Distinct populations of high-M_r mucins secreted by different human salivary glands discriminated by density-gradient electrophoresis

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High-M_r mucins [mucin glycoprotein 1 (MG1)] isolated from human saliva from the individual salivary glands were chemically characterized. The carbohydrate content of MG1 derived from palatal (PAL), submandibular (SM) and sublingual (SL) saliva was typical of mucins but showed heterogeneity, especially in the amount of sialic acid and sulphated sugar residues. The physicochemical properties of native MG1s make conventional SDS/PAGE and ion-exchange chromatography unsuitable for investigating differences between individual samples. Recently a density-gradient electrophoresis (DGE) device has been developed, primarily for separation based on the charge of entire cells or cell organelles [Tulp, Verwoerd and Pieters (1993) Electrophoresis **14**, 1295–1301]. We have used this apparatus to study the high-M_r salivary mucins. Using DGE, the MG1s of individual glands were seen to have clearly distinct electrophoretic mobilities, as monitored by ELISA using MG1-specific monoclonal antibodies. Even within a particular MG1 preparation, subpopulations could be distinguished. DGE analysis of a chemically and enzymically modified MG1 series, followed by ELISA and dot-blot detection using specific monoclonal antibodies, lectins and high-iron diamine staining, suggests that the high electrophoretic mobility of PAL-MG1 is mainly the result of a high sulphate content, whereas the SL subpopulations differ mainly in binding type and amount of sialic acid. SM-MG1 most resembles the low-mobility subpopulation of SL-MG1, except that it has a lower sulphate content. In conclusion, DGE appears to be a powerful method for analysis of native mucin; it has been used to demonstrate that MG1s from the various salivary glands are biochemically much more diverse than was previously assumed.

INTRODUCTION

Mucin-type glycoproteins are the most prominent constituents of the secretions that cover the sensitive epithelia, which need special protection against the environment. Knowledge of the function of these complex molecules is limited to the generally accepted idea that they are mainly protective (for a review see Strous and Dekker [1]. Moreover, modifications of mucins have been associated with pathological conditions. Diseases such as cystic fibrosis [2–5], those of the colon and intestine [6,7] and malignancy of the gastrointestinal or respiratory tract are attended by dramatic changes in mucin composition [8–10]. Elucidation of their function at the molecular level requires an understanding of the biochemical structure of these molecules, to which molecular cloning of mucin cDNAs have contributed greatly in recent years [11–17]. The protein backbone of mucins contains a variable number of repeating sequences. In each repeat, high levels of serine and threonine residues allow a high degree of glycosylation, mainly O-linked oligosaccharides (up to 90% by mass), and high numbers of proline residues result in stretched configurations. An increasing number of mucins have been described that also contain N-linked glycans [13,18–20]. As deduced from the *MUC* genes described so far, all the *N*-glycan sites are situated at translation regions 5′ or 3′ to the central tandem-repeat region [11–17].

Mucins are the major constituents of human saliva and are recognized as two structurally and functionally distinct types: high-M_r mucins [mucin glycoprotein 1 (MG1); M_r over 10^6] and low-M_r mucins (MG2; M_r about 125000) [17,21,22]. They are produced by the (sero)mucous glands: submandibular gland (SM), sublingual gland (SL), and a large number of minor glands of the palate (PAL), cheeks and lips. Most of the available structural data on salivary mucins are derived from studies on human whole saliva or human SM–SL saliva [21,23,24]. Little is known about potential differences between mucin populations synthesized by the individual salivary glands. Structural analysis of MG1 is greatly hampered by the size of the molecules. Potential differences between MG1s present in saliva from the different glands were suggested by immunochemical analysis, which showed different levels of expression of antigenic determinants [25], and by CsCl-density-gradient centrifugation, which revealed in particular that PAL mucins differ in buoyant density from SL, SM and labial mucins [26]. In the present study MG1, isolated from separately collected PAL, SM and SL saliva, has been characterized chemically and electrophoretically.

Because of the size of MG1 molecules, conventional SDS/PAGE cannot be used to elucidate differences between individual populations, as they do not even enter the stacking gel. Recently, a density-gradient electrophoresis (DGE) apparatus was developed, primarily for separation based on the charge of entire cells or cell organelles [27]. It is a small and simple alternative to the free-flow electrophoresis apparatus described by Hannig et al. [28]. DGE achieves zonal migration of negatively charged material upwards against gravity in a Ficoll gradient. With this device, a fine resolution between species-specific erythrocytes and between early and late endosomes of Mel JuSo cells has been

Abbreviations used: DGE, density-gradient electrophoresis; PAL, palatal; SL, sublingual; SM, submandibular; MG1, mucin glycoprotein 1 (high-M_r mucin); HID, high-iron diamine; mAb, monoclonal antibody.

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achieved [26,29]. We have used this apparatus to study MG1s isolated from individual glandular salivas. It was found that mucins from different mucous salivary glands display differential electrophoretic mobility, in line with differences in chemical composition. The origin of these differences was further studied by DGE of a chemically and enzymically modified MG1 series, followed by immunochemical detection, lectin-affinity detection and high-iron diamine (HID) sulphate staining. Type-specific differences in electrophoretic mobility could mainly be attributed to differences in the amount of sialic acid and sulphated sugar present. Another charge-based separation method, i.e. ion-exchange chromatography, which has been successfully used for the separation of reduced mucin subunits [4,30], proved to be inadequate for separation of populations of native mucin molecules.

EXPERIMENTAL

Collection of saliva

SM saliva was collected without stimulation from the orifice of the Wharton duct, using a custom-fitted device. The same device was simultaneously used to collect SL saliva from the smaller lateral ductal orifices of the SL glands. PAL secretions were collected using a custom-fitted device covering the soft as well as the hard palate. All saliva samples were collected from one person (bloodgroup A, non-secretor).

Isolation of MG1

MG1 was isolated from saliva by ultracentrifugation (100000 *g*; 24 h) under dissociative conditions, followed by gel filtration (Sephacryl HR-500; Pharmacia, Uppsala, Sweden) in 50 mM Tris/HCl/4 M guanidinium chloride, pH 7.4 [31]. The mucin-containing fractions were pooled, dialysed against double-distilled water and stored as aliquots at −20 °C. The desired amount of mucin was chemically or enzymically modified as described below or used unmodified.

Chemical composition of isolated mucins

Protein was determined using the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL, U.S.A.) according to the manufacturer's instructions. Carbohydrate compositions were estimated essentially as described by Savage et al. [32]. Sulphate content was determined by the method of Terho and Hartiala [33].

Chemical and enzymic modification of mucins

Isolated MG1 was desialylated by mild acid treatment (0.01 M HCl at 80 °C, for 1 h). Carbohydrate analysis revealed that this treatment removed all sialic acid residues, less than 20 % of the fucose residues and no other sugar residues [25]. As apparent from HID detection (see below) and verified by sulphate determination [33], this treatment also resulted in removal of over 80 % of sulphate residues. Desialylation without affecting sulphate residues was achieved by incubation with 0.1 unit of neuraminidase (*Vibrio cholerae*; EC 3.2.1.18; Boehringer-Mannheim) in 50 mM citric/acetate, pH 5.5, containing 1 mM $CaCl_2$, at 37 °C for 16 h. Controls were performed in parallel using the same buffers but without acid or enzyme respectively. Although the removal of sialic acid residues appeared to be complete for the positive-control glycoprotein, fetuin, up to 30 % of residual sialic acids could be detected in PAL-MG1 by chemical analysis. The SL-MG1 and SM-MG1 preparations were less refractory towards neuraminidase treatment.

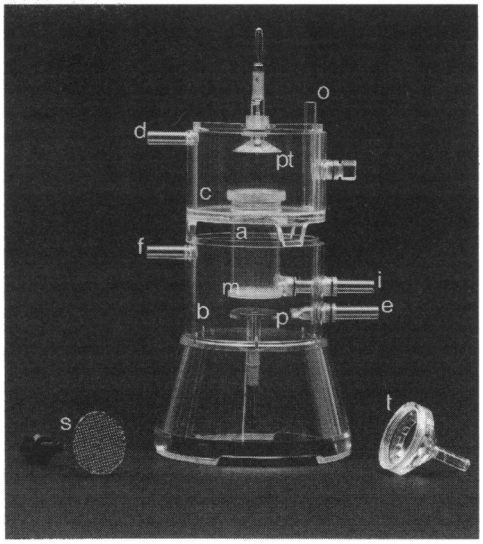

Figure 1 Photograph of the DGE apparatus

The apparatus is made of Perspex. The electrophoresis tube (a) is separated hydrodynamically but not electrically from the bottom reservoir (b) by a cationic permeable membrane (m) at the bottom of the tube. The membrane is held in place by a threaded fixture. In the cathodic reservoir is a non-gassing palladium circular electrode (p). With the top cone (t) screwed to the electrophoresis tube, the tube is filled completely via inlet (i). The gradient and sample are added via the top cone by letting liquid drip out from inlet (i). The top cone is replaced by a circular stainless-steel sieve (s) to allow rapid filling of the top reservoir (c) via the standing tube (o). Next the sieve is replaced by the top circular platinum electrode (pt). The reservoirs (b and c) can be replenished with buffer via inlets (o) and (e), while the fluid leaves via overflows (d) and (f).

DGE

The device (Figure 1), sample layering, electrophoresis and fractionation have been described in detail by Tulp et al. [27]. Briefly, the mucin sample was lyophilized and resuspended in 0.5 ml of electrophoresis medium (10 mM triethanolamine, 10 mM acetic acid, 1 mM EDTA, 0.25 M sucrose, final pH 7.4) containing 5 % Ficoll. The sample (0.1–0.5 mg dry weight in 0.5 ml of electrophoresis medium) was introduced into the apparatus on a linear gradient of 6 ml ranging from 10 to 6 % Ficoll over a 10 % Ficoll cushion and overlayered with a linear gradient of 9.5 ml ranging from 4 to 0 % Ficoll. A cone set-up was used for very precise thin sample layering and fractionation. Electrophoresis was initiated by a constant current regimen of 10 mA, and after a separation time of 60 min the material was fractionated into 35 fractions of six drops.

Ion-exchange chromatography

The mucin sample was lyophilized, resuspended in starting buffer (10 mM Bistris, pH 6.8) and subjected to high-performance ion-exchange chromatography on a Pharmacia Mono Q HR 5/5 column using the Pharmacia FPLC system, without prefilters to prevent retardation of mucins by the filters. The column was eluted at a flow rate of 0.5 ml/min with a linear gradient of starting buffer containing 0–0.5 M NaCl, followed by a wash with 1 M NaCl. Fractions (0.5 ml) were collected and portions were analysed by ELISA as described below.

Immunochemical detection

After DGE and ion-exchange chromatography, aliquots of each fraction were serially diluted and analysed by ELISA with

monoclonal antibodies (mAbs) F2 and E9. A detailed description of the method and anti-mucin mAbs used has been published [25,26]. Both mAbs recognize human MG1; mAb F2 is directed to a neuraminidase- and papain-insensitive epitope, and mAb E9 is directed to a sialic acid-containing epitope.

Lectin staining

Aliquots (20 μl) of each DGE fraction were applied to nitrocellulose filters using a Bio-Dot apparatus (Bio-Rad) by drying at room temperature, and washed once with 50 mM Tris/HCl, pH 7.5, containing 150 mM NaCl. The filters were subsequently removed from the apparatus, incubated in blocking buffer and further processed using digoxigenin-labelled lectins according to the manufacturer's (Boehringer-Mannheim) protocol. The following lectins were used: MAA from *Maackia amurensis*, which specifically binds α2-3NeuAc [34] and SNA from *Sambucus nigra*, which has a high specificity for α2-6NeuAc bound to galactose or *N*-acetylgalactosamine [35]. Fetuin and asialofetuin controls were included on the same blots.

HID staining

Dot-blots prepared as described above were stained for 1 h in freshly made HID solution containing 120 mg of *NN*-dimethyl-*m*-phenylenediamine and 20 mg of *NN*-dimethyl-*p*-phenylenediamine in 50 ml of distilled water, supplemented with 1.4 ml of $FeCl_3$ of the National Formulary solution (180 mg of Fe^{3+}) (essentially as described by Thornton et al. [4], modified from a histochemical staining method described by Spicer [36]). After the dot-blots had been washed thoroughly in water, the sulphomucins were stained purple–black.

RESULTS

Chemical analysis of human salivary-gland MG1 species

Human saliva samples were collected with special custom-fitted adapters from SM, SL and PAL glands from one subject (bloodgroup A, non-secretor). The high-M_r mucin, MG1, was isolated from each on the basis of size by centrifugation and gel-filtration chromatography. The three mucin preparations were characterized by determining the protein, carbohydrate and sulphate content and the relative composition of the carbohydrate moieties, and calculating the number of sialic acid and sulphate residues (Table 1). The chemical composition of each of the preparations was typical of mucin glycoproteins with respect to the sugar/protein ratio. However, substantial differences in sugar composition and sulphate content were apparent between the preparations from individual salivary glands. In particular, the terminal sugar residue, sialic acid (NeuAc), together with the sulphate content determined the glandular differences. PAL-MG1 expressed relatively low amounts of sialic acid; SL-MG1 and SM-MG1 contained two- and three-fold the amount respectively. Minor differences were found for the other sugars. The fucose content appeared to be reciprocal to the sialic acid content. Also striking were the differences in sulphate content. In comparison with MG1 from SL or SM, that derived from PAL contained a very high number of sulphate residues. Assuming an M_r of 10^6, over 1200 sulphate residues were calculated to be present per molecule of PAL-MG1.

Table 1 Chemical composition of MG1 species

The values in parentheses are number of residues, assuming an M_r of 1000000.

	Composition of MG1 (% by mass)		
Components	PAL	SM	SL
Protein	11.6	13.4	15.0
Carbohydrate	75.6	84.4	82.6
Man	0.6	0.4	0.6
Gal	23	22	22
GalNAc	9	11	11
GlcNAc	25	23	28
Fuc	12	8	10
NeuAc	6 (194)	20 (647)	11 (359)
Sulphate	11.9 (1239)	1.2 (135)	2.1 (219)

Table 2 Characteristics of the glandular MG1 species

++, Strong reactivity: +, moderate reactivity; ±, weak reactivity; +/−, −/+, partial reactivity; −, no reactivity.

Characteristic	PAL-MG1	SM-MG1	SL-MG1
Mobility	Fast	Moderate	Heterogeneous
F2 epitope	++	+	++
F9 epitope	−	++	+/−
HID stain	++	±	+
MAA recognition	++	+	−/+
SNA recognition	+	++	++

DGE of human salivary-gland MG1 species

The differences in negative charge of the MG1 species, already suggested by the sulphate and sialic acid content, were exploited by DGE. After DGE of the MG1 preparations, the fractions were monitored by ELISA using two MG1-recognizing antibodies, F2 and E9. MAb F2 is directed to a neuraminidase- and papain-insensitive epitope, and mAb E9 to a sialic acid-containing epitope. As in previous experiments [25], PAL-MG1 was characterized by high activity towards mAb F2 and very low reactivity, if any, to mAb E9. SM-MG1 and SL-MG1 could be detected by both mAbs. Their relative immunoreactivities, however, appeared to be reciprocal, i.e. SM-MG1 was recognized more effectively by mAb E9, whereas SL-MG1 was recognized more efficiently by mAb F2 (Table 2).

Figure 2 shows the electrophoretic mobility profiles. The three MG1 populations displayed clearly distinct electrophoretic mobilities. The highest mobility was observed for PAL-MG1 (Figure 2a), running right at the front of the electrophoretic tube, as visualized by immunoreactivity with mAb F2. The mobility of SM-MG1 was more moderate (Figure 2b), and that of SL-MG1 was distributed over a broad area of the electrophoretic tube (Figure 2c), as monitored by mAbs F2 and E9. Of the three mucin preparations, SL-MG1 was most clearly composed of subpopulations with different electrophoretic mobilities. It appeared that, of the SL mucins, the subpopulation with the lowest electrophoretic mobility possessed epitopes for both F2 and E9 mAbs, whereas that with higher mobility was preferentially recognized by mAb F2.

To assess the chemical basis of the observed differences in electrophoretic mobility, aliquots of each fraction were dotted on to nitrocellulose and analysed by the sialic acid-recognizing lectins SNA and MAA, which are specific for α2-6NeuAc and α2-3NeuAc respectively [34,35], and by HID staining, specific for sulphate-containing mucins [36] (Table 2). PAL-MG1 was

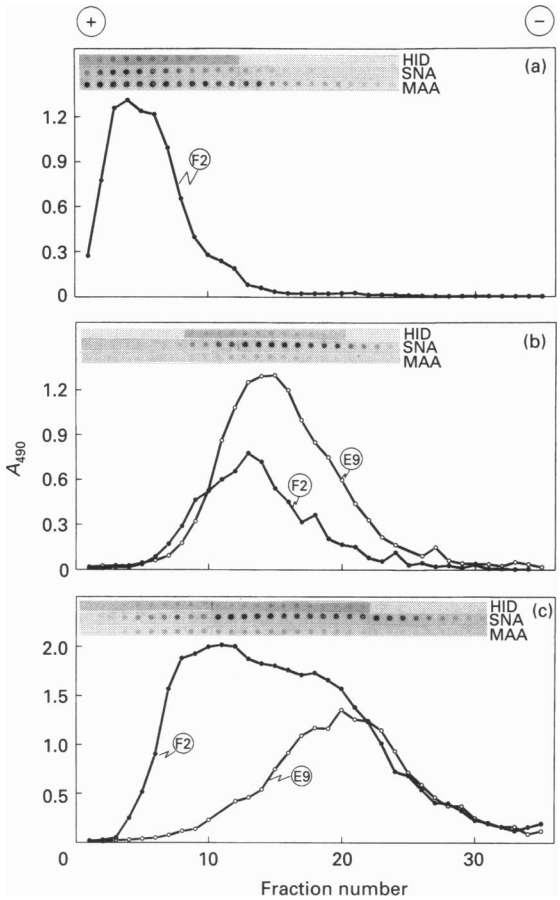

Figure 2 DGE of glandular MG1

DGE mobility profiles of PAL-MG1 (**a**), SM-MG1 (**b**) and SL-MG1 (**c**). The total content of the electrophoresis tube (+, anodic side; −, cathodic side) was fractionated and each fraction was probed with mAbs F2 (●) and E9 (○) as indicated. Above the electrophoretic mobility profiles are shown dot-blots stained with lectins SNA and MAA, specific for α2-6NeuAc and α2-3NeuAc respectively, and HID, specific for sulphate-containing mucins.

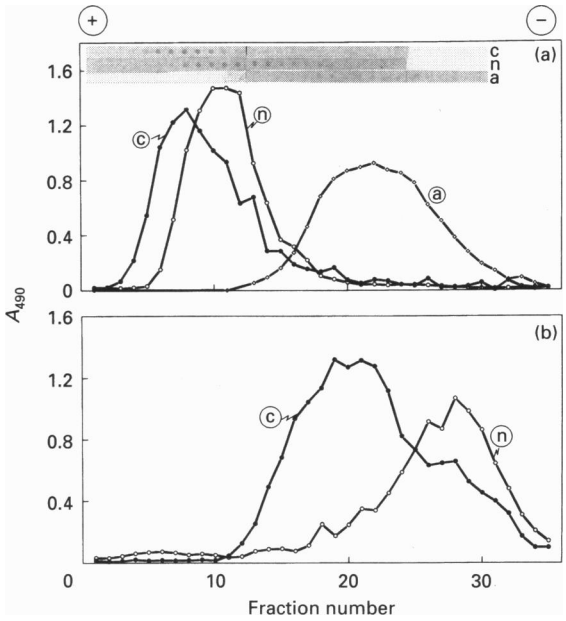

Figure 3 DGE of modified MG1

(**a**) DGE mobility profiles of PAL-MG1 without treatment as control (c: ●) and after treatment with neuraminidase (n: ○) or mild acid (a: ◇). The total content of the electrophoresis tube (+, anodic side; −, cathodic side) was fractionated and each fraction was probed with mAb F2. Above the electrophoretic mobility profiles are shown the corresponding HID-stained dot-blots (c, n, a) specific for sulphate-containing mucins. (**b**) DGE mobility profiles of SM-MG1 before (c: ●) and after neuraminidase (n: ○) treatment, probed with mAb F2. Mild-acid-treated SM-MG1 had a profile similar to that obtained by neuraminidase treatment (not shown).

recognized by both lectins and stained by HID (Figure 2a); thus mucins from this gland contain sulphate and both types of sialic acid residue (α2-3NeuAc and α2-6NeuAc) on their oligosaccharide side chains. For SM-MG1 a very minimal reaction with HID was detected, and SNA (recognizing α2-6NeuAc) bound to a significantly greater extent than MAA (recognizing α2-3NeuAc) (Figure 2b). SL-MG1 appeared to be positive for both HID staining and SNA binding over the complete area in the DGE profile, but only a subpopulation showed MAA affinity (Figure 2c). The subpopulation of SL-MG1 that displayed the E9 epitope was marginally reactive with the MAA lectin, suggesting that the neuraminidase-sensitive E9 epitope contains α2-6NeuAc rather than α2-3NeuAc.

Ion-exchange chromatography

For comparison, the MG1 preparations were also analysed by high-performance ion-exchange chromatography, and each fraction was subsequently monitored by ELISA, as for DGE. Under conditions in which tryptic digestion of reduced mucins results in fairly good separation with high recovery (see also Thornton et al. [30]), less than 1% of the native mucins could be retrieved from the column, even at very high salt concentrations (not shown).

Chemical and enzymic modification of MG1

From the differences in electrophoretic mobility between MG1s from different glands, the question of the origin of the negative charge arose; was it mainly due to the sulphate or sialic acid content? To investigate this, MG1 preparations were treated with mild acid (0.01 M HCl, 80 °C for 1 h), which is known to remove all sialic acid residues from MG1s while having only marginal effect on the other sugar residues. Chemical analysis revealed complete removal of sialic acids [25]. The chemically modified MG1 appeared to retain full reactivity with mAb F2, whereas recognition by mAb E9 was completely lost (results not shown). This was to be expected because of the requirement for the sialic acid residue in the E9 epitope. This chemical treatment also removed a major proportion of the sulphate residues (over 80% as determined by chemical analysis), shown by the low reactivity with HID (Figure 3a). The residual HID staining of mild-acid-treated PAL-MG1 was clearly less than that observed with untreated SM-MG1 (results not shown). These mild-acid-treated MG1 preparations were compared with MG1 samples treated with neuraminidase. The latter treatment removed substantial amounts of sialic acid without having any effect on the sulphate content. Figure 3 shows the mobility on DGE as assayed by ELISA using mAb F2. A marked loss of mobility was produced by mild acid treatment (resulting in asialodesulphomucins) of PAL-MG1, whereas neuraminidase (resulting in asialomucins) had only a moderate effect on mobility (Figure 3a). Clearly, the

sulphate content of PAL-MG1 contributes most to the high electrophoretic mobility. Reducing the negative charge on SM-MG1 by either mild acid treatment or neuraminidase treatment decreased the electrophoretic mobility to the same extent (Figure 3b). Almost the same result was obtained for SL-MG1, except that the differences between the two modifications were only marginal (results not shown). This suggests a major contribution by the sialic acid residues to the electrophoretic mobility of SM-MG1 and SL-MG1. After diminution of negatively charged sialic acid and sulphate residues, PAL-MG1 still displayed a substantially higher electrophoretic mobility than SM-MG1 or SL-MG1. This originates in part from residual sulphate content but other structural or physicochemical properties might also be involved.

DISCUSSION

Immunochemical analysis of salivas from individual glands suggested gland-specific differences in MG1s [25]. Furthermore, the buoyant density of PAL-MG1 differs from that of SM-MG1 and SL-MG1 [26]. In the present paper we have confirmed and expanded the known heterogeneity of MG1 in salivas from different glands by chemical and electrophoretic analysis of isolated MG1 populations (Tables 1 and 2). PAL-MG1 is characterized by a high sulphate content on the oligosaccharide side chains; by mass it is about 5 to 10 times that in SL-MG1 and SM-MG1 respectively. Of the three MG1 species studied, SM-MG1 has the highest percentage of sialic acid. Making use of DGE, human salivary mucins could be differentiated according to their charge. PAL-MG1 appeared to exhibit the highest electrophoretic mobility mainly as the result of its high sulphate content (Figure 3a). With respect to the differences in mass between sulphate and sialic acid, the number of sulphate residues in terms of negative charge of PAL-MG1 is about six times that of sialic acid residues. Although well recognized by SNA and even better by MAA, the sialic acid content seems to make only a minor contribution to the electrophoretic mobility of PAL-MG1 (Figure 2a). Binding by MAA and SNA lectins is a qualitative indication of the presence of α2-3NeuAc and α2-6NeuAc types respectively (see Tables 1 and 2). SM-MG1 contained oligosaccharide side chains with α2-3NeuAc and α2-6NeuAc residues, but very little sulphate (Figure 2b). SL-MG1 populations displayed heterogeneous electrophoretic mobility, all of them expressing sulphate and α2-6NeuAc but only a subpopulation containing α2-3NeuAc (Figure 2c). This divided SL-MG1 into two main groups, specified by reactivity to either α2-3NeuAc-specific lectin MAA or mAb E9, recognizing the α2-6NeuAc-containing epitope. Diminution of the negatively charged residues of the carbohydrate moiety (sialic acid residues and sulphate) did not completely abolish the electrophoretic differences between the mucin preparations. The persisting higher electrophoretic mobility of mild-acid-treated PAL-MG1 can partially be ascribed to the presence of residual sulphate groups. As mild-acid-treated PAL-MG1 contains less sulphate than untreated SM-MG1, this points to underlying structural differences (Figure 3). Thus in addition to differences in immunoreactivity and buoyant density, the salivary-gland MG1s displayed variable amounts of the charged residues sulphate and sialic acid. Even within MG1 populations derived from one and the same glandular secretion, subpopulations were apparent with differences in immunoreactivity as well as electrophoretic mobility (Figure 2c). It is not yet known whether these differences arise from post-translational modification, regulated by glycosyltransferases and sulphotransferases, or emerge from differences in the polypeptide backbone of the MG1 molecules or a combination of both.

The absence of MAA lectin binding to the SL-MG1 subpopulation recognized by mAb E9 suggests that the prerequisite of a sialic acid residue in its epitope [25] can be restricted to an α2-6NeuAc. Moreover, from the absence of mAb E9 reactivity towards PAL-MG1, it became apparent that this sialic acid residue is not by itself the epitope, despite the decent amounts of α2-6NeuAc present (Figure 2a).

The structural differences observed between mucins obtained from the individual salivary glands of a single person point to physiologically different functions. This heterogeneity of salivary mucins allows local variations in the composition of mucous layers covering the various oral surfaces. In particular, the high sulphate content of the palatal mucins leads to various speculations. First, the high viscosity and adhesiveness of palatal saliva, which is necessary for adherence to palatal epithelial cells, might be affected by sulphate, possibly in an analogous way to sulphated Lewis^a antigen-mediated binding to selectins on endothelial cells [37]. Secondly, modifications of sulphomucins have been associated with pathological conditions, such as cystic fibrosis [2–5]. In gastric adenocarcinomas, ulcerative and ischaemic colitis, and Crohn's disease, mucins with a higher percentage of sulphate and less sialic acid have been observed [6]. In addition to sulphate, sialic acid and other carbohydrate signals have been implicated in malignancy of the gastrointestinal and respiratory tract [8–10]. Elucidation of these processes requires an understanding of the biochemical structure of the molecules involved.

The present paper demonstrates the usefulness of the DGE apparatus for analysis of high-M_r mucins. Until now, a charge-dependent analysis of high-M_r mucins was hampered by the size of the molecules and had to be preceded by proteolytic digestion [4,30]. The present method is not destructive and can be used on both a semipreparative and an analytical scale. After fractionation of the total electrophoretic medium, each fraction can be subjected to a variety of analytical methods, and subpopulations can be isolated. After reduction and fractionation of these subpopulations into glycopeptide subunits, high-performance ion-exchange chromatography can be used, for instance for investigating whether mucin subpopulations are composed of unique subunits. It is evident that this method will also be of great value for research into mucins other than those of the human saliva. Thus DGE is a useful additional charge-separation method for structural analysis of high-M_r molecules, such as native mucins, that are not easily analysed by conventional SDS/PAGE or ion-exchange chromatography. Because the latter is successfully used for the separation of reduced mucin subunits [4,30], DGE and ion-exchange chromatography are complementary techniques valuable for mucin research.

We thank Ms. Petra van der Keybus and Ms. Carolien Koeleman, Department of Medical Chemistry, Vrije Universtiteit Amsterdam, for expert assistance with FPLC and for excellent carbohydrate composition analysis respectively.

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Received 2 March 1995; accepted 24 March 1995