PEP-dependent phosphorylation of fructose in the presence of membranes of fructose-grown cells of LG-1 X^r. The purified proteins were first analyzed by acrylamide gel electrophoresis by the method of Davis (10). HPr isolated from *S. mutans* LG-1 or LG-1 X^r always gave the same electrophoretic pattern (Fig. 1); however, this HPr was somehow different from that isolated from *S. salivarius*, although both proteins exhibited cross-reactivity (see Fig. 1, gels 6 and 7). The presence of two protein bands on the gel did not indicate the presence of a contaminant since the same preparations gave a single band when analyzed by IEF in gel and by SDS-polyacrylamide gel electrophoresis in the presence of urea. It could be due to the presence of HPr(ser)P as reported by Deutscher and Saier (11). However, none of these purified HPrs were able to catalyze the PEP-dependent phosphorylation of fructose in the presence of EI and membranes of fructose-grown cells of LG-1 X^r (Table 2). We thus concluded that *S. mutans* LG-1 X^r did not possess a fructose-specific HPr after growth on fructose.

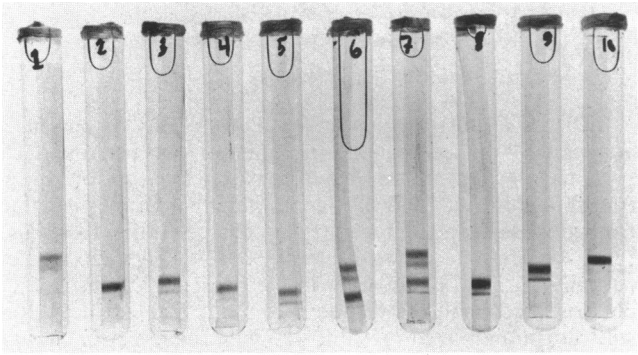

FIG. 1. Polyacrylamide gel electrophoresis of HPr. Electrophoresis was performed by the method of Davis (10) in 7.5% polyacrylamide gel. Protein (ca. 10 to 20 μg) was deposited on each gel. After electrophoresis (2 mA per gel), the gels were stained with Coomassie brilliant blue R250 for 2 h at room temperature and destained in a solution of acetic acid (10%) and methanol (50%). Gels: 1, HPr from glucose-grown cells of *S. salivarius*; 2, HPr from glucose-grown cells of strain LG-1; 3, HPr from glucose-grown cells of LG-1 X^r; 4, HPr from fructose-grown cells of LG-1; 5, HPr from fructose-grown cells of LG-1 X^r; 6, mixture of HPr isolated from glucose-grown cells of *S. salivarius* and LG-1 (gels 1 and 2); 7, mixture of HPr isolated from fructose-grown cells of LG-1 and glucose-grown cells of *S. salivarius* (gels 1 and 4); 8, mixture of HPr isolated from glucose-grown cells of LG-1 and LG-1 X^r (gels 2 and 3); 9, mixture of HPr from fructose-grown cells of LG-1 and LG-1 X^r (gels 4 and 5); 10, mixture of HPr from glucose-grown cells of LG-1 and fructose-grown cells of LG-1 X^r (gels 2 and 5).

Purification of a fructose-specific factor. As a consequence of the above results, we searched for the presence of another factor which was necessary for the PEP-dependent phosphorylation of fructose by isolated membranes of fructose-grown cells of strain LG-1 X^r. The purification procedure of this factor (III^{fru}) and the yield from each step are shown in Table 3. The preparation obtained after filtration on Sephadex G-75 still contained some minor contaminants that could be removed by preparative electrophoresis. This last step of purification, however, resulted in a decrease in the specific activity of III^{fru}. After complete purification, the protein was stored at −20°C in 15% glycerol, but its activity decreased rapidly, being null after 6 months of storage.

Purity of the preparation. Homogeneity of III^{fru} was confirmed by polyacrylamide gel electrophoresis by the method of Davis (10) (Fig. 2) and SDS-urea-gel electrophoresis (Fig. 3).

Involvement of III^{fru} in the PEP-dependent phosphorylation of fructose. The PEP-dependent phosphorylation of fructose by membranes of fructose-grown cells of LG-1 X^r required, in addition to EI and HPr, the presence of III^{fru} (Table 4). There was no activity in the absence of PEP. ATP could not substitute for PEP.

TABLE 2. Fructose phosphotransferase activity supported by purified HPr isolated from glucose or fructose-grown cells

S. mutans strain used[a]	Growth sugar	Sp. act (nmol of fructose phosphate per min per mg of membrane protein) measured with membranes isolated from fructose-grown cells of[b]	
		LG-1	LG-1 X^r
LG-1	Glucose	51.9	1.3
	Fructose	47.6	2.0
LG-1 X^r	Glucose	77.2	2.4
	Fructose	57.9	2.4

[a] For isolation of HPr. HPr was purified to homogeneity, as described in the text, from glucose- and fructose-grown cells of LG-1 and LG-1 X^r.

[b] The reaction medium was composed of 50 mM sodium phosphate (pH 7.0), 1 mM [*U*-^{14}C]fructose (0.1 Ci/mol), 4 mM $MgCl_2$, 2 mM PEP, 2 mM 2-ME, 10 mM NaF, ca. 1,000 U of EI, 50 μg of HPr, and 25 μl of membranes (25 to 50 μg of protein) in a total volume of 600 μl. After 30 min at 37°C, the phosphorylated product was isolated by precipitation with $BaBr_2$ (43) and counted for ^{14}C in a Beckman LS7500 liquid scintillation counter.

TABLE 3. Purification of factor III^{fru}[a]

Fraction	Total protein (mg)	Total activity (U^b)	Sp act (U/mg of protein)	Yield (%)	Purification (fold)
Membrane-free cellular extract	543.5	33,500	62	100	1
DEAE-cellulose	47.6	31,500	660	94	10.6
Hydroxylapatite	13.3	29,300	2,200	87	35.5
Sephadex G-75	3.3	16,900	5,200	50	83.8
Preparative electrophoresis	1.3	5,800	4,700	17	75.8

[a] From 123 g of cells (wet weight).

[b] One unit (U) of III^{fru} allowed the formation of 1 nmol of fructose phosphate per min at 37°C. The activity of III^{fru} in fractions was estimated by measuring the rate of phosphorylation of [^{14}C]fructose (1 mM; 0.1 Ci/mol) in the presence of partially purified HPr and EI isolated from *S. salivarius* (see text) and membrane fragments (≃400 μg of proteins) obtained from fructose-grown cells of strain LG-1 X^r. Care was taken to ensure that the activity increased linearly with the amount of the tested fraction added to the assay medium. The reaction was conducted at 37°C for 30 min.

Molecular weight of III^{fru} The molecular weight of the native protein was determined by using Ultrogel AcA34 molecular exclusion chromatography at 4°C. A graphical estimation of the molecular weight gave a value of 51,000. The protein was shown to have a subunit molecular weight of 12,600 by SDS-urea-polyacrylamide gel electrophoresis.

Electrofocusing with III^{fru}. The preparation obtained after preparative electrophoresis gave only one band when subjected to IEF in a gradient ranging from pH 3 to 10. The isoelectric point of III^{fru} was ca. 3.7.

Effect of III^{fru} on the phosphorylation of other PTS sugars. The PEP-dependent phosphorylation of other PTS sugars was measured with membranes of fructose-grown cells of LG-1 X^r. The addition of III^{fru} to the reaction medium did not modify any activity but the fructose phosphotransferase activity (Table 5). This suggests that III^{fru} is specific for the PEP-dependent phosphorylation of fructose.

Identification of the reaction products. Extracts of fructose- and glucose-grown cells of LG-1 and LG-1 X^r were assayed for 1-PFK and 6-PFK activities. After growth on glucose, strains LG-1 and LG-1 X^r contained four to five times more 6-PFK than 1-PFK activity (Table 6). Growth of both strains at the expense of fructose resulted in a four- to fivefold increase of 1-PFK activity and a two- to threefold increase in 6-PFK activity. These findings provided the first indication that the two fructose PTS systems did not phosphorylate fructose on the same carbon. This was confirmed by analysis of the reaction products by paper chromatography. The product formed by membranes of lactose-grown cells of strain LG-1 (containing mainly the constitutive fructose PTS that only needed EI and HPr for catalytic activity) had the same migration as fructose 6-phosphate (Fig. 4A). Treatment of the reaction product, before chromatography, with commercial 6-PFK resulted in the formation of a substance that comigrated with fructose diphosphate (Fig. 4B). Analysis of the products obtained with membranes of fructose-grown cells of LG-1 X^r (containing the fructose PTS that needs III^{fru} for catalytic activity and residual activity of the other fructose PTS) showed one broad peak that corresponded to fructose 1-phosphate and a small peak that had the same migration as fructose 6-phosphate (Fig. 4C). Treatment of the mixture with 6-PFK did not affect the peak that comigrated with fructose 1-phosphate but resulted in a decrease of the peak of fructose 6-phosphate and the formation of fructose diphosphate (Fig. 4D).

Effect of the energy source on the level of EII^{fru} and III^{fru} activities. We have measured fructose phosphotransferase activity with membranes of strain LG-1 X^r after cell growth on various sugars. Activity was measured in the presence and absence of membrane-free cellular extract obtained from fructose-grown cells of strain LG-1 X^r (Table 7). It was found that the membranes of fructose- and sucrose-grown cells possessed similar levels of EII^{fru} activity. Glucose- and lactose-grown cells exhibited, respectively, 35 and 22% of the activity found in fructose-grown cells. In all cases, the phosphorylation of fructose was dependent on the presence of III^{fru}. Membranes of sorbitol- and mannitol-grown cells were much less active.

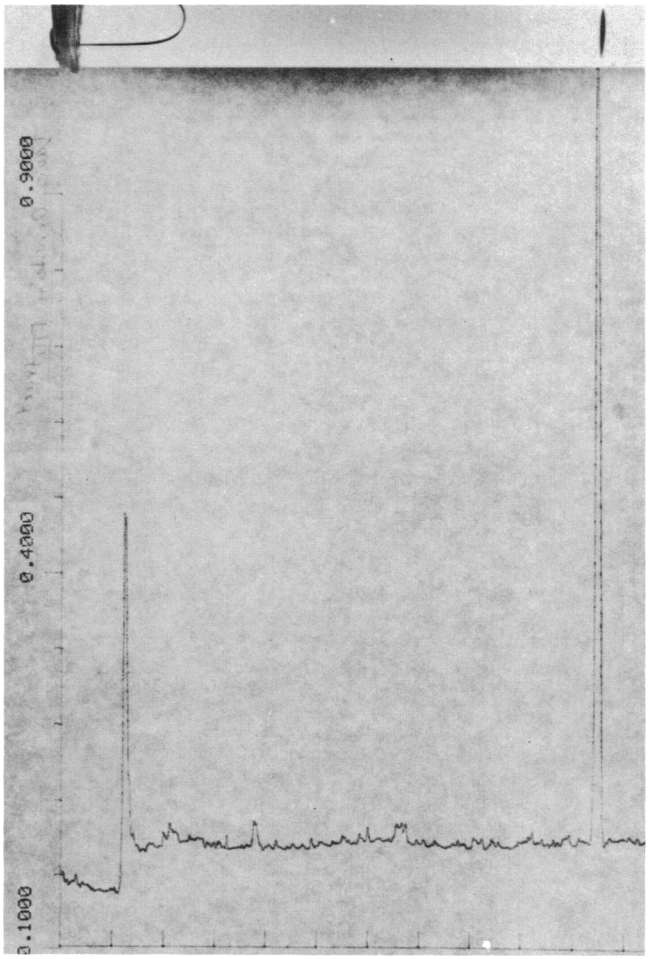

FIG. 2. Gel electrophoresis and electrophoretogram of purified III^{fru}. Electrophoresis was performed by the method of Davis (10) in 7.5% polyacrylamide gels. The gel was stained with Coomassie blue R250 and scanned at 595 nm with a Beckman DU-8 spectrophotometer.

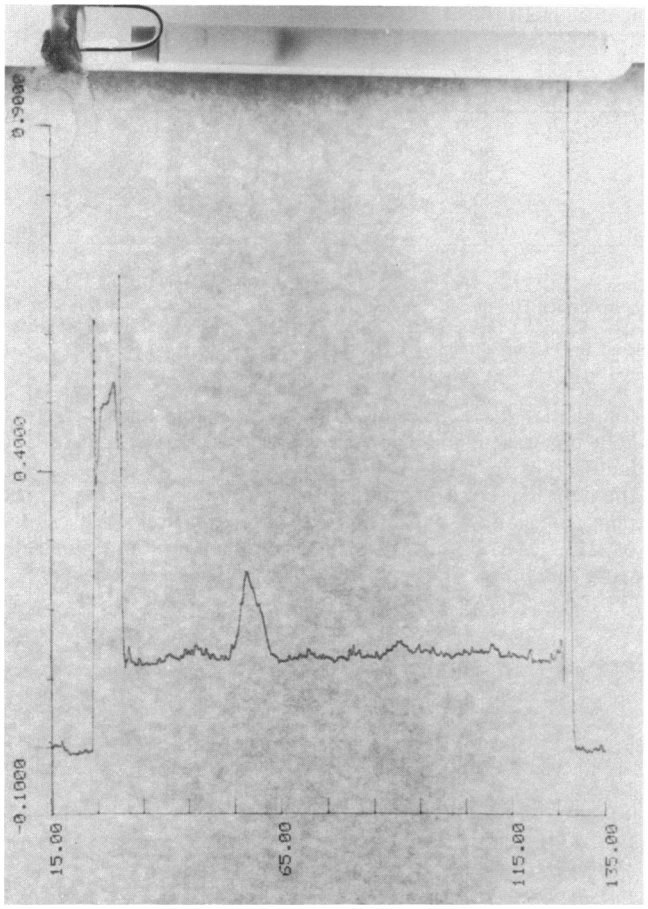

FIG. 3. SDS-polyacrylamide gel electrophoresis in the presence of urea and an electrophoretogram of purified III^{fru}. Protein sample (≈10 μg) was electrophoresed on a 12.8% SDS-polyacrylamide gel containing 8 M urea and was stained with Coomassie blue R250. The gel was scanned at 595 nm with a Beckman DU-8 spectrophotometer.

Similar complementation experiments were performed to detect the presence of III^{fru} in the cellular extract of cells grown on various sugars. In these experiments, membrane-free extracts obtained from fructose-, glucose-, lactose-, sucrose-, and sorbitol-grown cells of strain LG-1 X^r were mixed with membranes of fructose-grown cells of this strain. Results (Table 8) showed that cellular extracts derived from fructose-, sucrose-, and glucose-grown cells supported the PEP-dependent phosphorylation of fructose. Cellular extracts derived from lactose- and sorbitol-grown cells were much less active. All of these experiments were performed with extracts containing comparable protein concentrations. These findings were supported by immunodiffusion experiments in which extracts of strain LG-1 X^r grown on various sugars were tested against anti-*S. mutans* LG-1 X^r III^{fru} immunoglobulins. Extracts from fructose-, glucose-, and sucrose-grown cells produced fused precipitin bands. No reaction was observed with extracts of lactose- and sorbitol-grown cells (Fig. 5).

TABLE 4. Requirements for the fructose phosphotransferase activity in *S. mutans* LG-1 X[a]

Reaction medium	Sp act (nmol of fructose phosphate/min per mg of membrane protein)
Complete reaction medium[b]	46.8
Reaction medium minus:	
III^{fru}	5.5
HPr	3.2
EI	2.6
PEP	0
PEP plus 2 mM ATP	4.9

[a] Experiments were performed with membranes of fructose-grown cells.
[b] The complete reaction medium consisted of 1 mM [*U*-^{14}C]fructose (0.1 Ci/mol), 4 mM $MgCl_2$, 2 mM PEP, 2 mM 2-ME, 10 mM NaF, 200 U of EI, 38 μg of HPr, 1.7 μg of purified III^{fru}, and 25 μl of membrane (protein concentration, ≈1 mg/ml) in a total volume of 600 μl. The rate of the reaction was limited by the amount of III^{fru} in the reaction medium.

TABLE 5. Specificity of factor III^{fru}[a]

Carbohydrate challenged (1 mM)	Sp act (nmol/min per mg of membrane protein)[b]	
	−Factor III^{fru}	+Factor III^{fru}
Fructose	12	102
Glucose	163	172
Mannose	207	222
Sucrose	134	140

[a] Experiments were performed with membranes of fructose-grown cells of strain LG-1 X^r.
[b] The reaction medium was as described in footnote *b* of Table 3, except that we used 3.5 μg of III^{fru}.

Search for the presence of III^{fru} in other bacteria. We looked for the presence of antigenic determinants of III^{fru} by immunodiffusion experiments in a number of bacterial species. All strains of *S. mutans* tested (GS-5, NCTC 10449, Ingbritt, DR0001, and LG-1) contained a component that cross-reacted with anti-*S. mutans* LG-1 X^r III^{fru} immunoglobulins. The immunodiffusion pattern of these strains indicated the identity of their III^{fru} and the protein isolated from strain LG-1 X^r (results not shown). No cross-reaction was obtained with extracts from the following bacteria: *S. salivarius* ATCC 25975, *Streptococcus sanguis* ATCC 10556, *Streptococcus faecium* ATCC 8043, *Streptococcus lactis* ATCC e11454, *Staphylococcus aureus* ATCC e6538, *Bacillus subtilis* ATCC e6051, *Lactobacillus casei* ATCC 4646, and *Escherichia coli* K-12 ATCC 23282 (results not shown).

DISCUSSION

The transport of fructose by an inducible PTS has been described for a wide range of strict and facultative anaerobic bacteria (12). To our knowledge, however, the occurrence of two fructose PTS systems was reported only for *Aerobacter aerogenes* (50) and *E. coli* (16). In *E. coli*, a low affinity system specified by the *ptsX* gene yields fructose 6-phosphate which is further metabolized to fructose 1,6-diphosphate by 6-PFK. Another system specified by the *ptsF* gene

TABLE 6. Fructose phosphate kinase activities

Strain	Growth sugar	Sp act (μmol/min per mg of protein)	
		6-PFK	1-PFK
LG-1	Glucose	0.94	0.26
LG-1 X^r	Glucose	1.10	0.21
LG-1	Fructose	2.20	0.96
LG-1 X^r	Fructose	2.98	0.97

is inducible and gives rise to the formation of fructose 1-phosphate. The fructose 1-phosphate formed is converted to fructose 1,6-diphosphate through the action of 1-PFK. Results presented in this paper suggested that a similar situation prevails in *Streptococcus mutans*. The activity of one system was detected with membranes of glucose-grown cells of strain LG-1 in the presence of EI and HPr. The X^r mutation seemed to result in a defect in this constitutive fructose PTS since very little fructose PTS activity was detected with membranes of glucose-grown cells of mutant LG-1 X^r in the presence of EI and HPr. A second fructose PTS was, however, discovered in mutant LG-1 X^r. The activity of this system and of 1-PFK increased after growth of the cells at the expense of fructose, suggesting that fructose was phosphorylated at the C-1 position by this second fructose PTS. This was confirmed by paper chromatography. We have also demonstrated that this system required the presence of a new protein factor (III^{fru}) to be active. In many bacterial species, the occurrence of an inducible fructose PTS was reported together with the presence of a fructose-specific phosphocarrier protein called FPr that substitutes for HPr (30, 36, 50–52). III^{fru} isolated from *S. mutans* could not, however, substitute for HPr and is thus different from FPr. Our results also showed that III^{fru} was not an HPr^{fru} as found in *M. capricolum* (30). Finally, immunodiffusion experiments indicated that only cellular extracts from *S. mutans* strains cross-reacted with III^{fru} of LG-1 X^r. This suggests that if the other bacterial strains tested possess a III^{fru}, it is structurally quite different from the protein of *S. mutans*.

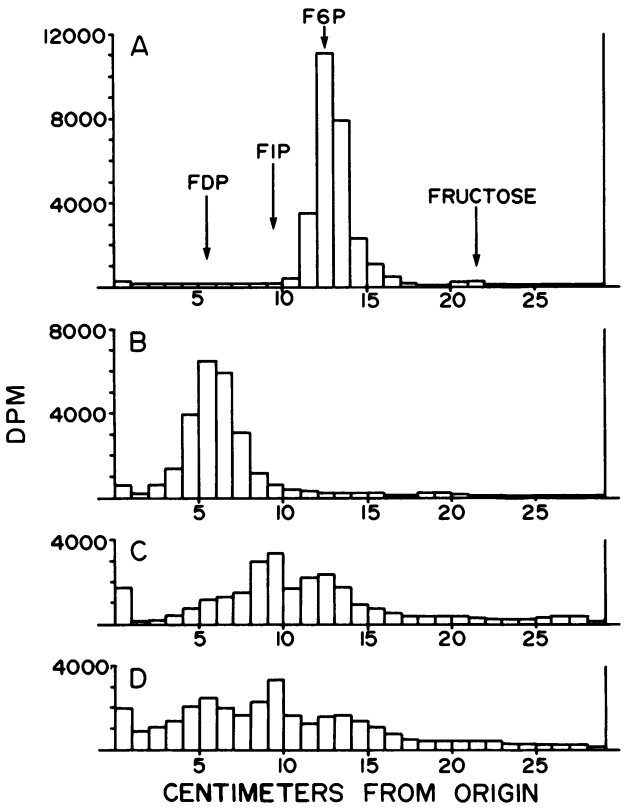

FIG. 4. Identification of the reaction products by paper chromatography. The PEP-dependent phosphorylation of [^{14}C]fructose was carried out in a mixture containing 1 mM [*U*-^{14}C]fructose (0.5 Ci/mol), 4 mM $MgCl_2$, 2 mM PEP, 2 mM 2-ME, and 10 mM NaF in a 5 mM triethanolamine buffer (pH 7.0). After 120 min of incubation at 37°C, the reaction was stopped by heating the mixtures at 100°C for 2 min. (A) Product obtained with membranes of lactose-grown cells of strain LG-1 in the presence of EI and HPr. (B) Same mixture as in (A) treated with 6-PFK before chromatography as described in the text. (C) Products obtained with membranes of fructose-grown cells of strain LG-1 X^r in the presence of EI, HPr, and membrane-free cellular extract of fructose-grown cells of LG-1 X^r (this cellular extract contained III^{fru}). (D) Same mixture as in (C) treated with 6-PFK before chromatography.

TABLE 7. Presence of EII^{fru} in membranes of LG-1 X^r grown on various sugars

Growth sugar	Activity (nmol of fructose phosphate per min per mg of membrane protein)[a]
Fructose	23.7
Sucrose	20.0
Glucose	8.5
Lactose	5.3
Sorbitol	2.9
Mannitol	2.5

[a] The reaction mixture contained 5 μl of membranes (6 mg of proteins per ml), 50 μl of membrane-free cellular extract (11 mg of protein per ml) of LG-1 X^r grown on fructose, a saturated amount of EI and HPr, 1 mM [*U*-^{14}C]fructose (0.1 Ci/mol), 2 mM PEP, 4 mM $MgCl_2$, 2 mM 2-ME, 10 mM NaF, and 50 mM sodium phosphate buffer (pH 7.0) in a total volume of 600 μl.

The molecular structure of III^{fru} isolated from *S. mutans* is very similar to the structure of the enzyme III specific for gluconate and isolated from *S. faecalis* (1). This protein has a molecular weight of 50,000 and subunits with molecular weights of 12,000. This is very similar to the molecular weight of III^{fru} described here (51,000 with subunits of 12,600). The other enzymes III so far isolated are structurally different. III^{lac}, isolated from *Staphylococcus aureus*, has a molecular weight of 35,000 and consists of three identical subunits (23), whereas other enzymes III each consist of a single polypeptide chain (26, 29).

We have detected the presence of III^{fru} and the corresponding EII^{fru} mainly in cells of strains LG-1 and LG-1 X^r grown on fructose, glucose, and sucrose. Although the presence of these proteins in glucose-grown cells was unex-

TABLE 8. Presence of factor III^{fru} in cellular extracts of LG-1 X^r grown on various sugars

Growth sugar	Sp act (nmol of fructose phosphate formed per min per mg of protein in the cellular extract)
Fructose	13.7
Glucose	5.7
Sucrose	4.5
Lactose	1.8
Sorbitol	0.4

[a] The reaction mixture contained 25 μl of membranes (4 mg of proteins per ml) from fructose-grown cells of LG-1 X^r, 50 μl of membrane-free cellular extract (≈10 mg of protein per ml) of LG-1 X^r grown on the indicated sugar, a saturated amount of EI and HPr, 1 mM [*U*-^{14}C]fructose (0.1 Ci/mol), 2 mM PEP, 4 mM $MgCl_2$, 2 mM 2-ME, 10 mM NaF, and 50 mM sodium phosphate buffer (pH 7.0) in a total volume of 600 μl.

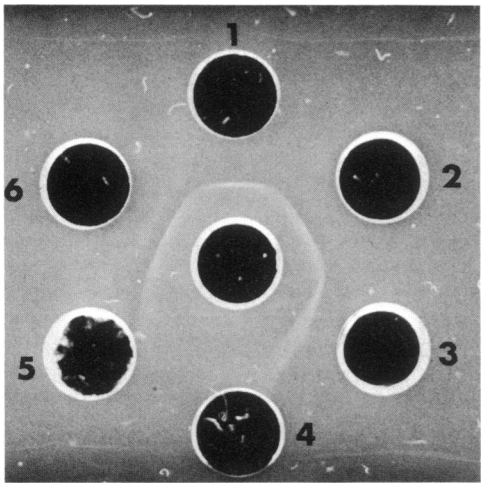

FIG. 5. Double-diffusion immunoprecipitation. Cells of strain LG-1 X^r were grown in 1 liter of TYE medium at the expense of various sugars. A crude extract was obtained by alumina grinding as described in the text. After centrifugation at 200,000 × *g* for 4 h, the extract was concentrated to 1 ml by ultrafiltration with an Amicon cell with a YM5 membrane. Each well received 10 μl of solution. Precipitation was performed for 24 h. Wells: 1, purified III^{fru}; 2, extract of fructose-grown cells; 3, extract of glucose-grown cells; 4, extract of lactose-grown cells; 5, extract of sorbitol-grown cells; 6, extract of sucrose-grown cells; center, anti-*S. mutans* III free immunoglobulins.

pected, the induction of this factor during growth of the cells on sucrose was not surprising. Indeed, it is known that *Streptococcus mutans* possesses extracellular enzymes that hydrolyze sucrose (7, 36). With nongrowing *S. mutans* cells, Tanzer et al. (43) have shown that 18% of the glucosyl portion of sucrose is polymerized into dextran. Similar results were obtained with growing cells (37). Thus, a sufficient amount of free extracellular fructose might arise from the hydrolysis of sucrose by the glucosyltransferase to induce III^{fru} and EII^{fru}.

The occurrence of multiple transport systems for a specific carbohydrate in oral streptococci is not unprecedented. Sucrose is transported by two PTS systems (40) and by a non-PTS transport system (14) in *S. mutans*. The transport of lactose in *Streptococcus salivarius* is ensured by a PTS and a non-PTS transport system (20). The existence of two glucose PTS systems was demonstrated in *S. salivarius* (46), *S. sanguis* (48), and *S. mutans* (47). *S. mutans* also possesses an active transport system for glucose which is energized by the proton motive force (21). Results presented in this paper revealed the presence of two fructose PTS systems in *S. mutans*. However, one may also assume that a non-PTS fructose transport system is present in this bacterium. The following results support this hypothesis: (i) it has been shown that under specific growth conditions, fructose phosphotransferase activity is repressed in *S. mutans* (14), and (ii) *S. mutans* possesses a fructokinase that allows the dissimilation of intracellular fructose (32).

The nature and the amount of carbohydrates available in the oral cavity fluctuate rapidly. This phenomenon of transient energy supply amounts to an ecological pressure resulting in the selection of certain microorganisms in the mouth. In this regard, the fact that oral streptococci possess multiple transport systems with a wide range of affinities for sugar uptake undoubtedly promotes their survival in dental plaque.

ACKNOWLEDGMENTS

We thank Noëlla Deslauriers, Marc Lavoie, Christian Mouton, and Luc Trahan for their critical review of the manuscript. We also thank Louis Turgeon for preparing the illustrations and Céline Bélanger for typing the manuscript.

This work was supported by grant MT 6979 from the Conseil de recherches médicales du Canada to Christian Vadeboncoeur, who is a scholar of the Fonds de la recherche en santé du Québec.

LITERATURE CITED

1. **Bernsmann, P., C. A. Alpert, P. Muss, J. Deutscher, and W. Hengstenberg.** 1982. A bacterial PEP-dependent phosphotransferase system: mechanism of gluconate phosphorylation in *Streptococcus faecalis*. FEBS Lett. **138**:101–103.
2. **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.
3. **Brown, A. T.** 1975. The role of dietary carbohydrates in plaque formation and oral disease. Nutr. Rev. **33**:353–361.
4. **Brudevold, F., A. Tehrani, F. Attarzadeh, J. van Houte, and J. Russo.** 1983. Enamel demineralization potential of dietary carbohydrates. J. Dent. Res. **62**:1218–1220.
5. **Calendar, R., and P. Berg.** 1966. Purification and physical characterization of tyrosyl ribonucleic acid synthetase from *Escherichia coli* and *Bacillus subtilis*. Biochemistry **5**:1681–1690.
6. **Calmes, R.** 1978. Involvement of phosphoenolpyruvate in the catabolism of caries-conducive disaccharides by *Streptococcus mutans*: lactose transport. Infect. Immun. **19**:934–942.
7. **Chassy, B. M., R. M. Bielanski, J. R. Beall, E. V. Porter, M. I. Krichevsky, and J. A. Donkersloot.** 1974. Extracellular invertase in *Streptococcus mutans* and *Streptococcus salivarius*. Life Sci. **15**:1173–1180.
8. **Colman, G., W. H. Bowen, and M. F. Cole.** 1977. The effects of sucrose, fructose, and a mixture of glucose and fructose on the incidence of dental caries in monkeys. Br. Dent. J. **142**:217–221.
9. **Conrad, R., and H. G. Schlegel.** 1977. Different degradation pathways for glucose and fructose in *Rhodopseudomonas capsulata*. Arch. Microbiol. **112**:39–48.
10. **Davis, B. J.** 1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
11. **Deutscher, J., and M. H. Saier, Jr.** 1983. ATP-dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphate carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. U.S.A. **80**:6790–6794.
12. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**:385–418.
13. **Dills, S. S., and S. Seno.** 1983. Regulation of hexitol catabolism in *Streptococcus mutans*. J. Bacteriol. **153**:861–866.
14. **Ellwood, D. C., and I. R. Hamilton.** 1982. Properties of *Streptococcus mutans* Ingbritt growing on limiting sucrose in a chemostat: repression of the phosphoenolpyruvate phosphotransferase transport system. Infect. Immun. **36**:576–581.
15. **Facklam, R. R.** 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. **5**:184–201.
16. **Ferenci, T., and H. L. Kornberg.** 1974. The role of phosphotransferase-mediated synthesis of fructose-1-phosphate and fructose-6-phosphate in the growth of *Escherichia coli* on fructose. Proc. R. Soc. London **187**:105–119.
17. **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.
18. **Gauthier, L., C. Vadeboncoeur, and D. Mayrand.** 1984. Loss of sensitivity to xylitol by *Streptococcus mutans* LG-1. Caries Res. **18**:289–295.
19. **Gibbons, R. J., and J. van Houte.** 1975. Dental caries. Annu. Rev. Med. **26**:121–136.
20. **Hamilton, I. R., and G. C. Y. Lo.** 1978. Co-induction of β-

galactosidase and the lactose-P-enolpyruvate phosphotransferase system in *Streptococcus salivarius* and *Streptococcus mutans*. J. Bacteriol. **136**:900–908.
21. **Hamilton, I. R., and E. J. St. Martin.** 1982. Evidence for the involvement of proton motive force in the transport of glucose by a mutant of *Streptococcus mutans* strain DR0001 defective in glucose-phosphoenolpyruvate phosphotransferase activity. Infect. Immun. **36**:567–575.
22. **Hanes, C. S., and F. A. Isherwood.** 1949. Separation of phosphonic esters on the filter paper chromatogram. Nature (London) **164**:1107–1112.
23. **Hays, J. B., R. D. Simoni, and S. Roseman.** 1973. Sugar transport. V. A trimeric lactose specific phosphocarrier protein of the *Staphylococcus aureus* phosphotransferase system. J. Biol. Chem. **248**:941–956.
24. **Koulourides, T., R. Bodolen, S. Keller, L. Mansson-Hing, J. Lastra, and T. Housch.** 1976. Cariogenicity of nine sugars tested with an intraoral device in man. Caries Res. **10**:427–441.
25. **Laurent, T. C., and J. A. Killander.** 1964. A theory of gel filtration and its experimental verification. J. Chromatogr. **14**:317–330.
26. **London, J., and S. Z. Hausman.** 1983. Purification and characterization of the III^{Xtl} phospho-carrier protein of the phosphoenolpyruvate-dependent xylitol:phosphotransferase found in *Lactobacillus casei* C183. J. Bacteriol. **156**:611–619.
27. **Lowry, O. H., N. H. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
28. **Maryanski, J. H., and C. L. Wittenberger.** 1975. Mannitol transport in *Streptococcus mutans*. J. Bacteriol. **124**:1475–1481.
29. **Meadow, N. D., and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Isolation and characterization of glucose-specific phosphocarrier protein (III^{Glu}) from *Salmonella typhimurium*. J. Biol. Chem. **257**:14526–14537.
30. **Mugharbil, U., and V. P. Cirillo.** 1978. Mycoplasma phosphoenolpyruvate-dependent sugar phosphotransferase system: glucose-negative mutant and regulation of intracellular cyclic AMP. J. Bacteriol. **133**:203–209.
31. **Orr, M. D., R. L. Blakley, and D. Panagou.** 1972. Discontinuous buffer systems for analytical and preparative electrophoresis of enzymes on polyacrylamide gel. Anal. Biochem. **45**:59–85.
32. **Porter, E. V., B. M. Chassy, and C. E. Holmlund.** 1980. Partial purification and properties of a mannofructokinase from *Streptococcus mutans* SL-1. Infect. Immun. **30**:43–50.
33. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate: sugar phosphotransferase system. Biochim. Biophys. Acta **457**:213–257.
34. **Reisner, A. H., P. Nemes, and C. Bucholtz.** 1975. The use of Coomassie blue G250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. Anal. Biochem. **64**:509–516.
35. **Righetti, P., and J. W. Drysdale.** 1971. Isoelectric focusing in polyacrylamide gels. Biochim. Biophys. Acta **236**:17–28.
36. **Saier, M. H., Jr., B. U. Feucht, and S. Roseman.** 1971. Phosphoenolpyruvate-dependent fructose phosphorylation in photosynthetic bacteria. J. Biol. Chem. **246**:7819–7821.
37. **Schachtele, C. F., A. E. Loken, and D. J. Knudson.** 1972. Preferential utilization of the glucosyl moiety of sucrose by a cariogenic strain of *Streptococcus mutans*. Infect. Immun. **5**:531–536.
38. **Schachtele, C. F., and J. A. Mayo.** 1973. Phosphoenolpyruvate-dependent glucose transport in oral streptococci. J. Dent. Res. **52**:1209–1215.
39. **Scheinin, A., K. K. Makinen, and K. Ylitalo.** 1976. Turku sugar studies. V. Final report on effect of sucrose, fructose, and xylitol diets on caries incidence in man. Acta Odontol. Scand. **34**:179–216.
40. **Slee, A. M., and J. M. Tanzer.** 1982. Sucrose transport by *Streptococcus mutans*. Evidence for multiple transport systems. Biochim. Biophys. Acta **692**:415–424.
41. **St. Martin, E. J., and C. L. Wittenberger.** 1979. Characterization of a phosphoenolpyruvate-dependent sucrose phosphotransferase system in *Streptococcus mutans*. Infect. Immun. **24**:865–868.
42. **Swank, R. T., and K. D. Munkres.** 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. Anal. Biochem. **39**:462–477.
43. **Tanzer, J. M., B. M. Chassy, and M. I. Krichevsky.** 1972. Sucrose metabolism by *Streptococcus mutans*, SL-1. Biochim. Biophys. Acta **261**:379–387.
44. **Vadeboncoeur, C.** 1984. Structure and properties of the phosphoenolpyruvate: glucose phosphotransferase system of oral streptococci. Can. J. Microbiol. **30**:495–502.
45. **Vadeboncoeur, C., M. Proulx, and L. Trahan.** 1983. Purification of HPr and enzyme I like proteins from the oral bacterium *Streptococcus salivarius*. Biochemical and immunochemical properties. Can. J. Microbiol. **29**:1694–1705.
46. **Vadeboncoeur, C., and L. Trahan.** 1982. Glucose transport in *Streptococcus salivarius*. Evidence for the presence of a distinct phosphoenolpyruvate: glucose phosphotransferase system which catalyses the phosphorylation of α-methylglucoside. Can. J. Microbiol. **28**:190–199.
47. **Vadeboncoeur, C., and L. Trahan.** 1983. Comparative study of *Streptococcus mutans* laboratory strains and fresh isolates from carious and caries-free tooth surfaces and from subjects with hereditary fructose intolerance. Infect. Immun. **40**:81–90.
48. **Vadeboncoeur, C., and L. Trahan.** 1983. Phosphoenolpyruvate-dependent phosphorylation of α-methylglucoside in *Streptococcus sanguis* ATCC 10556. Can. J. Microbiol. **29**:833–836.
49. **Van Houte, J.** 1980. Bacterial specificity in the etiology of dental caries. Int. Dent. J. **30**:305–326.
50. **Walter, R. W., and R. L. Anderson.** 1973. Evidence that the inducible phosphoenolpyruvate: D fructose 1-phosphotransferase system of *Aerobacter aerogenes* does not require "HPr". Biochem. Biophys. Res. Commun. **52**:93–97.
51. **Waygood, E. G.** 1980. Resolution of the phosphoenolpyruvate: fructose phosphotransferase system of *Escherichia coli* into two components: enzyme $II^{fructose}$ and fructose-induced HPr-like protein (FPr). Can. J. Biochem. **58**:1144–1146.
52. **Waygood, E. B., N. Meadow, and S. Roseman.** 1979. Modified assay procedures for the phosphotransferase system in enteric bacteria. Anal. Biochem. **95**:293–304.