Physical characterization of the MUC5AC mucin: a highly oligomeric glycoprotein whether isolated from cell culture or *in vivo* from respiratory mucous secretions

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We have isolated the high- M_r mucins from growth medium of the early stage of an HT-29 cell culture by gel chromatography and isopycnic density gradient centrifugation. The mucins (buoyant density 1.34–1.44 g/ml) were reactive with an anti-peptide antiserum (MAN-5AC1) raised against a sequence from within the MUC5AC mucin. Similar antisera raised against the MUC2 and MUC5B mucins were not reactive. The MUC5AC reduced-mucin subunits exhibited a homogeneous charge distribution on anion-exchange chromatography, but appeared as two bands, one major and one more minor, after agarose gel electrophoresis. The unreduced mucins had an average M_r in excess of 40 MDa and were visualized in the electron microscope as large, fine filamentous threads (many microns in length) that after reduction were greatly reduced in size (number average length 570 nm). Agarose gel electrophoresis of unreduced MUC5AC mucins identified a major band just entering the gel

with evidence of a 'ladder' of faster-migrating minor bands. Partial reduction of the mucins increased the proportion of the faster bands and at least 16 could be discriminated. M_r measurements showed that these bands differed by single monomer units. The mucins behaved as very stiff extended structures in solution and this characteristic might explain the poor separation of different-sized oligomers in sedimentation-rate experiments. The cell-culture mucin preparation had similar characteristics of charge and buoyant density to MUC5AC mucins from respiratory secretions *in vivo*. In addition the MUC5AC mucin from respiratory tract secretions exhibited similar behaviour, reduced and unreduced on agarose gel electrophoresis, indicating that the mucin has a similar molecular phenotype *in vivo* and *in vitro*.

Key words: light scattering, mucus, O-linked glycosylation.

INTRODUCTION

Gel-forming mucins are responsible for the rheological properties of the mucus gels found on the epithelial surfaces of the body. These macromolecules are high- M_r O-linked glycoproteins (5–30 MDa) that have an oligomeric structure stabilized by disulphide bonds [1–5]. Electron microscopy and hydrodynamic studies have shown that the oligomeric mucins from gastric, respiratory and cervical secretions are predominantly linear, apparently flexible threads that behave as random coils in dilute solution [2,6–10]. The mucus from these tissues can be solubilized by non-covalent-bond-breaking solvents, suggesting that entanglement is a key feature in gel formation. Moreover, treatment with reducing agents, which depolymerizes the mucins, disrupts the gel structure more rapidly. The size (i.e. extent of oligomerization) of the mucins will therefore have a critical effect on the nature of the gel.

Nine mucin genes have so far been identified in humans: *MUC1*–*MUC4*, *MUC5AC*, *MUC5B*, *MUC6*, *MUC7* and *MUC8* (for a review see [11]). Four of these genes (*MUC2*, *MUC5AC*, *MUC5B* and *MUC6*) are part of a gene cluster on chromosome 11 [12] and are believed to encode for oligomeric gel-forming mucins. Moreover, their deduced polypeptides share similarities in the N- and C-termini, particularly with respect to conservation of cysteine residues [13–20]. Some of these cysteine residues are

believed to form the intermolecular disulphide bonds by which the mucins oligomerize. Indeed, cysteine residues present within homologous regions of porcine submaxillary mucins have been shown to be involved in the formation of intermolecular disulphide linkages [21–23].

It is increasingly apparent that mucous secretions contain a mixture of different oligomeric mucins and the majority of the biochemical and physicochemical studies reported in the literature have been carried out on mixtures of different MUC gene products. However, some biochemical analyses have been performed on the MUC2, MUC5AC and MUC5B mucins isolated from *in vivo* mucous secretions [24–32]. In particular, the MUC5AC mucin has been shown to be an important component of respiratory tract mucus, and has also been found in gastric and cervical secretions [24–27,30]. In comparison with MUC5B the MUC5AC, mucins isolated from cervical and tracheo-bronchial secretions have lower average sedimentation rates. This was interpreted as indicating that MUC5AC was a smaller, less oligomerized, mucin compared with MUC5B [25,30]. However, in this study we report a detailed characterization of a MUC5AC mucin purified from an HT-29 cell line and show that it is highly oligomerized with an extended conformation in solution. Furthermore, it has similar physical and chemical characteristics to MUC5AC molecules found in respiratory secretions. The physical properties of this mucin would make it an ideal gel-forming molecule.

Abbreviations used: GdmCl, guanidinium chloride; DTT, dithiothreitol; PAS assay, periodate–Schiff's assay.

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MATERIALS AND METHODS

Isolation and purification of the MUC5AC mucin from cell culture

The human intestinal cell line HT-29 was cultured in RPMI 1640 medium with L-glutamine (Gibco-BRL, Paisley, UK) containing 10% foetal calf serum for 5 days until confluence. The medium was collected and diluted with an equal volume of 8 M guanidinium chloride (GdmCl) prior to gel chromatography on Sepharose CL-2B. The column (90 cm × 2.6 cm) was eluted at a flow rate of 24 ml/h with 4 M GdmCl and fractions (8 ml) were analysed for carbohydrate and for reactivity with the MAN-5ACI antiserum (see below). The MUC5AC mucins that were eluted in the void volume of the column (results not shown) were pooled and subjected to isopycnic density gradient centrifugation in CsCl/4 M GdmCl. Centrifugation was performed at a starting density of 1.4 g/ml in a Beckman 70Ti rotor at 93 000 *g* at 15 °C for 69 h and after centrifugation tubes were emptied from the top.

Preparation of reduced mucins

Reduced mucins were prepared as follows. Whole mucins were dialysed into GdmCl or urea reduction buffer (6 M GdmCl or 6 M urea containing 0.1 M Tris/5 mM EDTA, pH 8.0) and treatment with 10 mM dithiothreitol (DTT) for 5 h at 37 °C. Iodoacetamide was added to a final concentration of 25 mM and the mixture left in the dark overnight at room temperature [1]. Alternatively mucins were reduced on nitrocellulose membranes after slot or Western blotting [33]. Briefly, the blotted membrane was washed in distilled water for a few minutes and incubated in GdmCl reduction buffer containing 10 mM DTT at room temperature for 15 min. After removing the DTT solution, the membrane was incubated in the same buffer containing 25 mM iodoacetamide at room temperature for 10 min and then washed twice (5 min) with distilled water.

Time course of reduction of the MUC5AC mucin

Purified mucin samples in urea reduction buffer were treated on ice with 1 mM DTT. At intervals during the reduction reaction aliquots were removed and treated with 25 mM iodoacetamide prior to agarose gel electrophoresis.

Anion-exchange chromatography

Reduced mucins were transferred into 6 M urea/10 mM piperazine, pH 5.0, containing 0.02% CHAPS by chromatography on a Pharmacia PD-10 column prior to separation by anion-exchange chromatography. Reduced mucins were then chromatographed on a Pharmacia Mono Q HR 5/5 column eluted with a linear gradient of 0–0.4 M lithium perchlorate/10 mM piperazine, pH 5.0, in 6 M urea containing 0.02% CHAPS [27].

Agarose gel electrophoresis

Reduced mucin subunits were electrophoresed in 1.0% (w/v) agarose gels in 40 mM Tris/acetate/1 mM EDTA, pH 8.0, containing 0.1% (w/v) SDS as described previously [34]. After electrophoresis, molecules were transferred to nitrocellulose membrane by vacuum blotting in 0.6 M sodium chloride/60 mM sodium citrate (transfer buffer) using a Pharmacia VacuGene XL at a suction pressure of 40 mbar for 2 h prior to detection of mucins using antibodies. For the analysis of the intact and partially reduced molecules 0.7% (w/v) agarose gels were employed. After electrophoresis the gels were washed for 10 min

in transfer buffer at pH 8.0 and then treated with 10 mM DTT for 15 min prior to vacuum transfer [33].

Rate-zonal centrifugation

Samples were layered on to preformed urea gradients (6–8 M) and centrifuged in a Beckman SW40 swing-out rotor at 202 000 *g* for 6 h at 15 °C. After centrifugation the tubes were emptied from the top [33].

Electron microscopy

Electron microscopy was performed as described previously [8]. In short, carbon films were made by evaporation of carbon on to freshly cleaved mica. The films were floated off on water and deposited on to 600-mesh grids. Specimens were prepared by spreading molecules (1 µg/ml) in a benzyldimethylalkylammonium chloride (BAC) monolayer on a hypophase of 50 mM magnesium acetate. The molecules were picked up on carbon-coated grids, which were stained subsequently with uranyl acetate and rotary-shadowed with platinum/tungsten. Molecules were visualized with a Jeol 1200 electron microscope at 120 keV. Contour lengths on photographically enlarged electron micrographs were measured with a Planix digital planimeter (Hall and Watts, London, U.K.).

Determination of mucin size distribution

Partially reduced mucins (approx. 1 s on ice) were chromatographed on a column of Sephacryl S-1000 (1 cm × 30 cm) eluted at a flow rate of 0.15 ml/min with 0.2 M NaCl. The column effluent was monitored with an in-line Dawn DSP laser photometer and a Wyatt 903 interferometric monitor to measure light scattering and sample concentration respectively. Light-scattering data were collected simultaneously at 18 angles between 10° and 151° and the data analysed according to Zimm [35].

Polyclonal antisera

Polyclonal antisera were raised against synthetic peptides coupled to keyhole limpet haemocyanin from specific sequences within the mucins MUC2, MUC5AC and MUC5B. The antisera used were MAN-5ACI [27] and MAN-5BI [28], which have been described previously, and MAN-2I. The MAN-2I antiserum is similar to the antiserum LUM2-3 [24] and was raised against a synthetic peptide NGLQPVRVEDPDGC in the non-tandem repeat of MUC2 towards the C-terminus. The antisera were used at the following dilutions: MAN-2I and MAN-5ACI, 1:10 000; and MAN-5BI, 1:2000.

Analytical methods

Total carbohydrate was determined using a PAS (periodate-Schiff's) assay after slot blotting of mucins on to nitrocellulose [36]. Immunoassays were performed after blotting on to nitrocellulose as described previously [37]. Immuno- and Western blots were visualized using horseradish peroxidase-labelled secondary antibodies in conjunction with an ECL[®] Western detection kit. Band intensities were measured using a Bio-Rad model GS 700 imaging densitometer.

Figure 1 CsCl/4 M GdmCl density gradient centrifugation of high- M_r macromolecules secreted into medium by HT-29 cells in culture

The void-volume fractions from Sepharose CL-2B chromatography of the cell-culture-medium extract were subjected to CsCl/4 M GdmCl density gradient centrifugation. Fractions were analysed for (a) carbohydrate with the PAS assay (●), density (---) and (b) reactivity with the MAN-5ACI antiserum (○). Fractions 7–12 were pooled for further study.

RESULTS

Mucin purification

The mucins secreted into the medium by the HT-29 cell line were isolated by a combination of gel chromatography and isopycnic density gradient centrifugation. The cell-culture medium was diluted with an equal volume of 8 M GdmCl and chromatographed in 4 M GdmCl on a Sepharose CL-2B column. The high- M_r mucins eluted in the void volume and were separated from the smaller proteins and glycoproteins in the medium that were eluted in the included volume of the column (results not shown). The mucins were further purified by CsCl/4 M GdmCl density gradient centrifugation (Figure 1a). A carbohydrate-rich peak was observed with a density in the range 1.34–1.44 g/ml, which is typical for the gel-forming mucins MUC5AC and MUC5B isolated from respiratory mucous secretions *in vivo* [5,25,26,30,32].

Mucin identification

To ascertain the identity of the high- M_r mucins secreted into the cell-culture medium the density gradient fractions were probed with a number of mucin-specific polyclonal antisera. A low level of reactivity was found with antisera recognizing the MUC2 (MAN-2I) and MUC5B (MAN-5BI) mucins (results not shown). In contrast, strong reactivity was observed with the MAN-5ACI antiserum and this coincided with the major carbohydrate peak (Figure 1b). Mucin-containing fractions 7–12 were pooled and used for further study.

Figure 2 Anion-exchange chromatography of reduced-mucin preparations

Mucins isolated from (a) HT-29 cell-culture medium and (b) and (c) respiratory mucous secretions from two individuals were reduced and alkylated and chromatographed on a Mono Q HR5/5 column. Fractions (0.5 ml) were analysed for carbohydrate (●) and reactivity with the MAN-5ACI antiserum (○). The nominal salt gradient is shown (---).

The mucin preparation was reduced and alkylated and analysed by anion-exchange chromatography on Mono Q (Figure 2a). The carbohydrate profile exhibited a major peak between fractions 33 and 40, accounting for approx. 90% of the total PAS response, and minor components at both lower and higher charge densities. The MAN-5ACI antiserum reactivity coincided with the major carbohydrate-containing peak. The anion-exchange profiles of the reduced MUC5AC mucins present in the respiratory secretions from two individuals are shown for comparison (Figures 2b and 2c). In all three cases the reduced MUC5AC mucins are eluted from the Mono Q column with a similar concentration of lithium perchlorate, indicating that the molecules were of a similar charge density.

The reduced, unfractionated cell-culture mucins were also subjected to 1% agarose gel electrophoresis and a Western blot was probed with either the PAS reagent or the MAN-5ACI antiserum (Figure 3). A single PAS-positive band was observed that was also reactive with MAN-5ACI (Figure 3, lanes A and B). However, prolonged exposure of the blot revealed two MAN-5ACI-reactive bands (Figure 3, lane C). The predominant faster-migrating band represents the MUC5AC monomer and

Figure 3 Agarose gel electrophoresis of reduced mucins secreted by HT-29 cells in culture

Reduced mucins were subjected to 1% (w/v) agarose gel electrophoresis and subsequently Western blotted on to nitrocellulose. The blot was analysed for carbohydrate (lane A) and for reactivity with the MAN-5ACI antiserum (lanes B and C). Lane C shows a longer exposure of lane B.

Figure 4 Agarose gel electrophoresis of unreduced MUC5AC mucins secreted by HT-29 cells in culture

Unreduced (lanes A and B) and reduced (lane C) mucins were subjected to 0.7% (w/v) agarose gel electrophoresis and subsequently Western blotted on to nitrocellulose. The blot was analysed for reactivity with the MAN-5ACI antiserum. Twice as much sample was loaded in lane B than in lane A. M, reduced monomer; D, putative dimer.

the more minor band a putative reduction-resistant dimeric or oligomeric species.

The coincidence of the MAN-5ACI antiserum reactivity with the major PAS-containing component in density gradient centrifugation, anion-exchange chromatography and agarose gel electrophoresis indicates that MUC5AC is the predominant high- M_r mucin secreted by the cells in culture. This preparation was then used to examine the physical properties of the MUC5AC mucin.

Physical characterization of the MUC5AC mucin

Unlike the high electrophoretic mobility of the reduced MUC5AC monomer the majority of the intact mucins barely electrophorese into a 0.7% agarose gel (Figure 4). Similar behaviour has recently been reported for the MUC2 mucin [33]. However, it was also possible to discern a ladder-like series of faster-migrating bands reactive with the MAN-5ACI antiserum and the intensity of these bands alternated between strong and weak.

The unreduced mucins were eluted in the void volume of Sephacryl S-1000 (results not shown) and had an average M_r in excess of 40 MDa, assessed by light scattering. Electron microscopy of the intact MUC5AC mucins showed that the majority of the molecules were very long polydisperse filamentous threads, many in excess of 10 μm (Figure 5). The size and coil-like nature of the mucins make accurate measurements of their lengths difficult. However, after reduction much shorter filaments were visualized with lengths of between 400 and 750 nm and a number average length of 570 nm (results not shown). These data indicate the role of disulphide bonds in the integrity of the unreduced mucin.

Treatment of the mucins with DTT for 5 h at 37 °C yielded mainly reduced mucin monomers and a smaller proportion of putative dimer/oligomer as assessed by agarose gel electrophoresis (Figure 3). In contrast, if the mucins were treated with a lower concentration of reducing agent (1 mM) for shorter periods and at low temperature (on ice), the high- M_r mucins were not depolymerized as extensively. For example, after 30 s of partial reduction the monomer and a range of putative oligomers were evident. However, after just 2 min the two major bands observed using more rigorous reduction conditions predominated (Figure 6). The ratio of the monomer to putative dimeric/oligomeric species increased with time and after 3 h was similar to that observed with prolonged reduction at a higher temperature (Figure 3). It should be noted that there is a loss of material with an increasing time of reduction.

A partially reduced MUC5AC preparation (treated as above for approx. 1 s) was chromatographed on a Sephacryl S-1000 column eluted with 0.2 M NaCl (Figure 7). The column effluent was monitored for MAN-5ACI antiserum reactivity (Figure 7a) and in-line by laser light scattering to obtain values of radii of gyration (R_g) and M_r for the mucins across the chromatographic distribution (Figures 7b and 7c). The partially reduced mucin preparation was polydisperse with M_r values in the range of 2–200 MDa (average M_r 20.3 MDa) and R_g values of 65–450 nm (average R_g 140 nm). Agarose gel electrophoresis on fractions across the size distribution reveals a direct correlation between mucin size and electrophoretic mobility (Figure 7d), with the smaller mucins migrating further into the agarose gel. These data suggest that each electrophoretic band represents a different-sized MUC5AC oligomer. Assuming that the difference between bands on the agarose gel is a single monomer unit, we calculated the weight-average molecular masses for the mucins in different column fractions using the agarose electrophoresis data. This was achieved by first determining by densitometry the relative strengths of the different electrophoretic bands in lanes 7, 8 and 9 (Figure 7d). Then, using the measured M_r (2.2 MDa) of the reduced MUC5AC monomer we calculated average M_r values of 3.7, 4.9 and 6.3 MDa for the molecules in the three tracks. These values are in reasonable agreement with the experimentally determined average M_r values of 3.7, 5.8 and 8.4 MDa, ascertained by light scattering. Interpreting the difference between the bands as dimeric units yields molecular masses much higher than the measured values.

Figure 5 Electron microscopy of MUC5AC mucins secreted by HT-29 cells in culture

The unreduced mucins were prepared for electron microscopy as described in the Materials and methods section. Scale bar, 500 nm.

Figure 6 Partial reduction of MUC5AC mucins secreted by HT-29 cells in culture

Mucins were reduced (1 mM DTT on ice), aliquots taken at various times, the reduction was terminated by alkylation, and samples were electrophoresed on a 0.7% (w/v) agarose gel. A Western blot of the gel was probed with the MAN-5A1 antiserum. Each lane represents a different time point (in min). M, reduced monomer.

The relationship between size and shape for molecules in solution can be expressed by the Mark–Houwink parameter α , which is obtained from the relationship $R_g = AM_r^\alpha$, where A is

a constant relating to the mass per unit length of the molecule. The parameter (α) can be used as an index for the stiffness of the molecules. For example, α would equal 1 for rod-like molecules, 0.5–0.55 for random coils and 0.33 for compact spheres. A plot of $\log R_g$ against $\log M_r$ across the included fraction of the chromatographic distribution (Figure 8; elution volume 12–16 ml) yields a value for α of 0.69, which indicates that the MUC5AC mucin behaves as a very stiff coil in solution. This finding might explain the previously observed slow sedimentation rate of the MUC5AC mucin from secretions *in vivo* [25,30]. One might predict that the highly extended nature of the mucin would give rise to a poor separation of different-sized oligomers on the basis of sedimentation rate. This was confirmed by subjecting partially reduced mucins (1 min reduction at low temperature) to rate-zonal centrifugation (Figure 9, top panel). Agarose gel electrophoresis on fractions across the resultant distribution showed that there is very little discrimination in sedimentation rate between monomers and larger oligomers (Figure 9, bottom panel).

We have shown that the buoyant density, charge density and sedimentation properties of the MUC5AC mucin from cell culture are similar to its counterpart from respiratory secretions *in vivo*. However these findings do not tell us if their macromolecular organizations are similar. To address this we subjected a respiratory mucin preparation to partial reduction as described above. Agarose gel electrophoresis (Figure 10) revealed a series of bands with comparable electrophoretic mobilities to those observed with the MUC5AC mucins secreted by HT-29 cells in culture. The major difference was that the bands from the

Figure 7 Sephacryl S-1000 gel chromatography of partially reduced MUC5AC mucins secreted by HT-29 cells in culture

The partially reduced mucins (treated with 1 mM DTT for 1 s on ice) were chromatographed on a Sephacryl S-1000 column and the effluent monitored for light scattering and refractive index (see the Materials and methods section). (a) Fractions were analysed for reactivity with the MAN-5ACI antiserum. V_0 and V_t indicate void and totally included volumes of the column, respectively. Also shown are the calculated values for (b) R_g and (c) M_r across the distribution. (d) Agarose gel electrophoresis was performed on aliquots of fractions across the chromatogram (elution volume 10–18 ml, lanes 1–9 respectively) and a Western blot was probed with the MAN-5ACI antiserum. Lane 10 shows the reduced monomer (M).

Figure 8 Relationship between the size and shape of the mucins

The partially reduced mucins were chromatographed on Sephacryl S-1000 and the column effluent was monitored for light scattering and refractive index as described in the Materials and methods section. The data used in this analysis are for the part of the distribution included on the column, i.e. molecules eluted between 12 and 16 ml (see Figure 7). The slope (α) of the log/log plot of the dependence of M_r and R_g yields a value of 0.69.

Figure 9 Rate-zonal centrifugation of partially reduced MUC5AC mucins secreted by HT-29 cells in culture

Partially reduced mucins (1 mM DTT for 1 min on ice) were centrifuged on a 6–8 M urea gradient and fractions were analysed (top panel) for reactivity with the MAN-5ACI antiserum. (Bottom panel) Agarose gel electrophoresis was performed on an aliquot from each fraction and a Western blot probed with the MAN-5ACI antiserum (fractions 4–11 gave a response). The arrow indicates the origin of electrophoresis.

Figure 10 Partial reduction of MUC5AC mucins present in a human respiratory mucous secretion

Mucins were reduced (1 mM DTT on ice) and aliquots were taken at various times, the reduction was terminated by alkylation and samples were electrophoresed on a 0.7% (w/v) agarose gel. A Western blot of the gel was probed with the MAN-5ACI antiserum. Each lane represents a different time point (in min). M, reduced monomer.

secretion *in vivo* were more diffuse (Figure 10). Again a loss of material was apparent with increasing time of reduction.

DISCUSSION

The MUC5AC mucin has been demonstrated to be one of a mixture of mucins present in the mucous secretions from cervix, stomach and the airways [24–27,30]. To date there have been no reports of the physical characterization of the intact mucin because so far it has not been possible to isolate it in pure form from secretions *in vivo*. However, we have previously purified the reduced MUC5AC subunit from respiratory secretions and measured its M_r (2.2 MDa) and size (R_g 57 nm) [27]. In the present study we have shown that the MUC5AC glycoprotein was the predominant high- M_r mucin secreted into the cell culture medium from an HT-29 cell line. These mucins exhibit similar characteristics in mass, charge and buoyant density to MUC5AC mucins found in respiratory and cervical secretions *in vivo* [25,30]. Furthermore, the mucins are large oligomers, which after treatment with reducing agents yield smaller monomeric units (subunits), as shown previously for this mucin from mucous secretions *in vivo* [25,27,30]. These data indicate that the MUC5AC mucins secreted from this cell line therefore provide a valid model for study *in vitro* of the physical properties of this mucin and in the future will provide a useful tool to investigate their biosynthesis, oligomerization and secretion.

Electron microscopy of the MUC5AC mucins indicates that they are very long linear flexible threads with many of the molecules in excess of 10 μ m in length. In contrast, the reduced mucin monomers are much smaller (approx. 570 nm), suggesting that the unreduced mucins contain at least 18 monomers. The large size of the mucins observed in the electron microscope is consistent with their behaviour in agarose gel electrophoresis where the unreduced MUC5AC mucins barely entered the gel. Furthermore the average M_r of the unreduced mucins (> 40 MDa) confirms their high degree of oligomerization. The gel chromatographic analysis yielded information on the conformation of the mucins in solution. The value for the shape-dependent Mark–Houwink parameter ($\alpha = 0.69$) suggests the

MUC5AC mucin adopts a rather stiff coil conformation in solution. This value is higher than that determined for other gel-forming mucin preparations [6,38,39], but it should be noted that these other studies were performed on mixtures of mucins, containing MUC5AC, MUC5B and possibly other mucins.

Previous studies on the size and mass distribution of MUC5AC mucins from cervical and respiratory tract secretions using rate-zonal centrifugation indicated that this glycoprotein had a lower average sedimentation rate than MUC5B mucins [25,30]. Indeed the MUC5AC mucin preparation studied here exhibits similar sedimentation behaviour to the MUC5AC mucins present *in vivo* (results not shown). In these earlier studies this finding was assumed to indicate that MUC5AC had a lower average apparent M_r than the MUC5B mucins. However, the data presented here indicate that this is not the case and the stiff nature of the MUC5AC mucins may explain the discrepancy in their apparent M_r values determined from sedimentation studies, since for rod-like molecules an increase in M_r is counterbalanced by an increase in frictional coefficient, resulting in little change in sedimentation rate with an increase in mass/size. This has been demonstrated clearly here for MUC5AC.

Agarose gel electrophoresis of the unreduced MUC5AC mucins identified that a small proportion of the mucins gave rise to a ladder-like series of bands. Gel chromatography combined with electrophoresis of partially reduced mucins suggested that these bands represented different-sized oligomers differing by single monomer units. It was possible to discern approx. 16 bands after electrophoresis on a 0.7% agarose gel; oligomers beyond this length are unresolved and migrate as a single band. The MUC5AC mucins isolated from both cell-culture medium and respiratory mucous secretions exhibit a similar series of bands after partial reduction. However, the mucins from cell culture give rise to sharper, less diffuse, bands. The similar banding pattern for the mucins from the two different sources confirms the finding from anion-exchange chromatography of the reduced mucins that the molecules have similar charge density. In contrast with the limited electrophoretic mobility of the majority of the unreduced mucins, that of the reduced mucin is much greater and two bands were observed, a major species and a slower-migrating more minor species. The major band has a similar electrophoretic migration to the reduced monomer prepared from respiratory secretions [5,27]. In contrast, the minor band would appear to represent a reduction-insensitive dimer; such species have been reported for the MUC2 mucin [31,40].

Currently it is thought that mucins oligomerize via the agency of disulphide bonds, firstly at their C-termini to form dimers and then via their N-termini to form further multimers, presumably containing an even number of monomer units. The agarose gel electrophoresis of the unreduced mucin highlights the presence of oligomers containing an odd number of monomers, which is in contradiction with the current model. The intensity of the ladder-like bands observed after electrophoresis alternates with those representing putative even-numbered oligomers being stronger than the odd ones, suggesting that the former species are more abundant. Presently we have no explanation for these observations.

In conclusion we have demonstrated that the MUC5AC mucin, whether isolated from the medium of a cell culture or from a respiratory secretion *in vivo*, has very similar characteristics and can be highly oligomerized, containing in excess of 20 monomer units. The great physical size of MUC5AC mucins strongly confirms their suitability as gel-forming molecules.

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