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# Lactose Transport in *Streptococcus mutans*: Isolation and Characterization of Factor III$^{lac}$, a Specific Protein Component of the Phosphoenolpyruvate-Lactose Phosphotransferase System

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**The transport of lactose in *Streptococcus mutans* is mediated via an inducible phosphoenolpyruvate-lactose phosphotransferase system. This system requires for catalytic activity a membrane fraction (enzyme II), two general proteins called enzyme I and HPr, and a soluble specific protein termed factor III$^{lac}$. This protein factor was purified from *S. mutans* ATCC 27352 by chromatographies on DEAE-cellulose, hydroxylapatite, Ultrogel AcA 34, and phosphocellulose. The purified protein migrated as a single band with a molecular weight of 10,000 on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and urea. The molecular weight calculated from the amino acid composition was 10,541. Gel filtration of the native protein gave a molecular weight of 41,500. Its isoelectric point was ca. 4.70. A specific antiserum was prepared against purified factor III$^{lac}$. Immunodiffusion experiments revealed that only cellular extracts from lactose-grown cells contained factor III$^{lac}$. A cross-reaction was observed with all of the *S. mutans* strains tested as well as with *Streptococcus sanguis* 10556, *Streptococcus lactis* 11454, and *Staphylococcus aureus* 6538. No precipitin band was observed with extracts of *Streptococcus salivarius*, *Streptococcus faecalis*, *Lactobacillus casei*, and *Bacillus subtilis*.**

The transmembrane transport of dietary carbohydrates from the oral cavity to the cytoplasm of acidogenic oral bacteria is a prerequisite for the production of organic acids that cause carious lesions (9). The transport of sugars in these bacteria is ensured, in many instances, by the phosphoenolpyruvate-sugar phosphotransferase system (PTS) which catalyzes concomitantly the transport and phosphorylation of sugars at the expense of phosphoenolpyruvate (PEP) (23). The molecular components of the PTS were identified mainly from studies performed with enteric bacteria and *Staphylococcus aureus* (11). In these microorganisms, the system is made of several catalytic proteins. Two are nonspecific and found mainly in the cytoplasm of the cell. They are enzyme I (EI) and the phosphocarrier protein HPr. The other components of the PTS are sugar specific. These are the membrane-bound enzyme II (EII) complex and the enzymes III (or factors III) that might be soluble or membrane bound.

In addition to its role in the transport of carbohydrates, the PTS of enteric bacteria is also involved in many regulatory functions (23). Recently, some studies have reported the control of lactose utilization by the glucose PTS in oral streptococci (15, 30, 31), indicating a regulatory role for the glucose PTS in these bacteria. However, enlightenments on the mechanisms involved in this control await further investigations on the molecular components of the glucose and the lactose PTS (lac PTS) in those bacteria. We have already demonstrated the presence of EI and HPr in oral streptococci (32). In this report, we establish the presence of a specific factor III$^{lac}$ in *Streptococcus mutans*. The procedure used for the purification of this factor from *S. mutans* 27352 to electrophoretic homogeneity is described. The protein was characterized by its molecular weight, isoelectric point, amino acids composition, and immunological properties.

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## MATERIALS AND METHODS

**Chemicals.** Trypticase was purchased from BBL Microbiology Systems, and yeast extract was purchased from Difco Laboratories. Ultrogel AcA 34 and AcA 44 were obtained from LKB Instruments, Inc.; DEAE-cellulose (DE-52) and phosphocellulose P11 were obtained from Whatman. Hydroxylapatite (Biogel HT), sodium dodecyl sulfate (SDS), and urea were purchased from Bio-Rad Laboratories. [1-$^{14}$C-glucose]lactose was from Amersham Corp. All other biochemicals and substrates were from Sigma Chemical Co.

**Organisms and growth conditions.** The following strains were used in this study: *S. mutans* 27352 (ATCC); *S. mutans* GS5-2, NCTC 10449, OZM 175 (R. Linzer); *S. mutans* DR0001; *Streptococcus salivarius* ATCC 25975 (I. R. Hamilton); *Streptococcus sanguis* ATCC 10556 (K. Komiyama); *Lactobacillus casei* ATCC 4646; *Escherichia coli* K12 58-161; *Streptococcus faecalis* ATCC 8043; *Streptococcus lactis* ATCC 11454; *Staphylococcus aureus* ATCC 6538; and *Bacillus subtilis* ATCC 6051. Cells were cultured at 37°C in a complex medium containing the following in 1 litre: 17 g of Trypticase, 3 g of yeast extract, 5 g of NaCl, 2.5 g of disodium phosphate, and 5 g of lactose. The sugar was sterilized by filtration (Millipore filter, 0.22 μm; Millex-GS) and added aseptically to the medium. To obtain large quantities of cells, cultures were grown with gentle stirring in six 20-liter pyrex bottles each containing 12 liters of medium. Each was inoculated with six 250-ml overnight cultures. Cells were harvested at the end of logarithmic growth by continuous flow centrifugation in a Beckman model J2-21 centrifuge with a rotor model JCF-Z. Seventy-two liters of culture gave ca. 130 g of wet cell paste that was washed twice with 1 liter of 10 mM potassium phosphate (pH 7.5) and kept at 5°C overnight or frozen at −20°C.

**Assay of the PEP-lac PTS activity.** Lac PTS activity was determined by two methods: measuring (i) the rate of

formation of [$^{14}$C]lactose phosphate and (ii), the release of *o*-nitrophenol from *o*-nitrophenyl-β-D-galactopyranoside (ONPG) phosphate in the presence of phospo-β-galactosidase. (i) The following reaction mixture (600 μl) was incubated at 37°C: 4 mM $MgCl_2$, 2 mM PEP, 10 mM NaF, 25 μl of EII lactose-containing membranes, ca. 1 U of EI, 10 μg of HPr, and a limiting amount of factor III$^{lac}$ in 50 mM sodium phosphate buffer (pH 7.0). The reaction was initiated by the addition of [$^{14}$C]lactose to a final concentration of 1 mM (0.05 to 0.1 μCi/μmol). After 30 min, the [$^{14}$C]lactose phosphate was separated by precipitation with 10 volumes of 0.03 M $BaBr_2$ in 90% ethanol (vol/vol) as previously described (32). (ii) The reaction mixture (500 μl) was similar to the one described by Calmes (2), except that partially or completely purified components of the PTS were added to the medium. It consisted of the following: 20 mM ONPG, 1 mM PEP, 1 mM $MgCl_2$, 10 mM NaF, an excess amount of EII lactose-containing membranes, EI, HPr, and partially purified 6-phospho-β-galactosidase from *S. mutans* ATCC 27352 (3), and a limiting amount of factor III$^{lac}$ in 20 mM potassium phosphate (pH 7.0). After an incubation of 30 to 60 min at 37°C, the reaction was stopped by the addition of 50 μl of cold 1 M $Na_2CO_3$. The solution was then centrifuged at 15,000 × *g* for 10 min at 4°C. The absorbance of the supernatant was determined at 420 nm in a Beckman DU-8 spectophotometer.

**Partial purification of EI and HPr.** EI and HPr were partially purified from glucose-grown cells of *S. salivarius* ATCC 25975 by the procedure of Vadeboncoeur et al. (32). We have shown in this laboratory that PTS proteins from *S. salivarius* and *S. mutans* exhibited cross-reactivity (unpublished data) and immunochemical similarities (32). Because our preliminary results have shown that *S. salivarius* did not possess a soluble factor III$^{lac}$, we decided to use partially purified EI of this strain to facilitate the detection of factor III$^{lac}$ isolated from *S. mutans*. The preparation of EI used in this study was obtained after successive chromatographies on DEAE-cellulose and hydroxylapatite and showed several bands when subjected to acrylamide gel electrophoresis. It was, however, free of EII, HPr, and factor III$^{lac}$.

**Preparation of cell-free extract.** Cells were ground with levigated alumina (3 g of alumina per 1 g of wet cell paste) for 20 min in a refrigerated mortar (−40°C). After this period, 2 ml of 10 mM potassium phosphate (pH 7.5) containing 14 mM 2-mercaptoethanol (2-ME)–1 mM EDTA for each 1 g of cells was added. This suspension was then sonicated with a sonicator model W350 (Heat System Ultrasonic, Inc.) at maximum power for 3× 2 min. Alumina was removed by a centrifugation of 3,000 × *g* for 5 min, and cell debris and cells were sedimented at 10,000 × *g* for 20 min. The resulting supernatant was designated as the crude extract (fraction I; Table 1).

**Isolation of EII lactose-containing membranes.** The crude extract obtained from lactose-grown cells of *S. mutans* 27352 was sedimented at 200,000 × *g* for 4 h. The supernatant of this high-speed centrifugation was used further for the purification of factor III$^{lac}$. The 200,000 × *g* pellet was washed three times with 50 ml of potassium phosphate (pH 7.5) containing 14 mM 2-ME–1 mM EDTA–0.5 M KCl. The membranes were finally suspended in the same buffer without KCl at a protein concentration of 20 mg/ml and stored at −20°C.

**Purification of factor III$^{lac}$.** All purification procedures were carried out at 4°C, and unless otherwise mentioned, all buffers contained 14 mM 2-ME–0.1 mM EDTA. The presence of factor III$^{lac}$ in column fractions was detected by measuring the rate of phosphorylation of [$^{14}$C]lactose in the presence of partially purified EI and HPr from *S. salivarius* and membrane fragments (EII) isolated from lactose-grown cells of *S. mutans* 27352. The supernatant of the first centrifugation of 200,000 × *g* was loaded on a DEAE-cellulose column (5 by 25 cm) previously equilibrated with 10 mM potassium phosphate (pH 7.5)–1 mM EDTA. The column was washed first with buffer and then with 1 liter of 0.1 M KCl. It was then eluted with a 3-liter gradient of 0.1 to 0.5 M KCl. Phospho-β-galactosidase activity was recovered in fractions 140 to 160 (Fig. 1) and was used without further purification in this study. This fraction was devoid of HPr, EI, and factor III$^{lac}$ activity. Factor III$^{lac}$ was recovered at 0.30 M KCl. Those fractions containing factor III$^{lac}$ were pooled (Fig. 1) and dialyzed against 10 mM potassium phosphate (pH 6.8). The dialyzed sample was layered on a column of hydroxylapatite (2.6 by 11 cm; mixed with 15% [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 100 ml of equilibration buffer and with a linear gradient (1 liter) of 10 to 100 mM potassium phosphate at a rate of 50 ml/h, and 5-ml fractions were collected. Those fractions containing factor III$^{lac}$ (Fig. 1) were concentrated by ultrafiltration with an Amicon cell with a YM5 membrane to a volume of 5 ml and then dialyzed against 10 mM potassium phosphate (pH 7.5) containing 0.1 M KCl. The concentrated sample was loaded onto a column of Ultrogel AcA 34 (2.6 by 95 cm; LKB) that had been equilibrated with 10 mM potassium phosphate (pH 7.5)–0.1 M KCl. The column was eluted with the same buffer at a rate of 30 ml/h, and 3-ml fractions were collected. At this stage of purification, factor III$^{lac}$ was more than 99% pure. The preparation was ultimately purified by chromatography on phosphocellulose in 10 mM potassium phosphate (pH 6.0). Under these conditions, factor III$^{lac}$ was collected in the run-through of the column. The active fractions were pooled, concentrated by ultrafiltration, and kept frozen at −20°C in the presence of 15% glycerol.

**Electrophoresis procedure.** Purified factor III$^{lac}$ was ana-

TABLE 1. Purification of factor III$^{lac}$ [a]

| Fraction | Total protein (mg) | Total units (U)[b] | Sp act (U/mg) | Yield (%) | Purification |
|---|---|---|---|---|---|
| Crude extract | 3,025 | 178 | 0.59 | 100 | 1 |
| Ultracentrifugation | 1,512 | 144 | 0.95 | 81.0 | 1.6 |
| DEAE-cellulose | 267 | 101 | 3.77 | 56.6 | 6.4 |
| Hydroxylapatite | 22.4 | 45 | 20.1 | 25.3 | 34 |
| Ultrogel AcA 34 | 7.2 | 42 | 58.3 | 23.6 | 99 |

[a] From 130 g of cells (wet weight).

[b] One unit of factor III$^{lac}$ is the amount of protein required to allow the phosphorylation of 1 nmol of ONPG per min at 37°C.

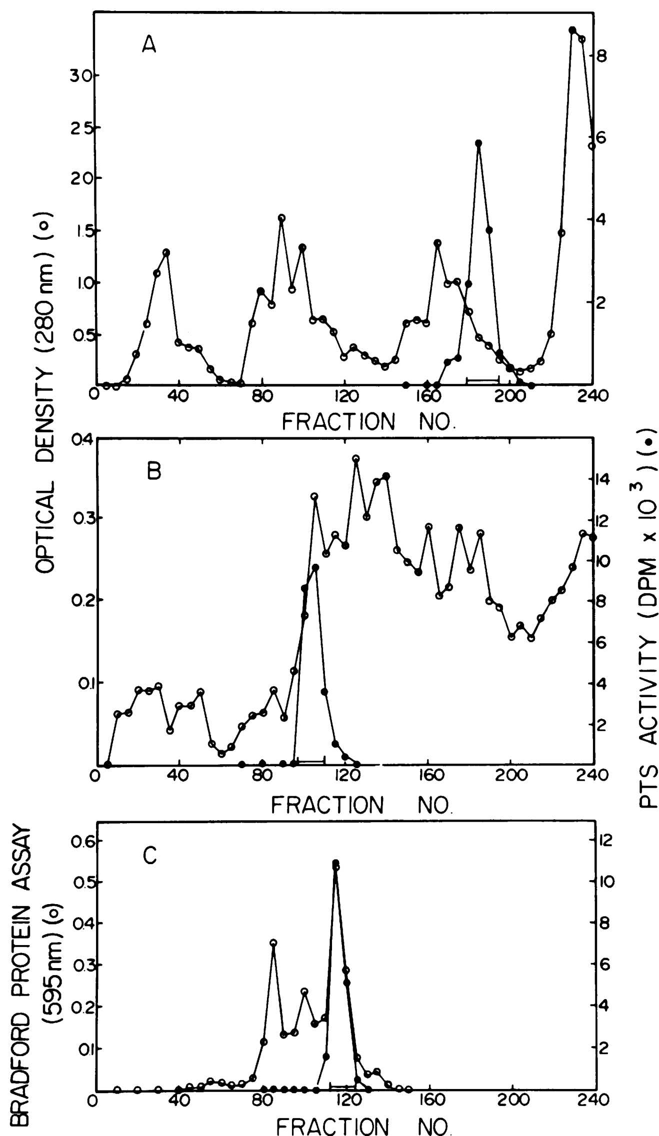



FIG. 1. Various steps of the purification of factor $III^{lac}$ from *S. mutans* 27352. (A) Chromatography on DEAE-cellulose. (B) Chromatography on hydroxylapatite. (C) Chromatography on Ultrogel AcA 34. The presence of factor $III^{lac}$ in column fractions was detected by measuring the PEP-dependent phosphorylation of lactose in the presence of EI, HPr, and EII lactose-containing membranes. Arrows indicate fractions that were pooled. See the text for more details.

lyzed by polyacrylamide gel electrophoresis by the method of Davis (5) and Laemli (13). Isoelectric focusing was carried out in gels by the method of Righetti and Drysdale (26) as described previously (32).

**Molecular weight estimation of factor $III^{lac}$.** Factor $III^{lac}$ was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of urea as described by Swank and Munkres (28). Partial cyanogen bromide cleavage products of sperm whale myoglobin from Pharmacia Fine Chemicals were used as molecular weight markers. Proteins were incubated 2 h at 37°C in the presence of 3% SDS–5% 2-ME–8 M urea in 0.01 M $H_3PO_4$ adjusted to pH 6.8 with Tris. The gels were stained with Coomassie brilliant blue R250. After destaining, the gels were scanned at 595 nm with a DU-8 Beckman spectrophotometer. The molecular weight of the native protein was estimated by gel filtration at 4°C on a Ultrogel AcA 44 column (2.5 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: ribonuclease A, chymotrypsinogen A, ovalbumin, and bovine serum albumin. The void volume was estimated with blue dextran. Molecular weight of factor $III^{lac}$ was determined as described by Laurent and Killander (14).

**Amino acid analysis.** Amino acids composition of purified factor $III^{lac}$ was obtained after hydrolysis at 110°C for 24 h in the presence of 3 N mercaptoethanesulfonic acid (22) or 6 N HCl. Lysosyme was run as the standard.

**Production of antibodies.** The production of antibodies and the purification of immunoglobulins G (IgGs) were conducted as described for the production of anti-*S. salivarius* EI IgGs (32).

**Immunochemical procedures.** Double-diffusion immunoprecipitation, crossed immunoelectrophoresis, and neutralization of the PEP-dependent phosphorylation of ONPG were conducted as described previously (32).

**Protein determination.** Proteins were measured either by the method of Bradford (1) or Lowry et al. (16). Protein determination of membrane preparations was carried out by the method of Lowry after a preincubation of the samples with 1 N NaOH (10 min, 37°C).

**Definition of specific activity.** One unit of EI catalyzes the formation of 1 μmole of phospho-HPr per min at 37°C. One unit of factor $III^{lac}$ is the amount of protein required to allow the phosphorylation of 1 nmol of ONPG per min at 37°C.

## RESULTS

**Preliminary evidence for the presence of factor $III^{lac}$ in *S. mutans*.** All measurements of lac PTS activity performed so far with *S. mutans* were realized with toluenized cells (2, 10). The use of such permeable cells does not, however, indicate how many components are required for the PEP-dependent phosphorylation of lactose or ONPG by the lac PTS. To clarify this point, we tried to measure the PEP-dependent phosphorylation of ONPG with isolated membranes of lactose-grown cells of *S. mutans* 27352 in the presence of partially purified EI, HPr, and phospho-β-galactosidase. We observed that this reaction medium did not allow any phosphorylation of ONPG. However, the addition of membrane-free cellular extract of lactose grown-cells resulted in the appearance of a yellowish color resulting from the hydrolysis of phospho-ONPG by phospho-β-galactosidase. This effect was not observed upon the addition to the reaction medium of extract from glucose- or fructose-grown cells. These results suggested that the lac PTS of *S. mutans* required, in addition to EI and HPr, a specific soluble component for catalytic activity.

**Purification of factor $^{lac}$ from *S. mutans* 27352.** The procedure for the purification of factor $III^{lac}$ from *S. mutans* 27352 is summarized in Table 1. The elution pattern of the various chromatographic steps is shown in Fig. 1. The preparation obtained after chromatography on Ultrogel AcA 34 was more than 99% pure as revealed by SDS-polyacrylamide gel electrophoresis. The preparation was purified to electrophoretic homogeneity (Fig. 2) by chromatography on phosphocellulose as described above. This step resulted in a significant loss of activity (ca. 50%). However, the purified protein could be thereafter stored at −20°C in the presence of 15% glycerol for several months without detectable loss of activity.

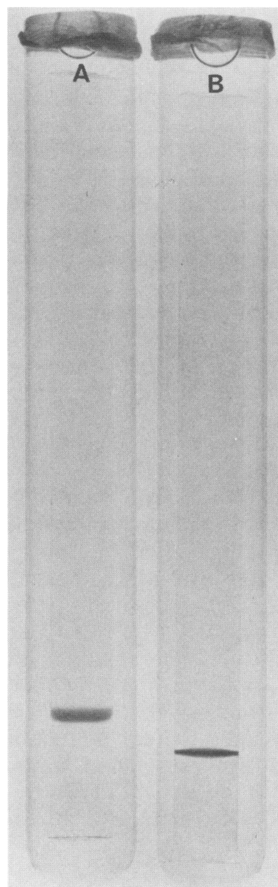



FIG. 2. Analytical electrophoresis on polyacrylamide gel. Samples of the fraction obtained after phosphocellulose chromatography (ca. 10 μg) were analyzed by (A) SDS gel electrophoresis as described by Laemli (13) and (B) discontinuous gel electrophoresis as described by Davis (5).

**Biochemical properties of factor III$^{lac}$.** The homogeneity of factor III$^{lac}$ was confirmed by polyacrylamide gel electrophoresis as described by Davis (Fig. 2), by SDS gel electrophoresis (Fig. 2), and by cross immunoelectrophoresis (Fig. 3). The purified factor III$^{lac}$ also migrated as a single band when subjected to polyacrylamide gel electrophoresis in the presence of SDS and urea, giving a molecular weight of 10,000. Molecular sieving on a calibrated column of AcA 44 gave a molecular weight of 41,500. This suggested that factor III$^{lac}$ of *S. mutans* consists of four identical polypeptides chain. Isoelectric focusing in gel of the sample that was pure according to the criteria mentioned above showed two bands very close to each other with a pI of 4.70 and 4.75. This was presumably caused by the low solubility of factor III near its isoelectric point. Indeed, we have observed that an overnight dialysis against 10 mM sodium acetate buffer at pH 5.0 resulted in a precipitation of the purified protein. Similar results were observed when purified factor III$^{lac}$ glucose from *Salmonella typhimurium* was analyzed by isoelectric focusing (17). The amino acid composition of factor III$^{lac}$ is listed in Table 2. Like factor III$^{lac}$ of *S. aureus* (12), the protein of *S. mutans* did not contain tryptophan residues. The molecular weight calculated from the sum of the amino acid residues (10,541) was very close to the value obtained by SDS-urea gel electrophoresis.

**Requirements for lac PTS activity.** The PEP-dependent phosphorylation of [$^{14}$C]lactose was measured in vitro with isolated membranes from lactose-grown cells, partially purified EI and HPr and purified factor III$^{lac}$. Omission of any one of these components prevented the phosphorylation of lactose at the expense of PEP (Table 3). Under our experimental conditions, the activity was proportional to the amount of factor III$^{lac}$ added to the reaction medium. PEP could not be replaced by ATP as the energy source. That factor III$^{lac}$ was essential for the lac PTS activity was also demonstrated by studying the effect of anti-*S. mutans* factor III$^{lac}$ IgGs on the PEP-dependent phosphorylation of ONPG. Indeed, Fig. 4 showed that the lac (ONPG) PTS activity, determined either with purified proteins or with crude cellu-

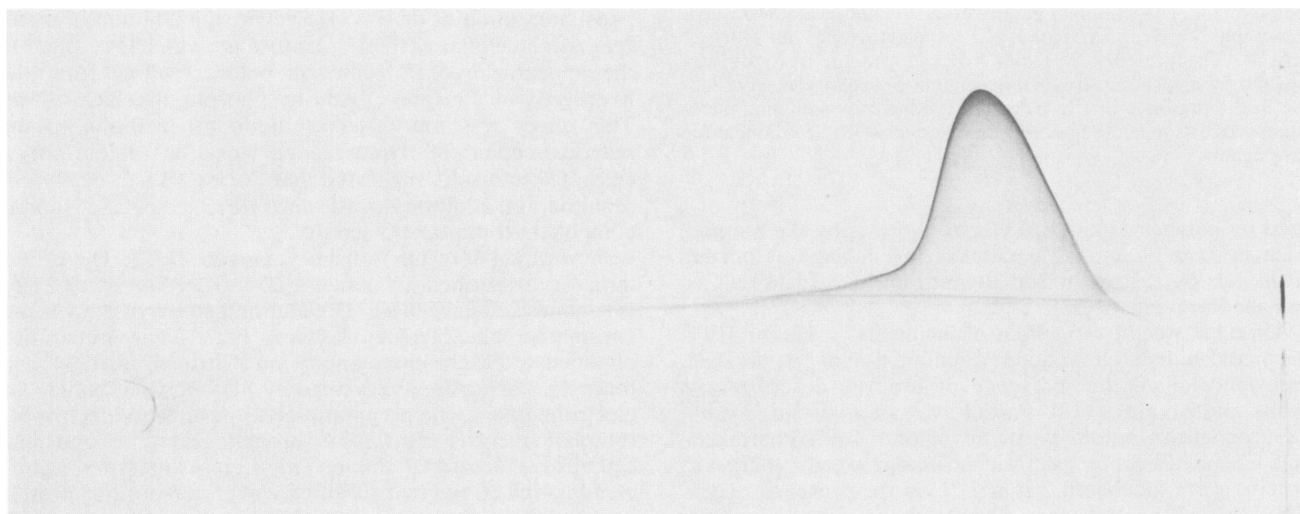


FIG. 3. Crossed immunoelectrophoresis of factor III$^{lac}$. Crossed immunoelectrophoresis was performed as described previously (32). The experiment was conducted with protein (300 μg) from the crude extract. The second-dimension gel contained 1.8 mg of anti-factor III$^{lac}$ IgGs.

TABLE 2. Amino acid analysis

| Amino acid | Factor $III^{lac}$ [a] | Lysosyme [b] |
|---|---|---|
| Asp | 12.0 | 15.1 (21) |
| Thr | 6.7 | 6.8 (7) |
| Ser | 3.8 | 9.2 (10) |
| Glu | 13.9 | 5.6 (5) |
| Pro | 0.5 | 1.6 (2) |
| Gly | 7.3 | 12.4 (12) |
| Ala | 10.6 | 11.9 (12) |
| Val | 4.3 | 5.6 (6) |
| Met | 3.7 | 2.2 (2) |
| Ileu | 4.7 | 5.2 (6) |
| Leu | 10.3 | 7.7 (8) |
| Tyr | 2.8 | 3.2 (3) |
| Phe | 2.3 | 2.8 (3) |
| His | 5.6 | 1.0 (1) |
| Lys | 5.1 | 6.0 (6) |
| Arg | 2.0 | 9.5 (11) |
| Trp | 0 | 7.1 (6) |

[a] Values are given without correction for loss during hydrolysis. Residues per 2 mol of arginine.
[b] Residues per 1 mol of histidine. Theoretical values as described by Penke et al. (22) are indicated in parentheses.

lar extracts, was neutralized by anti-factor $III^{lac}$. No inhibition was observed in the presence of preimmune serum.

**Immunoprecipitation experiments.** We have looked for the presence of factor $III^{lac}$ in cellular extracts of cells grown on various sugars by immunodiffusion experiments. Factor $III^{lac}$ was detected only in the extract of lactose-grown cells (Fig. 5B). No precipitin band was formed with extracts from sucrose-, glucose-, fructose-, and mannitol-grown cells. A cross-reaction was also observed with cellular extracts of lactose-grown cells of *S. mutans* GS5-2, 10449, LG1, Ingbritt, DR0001, and OMZ 175, *S. sanguis*, *S. lactis*, and *S. aureus* (Fig. 5A, C, and D). In some strains, two precipitin bands were observed, indicating that factor $III^{lac}$ may exist in multiple forms. Only one band was observed with the homologous *S. mutans* 27352. Precipitin bands were not detected with extracts of *S. salivarius*, *S. faecalis*, *L. casei*, *B. subtilis*, and *E. coli* (Fig. 5C and D). This last strain does not possess a lac PTS and was used as a control.

TABLE 3. Requirements for the lac PTS activity in *S. mutans* 27352

| Omission from the complete reaction medium [a] | Factor $III^{lac}$ (μg) | Activity [b] |
|---|---|---|
| None | 0 | 0 |
| None | 5.5 | 76.4 |
| None | 11 | 168.2 |
| None | 22 | 275.6 |
| PEP | 11 | 0 |
| PEP (+ 2 mM ATP) | 11 | 0 |
| EII | 11 | 0 |
| EI | 11 | 0 |
| HPr | 11 | 0 |

[a] The complete reaction medium (600 μl) consisted of the following: 4 mM $MgCl_2$, 2 mM PEP, 10 mM NaF, 25 μl of EII lactose-containing membrane, 1 U of EI, and 10 μg of HPr in a 50 mM sodium phosphate buffer (pH 7.0).
[b] Activity is expressed as nanomoles of [$^{14}C$]lactose phosphorylated per 30 min at 37°C.

## DISCUSSION

The inability of the $EII^{lac}$ to catalyze the PEP-dependent phosphorylation of ONPG in the presence of purified EI and HPr provided the first evidence for the requirement of another soluble component for the transport of lactose by the lac PTS in *S. mutans*. This protein component, termed factor $III^{lac}$, was purified to electrophoretic homogeneity. Its molecular weight obtained by analytical gel filtration chromatography was 41,500. The size of the subunits was 10,000 as determined by urea-SDS-polyacrylamide gel electrophoresis. This method (28) was shown to be more accurate for measuring the size of low-molecular-weight protein than the classical method of Weber and Osborn (33). A value of 10,541 was also obtained from the sum of the amino acids residues. These results suggest that factor $III^{lac}$ of *S. mutans* contains four subunits and is thus different from factor $III^{lac}$ of *Staphylococcus aureus*, which has a trimeric structure with a molecular weight of 33,000 to 35,000 (6, 12). These proteins, however, demonstrated a cross-reaction of partial identity in double-immunodiffusion experiments. The precipitin band produced by the *Staphylococcus aureus* protein

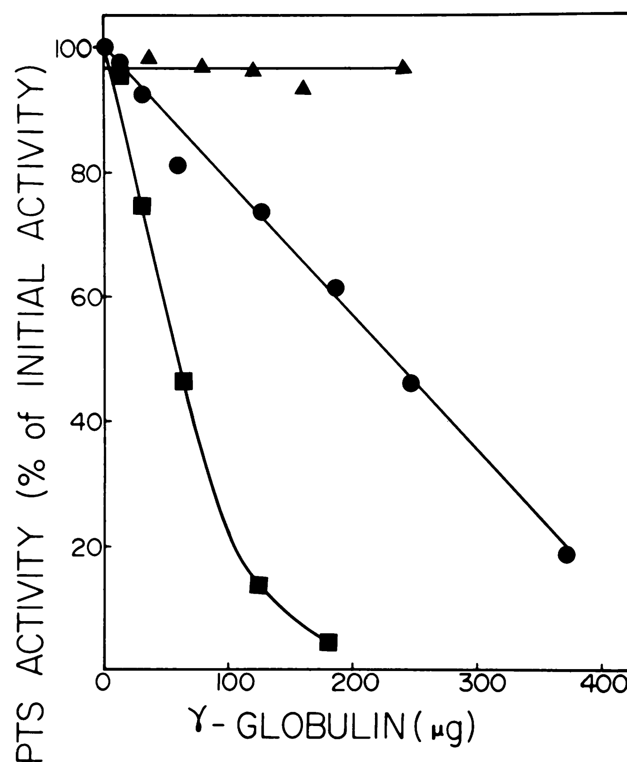



FIG. 4. Neutralization of the PEP-dependent phosphorylation of ONPG by anti-*S. mutans* factor $III^{lac}$ γ-globulins. Neutralization of PTS activity in a crude extract of *S. mutans* (■). Neutralization of PTS activity with purified factor $III^{lac}$ of *S. mutans* (●). Control was done with preimmune serum (▲). The reaction medium (500 μl) contained the following: 1 mM PEP, 1 mM $MgCl_2$, 10 mM NaF, an excess amount of EII lactose-containing membranes, ca. 1 U of EI, 10 μg of HPr, a limiting amount of purified factor $III^{lac}$ or crude extract, and partially purified 6-phospho-β-galactosidase. After incubation for 5 min at 37°C in the presence of IgGs, the reaction was initiated by the addition of 20 mM ONPG. After 30 min, the reaction was stopped by the addition of 50 μl of cold 1 M $Na_2CO_3$. The solution was then centrifuged at 15,000 × *g* for 10 min at 4°C. The absorbance of the supernatant was determined at 420 nm in a Beckman DU-8 spectrophotometer.

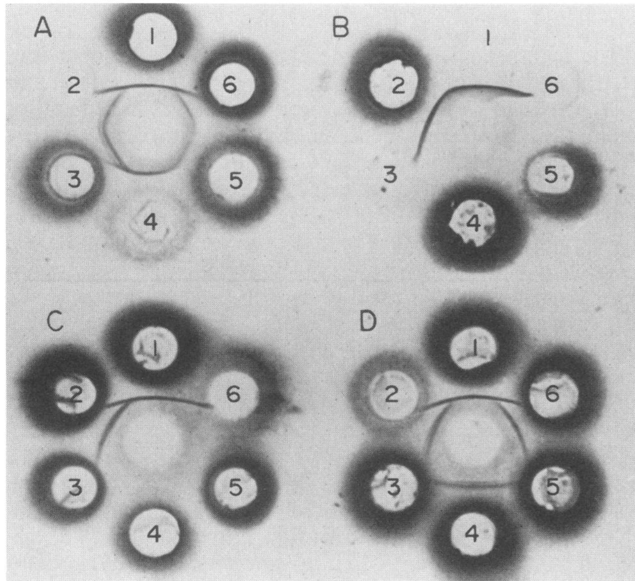
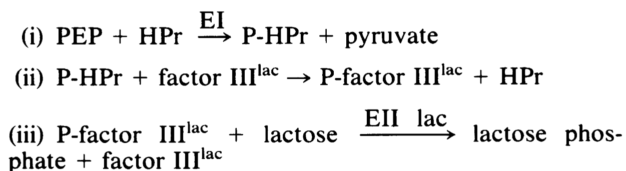



FIG. 5. Double-diffusion immunoprecipitation. Cells were grown in tryptone-yeast extract medium, and crude extracts were prepared by alumina grinding. Each well received 10 μl of solution. (A) Cells of different strains of *S. mutans* grown on lactose in each well were as follows: 1, 27352; 2, GS5-2; 3, 10449; 4, LG1; 5, Ingbritt; and 6, DR0001. (B) Cells of strains 27352 grown on various sugars in each well were as follows: 1, purified factor $III^{lac}$; 2, lactose; 3, sucrose; 4, glucose; 5, mannitol; and 6, fructose. (C) Cells of different species grown on lactose in each well were as follows: 1, *S. mutans* 27352; 2, *Staphylococcus aureus*; 3, *L. casei*; 4, *E. coli*; 5, *B. subtilis*; and 6, preimmune serum. (D) Streptococcal cells grown on lactose in each well were as follows: 1, *S. mutans* 27352; 2, *S. mutans* OMZ 175; 3 *S. salivarius*; 4, *S. sanguis*; 5, *S. faecalis*; and 6, *S. lactis*.

intersected the *S. mutans* band, suggesting that the protein of *Staphylococcus aureus* contained a smaller number of antigenic determinants than the *S. mutans* protein (8). Our results also showed that factor $III^{lac}$ was specifically induced in the presence of lactose and was present in all of the *S. mutans* strains tested. The fact that no cross-reaction was observed with *S. salivarius* agrees with our observation that the PEP-dependent phosphorylation of lactose by membranes of lactose-grown *S. salivarius* cells only required EI and HPr. Since *S. salivarius* possesses a non-PTS lactose transport system in addition to a lac PTS (10), and since it does not possess a factor $III^{lac}$, we may assume that the control of lactose utilization in this strain calls for different mechanisms than in *S. mutans*.

Experiments performed with purified proteins of the PTS indicated that the vectorial phosphorylation of lactose by the lac PTS in *S. mutans* required the participation of EII, EI, HPr, and factor $III^{lac}$. Based on the results obtained with the factor $III^{lac}$ of *S. aureus* (12), the reaction catalyzed by the lac PTS in *S. mutans* may be summarized as follows:



(i) $\text{PEP} + \text{HPr} \xrightarrow{\text{EI}} \text{P-HPr} + \text{pyruvate}$

(ii) $\text{P-HPr} + \text{factor III}^{lac} \rightarrow \text{P-factor III}^{lac} + \text{HPr}$

(iii) $\text{P-factor III}^{lac} + \text{lactose} \xrightarrow{\text{EII lac}} \text{lactose phosphate} + \text{factor III}^{lac}$

Relevant to the ecology of dental plaque, our results will be helpful in elucidating the mechanisms that account for the control of sugar utilization in oral streptococci. This control is of importance since in the oral cavity, oral streptococci are sporadically exposed to various amounts of miscellaneous carbohydrates. To survive, these microorganisms must select, during the periods of energy supply, the most beneficial nutrients and control their entry to maintain the intracellular metabolic balance. Recent studies have shown that the glucose PTS of oral streptococci is involved in the control of sugar utilization (15, 30, 31). In particular, the preferential dissimilation of glucose over lactose by streptococcal cells grown in mixture is abolished in glucose PTS-negative mutants affected in a membrane component of the PTS (15, 30, 31). Recently, the central role of the EII mannose in this regulatory process was confirmed (30). The molecular mechanism by which this control is achieved in oral streptococci remains, however, unclear. According to what is presently known about the control of sugar utilization in the *Streptococcus* genus and in other gram-positive bacteria, the regulatory mechanisms that might be anticipated are inducer exclusion (4, 7, 27, 29) and inducer expulsion (24, 25, 29). In enteric bacteria, lactose exclusion is ensured by allosteric regulation of the lactose permease by a protein component of the glucose PTS termed factor III glucose (18–21). Oral streptococci, however, do not possess this protein (30), so different mechanisms must be considered to explain inducer exclusion in *S. mutans*. In addition, as described in the work of Liberman and Bleiweis (15) on *S. mutans* GS5 and of Dills and Seno (7), the mechanism of inducer expulsion does not seem to operate in *S. mutans*. Our finding that *S. mutans* possesses an inducible specific factor $III^{lac}$ should aid in our understanding of the control of lactose utilization in this cariogenic bacterium.

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