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Mannitol Transport in *Streptococcus mutans*

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A hexitol-inducible, phosphoenolpyruvate-dependent phosphotransferase system was demonstrated in *Streptococcus mutans*. Cell-free extracts obtained from mannitol-grown cells from a representative strain of each of the five *S. mutans* serotypes (AHT, BHT, C-67-1, 6715, and LM7) were capable of converting mannitol to mannitol-1-phosphate by a reaction which required phosphoenolpyruvate and Mg^{2+}. Mannitol and sorbitol phosphotransferase activities were found in cell-free extracts prepared from cells grown on the respective substrate, but neither hexitol phosphotransferase activity was present in extracts obtained from cells grown on other substrates examined. A heat-stable, low-molecular-weight component was partially purified from glucose-grown cells and found to stimulate the mannitol phosphotransferase system. Divalent cations Mn^{2+} and Ca^{2+} partially replaced Mg^{2+}, while Zn^{2+} was found to be highly inhibitory.

The ability to utilize mannitol or sorbitol as a primary energy source for growth is one of the characteristics used to distinguish the group of cariogenic bacteria referred to as *Streptococcus mutans* from other oral streptococci (23). Previous studies in this laboratory (6) and elsewhere (7) demonstrated that various strains of *S. mutans* possessed separate and inducible mannitol-1-phosphate and sorbitol-6-phosphate dehydrogenases (EC 1.1.1.17 and EC 1.1.1.140, respectively), which converted the respective phosphorylated substrate to fructose-6-phosphate. The mechanism by which hexitols are initially phosphorylated by this group of organisms, however, is unknown. In *Escherichia coli* (14), *Salmonella typhimurium* (3), and *Clostridium thermocellum* (15), mannitol is initially transported into the cell by an inducible phosphoenolpyruvate (PEP)-dependent phosphotransferase system and is subsequently converted to fructose-6-phosphate by an inducible mannitol-1-phosphate dehydrogenase.

Roseman (12, 17) and others (15, 16, 20) have also shown that a number of facultative anaerobes transport various other carbohydrates into the cell by means of a PEP phosphotransferase system. Transport and concomitant phosphorylation of the substrate occurred as a result of at least two enzymatic reactions. Initially, a phosphate group was transferred from PEP to a low-molecular-weight, histidine-containing, heat-stable protein by a constitutive enzyme (I) of the phosphotransferase system. Phosphorylated heat-stable protein then served as a phosphate donor for the substrate in a reaction catalyzed by an inducible, substrate-specific, membrane-bound enzyme complex (II).

In this study it will be shown that *S. mutans* possesses a hexitol:PEP phosphotransferase system in which at least one component is inducible.

MATERIALS AND METHODS

Bacterial strains and growth media. Cultures of *S. mutans* strains AHT, BHT, C-67-1, 6715-14, and LM7 were obtained from R. M. McCabe, National Institute of Dental Research, Bethesda, Md., and stored at 4 C in fluid thioglycolate broth containing horse meat infusion (20% vol/vol) and excess $CaCO_3$. Transfers were made to fresh medium every 2 months. Cultures were routinely grown in an enriched medium containing: tryptone, 5.0 g; yeast extract, 5.0 g; K_2HPO_4, 5.0 g, and 0.05% (vol/vol) Tween 80 per liter of distilled water. Growth substrates such as glucose or mannitol were autoclaved separately and added aseptically to the medium. Solid enriched medium was prepared by adding 15 g of agar per liter to the medium prior to sterilization. The final pH was 7.4.

Chemicals. Mannitol-1-phosphate and sorbitol-6-phosphate were obtained from the Sigma Chemical Co., St. Louis, Mo., as the barium salts, and the barium was removed prior to use by treatment of the compounds with Sigma Dowex 50 W (1.5 g of resin per 100 mg of hexitol phosphate). For certain studies, as indicated in the text, mannitol was purified by recrystallization twice from deionized water, and sorbitol was purified by the method of Horwitz and Kaplan (11). PEP (tricyclohexylamide salt) and nicotinamide adenine dinucleotide (NAD) were purchased from Sigma Chemical Co., St. Louis, Mo. [U-^{14}C]mannitol (22 mCi/mmol), [U-^{14}C]sorbitol (185

mCi/mmol), and [U-^{14}C]glucose (180 mCi/mmol), were purchased from ICN Isotope and Nuclear Division, Irvine, Calif.

Preparation of cell-free extracts. Early stationary-phase cultures of *S. mutans* were obtained for enzyme assays following incubation for 18 h at 37 C in enriched medium supplemented with growth substrate (0.5% vol/vol). Cells were harvested by centrifugation at 10,400 × *g* at 4 C. The cells were washed twice with an equal volume of 0.05 M potassium phosphate buffer (PEM) containing ethylenediaminetetraacetic acid (EDTA), 1.0 mM, and 2-mercaptoethanol, 14 mM, pH 7.5. The cells were then resuspended at a concentration of 3.0 g (wet weight) in 8.0 ml of PEM buffer. Cells were broken by sonic treatment for 30 min at 4 C using a Branson model W185 sonifier operating at 185 W. The disrupted cell suspension was centrifuged at 31,000 × *g* for 30 min at 4 C, and the supernatant fluid was retained for enzyme assays. Cell-free extracts prepared as described above contained 20 to 25 mg of protein per ml as determined by the biuret reaction (10). Bovine serum albumin was used as a protein standard.

Hexitol phosphate dehydrogenase assays. The method used to measure mannitol-1-phosphate dehydrogenase (EC 1.1.1.17) and sorbitol-6-phosphate dehydrogenase (EC 1.1.1.140) activity was identical to that described previously (6). The reduction of NAD was followed continuously at 340 nm with a Gilford 2400-2 recording spectrophotometer. Enzyme units and specific activity were also calculated as described previously (6).

Mannitol phosphotransferase assay (spectrophotometric). The PEP-dependent formation of mannitol-1-phosphate from mannitol was measured by a modification of the procedure described by Patni and Alexander (15). Prior to use, the cell-free extract was treated for 30 min at 37 C with 0.05 unit of NADase (EC 3.2.2.5 from *Neurospora crassa*, Sigma Chemical Co., St. Louis, Mo.) per 25 mg of protein. The reaction was stopped by chilling the mixture in ice. This procedure prevented the conversion of mannitol-1-phosphate to fructose-6-phosphate by endogenous NAD and mannitol-1-phosphate dehydrogenase. The NADase-treated extract was then dialyzed against 5 volumes of PEM buffer and concentrated to 25 mg of protein per ml on an Amicon PM10 ultrafiltration membrane under 60 lb/in^2 of nitrogen at 4 C.

The phosphotransferase reaction was initiated by the addition of 1.0 ml of the NADase-treated extract to the following reaction mixture: mannitol, 2.0 mM; PEP, 5.0 mM; $MgCl_2$, 0.5 mM; and deionized water, to a final volume of 2.0 ml. The mixture was incubated for 60 min at 37 C, boiled 10 min, and centrifuged at 1,200 × *g* at room temperature for 15 min to remove the resulting precipitate. Mannitol-1-phosphate formed was measured by using the boiled supernatant fraction (0.2 to 0.5 ml) as a source of mannitol-1-phosphate in the assay mixture described above for mannitol-1-phosphate dehydrogenase activity. Cell-free extract obtained from mannitol-grown cells of *S. mutans* was employed as a source of the enzyme. The reaction was followed to completion at 340 nm using a Gilford 2400-2 recording spectrophotometer. The amount of mannitol-1-phosphate contained in the boiled fraction was estimated from a standard curve relating final absorbance at 340 nm to mannitol-1-phosphate concentration.

Mannitol phosphotransferase assay (^{14}C radioisotope). The method of Tanaka et al. (22) for the determination of mannitol-1-phosphate formed from ^{14}C-labeled mannitol was modified as follows. The reaction mixture contained: [U-^{14}C]mannitol, 0.024 mM (4 μCi/mmol); PEP, 5.0 mM; $MgCl_2$, 10.0 mM; 2-mercaptoethanol, 14.3 mM; Tris-hydrochloride buffer, pH 7.6, 10.0 mM; and cell-free extract (600 to 800 μg of protein) in a total volume of 0.2 ml. After addition of the cell-free extract, the reaction was allowed to proceed 30 min at 37 C. Fifty microliters of 1 M mannitol was added to stop the reaction, and the samples were chilled in ice. A 20-μl amount of each sample was placed on duplicate diethylaminoethyl (DEAE) filter paper disks (Whatman DE81, 2.4 cm) and allowed to dry. Disks were washed with 200 ml of deionized water, dried at 100 C for 15 min, and counted in Aquasol liquid scintillation fluid (New England Nuclear, Boston, Mass.) in a Packard Tricarb scintillation spectrometer (model 3375). Data are expressed as nanomoles of mannitol-1-phosphate formed in 30 min per milligram of protein. The reaction was linear for 40 min and was proportional to enzyme concentration under the conditions employed. Glucose and sorbitol phosphotransferase activities were measured by substituting [U-^{14}C]glucose or [U-^{14}C]sorbitol, respectively, for mannitol in the above assay.

Preparation of soluble and particulate fractions. Cell-free extracts were prepared from glucose- or mannitol-grown cells of *S. mutans* 6715-14 as described previously except that, after sonic treatment, the suspension was centrifuged at 10,000 × *g* for 20 min at 4 C. The supernatant fluid (9.0 ml) was then centrifuged at 106,000 × *g* for 120 min at 4 C in a model L2-65B ultracentrifuge in order to insure that a particle-free soluble preparation was obtained. The soluble fraction was placed in a separate tube, and the particulate fraction was resuspended in 9.0 ml of PEM buffer. Both fractions were centrifuged twice again at 106,000 × *g* for 60 min. The particulate fraction was resuspended in 2.0 ml of PEM buffer. Forty-microliter portions of each fraction were assayed individually and in various combinations for [^{14}C]glucose and [^{14}C]mannitol phosphotransferase activity.

Partial purification of the heat-stable component. The method employed consisted of the heat and acid precipitation steps described by Kundig and Roseman (13) for the purification of heat-stable protein from *E. coli*. *S. mutans* 6715-14 was grown 18 h in 40 liters of enriched medium containing 0.5% glucose. Cells were harvested by centrifugation and washed twice with 500 ml of 0.05 M potassium phosphate buffer, pH 7.5. Approximately 148 g (wet weight) of packed cells were obtained. A cell-free extract was prepared by sonic treatment of 15-g samples of cells resuspended in 40 ml of 0.05 M potassium phosphate, pH 7.5. Two grams of glass beads (5-μm diameter, Heat Systems Ultrasonics, Inc., Plainview,

N.Y.) was employed to facilitate cell breakage. After a 30-min treatment, the disrupted cell suspension was centrifuged at 18,000 × g for 15 min at 4 C. The supernatant fluid was again centrifuged at 31,000 × g for 30 min at 4 C. The 31,000 × g supernatant fraction was placed in a boiling water bath for 15 min, and then centrifuged at 18,000 × g for 30 min at 4 C to remove the precipitate. The pH of the supernatant fluid (approximately 200 ml) was adjusted to 1.0 by the dropwise addition of concentrated hydrochloric acid while maintaining the temperature at 4 C. The mixture was allowed to stir 30 min in an ice bath and then centrifuged at 18,000 × g for 5 min. The supernatant fluid was discarded, and the precipitate was washed with 185 ml of cold 0.01 N HCl. Finally, the precipitate was resuspended in 175 ml of 0.5 M potassium phosphate buffer, pH 7.6, and allowed to stir at room temperature for 24 h. The remaining precipitate was removed by centrifugation at 18,000 × g for 15 min, and the supernatant fluid was dialyzed against four changes of deionized water (2 liters each), for a total of 48 h. The dialysate was concentrated by ultrafiltration using an Amicon UM2 membrane. The final preparation contained 13.3 mg of protein per ml and was stored at −15 C.

RESULTS

Mannitol phosphotransferase activity. Cell-free extracts obtained from mannitol-grown cells of *S. mutans* 6715-14 were found to be capable of phosphorylating mannitol in a reaction that was dependent upon PEP (Table 1). Adenosine triphosphate (ATP) could not effectively replace PEP as the phosphate donor (Table 1). The reaction, as measured by the spectrophotometric assay, exhibited a broad pH optimum that extended between pH 6.4 and 8.0. Phosphotransferase activity was highest when extracts were assayed in phosphate buffer containing EDTA and 2-mercaptoethanol (PEM, Materials and Methods). The activity in 50 mM Tris-hydrochloride or 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffers containing 1.0 mM EDTA and 14 mM 2-mercaptoethanol (pH 7.5) was only 42% and 35%, respectively, of that observed in PEM. Enzyme activity was not stable to freezing and thawing, and fresh extracts were, therefore, prepared routinely and used throughout this study.

TABLE 1. *Formation of mannitol-1-phosphate by Streptococcus mutans 6715-14*[a]

Assay condition	Mannitol-1-PO_4 formed (nmol/mg of protein in 60 min)
Complete	31.0
No PEP	1.0
No mannitol	2.0
ATP[b]	1.0

[a] Cell-free extracts obtained from mannitol-grown cells of *S. mutans* 6715-14 were assayed for the ability to form mannitol-1-phosphate from mannitol by the spectrophotometric assay described.

[b] ATP was substituted for PEP (at the same concentration) in the complete system.

Effect of growth substrate on enzyme activity. Assays for hexitol phosphotransferase and hexitol phosphate dehydrogenase activities from cell-free extracts of *S. mutans* 6715-14 grown on various substrates gave preliminary evidence for the inducible nature of both enzyme activities. The data in Table 2 show that extracts derived from mannitol-grown cells contained appreciable levels of mannitol phosphotransferase activity and mannitol-1-phosphate dehydrogenase activity. Similarly, sorbitol phosphotransferase and sorbitol-6-phosphate dehydrogenase activities were high only in cell-free extracts derived from sorbitol-grown cells. Growth of the organism on substrates other than mannitol or sorbitol produced only low levels of the enzymes associated with hexitol transport.

In vitro complementation of the phosphotransferase system. Further evidence for the inducibility of a component of the mannitol phosphotransferase system was obtained by studying in vitro complementation of cell-free extracts obtained from glucose- and mannitol-grown cells of *S. mutans* 6715-14. Extracts were fractionated into soluble and particulate fractions by ultracentrifugation. The fractions were assayed individually and in various combinations for mannitol and glucose phosphotransferase activity. The data in Table 3 show that significant activity for glucose phosphorylation was found in the soluble fractions of extracts obtained from either glucose- or mannitol-grown cells. It should be noted that a substantial portion of this activity is probably due to the presence of endogenous pyruvate kinase and adenosine 5'-diphosphate (ADP) in the cell-free extract which generate ATP in the presence of PEP. A hexokinase also present in such extracts (B. Chassy, personal communication) would then produce glucose-6-phosphate. Such hexokinase activity, however, does not affect the results obtained in this experiment for mannitol phosphorylation, since the combination of glucose-soluble and glucose-particulate fractions was not capable of phosphorylating a significant amount of mannitol. Unexpectedly, mannitol phosphotransferase activity was detected at a high level in the soluble fraction obtained from mannitol-grown cells. It was also observed that the addition of either soluble or particulate glucose fraction to the mannitol-soluble fraction resulted in marked inhibition of mannitol phos-

TABLE 2. *Effect of growth substrate on enzyme induction in Streptococcus mutans 6715-14*[a]

Growth substrate	Mannitol phosphotransferase[b]	Mannitol-1-PO_4 dehydrogenase units/mg of protein	Sorbitol phosphotransferase[c]	Sorbitol-6-PO_4 dehydrogenase units/mg of protein[d]
Glucose	0.19	0.016	0.02	0
Mannitol[e]	2.57	0.412	0.10	0.043
Sorbitol[f]	0.36	0.066	1.25	0.290
Sucrose	0.29	0.112	0	ND
Fructose	0.24	0.069	0.02	ND
Lactose	0.22	0.061	0	ND
Galactose	0.35	0.095	0.18	ND
Mannose	0.31	0.105	0.10	ND

[a] Cell-free extracts were prepared as per Materials and Methods from cells grown in enriched medium supplemented with 0.5% of the indicated substrate.
[b] Nanomoles of [^{14}C]mannitol-1-PO_4 formed per milligram of protein in 30 min at 37 C.
[c] Nanomoles of [^{14}C]sorbitol-6-PO_4 formed per milligram of protein in 30 min at 37 C.
[d] ND, Not determined.
[e] Recrystallized 2 times from water.
[f] Purified by the method of Horwitz and Kaplan (11)

TABLE 3. *In vitro complementation of glucose- and mannitol-grown cell fractions of S. mutans 6715-14*

Cell fraction[a]	Phosphotransferase activity[b]	
	[^{14}C]glucose	[^{14}C]mannitol
Glucose (S)	0.91	0.26
Glucose (P)	0	0.10
Mannitol (S)	0.75	3.28
Mannitol (P)	0	0.27
Glucose (S) + glucose (P)	1.43	0.26
Glucose (S) + mannitol (S)	1.45	1.94
Glucose (S) + mannitol (P)	1.32	2.57
Glucose (P) + mannitol (S)	1.07	2.46
Glucose (P) + mannitol (P)	0	0.14
Mannitol (S) + mannitol (P)	1.03	3.10

[a] Cell fractions were prepared by ultracentrifugation as described in Materials and Methods. (S = 106,000 × *g* soluble fraction; P = 106,000 × *g* pellet fraction.) Forty microliters of each indicated fraction was employed as a source of enzyme. The protein concentrations for the glucose-soluble, glucose-particulate, mannitol-soluble and mannitol-particulate fractions were 23.6, 10.0, 18.3, and 9.0 mg/ml, respectively.
[b] Nanomoles of [^{14}C]phosphorylated substrate formed per milligram of protein in 30 min at 37 C.

photransferase activity. The reason for this inhibitory effect is not clear, and an explanation must await further investigation. Most importantly, the particulate fraction from mannitol-grown cells was found to complement the soluble fraction obtained from glucose-grown cells when assayed for mannitol phosphotransferase activity. A fivefold increase in activity over the additive value of each fraction was observed.

Occurrence of a heat-stable component. Treatment of cell-free extract obtained from glucose-grown cells of *S. mutans* 6715-14 by boiling and acid precipitation resulted in a preparation which was capable of stimulating the phosphorylation of mannitol by cell-free extracts obtained from mannitol-grown cells (Fig. 1). The molecular weight for the heat-stable component has not been determined. However, successive filtration of the preparation through Amicon ultrafiltration membranes showed that the activity passed membranes which had molecular weight retentions of approximately 30,000 and 10,000 (PM 30 and UM 10 membranes, respectively), but was retained by a membrane with a 1,000-molecular-weight retention (UM 2). Thus, the heat-stable component is a low-molecular-weight compound.

Effect of divalent cations. The phosphotransferase reaction was dependent upon Mg^{2+}. The chloride and sulfate salts of this cation were equally effective. Ca^{2+}, at concentrations up to 10 mM, could replace Mg^{2+}, yielding essentially the same level of phosphorylation of mannitol (Fig. 2). Concentrations of Ca^{2+} in excess of 10 mM, however, were much less effective in this capacity. Mn^{2+} also partially replaced Mg^{2+} (90% as effective) at a concentration of 10 mM (data not shown). Zn^{2+}, on the other hand, inhibited the *S. mutans* phosphotransferase system. Figure 3 shows that 50% inhibition was achieved by the addition of approximately 2.0 mM Zn^{2+} to the mannitol phosphotransferase assay containing Mg^{2+} (10 mM). The addition of Zn^{2+} did not alter the pH of the reaction mixture under the conditions employed. It was not possible under the assay conditions employed to extend the curve to

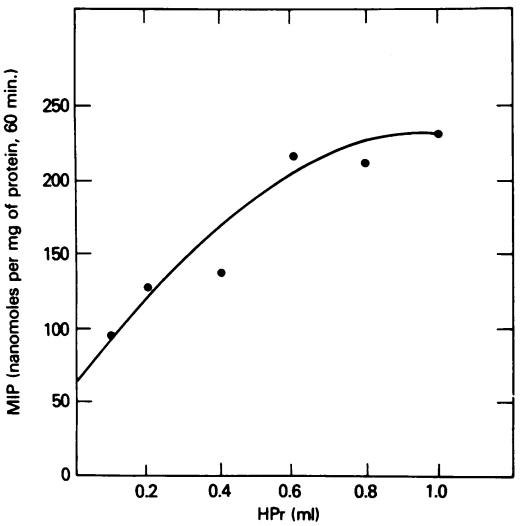

FIG. 1. *Effect of partially purified heat-stable component on mannitol-1-phosphate formation. A heat-stable component was isolated and partially purified from glucose-grown cells of S. mutans 6715-14. Portions of this heat-stable protein (HPr) were added to the spectrophotometric assay for mannitol phosphotransferase activity. An extract from mannitol-grown cells of S. mutans 6715-14 served as the source of phosphotransferase enzymes. The heat-stable preparation contained 13.3 mg/ml of protein.*

100% inhibition, since concentrations of Zn^{2+} greater than 10 mM produced a precipitate in the reaction mixture.

Enzyme activity in various strains of S. mutans. It is now well established that *S. mutans* represents a heterogeneous group of organisms that is comprised of at least five serotypes (4). It was of interest to determine whether the mannitol phosphotransferase system was present in all serotypes. Accordingly, a representative strain of each of the *S. mutans* serotypes was grown in enriched medium supplemented with mannitol, and cell-free extracts were examined for the presence of a mannitol: PEP-dependent phosphotransferase system and mannitol-1-phosphate dehydrogenase activity (Table 4). All extracts examined contained significant levels of mannitol phosphotransferase activity. Mannitol-1-phosphate dehydrogenase activity, also, was associated with all cell-free extracts examined.

DISCUSSION

Experiments presented here show that a representative strain of each of the five Bratthall serotypes (4) of *S. mutans* possesses mannitol phosphotransferase activity when the organisms are cultured on media containing mannitol as an energy source (Table 4). Phosphorylation was dependent on PEP, and ATP could not replace PEP as the phosphate donor (Table 1). In addition to hexitol phosphotransferase activity, mannitol- or sorbitol-adapted cells of *S. mutans* 6715-14 were also found to possess mannitol-1-phosphate or sorbitol-6-phosphate dehydrogenase activity, respectively (Table 2), as reported previously (5, 6, 7). The significance, if any, of the observed quantitative differences in the levels of activity of these enzymes in the various strains is not known. However, strain differences were observed by Brown and Patterson (5) who reported that *S. mutans* FA-1 possessed a higher level of mannitol-1-phosphate dehydrogenase than other strains examined. Hexitol phosphotransferase activity was not observed in cells of *S. mutans* 6715-14 adapted to substrates other than mannitol or sorbitol (Table 2), as would be predicted for inducible enzyme systems. In contrast, Schachtele and Mayo (20) showed that the glucose phosphotransferase system in *S. mutans* 6715 is constitutive.

In vitro complementation experiments (Table 3) provided further evidence for the inducibility

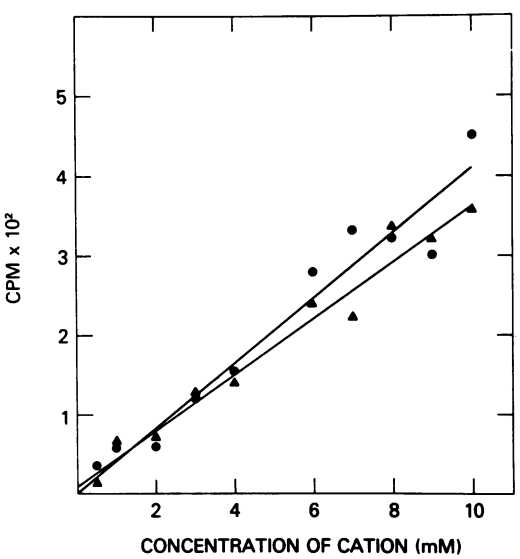

FIG. 2. *Effect of magnesium and calcium on mannitol phosphotransferase activity. Mannitol-grown cells of S. mutans 6715-14 were washed and suspended in 0.05 M Tris-hydrochloride buffer (TM) containing 1.4 × 10^{-2} M 2-mercaptoethanol, pH 7.5, since Tris-hydrochloride was reported to bind Mg^{2+} and Ca^{2+} only weakly (9). Cell-free extract was then prepared and used as a source of phosphotransferase enzymes in the [^{14}C]radioisotope assay to which various concentrations of magnesium (●) or calcium (▲) were added. Slopes were estimated by least-square analysis.*

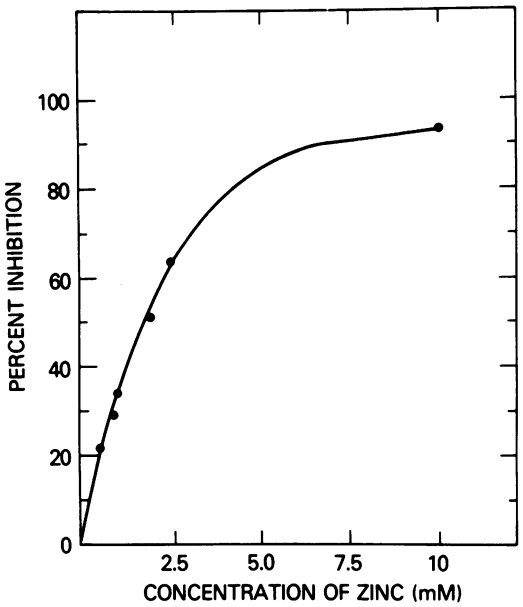

FIG. 3. *Inhibition of mannitol phosphotransferase activity by zinc. In addition to magnesium (10 mM), various concentrations of zinc chloride were added to the ^{14}C radioisotope assay for phosphotransferase activity. Cell-free extract obtained (as described in Fig. 2) from mannitol-grown cells of S. mutans 6715-14 served as a source of phosphotransferase enzymes. Data are expressed as percentage of inhibition when compared to the control in which no zinc is added.*

of at least one component of the mannitol phosphotransferase system in *S. mutans* 6715-14. This was shown by the fivefold increase in activity when a particulate fraction (106,000 × *g* pellet) from mannitol-grown cells complemented the soluble fraction (106,000 × *g* supernatant fraction) from glucose-grown cells. The particulate fraction derived from glucose-grown cells could not substitute for the corresponding mannitol-derived fraction, suggesting that the component which is specific for mannitol phosphorylation resides only in the cell envelope of mannitol-adapted cells. This component may be analogous to the substrate-specific, membrane-bound enzyme II complex found in *E. coli* (17).

Somewhat unexpectedly, a high level of mannitol phosphotransferase activity was associated with the soluble fraction obtained from mannitol-grown cells (Table 3). This activity might be explained by: (i) the presence of contaminating enzyme II complex released from the cell envelope during sonic treatment, or (ii) the presence of a mannitol-specific soluble component. The latter possibility seems unlikely in view of the complementation observed between the particulate fraction derived from mannitol-grown cells and the soluble fraction obtained from glucose-grown cells.

The data described thus far show that hexitol transport in *S. mutans* is mediated by an inducible, PEP phosphotransferase system, and that this system is similar to the one described for *E. coli* (1, 12, 17) and other facultative anaerobes (16). However, certain subtle but perhaps important differences have been observed with respect to metal requirements. Mg^{2+} is required by the *E. coli* glucose phosphotransferase system (12, 17), and a recent report (21) indicated that Mg^{2+} is involved in the phosphorylation of enzyme I in *Staphylococcus aureus*. Kundig et al. (12) reported that Mn^{2+}, Zn^{2+}, and Co^{2+} could each partially replace Mg^{2+} in the *E. coli* glucose phosphotransferase system, while Ca^{2+} and Cu^{2+} were highly inhibitory. Roseman (18) found that Ca^{2+} also was important in the formation of active, stable enzyme II complexes.

With respect to the *S. mutans* glucose phosphotransferase system, Schachtele (19) reported that Mn^{2+}, Co^{2+}, and Zn^{2+} could partially replace Mg^{2+}. In our experiments on the mannitol phosphotransferase system in *S. mutans*, Ca^{2+} was found to replace Mg^{2+} at concentrations up to 10 mM (Fig. 2), but Zn^{2+} was markedly inhibitory (Fig. 3). This effect of Zn^{2+} on *S. mutans* agrees with results obtained with intact cells of *E. coli* (2) and *Pseudomonas aeruginosa* (8) in which transport of sugars and amino acids was shown to be inhibited by Zn^{2+}

TABLE 4. *Mannitol phosphotransferase and mannitol-1-PO_4 dehydrogenase activity in various strains of Streptococcus mutans*[a]

Strain	Serotype[b]	Mannitol phosphotransferase[c]	Mannitol-1-PO_4 dehydrogenase (units per mg of protein)
AHT	a	0.82	0.317
BHT	b	1.29	0.753
C-67-1	c	2.29	0.584
6715	d	2.42	0.912
LM7	Lancefield Group E	1.27	0.402

[a] Cell-free extracts were prepared as per Materials and Methods from cells grown in enriched medium supplemented with 0.5% mannitol.

[b] As described previously by Bratthall (4).

[c] Nanomoles of [^{14}C]mannitol-1-PO_4 formed per milligram of protein in 30 min at 37 C.

Differences between our results and those reported by others (12, 19) might be attributed to variations in methodology or in unique properties of different transport systems. The possibility that the ability of *S. mutans* to utilize Ca^{2+} may represent an ecological adaptation is of interest but must remain speculative at this time.

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