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Heparin-Inhibitable Basement Membrane-Binding Protein of *Streptococcus pyogenes*

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Solubilized surface proteins of *Streptococcus pyogenes* serotype M6 were found by indirect immunofluorescence assays to bind selectively to proteoglycan-containing regions of basement membranes of kidney and cardiac muscle in vitro. Epithelial, endothelial, and interstitial cells were unstained. Binding of streptococcal protein to basement membranes was competitively inhibited by heparin and, to a lesser extent, by heparan sulfate. Weak inhibition was also observed with other glycosaminoglycans, including dermatan sulfate, chondroitin sulfate, and hyaluronic acid. Type IV collagen, gelatin, serum fibronectin, glucuronic acid, and a selection of monosaccharides had no significant effects on binding. The heparin-inhibitable basement membrane-binding protein was purified by affinity chromatography on heparin-Sepharose 6-B. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and urea dissociated the affinity-purified protein into two polypeptides of 9,000 and 15,000 M_rs. Chemical analyses revealed that the purified protein was devoid of cysteine, amino and neutral sugars, and phosphate. Thus, the polypeptides are not glycosylated or complexed with trace amounts of lipoteichoic acid or polysaccharide. Binding of purified protein to tissue was determined by direct radioassay and indirect immunofluorescence and was inhibitable by heparin. Although the in vivo effects of this streptococcal component remain to be determined, its deposition on basement membranes in vitro supports the hypothesis that it contributes to the pathogenesis of poststreptococcal glomerulonephritis or acute rheumatic fever.

The etiologic role of *Streptococcus pyogenes* in acute rheumatic fever and acute poststreptococcal glomerulonephritis is well established; however, the mechanisms of bacterial virulence in these diseases are poorly understood (15, 25). One current hypothesis involves direct binding of bacterial adhesins or exotoxins to components of cardiac muscle and kidney. These substances, released by streptococci at remote sites of infection such as the nasopharynx or skin, may be carried via the blood stream to other tissues, where they accumulate on the basement membranes. Histopathology may then arise from direct toxicity of the streptococcal component, from its in situ reaction with antibodies and complement, or from a combination of these mechanisms (1, 15, 23).

Several binding interactions between intact group A streptococci and components of animal tissue have been reported. *S. pyogenes* surfaces bind fibronectin (20, 21), laminin (26), fibrinogen (11, 29), nonspecific immunoglobulins A and G (10, 12), α_2-macroglobulin (17), β_2-microglobulin (13), and albumin (12). Although the consequences of these binding phenomena for host-parasite relationships are not known, they are believed to promote streptococcal adherence to mucous membranes (20) or to mask and protect bacterial surfaces from host defense mechanisms (29). In addition to surface adhesins, several extracellular toxins of *S. pyogenes* are known to bind to and damage host cells (for a review, see reference 27).

We reported previously (23, 24) on extracted surface proteins of *S. pyogenes* M serotypes 6 and 12 that bind specifically to basement membranes of muscle and kidney tissues in vitro. The proteins, which behaved as a large aggregate during chromatographic manipulations, were dissociated into four proteins with apparent M_rs of 10,000, 15,000, 17,000, and 19,000 upon electrophoresis in sodium dodecyl sulfate (SDS). Cardiac muscle-binding activity was apparently associated with the 19,000-M_r protein. In this report, we describe the inhibition of the binding of the streptococcal protein to cardiac muscle and kidney by heparin and the affinity purification and partial characterization of the heparin-binding protein.

MATERIALS AND METHODS

Bacteria and culture conditions. *S. pyogenes* serotype M6 (ATCC 12348) was grown in dialyzed tryptic soy broth supplemented with 0.1% yeast extract (Difco Laboratories, Detroit, Mich.). All cultures (1 liter of medium in a 2-liter Erlenmeyer flask or 20 liters of medium per 30-liter carboy) were incubated statically at 37°C. Cell growth was monitored with a Klett-Summerson colorimeter with a no. 66 filter. Cultures in mid-logarithmic to early-stationary phases of growth were harvested by centrifugation at 10,000 × *g* for 15 min at 4°C. The cells were washed twice with phosphate-buffered saline (PBS) (0.01 M NaH_2PO_4-Na_2HPO_4 [pH 7.2] containing 0.15 M NaCl).

Alkali extraction of *S. pyogenes*. Bacteria were extracted sequentially with organic solvents and alkali as previously described (23). After centrifugation at 4°C, the supernatant fluid was collected, dialyzed against water, and lyophilized.

IIF assay. Rabbit anti-*S. pyogenes* sera were titrated by indirect immunofluorescence (IIF) assay on 2-μm-thick, cryostat-cut sections of fresh cynomolgus monkey tissue and on sections of tissue that were pretreated with bacterial cell extracts as previously described (23). Naturally occurring, heart-reactive antibodies (24, 25a) were removed from normal and immune rabbit sera by absorption with homogenized human heart.

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Radiolabeling of bacterial extracts. Bacterial cell extracts (150 mg [dry weight] per 30 ml of 0.1 M Na_2HPO_4 [pH 9.0]) were incubated with 25 mCi of [^{3}H]acetic anhydride (5.5 mCi/mmol) and stirred for 18 h at 4°C. The solution was dialyzed against distilled water to remove nonprotein reactants and lyophilized. The tritium-labeled bacterial proteins had a specific radioactivity of 4.6×10^6 cpm/mg (dry weight).

Tissue preparation. Cardiac muscle which had been freshly obtained from cynomolgus monkeys was cut into 5-mm pieces and blended in cold PBS with a Sorvall Omnimixer (Ivan Sorvall, Inc., Norwalk, Conn.) as described elsewhere (23). The homogenate was passed through three layers of cotton gauze and centrifuged at 105,000 × *g* for 1 h. The pellet of cardiac muscle fragments was washed twice with PBS and stored at −20°C.

Direct binding assay. Radiolabeled streptococcal components were tested for direct binding activity to mammalian tissue components as previously described (23). Briefly, glass scintillation vials (7-ml minivials) containing dried cardiac muscle fragments were incubated with 0.3 ml of PBS containing 1% bovine serum albumin. After 30 min, the excess fluid was removed, and the moist heart material was incubated for 60 min with ^{3}H-labeled bacterial extract (0.2 ml of buffer containing 0.25 to 1.0 mg of bacterial components). The vials were rinsed four times with 4 ml of PBS, and the bound radioactivity was determined by liquid scintillation spectrometry.

Selected substances were tested as competitive inhibitors of the binding of radiolabeled bacterial components to cardiac tissue. Various concentrations of inhibitors were added to the amount of ^{3}H-labeled streptococcal components indicated in Table 1. The mixtures were incubated for 60 min at 4°C and assayed as described above.

Electrophoresis. SDS-polyacrylamide gel electrophoresis was performed by a modification of the procedure of Weber and Osborn (28). Vertical gels (1.5 by 140 mm) containing 12.5% acrylamide and 6 M urea were run at 30 mA per slab for 20 to 22 h at 17°C. Lysozyme (14,300 daltons), lactoglobulin (18,000 daltons), chymotrypsinogen (25,000 daltons), ovalbumin (45,000 daltons), and bovine serum albumin (67,000 daltons) were used as molecular weight standards. Gel fluorography was performed with En^{3}Hance (New England Nuclear Corp., Boston, Mass.). Dried gels were exposed to Kodak X-Omat R film (Eastman Kodak Co., Rochester, N.Y.) for 24 to 30 h at −70°C to detect radiolabeled proteins.

Component of the *S. pyogenes* extracts were also detected by Western blot (immunoblot) assays with rabbit anti-*S. pyogenes* sera according to the procedures of Burnette (4).

Preparation of heparin–Sepharose 6-B and chondroitin sulfate–Sepharose 6-B. Heparin and chondroitin sulfate were immobilized on Sepharose 6-B by the divinyl sulfone-coupling procedure of Fornstedt and Porath (8). The resulting gels were washed extensively with water, equilibrated in PBS at pH 7.4, and stored at 4°C. Between 2 and 3 mg of ligand per ml of Sepharose 6-B gel was obtained with this procedure, as calculated from the quantity of unreacted ligand after dialysis.

Heparin-Sepharose binding assay. To measure the binding of streptococcal components to heparin-Sepharose or chondroitin sulfate-Sepharose, 50 μl of a 50% suspension of affinity gel in PBS and 2% bovine serum albumin was added to siliconized test tubes (6 by 40 mm) containing 50 μl of ^{3}H-labeled streptococcal proteins (0.25 to 15 mg/ml in PBS at pH 7.4). The suspension was incubated at room temperature for 60 min, with frequent mixing. The gels were collected by low-speed centrifugation, washed seven times with 0.6 ml of PBS, and transferred to a 15-ml liquid scintillation vial containing 1.0 ml of 2% SDS. After incubation for 30 min at 56°C, 15 ml of scintillation fluid was added, and the sample was counted.

Affinity purification of streptococcal components. *S. pyogenes* extract (40 mg) was dissolved in 4.0 ml of PBS and centrifuged at 12,000 × *g* at 4°C for 30 min. The supernatant fluid was added to 5 ml of heparin-Sepharose (settled gel volume) and stirred at 4°C for 2 h. The gel was washed extensively with PBS and then with deionized water on a sintered-glass funnel. The bound streptococcal components were eluted with ice-cold 0.01 M NaOH (pH 12). The eluate was immediately neutralized with HCl and lyophilized. The dried material was reconstituted with 5 ml of 0.1 M sodium phosphate buffer at pH 9 and labeled with 5 mCi of [^{3}H]acetic anhydride as previously described (23). In later experiments, the beads were washed five times with 10 volumes of 0.1% SDS prior to elution with NaOH.

Chemical analysis of binding protein. Amino acid analysis was performed on an amino acid analyzer (120C; Beckman Instruments, Inc., Fullerton, Calif.) by the method of Moore and Stein (16). The protein was hydrolyzed in sealed vials for 24 to 28 h at 105°C in 6 N HCl under an atmosphere of nitrogen. The mixture was evaporated to dryness at reduced pressure and dissolved in water. No attempt was made to estimate the tryptophan, glutamine, or asparagine content.

Other procedures. Anti-*S. pyogenes* sera were prepared by the intravenous and intradermal immunization of New Zealand White rabbits with disrupted bacteria or isolated proteins as previously described (23). Monospecific antiserum to *S. pyogenes* type 6 M protein was obtained from Richard Facklam, Centers for Disease Control, Atlanta, Ga. Bacterial extracts were subjected to phosphate analysis by the procedure of Eibl and Lands (6). Protein was measured by the method of Hartree (9), with bovine serum albumin as a standard. Dry weight was determined on preparations that were dialyzed against distilled water and lyophilized.

RESULTS

Ligand inhibition studies. Previous studies (23, 24) demonstrated that a water-soluble protein from *S. pyogenes* selectively and specifically binds in vitro to basement membranes of cardiac muscle and kidney. Additional IIF studies (Fig. 1) revealed that the streptococcal components were deposited in a smooth, linear pattern along the basement membranes, giving the structure a double-tracked appearance in kidney sections (Fig. 1A). The bacterial components did not bind to epithelial or endothelial cells of the kidney but accumulated on the proteoglycan-rich regions that connect these cells to the underlying connective tissue. The basement membrane in the sarcolemmal sheaths of cardiac muscle bound the antigens in a more homogeneous, linear pattern (Fig. 1B). The anti-*S. pyogenes* serum did not react directly with basement membrane structures of tissue sections that were not treated with extracts of *S. pyogenes*. In control experiments with anti-basement membrane sera, the basement membranes exhibited linear, homogeneous staining patterns.

To define this binding interaction, several glycosaminoglycans and proteins were tested for their abilities to competitively inhibit the binding of ^{3}H-labeled streptococcal proteins to cardiac muscle (Table 1). Gelatin, serum fibronectin, and type IV collagen were ineffective inhibitors; however, a variety of the glycosaminoglycans showed inhibitory activity. At 2 mg/ml, heparin and heparan sulfate were more

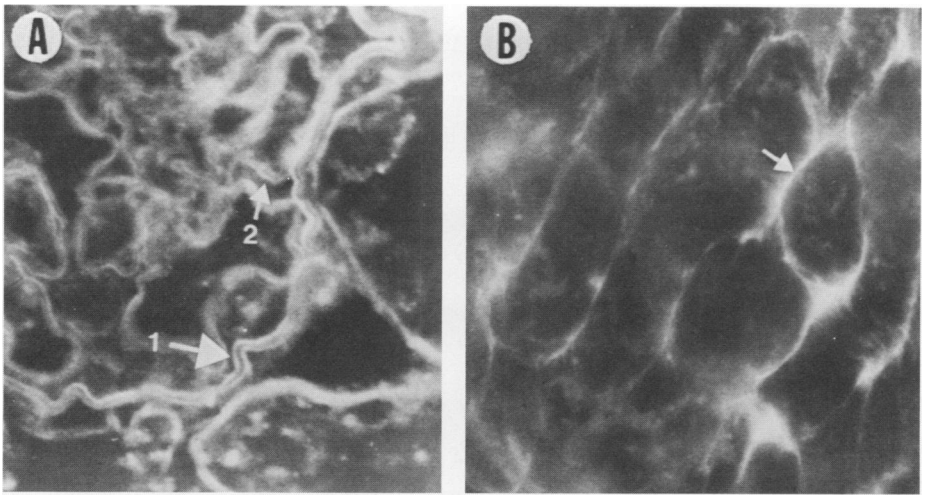

FIG. 1. Micrographs of monkey kidney (A) and cardiac muscle (B) stained by IIF. Tissue sections were incubated with soluble streptococcal components (1 mg/ml) and then with rabbit anti-*S. pyogenes* cell extract serum (1:80 [vol/vol]). (A) The basement membranes of Bowman's capsule (arrow 1) and glomerular capillaries (arrow 2) show double-tracked staining. (B) The basement membrane of the sarcolemmal sheath appears homogeneously stained (arrow). Magnification, ×1,000.

effective than chondroitin sulfate, dermatan sulfate, and hyaluronic acid. Binding of the protein to cardiac muscle was not inhibited by 10 mM *N*-acetylglucosamine, *N*-acetylgalactosamine, glucosamine, glucuronic acid, fucose, or galactose.

The effect of increasing quantities of heparin and chondroitin sulfate on the binding of radiolabeled *S. pyogenes* proteins to cardiac muscle is shown in Fig. 2. Heparin exerted a maximum inhibition of 73% at a concentration of 14 mg/ml, whereas a similar amount of chondroitin sulfate inhibited only 17% of the protein binding. Higher concentrations of heparin were not tested for inhibition of the residual binding of radioactivity. Instead, the inhibitory effects of heparin were confirmed in IIF assays on sections of cardiac muscle and kidney in which binding of *S. pyogenes* components to basement membranes was completely inhibited by 10 mg of heparin per ml. Immunofluorescence staining was diminished but not eliminated by the addition of chondroitin sulfate.

TABLE 1. Inhibition of cardiac muscle-binding activity of streptococcal components by glycosaminoglycans and selected protein[a]

Inhibitor	Concn (mg/ml)	% Inhibition (mean ± SEM)
Fibronectin (serum)	1	1 ± 1
Collagen IV	10	0
Gelatin	10	0
Chondroitin 4- and 6-sulfate	2	9 ± 2
	10	17 ± 3
Dermatan sulfate	2	4 ± 1
	10	28 ± 4
Hyaluronic acid	2	10 ± 2
	10	38 ± 9
Heparan sulfate	2	26 ± 5
	10	36 ± 7
Heparin	2	57 ± 5
	10	68 ± 4

[a] ^{3}H-labeled streptococcal components (1.0 mg/ml) were incubated with the indicated concentrations of inhibitors for 30 min at 4°C and then assayed for heart-binding activity by direct binding assay. Percent inhibition was determined relative to the control containing no inhibitor (100% binding).

Since the inhibitory activity of heparin in these binding assays could have resulted from its interaction with either cardiac muscle components or bacterial proteins, an experiment was conducted in which heparin was used to block the binding sites on the cardiac muscle. The tissue was preincubated with a large excess of heparin (4 mg in 100 μl), washed with PBS, and incubated with radiolabeled *S. pyogenes* proteins. Only 10% inhibition was observed under these conditions, indicating that the effect observed in the competitive inhibition experiment (Fig. 2) was due predominantly to the interaction of heparin with the streptococcal components.

Affinity-purification of basement membrane-binding protein. Heparin-coated beads of Sepharose 6-B readily bound

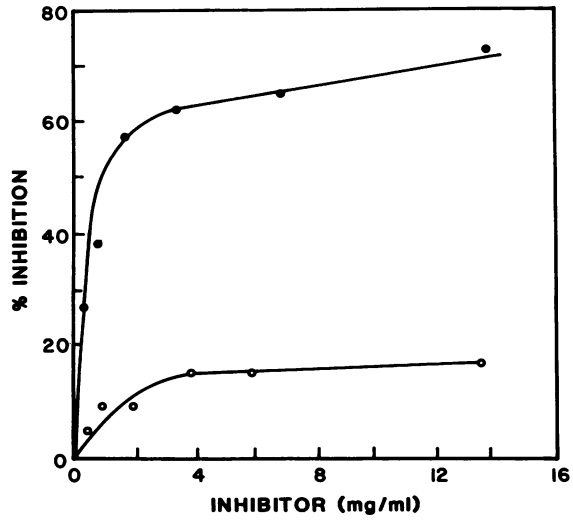

FIG. 2. Inhibition of cardiac muscle-binding activity by heparin (●) and chondroitin sulfate (○). ^{3}H-labeled streptococcal protein (400 μg in 0.2 ml) was incubated with the indicated amount of inhibitor for 1 h at 4°C and then assayed for binding to muscle components. Binding activity in the absence of inhibitors was designated as 100%.

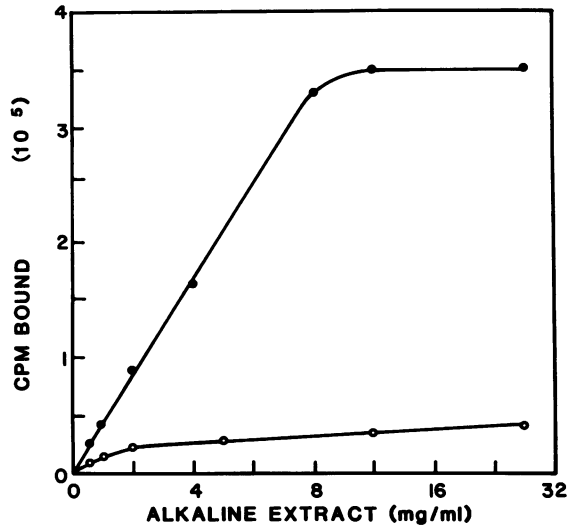

FIG. 3. Binding of ^{3}H-labeled streptococcal protein to heparin–Sepharose 6-B (●) and chondroitin sulfate–Sepharose 6-B (○) beads. The indicated amounts of bacterial protein were incubated with 50 μl of 50% bead suspension for 1 h at room temperature.

^{3}H-labeled streptococcal components (Fig. 3). At saturating concentrations of bacterial components, heparin-Sepharose bound nearly 10 times more radioactivity than comparable quantities of chondroitin sulfate-Sepharose and glycosaminoglycan-free Sepharose. To verify that these streptococcal components also bind to muscle tissue, a solution of ^{3}H-labeled bacterial components (0.2 mg in 50 μl) was absorbed with 50 μl of heparin-Sepharose beads and then assayed on cardiac muscle. Although only 2% of the total radioactivity of the initial preparation was bound by the heparin-Sepharose, 57% of the tissue-binding activity was removed. Residual binding activity of the extract was further reduced by additional absorptions.

Because affinity chromatography could be a highly effective and convenient way to purify these streptococcal components, several procedures were evaluated for their ability to dissociate the ^{3}H-labeled proteins from the heparin-Sepharose beads. Table 2 shows the quantities of radioactivity eluted from the affinity matrix and the binding activities of the streptococcal components in subsequent cardiac muscle assays. High yields of both radioactivity and heart-binding activity were obtained when the beads were eluted with 0.005 M NaOH at pH 12. Urea, NaCl, and SDS eluted various amounts of radioactivity from the affinity matrix; however, the heart-binding activity remained absorbed to the heparin-Sepharose. Therefore, subsequent affinity chromatography of *S. pyogenes* extracts employed sequential elution steps. First, the weakly associated streptococcal components were eluted from the heparin-Sepharose with 0.1% SDS at room temperature. After residual SDS was removed from the affinity matrix with water, the cardiac muscle-binding components were eluted with NaOH at pH 12. An SDS-polyacrylamide gel electrophoresis analysis of ^{3}H-labeled proteins purified in this manner is shown in Fig. 4. Two bands (15,000 and 9,000 M_r) are visible in both Coomassie blue-stained gels (Fig. 4A) and fluorographs (Fig. 4B). These two bands were also obtained when unlabeled proteins were affinity purified, indicating that the acetyl substituents did not alter the relative mobilities of the proteins. The final yield was 2 μg of protein per mg of the initial streptococcal extract or 1 μg of protein per g of dry bacteria.

TABLE 2. Elution of streptococcal components from heparin-Sepharose

Eluent and concn	% Release (cpm)[a]	% Binding to heart[b]
NaCl		
1.0 M	28	10
3.0 M	40	ND[c]
Urea		
4.0 M	11	0
8.0 M	25	5
Heparin (30 mg/ml)	34	ND
SDS[d]		
0.1%	71	9
1.0%	70	9
NaOH		
0.001 M (pH 11)	28	ND
0.005 M (pH 11.5)	72	18
0.010 M (pH 12)	90	49
0.100 M (pH 13)	95	47

[a] ^{3}H-labeled alkali-extracted (at pH 11.5) components of *S. pyogenes* (2.5 mg) were incubated with 0.5 ml of settled heparin-Sepharose gel for 1 h at 4°C. After removal of the nonadhereent components, a 20% suspension of the activated gel was made, and 50-μl samples were transferred to 200 μl of eluent (final concentration is indicated). After 1 h at 22°C, the percent count per minute released was determined by counting the supernatant fluid. The quantity eluted by 1 M NaOH was designated as 100%.

[b] Heart-binding activity of each sample was determined by the direct binding assay with heart cell fragments. The material eluted from the affinity matrix by NaCl, SDS, or urea was dialyzed against PBS for 48 h at 4°C to remove the reagent before it was assayed for binding activity. Percent binding = (total counts per minute bound/total counts per minute added) × 100.

[c] ND, Not determined.

[d] At 56°C for 1 h.

Self-aggregation of the 9- and 15-kilodalton polypeptides precluded their separation and further characterization. Gel filtration chromatography reported previously (23) indicated that aqueous solutions contained polypeptide aggregates of 100 to 300 kilodaltons. The binding proteins were readily dissociated by 2% SDS and separated by preparative SDS-urea-polyacrylamide gel electrophoresis; however, biological activity was not recovered upon removal of SDS by dialysis, extraction with acetone-triethylamine-acetic acid-water (85:5:5:5, vol/vol/vol/vol) or absorption onto Amberlite XAD-2 beads (Sigma Chemical Co., St. Louis, Mo.).

Characterization of purified protein. The affinity-purified proteins retained their binding activities for basement membranes and heparin. Reapplication of the material to heparin-Sepharose resulted in complete absorption of radioactivity and cardiac muscle-binding activity. Binding of isolated proteins to monkey tissue was readily detected in the direct radioassay and IIF assay (Fig. 5). The IIF staining pattern was identical to that obtained with the unfractionated bacterial extract (Fig. 1).

The possibility that heparin-inhibitable basement membrane-binding protein is streptococcal M protein was tested with Western blot assays. A serotype M6-specific antiserum stained several components in the alkali extract of *S. pyogenes* serotype M6 (Fig. 6, lane 1) but did not react with the heparin-Sepharose-purified proteins (Fig. 6, lane 2), indicating that the basement membrane-binding polypeptides are not amino-terminal fragments of M protein.

The amino acid composition of the heparin-Sepharose-

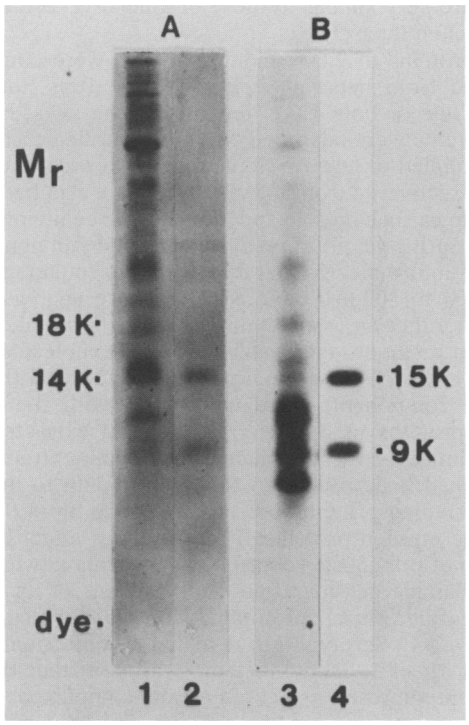

FIG. 4. Separation of streptococcal components by SDS-urea-polyacrylamide gel (12.5%) electrophoresis. Lanes: 1 and 3, 50 μg of ^{3}H-labeled, alkali-extracted components of *S. pyogenes* serotype M6; 2 and 4, ^{3}H-labeled streptococcal components purified on heparin–Sepharose 6-B. (A) Coomassie blue stain. (B) Fluorograph. M_rs (in thousands) are indicated to the left of lane 1 and to the right of lane 4. Migration of the tracking dye is indicated at the bottom of the gel.

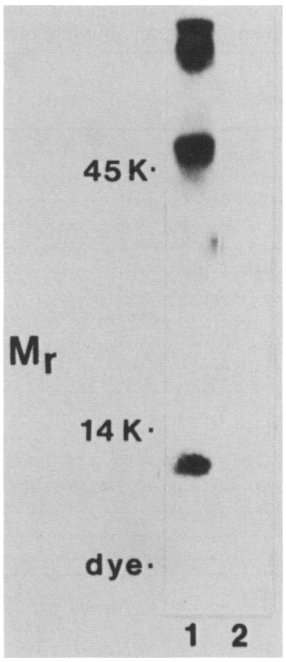

FIG. 6. Western blot of streptococcal components against antiserum to *S. pyogenes* type 6 M protein. Lanes: 1, 50 μg of alkali-extracted components of *S. pyogenes* serotype M6; 2, 14 μg of purified basement membrane-binding protein. M_rs (in thousands) are indicated to the left of lane 1. Migration of the tracking dye is indicated at the bottom of the gel.

purified protein is shown in Table 3. Aspartic acid, glutamic acid, glycine, alanine, and lysine were the predominant constituents. Half-cystine, neutral and amino sugars, and organic phosphate were not detected (less than 0.1%). Thus, it can be concluded that the preparation was free of bacterial polysaccharide and lipoteichoic acid and that the polypeptides do not contain oligosaccharide moieties.

DISCUSSION

Binding of protein components of group A streptococci to basement membranes of cardiac muscle and kidney was

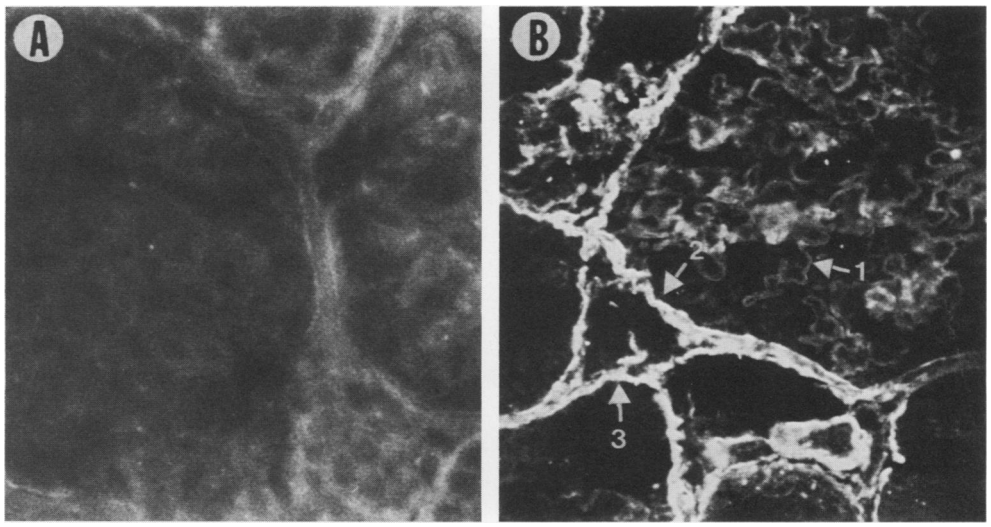

FIG. 5. Micrographs of monkey kidney sections stained by IIF. (A) Control tissue section incubated with rabbit anti-*S. pyogenes* serum (1:10 [vol/vol]) and then with fluorescein isothiocyanate-conjugated goat antibodies to rabbit immunoglobulin G. (B) Tissue section treated with purified binding protein followed by the serological reagents described for panel A. Basement membranes stained: arrow 1, glomerular; arrow 2, Bowman's capsule; arrow 3, tubular. Magnification, ×400.

TABLE 3. Amino acid composition of purified streptococcal basement membrane-binding protein

Residue	No. of residues/ 100 amino acid residues
Aspartic acid	10
Threonine	5
Serine	7
Glutamic acid	11
Proline	4
Glycine	10
Alanine	11
Half-cystine	0
Valine	7
Methionine	1
Isoleucine	5
Leucine	7
Tyrosine	2
Phenylalanine	2
Lysine	9
Histidine	2
Arginine	5
Tryptophan	ND[a]

[a] ND, Not determined.

inhibited in vitro by heparin and, to a lesser extent, by heparan sulfate. IIF staining patterns on thin sections of kidney treated in vitro with these bacterial extracts indicate that the streptococcal protein binds to the proteoglycan-rich regions of basement membranes. This may involve the laminae rarae of the basement membrane or the epithelial and endothelial cell membranes immediately adjacent to these structures. The purified streptococcal protein had a tissue-binding activity similar to that of the unfractionated cell extract and was inhibited by adsorption to heparin-Sepharose.

The binding of the streptococcal protein to heparin–Sepharose 6-B was not reversed by the addition of 3 M NaCl, 1% SDS, or 8 M urea. This characteristic permitted a rapid one-step purification in which other bacterial components, either coaggregated with the heparin-binding protein or weakly bound to the affinity support, were eluted first with 0.1% SDS. After residual SDS was removed by washing the beads with water, the heparin-binding protein was eluted with NaOH at a pH of 12. The effect of alkali indicates that binding is mediated largely by ionic interactions between free amino groups on the streptococcal protein and the sulfate and carboxyl groups of heparin; the positively charged groups on the protein are neutralized at a pH of 12. The apparent preference of the protein for heparin over other glycosaminoglycans tested may reflect the higher level of O and N sulfation in heparin. Heparin averages three sulfate groups per repeating disaccharide unit, whereas chondroitin sulfate, dermatan sulfate, and heparan sulfate contain only one; hyaluronic acid is not sulfated. Of the glycosaminoglycans tested, only heparin and heparan sulfate contain *N*-sulfate groups. Alternatively, the uronic acid content of the glycosaminoglycans may affect the binding of the streptococcal protein. Heparin contains a higher ratio of L-iduronic–D-glucuronic acids than heparan sulfate and dermatan sulfate, whereas chondroitin sulfate and hyaluronic acid contain only D-glucuronic acid (19, 30). Binding of the protein to cardiac muscle and heparin-Sepharose was not inhibited by 10 mM *N*-acetylglucosamine, *N*-acetylgalactosamine, glucosamine, glucuronic acid, glucose, or galactose. Interestingly, the binding characteristics of the streptococcal protein are very similar to those of a heparin lectin isolated from chicken liver (5).

Two proteins of 9,000 and 15,000 M_rs were consistently recovered from heparin–Sepharose 6-B. It is not known whether one or both have heparin-binding sites; however, both proteins were adsorbed when the purified components were reapplied to heparin-Sepharose. Likewise, the protein that was recovered from the affinity matrix also had binding activity to cardiac muscle and kidney. The behavior of these proteins during gel filtration chromatography in nondenaturing conditions indicates that they form molecular aggregates of 100,000 to 300,000 daltons (23). These aggregates may represent either an association of both heparin-binding proteins in a common aggregate or separate molecular aggregates of each. Also, serological assays indicated that other bacterial components were complexed with the heparin-binding proteins in the original bacterial extracts. In IIF assays, antisera to whole bacteria and alkali-extracted components had high antibody titers (160 to 320) to unpurified heparin-binding proteins but low antibody titers (less than 80) to the purified proteins. These findings affect the interpretation of previously reported experiments in which SDS-polyacrylamide gel electrophoresis analysis of an SDS extract of cardiac muscle pretreated with radiolabeled proteins of *S. pyogenes* serotype M6 revealed a component with an apparent M_r of 19,000 (23). It now appears that this latter component represents a contaminant complexed with the basement membrane-binding protein. The bacterial protein bound to the tissue glycosaminoglycans in the in vitro assay was probably not solubilized by the SDS and consequently went undetected (23). The ability of the binding protein to link other streptococcal components to mammalian tissue may be important in streptococcal pathogenesis.

Chemical analyses of the heparin-Sepharose-purified proteins revealed no half-cystine, amino or neutral sugars, or phosphate substituents. The basic amino acids lysine, histidine, and arginine constitute 17% of the amino acid residues. Although the distribution of these amino acids in the two proteins is not known, the binding affinity of the proteins predicts that they are arranged in a manner complementary to the charge density of the glycosaminoglycan either through primary amino acid sequence or via tertiary structure. For example, a cationic domain on streptococcal M protein that appears to mediate the binding of the protein to polyanionic lipoteichoic acid in the cell wall of the same bacterium has been detected (18). Although the M protein does not have a remarkably high proportion of basic amino acids, sequence analysis shows regions rich in lysine (3, 18). The basement membrane-binding protein reported here, however, does not appear to be the M protein. Western blot assays with antibodies specific for *S. pyogenes* type 6 M protein indicated that the M protein was present in the initial streptococcal extract but was absent in the purified preparation. Although these antibodies are specific for the amino-terminal portion of the M protein, it is very unlikely that the carboxy-terminal portion would be present in our extracts, since it appears to be covalently attached to peptidoglycan (7). Also, the amino acid composition of the basement membrane-binding protein is vastly different from that reported for M-protein fragments (3).

The participation of the basement membrane-binding protein in acute rheumatic fever and acute poststreptococcal glomerulonephritis remains to be established. Although the pathogeneses of these diseases are still inexplicable, direct binding of streptococcal components to host tissues must be considered as a possible contributing mechanism. Strepto-

coccal antigens have been observed in the gomeruli of patients with acute poststreptococcal glomerulonephritis (2, 14) and in rabbits with *Streptococcus mutans*-induced nephritis (1, 22). Further study of the in vitro and in vivo interactions of the basement membrane-binding proteins of *S. pyogenes* with animal tissues should lead to a better understanding of the virulence properties of these bacteria.

ACKNOWLEDGMENTS

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