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Selective Adsorption of Heterophile Polyglycerophosphate Antigen from Antigen Extracts of *Streptococcus mutans* and Other Gram-Positive Bacteria

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Hot saline extracts of *Streptococcus mutans* have been shown to contain antigenic substances which occasionally react nonspecifically with some antisera against whole cells of various serological groups and types of streptococci. Chromatography of the extract of *S. mutans* strain MT703 (serotype *e*) on a diethylaminoethyl-Sephadex A-25 column gave two principal antigens. One antigen was eluted without adsorption to the resin and was identified as the serotype-specific polysaccharide. The other antigen, which contained a large quantity of phosphorus, was adsorbed to and released from the resin by gradient elution. It was reactive against the antisera specific for polyglycerophosphate (PGP) from group A *Streptococcus pyogenes* and/or *S. mutans* strain Ingbritt (type *c*). The PGP antigen was further purified by gel filtration with Sephadex G-75. Two peaks, PGP-1 and PGP-2, were obtained. Each possessed the same antigenic specificity to anti-PGP serum as shown by immunodiffusion. Chemical analyses revealed that the molar ratio of phosphorus to glycerol in both was about 1:1, although the protein content between the two was significantly different. PGP antigen was found to be widely distributed in hot saline extracts from various gram-positive bacteria, with a few exceptions. However, all gram-negative bacteria examined were free of PGP. The PGP in the hot saline extracts of various gram-positive bacteria possessed an essentially identical antigenic specificity. The addition of diethylaminoethyl-Sephadex A-25 resin to hot saline extracts successfully removed the cross-reacting PGP antigen. After adsorption of the extract from *S. mutans*, the supernatant contained only type-specific polysaccharide antigen, except type *b*, in which both type *b*-specific polysaccharide and PGP antigens were adsorbed with the resin. This simple procedure should be useful for the removal of the PGP-type teichoic acid from antigen extracts of bacteria that contain uncharged polysaccharides.

The immunological specificity of bacterial cells is a property that has made a significant contribution to their classification. In the case of many streptococci, crude antigen-containing extracts of whole cells are used. Some of these procedures use dilute acid (17), formamide (7), and saline (26) at temperatures of 100°C or above. The saline extraction method of Rantz and Randall (RR extracts) has been frequently used in clinical laboratories and experimental investigations (10, 11, 16, 25, 26) because of its simplicity and reproducibility. Although the method has provided useful extracts for studies on the serotype-specific antigens of *Streptococcus mutans* (10), we have found that these extracts also contain substances that cross-react with anti-polyglycerophosphate (PGP) serum in addition to antiserum to the type-specific polysaccharide antigen. Therefore, removal of PGP from these extracts is necessary to identify the specificity of the polysaccharide. The present paper describes procedures for the removal of the PGP with anionic exchange resin from crude antigen extracts and the presence of PGP antibody in rabbit antisera to *S. mutans* and many other gram-positive bacteria.

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MATERIALS AND METHODS

Bacterial strains. Most of the streptococcal strains used in the present study were kept in the lyophilized state in our laboratories. A strain of *Micrococcus citreus* was obtained from the Midwest Culture Service (Terre Haute, Ind.) and designated as MCS645 according to its code number. Many strains of other species were generously given by T. Umemoto (New York State University School of Dentistry at Buffalo, Buffalo), B. Guggenheim

(Dental Institute, University of Zurich, Zurich, Switzerland), and N. Masuda (Osaka University Dental School, Osaka, Japan). All strains were grown in Todd-Hewitt broth (Difco).

Extraction of the antigen. Routine extraction of antigen from lyophilized cells was done according to a modified procedure of Rantz and Randall (26). Cells were suspended in 20 mg (dry weight) per ml of saline and autoclaved at 120°C for 20 min. They were centrifuged, and the supernatant was used as a crude extract (RR) for serological investigation. The PGP from group A *S. pyogenes* strain Richard (type 3) was prepared as previously described (22).

Purification of the cross-reacting antigen. The chemical nature of the cross-reacting antigen was investigated in *S. mutans* MT703 (serotype *e*) extract. The hot saline extract was applied to a column of diethylaminoethyl (DEAE)-Sephadex A-25, and three antigenic peaks (fractions *e*I, *e*II, and *e*III) were obtained (11). The first two peaks were composed of type *e*-specific polysaccharide antigen, whereas the third antigen (*e*III) contained a large quantity of phosphorus and showed a strong precipitin reaction against specific PGP antiserum (vide infra). Consequently, *e*III was purified on a Sephadex G-75 column.

Fraction *e*III (20 mg, dry weight) was suspended in 2 ml of saline. After vigorous shaking, the suspension was centrifuged to remove insoluble materials. The supernatant was applied to a Sephadex G-75 column (55 by 2 cm). The column was eluted with 0.15 M saline, and the fractions (5 ml/tube) were collected. Total phosphorus and protein were estimated for each fraction. Fractions that reacted with PGP-IB antiserum (see below) were monitored by the capillary precipitin test as described previously (11).

Antisera. One lot of antiserum against whole cells of *S. mutans* strain Ingbritt (IB) (serotype *c*) was found to be specific for PGP and free from anti-type *c*-specific antibody (9). This was designated as PGP-IB antiserum. Another reference antiserum specific for PGP of group A *S. pyogenes* (PGP-A antiserum) was kindly supplied by T. Myoda, Du Pont Institute. Furthermore, the antiserum against whole cells of *S. mutans* strain MT703 contained significant PGP antibody in addition to type *e* polysaccharide antibody (11, 12). Serotype-specific antisera (types *a* to *g*) were prepared in this laboratory by the absorption of 1 ml of serum with 50 mg (dry weight) of group A streptococcal cells or provided by B. Perch (Statens Seruminstitut, Copenhagen, Denmark) and N. Masuda. Antiserum against Lancefield group E streptococcal cells was obtained from the Center for Disease Control, Atlanta, Ga. Antiserum specific for glucan and dextran T-2000 was obtained by immunizing rabbits with sucrose-grown cells of *S. mutans* strain MT123 (9).

Immunological procedures. Capillary precipitin tests, immunodiffusion, and immunoelectrophoresis were carried out as previously reported (11).

Chemical analyses. To determine the composition of the material that cross-reacted with PGP antisera, the samples were hydrolyzed in 2 N HCl for 18 h at 100°C in sealed ampoules and dried in vacuo, and samples of the hydrolysates were spotted on precoated silica gel plates (Brinkmann Instruments Inc., Westbury, N.Y.). The plates were developed with the solvent system of *n*-propanol–ammonia–water, 6:1:3 (vol/vol/vol) (5). The components were located by spraying with aniline-phthalate (Brinkmann) and potassium permanganate-sulfuric acid (6).

For quantitative estimation of glycerol, the hydrolysates obtained above were further hydrolyzed with alkaline phosphatase (Sigma) to convert the residual glycerophosphate into glycerol and phosphate (5). The glycerol content was determined enzymatically (Glycerol Stat-Pak, Calbiochem, La Jolla, Calif.). Protein was estimated by using the Folin phenol reagent, total hexose was estimated by the authrone reagent, and total phosphorus was estimated by ammonium molybdate-ascorbic acid in sulfuric acid, as previously described (18).

RESULTS

Characterization of RR extract of the whole cells of *S. mutans* strain MT703 (serotype *e*). To determine the number and specificity of the antigenic components in a crude RR extract, the extract of *S. mutans* MT703 was subjected to electrophoresis in veronal-hydrochloride buffer (pH 8.25) and then tested with unadsorbed MT703 antisera (lot-R545) and against PGP derived from group A *S. pyogenes*. Figure 1 shows that the antigen extract gave two precipitin arcs against anti-MT703 serum. One antigen showed a slight movement toward the cathode, whereas the second antigen showed extensive movement toward the anode. The second antigen gave a clear precipitin arc against PGP-A (and PGP-IB) antiserum, with the same mobility as the arc developed against MT703 antiserum. This behavior, and the pres-

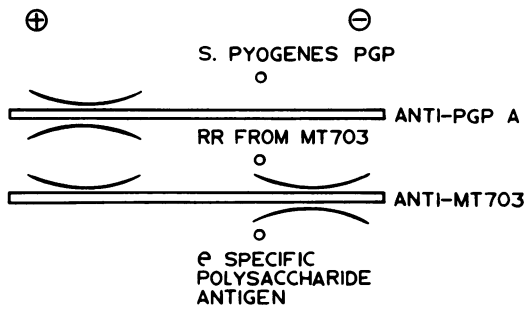

FIG. 1. *Diagram of immunoelectrophoresis of PGP from group A S. pyogenes, autoclave extract of S. mutans strain MT703 (serotype e) in saline (RR extract), and purified type e antigen from MT703 cells against PGP-A antiserum and the unadsorbed MT703 whole-cell antiserum. Electrophoresis was carried out at 6 mA for 1 h. Noble agar (Difco, 1%) was melted in barbital-hydrochloride (pH 8.25; ionic strength, 0.05) buffer.*

ence of a negative charge, indicated the presence of a PGP-like antigen. Immunodiffusion tests in agar gel demonstrated that the RR extract and PGP from group A *S. pyogenes* formed a fused precipitin line against PGP antisera (data not shown). The precipitin arc that moved to the cathode was shown in previous studies to be the type *e*-specific polysaccharide (11).

Separation and purification of PGP antigen from the RR extract. The RR extract of MT703 cells was separated into three antigenic components, *e*I, *e*II, and *e*III, as shown previously (11). Antigens *e*I and *e*II were the type *e*-specific antigens and had the same antigenic specificity, whereas *e*III contained phosphorus and gave a positive immunological reaction only against PGP antisera.

*e*III was further fractionated by gel filtration with Sephadex G-75 (Fig. 2). The fractions that gave a positive precipitin reaction against PGP antiserum were combined and designated as PGP-1 and PGP-2. The recoveries of PGP-1 and PGP-2 were 16 and 10% of the starting material (*e*III), respectively. Both fractions had the same immunological specificity against anti-PGP-A antiserum (Fig. 3), and PGP-1 reacted with both PGP-IB and PGP-A antisera.

Chemical analyses revealed that PGP-1 and PGP-2 were composed of glycerol, phosphorus, protein, and total hexoses in a ratio (weight basis) of 2.9:1.0:1.5:1.0 and 2.5:1.0:8.8:1.1, respectively. It is of interest that the molar ratio of glycerol and phosphorus of both antigens was about 1:1, although the protein content of PGP-2 was significantly higher than that of PGP-1. The minimum concentrations of PGP-1 and PGP-2 that gave a definite positive reaction against PGP-IB antiserum in the capillary precipitin test were 0.001 and 0.01 μg/μl, respectively.

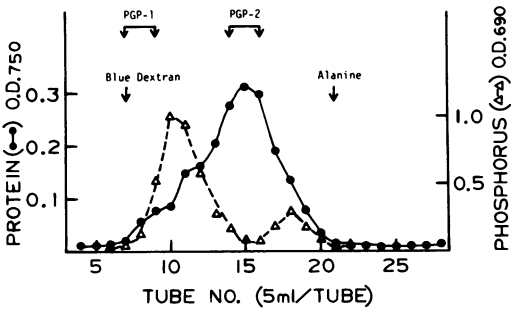

FIG. 2. *Gel filtration on Sephadex G-75 column of antigen eIII reactive with PGP-IB antiserum. eIII was obtained by applying the RR extract of MT703 on a DEAE-Sephadex A-25 column as described previously (11). The Sephadex G-75 column (2 by 55 cm) was eluted with saline; the fractions reacting with PGP-IB antiserum by capillary precipitin test are marked by brackets.*

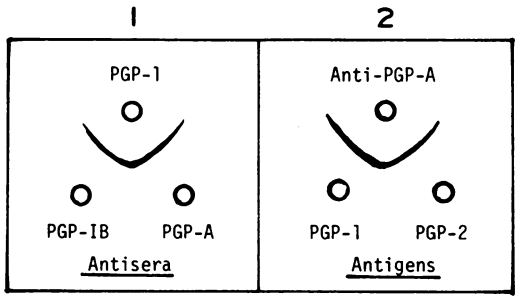

FIG. 3. *Diagram of immunodiffusion in agar gel showing the identity of immunological specificity between PGP-IB and PGP-A antisera and between PGP-1 and PGP-2.*

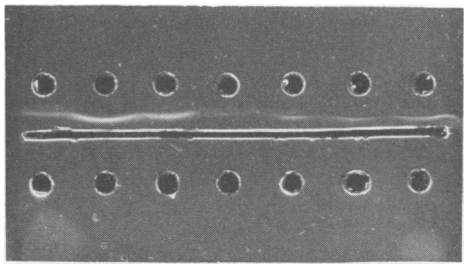

FIG. 4. *Immunodiffusion showing the presence of a common antigen in 10 μl of RR extracts (upper holes) from different serotypes of S. mutans cells and the extracts after adsorption (lower holes) with DEAE-Sephadex A-25. From left to right: RR extract from S. mutans strains HS6 (a), FA1 (b), Ingbritt (c), B13 (d), MT703 (e), OMZ175 (f), K1R (g). A 100-μl amount of PGP-IB antiserum was used.*

Presence of PGP in the other serotypes of *S. mutans*. Crude antigen extracts of *S. mutans* cells that belong to serotypes *a* to *g* were prepared by the RR procedure. Immunodiffusion tests revealed that all the extracts reacted with anti-PGP serum (Fig. 4, upper section). The continuous precipitin line suggested that a similar immunological specificity was present in each extract.

Specific adsorption of PGP from the crude antigen with DEAE-Sephadex A-25. Previous findings (9, 11, 18, 19, 23) and Fig. 1 show that PGP components from *S. mutans* are highly negatively charged due primarily to a high content of phosphorus. Therefore, it was considered that PGP would bind tightly to an anionic exchange resin, such as DEAE-Sephadex A-25, and would be released by a solution of higher ionic strength and/or an elution buffer of lower pH. On the other hand, most *S. mutans* polysaccharide antigens are weakly charged and show

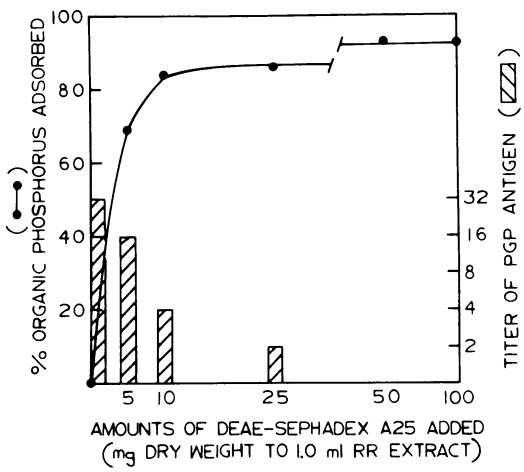

FIG. 5. *Adsorption of PGP-antigen from RR extract of S. mutans MT703 with DEAE-Sephadex A-25. One milliliter of RR extract was mixed with 5, 10, 25, 50, and 100 mg of A-25. The supernatant was assayed for organic phosphorus, and the antigenic titer against PGP-A antiserum was determined by the capillary precipitin test.*

no adsorption to Sephadex A-25 (9, 11, 18, 19, 23). These properties prompted us to use the A-25 resin as a specific adsorbant of PGP-containing polymers.

One milliliter of crude MT703 extract was added to 10-, 25-, 50-, 75-, and 100-mg (dry weight) quantities of commercial DEAE-Sephadex A-25 powder without pretreatment. After being mixed several times at room temperature, the mixture was held until the resin settled. The supernatant was then examined by the precipitin test for reactivity against PGP-A antiserum.

Figure 5 shows that the minimum amount of A-25 to completely remove the PGP antigen from the crude extract was 50 mg to 1 ml of extract. No reduction in the type-specific polysaccharide antigen was observed. Selective adsorption of PGP by this procedure from the crude extract of the other serotypes of *S. mutans* cells is shown by the absence of any precipitin lines in the lower section of Fig. 4. In numbers 1 and 5 (upper section) a second faint precipitin line can be seen. Figure 2 shows that two fractions that possessed different molecular weights were present in the extract. These possessed an identical specificity (Fig. 3) and are responsible for the two precipitin lines seen.

Table 1 shows that the various crude extracts adsorbed with A-25 lost precipitin reactivity against PGP-IB antiserum. Table 1 also shows that the unadsorbed antiserum against *S. mutans* MT703 (serotype *e*) whole cells contained the cross-reacting PGP antibody. This was supported by the fact that although crude extracts of all serotypes reacted with the antiserum, A-25-treated extracts, except homologous type *e* extracts, did not react with the antiserum. Adsorption of the antiserum with group A *S. pyogenes* cells (Table 1) or group E streptococcal cells (data not shown) resulted in a loss of cross-reactivity with the crude extracts, and only those extracts possessing the homologous type-specific *e* polysaccharide remained active in the capillary precipitin test.

Table 2 shows that all serotype-specific antigens except type *b* antigen remained fully active after adsorption of the crude antigen extracts with A-25 resin. Type *f* RR extracts adsorbed with A-25 were found to cross-react with antiglucan serum (9).

Distribution of PGP in the crude antigen extract from various bacteria. The presence or absence of antigenic substance in RR extracts from various bacteria was examined using PGP-IB antiserum. It was found that most gram-positive bacteria contained PGP. Bacteria containing PGP were the following: *Streptococcus* group A (Richards), group C (26Rp66), group D (ATCC 9790, ATCC 8307), group E (K129, K131, Newson), group H and/or *S. sanguis* (Challis, Hockley, Channon, ATCC 10556, OMZ9, ST3), group G (9603), group L (D167A), group N (C559, ATCC 9936), and group R (735); *S. salivarius* (SS2); *S. bovis* (8177); *S. mitis* (ATCC 903); *L. plantarum* (ATCC 8014); *L. casei* (ATCC 4646); *L. fermentum*; and *Staphylococcus aureus* (209P). The following bacteria

TABLE 1. *Demonstration of cross-reacting anti-PGP in the antiserum against S. mutans MT703 whole cells (serotype e)*

RR extract from strain (serotype):	Capillary precipitin reaction					
	Anti-MT703 (unadsorbed)		Anti-MT703 adsorbed with group A *S. pyogenes* cells		Anti-PGP-IB	
	RR	RR + A-25	RR	RR + A-25	RR	RR + A-25
AHT (*a*)	3+	–	–	–	3+	–
HS6 (*a*)	3+	–	–	–	3+	–
BHT (*b*)	2+	–	–	–	3+	–
FA1 (*b*)	2+	–	–	–	3+	–
IB (*c*)	3+	±	–	–	3+	–
C67-1 (*c*)	3+	–	–	–	3+	–
B13 (*d*)	2+	–	–	–	2+	–
MT703 (*e*)	4+	4+	4+	4+	3+	–
SE3 (*e*)	3+	3+	3+	3+	3+	–
SE11 (*f*)	2+	–	–	–	2+	–
OMZ175 (*f*)	2+	–	–	–	2+	–
K1R (*g*)	+	–	–	–	2+	–

TABLE 2. *Immunological reactivity of RR extract of seven serotypes of S. mutans with or without adsorption by DEAE-Sephadex A-25*

RR extract from strain (serotype):	Antiserum against:	Immunological reaction against homologous antiserum	
		Nonadsorbed RR extract	RR extract after adsorption with A-25
AHT (*a*)	HS6 (*a*)	3+	3+
HS6 (*a*)	HS6 (*a*)	2+	3+
BHT (*b*)	FA1 (*b*)	4+	–
FA1 (*b*)	FA1 (*b*)	4+	–
OMZ51 (*b*)	FA1 (*b*)	4+	–
OMZ71 (*b*)	FA1 (*b*)	4+	–
IB (*c*)	MT3 (*c*)	3+	3+
C67-1 (*c*)	MT3 (*c*)	3+	3+
GS5 (*c*)	MT3 (*c*)	3+	3+
B13 (*d*)	B13 (*d*)	3+	3+
OMZ176 (*d*)	B13 (*d*)	3+	3+
MT703 (*e*)[a]	MT703 (*e*)	4+	4+
LM7 (*e*)[a]	MT703 (*e*)	4+	4+
SE3 (*e*)[a]	MT703 (*e*)	3+	3+
SE11 (*f*)[b]	SE11 (*f*)	2+	2+
OMZ175 (*f*)[b]	SE11 (*f*)	3+	2+
MT557 (*f*)[b]	SE11 (*f*)	3+	3+
EN19-1 (*f*)[b]	SE11 (*f*)	3+	3+
K1R (*g*)	OMZ65 (*g*)	3+	3+
OMZ65 (*g*)	OMZ65 (*g*)	3+	3+

[a] These antigens (both with or without adsorption by DEAE-Sephadex A-25) also reacted with anti-Lancefield group E serum (11).

[b] These antigens (both with or without adsorption by DEAE-Sephadex A-25) also reacted with antiglucan serum (9).

contained no PGP: *Actinomyces naeslundii* (ATCC 12104); *Actinomyces viscosus* (ATCC 19246, OMZ105); *Streptococcus* group O (B357, B361); *M. luteus* (NCTC 2665); *M. citreus* (MCS 465); *Proteus mirabilis* (OMZ109); *Escherichia coli* (OMZ116, E16); *Serratia marcescens* (OMZ144); *Neisseria perflava* (OMZ143); *Leptotrichia buccalis* (ATCC 19616); and *Fusobacterium nucleatum* (ATCC 25586). Furthermore, immunodiffusion tests showed that the immunological specificities of PGP antigens were identical among different species of streptococci (Fig. 6). RR extracts from group D and N streptococci gave no precipitin line against PGP antisera on immunodiffusion plates, although these antigens were reactive with PGP antisera by the capillary precipitin method (Table 3). However, no PGP activity was demonstrated in the extracts of group O *Streptococcus, Micrococcus* spp., and *Actinomyces* spp., although each is gram positive. Gram-negative bacteria examined were found to be PGP negative. Although an RR extract of each *Micrococcus* spp. did not give a positive reaction against the PGP antisera, the RR extract of *M. citreus* MCS645 grown in Trypticase soy broth (BBL) fortified with 10 g of glycerol, 3 g of K_2HPO_4, and 2 g of KH_2PO_4 per liter of medium gave a positive reaction by the capillary precipitin test.

DISCUSSION

The presence of PGP as a common antigenic component among many gram-positive bacterial species has been demonstrated in crude antigen extracts obtained by different methods (3, 4, 15, 20, 23, 28, 35). This type of immunological factor has been termed heterophile or heterogenic (15) or Forssman antigen (32) because of its wide distribution among the bacteria.

During studies on the immunological specificities of the various serotypes of *S. mutans*, we frequently found that antisera obtained by immunizing rabbits with whole cells of *S. mutans* showed cross-reactions with heterologous antigen extracts of various types.

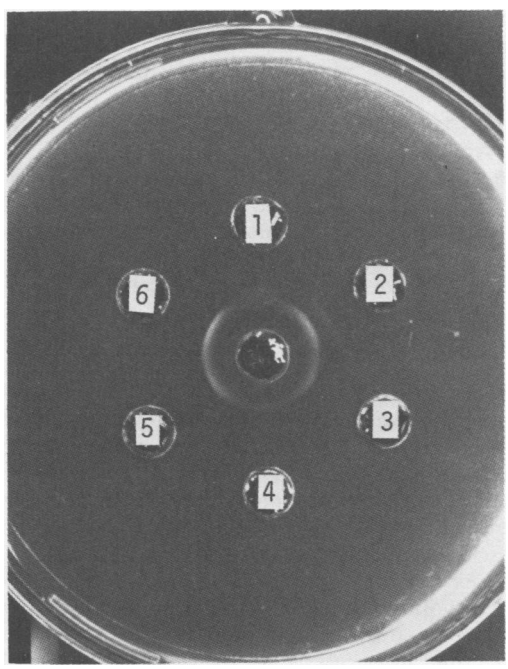

FIG. 6. *Immunodiffusion showing the immunological identity of PGP in the crude antigens from different streptococci. Center well: PGP-IB antiserum (20 μl). Peripheral wells: RR extract (15 μl) from group A S. pyogenes Richards (1), S. mutans MT703 (3), group H Channon (4), group G 9603 (5), and group C 26Rp66 (6). (2) is a group E antigen preparation (Difco lot 612134).*

Our previous study (11) revealed that RR extracts from type *e* *S. mutans* MT703 cells contained nucleoproteins and cross-reacting PGP, in addition to serotype-specific polysaccharide antigen. The extracts obtained with hot water (23) or trichloroacetic acid (9, 18, 19) also contained PGP. Thus, the selective removal of cross-reacting PGP is necessary to obtain the type-specific polysaccharide antigen. Otherwise, PGP may mislead or confuse the results of grouping or typing tests based on immunological specificities. In this connection, Gibbons et al. (8) reported that the antisera prepared against six strains of *S. mutans* designated later as serotype *a*, *b*, and *c* cross-reacted with seven formamide antigen extracts in varying degrees by the capillary precipitin test. Their failure to demonstrate a type differentiation was probably due to the presence of PGP components in these crude formamide extracts. This cross-reaction of *S. mutans* due to PGP was recently demonstrated by Mukasa and Slade (23) and Chorpenning (3), and Rosan and Knox have reported (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, E 49, p. 71) cross-reactions of group D, H, and N streptococci due to lipoteichoic acid. We have also found that a commercial grouping antigen of Lancefield group E streptococci reacted strongly with PGP-IB and PGP-A antisera.

An anionic exchanger, DEAE-Sephadex A-25, has been used to separate neutral polysaccharide and acidic substances such as PGP and/or nucleoprotein complexes for the chromatographic purification of various streptococcal antigens. Therefore, a batchwise adsorption of these acidic materials from RR extracts was attempted to remove heterophile PGP antigens. All PGP-positive materials in these extracts were completely adsorbed with A-25 and were released with increased molarity and/or by lowering the pH of the elution buffer. The immunologically active material was further purified by gel filtration and chemically characterized as glycerol-phosphate polymers with variable amounts of proteins and hexose (Fig. 1). As far as *S. mutans* is concerned, the type-specific polysaccharide antigen remained fully active even after treatment with A-25, except for that of serotype *b*. Previous results (24) showed that the type *b* polysaccharide antigen possessed a negative charge, probably because of its phosphorus content, as compared with that of other serotype-specific antigens. It can be expected that most streptococcal carbohydrate group antigens will remain unadsorbed with A-25. For example, A-25-absorbed RR extracts of groups A, C, E, and G reacted with their homologous grouping antisera (data not shown). Extracts from other streptococcal groups were not examined. However, antigens from groups D and N were absorbed with A-25, because their grouping antigens are glucose- or galactose-substituted teichoic acids (15, 34, 35). It is of interest that RR extracts from group D and N streptococci were reactive with PGP-IB and PGP-A antisera by the capillary precipitin test but not by the gel diffusion method. This may be a reflection of the immunological specificity of the substituted sugars, although the backbone structure, i.e., PGP, in the group D and N glycerol teichoic acids was reactive by the capillary precipitin test because of the high sensitivity of the method.

McCarty (20) described that most hot acid extracts of group H streptococcal cells examined were lacking PGP antigen. In our experience, however, RR extracts from six strains of group H streptococci/*S. sanguis* contained antigenic substances that cross-reacted with PGP antibody. Also, the presence of lipoteichoic acid has been reported in group H streptococci (Rosan and Knox, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, E 49, p. 71). In contrast to group H streptococci, PGP antigen could not be demonstrated in two RR preparations from group O streptococci, *M. luteus* and *M. citreus*. These results agree with the findings of McCarty (20). On the other hand, Sharpe et al. (28) failed to demonstrate PGP antigen in Lancefield extracts from various streptococci. The variation in these results is probably due in large measure to a difference in the extraction method and the relative activity of the PGP antibody used.

It is of interest that *M. citreus* cells were induced to synthesize a substance that reacted with PGP antisera when grown in Trypticase soy broth to which glycerol and phosphate were added.

Lipoteichoic acid has been postulated (13) to be one of the factors that cause bone resorption in peridontal disease. However, *A. viscosus* and *A. naeslundii*, which cause periodontal disease in experimental animals, were not found to contain PGP (see Results).

The presence of PGP was detected some years ago in association with the group D streptococcal protoplast membrane (29, 31) and the membrane fraction of various gram-positive bacteria (4, 15, 30, 33, 35). However, the adsorption of streptococcal PGP antibodies by streptococcal cells of groups A and E and *S. mutans* of types *a*, *c*, *e*, and *f* suggests that the immunological specific components of PGP reach the surface of the cell (9, 11, 12). Such a surface location for

the specific components of lipoteichoic acid of *Lactobacillus fermentii* and *L. casei* has been established (15, 33, 35), and, more recently, it has been found in the culture fluid of lactobacilli and oral streptococci (14, 21). An in situ difference for the immunological behavior among the streptococci, however, is indicated by the lack of agglutination of some *S. mutans* strains by PGP antibody, although these strains can adsorb the antibody. For example, antibody to PGP-IB and PGP-A did not agglutinate *S. mutans* type *a*, *b*, *d*, and *g* cells or Lancefield group A, E, and G cells. All these, however, adsorbed PGP antibody from whole-cell serum. These results may be due to a difference in the quantity of the immunologically specific component of PGP at the surface of these cells, or the penetration of antibody to a subsurface location in those cells that do not agglutinate.

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