0019-9567/78/0019-0160$02.00/0
INFECTION AND IMMUNITY, Jan. 1978, p. 160-169
Vol. 19, No. 1

Printed in U.S.A.

Antigens of *Streptococcus mutans*: Isolation of a Serotype-Specific and a Cross-Reactive Antigen from Walls of Strain V-100 (Serotype *e*)†

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Received for publication 29 August 1977

Two cell wall-associated polysaccharide antigens were extracted from purified cell walls of *Streptococcus mutans* serotype *e* strain V-100. One of these purified antigens (I) is specific for serotype *e*, whereas the other (II) has antigenic determinants reactive with both heterologous anti-serotype *c* serum (GS-5) and the homologous (*e*) serum. When crude formamide extracts of V-100 cell walls were loaded onto a Cellex-D column and eluted with a linear gradient of ammonium carbonate (0.02 to 0.40 M), the two products mentioned above could be recovered. The purified, antigenically reactive products (I and II) were each composed only of rhamnose and glucose in approximately a 2:1 molar ratio. Immunoelectrophoresis of the crude formamide extract, peak I, and peak II showed the purified fractions to have opposite mobilities and the crude extract to have a mobility that encompassed both purified peaks when reacted with homologous antiserum (V-100). When these three fractions were immunoelectrophoresed and reacted with heterologous anti-serotype *c* serum (GS-5), only the anodic portion of the crude V-100 formamide extract and purified peak II formed precipitates. Ouchterlony analysis with homologous antiserum produced precipitin patterns between the crude formamide extract and both purified peaks, indicating complete identity. However, only crude extracts of V-100 and the purified peak II material reacted with heterologous (*c*) antiserum; peak I did not cross-react in these Ouchterlony assays. Hapten inhibition studies revealed that a β-glucosyl moiety is the immunodeterminant for serotype *e* and is present on each purified fraction. The basis of the cross-reaction between anti-*c* sera and the purified antigen II of *e* is discussed.

Streptococcus mutans has been shown to be an important agent involved in the development of dental caries (6, 8, 10, 13). Bratthall (2, 3) and Perch et al. (19) divided this cariogenic species into seven serotypes (*a* through *g*) on the basis of the electrophoretic mobilities of Lancefield extracts and certain biochemical characteristics. Various investigators have examined the immunochemical natures of the determinant antigens for serotypes *a* (17, 22), *b* (18, 23), *c* (14, 24), *d* (15), *e* (12), *f* (11), and *g* (13). Although organisms of serotype *e* are much less common in the oral cavity than certain other serotypes, they are of interest due to the similar composition of cell walls of serotype *e* and serotype *c* organisms, as well as the unique immunological relationship of serotype *e* to serotype *c* and Lancefield group E. Briefly, extracts of serotype *e* are reported to cross-react with both serotype *c* (3) and Lancefield group E (3, 12) antisera, but there does not exist a cross-reaction between serotype *c* extracts and Lancefield group E immunoglobulins (3).

This paper describes the isolation, purification, and partial characterization of two polysaccharide antigens from purified cell walls of *S. mutans* V-100. One of these antigens is specific for serotype *e*, whereas the other cross-reacts with antibodies induced by serotype *c*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. For this study strains V-100 and B-2 of serotype *e* were used. These strains were obtained from R. McKinney (Center for Disease Control, Atlanta, Ga.). Each strain was characterized as to its ability to ferment certain key carbohydrates, including sorbitol and mannitol, and immunological specificity, using standard antiserum to V-100 obtained from R. McKinney. Antiserum to another serotype *e* strain (B66) was prepared and reacted with V-100 and B-2, but failed to react with extracts of all other *S. mutans* serotypes. Large-batch growth and harvesting of cells were as previously described (22).

Extraction of antigens from whole cells. To determine which extraction technique gave the highest

† Florida Agricultural Experiment Station Journal Series no. 709.

relative yield of serotype-specific antigen, four methods were compared. In each case, 20 mg of lyophilized whole cells of V-100 was extracted (see below), dialyzed against 200 volumes of distilled water, lyophilized, and finally brought up to 1 ml in distilled water.

The four methods that were compared were mild phosphate buffer extractions, the Lancefield acid estraction, cold 10% trichloroacetic acid extraction, and hot formamide extraction. Each of these extractions was done as described previously (24).

Extraction of cell wall antigens by formamide. Cell walls of V-10 and B-2 were purified according to the technique of Bleiweis et al. (1), using glass-bead disruption in a Braun tissue homogenizer followed by treatments with ribonuclease, deoxyribonuclease, and trypsin. The purified cell walls were then extracted with hot formamide as previously described (24), using a modification of the technique of Fuller (9).

Purification by gel filtration. Bio-Gel P-100 (100 to 200 mesh) (Bio-Rad Laboratories, Richmond, Calif.) was hydrated in distilled water and packed into columns (2.7 by 90 cm). The column was equilibrated, and samples (100 mg) were eluted with 0.85% NaCl. Fractions (2 ml) were collected in a Gilson Mini-Escargot fraction collector (Gilson Medical Electronics, Inc., Middleton, Wis.) at a flow rate of 10 ml/h. Fractions were analyzed for absorbancy at 220 nm to detect common polysaccharide chromophores, rhamnose, and antigenicity by capillary precipitation.

Purification by diethylaminoethyl-cellulose column chromatography. The antigenic fractions from the gel chromatographic column were pooled, dialyzed, lyophilized, and then loaded onto a Cellex-D (Bio-Rad Laboratories) column (2.7 by 40 cm). The column was washed extensively with 0.02 M ammonium carbonate buffer and then with 0.45 M ammonium carbonate buffer. Finally, the column was equilibrated with 0.02 M ammonium carbonate buffer before the sample was loaded. The samples were eluted with a linear gradient of 0.02 to 0.40 M ammonium carbonate buffer (pH 8.9) or 0.02 to 0.20 M ammonium carbonate buffer (pH 8.9), depending on the extent to which the sample had been purified. Selected 2-ml fractions were dialyzed and assayed for absorbance at 220 nm, rhamnose, and antigenicity. Antigenic fractions were pooled, dialyzed, and lyophilized before further analysis.

Quantitative chemical analyses. The method of Chen et al. (5) was used to determine the amount of phosphorus in purified samples. Rhamnose was determined by the method of Dische and Shettles (7). Glucostat and Galactostat reagents (Worthington Biochemicals Corp., Freehold, N.J.) were used to determine the amounts of glucose and galactose, respectively. The amount of glycerol present was determined by use of the glycerol Stat-Pak (Calbiochem, Atlanta, Ga.). Amino acids and amino sugars were measured on a JEOL model JLC-6AH automated amino acid analyzer (JEOL, Inc., Cranford, N.J.). All sample hydrolysates for the quantitative assays were prepared as previously described (22).

Preparation of rabbit antisera. Organisms of strain V-100 to be used in immunizations were grown in centrifuge bottles (200 ml) for 18 h at 37°C in Todd-Hewitt broth (Difco, Detroit, Mich.), pH 7.0, with 2% glucose added. The cells were pelleted by centrifugation and washed twice with distilled water. The washed pellet was then resuspended in 20 ml of distilled water and lyophilized. The lyophilized cells were brought up to a concentration of 5 mg/ml of sterile saline, which served as a stock suspension. Before immunization, an emulsion composed of equal volumes of stock suspension and Freund incomplete adjuvant (Difco, Detroit, Mich.) was prepared. New Zealand white male rabbits (3 kg) were given four equal subcutaneous injections on the days outlined below. The inoculations were in the regions of the cervical, axillary, and inguinal lymph nodes. Each animal was also administered intramuscularly 1 ml (50 mg/ml) of Liquamycin (oxytetracycline-HCl, Pfizer, Inc., New York, N.Y.) at the time of immunization. On days 1 and 5 the animals were given injections totaling 1 mg (dry weight) of whole cells. On days 9 and 12 the total dose was increased to 2 mg (dry weight) of whole cells. Small samples of blood were removed on days 7, 9, 12, and 14 and examined for the presence of antibody, using capillary precipitin and Ouchterlony techniques. Those rabbits having a sufficient antibody titer were bled from the heart and sacrificed. The antisera were concentrated by precipitation with 4.0 M ammonium sulfate (0.5 ml/1.0 ml of antiserum), dialyzed, and frozen in 0.2-ml samples.

Immunological assays. The details of the methods used for the quantitative precipitin assay and the quantitative precipitin inhibition assay were presented previously (22).

Gel diffusion and immunoelectrophoresis. A 2× stock barbital buffer solution was prepared containing 15.85 g of sodium barbital with 1.9 ml of concentrated HCl and brought up to 1 liter with distilled water (pH 8.2). A mixture of 0.75% agarose in 1× barbital buffer was used for preparing gels to be used in immunoelectrophoresis and double-diffusion analyses.

Slides for immunoelectrophoresis were prepared by pipetting 3 ml of the melted agarose mixture onto a 7.6- by 3.8-cm slide. A Shandon gel cutter (Colab) was used to punch the well and trough patterns in the solidified agar. The wells were filled with appropriate antigen extracts, and slides were placed into a Shandon immunoelectrophoresis apparatus (Colab) containing 1× barbital buffer. A Krohn-Hite model UHR-220 power supply was used to generate 10 mA/slide for 50 min at 4°C. After electrophoresis, the troughs were filled with the appropriate antisera and incubated at 4°C for 18 to 24 h.

Agar plates to be used for gel diffusion by the Ouchterlony technique were prepared by pipetting 3 ml of the same agrose-buffer mixture into a no. 1006 petri dish (50 by 12 mm) (Falcon Plastics, Oxnard, Calif.). Wells (0.050 ml) were cut from the solidified gel.

Lowry protein assay. Total protein precipitated in the heterologous quantitative precipitin and heterologous hapten inhibition experiments was determined in triplicate according to the method of Lowry et al. (16).

Chemicals. Organic solvents, acids, and most common salts were obtained from Mallinckrodt (Scientific Products, Chamblee, Ga.). Amino acids, amino sugars, L-rhamnose, maltose, cellobiose, β-methyl-D-glucopyranoside, α-methyl-D-glucopyranoside, and agarose

were obtained from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Comparison of extraction techniques. Preliminary investigations were done to determine which of four common extraction techniques gave the highest relative yield of antigen: hot phosphate buffer (100°C, 10 min), cold 10% trichloroacetic acid (4°C, 24 h), hot HCl by the Lancefield method, and hot formamide (180°C, 30 min). In each technique 20 mg of lyophilized whole cells of *S. mutans* V-100 was extracted as described by Wetherell and Bleiweis (24). The extracts were dialyzed, lyophilized, and brought up to 1 ml in distilled water. Extraction by hot formamide gave the highest relative yield of antigenic material when examined by double-diffusion in gel, and this technique was selected as the method of choice in extracting antigenic material.

Extraction of antigen from purified cell walls. The cellular location of the serotype *e* antigen and determination of its presence in one other serotype *e* strain were studied. Hot formamide extracts of purified cell walls of two serotype *e* strains (V-100 and B2) and two serotype *c* strains (GS-5 and JC-2) were compared with respect to antigenic reactivity with anti-V-100 serum. An antigen possessing identical determinants is present in each serotype *e* strain, but appears to be lacking in each serotype *c* strain (Fig. 1). The electrophoretic mobilities of antigenic cell wall extracts of strains V-100 and B-2 were identical (not shown) and were similar to those reported in the literature for B-2 (2) and MT-703 (12). The mobility of the V-100 extract is shown in Fig. 6.

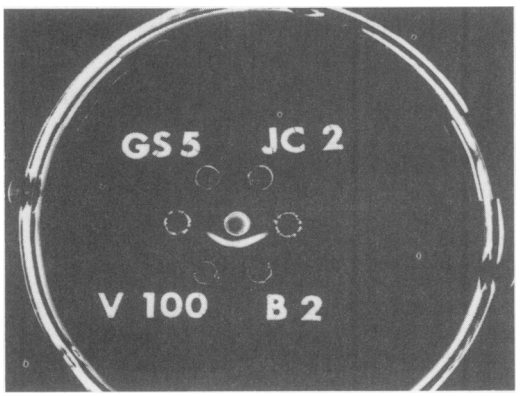

FIG. 1. *Immunodiffusion in gel showing precipitin patterns formed by formamide extracts of purified cell walls of two serotype e strains (V-100 and B-2) and two serotype c strains (GS-5 and JC-2). Anti-V-100 serum was used in the center well and forms bands of identity with serotype e strains, but fails to react with serotype c extracts.*

It is apparent the antigen extracted by hot formamide is cell wall localized in the serotype *e* strains studied here.

Purification of a cross-reactive and serotype-specific antigen. Purified cell walls of V-100 were extracted using the hot formamide technique, and extracts were loaded onto a Bio-Gel P-100 column which was eluted with 0.85% sodium chloride. Selected 2-ml fractions were dialyzed and examined for absorbancy at 220 nm, rhamnose, and reactivity with homologous antiserum. Each of these characteristics was found to be associated with a single peak (Fig. 2). The symmetry of this peak suggests that the antigenic fragments extracted were of approximately the same molecular size. Fractions 85 to 145 were pooled, dialyzed, and lyophilized.

The material recovered from the Bio-Gel P-100 column was reloaded onto a Cellex-D column and eluted with a continuous linear gradient of 0.02 to 0.40 M ammonium carbonate. Figure 3 shows the profile of selected, dialyzed fractions examined only for absorbancy at 220 nm. Fractions 35 to 53 (peak I) and 54 to 85 (peak II) were pooled separately, dialyzed, and lyophilized.

The recovered peak I material was loaded onto a Cellex-D column, but this time the linear gradient was 0.02 to 0.20 M ammonium carbonate (Fig. 4a). A single sharp peak was obtained (fractions 35 to 50) that contains absorbancy at 220 nm, rhamnose, and all antigenic reactivity with anti-V-100 serum.

The material of peak II also was reloaded onto a Cellex-D column with a linear gradient of 0.02 to 0.2 M ammonium carbonate (Fig. 4b). Absorbancy at 220 nm, rhamnose, and antigenicity again were found in a broad region located between fractions 40 and 100. This material was not further purified and is termed, collectively, peak II.

The recycled fractions comprising peaks I and II were pooled separately, dialyzed, and lyophilized.

Immunological characteristics of antigenic fractions. Peaks I and II and crude formamide extracts of V-100 were compared with respect to their reactivity with anti-V-100 sera (Fig. 5a). All three fractions show immunological identity using the homologous antiserum. However, when these same three fractions are reacted with antiserum to a serotype *c* organism (GS-5), the crude formamide extract of V-100 and the purified peak II material show identity, but the material of purified peak I fails to react (Fig. 5b). In an attempt to determine if the nonreactivity of peak I was due to limiting con-

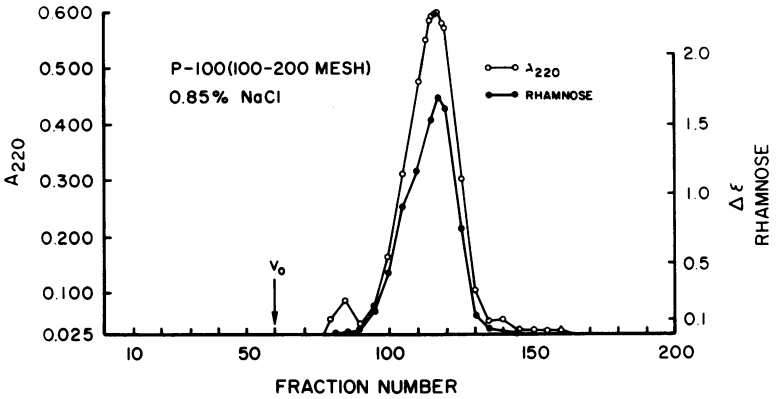

FIG. 2. *Elution profile of crude formamide extract of purified V-100 cell walls on Bio-Gel P-100 (100 to 200 mesh). Samples (100 mg) were loaded, and fractions (2 ml) were eluted with 0.85% sodium chloride. Selected fractions were examined for absorbance at 220 nm (A_{220}) (○) and rhamnose (●). All antigenic reactivity was located under the large single peak (fractions 85 to 145).*

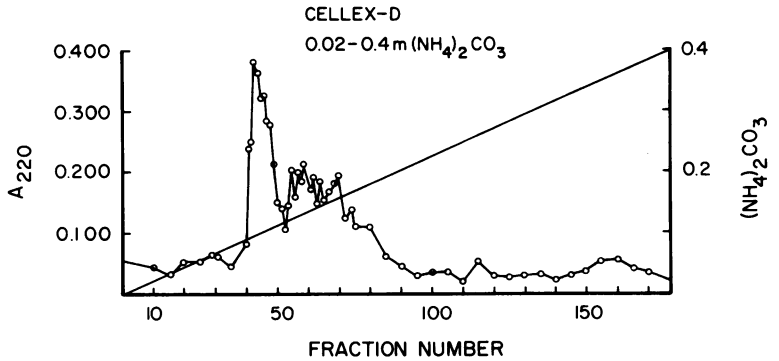

FIG. 3. *Elution profile of antigenic material recovered from Bio-Gel P-100 on Cellex-D. Samples (50 to 75 mg) were loaded, and fractions (2 ml) were eluted with a linear gradient of 0.02 to 0.40 M ammonium carbonate (pH 8.9). Selected fractions were dialyzed and examined for absorbance at 220 nm (A_{220}) (○). Antigenicity with homologous antiserum was found between fractions 40 and 80. The antigenic regions were divided into: I, fractions 35 to 53; and II, fractions 54 to 85. These regions were pooled, dialyzed, and lyophilized separately.*

centrations, several concentrations ranging from 0.04 to 80.0 mg/ml were reacted with antiserotype *c* serum. There was no detectable precipitin reaction at any of these concentrations.

When these three antigenic fractions are immunoelectrophoresed and reacted with homologous (V-100) antiserum, the precipitin patterns obtained can be described as follows: peak I shows slight cathodic mobility and peak II slight mobility towards the anode; the crude formamide extract of V-100 appears to be mobile, with regions that encompass both peaks I and II. When this experiment is repeated using anti-GS-5 serum, precipitin bands are formed as shown in Fig. 6. Anti-GS-5 serum fails to react with purified peak I, but does react with the anodically mobile part of the crude V-100 extract and the purified peak II material.

These results clearly indicate the presence in V-100 cell walls of a serotype-specific antigen for *e* (peak I) and cross-reactive antigen (peak II) reacting with both anti-*c* and anti-*e* sera. Figure 7 illustrates the partial identity obtained when *c* and *e* (peak II) polysaccharides are reacted with anti-*c* (GS-5) serum. On the other hand, there was no detectable reaction in either Ouchterlony analysis or immunoelectrophoresis between the purified serotype *c* (GS-5) polysaccharide antigen (24) and anti-V-100 serum.

Chemical characteristics of antigenic fractions. Table 1 lists the chemical composition of purified cell walls of *S. mutans* V-100. The chemical compositions of the V-100 crude formamide extract, peak I, and peak II are presented in Table 2. Rhamnose and glucose were the only detectable components of the purified

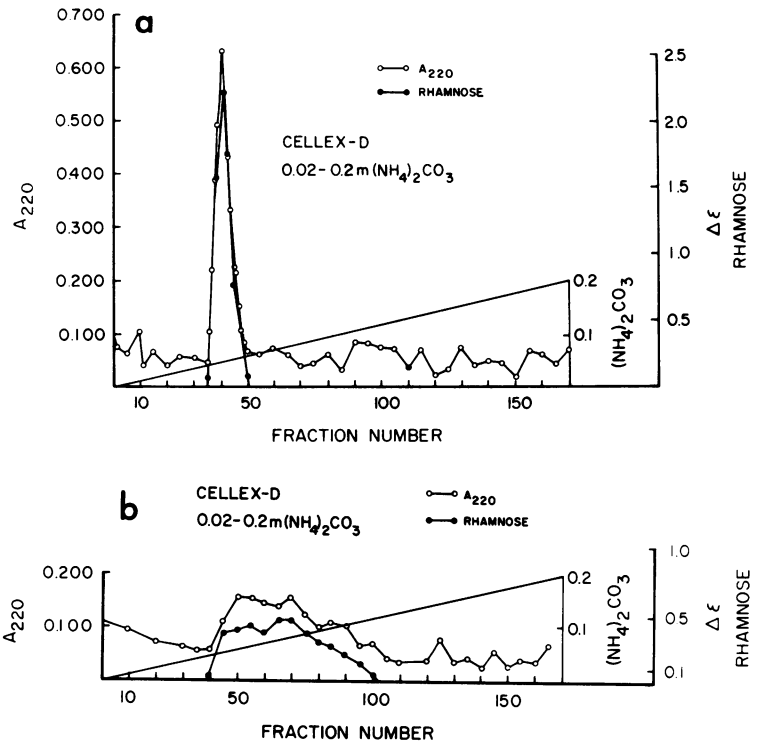

FIG. 4. *Elution profiles of pooled regions (I, II) of earlier Cellex-D elution (Fig. 3) on Cellex-D. Each pooled region was eluted with a Linear gradient of 0.02 to 0.20 M ammonium carbonate and examined for absorbance at 220 nm (A_{220})* (○) *and rhamnose* (●). *Pooled region I (a) gave a single sharp antigenic peak, whereas region II (b) produced a much broader profile that was also antigenic.*

antigens. The ratios of rhamnose to glucose in these preparations and in the purified cell walls were quite similar.

Quantitative precipitin reaction and hapten inhibition studies. A quantitative precipitin experiment was done to determine the equivalence point of the antiserum to be used for hapten inhibition studies. Varying concentrations of crude formamide extract, peak I, and peak II were incubated for 4 days with anti-V-100 serum. The precipitates were then washed and assayed for antibody protein content. Controls for nonspecific precipitating antibody were run in triplicate, with equivalence found to be at approximately 25 μg for each of the three antigenic products derived from V-100.

A preliminary quantitative precipitin inhibition assay was set up in triplicate with 25 μmol of several potential inhibitors and 25 μg of crude formamide extract to indicate which inhibitors were most effective. Considerable inhibition was obtained using β-methyl-D-glycopyranoside (65%) and the β-linked diglucoside D-cellobiose (88%). Only negligible inhibition was measured when α-methyl-D-glucopyranoside (17%) and the α-linked diglucoside maltose (24%) were used. There was no detectable inhibiton by rhamnose. These data are in agreement with hapten determinations of serotype *e* strain MT-703 reported by Hamada and Slade (12), who also found a β-glucoside to be the main determinant for this serotype.

On the basis of these data, a second quantitative precipitin inhibition assay was performed with varying concentrations of α- and β-methyl-D-glucopyranoside as well as maltose and cellobiose as potential inhibitors. However, this time inhibitions of the reactions between anti-V-100 serum and purified peak I (25 μg) or peak II (25 μg) were measured. Cellobiose gave the greatest inhibition (99%) at the lowest concentration (50 μmol) of all the inhibitors tested (Fig. 8a). In general, the β-linked anomers gave greater inhibition at any given concentration than either of the α-linked moieties.

At this stage in our investigations, we had isolated two polysaccharide antigens from V-100 with determinants for serotype *e*. One of these products (peak II) was further characterized by its unique ability to cross-react with anti-*c* serum

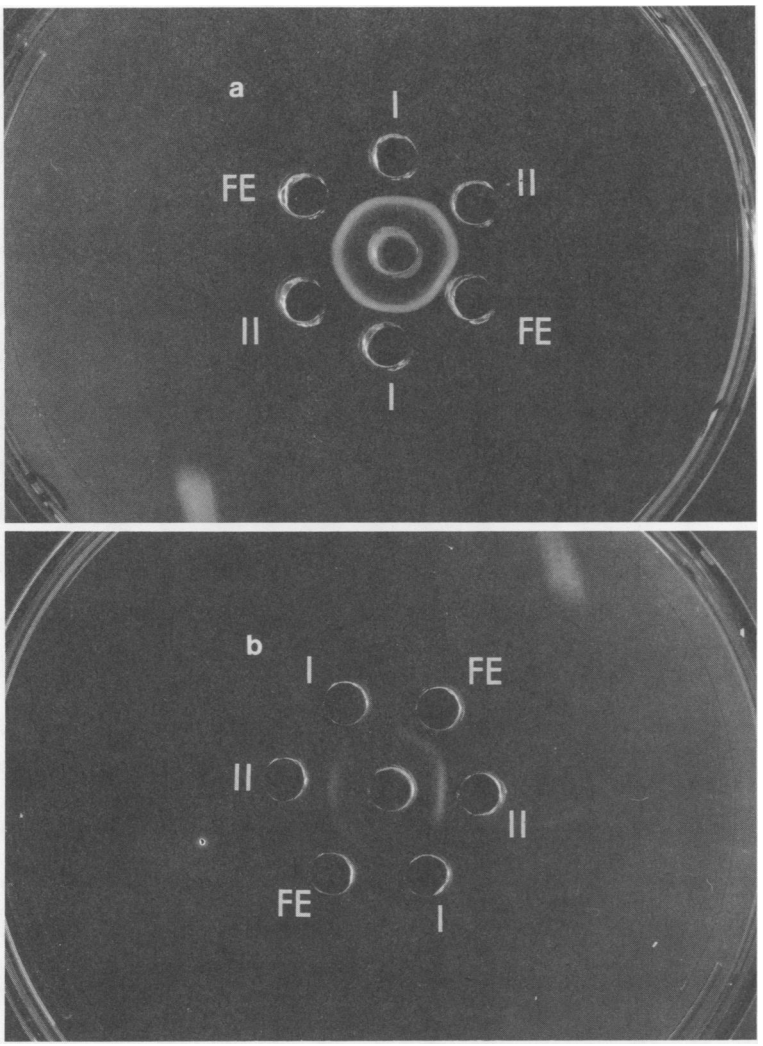

FIG. 5. *Double diffusion in gel of peak I (I), peak II (II), and crude formamide extract of V-100-purified cell walls showing precipitin bands formed when reacted with (a) anti-V-100 serum and (b) anti-serotype-c serum (GS-5).*

(Fig. 6). This serum, prepared using strain GS-5, is quite specific for α-glucosyl determinants. Figure 8b, a homologous precipitin inhibition study using various concentrations of potential inhibitors at immunological equivalence for this antibody and purified *c* polysaccharide antigen (24), clearly demonstrates a strong affinity by this antibody for the α-linked glucosyl determinant. Almost complete inhibition was obtained using just 3 μmol of maltose. It appeared reasonable to assume that such a determinant could exist in the V-100 peak II product, allowing the cross-reaction observed. To attempt to support this hypothesis, heterologous quantitative precipitin inhibition studies were undertaken.

First, a heterologous quantitative precipitin experiment was done with anti-serotype *c* sera and varying concentrations of V-100 peak I or II purified antigens. At equivalence, 47 μg of antibody protein was precipitated by approximately 8 μg of purified peak II antigen. Purified peak I antigen failed to react at detectable levels with the heterologous serum.

When a heterologous hapten inhibition study was performed at equivalence, it was found that maltose and cellobiose were apparently equally effective in inhibiting the reaction between *c* antibody and *e* antigen (peak II), as illustrated in Fig. 9. Since each inhibitor is a diglucoside linked by means of a 1$\rightarrow$4 glycosidic bond, the lack of

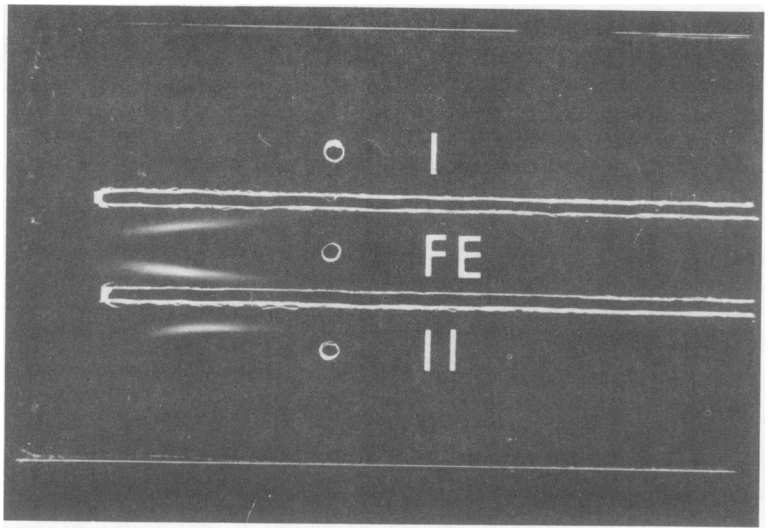

FIG. 6. *Immunoelectrophoresis of peak I (I), peak II (II), and crude formamide extract (FE) of V-100 purified cell walls showing precipitin bands formed when reacted with anti-serotype-c serum (GS-5).*

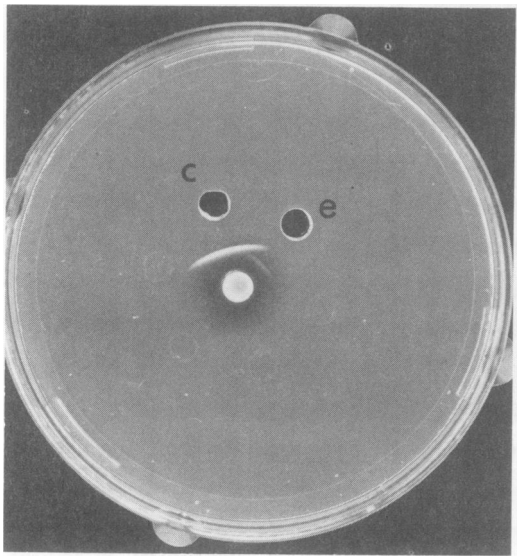

FIG. 7. *Double diffusion in gel of c and e (peak II) polysaccharides showing precipitin bands obtained when reacted with anti-c (GS-5) serum. Partial identity is clearly observed.*

substantial difference in inhibitory effect indicates that the anomeric linkage plays little or no role in the cross-reactive phenomenon. However, the fact that significant inhibition was obtained at 50 μmol of each dissacharide indicates a probable role for a glucose hapten in cross-reactions between *c* immunoglobulins and *e* antigens.

TABLE 1. *Chemical composition of purified cell walls of S. mutans V-100*

Major component[a]	Purified walls	
	μg/mg	μmol/mg
Rhamnose	226.3	1.38
Glucose	111.0	0.62
Galactose	8.1	0.05
Glucosamine	75.6	0.35
Muramic acid	56.5	0.23
Alanine	107.6	1.22
Lysine	53.2	0.36
Glutamic acid	56.2	0.38
Threonine		tr[b]
Aspartic acid	14.3	0.11
Glycerol	ND[c]	ND
Phosphorus	1.0	0.03
Total recovery	709.8	

[a] Amino sugars are reported as acetylated derivatives.
[b] Trace amount not measured.
[c] ND, Not determined.

DISCUSSION

Bratthall (2) demonstrated the cross-reactivity of *S. mutans* serotypes *c* and *e*. Furthermore, serotype *e* cross-reacts with sera to Lancefield group E organisms. This latter cross-reaction is not exhibited when serotype *c* extracts are used. Analysis of the Lancefield group E antigen (21), a cell wall-localized polysaccharide, revealed a rhamnose-to-glucose ratio of 2:1, and a β-D-glucose moiety was determined to be the immunodeterminant sugar. The presence of α-glucosyl

TABLE 2. *Chemical compositions of various fractions of S. mutants V-100 derived by formamide extraction of purified walls*[a]

Compound	Crude extract[b]			Peak I			Peak II		
	Amt		Molar ratio	Amt		Molar ratio	Amt		Molar ratio
	μg/mg	μmol/mg		μg/mg	μmol/mg		μg/mg	μmol/mg	
Rhamnose	525.4	3.20	2.7	516.9	3.15	2.3	451.8	2.75	2.1
Glucose	214.0	1.19	1.0	243.5	1.35	1.0	231.2	1.28	1.0

[a] Chemical compositions were not corrected for waters of hydration or ash contents. Instrumental analyses of each fraction failed to detect galactose, a cell wall component (Table 1), the various peptidoglycan components (e.g., muramic acid, glucosamine, alanine, lysine, and glutamic acid), or other amino acids. Chemical analyses yielded only trace amounts of phosphorus. In view of these analyses, it is assumed that water and mineral contents compose the remainder of the samples. Similar studies with other streptococcal antigens (22–24) have shown totals ranging from 70 to 85%, indicating variable contents of water or ash.

[b] See text for extraction technique used.

haptenic groups in strain GS-5 (24) would explain the inability of *S. mutans* serotype *c* to react strongly with antisera to Lancefield group E. The possible presence in *S. mutans* serotype *e* wall polysaccharides of both anomeric linkages of glucose was an attractive basis for observed cross-reactions with anti-*c* sera. However, as will be discussed below, this apparently is not the case.

Two cell wall-associated polysaccharide antigens were isolated from purified cell walls of *S. mutans* serotype *e* strain V-100. These products (peaks I and II) likewise contained rhamnose and glucose in a 2:1 molar ratio, and hapten inhibition studies revealed a β-glucosyl moiety to be the probable immunodeterminant moiety on each antigenic polysaccharide. These data support the report by Hamada and Slade (12) in which they characterized a polysaccharide antigen of similar composition and immunological specificity from another serotype *e* organism (strain MT703).

Ouchterlony double-diffusion analysis performed with the V-100 crude formamide extract and purified peak I and II material gave reactions of identity with homologous antiserum (Fig. 5a). These results indicate that the serotype-specific determinant for *e* is present in each purified product. Immunoelectrophoresis, however, revealed distinct differences in mobility, with peak I moving toward the cathode and peak II toward the anode. Even slight mobility by neutral polysaccharides is unexpected, so complete chemical analyses of each antigenic product were done. Components of protein, peptidoglycan, and polyglycerophosphate were absent in each case. Only rhamnose and glucose could be determined. Nevertheless, it is likely the two products are somewhat different in terms of their basic structural properties, despite their identical chemical compositions. Peak I material failed to react with heterologous antiserum in both Ouchterlony and immunoelectrophoretic analyses and should contain only the serotype-specific antigen for *e*. Peak II material, however, reacted with anti-*c* serum in each assay and apparently contains the common determinant responsible for the previously observed cross-reactivity.

With the isolation and purification of a cross-reactive antigen (peak II) from serotype *e* extracts, the reaction of antibodies made against serotype *c* organisms with extracts of serotype *e* cells can be explained. But, why does the reverse reaction fail to occur? We have consistently failed to obtain cross-reactions using anti-*e* serum and various types of extracts of serotype *c* cells or cell walls. The serological data presented in this paper suggest that perhaps there exists a common haptenic determinant in cell walls of both serotypes. One possible explanation is that the common determinant is not immunogenic in serotype *e* walls, whereas its position in serotype *c* walls allows good antibody production. This explanation supports an earlier hypothesis in which we proposed that serotype *e* may possess a unique antigen, and another haptenic determinant that is cross-reactive with anti-serotype *c* serum (24). This hypothesis seems to be supported by the findings of Pittman et al. (20). These workers found that when rabbit anti-*e* serum was absorbed with serotype *c* cells there was little loss in homologous titer. However, when anti-*c* serum was absorbed with serotype *e* whole cells the homologous titer was "considerably reduced." These are the results one might expect if no antibodies were made against the common hapten in serotype *e* walls; naturally, little absorption of antibody could occur using serotype *c* whole cells. On the other hand, since the cross-reactive hapten is present in serotype *e* walls, absorption of antiserotype *c* sera with *e*

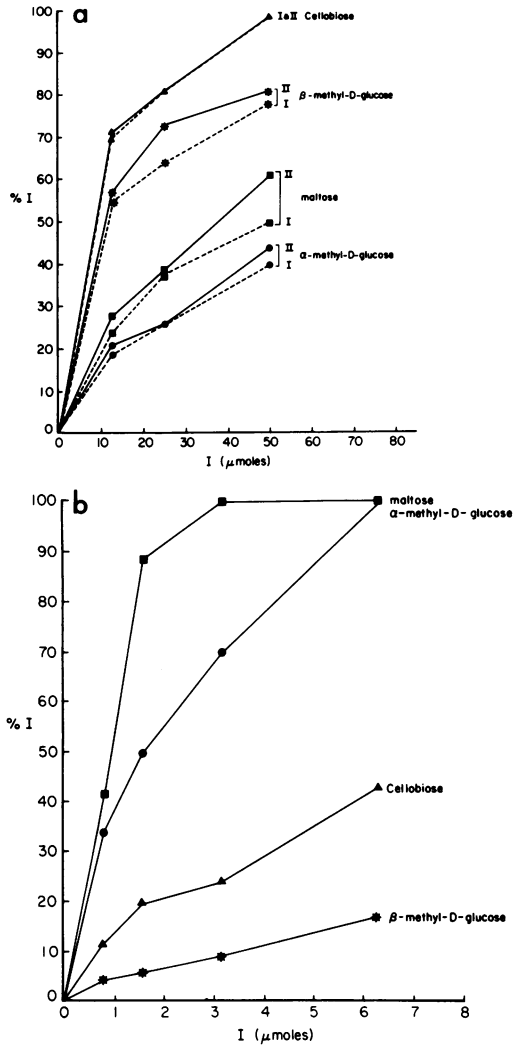

FIG. 8. *Inhibition by selected haptens at varying concentrations of the quantitative precipitin reaction between (a) purified serotype e peak I and II material and antiserum against S. mutans V-100, and (b) purified serotype c polysaccharide antigen (previously referred to as c peak II in reference 24) and antiserum against S. mutans GS-5 (serotype c).*

whole cells would result in a considerable loss of homologous serotype *c* titer.

As demonstrated previously (24) and in greater detail here (Fig. 8b), the anti-serotype *c* serum is quite specific for the α-anomeric linkages, especially the α-1→4 linkage of maltose. This sensitivity is demonstrated by the ability of very small amounts of maltose (~3 μmol) to inhibit to a high degree (>99%) the equivalence precipitin reaction between anti-serotype *c* serum and purified serotype *c* polysaccharide antigen. In an attempt to further clarify the nature of

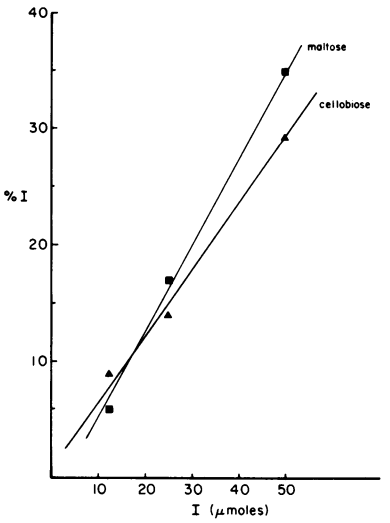

FIG. 9. *Hapten inhibition by varying concentrations of matose and cellobiose added to the reaction, at equivalence, between V-100 peak II antigen and anti-c serum (GS-5).*

the cross-reactive antigen present in peak II material, heterologous precipitin studies were performed. When a heterologous quantitative precipitin analysis was done using anti-serotype *c* serum and varying concentrations of peak I or II material, it was found that only peak II reacted. This reaction was weak, with only 47 μg of protein precipitated at equivalence by 8 μg of peak II antigen. Further investigation by the hapten inhibition technique showed that there was no significant difference between the ability of maltose and that of cellobiose to inhibit the heterologous reaction between anti-*c* serum and peak II material (Fig. 9). The result of this experiment would tend to suggest that the anomeric nature of the haptenic linkage may not be of sole significance; rather, the specificity of the heterologous reaction may be directed towards the type of glycosidic linkage present in the hapten (e.g., 1-3 versus 1-4 versus 1-6). Studies are in progress to determine more specifically the chemical structures of both the serotype *c* and serotype *e* antigenic polysaccharides.

ACKNOWLEDGMENTS

This investigation was supported by grant BC-01440 from the Florida Agricultural Experiment Station and by Public Health Service grant DE-02901-09 from the National Institute of Dental Research.

We thank E. M. Hoffmann for helpful comments during the preparation of this manuscript and S. Hurst for his technical assistance.

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