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Preparation of a Sialic Acid-Binding Protein from *Streptococcus mitis* KS32AR

PATRICIA A. MURRAY,[1]† MICHAEL J. LEVINE,[1]* MOLAKALA S. REDDY,[1] LAWRENCE A. TABAK,[1,2] AND E. JAMES BERGEY[1]

Departments of Oral Biology[1] and Endodontics,[2] School of Dental Medicine, State University of New York at Buffalo, Buffalo, New York 14214

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A recent report has identified a lectin on the surfaces of several strains of *Streptococcus mitis* and *Streptococcus sanguis* with specificity for an *N*-acetylneuraminic acid α2,3-galactose-β1,3-*N*-acetylgalactosamine sequence (P. A. Murray, M. J. Levine, L. A. Tabak, and M. S. Reddy, Biochem. Biophys. Res. Commun. 106:390–396, 1982). In the present study, purification and characterization of this sialic acid-binding protein (SABP) was begun. A clinical isolate of *S. mitis* was grown to mid stationary phase in synthetic FMC medium and then extracted with lithium 3,5-diiodosalicylate. Lyophilized extract was subjected to gel filtration on a Sephadex G-200 column, giving four protein peaks (A to D). Peak B, shown by hemagglutination assay to contain SABP, was next subjected to affinity chromatography on a Sepharose-4B matrix coupled to fetuin glycopeptides. After an extensive washing, peak B materials bound to the affinity matrix were eluted with buffered *N*-acetylneuraminic acid. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2-mercaptoethanol on 7.5% gels of affinity-purified materials revealed components of 96, 70, and 65 kilodaltons (kDa). Without reducing agent, only the 65-kDa band and materials which did not penetrate the gel were visualized, suggesting that the 96- and 70-kDa components were disulfide linked. The chemical cross-linking agent, disuccinimidyl suberate, was used to demonstrate specific interactions between the SABP preparation and [^{14}C]fetuin glycopeptides. After cross-linking, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography revealed the 96- and 70-kDa components, indicating that the SABP is at least bivalent. These findings support our previous suggestion that human salivary glycoproteins facilitate clearance of selected oral streptococci via specific interactions between sialic acid-containing oligosaccharides and a carbohydrate-binding protein on the bacterial cell surface.

The ability of a microorganism to adhere to oral surfaces is a prerequisite for successful colonization and subsequent plaque-mediated diseases (18). The failure of bacteria to adhere results in their being swept by mechanical means, such as swallowing and coughing, amid the fluids which continuously bathe the oral tissues. Although it is recognized that surface components of oral bacteria participate in adherence and clearance phenomena (35), their properties remain largely obscure. Several oral bacteria have been shown to contain surface adhesins or binding proteins, and a lectinlike mechanism of adherence has been proposed on the basis of the inhibition of attachment by specific sugars (2, 3, 7, 10, 14, 16, 24, 36, 42). The purification and characterization of these binding proteins provides a more complete understanding of their role in host-parasite interactions.

Streptococcus sanguis and *Streptococcus mitis* are among the earliest colonizers of the tooth surface (6); consequently, these microorganisms appear to have a fundamental role in the cascade of events leading to the formation of dental plaque. Although available evidence indicates that the adherence of streptococci to saliva-coated hydroxyapatite surfaces is complex and involves both specific and nonspecific types of interactions, the exact molecular mechanisms are not fully understood. Electrostatic (4, 19) and hydrophobic (9, 15, 30, 34) forces are clearly involved in streptococcal salivary pellicle binding, but these types of interactions do not fully account for the tissue specificity observed in bacterial adherence. However, recent studies on the interactions of *S. sanguis* and salivary glycoproteins have begun to elucidate additional mechanisms of attachment involving a bacterial recognition system which may function to align salivary glycoproteins with the bacterial surface (18, 24, 31). Earlier studies suggested that the carbohydrate moieties of salivary mucins play a role in both bacterial adherence and clearance (17) and that, in particular, sialic acids may be determinants necessary for certain types of saliva-bacteria interactions (29). Later investigations (23, 45) with human salivary mucin have shown that the agglutination of *S. sanguis*, but not that of *S. mutans*, is dependent upon the presence of nonreducing terminal *N*-acetylneuraminic acid (NeuAc). More recently, we have presented evidence for a lectin on the surface of several *S. sanguis* and *S. mitis* strains with specificity for an *N*-acetylneuraminic acid α2,3-galactose-β1,3-*N*-acetylgalactosamine (NGG) sequence (31). The significance of this is suggested by the fact that the major acidic oligosaccharide of the lower-molecular-weight human salivary mucin has this trisaccharide structure (37). Thus, interactions between a trisaccharide of human salivary mucin and a bacterial lectin may promote clearance of streptococci and may represent a nonimmune protective mechanism in the oral cavity (46). In this study, we describe the purification and preliminary characterization of a sialic acid-binding protein (SABP) from *S. mitis* KS32AR.

(Preliminary results of this work have been presented previously [P. A. Murray, M. J. Levine, L. A. Tabak, and M. S. Reddy, Proc. Annu. Meet. Am. Assoc. Dent. Re-

* Corresponding author.
† Present address: Department of Stomatology, School of Dentistry, University of California at San Francisco, San Francisco, CA 94143.

search, Cincinnati, Ohio, 17 to 20 March 1983, in J. Dent. Res. 62:257, 1983.])

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MATERIALS AND METHODS

Materials. Bovine fetuin (type II) was obtained from Calbiochem-Behring, La Jolla, Calif., and from GIBCO Laboratories, Grand Island, N.Y. Pronase was obtained from Calbiochem-Behring. HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), phenylmethanesulfonyl fluoride, NeuAc (type VII), *N*-acetylneuramin–lactose, and *N*-(*p*-aminophenyl)-oxamic acid–agarose were purchased from Sigma Chemical Co., St. Louis, Mo. [^{14}C]formaldehyde (52.4 mCi/mmol) and En^{3}Hance were obtained from New England Nuclear Corp., Boston, Mass. Lithium 3,5-diiodosalicylate (LIS) was purchased from Eastman Kodak Co., Rochester, N.Y. Sephadex resins, Sepharose-4B, and molecular weight standards were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Disuccinimidyl suberate was from Pierce Chemical Co., Rockford, Ill.

General chemical and analytical methods. General estimations of protein and neutral sugars were made by the procedure of Lowry (27) and the anthrone reaction (39), respectively. Polyacrylamide gel electrophoresis (PAGE) was performed on 7.5% sodium dodecyl sulfate (SDS) disk or slab gels by the method of Weber and Osborn (49). Samples were prepared for electrophoresis by heating at 100°C for 3 to 5 min in 0.01 M sodium phosphate (pH 7.0) containing 2% SDS with or without 5% (vol/vol) 2-mercaptoethanol. After electrophoresis, proteins were identified by staining with Coomassie blue (12). Molecular weights were calculated from plots of the log molecular weights versus the relative mobility of standard reference proteins. The following reference proteins (molecular weights) were used: unreduced immunoglobulin G (IgG), 150,000; phosphorylase *b*, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; and trypsin inhibitor, 20,000.

Preparation of glycopeptides. Fetuin glycopeptides containing the NGG sequence were obtained by the methods of Spiro and Bhoyroo (44). For this purpose, 1.5 g of fetuin (25 mg/ml) was digested at 37°C with 1% (wt/vol) pronase in 0.15 M Tris-acetate buffer (pH 7.8) containing 1.5 mM calcium acetate. After 72 h, this digest was fractionated on columns of Sephadex G-50 (2.5 by 100 cm) in 0.1 M pyridine acetate (pH 5.1) to separate N-glycosidic units from the smaller O-glycosidic units. Elution was at 20 ml/h at 4°C with a fraction size of 5.5 ml. Columns were monitored for hexose by the anthrone method (39), for hexosamines on an amino acid analyzer after hydrolysis in 2 N HCl for 6 h at 100°C (38), and for sialic acids (48) after acid hydrolysis (with 0.05 N H_2SO_4 for 1 h at 80°C). *N*-Acetylgalactosamine-containing glycopeptides were further fractionated on columns of Sephadex G-25 (1.5 by 120 cm) in 0.1 M pyridine acetate (pH 5.1) at 4°C. Fractions of 4.0 ml each were collected, and portions were monitored as described above. NGG glycopeptides were purified by chromatography on columns (1.25 by 80 cm) of DE-52 cellulose equilibrated with the 0.002 M pyridine acetate buffer (pH 5.1) and by using a linear pyridine acetate gradient to 0.15 M (44). The composition of NGG glycopeptides was confirmed by chemical analyses by methods previously described (38).

Labeling of NGG glycopeptides by reductive methylation. NGG glycopeptides (5 mg) were methylated with [^{14}C]formaldehyde (1.3 μmol at 0.94×10^8 cpm/μmol) by the method of Jentoft and Dearborn (20). Unreacted products were removed by gel filtration on columns (1.5 by 80 cm) of Bio-Gel P4 (200/400 mesh; Bio-Rad Laboratories, Richmond, Calif.) in 0.1 M pyridine acetate (pH 5.1). Columns were monitored by scintillation spectroscopy, and the appropriate fractions were pooled and lyophilized. The specific activity of the prepared ^{14}C-labeled NGG glycopeptides was 2.5 μCi/mg. Incorporation of ^{14}C-labeled methyl groups onto lysine was verified by hydrolyzing labeled materials (with 6 N HCl for 28 h at 105°C) and examining the hydrolysates for mono- and dimethyllysine by paper chromatography with 2-propanol–ammonia–water (8:1:1, vol/vol/vol) as the solvent system (22).

Bacteria and culture conditions. *S. mitis* KS32AR (obtained as a fresh clinical isolate from a 24-h human dental plaque [32]) was used in these studies. This strain has previously been identified both as *S. sanguis* biotype 2 on the basis of biochemical tests (5, 11) and as a dextran-producing *S. mitis* because of the presence of a cell-associated neuraminidase (32). For study, a 1% inoculum of bacteria was grown for 16 to 18 h (mid to late stationary phase) at 37°C in FMC chemically defined medium freshly buffered with 0.02 M each of sodium carbonate and sodium bicarbonate (47). Cells were harvested at 4°C by centrifugation at 12,000 × *g* for 10 min and were washed three times in cold 0.05 M Tris hydrochloride (pH 7.5). The yield was approximately 2.5 g of cells per liter (wet weight).

LIS extraction of bacteria and gel filtration of extract on Sephadex G-200. Bacteria were suspended in 0.3 M LIS in 0.05 M Tris hydrochloride (pH 7.5) containing 100 μM phenylmethylsulfonyl fluoride at 2×10^{10} bacteria per ml and were extracted as previously described (31). Examination of the bacterial pellet by phase-contrast microscopy verified that LIS extraction did not rupture the cell walls. Lyophilized LIS extract (150 mg) was dissolved in 0.1 M Tris hydrochloride (pH 7.5) (equilibration buffer) by shaking overnight at 4°C (20 mg/ml), centrifuged at 12,000 × *g* for 30 min at 4°C to remove insoluble material, and then subjected to gel filtration chromatography on columns of Sephadex G-200 (1.5 by 110 cm). Elution in equilibration buffer was achieved at 8 ml/h at 4°C with a fraction size of 4.5 ml. Portions of the collected fractions were monitored for protein at A_{280}. The appropriate tubes were then pooled, exhaustively dialyzed against distilled water, and lyophilized.

Preparation of affinity matrices. NGG glycopeptides were covalently coupled to Sepharose-4B after activation with cyanogen bromide (28) with 5 mg of NGG glycopeptides per ml of resin in 0.1 M $NaHCO_3$ (pH 8.3) containing 0.5 M NaCl (coupling buffer). After being mixed for 16 h at 4°C, unreacted sites were blocked by addition of an equal volume of 1.0 M ethanolamine in coupling buffer and shaking overnight at 4°C. Excess adsorbed protein was removed from resin by alternate washings (5 bed volumes) with 0.1 M sodium acetate (pH 5.0), containing 0.5 M NaCl, and coupling buffer. Combined washings were desalted by elution with water on columns (1.5 by 90 cm) of Sephadex G-10. The NGG glycopeptide affinity matrix contained approximately 375 μg of NeuAc per ml of gel and represented a coupling efficiency of 75%.

Affinity chromatography. Sephadex G-200 pool B was dissolved (3 mg/ml) in 0.05 M Tris hydrochloride (pH 7.4) with 0.15 M NaCl, centrifuged at 12,000 × *g* for 30 min at 4°C to remove any insoluble material, and incubated with NGG

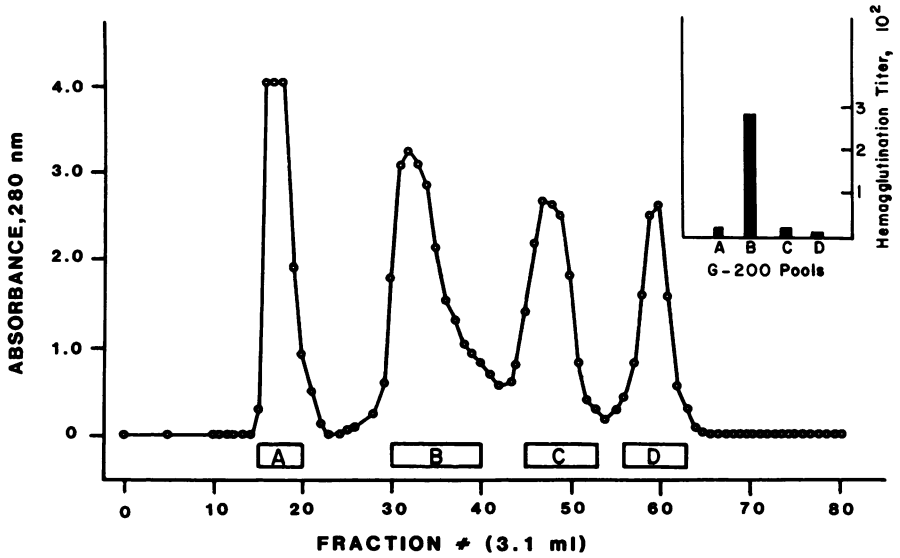

FIG. 1. Gel filtration of LIS-extracted *S. mitis* KS32AR on Sephadex G-200. The boxed letters represent the pooled materials. Inset, pooled materials were assayed for lectin activity by their ability to hemagglutinate guinea pig erythrocytes.

glycopeptide–Sepharose-4B (1:1, vol/vol) on an end-over-end rotary mixer for 16 h at 4°C. This mixture was then transferred to a column (1.5 by 4.5 cm) and washed with 0.05 M Tris hydrochloride (pH 7.4) with 0.15 M NaCl until the A_{280} was zero. Materials bound to the NGG glycopeptide column were eluted with 0.05 M Tris hydrochloride (pH 7.4) with 0.15 M NaCl containing 0.1 M NeuAc. Fractions were monitored for protein at A_{280}. The appropriate fractions were pooled, dialyzed, and lyophilized.

Hemagglutination assay. The successive lectin purification steps were monitored by a hemagglutination assay using a 3% (vol/vol) suspension of washed guinea pig erythrocytes in phosphate-buffered saline (31). Each 25-μl amount of a twofold serial dilution of lectin solution and an equal volume of erythrocyte suspension were incubated, after a gentle mixing, for 1 h at room temperature and scored. The hemagglutination titer represented the reciprocal of the highest dilution that gave agglutination upon visual inspection. Specific activity is defined as the hemagglutination titer per mg of binding protein.

Cross-linking ^{14}C-labeled NGG glycopeptides to streptococcal lectin. Sephadex G-200 pool B, 200 μg containing 65 μg of protein, (see Fig. 1) was dissolved in 50 μl of 0.05 M HEPES (pH 7.4) and incubated with 10^5 cpm of ^{14}C-labeled NGG glycopeptides in the presence and absence of a 100-fold excess of unlabeled NGG glycopeptides for 1 h at 37°C. The reaction mixture was cooled on ice to 24°C, and 5 μl of freshly prepared 0.05 M disuccinimidyl suberate was added. After 5 min, an equal volume of 0.1 M ammonium acetate was added to quench the reaction. The samples were immediately prepared for analysis by SDS-PAGE on 7.5% gels in the presence of 5% (vol/vol) 2-mercaptoethanol. Fluorography was carried out on dried gels with En^{3}Hance using LKB Instruments, Inc. Ultrafilm at −70°C; gels were developed for 4 to 6 weeks.

Preparation of bacterial neuraminidase. To demonstrate that the SABP was distinct from a sialidase (neuraminidase), a partially purified neuraminidase was prepared from *S. mitis* KS32AR. Bacteria were grown for 18 h in FMC medium, harvested by centrifugation, and washed three times with 0.01 M sodium phosphate buffer (pH 6.5) containing 0.15 M NaCl. Cells were then suspended in this washing buffer containing 0.15% (wt/vol) *N*-acetylneuramin–lactose at 4×10^9 cells per ml. After incubation at 37°C for 3 h to allow for enzyme secretion (32), the suspension was filtered (0.45-μm pore size; Millipore Corp., Bedford, Mass.) to separate the bacterial cells. The filtrate was dialyzed against 0.05 M sodium acetate (pH 5.5) containing 2 mM $CaCl_2$ and 0.2 mM disodium EDTA and applied to an affinity column (0.5 by 15 cm) of *N*-(*p*-aminophenyl)-oxamic acid–agarose equilibrated with the same buffer. Elution of neuraminidase was accomplished by increasing the ionic strength and raising the pH with a 0.1 M $NaHCO_3$ (pH 9.1) buffer (8). The flow rate was adjusted to 30 ml/h, and the eluate was collected as 0.5-ml fractions into tubes containing 10% (vol/vol) sodium acetate and monitored at an A_{280}.

Neuraminidase assay. Bacterial fractions from *S. mitis* KS32AR were examined for neuraminidase activity by their ability to hydrolyze [^{3}H]*N*-acetylneuramin–lactose. This procedure has previously been described in detail (13). *N*-acetylneuramin–lactose was prepared to a specific activity of 3.4 mCi/mmol (32).

RESULTS AND DISCUSSION

Purification of lectin. In a typical experiment, 30 mg of lyophilized LIS extract was obtained per liter of bacterial culture. Approximately 35% of the extract was protein, as determined by the method of Lowry (27), using bovine serum albumin as a standard. Gel filtration chromatography of the LIS extract on Sephadex G-200 is shown in Fig. 1, in which four protein peaks are visible. Using a hemagglutination assay to monitor lectin purification (Fig. 1), we found that the hemagglutination activity in pool B was approximately 60-fold greater than that of the LIS extract (Table 1). Pool B was then subjected to affinity chromatography on NGG glycopeptides-Sepharose (Fig. 2). Pool B1 represents the nonadherent material. After an extensive washing, materials bound to the column were eluted with 0.1 M NeuAc in 0.05 M Tris hydrochloride (pH 7.4) with 0.15 M NaCl. This pool, designated B2, represented the SABP preparation. This SABP preparation was purified approximately 10,000-fold with a hemagglutination activity of 28,858 (Table 1).

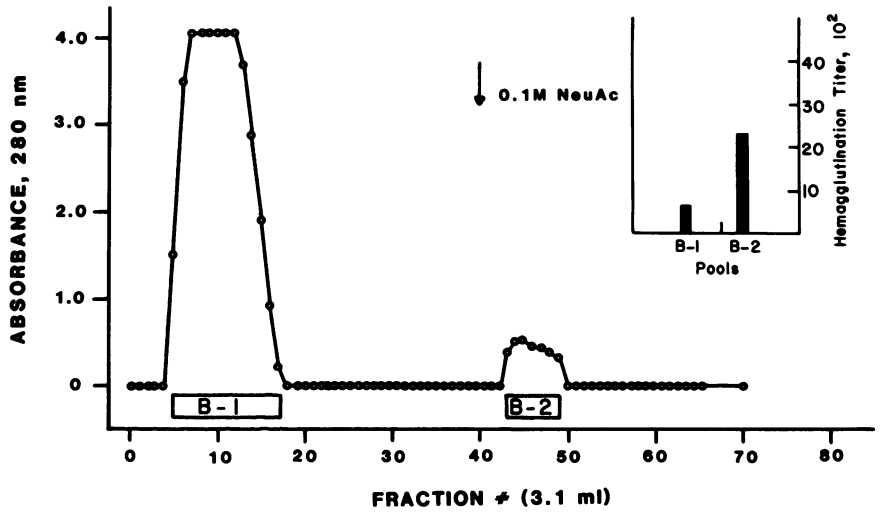

FIG. 2. Affinity chromatography of Sephadex G-200 pool B on NGG glycopeptide–Sepharose-4B. The peaks were monitored at A_{280}, and specifically bound materials were eluted with 0.1 M NeuAc in 0.05 Tris hydrochloride (pH 7.4), containing 0.15 M NaCl. (Inset) Pooled materials were assayed for lectin activity by their ability to hemagglutinate guinea pig erythrocytes.

SDS-PAGE of lectin preparation. SDS-PAGE analyses with 2-mercaptoethanol of pool B2 and the crude LIS extract are shown in Fig. 3. With Coomassie blue staining, a complex protein pattern was obtained with the crude LIS extract, whereas only three bands were seen with the SABP preparation. The SABP did not stain with periodic acid-Schiff reagent. The estimated sizes of the three polypeptide components in pool B2 were calculated to be 65, 70, and 96 kilodaltons (kDa). When pool B2 was examined in the absence of reducing agent, only the 65-kDa component, as well as material that did not penetrate the 7.5% gel matrix, was seen. SDS-PAGE of SABP on 5% gels without 2-mercaptoethanol demonstrated a band with an M_r of ~200 kDa in addition to the 65-kDa component. These data suggest that the 70- and 96-kDa components are disulfide-linked subunits associated noncovalently with the 65-kDa peptide.

Effects of repeated subculture. During a period of 2 months, *S. mitis* KS32AR was subcultured 60 times in FMC synthetic medium. No changes were observed in its reactivity in various biochemical tests (32) after the 60 passages. The original isolate, as well as the 18-h broth cultures from every sixth subculture, was tested for SABP activity by the hemagglutination inhibition assay. SABP activity after 60 transfers was comparable to that of the original isolate, indicating that repeated in vitro subcultures in synthetic FMC medium did not alter or affect the presence of lectin activity. However, other studies of *S. sanguis* have demonstrated the effect of growth conditions on the bacterial surface. For example, Liljemark and Bloomquist (26) found that an adherence-blocking component from *S. sanguis* was often no longer detectable after several passages of fresh isolates. Westergren and Olsson (50) also found that the hydrophobic properties of *S. sanguis* can diminish with repeated subcultures in vitro; this most probably reflects changes of different biochemical structures on the bacterial

TABLE 1. Purification of an SABP from *S. mitis* KS32AR

Prepn	Total protein (mg)	Total activity hemagglutination U[a]	Sp act U/mg of protein	Purification (fold)
LIS extract	150	4.5×10^4	2.7	1
Pool B	23	4.3×10^4	166	61.5
Pool B2	1.5	3.8×10^4	28,858	10,685

[a] A hemagglutination unit is defined as the reciprocal of the highest dilution of lectin which produced visible agglutination of guinea pig erythrocytes.

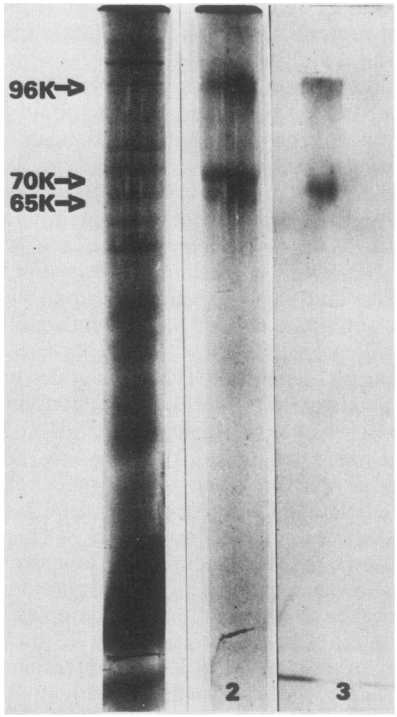

FIG. 3. SDS-PAGE analysis on 7.5% acrylamide gels in the presence of 2-mercaptoethanol. Lanes: 1, 65 μg of protein of crude LIS extract from *S. mitis* KS32AR; 2, 73 μg of protein of SABP (pool B2, Fig. 2); 3, 66 μg of protein of Sephadex G-200 pool B (Fig. 1) incubated with ^{14}C-labeled NGG glycopeptides (2×10^4 cpm) and subjected to the cross-linking agent disuccinimidyl suberate. The molecular weights of the three polypeptide components in pool B2 (in kilodaltons) are shown to the left of the gel.

TABLE 2. Streptococcal lectin versus enzyme: evidence for two different molecules

Parameter	Presence or reaction of[a]:	
	Lectin[b]	Neuraminidase[c]
Presence in organism		
S. sanguis G9B and 10556	+	−
S. mitis KS32AR and 10557	+	+
Extraction with LIS	+	−
Elution on Sephadex G-200		
Excluded	−	+
Included	+	−
Heat lability[d]	−	+

[a] +, Present or positive; −, absent or negative.
[b] Reference 31.
[c] Reference 32.
[d] Neuraminidase activity of whole cells was inactivated at 80°C for 45 min (31).

cell wall. For our studies, a chemically defined medium was used. It may be that continuous passages in a more complex medium or for a longer time produce surface changes that result in loss of lectinlike activity.

Characterization of SABP by covalent cross-linking with ^{14}C-labeled NGG glycopeptides. To identify which of the components in the SABP preparation contained carbohydrate-binding sites, Sephadex G-200 pool B (Fig. 1) was incubated with ^{14}C-labeled NGG glycopeptides (±100-fold molar excess of unlabeled NGG glycopeptide). After 1 h at 37°C, the covalent cross-linking agent, disuccinimidyl suberate, was added. If the glycopeptides were bound to the SABP, they would be cross-linked by the disuccinimidyl suberate and the ^{14}C-labeled glycopeptide-adhesin complex(es) and then identified by fluorography after SDS-PAGE. The results of this experiment are depicted in Fig. 3, lane 3. Two radiolabeled bands which comigrated with the 96- and 70-kDa components are shown. These results indicate that the two disulfide-linked constituents of the lectin contain carbohydrate-binding sites. The specificity of these interactions was verified by running this experiment in the presence of a 100-fold excess of unlabeled glycopeptide. Under these conditions, no radiolabeled bands were visualized. The fact that the 65-kDa component was not radiolabeled indicates that this component does not contain a carbohydrate-binding site and confirms the earlier explanation that this band is either a noncovalently linked subunit of the lectin or, alternately, may represent a protein contaminant.

Preparation of *S. mitis* neuraminidase. Because *S. mitis* strains are known to possess a cell-associated, substrate-inducible neuraminidase (32), it was necessary to rule out the possibility that the SABP was a neuraminidase. An active enzyme preparation was prepared from the concentrated culture supernatant of strain KS32AR with *N*-(*p*-aminophenyl)-oxamic acid-agarose as an affinity matrix (8). This chromatography procedure resulted in a 160-fold purification of the neuraminidase. When the neuraminidase preparation was subjected to gel filtration on Sephadex G-200, enzyme activity was eluted in the void volume fractions (in contrast to SABP, which was in the included fractions [Fig. 1, pool B]). In addition, neuraminidase was not extracted with LIS (31) but remained (albeit decreased) with the cell pellet. Previously, we have reported the presence of SABP in *S. sanguis* strains that do not produce neuraminidase (31). Collectively, these data suggest that the neuraminidase and SABP from *S. mitis* KS32AR are two distinct molecules (Table 2).

In summary, this study was undertaken to purify and to begin characterizing a component on the surface of *S. mitis* KS32AR that appears to specifically interact with the sialic acid-containing oligosaccharides of salivary glycoproteins. This SABP was proteinaceous in nature and contained at least two disulfide-linked subunits of 96 and 70 kDa. Studies using the chemical cross-linking agent, disuccinimidyl suberate, confirmed the bivalent nature of SABP, since each subunit was shown to bind the NGG sequence. Studies from several laboratories have demonstrated the presence of adhesins on the surface of *S. mitis* or *S. sanguis* (1, 21, 33, 40, 41, 43). The mechanism by which these adhesins interact with saliva molecules is not completely understood. Indeed, the presence of at least two lectinlike adhesions on the surface of *S. mitis* 10557 has been reported. These include a galactose-binding lectin (smaller than the SABP) (33) and a lectin interactive with the NGG sequence (32). Both the galactose-binding protein (33) and SABP (3) have been shown to interact with the proline-rich glycoprotein of human parotid saliva. The presence of terminal NeuAc and galactose on the triantennary oligosaccharides of the proline-rich glycoprotein (25, 38) suggests that different ligands on a single saliva molecule may interact with more than one adhesin on the same bacterium. It is apparent that primary structural determinants on saliva molecules (e.g., a defined oligosaccharide sequence) can be recognized by some bacterial adhesins. In addition, conformational determinants on saliva molecules may be important in saliva-bacteria interactions. It can be speculated that primary structural determinants function in clearance phenomena, whereas conformational determinants play a role in the adherence of bacteria to saliva-coated enamel or mucosal surfaces. These conformational determinants could form de novo as the result of surface binding, or they might be buried within the suprastructure of the molecule and become accessible only upon binding to an oral surface. Site-specific reagents such as SABP could be used to examine the availability of structural or conformational determinants or both on saliva molecules before and after their binding to tissue surfaces.

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