Glycoconjugate Journal (1996) **13:** 839–847

MUC5AC, but not MUC2, is a prominent mucin in respiratory secretions

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Received 6 November 1995, revised 18 December 1995

Airway mucus was collected from healthy and chronic bronchitic subjects. The chronic bronchitic sputum was separated into gel and sol phase by centrifugation and mucins were isolated using isopycnic density-gradient centrifugation in CsCl. The presence of the MUC5AC and MUC2 mucins was investigated with antisera raised against synthetic peptides with sequences from the respective apoproteins. The gel and sol phase of chronic bronchitic sputum as well as healthy respiratory secretions were shown to contain MUC5AC whereas the MUC2 mucin could not be detected. Rate-zonal centrifugation showed that the MUC5AC mucin was large, polydisperse in size and that reduction yielded subunits. Ion-exchange HPLC revealed the presence of two subunit populations in all secretions, the MUC5AC subunits always being the more acidic. MUC5AC is thus the first large, subunit-based, gel-forming respiratory mucin identified and this glycoprotein is biochemically distinct from at least one other population of large, gel-forming mucins also composed of subunits but lacking a genetic identity.

Keywords: mucin, trachea, secretion, MUC5AC, MUC2

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CF, cystic fibrosis; DFP, di-isopropylphosphofluoridate; DTT, dithiothreitol; EDTA, ethylenedinitrilotetraacetic acid; NEM, *N*-ethylmaleimide; PAS, periodic acid/Schiffs; PMSF, phenylmethylsulphonyl fluoride; Tris, Tris(hydroxymethyl)aminomethane; VNTR, variable number of tandem repeats.

Introduction

The mucociliary transport system is the mechanism by which the normal airways are kept free of inhaled particles and potential pathogens. In hypersecretory diseases such as chronic bronchitis and CF, over-production of mucus causes this mechanism to break down leading to colonization of the airways by pathogenic bacteria and eventually to tissue damage. The large mucus-forming mucins are produced by at least two populations of secretory cells in the airways, the goblet cells in the surface epithelium and the mucous cells in the submucosal glands. These glycoproteins have a molecular mass between 10–30 MDa and are composed of subunits linked end-to-end by disulfide bonds [1–3]. The subunits contain alternating regions of heavy and light substitution with O-linked glycans. The proteinase-resistant highly glycosylated regions are rich in serine, threonine and proline and are flanked by sequences sensitive to proteolytic cleavage [4]. Recent studies have indicated the presence of at least two distinct populations of mucins in tracheobronchial secretions [5]. The two mucins differ in their amino acid compositions and thus appear to have different apoproteins.

Several mucin genes have been cloned and fully or partially sequenced suggesting, so far, the existence of nine different human mucin apoproteins most of which contain characteristic long stretches of tandemly repeated motifs rich in serine, threonine and proline flanked by cysteine-rich regions. The complete amino acid sequences for MUC1 [6], MUC2 [7] and MUC7 [8] have been reported whereas only partial sequences are known for MUC3 [9], MUC4 [10], MUC5AC [11–13], MUC5B

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[14], MUC6 [15] and MUC8 [16]. For a review, see [17]. The *MUC4*, *MUC5AC* and *MUC5B* genes were cloned from tracheobronchial cDNA libraries [10, 18, 14] and *in situ* hybridization and Northern blot analysis have suggested that these genes are expressed in the airways [19]. Several workers have shown that *MUC2* is expressed in nasal polyps [20] and in the airways of patients with cystic fibrosis [21] as well as chronic bronchitis [22]. In rats, the expression of *MUC2* increases in response to exposure to SO_2 [23] suggesting that this mucin is synthesized in response to irritation but the mature MUC2 mucin has not been identified in respiratory secretions. Likewise, no studies have given the gel-forming, subunit-based respiratory mucins a genetic identity, although a preliminary study has suggested that MUC5AC is one candidate [24]. The aim of this investigation is to determine whether MUC2 and/or MUC5AC are present as large, subunit-based glycoproteins in airway secretions from individuals with healthy airways and patients with chronic bronchitis.

Materials and methods

Materials

Guanidinium chloride (practical grade), DFP and CHAPS were from Fluka. Stock solutions (approximately 8 M) of guanidinium chloride were treated with charcoal and filtered through a PM 10 filter (Amicon) before use whereas DFP was stored as a 100 mM solution in propan-1-ol at −20 °C. DTT was bought from Merck, NEM from BDH Chemicals and iodoacetamide from Sigma. The Mono Q HR 5/5 column was obtained from Pharmacia. All other reagents were of A. R. or equivalent quality.

Preparation of polyclonal antisera and ELISA

Synthetic peptides with the sequence RNQDQQGPFKMC present in the C-terminal region [12, 13] and in both stretches of 'unique' sequence flanking a tandem repeat region [11] of the *MUC5AC* gene and the sequence NGLQPVRVEDPDGC present on the C-terminal side of the large tandem repeat region of the *MUC2* gene were conjugated with keyhole limpet haemocyanin and used to prepare polyclonal sera in rabbits, referred to as LUM5-1 and LUM2-3 respectively. Initially, rabbits were injected intracutaneously with 370 µg of peptide mixed with Freund's complete adjuvant. A booster dose of 250 µg of peptide mixed with Freund's incomplete adjuvant was given after 3 weeks and the animals were bled after a further 2–3 weeks. Samples were coated overnight onto ELISA-plates (Falcon) after appropriate dilution in 6 M guanidinium chloride (isopycnic and rate-zonal gradients) or undiluted (ion-exchange HPLC) and antibody reactivity was measured using an alkaline phosphatase-conjugated secondary antibody with nitrophenylphosphate as a substrate. The antisera were used without any prior preadsorption steps at a dilution of 1:2000 and 1:1000 for LUM5-1 and LUM2-3, respectively. All samples studied using the LUM2-3 antiserum were subjected to reduction by the addition of 6 M guanidinium chloride, 10 mM Tris-HCl buffer, pH 8.0 containing 10 mM DTT for 5 h at 37 °C before analysis.

Collection of normal respiratory tract secretions and chronic bronchitic sputa

Normal respiratory tract secretions were collected as described previously [1]. The donors were three healthy children undergoing minor dental surgery for which they required tracheal intubation but no premedication with antimuscarinic drugs. All children were non-smokers and free of active respiratory tract disease. Secretions were aspirated from the tracheal surface and subsequently washed out with ice-cold 6 M guanidinium chloride, 5 mM Na_2EDTA, 10 mM sodium phosphate buffer, pH 6.5 (extraction buffer) containing 5 mM NEM and 0.1 mM PMSF and stored at 4 °C.

Sputum from two chronic bronchitic patients was collected and frozen at −20 °C as described previously [3]. Samples were thawed in the presence of an equal volume of ice-cold 0.2 M sodium phosphate buffer, pH 7.4 containing 10 mM Na_2EDTA, 10 mM NEM and 2 mM DFP. Samples were then subjected to centrifugation in a Beckman L70 Optima ultracentrifuge (Beckman 50.2Ti rotor, 36 000 rpm, 4 °C, 45 min) to separate the gel from the sol phase. Extraction buffer (50 ml), containing 5 mM NEM and 0.1 mM DFP was then added to the gel phase material and the sample stirred overnight in the cold. The extracts were centrifuged as above, the supernatants retained and the pellet re-extracted until four supernatants were obtained. For one sample, the supernatants were pooled, while in the other, mucins were isolated from the individual four supernatants. The remaining pellet was finally solubilized by reduction in 6 M guanidinium chloride, 5 mM Na_2EDTA, 10 mM DTT, Tris-HCl buffer, pH 8.0, 5 h, 37 °C and alkylated by the addition of iodoacetamide (2.5 molar excess over dithiothreitol) for 15 h in the dark. The sample was then centrifuged in a Beckman J21 centrifuge (Beckman JA20 rotor, 18 000 rpm, 4 °C, 45 min) and the supernatant retained.

Purification of mucins and mucin subunits

Mucins were isolated using isopycnic density-gradient centrifugation in CsCl/4 M guanidinium chloride in a Beckman L70 Optima centrifuge (normal secretions; Beckman 70.1 Ti rotor, 40 000 rpm, 15 °C, 80 h, initial density 1.40 g ml^{-1}, gel and sol phases from chronic bronchitic sputum: Beckman 50.2 Ti rotor, 36 000 rpm, 15 °C, 80 h, initial density 1.39 g ml^{-1}). Mucin-containing fractions were pooled (Fig. 1a, c and e) and subjected to a

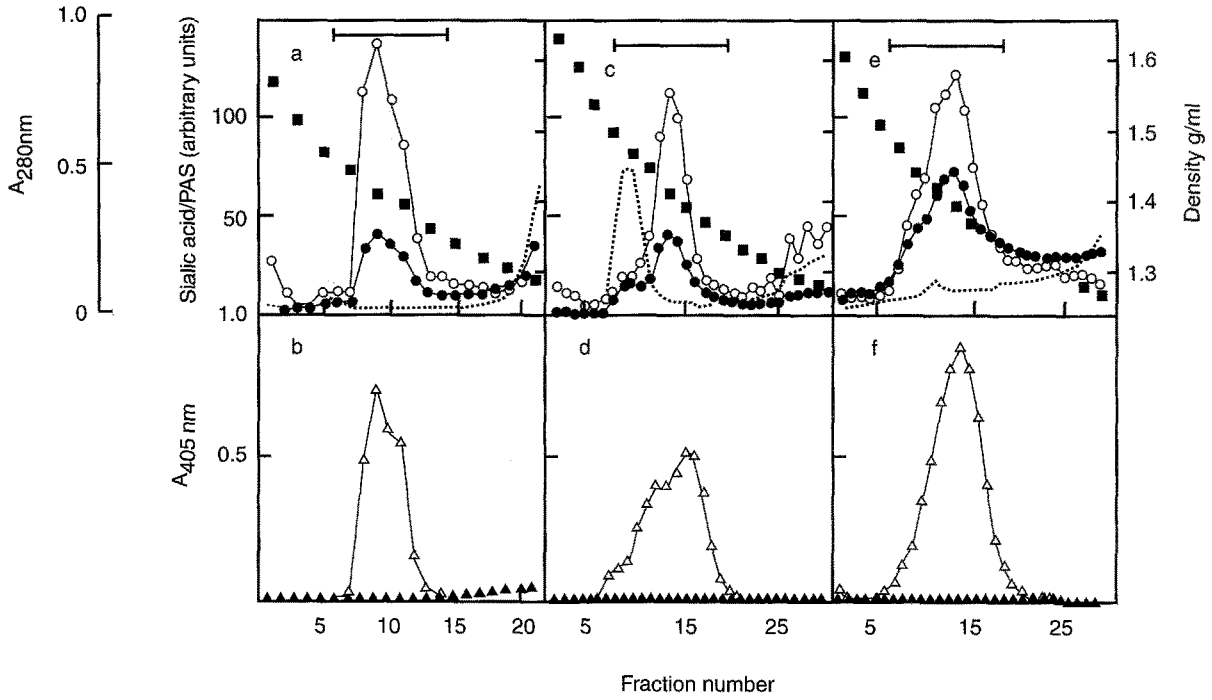

Figure 1. Isopycnic density-gradient centrifugation in CsCl/4 M guanidinium chloride of the respiratory tract secretions from a healthy individual (a, b), the gel phase (c, d), and the sol phase (e, f) of the sputum from an individual with chronic bronchitis. (a, b) Secretions from healthy individuals were extracted with 6 M guanidinium chloride/10 mM sodium phosphate buffer, pH 6.5 containing protease inhibitors and subjected to density-gradient centrifugation in a Beckman L-70 Optima centrifuge (40 000 rpm, 15 °C, 80 h, Beckman 70.1Ti rotor, initial density 1.40 g ml^{-1}). (c–f) Sputum from patients with chronic bronchitis was separated into a gel and a sol phase by centrifugation. The gel (c, d) was solubilized using 6 M guanidinium chloride/10 mM sodium phosphate buffer, pH 6.5 containing protease inhibitors and the sol phase (e, f) was dialysed against this solvent. The samples were then subjected to density-gradient centrifugation in a Beckman L-70 Optima centrifuge (36 000 rpm, 15 °C, 80 h, Beckman 50.2Ti rotor, initial density 1.39 g ml^{-1}). Fractions were analysed for density (■), absorbance at 280 nm (- - -), sialic acid (●), PAS reactivity (○), (a, c, e) and reactivity with the LUM2-3 (▲) and LUM5-1 (△) antisera, (b, d, f). Fractions were pooled according to the horizontal bars.

second isopycnic density-gradient step (as above) in CsCl/0.5 M guanidinium chloride (normal secretions: initial density 1.52 g ml^{-1}; gel and sol phases from chronic bronchitic sputum: initial density 1.50 g ml^{-1}). All solvents contained 5 mM Na_2EDTA and 10 mM sodium phosphate buffer, pH 7.4. In addition, the 0.5 M guanidinium chloride gradients contained 0.01% CHAPS.

Subunits were prepared from the cognate mucins by reduction of disulfide bonds in 6 M guanidinium chloride, 5 mM Na_2EDTA, 10 mM dithiothreitol, 10 mM Tris/HCl buffer, pH 8.0 for 5 h at 37 °C and subsequent alkylation was performed with iodoacetamide (2.5 molar excess over dithiothreitol) for 15 h at room temperature in the dark.

Isolation of human colonic mucin subunits

Human colonic subunits were prepared as described by Carlstedt *et al.* [25]. Briefly, tissue was scraped with a glass microscope slide to remove the surface epithelium and the adherent mucus layer. After the addition of DFP (final concentration: 1 mM), ice-cold extraction buffer was added and the sample was stirred overnight in the cold. The sample was then centrifuged in a Beckman J21 centrifuge (Beckman JA20 rotor, 18 000 rpm, 4 °C, 45 min) and the pellet re-extracted three times as above. 6 M guanidinium chloride, 5 mM Na_2EDTA, 10 mM DTT, 10 mM Tris-HCl buffer, pH 8.0 was then added to the 'insoluble' pellet and incubated for 5 h at 37 °C followed by iodoacetamide (2.5 molar excess over DTT) for 15 h at room temperature in the dark. The solubilized material was subjected to density-gradient centrifugation in CsCl/4 M guanidinium chloride and the subunits isolated as a sharp band between the densities of 1.37 and 1.44 g ml^{-1} (data not shown).

Rate-zonal centrifugation

Rate-zonal centrifugation was carried out according to Sheehan and Carlstedt [26]. Briefly, gradients of 6–8 M guanidinium chloride were prepared using a Hoeffer gradient maker and an LKB 2232 microperpex pump at

a flow of 40 ml h^{-1} in 14 ml centrifuge tubes. Solutions (100–200 µl) of whole mucins or mucin subunits were layered on top of the gradients and the tubes were spun in a Beckman L-70 Optima centrifuge (Beckman SW 41 Ti rotor for 2 h 45 min at 40 000 rpm, 20 °C). Fractions (500 µl) were collected from the top of the tubes and analysed for antibody reactivity and carbohydrate using the PAS method.

Ion-exchange HPLC of reduced subunits

Ion-exchange HPLC was performed on a Mono Q HR 5/5 column as described by Davies *et al.* [3]. The column was eluted with 6 M urea, 10 mM-piperazine/perchlorate buffer, pH 5.0 containing 0.1% CHAPS at a flow rate of 0.5 ml min^{-1} for 10 min followed by a linear gradient over 60 min to a final concentration of 0.4M-$LiClO_4$ in starting buffer. Fractions (0.5 ml) were collected and analysed for carbohydrate and antibody reactivity.

Analytical methods

Densities were measured using a Carlsberg pipette as a pycnometer. Sialic acid was measured with an automated version [27] of the original method [28]. Carbohydrate was also determined by slot-blotting aliquots of fractions onto nitro-cellulose membranes and staining with PAS [29]. Staining was evaluated using a scanning densitometer (Hoeffer) in the transmittance mode and aliquots were within the linear range of response as verified by comparison with a known amount of a standard respiratory mucin.

Results

Purification of mucins

In respiratory tract secretions from three healthy individuals, the major mucin population, as shown by analyses for sialic acid and PAS reactivity, occurred at a buoyant density of approximately 1.41 g ml^{-1} in CsCl/4 M guanidinium chloride and a representative example is shown in Fig. 1a. In addition, some sialic acid and carbohydrate-containing material with a high absorbance at 280 nm was found at the top of the gradient. These findings are similar to those obtained previously for secretions from healthy individuals [1, 3]. Reactivity with the LUM5-1 antiserum coincided with the mucin peak (Fig. 1b). The LUM2-3 antiserum did not react with the main mucin band in any of the samples but some reactivity was found with the low-density material at the top of the gradient (Fig. 1b).

Chronic bronchitic sputum from two individuals was centrifuged to separate the gel from the sol phase and the gel phase was extracted several times with 6 M guanidinium chloride buffer. In one of the samples, the extracts were pooled whereas in the other, each extract was treated individually. In both samples, the mucins were similar in appearance to those from normal secretions in CsCl/4 M guanidinium chloride (Fig. 1c). The main mucin population had a buoyant density of 1.41 g ml^{-1}, similar to that seen previously for mucins from chronic bronchitic sputum [3]. The mucins were partially separated from a peak with a high absorbance at 280 nm, at a density expected for DNA [30]. Reactivity with the LUM5-1 antiserum was associated with the main mucin band and the distribution appeared to be partially separated into two populations (Fig. 1d). No reactivity with the LUM2-3 antiserum could be detected. In the sample where the gel extracts were treated separately, 72% of the material was found in the first extract and this material was similar in appearance to that of the chronic bronchitic sample shown above (results not shown). The 'insoluble' residue, remaining after the guanidinium chloride extractions, was solubilized by reduction/alkylation and, in both samples, comprised 1–2% of the total. In CsCl/4 M guanidinium chloride, these reduced/alkylated mucin subunits occurred as a unimodal peak at a buoyant density of 1.41 g ml^{-1} and reacted with the LUM5-1 but not the LUM2-3 antiserum (data not shown). The mucins in the sol phase of the chronic bronchitic sputum comprised approximately 20% of the total in each sample. Mucins from both individuals occurred as a broad distribution between 1.32 g ml^{-1} and 1.52 g ml^{-1} (Fig. 1e). Reactivity with the LUM5-1 antiserum was found over the main mucin band but there was no reactivity with the LUM2-3 antiserum (Fig. 1f).

The major mucin populations from the CsCl/4 M guanidinium chloride gradients were pooled and centrifuged in CsCl/0.5 M guanidinium chloride. In both the normal (Fig. 2a) and chronic bronchitic gel secretions (Fig. 2c), the mucins appeared as a unimodal peak between the buoyant densities of 1.42–1.49 g ml^{-1}, well separated from DNA. The reactivity with the LUM5-1 antiserum coincided with the analyses for sialic acid and carbohydrate (Fig. 2b and d). Secretions from the sol phase contained a major mucin population with a buoyant density between 1.40 and 1.50 g ml^{-1} (Fig. 2e). In addition, there was a shoulder on the high-density side of the distribution, as shown by analysis for sialic acid and PAS reactivity. The LUM5-1 antiserum reacted mainly with the major mucin population, and there was relatively less reactivity over the high-density shoulder (Fig. 2f). No reactivity with any of the samples was seen with the LUM2-3 antiserum.

Characterization of the LUM5-1 and LUM2-3 antisera

Mucins from the gel phase of chronic bronchitic sputum and normal secretions after density-gradient centrifugation in CsCl/0.5 M guanidinium chloride, as well as mucin subunits from the insoluble mucin complex from human colon, were used to characterize the LUM5-1 and LUM2-

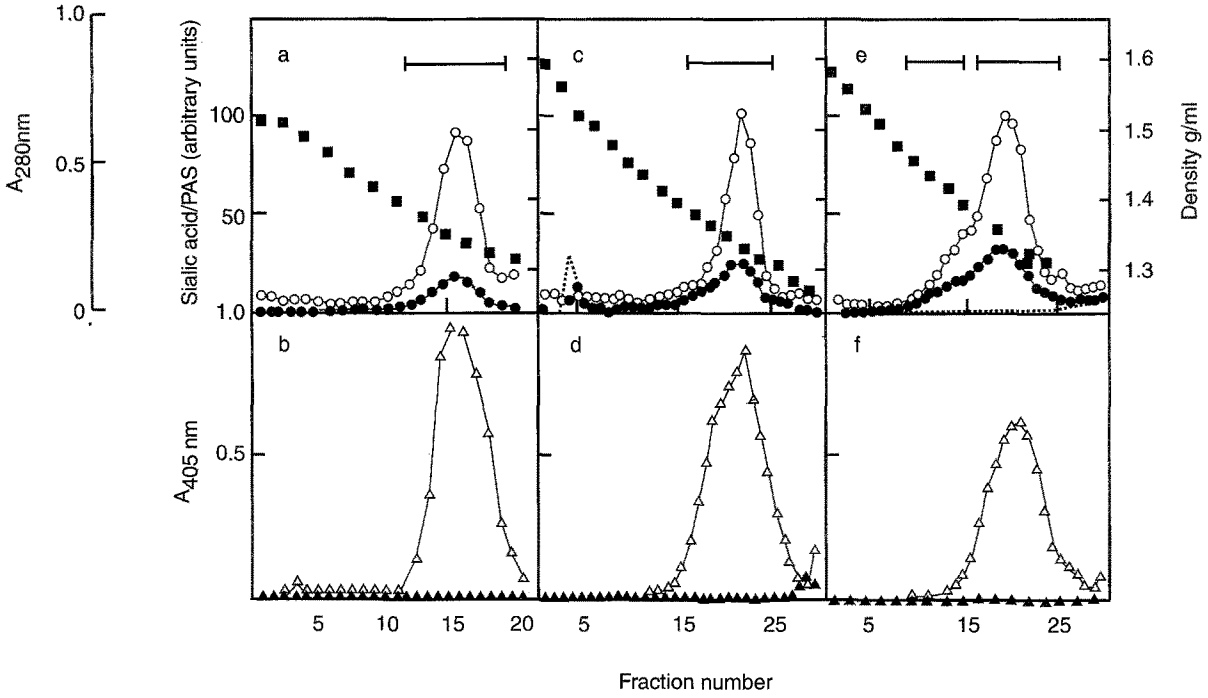

Figure 2. Isopycnic density-gradient centrifugation in CsCl/0.5 M guanidinium chloride of mucins from normal respiratory tract secretions (a, b), the gel phase (c, d) and the sol phase (e, f) of sputum from an individual with chronic bronchitis. Mucin samples pooled as shown by the horizontal bars in Fig. 1 were subjected to density-gradient centrifugation in 0.5 M guanidinium chloride/CsCl containing 0.01% CHAPS in a Beckman L-70 Optima centrifuge (40 000 rpm, 15 °C, 80 h, Beckman 70.1Ti rotor, starting density 1.52 g ml^{-1} (a) and 36 000 rpm, 15 °C, 80 h, Beckman 50.2Ti rotor, initial density 1.50 g ml^{-1} (c and e)). Fractions were analysed for density (■), absorbance at 280 nm (---), sialic acid (●), PAS reactivity (○) (a, c, e) and reactivity with the LUM2-3 (▲) and LUM5-1 (△) antisera (b, d, f). Fractions were pooled according to the horizontal bars.

3 antisera. The respiratory tract mucins reacted in a concentration-dependent manner with the LUM5-1 antiserum (Fig. 3a) but showed no reactivity with the LUM2-3 antiserum (Fig. 3b). In contrast, subunits from human colon reacted in a concentration-dependent manner with the LUM2-3 antiserum (Fig. 3b) but did not react with the LUM5-1 antiserum (Fig. 3a).

Size distribution of whole mucins and reduced subunits

Whole mucins and reduced subunits were subjected to rate-zonal centrifugation in order to investigate the size of the macromolecules. Normal respiratory secretions contained mucins with a broad range of molecular sizes and the LUM5-1 antiserum reacted over the entire distribution as well as with a small amount of glycoproteins voided at the bottom of the tube (Fig. 4a). Subunits were much smaller as shown by both PAS and MUC5AC reactivity (Fig. 4b). Mucins from the gel phase of chronic bronchitic sputum were similar in appearance to those from normal secretions (Fig. 4c). Subunits were, again, smaller than the cognate mucins and reacted with the LUM5-1 antiserum (Fig. 4d). Mucins from the 'low-density' population from the sol phase of chronic bronchitic sputum were smaller than those from the gel and the LUM5-1 antiserum reacted over the entire distribution (Fig. 4e). Subunits were similar in size to those from the gel (Fig. 4f).

Ion-exchange HPLC of reduced subunits

Subunits from the various mucin preparations were subjected to ion-exchange HPLC in order to study the charge density of the glycoproteins. In two samples of normal secretions, PAS reactivity revealed the presence of two major partially separated populations (Fig. 5a, b). The more acidic one reacted with the LUM5-1 antiserum whereas the earlier-eluting component showed no reactivity. In the third sample, the predominant population reacted with the LUM5-1 antiserum and there was very little of the earlier eluting population (Fig. 5c). The gel phase samples from chronic bronchitic sputum also contained two populations of reduced subunits (Fig. 5d, e), similar in appearance to those from the normal secretions. The low-density population from the density-gradient of the sol phase mucins contained only the late-eluting component which reacted with the LUM5-1 antiserum (Fig. 5f). Subunits from the high density shoulder in the sol showed a similar elution pattern but the reactivity with the LUM5-1 antiserum relative to PAS was much lower (results not shown).

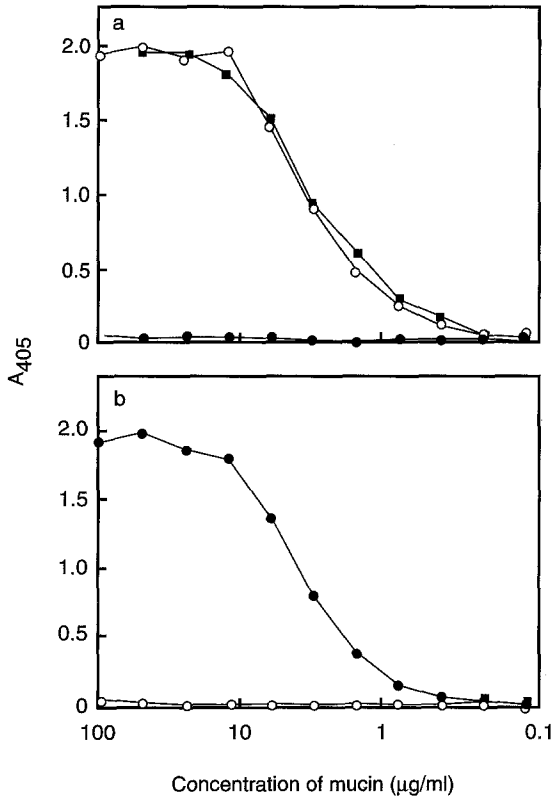

Figure 3. Reactivity with the MUC5AC antiserum of normal and chronic bronchitic gel phase mucins and reduced subunits from the 'insoluble' mucin complex from human colon (a) and reactivity with the MUC2 antiserum of reduced subunits from normal and chronic bronchitic gel phase mucins and reduced subunits from the 'insoluble' mucin complex from human colon (b). Mucins from normal secretions (■), the gel phase of chronic bronchitic sputum (○) and reduced subunits from the 'insoluble' mucin complex from human colon (●) were coated onto microtitre plates and tested for reactivity against the LUM5-1 and LUM2-3 antisera with ELISA. Absorbance at 405 nm was plotted against log mucin concentration. (Filled squares are masked by the open circles in (b))

Discussion

MUC1, *MUC2*, *MUC4*, *MUC5AC* and *MUC5B* are all expressed in the airways but no studies have established which are the large, subunit-based, gel-forming mucins in airway secretions. MUC1 is a monomeric glycoprotein associated with the cell membrane [31] whereas the much larger MUC2 mucin is present in the 'insoluble' glycoprotein complex in human colon [32, 33]. Since only partial sequences are available for the remaining three, it is not possible to predict whether these genes code for any of the large, gel-forming mucins present in respiratory secretions. Here, antibodies against synthetic peptides with sequences present in the non-VNTR regions of the MUC5AC and MUC2 apoproteins were raised and used as tools to detect and characterize the cognate mature mucins in respiratory secretions without prior deglycosylation.

Density-gradient centrifugation revealed the presence of MUC5AC mucins both in secretions from normal individuals and the gel phase, sol phase and extraction residues from chronic bronchitic sputum. In the gel phase, the distribution of MUC5AC was partially separated into two populations while the sol phase, in addition to the major MUC5AC-reactive population, contained a shoulder of 'high-density' mucins with less MUC5AC reactivity. The buoyant densities found here are in good agreement with those obtained previously for mucins from non-diseased airways, chronic bronchitic sputum and cystic fibrosis patients [1–3]. Thus it appears that, in addition to being expressed [19, 20], the MUC5AC mucin is actually present in airway secretions both in 'normal' and chronic bronchitic subjects. Furthermore, since MUC5AC is present in the gel phase it seems likely that this mucin participates in the formation of the mucus gel. In contrast, the major mucin population from both 'normal' secretions and chronic bronchitic sputum, showed no reactivity with the LUM2-3 antiserum, suggesting that the MUC2 mucin, if present at all, occurs in small amounts. However, some reactivity was seen over low-buoyant density material. The reason for this is not known although it is possible that the antiserum cross-reacts with a protein or that a non-glycosylated/less-glycosylated MUC2 apoprotein is indeed present in the secretions. Since the MUC2 mucin occurs as an 'insoluble' complex in human colon [31, 32], the extraction residues from chronic bronchitic sputum were investigated for the presence of MUC2 but again, the MUC2 mucin could not be detected. These findings are unexpected since it has been shown that *MUC2* is expressed in the airways [19, 22, 34, 35] although Voynow and Rose, [20] showed that the level of expression of *MUC2* in nasal polyps was much lower than that of *MUC5AC*. It has been suggested that the expression of *MUC2* is increased in chronic bronchitis [23] and on exposure to tobacco smoke [36] but our findings indicate that the observed upregulation of *MUC2* gene *expression* in disease does not result in a significant *secretion* of the fully glycosylated MUC2 mucin. However, more examples, including secretions from normal adults and CF patients, are needed to verify how general this observation is.

Subunits from the 'insoluble' complex from human colon as well as mucins from normal secretions and the gel phase of chronic bronchitic sputum were used to study the specificity of the antibodies used in these studies. The LUM5-1 antiserum reacted with the normal and chronic bronchitic mucins but not with the colonic mucins whereas the reverse was true for the LUM2-3 antiserum. This suggests that the antibodies recognize the MUC5AC and MUC2 gene products, respectively, without any significant cross-reactivity. The lack of reactivity of the LUM2-3 antiserum with a putative MUC2 mucin

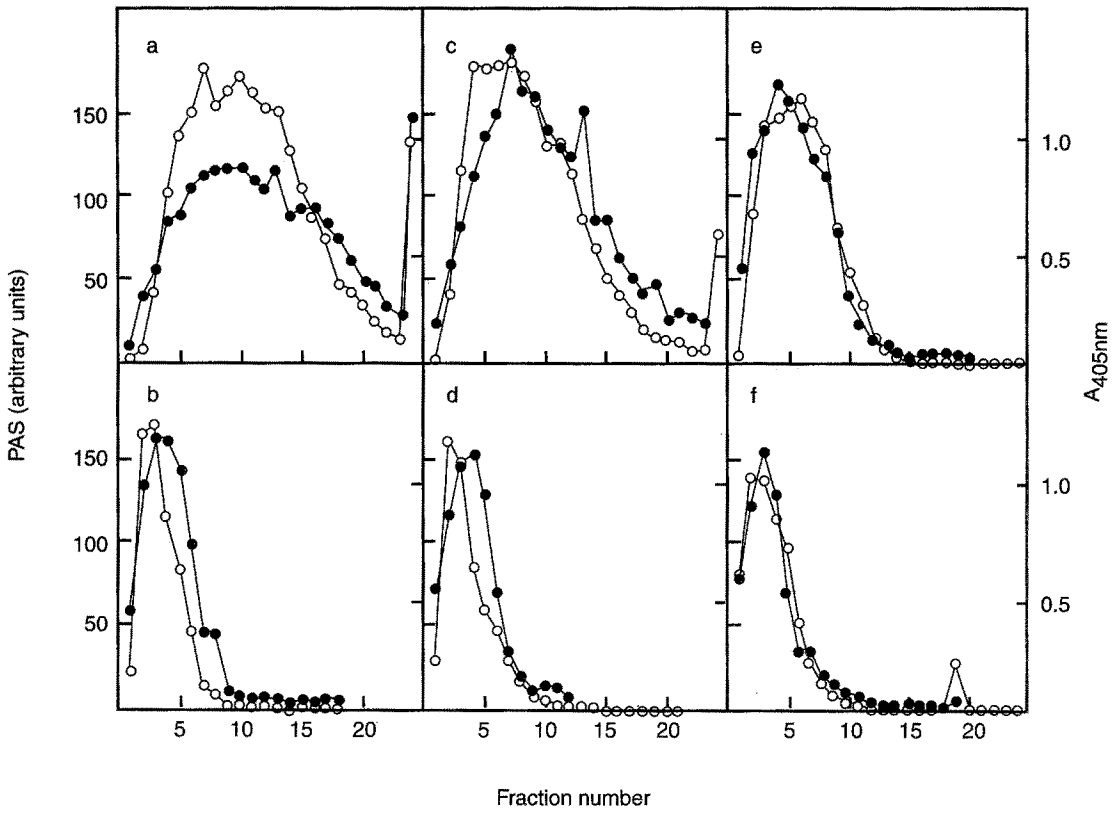

Figure 4. Rate-zonal centrifugation of whole mucins (a, c, e) and reduced subunits (b, d, f) from (a, b) normal respiratory tract secretions, (c, d) gel phase mucins from chronic bronchitic sputum and (e, f) sol phase mucins from chronic bronchitic sputum. Mucins obtained as shown in Fig. 2 and the cognate subunits were layered on top of linear 6-8 M guanidinium chloride gradients and subjected to rate-zonal centrifugation in a Beckman L-70 Optima centrifuge (2 h 45 min, Beckman SW40.1Ti rotor). Fractions (500 μl) collected from the top of the tubes were analysed for PAS reactivity (●) and reactivity with the LUM5-1 antiserum (○).

in respiratory secretions could, in principle, be explained by epitope masking and/or that the MUC2 apoprotein is 'processed' in a different manner in the airways. However, three other antisera raised against peptide sequences (two located N-terminal and one located C-terminal to the tandem repeat region) all reacted with colonic but not with the respiratory mucins making this possibility less likely (unpublished results).

Rate-zonal centrifugation showed that the MUC5AC mucins from the 'normal' and chronic bronchitic secretions were polydisperse and had similar size distributions. Reduced subunits were smaller and more homogeneous in size showing that the MUC5AC mucin is composed of subunits. The sol samples contained mucins which were smaller than those in the gel, reacted with the LUM5-1 antiserum and gave rise to subunits on reduction. The MUC5AC mucins in the sol phase thus appear to be smaller than those in the gel phase while the subunits are of a similar size.

To investigate the charge density of the MUC5AC mucin, reduced subunits from the mucin were subjected to ion-exchange HPLC revealing the presence of two major populations in both the 'normal' and chronic bronchitic secretions. One population was relatively more acidic and reacted with the LUM5-1 antiserum whereas the less acidic one showed no reactivity. The chronic bronchitic sol phase samples contained mainly the former population. 'Normal' and chronic bronchitic secretions thus contain two major populations of subunits, one of which is based upon the MUC5AC apoprotein whereas the other represents an, as yet, unidentified gene product. The existence of two major respiratory mucins is in keeping with the finding of Thornton *et al.* [5] where subunits from respiratory secretions were separated into two populations using agarose gel electrophoresis. Respiratory mucins are produced from two sources in the airways, the goblet cells and the submucosal glands and it is appealing to suggest that the two mucin populations identified biochemically are the products of these two secretory cell populations.

In summary, we have shown that MUC5AC apoprotein is present as a major mucin in airway secretions. The mucin is large, composed of subunits and is apparently gel-forming. In addition, another high molecular mass, gel-forming mucin is present, the identity of which has yet to be established. The MUC2 mucin was not found in

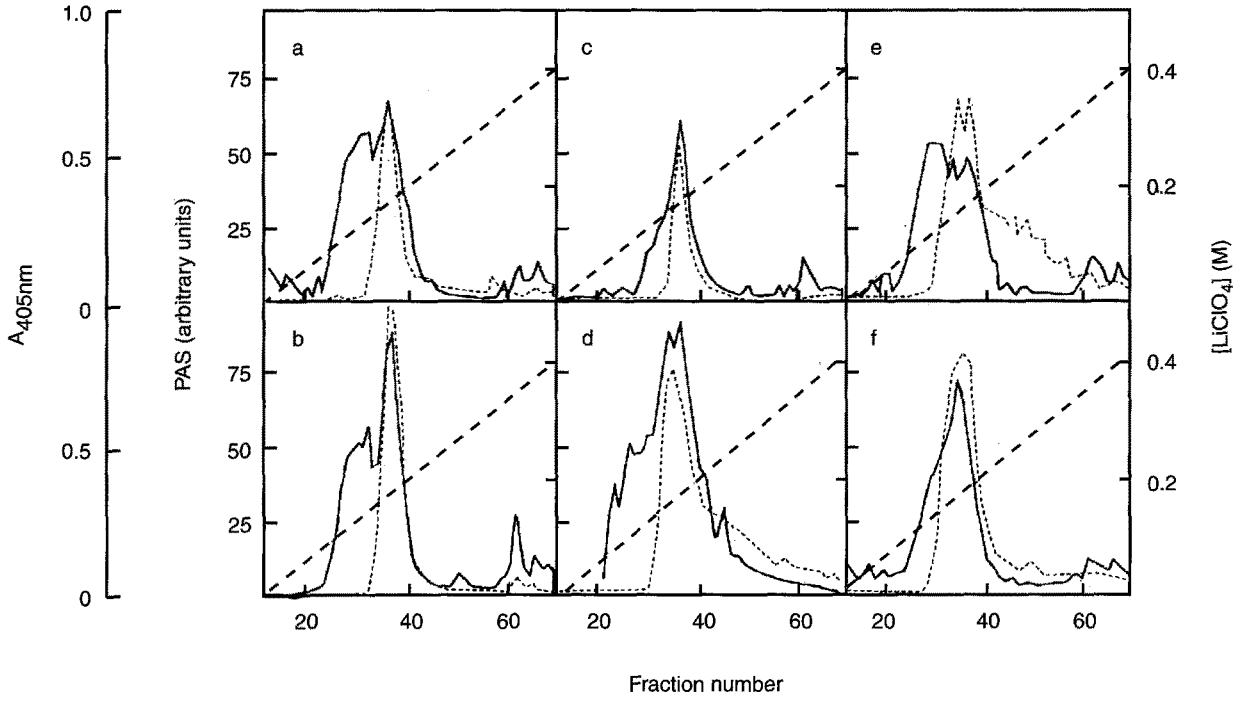

Figure 5. Ion-exchange HPLC on a Mono Q column of reduced subunits from normal respiratory tract secretions (a, b, c), gel phase mucins from chronic bronchitic sputum (d, e) and sol phase mucins from chronic bronchitic sputum (f). Subunits prepared by reduction/alkylation of the cognate mucins were dialysed against 6 M urea, 10 mM-piperazine/perchlorate buffer, pH 5.0 containing 0.1% CHAPS (starting buffer) and chromatographed on a Mono Q column. The column was eluted with starting buffer for 10 mins followed by a linear gradient over 60 min to a final concentration of 0.4 M $LiClO_4$ in starting buffer. Fractions (0.5 ml) were collected and analysed for PAS (-) and LUM5-1 reactivity (- - -).

either normal individuals or patients with chronic bronchitis suggesting that the observed upregulation of *MUC2* expression in disease does not result in a significant secretion of the MUC2 mucin.

Acknowledgements

We thank our laboratory assistants Anders Hansson and Annika Böök for their contribution to this work and gratefully acknowledge support from the Swedish Medical Research Council (grant nos 7902, 9823, 9711), The Medical Faculty of Lund, The Swedish National Association against Heart and Chest Diseases, The Smokeless Tobacco Research Council, Inc. (USA), Centrala försöksdjursnämnden (CFN), Greta and Johan Kocks Stiftelse, Stiftelsen Lars Hiertas Minne, Tore Nilsons Fond för Medicinsk Forskning, Stiftelsen Riksförbundet Cystisk Fibros, the Swedish Association against Asthma and Allergy and Alfred Österlunds Stiftelse.

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