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Affinity Purification and Characterization of Protease-Susceptible Antigen I of *Streptococcus mutans*

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An antigenic component (antigen I) of the cell surface of *Streptococcus mutans* has been purified from culture supernatants and shown to be immunologically identical to the protease-susceptible moiety of antigen I/II. Ion-exchange and gel filtration chromatography failed to yield a physicochemically homogeneous product. Immunoabsorbent chromatography on single and tandem columns containing immobilized antibodies to antigens I/II and II yielded identical products which were homogeneous in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and which when injected into rabbits induced monospecific antisera to antigen I. This antigen consisted of approximately 70% protein. Its molecular weight was estimated as 150,000, and the isoelectric point was estimated to be 5.1. Immunofluorescence microscopy using monospecific antiserum to antigen I showed that a similar antigen was present on cells of *S. mutans* serotypes *a*, *c*, *d*, *e*, *f*, and *g*, but not *b*.

In an investigation of the antigenic structure of *Streptococcus mutans*, we have previously described cell surface antigens I, II, and III, which are also present in culture fluids (19). Antigens I and II appear to be two determinants present on one molecule, now known as antigen I/II. The antigen I determinant can be removed by treatment with trypsin or pronase, and the protease-resistant antigen II has thereby been purified and characterized (15). Purification of antigen I has proved more difficult, since it has not been possible to dissociate it in intact form from the purified antigen I/II by physical, chemical, or enzymatic methods. However, its existence as a separate entity from antigens I/II and II has been observed during chromatographic purification of antigen I/II. Further, some final preparations of antigen I/II, when examined by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (SDS), have shown contamination with a protein (band C) possessing only antigen I activity (15).

Antigen I has now been partially purified from *S. mutans* culture supernatants by ion-exchange chromatography and purified to homogeneity by using affinity chromatography on single and tandem immunoabsorbent columns. This paper describes these procedures, together with an analysis of purified antigen I and its distribution among oral streptococci.

MATERIALS AND METHODS

Organisms and cultural conditions. Antigens were prepared from *S. mutans* Guy, grown in 5-liter flasks of medium incubated at 37°C for up to 65 h (15). The medium was either a dialysate of Oxoid tryptone, yeast extract, and acid casein hydrolysate, supplemented with glucose and salts (7), or a semidefined medium (1) based on Acidicase peptone 2, low salt (BBL Microbiology Systems). Antigens were precipitated from the culture supernatant with 3 M ammonium sulfate. Alternatively, antigens were extracted from the 3 M ammonium sulfate precipitate of supernatant obtained from a 400-liter culture supplied by the Centre for Applied Microbiology and Research, Porton, Salisbury, Wilts, U.K., also using a tryptone-yeast dialysate medium.

Other organisms used for immunofluorescence have been described previously (14), and were grown in Todd-Hewitt broth for 24 h at 37°C.

Column chromatography. Culture supernatant antigens were separated by chromatography on diethylaminoethyl (DEAE)-cellulose equilibrated with 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4) in a column 5 to 10 cm long and 5 cm in diameter. After eluting with the starting buffer at a flow rate of 80 ml/h, a step of 0.1 M NaCl in starting buffer was applied. Fractions eluted with starting buffer and containing antigen I were dialyzed against water and lyophilized. Antigen I/II was then eluted with 0.1 M NaCl. Some preparations of antigen I were subjected to repeated chromatography on DEAE-cellulose in 0.01 M Tris-hydrochloride (pH 8) in a smaller column (15 by 1.6 cm), using a gradient of 0 to 0.5 M NaCl. This did not result in any substantial improvement in purification.

Partially purified antigen I was further fractionated by gel filtration on a column (85 by 2.6 cm) of Ultrogel AcA 22 (LKB) in 0.1 M Tris-hydrochloride–0.5 M NaCl (pH 7.4). A flow rate of 10 ml/h was used, and 5-ml fractions were collected.

Preparation and use of immunoabsorbents. An

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immunoglobulin G fraction of 5 ml of rabbit antiserum to antigen I/II or to antigen II was obtained by chromatography on DEAE-cellulose (10 by 1.6 cm) in 0.0175 M phosphate (pH 6.5). The starting buffer peak yielded approximately 30 mg of immunoglobulin G which contained precipitating antibody activity identical to that of the original serum. This was dialyzed against 0.1 M $NaHCO_3$–0.5 M NaCl (pH 8.3) and coupled to 3 g of CNBr-activated Sepharose 4B (Pharmacia) as described by the manufacturers. The immunoabsorbents were packed into columns approximately 5 by 1.6 cm and washed with two cycles of 0.1 M borate–1 M NaCl (pH 8) and 0.1 M acetate–1 M NaCl (pH 4), followed by 6 M guanidinium chloride. After washing with 0.1 M phosphate–0.5 M NaCl (pH 7.4), the columns were ready for use.

Samples were applied in 1 to 2 ml of 0.1 M phosphate–0.5 M NaCl (pH 7.4) and eluted at a flow rate of 10 ml/h, first with the starting buffer, then with 0.2 M glycine hydrochloride–0.5 M NaCl (pH 2.8), and finally with 6 M guanidinium chloride, using at least 2 column volumes (20 ml) for each eluent. Fractions of 1 ml were collected, and their absorption at 280 nm was measured. The columns could be reused after re-equilibrating with the starting buffer.

Other antigens. *S. mutans* antigens I/II, II, and III were purified as described previously (14, 15).

Antisera. Rabbit antisera were raised against unfractionated culture supernatant antigens and against purified antigens I/II and II (15). Antisera to antigen I were raised similarly by injecting about 1 mg of gel filtration-purified antigen I, or about 0.1 mg of affinity-purified antigen I. The first dose was given intramuscularly in Freund complete adjuvant, and the second dose was given 3 to 4 weeks later, subcutaneously, in Freund incomplete adjuvant. Blood was taken after 3 further weeks.

Immunological methods. Immunodiffusion (ID), immunoelectrophoresis (IEP) and single radial ID for the identification of antigens in chromatographic fractions were performed in 1% agarose gel (15, 19). Indirect immunofluorescence was performed on air-dried smears of streptococci using serial twofold dilutions of test antisera and a 1:20 dilution of fluorescein-conjugated sheep anti-rabbit immunoglobulin (Wellcome Reagents Ltd.) (14).

Chemical analyses. Protein was assayed by the Lowry method (12), using bovine serum albumin as a standard. Carbohydrate was assayed by the phenol-sulfuric acid reaction with glucose as a standard (6). Amino sugar was assayed using 3-methyl-2-benzothiazolinone hydrazine hydrochloride (Sigma) and a glucosamine standard (24). Phosphorus was assayed by the method of Chen et al. (4).

Amino acid analysis was carried out on 70 μg of affinity-purified antigen I protein, hydrolyzed in 6 N HCl at 105°C for 17 h in the presence of thioglycolate and under nitrogen, and then analyzed on an LKB Amino Acid Analyzer.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the Laemmli buffer system (10). Molecular weights were estimated using ^{14}C-methylated protein standards (Radiochemical Centre, Amersham, U.K.) detected by autoradiography. Two-dimensional immunoisoelectric focusing was performed in polyacrylamide gels (21).

RESULTS

Extraction of free antigen I from culture supernatants and its partial purification. The separation of antigen precipitated from about 12 liters of culture supernatant by DEAE-cellulose chromatography is shown in Fig. 1. When the fractions were tested in single radial ID against antisera specific for antigens I/II and II, it was observed that fractions eluted with starting buffer reacted with the former but not the latter antiserum and therefore contained antigen I free of antigen II. However, the precipitin reaction was weak, and the ultraviolet absorption profile, which was relatively strong, did not entirely coincide with the distribution of antigen, so it was clear that the antigen constituted only a minor component. Antigen I-positive fractions were combined, dialyzed, and lyophilized to yield 28 mg, in this example.

The yields of three such columns were combined and further chromatographed by gel filtration on Ultrogel AcA 22 (Fig. 2). Fractions containing antigen I were distributed over a wide zone, in which V_e/V_0 extended from 1.85 to 2.32, corresponding to a molecular weight range of approximately 70×10^3 to 300×10^3. In the typical experiment illustrated, 6.7 mg of pooled lyophilized material was obtained from 38 mg of starting sample.

This gel filtration-purified antigen I consisted of 28% protein, 44% carbohydrate, 9.2% amino sugar, and 0.31% phosphorus. In ID against polyspecific *S. mutans* antiserum, it showed partial identity with antigen I/II and nonidentity with antigen II (Fig. 3A). In IEP, it reacted with antiserum to antigen I/II, but not with antiserum to antigen II, and it migrated more slowly than antigens I/II and II (Fig. 3B). When injected into rabbits it yielded an apparently monospecific antiserum, which reacted with the im-

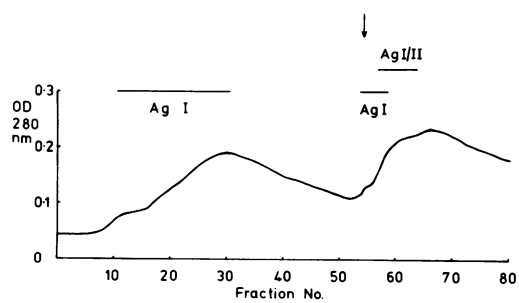

FIG. 1. *DEAE-cellulose chromatography of crude culture supernatant antigens in 0.01 M Tris-hydrochloride (pH 7.4). Arrow indicates step to 0.1 M NaCl; bars indicate distributions of antigens I and I/II.*

munizing antigen I and with antigen I/II to give a line of identity, but not with antigen II (Fig. 3C).

Thus antigen I purified by gel filtration appeared to be antigenically homogeneous. However, whereas its antigenic activity was destroyed by treatment with trypsin or pronase (not shown), its elution pattern on Ultrogel AcA 22 (Fig. 2) was not consistent with that of a single protein species, and in SDS-PAGE, several weak bands staining with Coomassie blue were observed (see below, Fig. 6 lane b). Clearly, therefore, this was not a chemically homogeneous substance suitable for analysis.

Immunoabsorbent separation of antigens I/II and I. It has previously been found that preparations of antigen I/II were sometimes contaminated with free antigen I, which was revealed as a faster-migrating band (band C) in SDS-PAGE (15) (see below, Fig. 6, lane a). This band C material was located in the low-molecular-weight tail of the antigen I/II peak on gel filtration, but was not satisfactorily resolved from antigen I/II by such means. Figure 4 shows, by IEP, the presence of an extra free antigen I line which reacted with antiserum to antigen I/II but not with antiserum to antigen II in the tail fraction of antigen I/II purified on Ultrogel AcA 22. This tail fraction was considered amenable to separation by immunoabsorbent chromatography.

Approximately 3 mg of this mixed antigen I and I/II was applied to a column containing immobilized antibody to antigen II (Fig. 5). Antigen I/II was retained by virtue of its antigen II determinant, whereas free antigen I passed through with the starting buffer. Antigen I/II was later eluted with 6 M guanidinium chloride. Both antigens were collected and dialyzed exhaustively against saline.

The chemical composition of this antigen I preparation is given in Table 1. Consistent with a protein content of approximately 70%, antigen I had an ultraviolet absorption peak at 270 to 280 nm, and absorbed strongly below 230 nm. E_{280} for a solution of 1 mg/ml (as protein) was

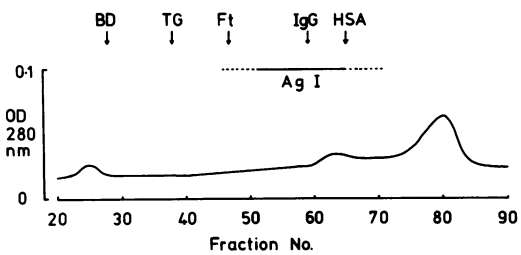

FIG. 2. *Ultrogel AcA 22 chromatography of partially purified antigen I. Bar shows the distribution of antigen I; arrows indicate elution position of: BD, blue dextran (V_0); TG, thyroglobulin (molecular weight, 670,000); Ft, ferritin (apoferritin, 430,000); IgG, immunoglobulin G (150,000); HSA, human serum albumin (67,000).*

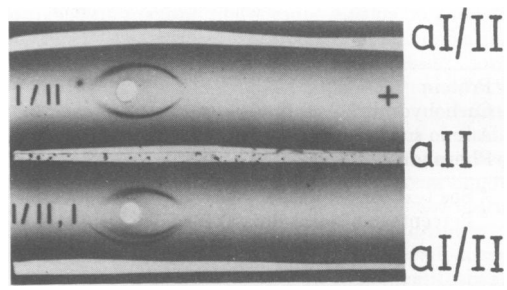

FIG. 4. *IEP analysis of two antigen I/II preparations, to show contamination of tail fraction with antigen I (lower well). Abbreviations as in Fig. 3.*

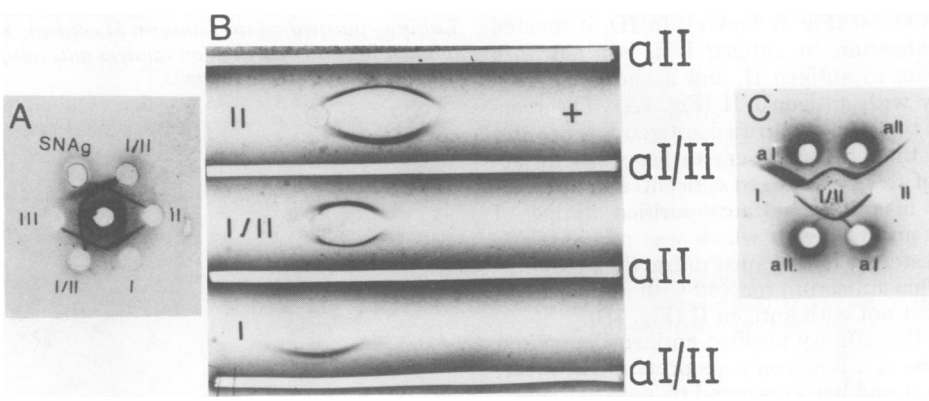

FIG. 3. *(A) ID analysis of antigen I compared with other antigens against polyspecific anti-S. mutans antiserum (center well). (B) IEP analysis of antigen I compared with antigens I/II and II. (C) ID analysis of antiserum to gel filtration-purified antigen I. I, Antigen I; I/II, antigen I/II; II, antigen II; III, antigen III; SNAg, unfractionated culture supernatant antigens. Prefix "a" indicates antiserum to the designated antigen.*

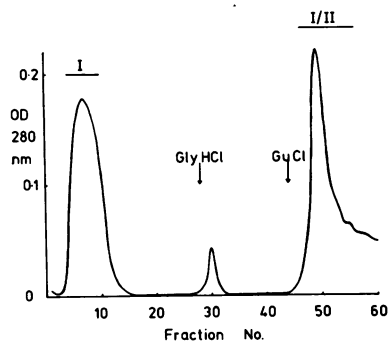

FIG. 5. *Affinity purification of antigen I from mixture with antigen I/II, by immunoabsorbent chromatography on an anti-antigen II column. Arrows indicate elution with: Gly HCl, 0.2 M glycine hydrochloride–0.5 M NaCl (pH 2.8); GuCl, 6 M guanidinium chloride. Bars show distribution of antigens I and I/II.*

TABLE 1. *Chemical composition of antigen I*

Substance	Purified by immunoabsorbent chromatography on:			
	Single column[a]		Tandem columns[a]	
	mg/ml	%[b]	mg/ml	%[b]
Protein	115	71	138	67
Carbohydrate	40	25	57	28
Amino sugar	6	4	11	5
Phosphorus	0	0	0.9	0.4

[a] See text for details.
[b] Percentage of total substances analyzed.

1.07, and the ratio E_{280}/E_{260} was 1.45. On SDS-PAGE, antigen I resolved into one major Coomassie blue-positive band corresponding to band C and with an estimated molecular weight of about 150,000 (Fig. 6, lane c). In ID, it reacted with antiserum to antigen I/II, but not with antiserum to antigen II, and it showed partial identity with antigen I/II (Fig. 7A). The reactions of the affinity-purified antigen I/II are also shown, though these were rather weak on account of the low antigen concentration. On injection in rabbits, affinity-purified antigen I yielded an antiserum which was monospecific when tested in IEP against unfractionated antigens. This antiserum reacted with antigens I/II and I, but not with antigen II (Fig. 7B).

Thus this affinity-purified antigen I appeared to represent a homogeneous protein. However, because it had been prepared by negative selection on an anti-antigen II column, the possibility remained that it could contain contaminants of the starting material which also passed through the immunoabsorbent. The presence of carbohydrate and amino sugar might be an indication of this. The use of a second immunoabsorbent column containing antibody to antigen I/II placed in series after the anti-antigen II column would provide a means of retaining free antigen I which had passed the first anti-antigen II column.

Purification of antigen I on tandem immunoabsorbent columns. Approximately 10 mg of antigen I partially purified from culture supernatant on DEAE-cellulose was applied to tandem immunoabsorbent columns of anti-antigen II and anti-antigen I/II linked in series in that order. After washing with starting buffer,

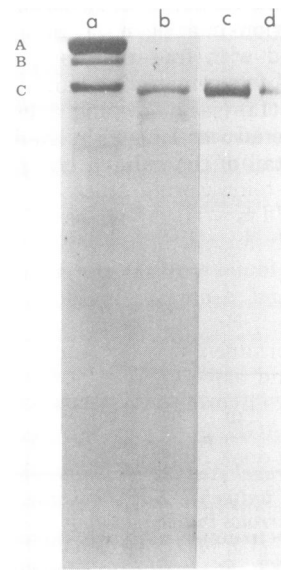

FIG. 6. *SDS-PAGE. Lane a, Antigen I/II preparation, to mark bands A, B, and C; lane b, antigen I incompletely purified by gel filtration; lane c, antigen I affinity purified on anti-antigen II column; lane d, antigen I affinity purified on tandem anti-antigen II and anti-antigen I/II columns.*

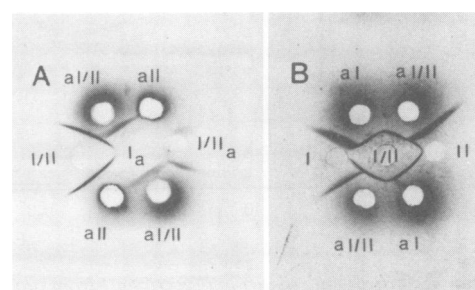

FIG. 7. *(A) ID analysis of affinity-purified antigens I (Ia) and I/II (I/IIa). (B), ID analysis of antiserum to affinity-purified antigen I. Abbreviations as in Fig. 3.*

the columns were separated for elution with dissociating agents (Fig. 8). Nonantigenic material passed through both columns (first double peak), whereas any antigen II, free or combined as antigen I/II, was retained on the first anti-antigen II column. Free antigen I was then retained on the second anti-antigen I/II column and was eluted from it with 6 M guanidinium chloride. This material, which coincided with the ultraviolet absorption peak, was collected and dialyzed exhaustively against saline. The anti-antigen II column was similarly eluted, and a large peak of opaque material overlapping with the distribution of weakly reactive antigen II (or I/II) was obtained.

The chemical composition of this positively selected affinity-purified antigen I was closely similar to that of the negatively selected antigen I (Table 1): both preparations contained similar proportions of protein, carbohydrate, and amino sugar. Amino acid analysis revealed a mixture of most common amino acids, except that methionine and cysteine were not detected, and tryptophan would have been destroyed during hydrolysis. Only low amounts of histidine and arginine were found, so that the acidic residues, glutamate and aspartate, predominated over basic residues. However, since amide nitrogen was not determined, it is not known how much glutamine and asparagine were present in the protein before hydrolysis.

In SDS-PAGE, positively selected antigen I also revealed one Coomassie blue-positive band, corresponding to band C and with an estimated molecular weight of 150,000 (Fig. 6, lane d). On isoelectric focusing, a single band was formed with an estimated isoelectric point at pH 5.0 to 5.2.

Presence of antigen I in oral streptococci. Indirect immunofluorescence using antiserum to affinity-purified antigen I was used to examine its occurrence among representatives of the different serotypes of *S. mutans* and other oral streptococci (Table 2). Strong cell surface fluorescence was seen not only with the homologous strain Guy, but also with other serotype *c* and also serotype *d*, *e*, *f*, and *g* organisms. Lower

TABLE 2. *Immunofluorescence reactions of streptococci with antiserum to antigen I*

Organism/strain	Serotype	Titer[a]
S. mutans		
Guy	*c*	6
Ingbritt	*c*	6
SR 25	*c*	6
SS 41	*c*	6
NCTC 10449	*c*	6
LM 7	*e*	6
T 93	*e*	6
OMZ 175	*f*	5
AHT	*a*	2
OMZ 61	*a*	3
OMZ 176	*d*	4
6715	*g*	5
Fa-1	*b*	0
Rat	*b*	0
S. sanguis OMZ 9		1
S. salivarius HHT		0
Streptococcus CHT		1

[a] Log_2 highest dilution, starting at 1:10, giving strong fluorescence. Control reactions were all completely negative.

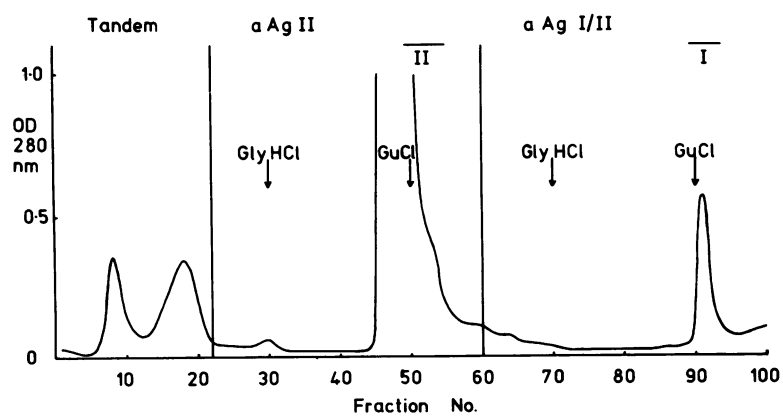

FIG. 8. *Affinity purification of antigen I from partially purified antigen I, by immunoabsorbent chromatography on tandem anti-antigen II and anti-antigen I/II columns. Columns were loaded and eluted with starting buffer while in series (fractions 1 to 21) and then separated for elution with dissociating agents (fractions 22 to 60; 61 to 100). Gly HCl, 0.2 M glycine hydrochloride-0.5 M NaCl (pH 2.8); GuCl, 6 M guanidinium chloride. Bars show distribution of antigens.*

titers were given against serotype *a* cells, and serotype *b* strains were negative. *Streptococcus sanguis*, *Streptococcus salivarius*, and *Streptococcus* CHT were weakly reactive or negative. No organism exhibited fluorescence if treatment with antiserum to antigen I was omitted or replaced by normal rabbit serum.

DISCUSSION

The recognition and identification of antigen I of *S. mutans* have previously depended on the use of two antisera, one having specificity for both antigens I and II, and the other being monospecific for antigen II. The former antiserum was raised against the dual antigen I/II prepared from cells or culture supernatants of *S. mutans*, whereas the latter was raised against antigen II that was prepared by treating antigen I/II with pronase (15). Thus antigen I was defined as the protease-susceptible component of antigen I/II and could be identified as a free substance reacting with antiserum to antigen I/II but not with antiserum to antigen II. In the absence of an antiserum monospecific for antigen I, the presence of antigen I in antigen I/II was demonstrated by the formation of a spur in gel ID against antigen I/II antiserum in comparison with antigen II (15). It has, however, proved impractical to obtain a satisfactory antiserum to antigen I by absorbing antiserum to antigen I/II with antigen II (which is available only in limited amounts) or with *S. mutans* cells or cell walls treated with pronase. It has therefore been necessary to obtain and purify antigen I free of antigen II, not only to characterize it, but also to raise a monospecific antiserum.

Antigen I free of antigen II has been identified in fractions of culture supernatant eluted from DEAE-cellulose under starting buffer conditions, and it has been further purified by gel filtration. The product was, however, not chemically homogeneous by the criterion of SDS-PAGE. Its protein content was low (<30%) and its carbohydrate content was high (>40%), although the only detectable antigen present was protease-susceptible antigen I. It elicited an apparently monospecific antiserum when injected into rabbits, which suggests that the contaminating substances were at most weakly immunogenic. The nature of these contaminants, and hence of their relation to antigen I and the reason for its polydisperse behavior on gel filtration, remains unclear. The low estimates of amino sugar and phosphorus appear to eliminate contamination with significant amounts of peptidoglycan or teichoic acid. Furthermore, contamination with high-molecular-weight substances derived from the culture medium can be eliminated because, among other things, either a dialysate or a semidefined medium was used.

Antigen I was successfully purified to homogeneity as judged by SDS-PAGE by affinity chromatography on immunoabsorbents. Starting with a mixture of antigens I/II and I arising as a by-product in the purification of antigen I/II, antigen I was obtained by retaining antigen I/II on an anti-antigen II column. A more satisfactory procedure, and one amenable to purification of antigen I from crude mixtures, was to use tandem columns, the first containing antibody to antigen II to retain antigen I/II and any free antigen II, and the second containing antibody to antigen I/II to retain free antigen I that had passed the first column. Nonantigenic material, or substances with other antigenic determinants, would pass both columns. Antigen I was then recovered from the second column. An essential requirement in this procedure is that the capacity of the columns, especially of the first anti-antigen II column, should not be exceeded. Otherwise, antigens I/II and II would be copurified with the antigen I. That this did not occur in these experiments was demonstrated by SDS-PAGE, in which antigen I was shown to be homogeneous, and by single radial ID, which showed that the antigen I fractions did not react with antiserum to antigen II. The substitution of antibody to antigen I, now that this has been raised, for antibody to antigen I/II in the second column would not overcome the difficulty arising from overloading, since it would also retain antigen I/II, though not antigen II. This substitution would, however, be a satisfactory alternative to the anti-antigen I/II column.

The two affinity-purified antigen I preparations were identical by ID and IEP and by SDS-PAGE, which showed that they corresponded to band C which was previously recognized as an antigen I moiety (15). They also gave reactions of identity with antigen I which had been incompletely purified by gel filtration.

The peptide molecular weight of 150,000 estimated by SDS-PAGE agrees reasonably well with the difference between antigen I/II (185,000) and antigen II (48,000). The preponderance of acidic over basic amino acid is consistent with an isoelectric point at pH 5.0 to 5.2. This property also agrees with the isoelectric points of antigen I/II (4.8) and antigen II (4.1) (15). However, the nature of the relationship bewteen antigens I and II, and how they are combined in antigen I/II, cannot be explained without detailed information on the structure of these molecules. It would also be interesting to know whether any of these molecules can bind to structural cell wall components such as peptidoglycan, teichoic acid, or polysaccharide, or whether the carbohydrate associated with anti-

gen I is related to cell wall or serotype polysaccharide.

Previous studies using immunofluorescence with antisera to antigen I/II and II (15) have indicated that antigen I was present in *S. mutans* serotypes *a*, *d*, and *g* which lacked antigen II, and possibly in serotypes *c*, *e* and *f* which possessed antigen II. Positive identification in the latter group was not possible because the presence of antigen II precluded additional identification of antigen I with antiserum to antigen I/II. However, the use of antiserum monospecific for antigen I has now confirmed its presence in *S. mutans* serotypes *c*, *e*, and *f* as well as in serotypes *a*, *d*, and *g*. The lower fluorescence titers given with *S. mutans* serotype *a* (and also with *S. sanguis* and *Streptococcus* CHT) may indicate that these organisms possess a more distantly related cross-reactive antigen rather than an identical one. However, more studies will be necessary to determine whether the antigen I of different organisms is truly identical with that isolated from *S. mutans* serotype *c*.

There has recently been another report of *S. mutans* antigens (20), one of which has a molecular weight (190,000) and isoelectric point (5.4) similar to those of antigen I or antigen I/II (15). Further tests will be necessary to determine whether this protein B is indeed identical with either antigen I or I/II. Protein B was also reported to cross-react with an antigen extracted from human heart tissue (9, 20). The finding, however, that antigens extracted from *S. mutans* can themselves bind to human heart tissue (23) raises a further complicating factor in the evaluation of cross-reactivity between streptococcal and heart antigens. Clearly, inability to bind to or cross-react with heart tissue will need to be demonstrated for any antigen that is proposed for potential human vaccination.

It has been known for some time that there are antigens shared by different serotypes of *S. mutans* (2). However, apart from glycerol teichoic acids (5), which are of frequent occurrence in gram-positive bacteria (25), and certain cross-reactions between serotype polysaccharides, e.g., *a-d* (3) and *c-e* (8), these have not been explained. The practice of employing extensive cross-absorption to obtain serotype-specific antisera has of necessity detracted from the recognition of non-serotype-specific protein antigens. Our experience, however, has been that protein antigens I, II, and III are distinctly more immunogenic, even if less abundant, then the polysaccharide in serotype *c* organisms, and therefore of potentially greater importance in immunity to infection. Studies on immunity to dental caries, particularly in rodents, have concentrated on the role of glucosyltransferase and serotype polysaccharide (22), which are involved in the adherence of *S. mutans* to the tooth surface (13). In rhesus monkeys, however, it appears that antibodies to glucosyltransferase, serotype polysaccharide, or lipoteichoic acid are not related to caries immunity (16, 17). Rather, antibodies to antigen I/II may be important (18); and there is now direct evidence that immunization of monkeys with antigen I/II induces significant protection against dental caries (11).

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Bowden, G. H., J. M. Hardie, and E. D. Fillery.** 1976. Antigens from Actinomyces species and their role in identification. J. Dent. Res. (Special Issue A) **55**:A192-204.
2. **Bratthall, D., and B.-M. Pettersson.** 1976. Common and unique antigens of *Streptococcus mutants*. J. Dent. Res. (Special Issue A) **55**:A60-64.
3. **Brown, T. A., and A. S. Bleiweis.** 1979. Chemical, immunochemical, and structural studies of the cross-reactive antigens of *Streptococcus mutans*. Infect. Immun. **24**:326-336.
4. **Chen, P. S., T. Y. Toribara, and H. Warner.** 1956. Micro-determination of phosphorus. Anal. Chem. **28**: 1756-1758.
5. **Chorpenning, E. W., H. R. Cooper, and S. Rosen.** 1975. Cross-reactions of *Streptococcus mutans* due to cell wall teichoic acid. Infect. Immun. **12**:586-591.
6. **Dubois, M., K. A. Gilles, J. K. Hamilton. P. A. Rebers, and E. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350-356.
7. **Fukui, K., Y. Fukui, and T. Moriyama.** 1974. Purification and properteis of dextransucrase and invertase from *Streptococcus mutans*. J. Bacteriol. **118**:796-804.
8. **Hamada, S., and H. D. Slade.** 1976. Purification and immunochemical characterization of type *e* polysaccharide antigen of *Streptococcus mutans*. Infect. Immun. **14**:68-76.
9. **Hughes, M., S. M. Machardy, A. J. Sheppard, and N. C. Woods.** 1980. Evidence for an immunological relationship between *Streptococcus mutans* and human cardiac tissue. Infect. Immun. **27**:576-588.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
11. **Lehner, T., M. W. Russell, and J. Caldwell.** 1980. Immunisation with a purified protein from *Streptococcus mutans* against dental caries in rhesus monkeys. Lancet **i**:995-996.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
13. **Mukasa, H., and H. D. Slade.** 1973. Mechanism of adherence of *Streptococcus mutans* to smooth surfaces. I. Roles of insoluble dextran-levan synthetase enzymes and cell wall polysaccharide antigen in plaque formation. Infect. Immun. **8**:555-562.
14. **Russell, M. W.** 1979. Purification and properties of a protein surface antigen of *Streptococcus mutans*. Microbios **25**:7-18.

15. **Russell, M. W., L. A. Bergmeier, E. D. Zanders, and T. Lehner.** 1980. Protein antigens of *Streptococcus mutans*: purification and properties of a double antigen and its protease-resistant component. Infect. Immun. **28**:486–493.
16. **Russell, M. W., J. Caldwell, and T. Lehner.** 1979. Antibody response to *Streptococcus mutans* antigens during immunisation against dental caries, p. 217–218. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks, Chertsey, U.K.
17. **Russell, M. W., S. J. Challacombe, and T. Lehner.** 1976. Serum glucosyltransferase-inhibiting antibodies and dental caries in rhesus monkeys immunized against *Streptococcus mutans*. Immunology **30**:619–627.
18. **Russell, M. W., S. J. Challacombe, and T. Lehner.** 1980. Specificity of antibodies induced by *Streptococcus mutans* during immunization against dental caries. Immunology **40**:97–106.
19. **Russell, M. W., and T. Lehner.** 1978. Characterization of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype *c*. Arch. Oral Biol. **23**: 7–15.
20. **Russell, R. R. B.** 1979. Wall-associated protein antigens of *Streptococcus mutans*. J. Gen. Microbiol. **114**:109–115.
21. **Schmidt-Ullrich, R., W. S. Thompson, and D. E. M. Wallach.** 1977. Antigenic distinctions of glycoproteins in plasma and mitochondrial membranes of lymphoid cells neoplastically transformed by simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **74**:643–647.
22. **Smith, D. L., M. A. Taubman, and J. L. Ebersole.** 1978. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in hamsters caused by homologous and heterologous serotypes of *Streptococcus mutans*. Infect. Immun. **21**:843–851.
23. **Stinson, M. W., R. J. Nisengard, and E. J. Bergey.** 1980. Binding of streptococcal antigens to muscle tissue in vitro. Infect. Immun. **27**:604–613.
24. **Tsuji, A., T. Kinoshito, and M. Hoshino.** 1969. Analytical chemical studies on amino augars. II. Determination of hexosamines using 3-methyl-2-benzo-thiazolinone hydrazine hydrochloride. Chem. Pharm. Bull. **17**: 1505–1510.
25. **Wicken, A. K., and K. W. Knox.** 1975. Lipoteichoic acids: a new class of bacterial antigens. Science **187**: 1161–1167.