A study of the intracellular and secreted forms of the MUC2 mucin from the PC/AA intestinal cell line

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In this study we present data on the entire population of MUC2 molecules secreted from and within the cell layer of an intestinal cell line. The molecular size distribution of the extracted molecules and their reactivity with two different MUC2 polypeptide antibodies indicated the presence of precursor and mature forms of the mucin. Oligomerized forms of the mucin were found in both the cell layer and medium; however, precursor forms were confined to the cell layer. Isopycnic density centrifugation gave good resolution of mature and precursor forms of MUC2 as assessed by agarose gel electrophoresis. Three different populations of MUC2 were identified: one at low density (>1.3 g/ml) containing the N-glycosylated, non-O-glycosylated polypeptide; a second at intermediate density (1.3–1.35 g/ml) which may represent partially O-glycosylated intermediates; and a third at high density (1.36–1.48 g/ml) containing the mature MUC2 mucins. Rate-zonal centrifugation and agarose electrophoretic analysis of the low-density fraction indicated that the N-glycosylated MUC2 polypeptide was present as putative monomer and dimer/oligomer species. The combination of isopycnic density gradient centrifugation with agarose electrophoresis provides a new and simple approach that allows us to follow the MUC2 gene product from polypeptide through to the mature glycosylated mucin.

Key words: mucins/MUC2/PC/AA cell line

Introduction

Mucins are high-Mr and heavily O-glycosylated macromolecules found on the luminal side of the epithelial surfaces; for instance in the mouth, respiratory tract, gastrointestinal tract, urogenital tract, and corneal surface. They are synthesized, stored, and secreted from cells in the epithelial surface layer and glands in the underlying submucosa. Mucins have been identified in both cell surface attached and fully released forms. A subfamily of the latter mucins, termed gel-forming mucins, are oligomeric, high Mr, thread-like structures assembled from a variable number of subunits via the agency of disulfide bonds. These glycoproteins are the major macromolecular components of mucus, and to date the MUC2, MUC5AC, and MUC5B mucins have been demonstrated to be members of this family (Tytgat et al., 1994; Carlstedt et al., 1995; Sheehan et al., 1996; Thornton et al., 1996, 1997; Wickström et al., 1998).

The mechanism(s) of oligomerization of gel-forming mucins is not fully elucidated. cDNA cloning data have identified domains at the C- and/or N-termini of a number of mucin polypeptides (MUC2, MUC5AC, MUC5B, and MUC6) that are involved in the oligomerization of this latter glycoprotein (Gum et al., 1994; Desseyn et al., 1997a,b; Keates et al., 1997; Toribara et al., 1997; Li et al., 1998; Van de Bovenkamp et al., 1998). By analogy to the vWF, it seems reasonable to propose that mucin subunits assemble by a similar mechanism. Recent data on porcine submaxillary mucin, which also shares similar homologies with vWF, suggest this may indeed be the case (Perez-Vilar et al., 1996, 1998; Perez-Vilar and Hill, 1998).

The early passages (8–20) of the intestinal PC/AA cell line makes copious quantities of mucins which are both stored within and secreted by the cells (Corfield et al., 1990; Corfield and Warren, 1996). We have previously identified the major mucin synthesized by this cell line as the product of the MUC2 gene (Sheehan et al., 1996). Furthermore, we demonstrated that the MUC2 mucins were present as fully glycosylated, highly oligomerized molecules within the cell layer, as well as, secreted into the medium (Sheehan et al., 1996). Where and how this extensive oligomerization takes place within the cell is obscure. However, there is growing evidence that a disulfide bond mediated MUC2 polypeptide dimerization proceeds O-glycosylation (Tytgat et al., 1994; Asker et al., 1995; Van Klinken et al., 1998) and that this occurs in the endoplasmic reticulum (Asker et al., 1998).

Currently, there is no single experimental approach that copes with the physical changes in the molecular characteristics in going from the MUC2 polypeptide to the final glycosylated and oligomerized mucin. In this work, using the human colonic adenoma cell line PC/AA, we describe an approach that allows the study of the total population of MUC2 molecules, both inside the cell and secreted into the culture medium.

Results

Two antibody probes were used to monitor for MUC2 in this study. One the monoclonal antibody 4F1 recognizes epitopes in the tandem repeat (TR) region (Devine et al., 1991, 1993; McGuckin et al., 1996). These epitopes would be expected to be covered by glycans in the mature MUC2 mucins; thus, this may only be a suitable probe for the polypeptide. The other is a polyclonal antiserum LUM2-3 that was raised against a peptide outside the TR region. Its activity is dramatically enhanced by reduction of disulfide bonds, indicating that these epitopes are buried within tertiary structural elements of the molecule (Carlstedt et al., 1995). This antiserum is an effective probe for the reduced MUC2 subunit regardless of its state of O-glycosylation.
Fig. 1. Sephacryl S-1000 gel filtration chromatography of MUC2 mucins from the PC/AA8 cell line. The unreduced cell layer extract (a) and medium (b) of the PC/AA8 cell line were dialyzed into 6 M urea and SDS was added to a final concentration 0.1% (w/v). Gel filtration chromatography was performed on a Sephacryl S-1000 column (1.2 × 36 cm) eluted with the same solvent at a flow rate of 0.16 ml/min. Fractions (∼0.8 ml) were collected and analyzed with two anti-MUC2 antibodies; LUM2-3 (solid squares) and 4F1 (solid triangles). The data are not quantitative since different volumes were blotted for each antibody assay.

The size distributions of secreted and intracellular forms of MUC2

The size distributions of MUC2 molecules in the GuHCl extracts of the cell layer and medium of the PC/AA cell line were examined by gel chromatography and rate-zonal centrifugation. Gel chromatography of the two extracts was performed on Sephacryl S-1000 (Figure 1). The MUC2 molecules extracted from the cell layer were recovered as two major populations, one in the void volume and the other in the included volume (Figure 1a). Only the molecules included on the column reacted with 4F1, whereas both components were detected with LUM2-3. In contrast, in the medium extract only the LUM2-3 probe gave a positive-signal and the majority of the MUC2 was eluted in the void volume of the column (Figure 1a).

Rate-zonal centrifugation of the two extracts was performed on 6–8 M GuHCl gradients (Figure 2). Analysis of the gradient fractions indicated that the 4F1-reactive molecules were found only in the cell layer extract, primarily as slow sedimenting species at the top of the gradient (Figure 2a). Again, no 4F1-reactivity was observed in the medium extract (Figure 2c). The MUC2 molecules in both the cell layer and medium, as detected by LUM2-3, were polydisperse and of higher sedimentation rate than the 4F1 reactive species. The polydispersity and sedimentation rates of the mucins as assessed by LUM2-3 were greatly lowered by reduction indicating their oligomeric nature (Figure 2b,d).
Rate-zonal centrifugation of the reduced cell layer extract performed for a longer time demonstrated a difference in sedimentation rate of the 4F1- and LUM2-3 reactive molecules, indicating the latter were of higher apparent mass (Figure 2e).

These data show that there is no 4F1-reactive material secreted into the medium, which contains only oligomerized, presumably fully mature MUC2 mucins. In contrast, while the majority of the cell layer MUC2 molecules appear as similarly oligomerized species there are smaller, less massive molecules strongly reactive with the 4F1 antibody. These are assumed to be non-, or partially, O-glycosylated MUC2 mucin precursors.

Separation of the different forms of the MUC2 mucin

Isopycnic density gradient centrifugation in 4 M GuHCl/CsCl has been used to separate mucins (density <1.3 g/ml) from non-mucin proteins and other glycoproteins (density <1.3 g/ml) (Carlstedt et al., 1983). Thus, we anticipated that this would be an effective technique to separate the mucin polypeptide and any partially glycosylated intermediates from the mature MUC2 mucin. The cell layer and medium extracts were therefore subjected to density gradient centrifugation in 4 M GuHCl/CsCl (Figures 3a, 4a). In both cases, the major MUC2-reactivity detected by LUM2-3 coincided with the major carbohydrate-containing peak. This material has a density range of 1.36–1.48 g/ml, which is the density observed previously for the mature mucins isolated from this cell line (Sheehan et al., 1996). In the low density fractions of the cell layer extract where we expected to find the MUC2 precursors predicted by the gel chromatography (Figure 1a) and rate-zonal centrifugation (Figure 2a), there was only a weak response with the two antibody probes. In contrast, Western blotting after agarose gel electrophoresis of these fractions (Figures 3b,c) demonstrated the presence of the putative MUC2 precursor species. As predicted these were not observed in the medium (Figure 4b). The 4F1-reactive species are found predominantly in the top three fractions of the CsCl gradient, and before reduction they appear as two bands that after reduction become a single more rapidly migrating band (Figure 3b). The same bands are also visualized with LUM2-3 (Figure 3c).

The molecules in the density range corresponding to mature MUC2 mucins (1.36–1.48 g/ml) in both cell layer and medium gave two bands after reduction, which were visualized only with LUM2-3 (Figure 3c & 4b). Without reduction bands were not observed in these fractions from the medium extract (Figure 4b). However bands with similar migration to the reduced MUC2 subunits and also some slower migrating species were observed in these fractions from the cell layer extract (Figure 3c).

In the cell layer (Figure 3c), but not the medium (Figure 4b), there is a LUM2-3-reactive population of MUC2 molecules in fractions of intermediate density (1.3–1.35 g/ml). No bands are visualized with the intact molecules, but two bands are observed after reduction. These bands are similar to those observed after reduction of the mature mucins but have a slightly slower electrophoretic migration.

Recovery of unreduced MUC2 mucins after agarose gel electrophoresis

The data presented above suggest that the unreduced oligomeric MUC2 mucins do not enter the agarose gel or do not transfer to nitrocellulose during vacuum blotting. To address this question, we performed agarose electrophoresis on the total extract from the cell layer and medium both before and after reduction (Figure 5). However, in these experiments the gel was subjected to a reducing agent after electrophoresis but prior to Western blotting. This treatment did not change the number of bands observed above with the 4F1 antibody (Figure 5, lanes 1, 2, 5, 6). No bands were observed in the medium fraction (Figure 5, lanes 1, 2), whereas the unreduced cell layer extract exhibited two major bands (Figure 5, lane 5), which after reduction became a single more rapidly migrating band (Figure 5, lane 6). In contrast, in both the unreduced medium (Figure 5, lane 3) and cell layer extracts (Figure 5, lane 7), LUM2-3 reactivity was observed as a major band that barely entered the gel. Reduction of both preparations prior to electrophoresis yielded the same two bands, one major and one more minor (Figure 5, lanes 4, 8), observed previously (Figures 3c, 4b). It should be noted that bands similar to those observed with 4F1 in the cell layer extract (Figure 5, lanes 5, 6) could be stained with LUM2-3 after prolonged development of the ECL reaction (data not shown) suggesting the precursor forms are a minor proportion of the total MUC2.
Fig. 4. Density gradient centrifugation on the medium of the PC/AA8 cell line. (a) Medium of the PC/AA8 cell line was mixed (1:1 dilution) with 6M GuHCl containing proteinase inhibitors. The molarity of GuHCl was adjusted to 4 M by adding solid GuHCl and thereafter subjected to isopycnic density gradient centrifugation at a starting density of 1.40 g/ml in CsCl/4 M GuHCl, in a Beckman Ti70.1 rotor at 40 000 r.p.m. for 68 h at 15 °C. After centrifugation the tubes were emptied from the top and fractions were analyzed for PAS (solid circles), absorbance at 280 nm (open circles) and reactivity with the two MUC2 antibodies LUM2-3 (solid squares) and 4F1 (solid triangles). The density of each fraction (dashed line) was determined by weighing. (b) Aliquots of fractions from across the density gradient (unreduced (U) and reduced(R)) were electrophoresed in 1.0% (w/v) agarose gels and blotted onto nitrocellulose membranes prior to detection with the MUC2 antibody LUM2-3. No 4F1 response was detected.

Analysis of the putative precursor forms of the MUC2 mucin

To study the low density, putative MUC2 precursor species in more detail fraction 2 from the density-gradient separation of the cell layer extract was subjected to agarose gel electrophoresis followed by Western blotting using carbohydrate specific probes (Figure 6). The bands identified with the 4F1 antibody, two before (Figure 6, lane 1, bands I, II) and one after reduction (Figure 6, lane 2, band III) are also stained with the lectin Concanavalin-A (Figure 6, lanes 3, 4) indicating the presence of N-linked glycans. However, there was no staining of these bands with a monoclonal antibody directed to the Tn antigen (data not shown). We attempted to separate these 4F1-staining components using rate-zonal centrifugation (Figure 7a). Aliquots of fractions across the rate-zonal gradient were subjected to agarose gel electrophoresis followed by Western blotting (Figure 7b) and showed that the slower migrating of the two 4F1-positive bands (Figure 6, band I) sediments more rapidly than the faster electrophoretic species (Figure 6, band II). Reduction had only a minor effect on the sedimentation profile, resulting in a slight decrease in the proportion of the faster sedimenting species (Figure 7a). In contrast, a more marked change was observed in electrophoretic migration (Figure 7c).

Fig. 5. Agarose gel electrophoresis of the cell layer extract and medium of the PC/AA8 cell line. Medium (U) unreduced (lanes 1 and 3) and (R) reduced (lanes 2 and 4) and cell layer extract unreduced (lanes 5 and 7) and reduced (lanes 6 and 8) were electrophoresed in a 1.0 (w/v) % agarose gel. The gel was then treated with 10 mM DTT prior to Western transfer onto nitrocellulose membrane and detection with two MUC2 antibodies; LUM2-3 (lanes 3–4, 7–8) and 4F1 (lanes 1–2, 5–6).

Fig. 6. Agarose gel electrophoresis of a low-density fraction from the density gradient centrifugation on the cell layer extract of PC/AA8 cell line. Fraction 2 from the density gradient, unreduced (U) and reduced (R), was electrophoresed in a 1.0% (w/v) agarose gel and blotted onto nitrocellulose membrane prior to detection with the MUC2 antibody 4F1 (lanes 1 and 2) and the lectin Con-A (lanes 3 and 4). Bands highlighted as I, II, and III are discussed in the text.
by the intestinal cell line PC/AA. One, a high-M r population, was two differently sized populations of MUC2 molecules produced the mature forms of the mucin, unlike here, are excluded from the mass and hydrodynamic volume of the MUC2 polypeptide.

Tytgat finding indicates that the latter molecules are partially or fully precursors are smaller than the LUM2-3–reactive species. This sedimentation studies on the intracellular these were precursor forms of MUC2 that were depleted in, or lacking, O-glycan chains. Sedimentation studies on the intracellular polypeptide of TR-region of the molecule. This suggested that

This is the first study to investigate the total population of MUC2 molecules. In combination with agarose gel electrophoresis it provides a powerful approach to study the mucins at all stages of their biogenesis. In analyzing the CsCl gradients using slot blotting there was a suppression of the antibody response with the precursor forms present in the low-density fractions. This is almost certainly due to the high amount of other non-mucin protein present in these fractions (as assessed by A280 measurements). The high protein content may decrease both the efficiency of binding of the MUC2 precursors to the nitrocellulose membrane employed for slot blotting and the binding of the antibodies to their epitopes.

Isoympnic centrifugation of the cell layer extract yields a clear separation between the mature mucins (density range 1.36–1.48 g/ml) and the putative polypeptide precursors (density > 1.3 g/ml). However agarose gel electrophoresis of the density gradient fractions indicated another population of oligomerized MUC2 molecules (density range 1.3–1.35 g/ml) that were reactive with LUM2-3 but not with 4F1. The lack of 4F1-reactivity suggests that they are glycosylated; however, their buoyant density indicates the level of glycosylation is lower than that of the mature subunits. Furthermore, these molecules had a slightly slower electrophoretic migration than did the mature subunits. The increased migration of the mature subunits may be due to the enhancement of the charge on the molecules by addition of sialic acid, which we have previously shown to be present on the mature mucins (Corfield et al., 1990, 1995; Vavasseur et al., 1994; Sheehan et al., 1996).

Previously, we have used agarose gel electrophoresis with reduced mucin preparations (Sheehan et al., 1996; Thornton et al., 1995b, 1996; 1997). However, in this study we have shown that they can also be used to distinguish between mature oligomeric mucins, their reduced subunits, and biosynthetic intermediates. Under our standard conditions (Thornton et al., 1995b), unreduced mature mucins do not give bands after Western blotting. However, treatment of the agarose gel after electrophoresis, but prior to Western blotting, with a reducing agent visualized a major band that barely entered the gel. This demonstrates that vacuum blotting prior to reduction of the molecules did not transfer the oligomerized mucins. None of the oligomerized species reacted with the 4F1 antibody.

Western blots of the unreduced cell layer extract demonstrated the presence of two distinct bands, which after reduction became a single more rapidly migrating band. These bands stain with 4F1, LUM2-3 and the lectin Con-A but not with a monoclonal antibody against the Tn antigen. This indicates that the MUC2 polypeptides in these bands are N-glycosylated but have not undergone (to any major extent) the first step of O-glycosylation (i.e., addition of GalNAc). We therefore propose that the single band observed after reduction represents a denatured, non-O-glycosylated MUC2 polypeptide. Whereas the two bands observed before reduction may represent "native" monomeric (fast migrating band) and dimeric/oligomeric (slower migrating band) forms of glycosylated subunits. In addition, the reduced subunits derived from molecules in the medium appear to be larger than those in the cell layer, thus indicating that some, though not all, of the subunits inside the cells are not fully mature in terms of glycosylation. The absence of 4F1 reactivity with the MUC2 mucins in the medium indicates that these molecules are heavily substituted with O-glycans. High-M r forms of MUC2 are found in both the cell layer and medium, and these are polydisperses, oligomeric assemblies stabilized by disulfide bonds.

We demonstrate here that isopycnic density gradient centri-fugation is very effective for separating the different populations of MUC2 molecules. In combination with agarase gel electrophoresis it provides a powerful approach to study the mucins at all stages of their biogenesis. In analyzing the CsCl gradients using slot blotting there was a suppression of the antibody response with the precursor forms present in the low-density fractions. This is almost certainly due to the high amount of other non-mucin protein present in these fractions (as assessed by A280 measurements). The high protein content may decrease both the efficiency of binding of the MUC2 precursors to the nitrocellulose membrane employed for slot blotting and the binding of the antibodies to their epitopes.

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the non-O-glycosylated MUC2 polypeptide. This interpretation is consistent with earlier findings which have reported that dimerization of the MUC2 polypeptide precedes its O-glycosylation (Asker et al., 1995). The putative monomer and dimer were partially separated by rate-zonal centrifugation suggesting that they are different in mass. However, after reduction there is no significant change in the sedimentation rate of the putative monomer (slower sedimenting band), and this would be consistent with the monomer/dimer hypothesis. While there is little change in the sedimentation behavior upon reduction there is a marked increase in the electrophoretic migration of the MUC2 polypeptide. The most likely explanation for this is that a 1% (w/v) agarose gel causes very little retardation of the mucin polypeptide. This is supported by the observation that electrophoresis in a 1.5% (w/v) agarose gel had a significantly increased retarding effect on the migration of the mature subunits though almost none on the precursor protein (data not shown). An increased binding of SDS to the fully reduced and denatured N- and C-termini might therefore explain the enhanced migration.

The two bands that are observed after agarose electrophoresis of the reduced mature mucins are as yet not fully explained. The major band is probably the monomer and the other more minor band may be a dimer as has been reported by others (Asker et al., 1995, 1998). Reduction insensitive MUC2 intestinal mucin complexes were originally demonstrated by Carlstedt and co-workers in both the human and the rat (Carlstedt et al., 1993, 1995; Hansson et al., 1994). Such complexes have more recently been proposed for MUC2 mucins from human intestinal cell culture (Axelsson et al., 1998). The site and nature of the reduction insensitive link between the mucin monomers are not yet established.

In conclusion, we have outlined procedures for identifying all forms of MUC2 within and secreted from an intestinal cell line. We demonstrate the presence of three distinctive categories of molecules: (1) the N-glycosylated protein core in monomeric and dimeric form, (2) partially glycosylated intermediates that are oligomerized, and (3) fully glycosylated, oligomerized species. These latter molecules are the only ones to be found in the medium. Our identification of these species suggests a distinctive step-wise assembly of this complex molecule and a “quality control” process operating on the secreted product.

Materials and methods

Guanidinium chloride (GuHCl), Schiff’s reagent, peroxidase-labeled Concanavalin-A, nitroblue tetrazolium, 5-bromo-4-chloro-3-indolylphosphate, goat anti-rabbit IgG horseradish peroxidase conjugate, goat anti-mouse IgM horseradish peroxidase, and alkaline phosphatase conjugate were purchased from the Sigma Chemical Co. (Poole, Dorset, UK). Urea, Tween 20, and CsCl were from BDH Ltd. (Dagenham, Essex, UK). Stock solutions of guanidinium chloride and urea were treated with charcoal before use. In addition urea solutions were treated with the mixed bed resin Amberlite MB3. Goat anti-rabbit IgG alkaline phosphatase conjugate was from Promega (Madison, WI). Agarose UltraPURE (electrophoresis grade) was from GIBCO-BRL (Paisley, Scotland). The enhanced chemiluminescence (ECL) Western detection kit was from Amersham International Plc (Buckinghamshire, UK).

Cell culture and collection of mucins

The PC/AA adenoma cell line was derived from a single, large, colonic tubular adenoma of 3–4 cm diameter that exhibited only mild dysplasia. The cells were continuously passaged in vitro at 37°C in the presence of 5% CO2 in air and 3T3 feeder cells on collagen type IV-coated T25 flasks. The PC/AA cells at passage 8 used in this study are non-tumorogenic in nude mice and have ultrastructural characteristics of colonic cells (Paraskeva et al., 1984). The cell layer was solubilized with 6 M GuHCl containing proteinase inhibitors, and the medium was mixed with an equal volume of 6 M GuHCl.

Antibodies and lectin

The following antisera were used in this study; a monoclonal antibody 4F1 (IgM ascites, 1:1000) was a kind gift from Dr. M. McGuckin (University of Queensland, Australia). This antibody was raised to a synthetic peptide corresponding to a single repeat of the MUC2 tandem repeat (TR) and recognizes two different sites, TPTP and PTPTT (Devine et al., 1991, 1993). A polyclonal antiserum LUM2-3 was a kind gift from Dr. I. Carlstedt (University of Lund, Sweden). This antiserum was raised against a synthetic peptide NGLQPVRVEDPGC in the non-TR of the molecule towards the C-terminus (Carlstedt et al., 1995). When using LUM2-3 for probing slot- or Western blots of unreduced mucins, the molecules are reduced on the membrane (as described below) prior to incubation with the antiserum. A monoclonal antibody against to Tn antigen, purchased from Dako A/S, was used at a dilution of 1:1000. The peroxidase-labeled lectin Concanavalin-A (Con A) was used at a concentration of 2 µg/ml.

Preparation of reduced mucins

Reduced mucins were prepared following dialysis of the whole mucins into GuHCl or urea reduction buffer (6 M GuHCl or 6 M urea containing 0.1 M Tris/5 mM EDTA, pH 8.0) and then treatment with 10 mM 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 (Carlstedt et al., 1983). Alternatively, mucins were reduced on nitrocellulose membranes after slot or Western blotting. Briefly, the blotted membrane was washed in distilled water for a few minutes and incubated in urea or GuHCl 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.

Gel chromatography

The cell layer extract and corresponding medium were dialyzed into 6 M urea and SDS was added to give a final concentration of 0.1% (w/v). Samples were subjected to gel filtration chromatography on a Sephacryl S-1000 column (1.2 × 36 cm) eluted with 6 M urea containing 0.1% SDS at a flow rate of 0.16 ml/min and 0.8 ml fractions were collected.

Rate-zonal centrifugation

Samples were layered on to preformed GuHCl or urea gradients (6–8 M). Intact and reduced cell layer and medium extracts were centrifuged in GuHCl gradients in a Beckman SW40 swing-out rotor at 40,000 r.p.m. for 2.5 h at 15°C. The reduced cell layer extract was also centrifuged under the same conditions except for the time which was 7 h. Low-density fractions from isopycnic density gradient centrifugation were centrifuged in urea gradients in a Beckman SW55 swing-out rotor at 55,000 r.p.m. for 5 h at 15°C. After centrifugation the tubes were emptied from the top.
Isopycnic density-gradient centrifugation

The cell layer extract and medium were centrifuged at a starting density of 1.40 g/ml in 4 M GuHCl/CsCl using a Beckman Ti70.1 rotor at 40 000 r.p.m. for 68 h at 15°C. After centrifugation tubes were emptied from the top.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed in 1% (w/v) agarose gels as described previously (Thornton et al., 1995b). After electrophoresis molecules were transferred to nitrocellulose membrane by vacuum blotting in 0.6 M sodium chloride/0.06 M sodium citrate 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, unreduced molecules the gel, after electrophoresis, was 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.

Analytical methods

Analysis of mucin was performed by using a periodate-Schiff (PAS) assay after slot blotting onto nitrocellulose (Thornton et al., 1995a). Detection was accomplished using either an ECL kit according to the manufacturer’s instructions or using the colored substrates nitroblue tetrazolium and ECL, enhanced chemiluminescence, respectively. For the analysis of the intact, unreduced molecules the Agarose gel electrophoresis molecules were transferred to nitrocellulose gel, after electrophoresis, was 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.

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Abbreviations

DTT, dithiothreitol; ECL, enhanced chemiluminescence; M r, relative molecular weight; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; GuHCl, guanidinium chloride; TR, tandem repeat; PAS, periodate-Schiff; Con-A, Concanavalin A; GalNAC, N-acetylgalactosamine.

References


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