Carbohydrate Research, 108 (1982) 111–122
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

PURIFICATION OF A LOW-MOLECULAR-WEIGHT, MUCIN-TYPE GLYCOPROTEIN FROM HUMAN SUBMANDIBULAR–SUBLINGUAL SALIVA

AKRAPORN PRAKOBPHOL, MICHAEL J. LEVINE*, LAWRENCE A. TABAK, AND MOLAKALA S. REDDY
Department of Oral Biology, School of Dentistry, State University of New York at Buffalo, Buffalo, New York 14226 (U.S.A.)

(Received September 4th, 1981; accepted for publication, December 7th, 1981)

ABSTRACT

A low-molecular-weight, monomeric, mucin-type glycoprotein (MG2) has been isolated from human submandibular–sublingual saliva. Initial purification involved sequential gel-filtration on Sephadex G-200 and Sepharose CL-2B, the latter in the presence of 6M urea. Fractions containing MG2 were next separated from contaminating secretory IgA by immunoaffinity chromatography or recycling through Sephadex G-200. Mucin fractions were ^{14}C-labeled by reductive methylation, and then the final purification-step entailed recycling radiolabeled materials through Sephadex G-200. Radiolabeling aided in the assessment of purity, as judged by SDS–PAGE and ion-exchange chromatography. The carbohydrate portion accounted for 69.6% of the recovered weight and was composed of *N*-acetyl-glucosamine, *N*-acetylgalactosamine, galactose, fucose, and *N*-acetylneuraminic acid. Sulfate was also present. The protein comprised 30.4% of the recovered weight with threonine, serine, proline, and glycine accounting for 75.2% of the total amino acids. The oligosaccharides were alkali-labile, indicating an *O*-glycosyl linkage to the peptide. The mucin was weakly acidic and had an estimated mol. wt. of 200 000–250 000.

INTRODUCTION

Salivary mucins are considered to play a major role in the nonimmune protection of the oral cavity[1]. Such functions include acting as a permeability barrier, lubrication, concentrating protective macromolecules at the tissue environmental interface, and modulation of the oral flora. By analogy with immunoglobulins[2], fibronectin[3–5], and collagens[6–8], it is proposed that the various functions of the mucin might be assigned to a specific structural domain[1]. Such assignment requires the structural characterization of highly purified mucin(s) in a form representative of its (their) natural secreted state. We have previously described the purification of monkey salivary mucin by methods that minimize degradation during handling and

*To whom all correspondence should be addressed.

purification[9]. Using a comparable approach with human salivary secretions, we report here the presence of at least two major mucin components in submandibular–sublingual secretions, and have isolated and partially characterized the lower-molecular-weight species. Additionally, radiolabeling by reductive methylation was used to facilitate isolation and assess purity.

EXPERIMENTAL

Materials. — Sephadex G-200, Sepharose 4B, and Sepharose CL-2B were obtained from Pharmacia Fine Chemicals (Piscataway, NJ 08854). DE-52 cellulose was purchased from Whatman Products Inc. (Clifton, NJ 07014), and [^{14}C]formaldehyde (51 mCi/mmol) from New England Nuclear Laboratory (Boston, MA 02118). Contaminants from [^{14}C]formaldehyde were removed by passage through columns of Dowex 1-X8 (AcO^-, 200–400 mesh) and the purified solution adjusted to a final concentration of 3.8 μmol/mL. Partially purified, colostral secretory immunoglobulin (sIgA) was obtained from Calbiochem-Behring Corp. (San Diego, CA 92112); 1-(2-hydroxyethyl)piperazine-2-(2-ethanesulfonic acid) (HEPES), dichloromethane, sodium cyanoborohydride, ovalbumin, and neuraminidase (*Clostridium perfringens* Type VI) were obtained from Sigma Chemical Co. (St. Louis, MO 63178).

Collection and handling of human submandibular–sublingual saliva (HSMSL). — HSMSL was collected from a single donor (25-years old and B blood-group active) and handled as previously described[10]. In selected instances, stimulated saliva was collected directly, from the ductal orifices of the labial and submandibular glands, with capillary tubes, and then deposited in 0.1M sodium phosphate buffer, pH 7.0, with 1% sodium dodecyl sulfate (SDS) and 1% (v/v) 2-mercaptoethanol for subsequent analysis by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

Analytical procedures. — Protein and neutral sugars were determined by the Lowry method[11] and anthrone reaction[12], respectively. Analyses of amino acids, amino sugars, neutral sugars, sialic acids, and sulfate were carried out as previously described[9].

Purification of human salivary mucin. — (*a*) *Gel filtration on Sephadex G-200.* This initial step of purification was performed as previously described[10,13].

(*b*) *Sepharose CL-2B filtration of peak A from Sephadex G-200 column.* Excluded materials (designated Peak A, 40 mg) from the Sephadex G-200 column were subjected to gel filtration on columns (1.5 × 160 cm) of Sepharose CL-2B equilibrated with 0.1M pyridine–acetate buffer, pH 6.0, in 6M urea. Each sample was suspended at a concentration of 8 mg/mL in the equilibrating buffer and dissolved overnight by gentle shaking at 4°. Undissolved materials were removed by centrifugation at 12 000*g* for 30 min at 4°. After being warmed to room temperature, the sample was applied and fractions of 3.3 mL were collected at room temperature at a flow rate of 3–5 mL/h. Fractions were monitored for protein and neutral sugars pooled as indicated in Fig. 1 (top), dialyzed extensively against distilled water, and lyophilized.

(*c*) *Removal of Secretory IgA.* sIgA was removed from Peak A-2 by immuno-

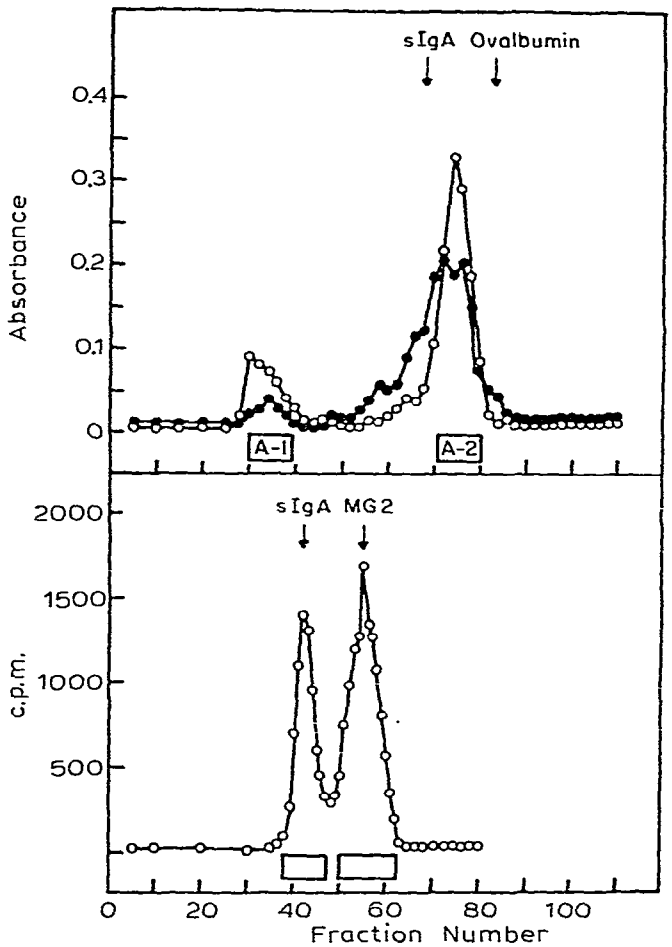

Fig. 1. (Top) Gel filtration on Sepharose CL-2B of void-volume materials from Sephadex G-200 column; (—○—○—) neutral sugars and (—●—●—) protein. Secretory IgA (M_r ~320 000) and ovalbumin (M_r ~46 000) were used to calibrate the column. (Bottom) Gel filtration on Sephadex G-200 of Peak A-2 (~24 mg). [^{14}C]sIgA (1.5 × 10^5 c.p.m.) and [^{14}C]MG2 (3.0 × 10^5 c.p.m.) were added to the sample as markers.

affinity chromatography. Rabbit anti-sIgA was prepared as previously described[10], or purchased from Accurate Chemical & Scientific Corp. (Code No.A092, Westbury, NY 11590). An IgG fraction obtained by sodium sulfate precipitation, followed by chromatography on DE-52 cellulose, was coupled to Sepharose 4B after cyanogen bromide activation[10]. The adsorbent was able to bind approximately 300 μg of sIgA (protein) per mL of gel. Samples of Peak A-2 (8–10 mg) were dissolved in 0.1M sodium hydrogencarbonate–0.5M sodium chloride buffer, pH 8.3 (equilibrating buffer), at a concentration of 8 mg/mL. After application to the affinity column (5-mL bed volume), the effluent flow was stopped for 1 h. Fractions of 1.7 mL were

then collected with the equilibrating buffer at 4°, at a flow rate of 15 mL/h. Eluates were monitored by absorbance at 230 nm, and unbound materials dialyzed extensively against distilled water, and lyophilized. Secretory IgA was identified in bound and unbound fractions by immunoelectrophoresis or SDS–PAGE. Mucin fractions were recycled until all sIgA was removed as evidenced by SDS–PAGE.

Alternatively, sIgA was separated from the lower-molecular-weight mucin by chromatography of Peak A-2 through columns (1.5 × 120 cm) of Sephadex G-200, equilibrated with 0.1M pyridine–acetate buffer, pH 6.0, in 6M urea, and then recycling the mucin fractions through the same column. Initially, [^{14}C]sIgA and [^{14}C]mucin (1.5–3.0 × 10^5 c.p.m. each) were mixed as markers* with the sample. Fractions of 1.4 mL were collected at room temperature and monitored by liquid-scintillation spectrometry with a Beckman LS 7000 scintillation counter. A typical elution profile is shown in Fig. 1 (bottom).

(*d*) *^{14}C-Radiolabeling by reductive methylation.* Mucin-containing fractions substantially free of SIgA were subjected to reductive methylation with [^{14}C]formaldehyde following the method of Jentoft and Dearborn[14]. In a typical experiment, the sample (10 mg) was dissolved, at a concentration of 5 mg/mL, in 0.1M HEPES buffer, pH 7.5, and shaken overnight at 4°. Fractions that contained sIgA (Peak A-2, Fig. 1, top) were dissolved in HEPES buffer containing 6M urea. A 0.2M solution of recrystallized sodium cyanoborohydride in 0.1M HEPES buffer (200 μL) was added to the reaction mixture (2 mL). The amount of formaldehyde to be added was at a 10-fold molar excess over the total amount of lysine residues (~0.1 μmol/mg) in the sample. [^{14}C]Formaldehyde (2.5 μmol at 10^8 c.p.m./μmol) was added and the mixture incubated for 1 h at 37°. Then unlabeled formaldehyde (7.5 μmol) was added and the incubation continued for another 23 h at 37°. Unreactive products were removed by gel filtration on columns (1.5 × 80 cm) of Bio-Gel P4 (200–400 mesh) equilibrated with 0.1M pyridine–acetate buffer, pH 5.1. Fractions were monitored by liquid-scintillation spectrometry. Materials that were eluted at the void volume were pooled and lyophilized. By use of these procedures, specific activities of approximately 1–2 × 10^6 c.p.m./mg were obtained. To verify the incorporation of [^{14}C]methyl groups into lysine, the labeled materials were hydrolyzed (6M hydrochloric acid, 28 h), 105°, and the hydrolyzates examined for mono- and dimethyllysine by paper chromatography (Whatman No. 1) in 8:1:1 (v/v) 2-propanol–ammonia–water[15].

(*e*) *Sephadex G-200 gel filtration of ^{14}C-labeled materials.* Final purification of the lower-molecular-weight mucin was achieved by fractionation of ^{14}C-labeled material on calibrated columns (1.5 × 120 cm) of Sephadex G-200 equilibrated with 0.1M pyridine–acetate buffer, pH 6.0, in 6M urea. Fractions (1.4 mL) were collected at room temperature. Eluates were monitored by liquid-scintillation spectrometry. Mucin fractions were pooled, dialyzed, and lyophilized.

*These markers were obtained in a separate experiment wherein Peak A-2 was subjected to ^{14}C-reductive methylation, and the material (both [^{14}C]sIgA and [^{14}C]mucin) recycled, as described above, through columns of Sephadex G-200. Final purification was assessed by 5% SDS–PAGE.

DE-52 Cellulose chromatography of [^{14}C]*MG2.* — The mucin (1.5×10^6 c.p.m./mg) was dissolved (5.6 mg/mL) in 10mM sodium phosphate, pH 7.0, in 6M urea, and the solution applied to a column (1.5 × 18 cm) of DE-52 cellulose equilibrated with the same buffer. After elution with the equilibrating buffer, a linear gradient was started (at Fraction 20) which consisted of 150 mL of equilibrating buffer and 150 mL of equilibrating buffer in 3.0M sodium chloride. Fractions (1.4 mL) were collected at room temperature and monitored by liquid-scintillation spectrometry (Fig. 2, top). Pooled fractions were dialyzed and lyophilized. The recovered material

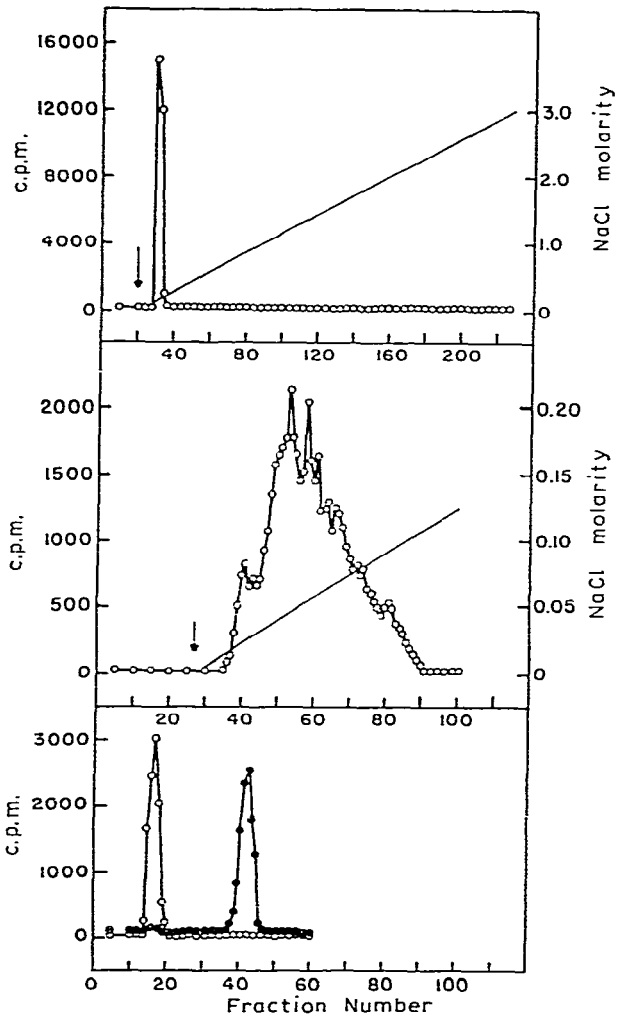

Fig. 2. (Top) DE-52 cellulose chromatography of purified [^{14}C]MG2 (5.6 mg, 1.5×10^6 c.p.m./mg). After elution with 10mM sodium phosphate buffer, pH 7.0, a linear gradient was started from 0 to 3.0M sodium chloride. (Middle) DE-52 cellulose chromatography of [^{14}C]MG2 using a shallow gradient from 0 to 0.25M sodium chloride. (Bottom) Sephadex G-200 gel filtration of [^{14}C]MG2 (2×10^5 c.p.m.) before (—○—○—) and after (—●—●—) alkali-borohydride treatment.

(5 mg) was then applied to another DE-52 cellulose column (1.5 × 18 cm). Following elution with the equilibrating buffer, the adsorbed material was subjected to a shallow linear gradient of 100 mL each of equilibrating buffer and equilibrating buffer in 0.25M sodium chloride (Fig. 2, middle). Fractions (1.4 mL) were collected and mucin samples recovered as previously described.

Characterization of the glycopeptide linkage. — The mucin (2×10^5 c.p.m.) was dissolved in 0.5 mL of 50mM sodium hydroxide–M sodium borohydride and the solution kept for 16 h at 45°. The excess borohydride was eliminated by titration with 4M acetic acid at 4° in the presence of 1-octanol to minimize foaming. The mixture was fractionated on a column (1.5 × 85 cm) of Sephadex G-200 equilibrated with 0.1M pyridine–acetate buffer, pH 6.0. Fractions (2.8 mL) were collected at room temperature and monitored by liquid-scintillation spectrometry (Fig. 2, bottom).

Electrophoretic procedures. — SDS–polyacrylamide gel electrophoresis was performed as previously described[9]. Glycoproteins were stained for protein with Coomassie Blue and for carbohydrate with the periodic acid–Schiff reagent[16]. Molecular weights were calculated from plots of the log molecular weight *vs.* relative mobility of standard proteins. The reference standards used were: porcine thyroglobulin subunit, 330 000; unreduced IgG, 150 000; phosphorylase a, 94 000; bovine serum albumin, 66 000; beef liver catalase subunit, 60 000; and chymotrypsinogen, 25 700. To locate radioactivity, the gels were cut into 1.5-mm slices and solubilized overnight in Soluene 350 (Packard, 1 mL) at 37°. After the addition of scintillant, the solubilized materials were allowed to equilibrate for 24 h before counting. Immunoelectrophoresis was carried out as previously described[17].

RESULTS

Isolation. — The excluded material obtained by fractionation of HSMSL on Sephadex G-200 contained mucin-like glycoproteins, sIgA, lysozyme, and group Cla (M_r 14 000) of the cysteine-containing phosphoproteins[10,13]. SDS–PAGE on 5% gels of these void-volume materials is shown in Fig. 3 (gels 1 and 2). The subunits of sIgA, namely secretory component (SC), H chain, and J or L chains can be seen (see gel 9 for reference[18]). In addition, two other glycoproteins (MG1 and MG2) were detected with the latter penetrating 5% gels.

To purify MG2, the void-volume materials from Sephadex G-200 were further fractionated on columns of Sepharose CL-2B. Filtration was carried out in the presence of 6M urea to facilitate solubility of the sample. Two major carbohydrate-containing peaks (A-1 and A-2) were obtained (Fig. 1, top). Analysis by SDS–PAGE revealed that MG1 and MG2 were separated (Fig. 3, gels 3–6). Also present in Peak A-2 was sIgA (Fig. 3, gel 5). This was verified by immunoelectrophoresis. Secretory IgA was removed by passing Peak A-2 through columns of anti-sIgA coupled to Sepharose 4B. It was found that unbound materials had to be recycled several times to completely separate sIgA from MG2. The data shown in Fig. 3 (gel 7) represent

MG2 obtained after three cycles through anti-sIgA columns. Alternatively, sIgA could be separated from MG2 by passing Peak A-2 through columns of Sephadex G-200 equilibrated with 6M urea in 0.1M pyridine–acetate, pH 6.0 (Fig. 1, bottom), and then recycling the mucin fractions until sIgA was removed, as evidenced by SDS–PAGE. [^{14}C]sIgA and [^{14}C]MG2 were included initially as markers to facilitate pooling of the fractions.

The next step of MG2 purification involved radiolabeling the unbound materials from the immunoaffinity columns, or the mucin fractions obtained as shown in Fig. 1, bottom. This was accomplished by ^{14}C-reductive methylation of lysine residues to give their mono- and di-methyl derivatives. This procedure offers several advantages[14]; it facilitates further characterization of MG2 by conserving material and, in addition, does not alter the overall charge and can be carried out at neutral pH. More importantly, radiolabeling permitted detection and removal of residual sIgA, as well as small amounts of low-molecular-weight peptides by gel filtration on Sephadex G-200 with urea of the desalted [^{14}C]MG2 reaction mixture. These smaller peptides ranged in size from 7 000 to 35 000 daltons, as determined by analysis of gel slices following SDS–PAGE.

Purity. — SDS–PAGE of purified MG2 in both 3% and 5% gels revealed a single band when stained with Coomassie Blue and the periodic acid–Schiff reagent. When gels containing [^{14}C]MG2 were sliced and counted, all the radioactivity was recovered at a position comparable to stained MG2. As an additional criterion of purity, [^{14}C]MG2 was fractionated on DE-52 cellulose equilibrated with 10mM sodium phosphate buffer, pH 7.0. All radioactivity was recovered as a single sharp

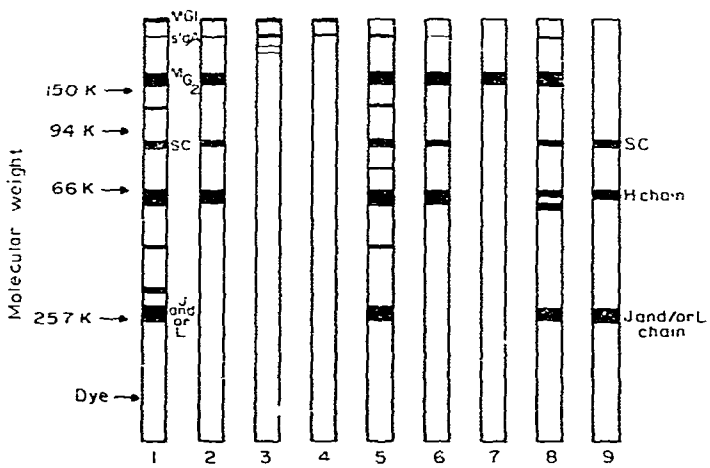

Fig. 3. SDS–PAGE on 5% gels; 50–100 μg of material were applied to each gel, and all samples were reduced with 2-mercaptoethanol. The gels were stained either with Coomassie Blue (CB) or the periodic acid–Schiff reagent (PAS). Gels: (1) G-200 Peak A (CB); (2) G-200 Peak A (PAS); (3) Peak A-1 (CB); (4) Peak A-1 (PAS); (5) Peak A-2 (CB); (6) Peak A-2 (PAS); (7) purified MG2 (CB or PAS); (8) material initially bound to anti-sIgA column (CB); and (9) sIgA (CB).

peak following the start of a sodium chloride gradient (Fig. 2, top). Subsequent fractionation of this material using a shallow gradient indicated that MG2 is weakly acidic and somewhat heterogenous with respect to charge (Fig. 2, middle). These experiments reinforce the value of radiolabeling as a sensitive adjunct in assessing samples for the presence of trace amounts of peptide contaminants.

Chemical composition and size. — The chemical composition of MG2 is given in Tables I and II. The molecule displays the major characteristics of mucin. It has a high content of carbohydrate consisting of *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, and galactose. Sialic acid was identified as *N*-acetylneuraminic acid after release by neuraminidase. Preliminary studies using recently published methods[20] indicate that a sulfate group is linked to C-4 of an *N*-acetylglucosamine residue. Mild-alkali treatment of [^{14}C]MG2, followed by reduction with sodium borohydride, resulted in the cleavage of oligosaccharides, as evidenced by a marked increase in the elution volume of radiolabeled peptide(s) (Fig. 2, bottom). These data suggest an *O*-glycosyl linkage of oligosaccharides to a peptide backbone. However, the additional presence of mannose (Tables I and II) cannot exclude the possibility that MG2 also contains asparagine-linked carbohydrate units.

The protein composition consisted of a high content of threonine, serine, proline (23.7%), and alanine (14.4%). A low content of basic amino acids was observed, and the proportion of sulfur-containing amino acids was negligible. The estimated M_r for MG2 ranged from 250 000 on 3% gels to 200 000 on 5% gels. Such variations in estimated size are commonly found for highly glycosylated molecules with varying acrylamide concentration of the gels[21]. The values obtained are consistent with the elution volume of MG2 on Sepharose CL-2B, which was between sIgA (M_r 320 000) and ovalbumin (M_r 46 600) (Fig. 1, top).

Further characterization. — Several experiments were performed to determine whether MG2 is associated with MG1, or if MG2 itself can be dissociated into subunits. When intact HSMSL was examined by SDS–PAGE on 5% gels, with or without 2-mercaptoethanol, there was no change in the relative mobility of MG2. This suggests the following: (a) MG2 is not disulfide-linked to MG1; and (b) MG2 does not contain disulfide-linked subunits. In an additional experiment, MG1 and MG2 could be separated by fractionating 20 mL of freshly collected HSMSL on columns (2 × 120 cm) of Sepharose CL-2B in 0.1M pyridine–acetate, pH 6.0. This separation in the absence of urea indicates that the two glycoproteins were not associated by hydrogen bonding. Finally, [^{14}C]MG2 was examined by gel filtration on Sephadex G-200 using 2M sodium chloride in 0.1M pyridine–acetate, pH 6.0. There was no appreciable difference in the elution volume as compared to the control, thus indicating the absence of subunits associated by electrostatic forces. Collectively, these data indicate that MG2 is not associated with MG1 and represents a monomeric species.

Direct analysis of freshly collected, submandibular and labial salivas by SDS–PAGE revealed that MG1 and MG2 were present in each secretion. Since labial saliva is derived from a pure mucus gland, MG1 and MG2 appear to be of mucus-cell origin.

TABLE I

COMPONENTS OF MUCIN (RESIDUES)

Component	*Residues/1000 Amino acid residues*
Aspartic acid	49
Threonine	209
Serine	162
Glutamic acid	69
Proline	237
Glycine	14
Alanine	144
Half-cystine	1.0
Valine	31
Methionine	1.1
Isoleucine	15
Leucine	28
Tyrosine	1.4
Phenylalanine	8.6
Lysine	14
Histidine	8.7
Arginine	6.9
N-Acetylglucosamine	157
N-Acetylgalactosamine	274
Mannose	28
Fucose	209
Galactose	321
N-Acetylneuraminic acid[a]	102
Sulfate	49

[a]Identified by thin-layer chromatography (Avicel, Analtech Inc., Newark, DE 19711), on prewashed plates (0.1M hydrochloric acid), in 1:2:1 (v/v) 1-butanol–1-propanol–0.1M hydrochloric acid[19], after release by *Clostridium perfringens* neuraminidase[13].

TABLE II

COMPOSITION OF MUCIN IN PERCENT

Component	%
Protein	22.1
N-Acetylglucosamine	7.3
N-Acetylgalactosamine	12.8
Mannose	1.0
Fucose	7.1
Galactose	12.0
N-Acetylneuraminic acid	6.9
Sulfate	1.1

DISCUSSION

Studies aimed at identifying and partially characterizing human salivary mucins have dealt primarily with high-molecular-weight species[22-24]. In one study[23] where salivary mucin was prepared by precipitation with cetyltrimethylammonium bromide, followed by gel filtration on Sephadex G-200, a lower-molecular-weight glycoprotein was also detected. The authors suggested that this represented a subunit of the higher-molecular-weight mucin dissociable by SDS. In the study presented here, we have isolated this lower-molecular weight glycoprotein (designated MG2), begun its characterization, and shown that it is not associated with the higher-molecular-weight species.

In general, studies aimed at the purification of a mucin encounter difficulties that can be attributed to the rheological properties of the molecule, namely, low solubility, high viscosity, elasticity, and adhesiveness. Depending upon the mucin source, these rheological properties are maintained in large part by combinations of disulfide, hydrogen, and hydrophobic bonds, and electrostatic forces. Consequently, disruption of these interactions becomes necessary to counteract the viscoelastic character of the mucin and facilitate its isolation. The rheological properties of the mucin can be enhanced by heterotypic complexing with other molecules. For example, List *et al.*[25] have shown that the viscosity of hog gastric mucin is increased by the addition of small amounts of human serum albumin. Mucin–lipid complexes have been described for bronchial mucins[26-29], with the lipid possibly contributing to the mucin's viscosity[28]. Identifying the existence of such complexes, and subsequently separating the mucin component, provides an additional problem. For example, mild chaotropes, such as caesium chloride or bromide, have been utilized to dissociate noncovalent interactions between IgA and gastric mucin[30], as well as lipids from bronchial mucin[29]. Additionally, 6M urea has been used to dissociate noncovalent interactions between lysozyme and human bronchial mucin[31]. Noncovalent interactions might explain why sIgA and MG2 did not dissociate during the initial gel-filtration of HSMSL on Sephadex G-200, but appeared to be separable on Sephadex G-200 in the presence of 6M urea. Noncovalent interactions between sIgA and MG2 may also account for the need to recycle materials through anti-sIgA affinity columns to complete their separation. It also appears that MG2 complexes with smaller salivary peptides. However, we cannot state at present whether dissociation of these complexes is affected by 6M urea, or reductive methylation, or both. The possible interaction of lipid-like molecules with purified MG2 was also examined. This appeared unlikely as 2:1 (v/v) chloroform–methanol extraction of [^{14}C]MG2 resulted in over 98% of the recoverable radioactivity being present in the aqueous layer, which, upon gel filtration on Sephadex G-200, gave an identical elution-volume as unextracted MG2. It should be pointed out that we cannot distinguish whether these heterotypic mucin-complexes were artificially induced by such manipulations as lyophilization. Studies are currently under way using unlyophilized, freshly collected

saliva to examine the nature of the interactions between mucin and other salivary constituents.

The potential role of salivary mucins in oral disease has been recently reviewed[1]. We have previously demonstrated that the lower-molecular-weight mucin from human, submandibular-sublingual saliva can agglutinate strains of *Streptococcus sanguis* and *Streptococcus mutans*[13]. Removal of sialic acid from the mucin resulted in a loss of agglutination of *S. sanguis*, but not *S. mutans*. Such interaction may be of importance in modulating the protective salivary-clearance of microorganisms from the oral cavity[1]. Conversely, a mucin coat on the tooth enamel or oral mucosa, or both, could promote the initial bacterial colonization of these surfaces, and subsequently lead to dental caries or periodontal disease. The high content of proline in this mucin could promote hydrophobic interactions with enamel or mucosa, allowing the oligosaccharide chains to project out into the oral environment for subsequent microbial attachment. The purification of human salivary mucins and their subsequent characterization are the first steps aimed at precisely defining the specificity of mucin interaction in the oral cavity.

ACKNOWLEDGMENTS

This research was supported, in part, by Public Health Service Grants 1 RL1-DE04971 and 1R23-DE04518 from the National Institute of Dental Research. L.A.T. was supported, in part, by a Young Investigator Award from the Cystic Fibrosis Foundation. The author would like to thank Mr. Hector A. Velasco for the photography and illustrations and Ms. Mildred Saks for typing the manuscript.

REFERENCES

1 L. A. Tabak, M. J. Levine, I. D. Mandel, and S. A. Ellison, *J. Oral Path.*, 11 (1982) 1–17.
2 E. A. Padlan, *Q. Rev. Biophys.*, 10 (1977) 35–65.
3 E. Rouslahti, E. G. Hayman, P. Kuvsela, J. E. Shively, and E. Engvall, *J. Biol. Chem.*, 254 (1979) 6054–6059.
4 G. Balian, E. M. Click, and P. Bornstein, *J. Biol. Chem.*, 255 (1980) 3234–3236.
5 D. D. Wagner and R. O. Hynes, *J. Biol. Chem.*, 255 (1980) 4304–4312.
6 E. J. Miller, *Mol. Cell. Biochem.*, 13 (1976) 165–192.
7 D. J. Prockop, K. I. Kivirikko, L. Tuderman, and N. A. Guzman, *N. Engl. J. Med.*, 301 (1979) 13–23; 77–85.
8 D. R. Eyre, *Science*, 207 (1980) 1315–1322.
9 M. C. Herzberg, M. J. Levine, S. A. Ellison, and L. A. Tabak, *J. Biol. Chem.*, 254 (1979) 1487–1494.
10 J. P. Shomers, L. A. Tabak, M. J. Levine, I. D. Mandel, and S. A. Ellison, *J. Dent. Res.*, 61 (1982) 764–767.
11 O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.
12 J. H. Roe, *J. Biol. Chem.*, 212 (1955) 335–343.
13 M. J. Levine, M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li, and T. van Dyke, *Infect. Immun.*, 19 (1978) 107–115.
14 N. Jentoft and D. G. Dearborn, *J. Biol. Chem.*, 254 (1979) 4359–4365.
15 W. M. Kuehl and R. S. Adelstein, *Biochem. Biophys. Res. Commun.*, 37 (1969) 59–65.
16 G. Fairbanks, T. C. Steck, and D. Wallach, *Biochemistry*, 10 (1971) 2606–2617.

17 M. J. LEVINE AND S. A. ELLISON, *Arch. Oral. Biol.*, 18 (1973) 839–853.
18 J. MESTECKY, R. KULHAVY, AND F. W. KRAUS, *J. Immunol.*, 108 (1972) 738–747.
19 E. SVENNERHOLM AND L. SVENNERHOLM, *Nature (London)*, 181 (1958) 1154–1165.
20 L. A. TABAK AND M. J. LEVINE, *Arch. Oral Biol.*, 26 (1981) 315–317.
21 J. P. SEGREST AND R. L. JACKSON, *Methods Enzymol.*, 28 (1972) 54–63.
22 J. SCHLAGER AND M. D. G. OATES, *Arch. Oral Biol.*, 16 (1971) 287–303.
23 M. M. BAIG, R. J. WINZLER, AND O. M. RENNERT, *J. Immunol.*, 111 (1973) 1826–1833.
24 I. OEMRASINGH AND P. A. ROUKEMA, *Arch. Oral Biol.*, 19 (1974) 615–626.
25 S. J. LIST, B. P. FINDLAY, G. G. FORSTNER, AND J. F. FORSTNER, *Biochem. J.*, 175 (1978) 565–571.
26 M. LHERMITTE, G. LAMBLIN, P. DEGAND, P. ROUSSEL, AND M. MAZZUCA, *Biochimie*, 59 (1977) 611–620.
27 R. HAVEZ AND G. BISERTE, *Hypersécrétion Bronchique, Coll. Int. Pathol. Thorac., Lille*, 1968, pp. 43–67.
28 R. W. LEWIS, *J. Theor. Biol.*, 61 (1976) 21–25.
29 K. R. BHASKAR AND L. REID, *J. Biol. Chem.*, 256 (1981) 7583–7589.
30 M. SPOHN AND I. MCCOLL, *Biochem. Biophys. Res. Commun.*, 79 (1977) 837–842.
31 L. A. TABAK AND M. J. LEVINE, unpublished observations.