INFECTION AND IMMUNITY, Dec. 1989, p. 3834–3840
0019-9567/89/123834-07$02.00/0

Vol. 57, No. 12

Purification of a *Streptococcus mutans* Protein That Binds to Heart Tissue and Glycosaminoglycans

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Received 26 June 1989/Accepted 11 September 1989

Proteins of *Streptococcus mutans* MT703 were isolated by differential filtration from chemically defined culture medium following growth of the bacteria. Incubation of this preparation with cryostat-cut sections of fresh rabbit cardiac muscle resulted in deposition of streptococcal components on basement membranes of sarcolemmal sheaths and capillary walls, as indicated by indirect immunofluorescence assay. Binding of radioiodinated streptococcal proteins to heart in vitro was time dependent and saturable. Unlabeled *S. mutans* proteins competitively inhibited 72% of heart binding by the radiolabeled proteins, indicating a high level of binding specificity. A selection of components common to tissue basement membranes was tested for their abilities to inhibit the binding of streptococcal proteins to heart tissue. Of the glycosaminoglycans, heparin was the most effective inhibitor, followed by heparan sulfate and chondroitin sulfate. Hyaluronic acid was not inhibitory. Of the glycoproteins tested, laminin and collagen type IV were weakly inhibitory, whereas fibronectin was ineffective. A single polypeptide was purified to homogeneity by affinity chromatography on a column of heparin-agarose. Gel filtration chromatography of the purified protein under nondissociating conditions showed a single component at 31 kilodaltons (kDa), whereas in sodium dodecyl sulfate-polyacrylamide gel electrophoresis one band appeared at 8 kDa. This indicates that the tissue-binding protein may either be a linear polypeptide or be released into the environment by the bacterium as a tetramer of the 8-kDa polypeptide. The purified protein had an isoelectric point of 9.5 and showed binding activity for basement membranes in thin sections of heart. Chemical analyses of the purified binding protein showed it to have high contents of lysine and alanine and to be devoid of half-cystine, methionine, tyrosine, histidine, and both neutral and amino sugars.

Streptococcus mutans is commonly found in supragingival dental plaque and is considered to be the major etiologic agent of pit and fissure caries in humans (5, 12, 13). In addition, it can enter the bloodstream as a consequence of trauma to oral mucosa and colonize heart valves and the endocardium, resulting in a subacute bacterial endocarditis (7, 16).

Immunization of rabbits with suspensions of disrupted *S. mutans* cells results in the production of serum antibodies that bind in vitro to human, rabbit, and monkey cardiac muscle (9, 19, 23). It was recently determined that these antibodies have specificities identical to those of autoantibodies found at low concentration in the sera of nonimmunized rabbits and are not cross-reactive with *S. mutans*. Thus, it is likely that increased production of heart-reactive antibodies during immunization resulted from injury of muscle fibers by streptococcal components that exposed hidden self-antigens and stimulated preexisting clones of self-reactive B lymphocytes (20, 21). The contribution of these autoantibodies to the pathogenesis of heart disease has not been determined; however, myocardial lesions have been observed in rabbits that were injected with *S. mutans* components (9, 20). These lesions appeared as perivascular infiltrations of polymorphonuclear cells and plasma cells. Immunofluorescence staining revealed granular deposits of immunoglobulins along sarcolemmal sheaths and capillaries; however, it was not clear whether the antibodies were bound directly to heart components or to streptococcal antigens previously deposited in the heart.

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Intravenous injection of disrupted *S. mutans* into rabbits also results in a severe nephritis, involving glomeruli, tubules, and interstitium (1, 19). The histopathologic and immunopathologic features of the disease resemble those of streptococcus-associated nephritides in humans, particularly the diffuse glomerular nephritis often associated with subacute bacterial endocarditis (1, 6). Immunofluorescence assays of monkey and rabbit tissues treated in vitro with extracts of *S. mutans* showed that this opportunistic pathogen contains proteins that bind directly to basement membranes of capillary walls and sarcolemmal sheaths of cardiac muscle and to basement membranes of renal tubules and glomeruli (18, 19).

The present study was undertaken to identify and characterize components of *S. mutans* that may contribute to its pathogenicity in heart disease. In this report, we describe the purification and properties of a protein that has binding activity for glycosaminoglycans and basement membrane-containing structures of cardiac muscle.

MATERIALS AND METHODS

Bacteria and culture conditions. *S. mutans* MT703 (serotype e) was grown in chemically defined medium (22). Sixty-liter cultures were grown in an 80-liter pilot plant fermentor (New Brunswick Scientific Co., Inc., Edison, N.J.) at 37°C with agitation. The culture was maintained at pH 6 by constant addition of 5 M NaOH. Bacterial growth was monitored spectrophotometrically at 600 nm. Cultures in late-logarithmic to early-stationary phases of growth were harvested by tangential flow filtration, using a filter with 0.2-μm pores (Millipore Corp., Bedford, Mass.). Extracel-

lular components were isolated from culture medium by ultrafiltration on a filter with a 10,000-molecular-weight pore size (Filtron Technology Corp., Clinton, Mass.). The retentate was adjusted to pH 7.3, dialyzed against water, and lyophilized.

Immunization. Heat-treated (70°C for 30 min) disrupted *S. mutans* cells were homogenized in complete Freund adjuvant at 4 mg of protein in 2 ml and injected intramuscularly into a goat biweekly for 6 months.

Absorption of antiserum with tissue. To remove any naturally occurring tissue-reactive antibodies, rabbit heart homogenate was added to goat anti-*S. mutans* serum at 1 mg of tissue protein per ml. The mixture was incubated at room temperature for 3 h and at 4°C overnight and then clarified by centrifugation.

Radioisotope labeling. Bacterial proteins were radiolabeled with $Na^{125}I$ (ICN Radiochemicals, Irvine, Calif.), using Iodo-Beads (Pierce Chemical Co., Rockford, Ill.). The bacterial components (3 mg of protein) in 1 ml of 0.01 M Na_2HPO_4–KH_2PO_4, pH 7.3, containing 0.15 M NaCl (phosphate-buffered saline [PBS]) were added to four Iodo-Beads that had been preincubated with 1 mCi of $Na^{125}I$ in 200 ml of PBS for 5 min. After 10 min, the solution was removed from the reaction tube and loaded on a column (0.8 by 20 cm) of Bio-Gel P-6DG (Bio-Rad Laboratories, Richmond, Calif.). The radioactive proteins eluted at the void volume were collected. The ^{125}I-labeled preparation had specific radioactivity of 3×10^7 cpm/mg of protein.

Tissue homogenate. Rabbit heart was shaved into ice-cold PBS and rinsed three times to remove blood components. The tissue suspension (20% [wt/vol]) was homogenized in a Waring blender and passed through three layers of cheesecloth to remove large clumps of connective tissue. After the resulting homogenate was made up to 0.2 mM phenylmethylsulfonyl fluoride and 0.05% sodium azide, it was ground in a Teflon mortar and pestle and heated at 65°C for 15 min, followed by cooling in ice. The tissue was washed four times with PBS and stored at −20°C.

Binding assay. Heart-binding activity of streptococcal proteins was determined by using a radioisotope assay. A suspension of homogenized tissue (50 μg of protein in 0.1 ml) was dispensed into wells of a 96-well vinyl assay plate (Costar, Cambridge, Mass.) and incubated at 4°C overnight. Tissue-coated wells and control wells without tissue were pretreated subsequently with 0.2 ml of PBS containing 1% bovine serum albumin for 1 h and 0.2 ml of PBS–0.05% Triton X-100 (PBS-Triton) for 20 min. The wells were then incubated with 0.1 ml of ^{125}I-labeled streptococcal proteins in PBS-Triton for 1 h and rinsed three times with PBS-Triton to remove unbound reactants, and the radioactivity was counted on a gamma radiation counter.

When selected substances were tested as inhibitors of the binding of radiolabeled streptococcal proteins to heart tissue, inhibitors were added to radiolabeled extracellular proteins (50 μg/ml) and assayed as described above after 1 h at room temperature.

Enzyme-linked immunosorbent assay (ELISA) was also used to determine binding to tissue by unlabeled bacterial components. The tissue-coated wells were incubated sequentially with 0.1 ml of bacterial components, goat anti-*S. mutans* serum (1:200), and rabbit anti-goat immunoglobulin G (IgG) conjugated to alkaline phosphatase (1:1,000). Each step was followed by two washes with PBS-Triton. The wells were incubated with 0.1 ml of *p*-nitrophenylphosphate (1 mg/ml) in 9.7% (vol/vol) diethylamine buffer, pH 9.8, for 20 min. The reaction was stopped by adding 0.05 ml of 1.5 M NaOH, and the A_{405} was determined with an ELISA reader (model EL310; BIO-TEK Instruments Inc., Burlington, Vt.). The wells without tissue were used as a control. Absorbance of the streptococcal antigen-treated tissue was corrected against absorbance of the control.

Affinity chromatography. Streptococcal components (2.5 g [dry weight]) were dissolved in 250 ml of PBS. The solution was adjusted to pH 7.3 and centrifuged at 12,000 × *g* at 4°C for 1 h. The supernatant was loaded on a column (1 by 17.5 cm) of heparin-agarose (Sigma Chemical Co., St. Louis, Mo.). The column was washed extensively with 0.01 M sodium phosphate, pH 7.3, containing 0.5 M NaCl. A continuous salt gradient from 0.5 to 1.2 M NaCl was used to elute the bound proteins. After dialysis and lyophilization, the column peaks were dissolved in 10 ml of PBS and rechromatographed on the heparin-agarose column. The eluted fractions were dialyzed and lyophilized.

Gel filtration chromatography. Molecular weights of proteins under nondissociating conditions were determined by using a column (1 by 30 cm) of Superose 6 (Pharmacia, Uppsala, Sweden) equilibrated with 10 mM phosphate, pH 7.2, containing 0.5 M NaCl, at a flow rate of 0.4 ml/min. The chromatography system included a high-performance liquid chromatography system (model 342; Beckman Instruments, Inc., Berkeley, Calif.) and a model HM/HPLC Holochrome detector (Gilson, Middletown, Wis.). The void volume and total volume of the column were determined by using blue dextran and acetone, respectively. Ferritin (440 kilodaltons [kDa]), aldolase (158 kDa), ovalbumin (45 kDa), chymotrypsin (25 kDa), and aprotinin (6.5 kDa), were used as molecular weight standards.

Antibody purification. IgG was fractionated by adding ammonium sulfate to goat anti-*S. mutans* serum (33% saturation). The resulting IgG was loaded on a column of CNBr-activated Sepharose 4B (Pharmacia), cross-linked to streptococcal protein (0.9 mg/ml), by the procedure specified by the manufacturer. After the column was washed with PBS containing 0.5 M NaCl, the specific antibodies were eluted with 3 M KSCN in 0.5 M NH_4OH. The antibodies were dialyzed against PBS before use.

Immunofluorescence assay. Cryostat-cut sections (3 μm thick) of fresh cardiac muscle from New Zealand White Rabbits were incubated with streptococcal components for 1 h, followed by 15 min of washing with PBS. The tissue was then incubated with diluted goat anti-*S. mutans* serum or affinity-purified antibodies for 1 h, followed by a 15-min wash in PBS. The tissue was then incubated for 1 h with fluorescein isothiocyanate-conjugated affinity-purified rabbit anti-goat IgG (1:20) (Organon Teknika, West Chester, Pa.), followed by a 15-min washing in PBS. For controls, tissue not pretreated with bacterial components was incubated with the same serological reagents.

Electrophoresis. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed with 13.5% acrylamide gels by the method of Laemmli (11). Lysozyme (14.3 kDa), lactoglobulin (18.4 kDa), carbonic anhydrase (29 kDa), ovalbumin (45 kDa), and bovine serum albumin (67 kDa) were used as molecular weight standards. Horse heart myoglobin (17 kDa) and its cleaved polypeptides (14.4, 8.2, 6.2, and 2.5 kDa) (Sigma) were also used as molecular weight standards. Gels were silver stained by the procedure of Morrisey (14).

The isoelectric point of the protein was determined with a 5% polyacrylamide gel containing 2% Ampholine 3.5-10 (LKB-Pharmacia, Bromma, Sweden) by the procedure provided by the manufacturer.

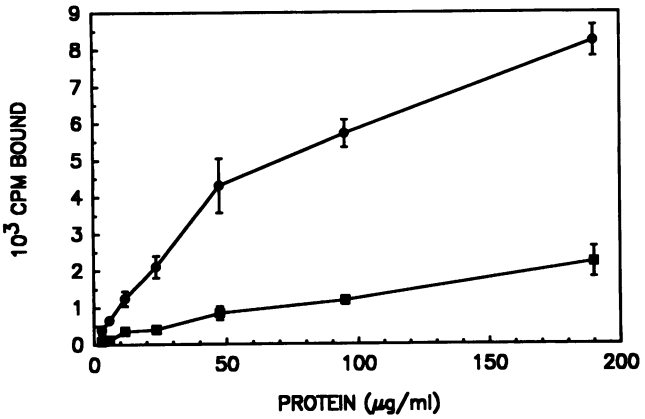

FIG. 1. Binding of radiolabeled protein of *S. mutans* to rabbit heart tissue. Symbols: ●, binding to tissue-coated well; ■, binding to tissue-free control well.

Chemical analysis. Purified protein was hydrolyzed by 6 N HCl at 110°C for 24 h under an atmosphere of nitrogen. The mixture was evaporated to dryness at reduced pressure and dissolved in water. Amino acid analysis was performed on an amino acid analyzer (model 6300A; Beckman Instruments, Inc., Fullerton, Calif.). No attempt was made to estimate the tryptophan, glutamine, or asparagine content.

Total protein was determined by using BCA protein assay reagent (Pierce), and carbohydrate was determined by using a phenol-sulfuric acid assay (3).

RESULTS

Heart binding. Components of *S. mutans* were isolated from culture medium, radiolabeled with $Na^{125}I$, and incubated with heart tissue. Binding activity for heart was proportional to the amount of available bacterial protein up to 50 μg/ml, at which saturation of tissue sites occurred (Fig. 1). At this concentration, the amount of heart-bound protein was 26 ng or 0.52% of the available radioactive proteins. Binding of the radiolabeled bacterial proteins to tissue-free control wells was low and did not show any evidence of saturation kinetics. Binding of streptococcal proteins to heart also showed time dependence (Fig. 2), with maximum binding occurring at 60 min.

To test the specificity of binding and to determine the effect of radioiodination on the binding activity of the bacterial proteins, unlabeled proteins were tested for competitive inhibition of the radiolabeled proteins in an isotopic dilution experiment (Fig. 3). Maximum inhibition of 72% was obtained at a fourfold excess of inhibitor (200 μg/ml against 50 μg/ml); further increases of inhibitor up to 24 to 1 did not result in increased inhibition. Although most of the initial protein binding was subject to competitive inhibition by unlabeled proteins, proteins previously bound to heart were not easily dissociated by incubating the preformed complexes with excess unlabeled proteins. In the latter experiment, the radiolabeled proteins were incubated with cardiac muscle before addition of the unlabeled preparation; only 41% of the radiolabeled proteins were released over 22 h by a 24-fold excess of the inhibitor. No radioactivity was released by tissue suspended in PBS solution only.

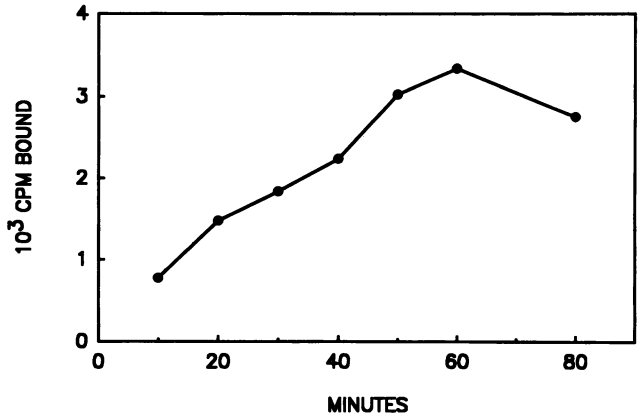

FIG. 2. Binding of radiolabeled streptococcal protein (50 μg/ml) to rabbit heart tissue over time.

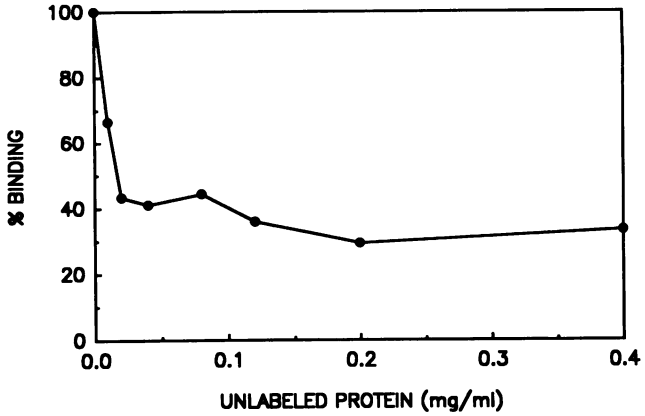

FIG. 3. Competitive inhibition of heart binding activity of radiolabeled streptococcal protein by unlabeled protein. Increasing amounts of unlabeled protein were mixed with 50 μg of the radiolabeled protein per ml before addition to tissue-coated wells.

Specificity of binding. Indirect immunofluorescence assays showed that *S. mutans* components bind selectively to sarcolemmal sheaths and capillaries of cardiac muscle (18; see Fig. 8). To identify the tissue components involved, selected glycoproteins and glycosaminoglycans commonly found in basement membranes were tested for their abilities to competitively inhibit binding of streptococcal proteins to heart. The substances were mixed with radiolabeled bacterial proteins and added to tissue-coated wells (Table 1). Strong inhibition was exerted by heparin and to a lesser extent by heparan sulfate and chondroitin sulfate. Hyaluronic acid showed no inhibitory activity. Dextran sulfate, used as a structural analog of glycosaminoglycans, was inhibitory at 1.0 mg/ml but not at 0.1 mg/ml. Among glycoproteins, laminin and collagen type IV showed inhibitory activity at 0.4 mg/ml but not at 0.1 mg/ml. Fibronectin did not inhibit binding of streptococcal components to heart tissue at the concentrations tested.

Purification. Because the inhibition assays indicated that heparin and other glycosaminoglycans may bind to proteins of *S. mutans*, chromatography on a column of heparin-agarose was used to affinity purify the heart-binding protein (Fig. 4). Bacterial components were eluted from the column with a sodium chloride gradient as two peaks, designated peak A and peak B. Peak A eluted between 0.50 and 0.65 M NaCl and contained major polypeptides at 20, 28, and 45 kDa, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 5). Peak B eluted between 0.9 and 1.0 M NaCl and contained a major polypeptide at 8 kDa and four minor polypeptides. The 8-kDa polypeptide was

TABLE 1. Effects of glycosaminoglycans and glycoproteins on binding of streptococcal components to heart tissue

Inhibitor	Amt (mg/ml)	Inhibition (%)[a]
Hyaluronic acid	0.1	3 ± 4
	1.0	0 ± 3
Heparin	0.1	62 ± 1
	1.0	64 ± 1
Heparan sulfate	0.1	33 ± 12
	1.0	33 ± 17
Chondroitin sulfate type A	0.1	17 ± 7
	1.0	24 ± 7
Dextran sulfate	0.1	0
	1.0	29 ± 8
Laminin	0.1	3 ± 5
	0.4	43 ± 3
Fibronectin	0.1	6 ± 9
	0.4	0
Collagen type IV	0.1	0
	0.4	30 ± 9

[a] Inhibition assays were done in duplicate; means and ranges are given.

purified to apparent homogeneity by a second passage through the heparin-agarose column (Fig. 5). When the affinity-purified protein was examined by gel filtration chromatography on a Superose 6 column under nondissociating conditions, a single component was eluted with an apparent molecular mass of 31 kDa (Fig. 6). This indicates that the heparin-binding protein of *S. mutans* is either a tetramer of the 8-kDa polypeptide or a linear polypeptide with nominal tertiary structure. Monospecific antibodies prepared to the 8-kDa polypeptide did not react with larger *S. mutans* components in Western immunoblot assays. This observation together with the precise and reproducible molecular mass value make it unlikely that the 8-kDa protein is a product of random proteolysis in the culture fluid. The purity of the binding protein was confirmed by detection of only one component in Western blot assays with rabbit anti-*S. mutans* sera and in isoelectric focusing experiments. The yield of the purified protein was 9.1 mg from 4.3 g of streptococcal protein derived from 60 liters of culture medium.

Characterization. The heart-binding activity of the proteins in the column fractions was determined by ELISA (Fig. 7), using goat anti-*S. mutans* serum and alkaline phosphatase-conjugated anti-goat IgG antibody. Peak B material and the purified 8-kDa protein bound strongly to heart, whereas peak A showed little binding activity.

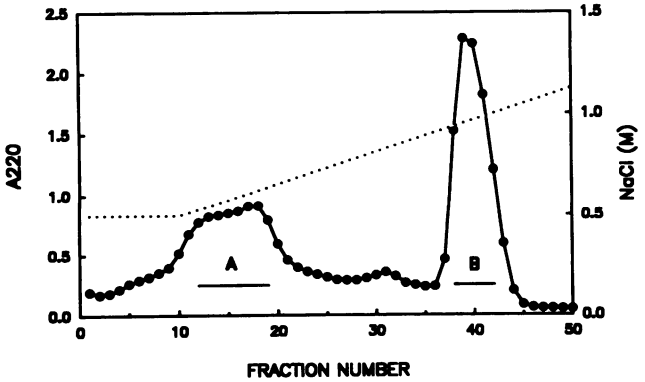

FIG. 4. Heparin-agarose affinity chromatography of streptococcal components. Fractions were pooled as indicated. The NaCl gradient is indicated by the dotted line.

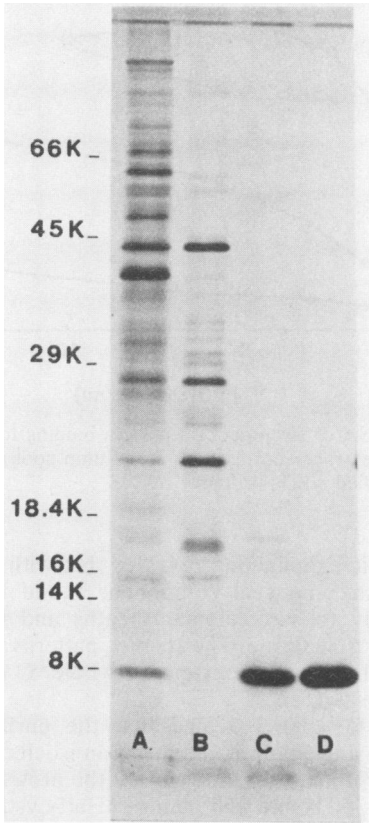

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions obtained from heparin-agarose chromatography. Lanes: A, crude extract; B, column pool A; C, column pool B; D, pool B after second column passage. Molecular weight values of standard proteins are shown.

To determine the structures of cardiac muscle that bind this streptococcal component; cryostat-cut sections of fresh rabbit heart were treated with the unfractionated streptococcal proteins or the 8-kDa protein and stained by indirect immunofluorescence, using affinity-purified goat antibodies to the 8-kDa protein as the primary serological reagent (Fig.

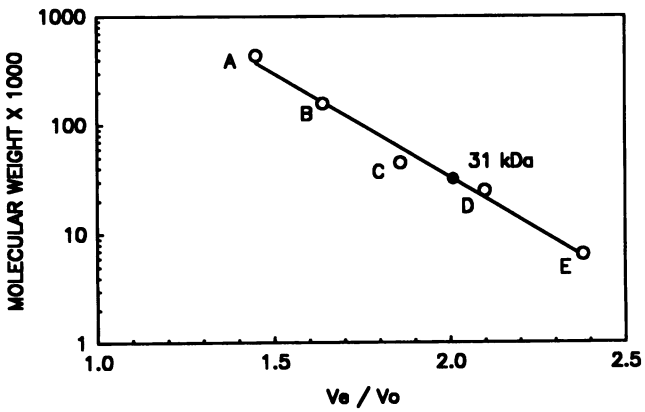

FIG. 6. Determination of molecular weight of the heparin-binding protein under nondissociating conditions. Molecular weight standard proteins: A, ferritin (440 kDa); B, aldolase (158 kDa); C, ovalbumin (45 kDa); D, chymotrypsin (25 kDa); E, aprotinin (6.5 kDa). V_0, Void volume; V_e, elution volume.

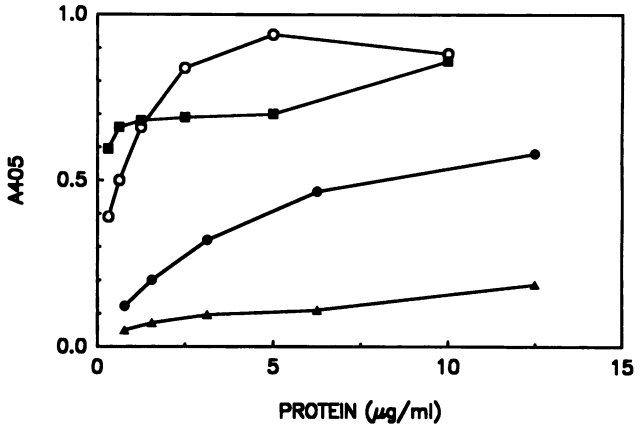

FIG. 7. ELISA of streptococcal protein binding to heart. Symbols: ●, crude extracellular extract; ▲, column pool A; ■, column pool B; ○, purified 8-kDa protein.

8). Microscopic examination revealed that with both preparations, the streptococcal component bound linearly and homogeneously to sarcolemmal sheaths and perivascular areas of the muscle tissue. The staining patterns were similar to those obtained when antisera to whole *S. mutans* was used in the assay (18).

Isoelectric focusing revealed that the purified binding protein was a single polypeptide with an isoelectric point of 9.5. Amino acid analysis showed that the protein contained large amounts of lysine and alanine. Half-cystine, methionine, tyrosine, and histidine were absent (Table 2). Neither neutral nor amino sugars were detected in the purified protein.

DISCUSSION

Binding of radioiodinated components of *S. mutans* MT703 to rabbit cardiac muscle showed saturation kinetics, time dependence, and sensitivity to competitive inhibition by unlabeled streptococcal and tissue components. These pharmacologic criteria indicate that binding of radiolabeled proteins (72%) to heart involved stereochemical specificity rather than nonspecific adsorption (8). Preformed complexes of heart and streptococcal components were only partially (<40%) dissociated by incubation with unlabeled streptococcal components over 24 h. This unexpectedly low value indicates that binding to cardiac muscle components may lead to conformational changes in the streptococcal protein that make competitive dissociation of complexes by free proteins difficult.

Except for hyaluronic acid, glycosaminoglycans inhibited binding of proteins of *S. mutans* to heart tissue. Inhibitory activity may be correlated with the density of anionic substituents of the molecule. The glycosaminoglycans are acidic polysaccharides containing both uronic acids and amino sugars. In addition, most glycosaminoglycans contain O- and N-linked sulfate groups (24). Heparin, a serum component, averages three sulfate groups per repeating dissacharide unit, whereas heparan sulfate, a structural analog present in tissue, contains only one. Hyaluronic acid is not sulfated. Successful dissociation of the binding protein from heparin-agarose by 1 M NaCl suggested that the binding is mediated largely by ionic interactions between oppositely charged polymers. This conclusion is substantiated by the properties of the purified streptococcal protein.

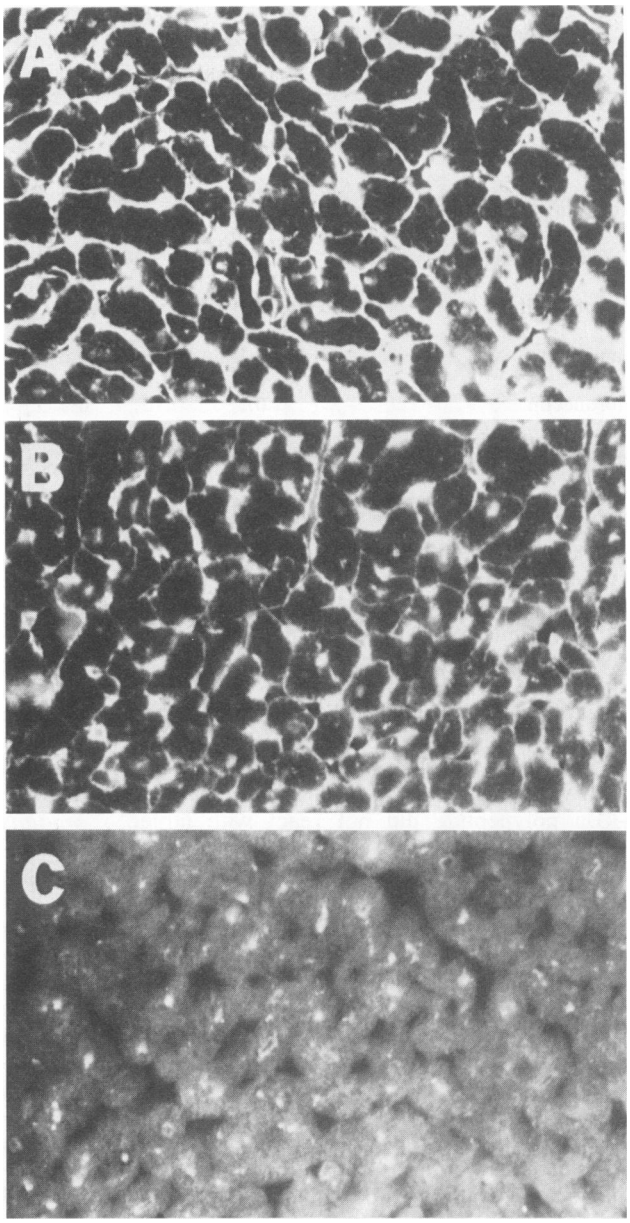

FIG. 8. Immunofluorescence staining of rabbit heart tissue pretreated with unfractionated streptococcal components (2 mg/ml) (A), purified 8-kDa protein (20 μg/ml) (B), and PBS without streptococcal components (C). Magnification, ×400.

Its isoelectric point of 9.5 and high content of lysine indicate that the binding protein has a net positive charge at physiological pH due to free epsilon amino groups. Therefore, it appears that sulfated glycosaminoglycans, such as heparan sulfate, in the basement membranes of animal tissues can serve as receptors for binding of streptococcal proteins. *Streptococcus pyogenes* also produces a heparin-inhibitable basement membrane-binding protein with similar physical properties (2). Although the exact relationship of these bacterial proteins remains to be determined, preliminary experiments have shown that affinity-purified polyclonal antibodies cross-react with purified proteins of both *Streptococcus* species.

The identities of the tissue structures which bind the 8-kDa protein were revealed by indirect immunofluores-

TABLE 2. Amino acid composition of the 8-kDa protein

Amino acid	% of total[a]
Aspartic acid/asparagine	8
Threonine	3
Serine	5
Glutamic acid/glutamine	11
Proline	2
Glycine	7
Alanine	19
Half-cystine	0
Valine	9
Methionine	0
Isoleucine	4
Leucine	6
Tyrosine	0
Phenylalanine	5
Histidine	0
Lysine	16
Arginine	5
Tryptophan	ND[b]

[a] Number of residues per 100 amino acid residues.
[b] ND, Not determined.

cence staining with affinity-purified antibodies to the protein. This antibody preparation was totally free of natural antibodies to heart components that frequently complicate interpretation of indirect immunofluorescence assays when whole sera are used (21). With purified antibodies, immunofluorescence assay of heart tissue treated with unfractionated streptococcal components and purified protein showed homogeneous, linear staining of sarcolemmal sheaths (Fig. 8), indicating that the 8-kDa protein binds to proteoglycan-rich connective tissue.

Heparan sulfate proteoglycans are ubiquitous in animal tissues, particularly on plasma membranes and the extracellular matrix comprising basement membranes. Their relatively large polyanionic and hydrated domains are believed to influence the chemical and physical properties of the pericellular regions (for reviews, see references 4 and 24). For example, they may control the access to cell membranes of regulatory molecules such as growth factors, hormones, and neurotransmitters and influence local cation balance, especially that of Ca^{2+} (15). Although in vivo effects of the purified streptococcal protein remain to be determined, it can be anticipated that binding of the 8-kDa protein to cell surfaces and basement membranes may exert direct cytotoxicity or interfere with passage of ions and proteins through basement membranes by altering the net charge. Polycationic substances, such as protamine sulfate, have been shown to cause structural alterations in glomerular epithelia of perfused kidneys. These changes are similar to those observed in some human glomerulonephritis cases and were largely reversed by subsequent perfusion with heparin (10, 17). An alternative or concurrent mechanism for pathogenesis may involve serological events. Binding of streptococcal proteins to tissue may result in in situ formation of immune complexes as antibody concentrations increase in the blood. Activation of complement through the classical or alternate pathways may lead to localized inflammation that is characteristic of streptococcus-associated heart disease.

The 8-kDa protein may also act as a carrier of other streptococcal virulence factors. The strong positive charge on the binding protein may cause it to associate with anionic streptococcal proteins and lipoteichoic acid on the bacterial surface and upon their release into the environment. The molecular aggregates may bind to the tissue and exhibit enhanced pathogenicity through their combined effects. Preliminary experiments indicate that the 8-kDa protein is present on the cell wall of *S. mutans* and can complex with lipoteichoic acid in solution. Additional studies are needed to define the mechanism for its release from the cell and the roles it plays in bacterial adherence to host surfaces and in pathogenesis in vivo.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant R01-DE05696 from the National Institute of Dental Research and a grant-in-aid from the American Heart Association.

We thank Earl J. Bergey for the amino acid analysis and Frank Watson for excellent technical assistance.

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