INFECTION AND IMMUNITY, Feb. 1991, p. 537–543
0019-9567/91/020537-07$02.00/0

Vol. 59, No. 2

Binding of a *Streptococcus mutans* Cationic Protein to Kidney In Vitro

SUK HO CHOI[1] AND MURRAY W. STINSON[1,2]*

Department of Microbiology, School of Medicine and Biomedical Sciences and School of Dental Medicine,[1] and Department of Oral Biology, School of Dental Medicine,[2] State University of New York at Buffalo, Buffalo, New York 14214

Received 25 June 1990/Accepted 20 November 1990

An 8-kDa protein, with binding activity for heparin and heparan sulfate of basal laminae of animal tissues, was isolated from *Streptococcus mutans* MT703 and purified to homogeneity. Binding of radioiodinated 8-kDa protein to rabbit kidney tissue in vitro showed a high degree of specificity, as indicated by saturation kinetics, time dependence, and competitive inhibition by unlabeled protein. Binding activity for kidney tissue was competitively inhibited by selected glycosaminoglycans and polyanions in the following order: heparin > dextran sulfate > heparan sulfate > chondroitin sulfate > lipoteichoic acid > keratan sulfate > hyaluronic acid. Binding of the streptococcal protein to rabbit kidney tissue was also strongly inhibited by protamine sulfate, polylysine, and a random copolymer of lysine and alanine. Among the monosaccharides tested at 50 mM, glucosamine 2,3- or 2,6-disulfate, glucuronic acid, glucose 6-phosphate, and glucose 6-sulfate inhibited 50% or more of the binding activity, whereas *N*-acetylglucosamine 3-sulfate, glucosamine 6-sulfate, *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylneuraminic acid, and a selection of neutral sugars were not inhibitory. The heparin-binding protein was detected on the cell wall of *S. mutans* and in the culture medium following growth. Several other species of streptococci produce an immunologically related protein of similar size.

Streptococcus mutans is an opportunistic pathogen of humans and is associated with dental caries (6, 14, 15), subacute bacterial endocarditis (7, 13, 17), and subacute bacterial endocarditis-associated glomerulonephritis (7, 13). Although the mechanism of pathogenesis is well understood in dental infections (6, 14), it is largely unknown in systemic diseases. Intravenous injection of *S. mutans* MT703 components into rabbits results in a severe nephritis and mild myocarditis in >90% (1, 20) and 25% (21) of the animals, respectively. Lesions contain complexes of streptococcal antigens, immunoglobulins, and complement (1, 19, 20). Although autoantibodies to heart and kidney tissue are produced by the rabbits, they do not cross-react with streptococci (22). Instead, the stimulus for their production appears to be tissue injury caused either by toxicity of streptococcal components or by inflammation elicited by immune complexes deposited in tissues. Several streptococcal antigens have been found to bind directly to heart and kidney components in vitro (3, 4, 19, 26).

We previously reported the isolation and chemical composition of an *S. mutans* protein that binds to basal laminae of heart tissue and to heparin (4). This heparin-binding protein (HBP) is rich in lysine and alanine and has a pI of 9.5. The present study was undertaken to define the interaction of the purified HBP with kidney tissue and to further characterize its binding specificity.

MATERIALS AND METHODS

Bacteria and culture conditions. *S. mutans* MT703 (serotype e) was grown in chemically defined medium (23) as previously described (4). This strain was isolated from dental plaque and was generously provided by S. Hamada, Osaka, Japan. Previous studies (1, 20) have shown that injection of disrupted *S. mutans* MT703 cells into rabbits results in severe glomerulonephritis.

Extraction of bacteria. *S. mutans* cells were washed with phosphate-buffered saline (PBS) (10 mM sodium phosphate, pH 7.2, containing 0.15 M NaCl) and suspended in water to 20% (wt/vol). The suspension was adjusted to pH 11.5 with 1 N NaOH and stirred at 4°C overnight. The supernatant fluid was collected following centrifugation and passed through a 0.22-μm-pore-size filter (Millipore) to remove residual cells. After the pH was adjusted to 7 with HCl, the extract was dialyzed against water and lyophilized.

Affinity chromatography. *S. mutans* HBP was purified from culture medium concentrates and cell extracts by chromatography on a column of heparin-agarose (4). Streptococcus components were eluted from the column with a concentration gradient of NaCl, with HBP appearing at 1 M NaCl.

Radioisotope labeling. Purified HBP was radiolabeled with $Na^{125}I$ (ICN Radiochemicals, Irvine, Calif.) by using Iodobeads (Pierce Chemical Co., Rockford, Ill.) as previously described (4). The specific radioactivity of the final protein solution was 7.6×10^7 cpm/mg of protein. Autoradiography of sodium dodecyl sulfate (SDS)-polyacrylamide gels showed a single band that corresponded to the 8-kDa protein band on silver-stained SDS-polyacrylamide gel electrophoresis (PAGE).

Preparation of kidney homogenate. Rabbit kidney was homogenized in PBS (20%, wt/vol) in a Waring blender, filtered through 80-gauge wire mesh, and stored as previously described (4).

Binding assay. Kidney tissue-binding activity of HBP was determined by a radioisotope assay (4). Briefly, plastic microtiter wells were coated with kidney homogenate and then incubated at room temperature with selected concen-

* Corresponding author.

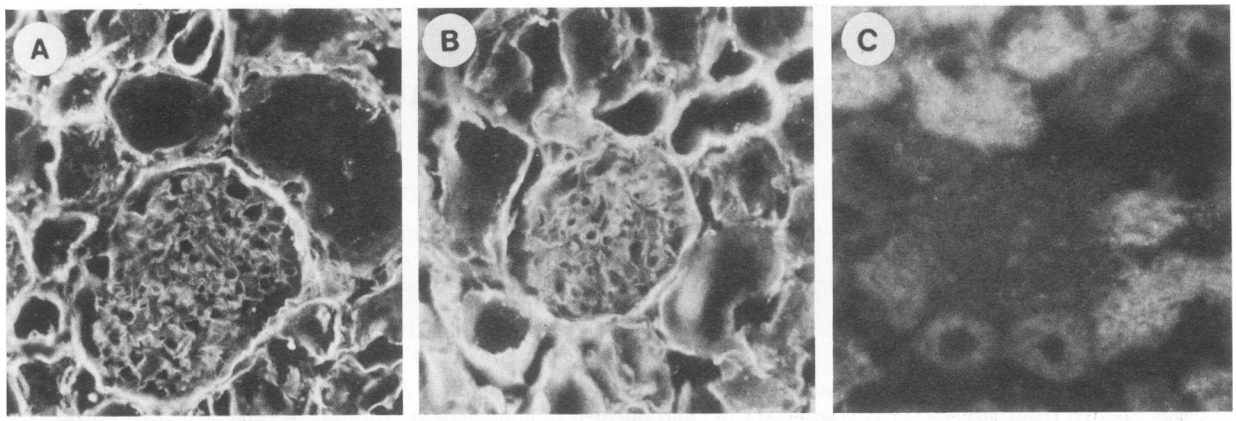

FIG. 1. Immunofluorescence staining of rabbit kidney tissue pretreated with unfractionated streptococcal components (1 mg/ml) (A), purified HBP (10 μg/ml) (B), and PBS without streptococcal components (C). Magnification, ×400.

trations of the radiolabeled HBP. After 1 h, the wells were washed with PBS, cut out of the plate, and counted on a gamma radiation counter (Beckman Instruments).

Potential inhibitors of HBP binding were mixed with ^{125}I-HBP and incubated for 1 h at room temperature before being tested in the binding assay. Inhibitors were also tested for their abilities to competitively dissociate the streptococcal protein from kidney tissue. In the latter assay, 1.5 μg of the ^{125}I-HBP in 0.1 ml of PBS was incubated for 1 h in tissue-coated wells. After excess protein was washed away with buffer, selected concentrations of inhibitor were added and the incubation was continued for another hour. The wells were washed three times with buffer, and the adherent radioactivity was counted.

Antibody purification. Immunoglobulins to HBP were purified from goat antiserum to *S. mutans* by ammonium sulfate precipitation and affinity chromatography (4). The stock solution of purified antibodies used in this study was 0.36 mg/ml.

Immunofluorescence assay. Cryostat-cut sections (3 μm thick) of kidney tissue, freshly collected from New Zealand White rabbits, were incubated sequentially with HBP, with affinity-purified antibodies (18 μg/ml), and with affinity-purified fluorescein-conjugated rabbit antibodies to goat immunoglobulin G (Organon Teknika Corp., West Chester, Pa.) (4).

SDS extraction of HBP. Selected species of streptococci (5×10^9 cells) were suspended in 0.5 ml of 2% SDS and heated at 100°C for 5 min. After centrifugation at 16,000 × *g* for 10 min, the supernatant fluids were analyzed by SDS-PAGE and Western blot (immunoblot) assay.

Electrophoresis. SDS-PAGE was performed with 13.5% acrylamide gels by the method of Laemmli (10). Lysozyme (molecular weight, 14,300), lactoglobulin (18,400), carbonic anhydrase (29,000), bovine serum albumin (BSA; 67,000), horse heart myoglobulin (16,950), and its cleaved polypeptides (14,400, 8,160, 6,210, and 2,510) (Sigma Chemical Co., St. Louis, Mo.) were used as molecular weight standards. Proteins were stained with silver by the procedure of Morrisey (16).

Western blot assay. Bacterial components were separated by SDS-PAGE and then electrotransferred to nitrocellulose paper (25). The resulting blot was incubated sequentially with 1% BSA, with goat antiserum to *S. mutans* or affinity-purified antibody (700 ng/ml), and with alkaline phosphatase-conjugated rabbit antibodies to goat immunoglobulin G, as previously described (4).

ELISA. *S. mutans* was suspended in PBS to a density of 10^9 cells per ml, and 0.1-ml aliquots were transferred to wells of a polystyrene 96-well plate. After 18 h at 4°C, the bacterium-coated wells were incubated sequentially with 1% BSA, affinity-purified antibodies to HBP (1.5 μg per ml), and enzyme-linked immunosorbent assay (ELISA) reagents (4).

Chemical analysis. Protein was determined by using BCA protein assay reagent (Pierce Chemical).

Materials. Carbohydrates, glycosaminoglycans, and cationic proteins were purchased from Sigma. Other chemicals were reagent grade and were obtained from local vendors.

RESULTS

Kidney-binding assays. To define the binding characteristics of HBP, cryostat-cut sections of rabbit kidney were pretreated with purified HBP and incubated sequentially with affinity-purified antibodies to HBP and fluorescein isothiocyanate-conjugated rabbit antibodies to goat immunoglobulin G. The immunofluorescence staining pattern (Fig. 1) indicates that HBP was deposited on the basal laminae of Bowman's capsule, glomerular capillaries, and tubules, but not on other renal components. Kidney sections pretreated with PBS instead of HBP did not stain with these antibodies. Similar staining patterns were also observed on thin sections of normal human, monkey, and mouse kidneys (data not shown).

Binding activity of the radiolabeled HBP was studied on rabbit kidney homogenates coated onto plastic microtiter wells. Figure 2 shows that binding of HBP to kidney tissue increased proportionately with the protein concentration, up to 15 μg/ml, after which binding sites on the tissue became saturated. On the basis of specific radioactivity, a maximum of 170 ng of streptococcal protein was bound to the kidney-coated wells. Control wells without kidney tissue did not bind significant amounts (<100 cpm or 1 ng) of radiolabeled protein. At concentrations below the saturating dose, 28% of the available protein bound to kidney tissue. A Scatchard plot of the binding data is also shown in Fig. 2 (inset). The apparent dissociation constant (K_d) is 0.62 μM or 5 μg of protein per ml. Binding of the radiolabeled protein to kidney tissue was time dependent, whereby 65% of binding oc-

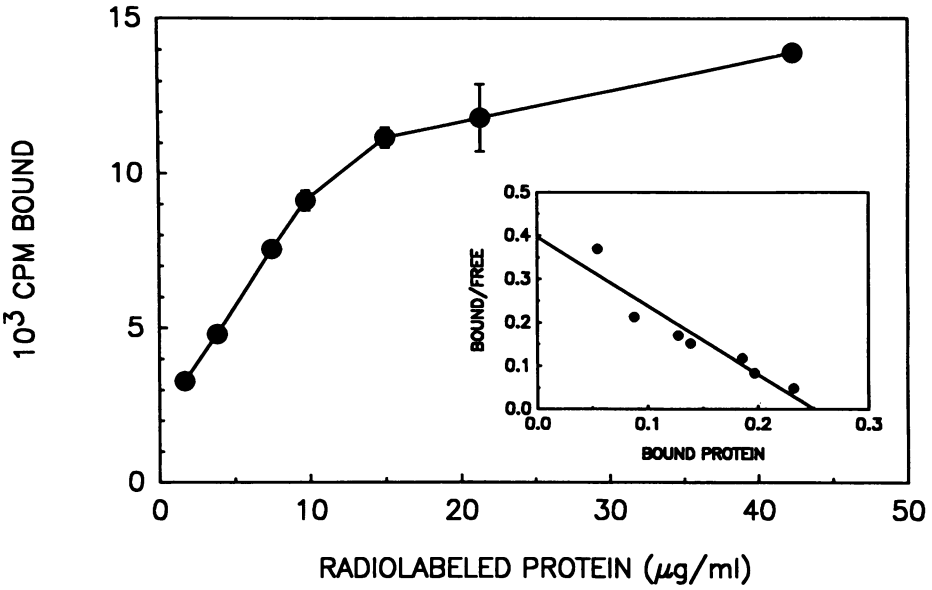

FIG. 2. Binding of the radiolabeled streptococcal HBP to kidney-coated microtiter wells after 1 h at room temperature. Less than 100 cpm bound to control wells coated only with BSA. A Scatchard plot of the binding data is shown in the inset.

curred within 10 min and reached saturation (100%) within 60 min (Fig. 3).

The specificity of HBP binding and the effect of the extrinsic radiolabel on binding activity was tested with unlabeled HBP in an isotopic dilution experiment (Fig. 4A). A sixfold excess of unlabeled HBP competitively inhibited 82% of the binding of radiolabeled HBP to kidney tissue. In addition, radiolabeled protein was competitively dissociated from preformed complexes with kidney tissue by unlabeled protein; 85% of the radiolabeled protein was released by a sixfold excess of competitor (Fig. 4B). Less than 5% of the complexes were solubilized during incubation with PBS.

Binding characteristics. Binding of streptococcal HBP to renal basal laminae (Fig. 1) indicated that the tissue glycosaminoglycans, such as heparan sulfate, chondroitin sulfate, and hyaluronic acid, may constitute the binding site. To determine the specificity of streptococcal HBP for tissue glycosaminoglycans, selected polyanions were tested as competitive inhibitors. Dextran sulfate and streptococcal lipoteichoic acid were also evaluated as binding inhibitors. The order of inhibitory activity among the glycosaminoglycans tested was as follows: heparin > heparan sulfate > dermatan sulfate > chondroitin sulfate > keratan sulfate >

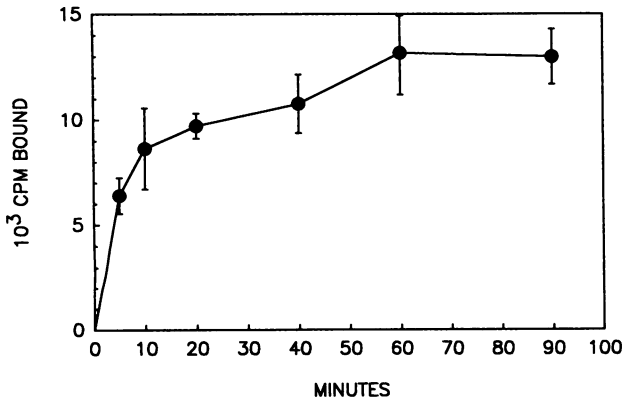

FIG. 3. Effect of time on binding of radiolabeled protein to kidney-coated microtiter wells at room temperature. HBP (1.5 μg) was added to each well and removed by washing at the indicated times.

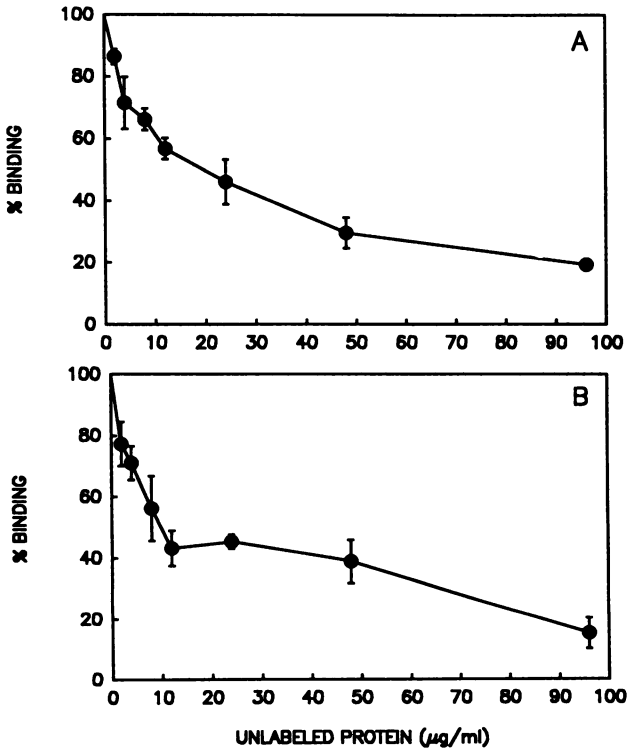

FIG. 4. Effect of unlabeled HPB on the binding of radiolabeled HBP (1.5 μg per well) to kidney tissue. (A) Competitive inhibition of binding; (B) competitive dissociation of preformed complexes.

TABLE 1. Inhibition of kidney-binding activity by glycosaminoglycans and polyanions

Inhibitors[a]	ID_{50} (μg/ml)[b]
Heparin	0.75
Heparan sulfate	1.7
Dermatan sulfate	5.3
Chondroitin sulfate type A	25
Chondroitin sulfate type C	35
Keratan sulfate	200
Hyaluronic acid	>1,000
Dextran sulfate	1.0
Lipoteichoic acid	100

[a] Inhibitors were added to the radiolabeled protein (10 μg/ml) at the level of 0.1, 1, 10, 100 and 1,000 μg/ml in duplicate and preincubated for 1 h before adding to tissue-coated wells.
[b] ID_{50}, 50% inhibitory dose.

hyaluronic acid (Table 1). No significant difference was observed between the inhibitory activities of chondroitin sulfate type A and chondroitin sulfate type C. Of the other substances tested, dextran sulfate inhibited binding as effectively as heparin whereas lipoteichoic acid was only weakly inhibitory. To further define the binding specificity of HBP, various monosaccharides were tested for their abilities to inhibit HBP binding to kidney tissue (Table 2). Strong inhibition (>50%) was exhibited by glucosamine 2,6-disulfate, glucosamine 2,3-disulfate, glucuronic acid, glucose 6-sulfate, and glucose 6-phosphate, whereas *N*-acetylglucosamine 6-sulfate and glucosamine 2-sulfate were weak inhibitors. *N*-acetylneuraminic acid, *N*-acetylglucosamine 3-sulfate, glucosamine 6-sulfate, glucose, galactose, mannose, *N*-acetylgalactosamine, and *N*-acetylglucosamine did not inhibit binding activity.

HBP was shown previously to be a basic protein (pI, 9.5) and to contain a large amount of lysine (16 residues per 100 residues) (4). Thus, several other basic proteins and polycations were tested for their abilities to inhibit binding of HBP to kidney tissue (Table 3). Polylysine, protamine, and a copolymer of lysine and alanine strongly inhibited binding activity. They were more effective inhibitors of binding than unlabeled HBP. Lysine, spermine, spermidine, BSA, cytochrome *c*, and lysozyme did not significantly inhibit binding activity.

Location and distribution of the HBP. Goat antibodies to HBP were purified from antiserum to *S. mutans* by affinity chromatography on a column of HBP coupled to CNBr-Sepharose. In Western blot assays of *S. mutans* extracts, the purified antibodies bound only to HBP (Fig. 5). The affinity-purified antibodies were also incubated with intact *S. mutans* cells to determine if HBP is expressed on the cell surface. The ELISA results (Fig. 6) show that the quantity of bound antibody was proportional to the number of bacteria. Control bacteria, treated with PBS instead of antibodies to HBP, did not react with the alkaline phosphatase-conjugated secondary antibodies. The antibodies to HBP did not bind to BSA-coated wells devoid of streptococci.

The natural distribution of HBP among different species of streptococci was indicated by Western blot assays of SDS extracts of bacteria (Fig. 7). A single polypeptide was detected in most extracts by using affinity-purified polyclonal antibodies. The molecular weights of the immunologically cross-reactive proteins ranged from 7,000 for *S. salivarius* to 9,000 for *S. sanguis*. The significance of the apparent quantitative differences in the HBP content of streptococcal extracts cannot be accurately determined from this experiment because several factors may be responsible for these differences, including the number of epitopes shared by the respective proteins, the quantity of proteins extracted from each species by SDS, the phase of bacterial growth, and the cultural conditions necessary for production of cross-reactive protein. For example, a cross-reactive protein was not detected in *S. pyogenes* M6 or *S. lactis* cells grown on chemically defined medium (Fig. 7) but was present in bacteria grown on tryptic soy broth supplemented with 0.1% yeast extract (Difco Laboratories, Detroit, Mich.). Thus, all species of streptococci tested (Fig. 7) produce a cross-reactive protein of similar molecular weight.

TABLE 2. Inhibition of binding activity by monosaccharides[a]

Inhibitor (50 mM)	% Inhibition (mean ± SD)
Glucuronic acid	69.9 ± 6.3
Glucosamine 2,6-disulfate	72.4 ± 3.3
Glucosamine 2,3-disulfate	66.0 ± 2.7
N-Acetylglucosamine 6-sulfate	29.6 ± 5.1
N-Acetylglucosamine 3-sulfate	5.7 ± 12.0
Glucosamine 2-sulfate	27.3 ± 0.4
Glucosamine 6-sulfate	0
N-Acetylneuraminic acid	2.2 ± 5.1
Glucose 6-sulfate	49.7 ± 3.4
Glucose 6-phosphate	61.4 ± 6.3

[a] Sugars were mixed with radiolabeled protein (10 μg/ml) for 1 h and incubated with immobilized kidney at pH 7.2. Data are expressed as the means of triplicate assays ± SD. The following neutral sugars exerted less than 5% inhibition: galactose, glucose, mannose, *N*-acetylgalactosamine, and *N*-acetylglucosamine.

TABLE 3. Inhibition of binding activity by polycations and basic proteins[a]

Inhibitor	Amt (μg/ml)	% Inhibition (mean ± SD)
BSA	100	2.2 ± 5.2
Cytochrome *c*	10	0
	100	8.5 ± 17.9
Lysozyme	10	26.8 ± 10.2
	100	14.1 ± 9.6
Lysine	100	8.6 ± 15.2
Spermine	10	8.9 ± 10.0
	100	0
Spermidine	10	16.1 ± 8.1
	100	5.7 ± 15.9
Protamine	10	90.7 ± 1.6
	100	96.9 ± 0.5
Polylysine	10	95.1 ± 1.0
	100	98.5 ± 0.2
Lysine/alanine copolymer	10	88.3 ± 2.5
	100	97.1 ± 0.2
HBP	10	43.1 ± 10.0
	100	83.3 ± 8.1

[a] Inhibitors were added to radiolabeled protein (10 μg/ml) before incubation with immobilized kidney. Inhibition data are expressed as the means ± SD of triplicate assays.

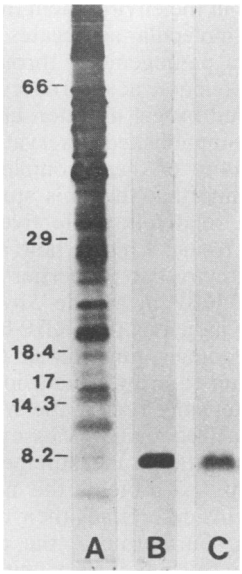

FIG. 5. Western blot of 10 μg of unfractionated streptococcal components (lane a) and 1 μg of purified HBP (lane B) detected with 200-fold-diluted anti-*S. mutans* serum and 120 μg of unfractionated streptococcal component detected with affinity-purified antibodies to HBP (lane C). Shown at left are kilodalton values of standard proteins.

DISCUSSION

Immunofluorescence assays show that purified HBP of *S. mutans* binds selectively to heparan-sulfate proteoglycan-containing structures (basal laminae) of kidney. Binding of radiolabeled protein to kidney tissue exhibits a high degree of specificity (8), as indicated by saturation kinetics, time dependence, and sensitivity to competitive inhibition by selected glycosaminoglycans and HBP. Specificity of binding is substantiated by competitive dissociation (83%) of preformed complexes between ^{125}I-HBP and kidney tissue by the native protein. This observation also indicates that the extrinsic radiolabel on the protein did not significantly change its binding properties. Although quantitative measurements indicated that only 25 to 30% of the available

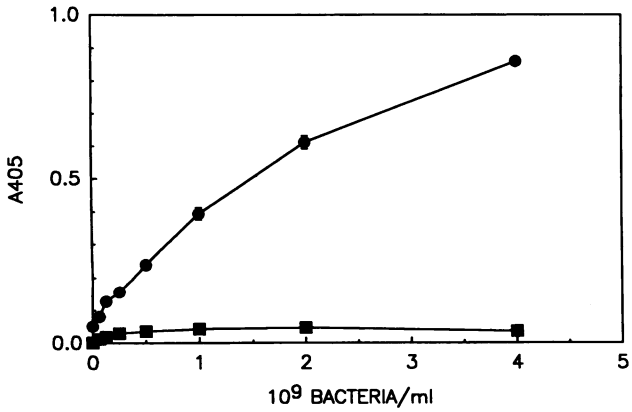

FIG. 6. Detection of HBP on the surface of *S. mutans* cells in the ELISA by using affinity-purified antibodies. ●, Bacteria treated with primary and secondary antibodies; ■, bacteria treated with secondary antibodies only.

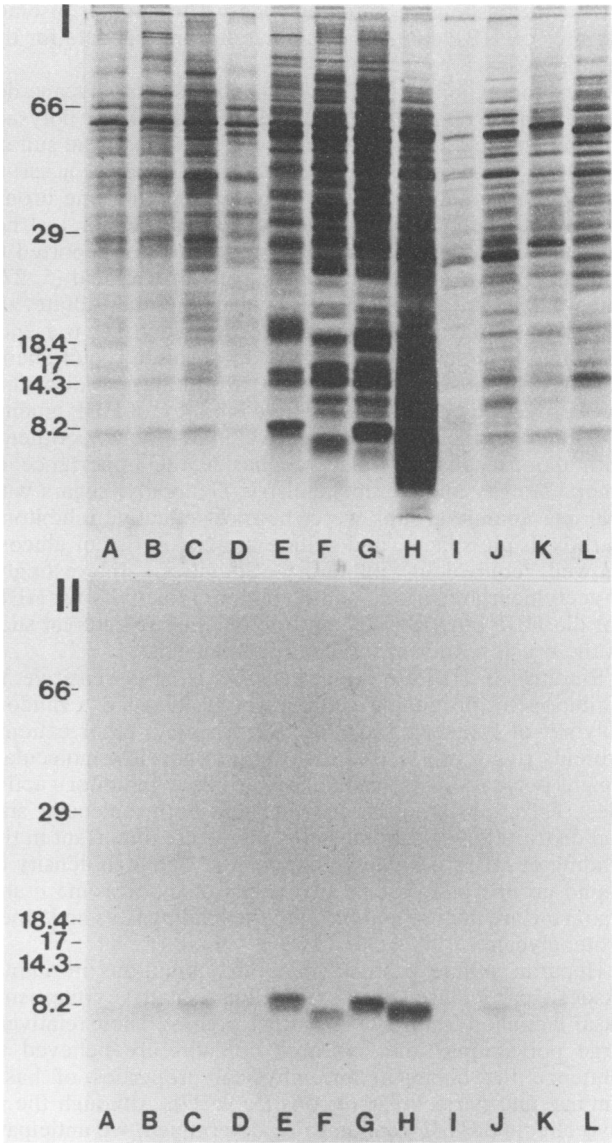

FIG. 7. Silver stain (I) and Western blot assay (II) of streptococcal extracts after SDS-PAGE. Lanes: A, *S. mutans* MT703, 60 μl; B, *S. mutans* 10449, 60 μl; C, *S. sobrinus* K1R, 60 μl; D, *S. cricetus* AHT, 60 μl; E, *S. sanguis* G9B, 20 μl; F, *S. salivarius*, 20 μl; G, *S. mitis* ATCC 9811, 7.5 μl; H, *S. pyogenes* ATCC 14289, 30 μl; I, *S. pyogenes* M6, 30 μl; J, *S. pyogenes* M12, 30 μl; K, *S. agalactiae* ATCC 13813, 60 μl; L, *S. lactis*, 20 μl. Shown at left are kilodalton values of standard proteins. Affinity-purified antibodies were used at a concentration of 700 ng/ml.

^{125}I-protein bound to kidney tissue, the contribution of the radiolabeling procedure to this apparent low binding activity is unclear because the percent activity of the unlabeled protein cannot be measured with the same degree of accuracy. Alternatively, the nature of the assay could also be responsible for this observation. The extensive washing procedure used to remove unbound reactants may also remove a large proportion of glycosaminoglycan-HBP complexes from the tissue homogenate on the plastic wells. Because of the heterogeneous nature of the ligand (homogenized kidney), the dissociation constant of 6.2×10^{-7} M should be considered an average of multiple interactions of

HBP with tissue glycosaminoglycans. The number of binding sites on HBP cannot be calculated from this data for the same reasons.

The binding activity of HBP for glycosaminoglycans depended on the density of sulfate substituents on the polysaccharide and to a lesser degree on the location of the sulfate group on the monosaccharide subunits. Heparin, a serum component, contains 2 to 3 sulfate groups and one uronic acid per disaccharide unit, whereas heparan sulfate, a structural analog present in kidney basal laminae, is reported to contain 0.2 to 3 sulfate groups and one uronic acid (5, 27). The weak inhibitors, hyaluronic acid and keratan sulfate, are devoid of sulfate and uronic acid, respectively. The presence of uronic acid substituents is not an absolute requirement, however, because dextran sulfate (two sulfates per disaccharide) proved to be a highly effective inhibitor of HBP binding to kidney tissue. The competitive inhibition experiments with monosaccharides also emphasize the importance of charge density and stereochemistry. Generally, sugars with multiple anionic groups were the most effective inhibitors. Carboxyl, phosphate, and sulfate groups on C-6 of glucose showed equivalent inhibitory activities. Interestingly, *N*-acetylneuraminic acid had no inhibitory activity for HBP, nor did HBP bind to sialic acid-rich kidney structures, such as the brush border of tubular epithelial cells.

Binding of HBP to kidney tissue is also sensitive to inhibition by protamine sulfate, polylysine, and a random polymer of lysine and alanine. Surprisingly, other cationic proteins (lysozyme and cytochrome *c*) and low-molecular-weight polycations (spermine) did not have inhibitory activities. These observations indicate that both molecular size and distribution of cationic substituents are important in the binding of HBP to kidney components. The high density of amino groups in spermine or the pIs of cytochrome *c* and lysozyme are not compatible with the binding sites on kidney proteoglycans.

Heparan sulfate proteoglycans are ubiquitous in animal tissues, particularly the extracellular matrix comprising basal laminae of kidneys and other organs. Their relatively large polyanionic and hydrated domains are believed to influence the chemical and physical properties of basal laminae and pericellular regions (5, 9, 27). Although the in vivo effects of HBP remain to be determined, we anticipate that its deposition on basal laminae may either exert direct toxicity on epithelial and endothelial cells or interfere with the passage of ions and proteins through glomerular capillaries by altering the net charge. Polycation substances, such as protamine sulfate, have been found to cause structural alterations in glomerular epithelia of perfused kidneys (2, 12, 18). This pathology is similar to that seen in some human glomerulonephritis cases and is reversed by subsequent perfusion with heparin (18). An alternative or concurrent mechanism for pathogenesis involves serological events in which binding of streptococcal HBP to kidney tissue leads to in situ formation of immune complexes as specific antibodies appear in the blood. Activation of complement through the classical or alternative pathways may lead to localized inflammation that is characteristic of delayed sequelae of streptococcal infections.

S. mutans HBP is present on the cell surface and is released into the culture medium during growth. Surface-localized HBP is readily dissociated from the cell by NaOH at pH 11.5 (3, 4), indicating that the protein is loosely associated with the cell wall. We suspect that the strong positive charge (pI 9.5) of HBP may cause it to associate with anionic streptococcal proteins and lipoteichoic acid on the cell surface and in the environment in which the bacteria are growing. These molecular aggregates may bind to tissues and exert enhanced pathogenicity through synergism. We have found that lipoteichoic acid forms complexes with HBP in vitro, as measured by gel filtration chromatography and isoelectrofocusing (unpublished observations). Although the kidney-binding activity of these complexes remains to be determined, it is important that this study showed lipoteichoic acid to be a relatively ineffective inhibitor of HBP activity for kidney tissue. Lipoteichoic acid also possesses kidney-binding and cytotoxic properties (11, 24).

The presence of HBP in all the *Streptococcus* species tested in this study indicates that HBP has an essential but still unknown physiologic function in these bacteria. Although it is tempting to draw conclusions from the differences in staining intensities seen in the Western blot (Fig. 7), it should be emphasized that this assay method does not permit accurate quantitative measurements. In addition to the relative amounts of protein, the number of epitopes shared by the proteins determines their reactivities with the primary antibodies. Furthermore, the quantity of protein extracted from the cells may vary with species, cultural conditions, or phase of growth. Further studies are necessary to identify the conditions that regulate synthesis of HBP by streptococci and to define the role of HBP in the pathogenesis of streptococcus-associated nephritides. Cationic antigens of *S. pyogenes* have been previously implicated in acute poststreptococcal glomerulonephritis (26).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant RO1-DE05696 from the National Institute of Dental Research.

We thank Xiaolei Zhang for her expert technical work and Nancy Hurley for her editorial assistance.

REFERENCES

1. **Albini, B., R. J. Nisengard, I. Glurich, M. E. Neiders, and M. W. Stinson.** 1985. *Streptococcus mutans*-induced nephritis in rabbits. Am. J. Pathol. **118**:408–418.
2. **Alvarez, J., L. Alvarez, C. Escudera, F. Gilsanz, S. de Oya, and J. L. Castillo-Olivares.** 1988. Hemodynamic and morphologic alterations after experimental administration of protamine sulfate. Am. J. Surg. **155**:735–740.
3. **Bergey, E. J., and M. W. Stinson.** 1988. Heparin-inhibitable basement membrane-binding protein of *Streptococcus pyogenes*. Infect. Immun. **56**:1715–1721.
4. **Choi, S. H., and M. W. Stinson.** 1989. Purification of a *Streptococcus mutans* protein that binds to heart tissue and glycosaminoglycans. Infect. Immun. **57**:3834–3840.
5. **Gallagher, J. T., M. Lyon, and W. P. Steward.** 1986. Structure and function of heparan sulphate proteoglycans. Biochem. J. **236**:313–325.
6. **Gibbons, R. J., and J. van Houte.** 1975. Dental caries. Annu. Rev. Med. **26**:121–126.
7. **Harder, E. J., C. J. Wilkowski, A. J. Washington, and J. E. Geraci.** 1974. *Streptococcus mutans* endocarditis. Ann. Intern. Med. **80**:364–368.
8. **Hollenberg, M. D., and P. Cuatrecasas.** 1979. Distinction of receptor from non-receptor interactions in binding studies, p. 193–214. *In* R. D. O'Brien (ed.), The receptors, a comprehensive treatise, vol. 1. Plenum Publishing Corp., New York.
9. **Kanwar, Y. S., A. Linker, and M. G. Farquhar.** 1980. Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate by enzyme digestion). J. Cell Biol. **86**:688–693.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Leon, O., and C. Panos.** 1983. Cytotoxicity and inhibition of

normal collagen synthesis in mouse fibroblasts by lipoteichoic acid from *Streptococcus pyogenes* type 12. Infect. Immun. **40**:785–794.
12. **Lin, W.-L.** 1988. Leakage of blood-retinal barrier due to damaging effect of protamine sulfate on the endothelium. Acta Neuropathol. **76**:427–431.
13. **Lockwood, W. R., L. A. Lawson, D. L. Smith, K. M. McNeill, and F. S. Morrison.** 1974. *Streptococcus mutans* endocarditis: report of a case. Ann. Intern. Med. **80**:369–370.
14. **Loesche, W. J.** 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. **50**:353–380.
15. **McGhee, R. J., and S. M. Michalk.** 1981. Immunobiology of dental caries: microbial aspects and local immunity. Annu. Rev. Microbiol. **35**:595–638.
16. **Morrisey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117**:307–310.
17. **Roberts, R. B., A. G. Krieger, N. L. Schiller, and K. C. Gross.** 1979. Viridans streptococcal endocarditis: the role of various species, including pyridoxal dependent streptococci. Rev. Infect. Dis. **1**:955–965.
18. **Seiler, M. W., M. A. Venkatachalam, and R. S. Cotran.** 1975. Glomerular epithelium: structural alterations induced by polycations. Science **189**:390–393.
19. **Stinson, M. W., P. K. Barua, E. J. Bergey, R. J. Nisengard, M. E. Neiders, and B. Albini.** 1984. Binding of *Streptococcus mutans* antigens to heart and kidney basement membranes. Infect. Immun. **46**:145–151.
20. **Stinson, M. W., R. J. Nisengard, M. E. Neiders, and B. Albini.** 1983. Serology and tissue lesions in rabbits immunized with *Streptococcus mutans*. J. Immunol. **131**:3021–3027.
21. **Stinson, M. W., F. J. Swartzwelder, R. J. Nisengard, and B. Albini.** 1987. Antigens shared by *Streptococcus mutans* and cardiac muscle: a re-evaluation, p. 424–434. *In* H. Koehler and P. T. LoVerde (ed.), Vaccines: new concepts and development. Longman Group Ltd., London.
22. **Swartzwelder, F. J., P. K. Barua, B. Albini, and M. W. Stinson.** 1988. Heart-reactive antibodies in rabbit anti-*Streptococcus mutans* sera fail to cross-react with *Streptococcus mutans*. J. Immunol. **140**:954–961.
23. **Terleckyj, B., N. P. Willett, and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11**:649–655.
24. **Tomlinson, K., O. Leon, and C. Panos.** 1983. Morphological changes and pathology of mouse glomeruli infected with a streptococcal L-form or exposed to lipoteichoic acid. Infect. Immun. **42**:1144–1151.
25. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
26. **Vogt, A., S. Batsford, B. Rodriguez-Iturbe, and R. Garcia.** 1983. Cationic antigens in post-streptococcal glomerulonephritis. Clin. Nephrol. **20**:271–279.
27. **Wight, T. M.** 1980. Vessel proteoglycans and thrombogenesis, p. 1–30. *In* T. N. Spaet (ed.), Progress in hemostasis and thrombosis, vol. 5. Greene and Stratton, Inc., New York.