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Distribution of Proteins Similar to III_H^{Man} and III_L^{Man} of the *Streptococcus salivarius* Phosphoenolpyruvate:Mannose-Glucose Phosphotransferase System among Oral and Nonoral Bacteria

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In *Streptococcus salivarius*, the phosphoenolpyruvate (PEP):mannose-glucose phosphotransferase system, which concomitantly transports and phosphorylates mannose, glucose, fructose, and 2-deoxyglucose, is composed of the general energy-coupling proteins EI and HPr, the specific membrane-bound EII^{Man}, and two forms of a protein called III^{Man}, with molecular weights of 38,900 (III_H^{Man}) and 35,200 (III_L^{Man}), that are found in the cytoplasm as well as associated with the membrane. Several lines of evidence suggest that III_H^{Man} and/or III_L^{Man} are involved in the control of sugar metabolism. To determine whether other bacteria possess these proteins, we tested for their presence in 28 oral streptococcus strains, 3 nonoral streptococcus strains, 2 lactococcus strains, 2 enterococcus strains, 2 bacillus strains, 1 lactobacillus strain, *Staphylococcus aureus*, and *Escherichia coli*. Three approaches were used to determine whether the III^{Man} proteins were present in these bacteria: (i) Western blot (immunoblot) analysis of cytoplasmic and membrane proteins, using anti-III_H^{Man} and anti-III_H^{Man} rabbit polyclonal antibodies; (ii) analysis of PEP-dependent phosphoproteins by polyacrylamide gel electrophoresis; and (iii) inhibition by anti-III^{Man} antibodies of the PEP-dependent phosphorylation of 2-deoxyglucose (a mannose analog) by crude cellular extracts. Only the species *S. salivarius* and *Streptococcus vestibularis* possessed the two forms of III^{Man}. Fifteen other streptococcal species possessed one protein with a molecular weight between 35,200 and 38,900 that cross-reacted with both antibodies. In the case of 9 species, a protein possessing the same electrophoretic mobility was phosphorylated at the expense of PEP. No such phosphoprotein, however, could be detected in the other six species. A III^{Man}-like protein with a molecular weight of 35,500 was also detected in *Lactobacillus casei* by Western blot experiments as well as by PEP-dependent phosphoprotein analysis, and a protein with a molecular weight of 38,900 that cross-reacted with anti-III^{Man} antibodies was detected in *Lactococcus lactis*. In several cases, the involvement of these putative III^{Man} proteins in the PEP-dependent phosphorylation of 2-deoxyglucose was substantiated by the inhibition of phosphorylation activity by anti-III^{Man} antibodies. No proteins cross-reacting with anti-III^{Man} antibodies were detected in enterococci, bacilli, and *E. coli*. In *S. aureus*, a membrane protein with a molecular weight of 50,000 reacted strongly with the antibodies. This protein, however, was not phosphorylated at the expense of PEP.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) simultaneously catalyzes the transport and phosphorylation of mono- and disaccharides, using PEP as the energy source (14, 18, 21). The PTS is composed of two cytosolic proteins, Enzyme I (EI) and HPr, which are involved in the transport of all PTS sugars, and a family of sugar-specific EII complexes consisting of three or four domains called A, B, C, and D that can be on a single protein or on separate polypeptides (21). Besides its role in the transport of carbohydrates, the PTS is also involved in the control of sugar utilization. In the family *Enterobacteriaceae*, the regulatory functions of the PTS have been chiefly assigned to the protein IIA^{Glc} (formerly III^{Glc}), specifically associated with the transport of glucose (14, 18, 24). Even though IIA^{Glc}-like domains have been identified in the transport proteins of some gram-positive bacteria, no regulatory functions such as those associated with IIA^{Glc} of *Escherichia coli* could be demonstrated for these domains (reviewed in reference 18).

In several streptococcal species, glucose is transported by a PTS that also recognizes mannose, fructose, and the nonmetabolizable analog 2-deoxyglucose (2DG) (6, 23). Biochemical characterization of this system in *Streptococcus salivarius*, referred to as the mannose PTS, indicated that a III^{Man} complex, corresponding to a IIA component according to the nomenclature proposed by Saier and Reizer (21), is involved in the transport of the mannose PTS substrates (2). The III^{Man} complex of *S. salivarius* is composed of two polypeptides with different molecular weights designated III_H^{Man} (molecular weight, 38,900) and III_L^{Man} (molecular weight, 35,200). Whether both forms of III^{Man} are necessary for the transport of the mannose PTS substrates remains to be determined. However, we have shown that spontaneous III_L^{Man}-deficient mutants do not exhibit diauxic growth when cultured in media containing mixtures of glucose and lactose, in contrast to the case for the wild-type strain (5). Moreover, these mutants expressed, after growth on glucose, several genes encoding cytoplasmic as well as membrane proteins that are repressed in the wild-type strain (3, 4, 10). These results suggest that the III^{Man} complex of *S. salivarius* might have important regulatory functions. A preliminary survey conducted with the cytoplasmic fraction of nine species representing five genera suggested that III^{Man} was not restricted to *S. salivarius* (2). In this study, the distribution of III^{Man}-like proteins was assessed in the

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TABLE 1. Bacterial strains

Bacterial species	Strain(s)[a]
B. stearothermophilus	ATCC 12980
B. subtilis	ATCC 6051
E. faecalis	ATCC 35550
E. hirae	ATCC 8043
L. casei	ATCC 4646
L. lactis	ATCC 19257, ATCC 11454
S. aureus	ATCC 6538
S. anginosus	ATCC 33397
S. bovis	ATCC 35034
S. constellatus	ATCC 27823
S. cricetus	ATCC 19642
S. ferus	ATCC 33477
S. gordonii	ATCC 10558
S. mitior	ATCC 903
S. mitis	ATCC 33399
S. mutans	Ingbritt,[b] DR0001,[c] ATCC 25175, T8,[d] GS-5[e]
S. oralis	ATCC 35037
S. parasanguis	ATCC 15912
S. pneumoniae	R800[f]
S. rattus	ATCC 19645, FA-1[g]
S. salivarius	ATCC 25975, ATCC 27945, ATCC 13419, 30.1,[g] ATCC 7073, 149.1[g]
S. sanguis	ATCC 10556, NY101[d]
S. sobrinus	ATCC 27352, ATCC 33478
S. suis	170-B[h]
S. thermophilus	ATCC 19258
S. vestibularis	ATCC 49124
E. coli	K-12

[a] All strains were obtained from the American Type Culture Collection (ATCC) except as noted.
[b] J. Sandham (Toronto, Ontario, Canada).
[c] I. R. Hamilton (University of Manitoba, Winnipeg, Canada).
[d] J. S. van der Hoeven (Nijmegen, The Netherlands).
[e] A. S. Bleiweis (University of Florida, Gainesville).
[f] J. P. Claverys (Centre de Recherche de Biochimie et de Génétique Cellulaires, Toulouse, France).
[g] Fresh isolates.
[h] D. Dubreuil (GREMIP, Université de Montréal, St.-Hyacinthe, Québec, Canada).

cytoplasmic and membrane fractions of 40 bacterial species representing seven genera.

MATERIALS AND METHODS

Organisms and growth conditions. The various strains used in this study are listed in Table 1. Cells were grown at 37°C (except for *Bacillus stearothermophilus* ATCC 12980, which was grown at 55°C) in TYE medium, which contains (per liter) 17 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 3 g of yeast extract (Difco Laboratories, Detroit, Mich.), 5 g of NaCl, and 2.5 g of disodium phosphate. Sugars were sterilized by filtration (with a 0.22-μm-pore-size Millipore filter) and added aseptically to the medium to a final concentration of 0.5%.

Preparation of the cytoplasmic and membrane fractions. Cells were broken by grinding with alumina in the presence of 10 mM potassium phosphate (pH 7.0) containing 0.1 mM phenylmethylsulfonyl fluoride, 14 mM 2-mercaptoethanol (2ME), and 1 mM EDTA (26). Cytoplasmic and membrane fractions were obtained by differential centrifugation as previously described (2).

Production of antibodies against III$_H^{Man}$ and III$_L^{Man}$. The purification and production of rabbit polyclonal antibodies against III$_H^{Man}$ were reported previously (2). A different approach was used to purify and produce polyclonal antibodies against III$_L^{Man}$. The purification steps were all performed at 4°C. The cytoplasmic extract obtained from 72 liters of culture of glucose-grown cells of *S. salivarius* ATCC 25975 was chromatographed on a HiLoad 16/10 Q Sepharose Fast Flow column (1.6 by 11 cm) (Pharmacia) equilibrated with 10 mM potassium phosphate (pH 7.5) (PP buffer). The column was washed first with 10 volumes of PP buffer and then with 5 volumes of the same buffer containing 0.1 M KCl. The column was then eluted with a 390-ml (0.1 to 0.6 M KCl) gradient in PP buffer at a flow rate of 180 ml/h. III$_H^{Man}$ and III$_L^{Man}$ were eluted in the same fractions at approximately 0.35 M KCl and were detected by using anti-III$_H^{Man}$ antibodies (these antibodies reacted with both forms of III^{Man} [2]). The fractions were pooled and dialyzed against PP buffer. The dialyzed sample was then applied to a Sephacryl S-200 column (2.6 by 66 cm) (Pharmacia) equilibrated with PP buffer containing 0.1 M KCl. The column was washed with 400 ml of the same buffer at a flow rate of 150 ml/h. III$_H^{Man}$ and III$_L^{Man}$ were eluted in the same fractions which were pooled and concentrated to approximately 15 mg of protein per ml. Pooled proteins were separated by two-dimensional polyacrylamide gel electrophoresis (PAGE) (16) with modifications. Samples (50 μl containing 700 to 800 μg of proteins) were mixed with 37.5 mg of urea and 100 μl of a solution containing 5.7% (wt/vol) urea, 8% (wt/vol) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 2% (vol/vol) 2ME, and 2% (vol/vol) ampholytes (pH 5 to 6) (Servalyt). The samples were applied to gels (9 cm long and 3 mm in diameter) containing 4% (wt/vol) acrylamide, 5.5% (wt/vol) urea, 2% (wt/vol) CHAPS, and 2% (wt/vol) ampholytes (pH 5 to 6). The electrofocusing was carried out at room temperature at 300 V for 18 h; this was followed by a 1-h run at 400 V. The second dimension was performed on a 10% acrylamide gel at 10°C, using currents of 50 mA per gel during migration in the stacking gel and 70 mA per gel during migration in the resolving gel. The proteins were then transferred to a nitrocellulose sheet according to the method of Towbin et al. (25). After staining with 0.1% Ponceau Red in 1% acetic acid for 5 min, the nitrocellulose was briefly destained in distilled water and the spot corresponding to III$_L^{Man}$ (previously identified with anti-III$_H^{Man}$ antibodies) was excised and kept at 4°C until used. Anti-III$_L^{Man}$ antibodies were obtained from New Zealand White female rabbits after immunization by multisite intradermal injection. Approximately 100 μg of two-dimensional PAGE-purified III$_L^{Man}$ mixed with complete Freund adjuvant was used for the first injection, and 50 μg of two-dimensional PAGE-purified III$_L^{Man}$ mixed with incomplete Freund adjuvant was used for the booster injection. The antisera were purified as previously described (2).

III^{Man} analysis by Western blotting (immunoblotting). Electrophoresis of cytoplasmic and membrane proteins was performed with a Protean II apparatus from Bio-Rad laboratories by the method of Laemmli (9), modified as previously described (2). Prior to electrophoresis, the samples were heated at 100°C for 3 min in the presence of 2% sodium dodecyl sulfate (SDS) and 5% (vol/vol) 2ME. Samples (100 μl) containing between 100 and 250 μg of proteins were deposited on the gel. Currents of 15 mA per gel (stacking gel) and 25 mA per gel (resolving gel) were applied. After electrophoresis, the proteins were transferred electrophoretically to a nitrocellulose membrane (pore size, 0.45 μm) according to the method of Towbin et al. (25). The immunodetection of proteins cross-reacting with anti-III$_L^{Man}$ or anti-III$_H^{Man}$ antibodies was carried out as described previously (19), except that 5% skim milk and anti-rabbit alkaline phosphatase antibodies were used instead of 1% gelatin and anti-rabbit gold conjugate, respectively.

Phosphorylation of proteins by [^{32}P]PEP. [^{32}P]PEP was prepared as described by Mattoo and Waygood (13), using partially purified PEP carboxykinase from *E. coli* K-12 HFr 3000. The enzyme was kindly provided by A. H. Goldie (University of Saskatchewan, Saskatoon, Canada). Cytoplasmic and membrane proteins were phosphorylated by [^{32}P]PEP as described by Waygood et al. (29) with slight modifications. Samples containing between 40 and 250 μg of proteins were incubated in a 60-μl volume for 6 to 15 min at room temperature in the presence of 0.1 mM [^{32}P]PEP (specific activity, 1.0×10^5 to 3.33×10^5 cpm/nmol), 2 μg of EI, and 3 μg of HPr, both proteins having been purified from *Streptococcus mutans* DR0001 (20). The reaction was stopped by the addition of 22.5 μl of a solution containing 188 mM Tris-HCl (pH 8.0), 6% (wt/vol) SDS, 30% (vol/vol) glycerol, 6% (vol/vol) 2ME, and 0.005% bromophenol blue. Samples (75 μl) were then loaded onto polyacrylamide gels (16 by 16 cm) with a 10% resolving gel and subjected to denaturing SDS-PAGE. The gels were 1 mm thick and were run at 25 mA per gel for 4 h. Autoradiography of dried gels was performed either at room temperature for 24 h or at −80°C for 6 to 12 h with Kodak X-ray films (X-Omat AR).

PTS assay. The reaction mixture (600 μl) contained 50 mM sodium phosphate (pH 7.0); 4 mM $MgCl_2$; 2 mM PEP; 5 mM 2ME; 10 mM NaF; 10 mM ^{14}C-labelled sugars (0.1 μCi/μmol); 250 μg of cytoplasmic proteins as a source of EI, HPr, and putative IIA proteins; and 200 μg of membrane proteins as a source of II complexes. After a preincubation of 30 min at 37°C, the labelled sugar was added and the reaction was allowed to continue for 2 h at 37°C. Under these conditions, the reaction rate measured with membrane and cytoplasmic fractions of *S. salivarius* ATCC 25975 was linear in time for at least 2 h, using 2DG or glucose as the substrate. The amount of phosphorylated sugar produced was determined as previously described (26). The procedure for inhibiting PTS activity by the anti-III^{Man} antibodies was the same, except that the reaction medium was incubated with either anti-III$_L^{Man}$ or anti-III$_H^{Man}$ antibodies for 30 min before adding the labelled sugars. For practical purposes, the inhibition studies were carried out with 1 mg of purified antibody (2). This corresponded to the amount of anti-III$_H^{Man}$ antibody required to inhibit the 2DG PTS activity of the reference strain by approximately 50%. No effort was made to maximize antibody inhibition with the other strains tested.

RESULTS AND DISCUSSION

The presence of III^{Man} in representative strains of several species was investigated by three different approaches: West-

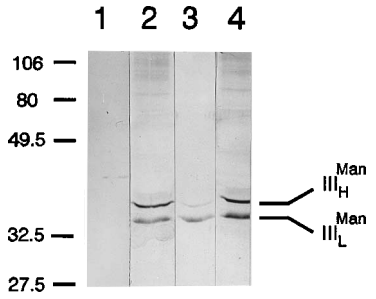

FIG. 1. Western blot analyses of a membrane-free cellular extract of *S. salivarius* ATCC 25975. Samples containing 100 μg of protein were electrophoresed in 10% acrylamide gels and electrophoretically transferred to a nitrocellulose sheet. The numbers on the left are molecular weights (in thousands). The lanes contain sample probed with preimmune serum (lane 1), anti-III_H^{Man} antibodies (lane 2), anti-III_L^{Man} antibodies (lane 3), and both antibodies (lane 4).

ern blot experiments, analysis of PEP-dependent phosphoproteins, and inhibition of PEP-dependent 2DG phosphorylation by anti-III^{Man} antibodies. For each test, the conditions were optimized with the reference strain *S. salivarius* ATCC 25975 and applied to the other species tested.

The reference strain: *S. salivarius* ATCC 25975. Figure 1 shows the results of Western blot experiments carried out with membrane-free cellular extracts of the reference strain probed with rabbit polyclonal antibodies produced against III_H^{Man} or III_L^{Man}. The results indicated that the anti-III_H^{Man} antibodies also reacted with III_L^{Man} (Fig. 1, lane 2) and that the anti-III_L^{Man} antibodies cross-reacted with III_H^{Man}, albeit very weakly (Fig. 1, lane 3). Identical results were obtained with membrane fractions (not shown).

The PEP-dependent phosphorylation of III^{Man} was conducted with membrane fractions and membrane-free cellular extracts of the tested strain in the presence of purified EI and HPr. As previously reported (2), two phosphoproteins with molecular weights corresponding to those of III_H^{Man} (38,900) and III_L^{Man} (35,200) were detected in membrane preparations (not shown) and in membrane-free cellular extract (Fig. 2, lane 1) prepared from glucose-grown cells of the reference strain.

TABLE 2. Effect of anti-III^{Man} antibodies on glucose and 2DG PTS activities[a]

Bacterial strains	2DG		Glucose	
	PTS activity	% Inhibition[c]	PTS activity	% Inhibition
S. salivarius ATCC 25975	134.0[b]	52.8	346.0	81.7
		30.5		52.3
S. salivarius ATCC 7073	346.0	81.7	419.0	46.6
		40.2		8.2
S. salivarius 149.1	237.3	61.2	89.5	60.2
		18.8		25.7
S. salivarius 30.1	81.7	42.3	67.1	—
		14.9		—
S. salivarius ATCC 13419	7.2	ND	6.6	ND
S. salivarius ATCC 27945	10.2	ND	141.6	48.6
				40.8
S. thermophilus ATCC 19258	94.0	—	96.5	—
S. vestibularis ATCC 49124	5.0	ND	13.2	ND
S. mutans Ingbritt	38.5	25.2	235.2	—
		61.1		—
S. cricetus ATCC 19642	184.0	10.1	250.1	—
		51.5		—
S. sanguis ATCC 10556	757.4	30.0	1263.0	77.1
		60.0		55.6
S. gordonii ATCC 10558	65.5	66.3	365.9	—
		52.8		—
S. anginosus ATCC 33397	164.4	68.0	297.7	8.2
		38.8		46.5
S. bovis ATCC 35034	871.4	93.4	1075.3	91.6
		12.9		39.6

[a] The reaction mixture (600 μl) contained 50 mM sodium phosphate (pH 7.0), 4 mM $MgCl_2$, 2 mM PEP, 5 mM 2ME, 10 mM NaF, 10 mM ^{14}C-labelled sugars (0.1 μCi/μmol), 250 μg of cytoplasmic extract as a source of EI, HPr, and EIII, and 200 μg of membranes as a source of EII and EIII. Activities are expressed as nanomoles of phosphorylated sugar per milligram of membrane per 120 min.

[b] Although 2DG is apparently only phosphorylated by the mannose PTS in the reference strain, we do not know whether this is the case in the other strains.

[c] The first value for each strain is the percent inhibition by 1.0 mg of anti-III_H^{Man} antibody. The second value for each strain is the percent inhibition by 1.0 mg of anti-III_L^{Man} antibody. —, no inhibition; ND, not determined.

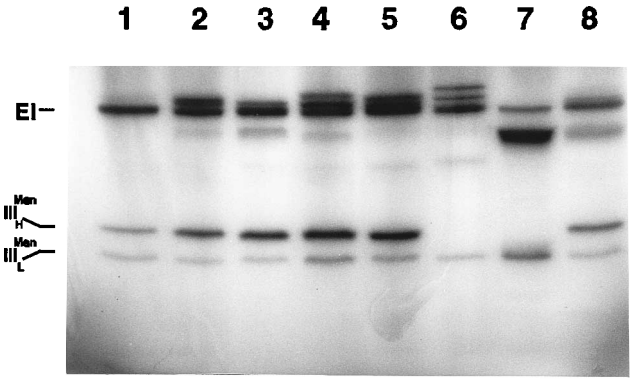

FIG. 2. Detection of III^{Man}-like proteins by [^{32}P]PEP-dependent phosphorylation. The samples were analyzed by SDS-PAGE, with the resolving gel containing 10% acrylamide. HPr is not retained in the gel under the conditions used. Prior to electrophoresis, the samples were incubated for 6 to 15 min in the presence of 0.1 mM [^{32}P]PEP (specific activity, 1.0×10^5 to 3.3×10^5 cpm/nmol) and purified HPr and EI. The bands corresponding to phospho-III_H^{Man} of the reference strain were identified on the gel by comparison of their molecular weights with the molecular weight of the III_L^{Man} proteins determined by Western blot analysis (2). Lane 1, soluble proteins of *S. salivarius* ATCC 25975; lane 2, membrane proteins of *S. salivarius* ATCC 7073; lane 3, membrane proteins of *S. salivarius* 30.1; lane 4, membrane proteins of *S. salivarius* 149.1; lane 5, membrane proteins of *S. salivarius* ATCC 13419; lane 6, membrane proteins of *S. salivarius* ATCC 27945; lane 7, membrane proteins of *S. thermophilus* ATCC 19258; lane 8, membrane proteins of *S. vestibularis* ATCC 49124.

The presence of a III^{Man}-like protein was ascertained by measuring the kinase activities of the mannose PTS and by studying the effect of anti-III^{Man} antibodies on these activities. As 2DG is specific for the mannose PTS (3, 17, 26, 27), we used it as a marker for the presence of this PTS in the tested strains. We also measured glucose PEP-dependent phosphorylation activities and tested the effect of anti-III^{Man} on these activities, as we wanted to determine whether glucose could be transported by a PTS distinct from the mannose PTS in other bacteria. The results shown in Table 2 do not indicate optimal activities, as the amount of ancillary proteins, particularly III^{Man}, required for maximum activity was not optimized and can vary from one species to another. The values, however, are indicative of the presence or the absence of a PTS that phosphorylates 2DG and/or glucose. Under the conditions described in Materials and Methods, the phosphorylation of 2DG by membranes of the reference strain was inhibited 50% by anti-III_H^{Man} antibodies and 30% by anti-III_L^{Man} antibodies. The phosphorylation of glucose was inhibited 82 and 52% by anti-III_H^{Man} and anti-III_L^{Man} antibodies, respectively.

The *S. salivarius* group. Oral streptococci can be divided into three main species groups: the *Streptococcus oralis* group, the *S. mutans* group, and the *S. salivarius* group (8). The *S. saliva-*

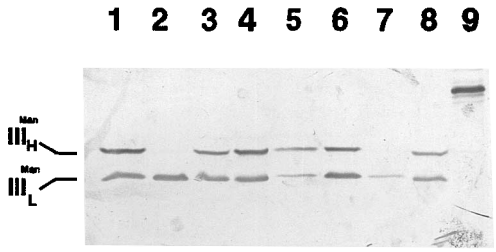

FIG. 3. Western blot analyses with anti-III$_H^{Man}$ antibodies. The results illustrated in lanes 1 to 8 were obtained with membrane-free cellular extracts. Identical results were obtained with the membrane fractions. The result illustrated in lane 9 was obtained only with the membrane fraction. Lane 1: *S. salivarius* ATCC 25975; lane 2, *S. salivarius* ATCC 27945; lane 3, *S. salivarius* ATCC 13419; lane 4, *S. salivarius* ATCC 7073; lane 5, *S. salivarius* 30.1; lane 6, *S. salivarius* 149.1; lane 7, *S. thermophilus* ATCC 19258; lane 8, *S. vestibularis* ATCC 49124; lane 9, *S. aureus* ATCC 6538. The membrane protein of *S. aureus* reacting with anti-*S. salivarius* III^{Man} antibodies migrated at a position corresponding to a molecular weight of 50,000, which is much higher than the molecular weights of III$_H^{Man}$ and of III$_L^{Man}$ of *S. salivarius*. Similar results were obtained with anti-III$_L^{Man}$ antibodies.

rius group comprises three closely related species: *S. salivarius*, *Streptococcus vestibularis*, and *Streptococcus thermophilus*. Western blot experiments conducted with five strains of *S. salivarius*, one strain of *S. vestibularis*, and one strain of *S. thermophilus* (Fig. 3) indicated that four strains of *S. salivarius* and the *S. vestibularis* strain possessed two peptides cross-reacting with anti-III$_H^{Man}$ as well as with anti-III$_L^{Man}$. The molecular weights of these peptides corresponded to those of III$_H^{Man}$ and III$_L^{Man}$ of the reference strain. Only one strain of *S. salivarius* (strain ATCC 27945) (Fig. 3, lane 2) did not possess both forms of III^{Man}. This strain possessed a single peptide with a molecular weight of 35,200, corresponding to the size of III$_L^{Man}$. The same result was obtained with the representative strain of *S. thermophilus* (Fig. 3, lane 7). As *S. salivarius* ATCC 27945 could grow at 45°C (result not shown), a phenotypic trait characteristic of the *S. thermophilus* species (8), strain ATCC 27945 might be *S. thermophilus* rather than *S. salivarius*. All the peptides detected by Western blot analysis in the strains belonging to the *S. salivarius* group were phosphorylated by PEP in the presence of EI and HPr (Fig. 2). Three out of four strains of *S. salivarius* that possessed the two forms of III^{Man} also possessed 2DG PTS activity that was inhibited by anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies (Table 2). These three strains also possessed glucose PTS activity, and in two of them (strain ATCC 7073 and strain 149.1), the activity was inhibited by the two sets of antibodies. Therefore, in at least three *S. salivarius* strains (strain ATCC 7073 and strains 149.1 and 30.1), the data for PTS activity and inhibition by antibodies were consistent with the presence of a mannose PTS similar to the system found in the reference strain ATCC 25975. As glucose PTS activity was not inhibited by the antibodies in strain 30.1, we propose that this strain possesses an additional glucose PTS, independent of the III^{Man} proteins. No significant 2DG or glucose PTS activity could be detected in *S. salivarius* ATCC 13419, even though this strain possessed both forms of III^{Man} and grew well on mannose and glucose (generation times were between 40 and 50 min at 37°C). One may speculate that this strain has lost or did not express to a detectable level the permease counterpart of the mannose PTS (protein IIC) and took up mannose and glucose by non-PTS transport systems. *S. salivarius* ATCC 27945 had barely detectable PEP-dependent phosphorylation activity for 2DG but possessed glucose as well as α-methyl-glucoside (not shown) PTS activities that were inhibited by anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies. As mentioned, this strain possessed only a III$_L^{Man}$-like protein. The results suggested that glucose is transported in *S. salivarius* ATCC 27945 by only one PTS which requires a IIA^{Glc}-like protein for activity. The molecular structure as well as the specificity of this system is reminiscent of the glucose PTS of the family *Enterobacteriaceae* (14, 18). Determination of whether the IIA^{Glc}-like protein of *S. salivarius* ATCC 27945 possesses regulatory functions similar to those reported for IIA^{Glc} of *E. coli* requires further study. The results obtained with *S. thermophilus* did not support the involvement of the III$_L^{Man}$-like protein detected in this species in the PEP-dependent phosphorylation of 2DG and glucose, as these activities were not influenced by the presence of anti-III^{Man} antibodies. The presence of alternative 2DG and glucose PTSs in this species that would have masked the inhibitory effect of the antibodies could not, however, be ruled out. Finally, no significant PTS activity was detected in *S. vestibularis*. Therefore, it was not possible to associate the III^{Man}-like proteins detected in *S. vestibularis* with the transport of 2DG and glucose.

TABLE 3. Presence of III^{Man}-like proteins in different strains of oral and nonoral gram-positive bacteria

Main group	Strain tested	MW (kDa)[a]	PEP-dependent phosphorylation[b]
S. mutans	*S. mutans* DR0001	38.9	+
	S. mutans ATCC 25175	35.5	+
	S. mutans T8	36.5	+
	S. mutans GS-5	35.5	+
	S. mutans Ingbritt	35.5	+
	S. sobrinus ATCC 27352	37.1	+
	S. sobrinus ATCC 33478	38.9	+
	S. cricetus ATCC 19642	35.5	+
	S. rattus FA-1	36.5	−
	S. rattus ATCC 19645	37.1	−
	S. ferus ATCC 33477	38.9	−
S. oralis	*S. oralis* ATCC 35037	36.5	−
	S. mitis ATCC 33399	35.5	−
	S. pneumoniae R800	36.5	+
	S. sanguis ATCC 10556	36.1	−
	S. sanguis NY101	36.1	−
	S. gordonii ATCC 10558	35.5	+
	S. parasanguis ATCC 15912	38.0	+
	S. anginosus ATCC 33397	36.5	+
	S. constellatus ATCC 27823	36.5	−
Other streptococci	*S. suis* 170-B	38.9	+
	S. bovis ATCC 35034	38.9	+
Lactic acid bacteria	*L. lactis* ATCC 11454	38.9	ND
	L. lactis ATCC 19257	38.9	ND
	L. casei ATCC 4646	35.5	+

[a] Molecular weight (MW) was determined by Western blot analysis of the cytoplasmic and membrane fractions, using polyclonal anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies.
[b] ND, not determined.

The *S. mutans* group. The *S. mutans* group consists of seven distinct species: *S. mutans*, *Streptococcus sobrinus*, *Streptococcus cricetus*, *Streptococcus rattus*, *Streptococcus ferus*, *Streptococcus macacae*, and *Streptococcus downei* (8). Western blot experiments conducted with five strains of *S. mutans*, two strains of *S. sobrinus*, two strains of *S. rattus*, one strain of *S. cricetus*, and one strain of *S. ferus* indicated that all of these species possessed a single polypeptide that cross-reacted with anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies. The molecular weight

of the peptide was variable among these species and ranged from 35,500 to 38,900 (Table 3). The proteins of *S. mutans*, *S. sobrinus*, and *S. cricetus* were phosphorylated at the expense of PEP in the presence of EI and HPr. Such phosphorylation could not be detected in the case of *S. rattus* and *S. ferus*, even when using homologous cellular extracts as a source of EI and HPr. Membranes from glucose-grown cells of *S. mutans* and *S. cricetus* catalyzed the PEP-dependent phosphorylation of 2DG in the presence of EI and HPr, and these activities were inhibited by anti-III^{Man} antibodies (Table 2). These membranes also catalyzed the PEP-dependent phosphorylation of glucose. However, this activity was not affected by the presence of anti-III$_H^{Man}$ or anti-III$_L^{Man}$ antibodies, indicating that alternative PTSs were involved in the transport and phosphorylation of glucose in these species. These results are consistent with those of Vadeboncoeur (26) and of Néron and Vadeboncoeur (15), who reported the presence of two distinct glucose PTSs in *S. mutans.*

The *S. oralis* group. The *S. oralis* group consists of ten species (8): *S. oralis*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Streptococcus sanguis*, *Streptococcus gordonii*, *Streptococcus parasanguis*, *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus intermedius*, and a "tufted-fibril group" now considered to be *Streptococcus crista* (7). Western blot experiments carried out with two strains of *S. sanguis* and one strain each of *S. oralis*, *S. mitis*, *S. pneumoniae*, *S. gordonii*, *S. parasanguis*, *S. anginosus*, and *S. constellatus* revealed that all of these species possessed a single polypeptide that cross-reacted with anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies. The molecular weights of the peptides were variable, ranging from 35,500 to 38,000 (Table 3). The III^{Man}-like proteins of *S. anginosus*, *S. gordonii*, *S. parasanguis*, and *S. pneumoniae* were phosphorylated at the expense of PEP in the presence of EI and HPr. No phosphorylated forms of the cross-reacting proteins found in the other species could be detected under the experimental conditions used in this study. Assays carried out with *S. sanguis*, *S. anginosus*, and *S. gordonii* indicated that these three species possessed 2DG PTS activity that was inhibited by anti-III^{Man} antibodies (Table 2). These results were consistent with the participation of a III^{Man}-like protein in the transport of the mannose PTS substrates in these species. Glucose PTS activities were also found in these three species. Anti-III^{Man} antibodies reduced these activities in *S. anginosus* and in *S. sanguis* but not in *S. gordonii*, suggesting that *S. gordonii* possesses a separate glucose PTS.

Other streptococci. We looked for the presence of III^{Man} in two other streptococcal species: *Streptococcus bovis* and *Streptococcus suis*. Genetically, *S. bovis* forms a distinct cluster with *Streptococcus equinus* and *Streptococcus alactolyticus*, whereas *S. suis* is distinct and displays no specific relationship with other streptococcal species (1). Western blot experiments as well as analysis of PEP-dependent phosphoproteins by SDS-PAGE indicated the presence in both species of a III^{Man}-like protein with a molecular weight of 38,900, corresponding to the molecular weight of III$_H^{Man}$ of the reference strain (Table 3). *S. bovis* possessed high levels of 2DG and glucose PTS activities (Table 2), and both activities were inhibited by anti-III^{Man} antibodies. These results are consistent with and complement those of Martin and Russell (11, 12), who reported glucose and 2DG PTS activities in *S. bovis* JB1. PTS activities in *S. suis* were not measured.

Other lactic acid bacteria. The presence of III^{Man} was also tested for in two strains of *Lactococcus lactis* and in one strain each of *Enterococcus faecalis*, *Enterococcus hirae*, and *Lactobacillus casei*. With the exception of the enterococci, these species possessed a protein that cross-reacted with anti-III^{Man} antibodies. A 38.9-kDa protein was detected in the two strains of *L. lactis* tested, whereas in *L. casei*, a protein with a molecular weight of 35,500 cross-reacted with anti-III^{Man} antibodies and was phosphorylated by PEP in the presence of EI and HPr (Table 3). The III^{Man}-like protein of *L. casei* was, however, found only in the membrane fraction. This result explains why in a preliminary study (2), we did not find a III^{Man}-like protein in this strain of *L. casei*, as we had only analyzed the cytoplasmic fraction. Our results are consistent with the recent finding of Veyrat et al. (28), who reported the presence of a mannose PTS in *L. casei* ATCC 393 that would be involved in catabolite repression.

Other gram-positive bacteria and *E. coli*. We did not find any protein that cross-reacted with anti-III^{Man} antibodies in *B. stearothermophilus*, *Bacillus subtilis*, and *E. coli*. We detected, however, in *Staphylococcus aureus*, a membrane protein with a molecular weight of 50,000 that strongly reacted with both anti-III$_H^{Man}$ and anti-III$_L^{Man}$ antibodies (Fig. 3, lane 9). We did not, however, find a PEP-dependent phosphoprotein having the same molecular weight.

Conclusion. The two forms of III^{Man} were detected only in two species, *S. salivarius* and *S. vestibularis*, both species belonging to the *S. salivarius* group of oral streptococci. Nevertheless, the presence of a III^{Man}-like protein was detected in several other streptococcal species and was also found in evolutionarily distant genera belonging to other clusters of gram-positive bacteria (22), such as the genus *Lactobacillus*. However, other genera of equivalent evolutionary distance, such as *Enterococcus* and *Bacillus*, did not seem to possess a protein homologous to III^{Man}. It is, therefore, difficult on the basis of our results to indicate with precision the appearance of an ancestral protein during the evolution of gram-positive bacteria.

Our results suggest that the type and number of glucose transport systems in streptococci are variable even among strains of the same species. Some strains, such as *S. salivarius* ATCC 25975, transport glucose mainly by the mannose PTS (2, 3, 17, 26), whereas other strains possess, in addition to the mannose PTS, an alternative PTS or non-PTS glucose transport system independent of III^{Man}. It appears, therefore, that generalizations about glucose transport in streptococci must be avoided and that each strain should be characterized individually.

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