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Structural and compositional differences between intracellular and secreted mucin of rat small intestine

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An investigation was undertaken to discover whether mucin purified from secretions in the lumen of rat small intestine differed in structure or composition from intracellular mucin purified from rat intestinal tissue. To do this, ligated loops were constructed *in situ* from previously washed intestinal segments and mucin purified separately from tissue homogenates or loop fluid. Secreted mucin (SM) differed from intracellular mucin (IM) by having a higher proportion of 'minor' mucin amino acids (aspartic acid, glutamic acid, glycine and alanine) and a lower proportion of 'major' amino acids (serine, proline and threonine). SM also contained less *N*-acetylgalactosamine and a small, but measurable, amount of mannose. Gel electrophoresis showed that SM penetrated the gel more readily and, unlike IM, gave a rather prominent, but diffuse, band having a midpoint position of M_r 200000. After reduction both IM and SM gave rise to the putative 'link' component of M_r 118000, and the 200000-M_r band of SM disappeared. SM was included to a greater extent than IM on Sepharose CL-2B chromatography, suggesting a smaller size. With the use of CsCl-density-gradient ultracentrifugation of SM, a lighter species [buoyant density (ρ) = 1.38 g/ml] enriched in the 200000-M_r component, was separated from a heavier, more glycosylated, species (ρ = 1.50 g/ml). Purified 200000-M_r component had a composition identical with that of the 118000-M_r 'link' component of IM, reacted in Western blots with an antibody specific for the 118000-M_r 'link' component, and after reduction gave rise to a 118000-M_r component on gel electrophoresis. Thus secreted mucin contains a 200000-M_r component which appears to represent a disulphide-linked dimer of the previously described 118000-M_r 'link' component of intracellular mucin.

INTRODUCTION

Rat intestinal mucin is made up of different high-M_r glycopeptides that polymerize and/or aggregate to form a viscoelastic gel (Fahim *et al.*, 1983). Some of the glycopeptides are covalently bonded through disulphide bridges to a putative 'link' component of M_r 118000. We have shown previously that the latter has an amino acid composition which differs from that of the higher-M_r glycopeptides (Fahim *et al.*, 1983). In particular, the 118000-M_r component is enriched in the 'minor' mucin residues (aspartic acid, glutamic acid, valine, glycine and alanine), whereas the large glycopeptides are significantly more enriched in the 'major' residues (serine, threonine and proline). When the 'link' component is removed experimentally from the native mucin by thiol reduction, the mucin dissociates into smaller glycopeptide structures.

Verdugo (1984) has shown that, after secretion, mucins rapidly expand in volume, owing to hydration, and layer themselves on to the surface of the epithelium. It is not known whether this dramatic structural change in mucins is accompanied, or followed, by significant chemical changes in the macromolecules. No investigators to our knowledge have purified both intracellular and secreted mucin from the same organ and compared them chemically. Less rigorous studies, comparing impure or partially purified mucin preparations, have given equivocal results. For example, Schrager & Oates (1971) reported that the molar ratios of carbohydrates of the total glycoproteins in human gastric secretions and gastric tissue extracts exhibited no differences. Woussen-Colle *et al.* (1975) also found no major differences in carbohydrates of canine gastric juice and gastric mucosal extracts. Fouad & Waldron-Edward (1980) found a lower carbohydrate content in partly purified mucins of dog gastric secretions than in mucins of the corresponding mucus cells. Those authors suggested that lysosomal hydrolases released into the medium from sloughed epithelial cells may have been responsible for the difference. Pearson *et al.* (1986) showed that native pig gastric mucin is cleaved into smaller molecules by the action of human pepsins, which attack 'naked' peptide regions of the mucin. This appears to be a normal early step in the solubilization of the gastric mucus gel (Allen, 1981).

In our own laboratory we have observed that secreted rat mucin retains the antigenic determinants of intracellular mucin (Forstner *et al.*, 1977; Roomi *et al.*, 1984), but that secreted radiolabelled glycoproteins tend to emerge from Sepharose CL-2B columns later than radiolabelled intracellular mucin (IM), suggesting that secreted mucin (SM) may be somewhat smaller in size (Forstner *et al.*, 1984). We have therefore speculated that

Abbreviations used: PBS, phosphate-buffered saline (0.01 M-NaH_2PO_4/0.15 M-NaCl, pH 7.0); ρ, buoyant density; d.g.u., density-gradient ultracentrifugation; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; IM, intracellular mucin; SM, secreted mucin; PAGE, polyacrylamide-gel electrophoresis; NEM, *N*-ethylmaleimide; PMSF, phenylmethanesulphonyl fluoride; PAS, periodate/Schiff.

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SM may undergo partial dissociation or degradation during, or shortly after, exocytosis.

In the present study we have purified IM and SM from washed rat intestine (ligated loops) and have compared the two mucins with respect to their size, composition, antigenicity, electrophoretic behaviour and selected structural features. Our data suggest that SM differs significantly from IM. Most of the changes in composition and structure take place within 1 h of secretion, with very little change thereafter up to 4 h. A surprising finding was the presence, in purified SM, of a non-covalently associated glycopeptide having an M_r of about 200000. This component was similar in composition and antigenic reactivity to the putative 'link' glycopeptide (M_r 118000) of IM (Fahim *et al.*, 1987). After reduction, the 200000-M_r band on SDS/PAGE generated a band at a position corresponding to M_r 118000, suggesting that the former represents a disulphide-linked dimer of the putative 'link' glycopeptide.

MATERIALS AND METHODS

Intestinal loops

Rats were anaesthetized in an ether chamber or by intraperitoneal injection of 10 mg of pentobarbital (Abbot Laboratories, Montreal, Que., Canada). The small intestine was washed free of luminal contents with warm (37 °C) sterile NaCl (0.15 M), followed by air, using a proximal catheter and a distal drainage tube. Ligated intestinal loops approx. 25 cm in length were constructed beginning distal to the ligament of Trietz.

Rats were killed by cervical dislocation 1–4 h later, the loops removed, and their contents drained. The loops were then opened and washed by swirling gently in a beaker containing 25 ml of 5 mM-EDTA/NaOH (pH 7.0)/1 mM-NEM/1 mM-PMSF. The luminal contents, together with the wash, were centrifuged, and the supernatant used for the purification of soluble SM. Epithelial scrapings were homogenized and centrifuged as described previously (Fahim *et al.*, 1983) and the supernatant used for purification of soluble IM.

Purification of mucin

Tissue or luminal supernatants were separately dialysed overnight against distilled water and then CsCl added to give a final density of 1.40 g/ml. Centrifugation at 150000 *g* for 72 h at 4 °C was performed in Polyallomer Quick-Seal centrifuge tubes (Beckman, Palo Alto, CA, U.S.A.) in a fixed-angle Ti 50.2 rotor (Beckman) placed in either a model L3-50 or a model L8-55 centrifuge (Beckman). The tubes were fractionated into eight equal parts, individually dialysed against water, and assayed for protein (A_{280}) and for sugar (PAS assay; Mantle & Allen, 1978). The PAS-positive (mucin-rich) fractions (4–7) were pooled and subjected to a second ultracentrifugation. The resultant mucin was contained within fractions 4–7.

Gel electrophoresis

Composite agarose/PAGE was performed with non-reduced or reduced mucin as described previously (Fahim *et al.*, 1983) and stained by the silver method of Switzer *et al.* (1979). SDS/PAGE was performed by the method of Laemmli (1970), and gels were stained by the method of Merill *et al.* (1981).

The 'link' component of rat intestinal mucin was isolated from preparative SDS/PAGE by electroelution. To do this, reduced and alkylated native mucin was subjected to SDS/PAGE; one track was stained with Coomassie Blue, and the link component was identified by its mobility (corresponding to 118000 M_r) on the gel. The band was then isolated from the other tracks, chopped into small pieces and placed in an ISCO (Lincoln, NE, U.S.A.) model 1750 'electrophoretic concentrator' apparatus. The electroeluted sample was then dialysed against distilled water and used for chemical analysis or for antibody production. For amino acid and sugar analysis, SDS was first extracted by the method of Henderson *et al.* (1979).

Amino acid and carbohydrate analyses

Amino acid analyses were performed on either a Durrum model D-500 analyser or on a Waters h.p.l.c. amino acid analysis system after hydrolysis of the samples in 5.5 M constant-boiling HCl at 110 °C for 20 h.

Carbohydrates were analysed either on a Varian Aerograph series 2100 or a Varian model 3700 gas chromatograph, using a 3% OV-210 column, by the method of Zanetta *et al.* (1972). A temperature program of 110–210 °C at 1 °C/min was used to separate the *O*-methyl glycoside derivatives.

Preparation of antibodies

Antibody to the intact (native) IM was prepared by subcutaneous injection of the mucin containing 40 μg of protein (mixed with Freund's complete adjuvant) into each of three New Zealand rabbits. Before the first injection, about 50 ml of blood was collected and the serum saved from each rabbit to serve as a control (pre-immune serum). Subcutaneous injections of mucin were repeated with Freund's incomplete adjuvant at 2 and 4 weeks, and thereafter every 2 months. Antiserum production was monitored either by Ouchterlony (1968) double immunodiffusion or by counter-immunoelectrophoresis (Kenny & Foy, 1975). The IgG fraction was harvested on a Protein A–Sepharose column, and was used as the antibody to rat mucin.

A second antibody used in these studies was developed in a similar fashion against the purified 118000-M_r 'link' glycopeptide of IM. This antibody was rendered monospecific by chromatography on columns of Reacti-gel (Pierce Chemical Co., Rockford, IL, U.S.A.) to which was bound purified 'link' component according to the manufacturer's instructions. Antiserum to the 'link' component was applied, and the unbound material was washed off the column with 25 ml of PBS, concentrated, and re-applied twice on the column. The column-bound material was eluted with 0.1 M-glycine/HCl, pH 2.8, neutralized immediately with Trizma and portions stored at −20 °C. Characterization and specificity of this antibody for the 118000-M_r 'link' glycopeptide was reported previously (Fahim *et al.*, 1987).

Other methods

Electrophoretic transfer of antigens and detection with antibody and ^{125}I-Protein A was performed by the immunoblot method of Towbin *et al.* (1979).

Radioimmunoassay of mucin was carried out by a solid-phase assay described previously (Roomi *et al.*, 1984).

Column (0.9 cm × 55 cm) chromatography of purified mucins was performed on Sepharose CL-2B, with PBS as

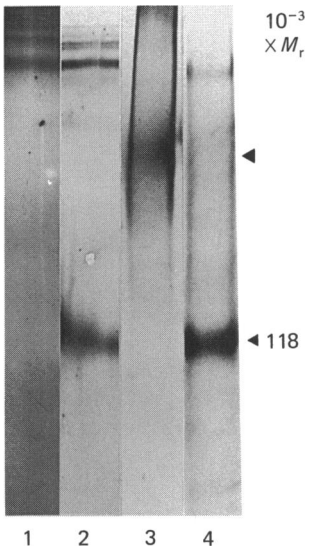

Fig. 1. Agarose/PAGE of IM and SM

Samples (approx. 2 μg of mucin protein per track) of intracellular (tracks 1 and 2) and secreted mucin (tracks 3 and 4) were loaded on the gel after heating for 2 min at 100 °C in 1% SDS, pH 6.8, without (tracks 1 and 3) and with (tracks 2 and 4) 0.3 M-2-mercaptoethanol. The arrowhead without a value points to the position of a band in SM corresponding to an M_r of approx. 200000.

Table 1. Amino acid composition of IM and SM

Amino acid values for IM are the means for three independent samples, and for SM of two samples at each time period. Reproducibility of samples was high (i.e. variation was less than ±10% of the mean).

	Composition (mol/100 mol)		
		SM	
Amino acid	IM	1 h	4 h
Asp	4.6	11.3	9.9
Thr	31.4	16.9	18.5
Ser	15.9	11.4	10.9
Glu	6.3	12.3	11.5
Pro	14.8	11.1	11.8
Gly	6.0	8.2	8.7
Ala	2.4	5.7	5.5
Val	3.1	3.3	3.8
Ile	3.2	2.2	3.4
Leu	3.1	4.1	5.6
Tyr	1.6	2.6	1.6
Phe	2.9	3.9	2.8
His	1.5	2.6	1.6
Lys	2.0	2.6	2.8
Arg	1.2	1.8	1.7

eluent. Bio-Gel P-100 columns were used in the isolation of the 200 component of SM, with elution by PBS.

RESULTS

Isolated loops of rat small intestine were constructed *in situ* after first flushing the intestine with warm sterile 0.15 M-NaCl to remove bacteria, adherent mucus and debris. At periods up to 4 h later, mucins were purified (in the presence of proteinase inhibitors) from the washed epithelial tissue to yield IM and from the luminal fluid to yield SM. In both cases the mucin appeared in CsCl gradients within the range $\rho = 1.35$–1.55 g/ml. By SDS/PAGE (not shown) and composite gels (Fig. 1, tracks 1 and 3) the mucins were free of low-M_r protein contaminants. The amino acid and carbohydrate composition of SM and IM revealed several surprising differences. At both 1 and 4 h the SM had less threonine, serine and proline (major amino acids) and more aspartic acid, glutamic acid, glycine and alanine (minor amino acids) than IM (Table 1). These differences suggested that SM might contain more poorly glycosylated peptide domains than IM. Very few differences were noted between 1 and 4 h samples, suggesting that, whatever process was responsible for the marked differences between IM and SM, it occurred during, or early after, the secretory event, and did not progress significantly thereafter.

Table 2. Carbohydrate composition of IM and SM

Values for are the means for three independent samples, and values for SM are for two samples at each time period.

	Composition (mol/100 mol)		
		SM	
Sugar	IM	1 h	4 h
Fucose	16.5	14.3	16.3
Galactose	27.0	29.7	30.1
GlcNAc	20.3	20.4	22.1
GalNAc	23.2	16.9	15.8
Sialic acid	12.6	16.7	11.9
Mannose	0.4	2.0	3.8

Carbohydrate analyses (Table 2) revealed that SM differed very little from IM, except that SM had less GalNAc and a measurable amount of mannose. The presence of mannose suggested that SM might contain more of the 118000-M_r 'link' component, since the latter has been shown in human, and more recently in rat intestinal mucin, to contain mannose (Mantle *et al.*, 1984; Fahim *et al.*, 1987). We could not rule out at this stage, however, that a large non-mucin (membrane or secreted) glycoprotein might also be contaminating the SM preparation. Apart from a higher sialic acid concentration seen at 1 h, the carbohydrate differences in SM between 1 and 4 h were unremarkable, suggesting that there had been little selective loss of sugar with time.

Gel electrophoresis was performed on IM and SM (4 h secretion samples) using composite agarose/acrylamide gels (Fig. 1). As in previous experiments (Fahim *et al.*, 1983), IM gave several bands near the top of the gel and, after reduction, a decrease in polymeric material and two faint bands of slightly greater mobility. A dark band at 118000 M_r marked the appearance of the putative 'link' component. The pattern of SM differed, however, since, before reduction, there was only a faint smear at the top of the gel, indicative of less polymeric mucin, and a prominent broad band having a midpoint

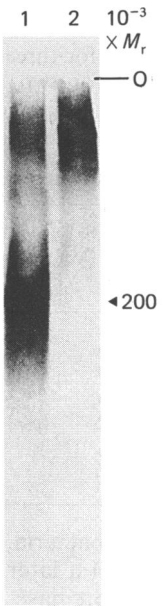

Fig. 2. Western blot of IM and SM

Approx. 3 μg (protein) of SM (track 1) and IM (track 2) were subjected to composite-gel electrophoresis under non-reducing conditions, followed by Western blot, using the antibody to intracellular mucin. O represents the origin of the gel.

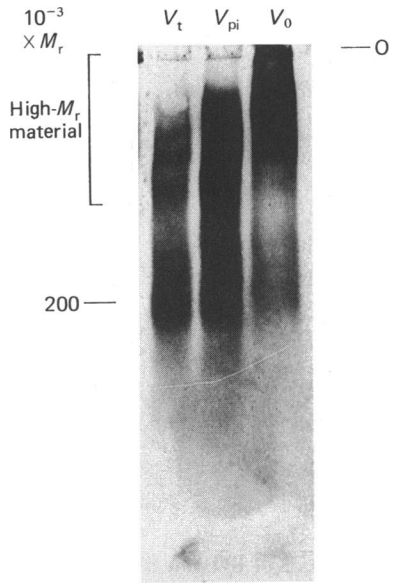

Fig. 3. Western blot of fractions from Sepharose CL 2B chromatography of SM

Approx. 3 μg of protein each of the void volume (V_0), partially included (V_{pi}) and totally included fractions (V_t) of Sepharose CL 2B were subjected to composite agarose/PAGE under non-reducing conditions, followed by Western blot, using the antibody to intracellular mucin. O represents the origin of the gel.

position corresponding to an M_r of about 200000. Some experiments with SM (non-reduced) also showed a faint band at 118000 M_r, although this is not shown in Fig. 1. After reduction, a faint band was seen near the top of the gel, the 200000-M_r band almost disappeared, and a band at 118000 M_r became prominent (Fig. 1, track 4). No other low-M_r bands were seen. Thus the 200000-M_r band was considered as the probable source of the 118000-M_r 'link' component in SM.

Since the 200000-M_r band appeared to be the major component of SM, experiments were undertaken to exclude the possibility of contamination and to explain the relationship of this band to the 118000-M_r 'link' component of IM. Both IM and SM (non-reduced) were subjected to a Western blot using a polyclonal antibody specific for rat intestinal mucin (IM) (Forstner *et al.*, 1977). Fig. 2 shows that both mucins, including the 200000-M_r band of SM, were reactive, suggesting that the 200000-M_r band was a mucin component rather than a high-M_r contaminant in the SM preparation.

In an attempt to isolate and analyse the 200000-M_r component and compare the two mucins more extensively, both IM and SM (1 h secretion samples) were subjected to Sepharose CL-2B chromatography (Table 3). As expected, IM appeared mainly in the void volume (V_0), with a shoulder trailing into the partially included volume (V_{pi}). In keeping with its greater mobility on SDS/PAGE and composite gels and presumed smaller size, SM showed a much greater retention by the column. About 60–70% of SM emerged in V_{pi} and almost 30% in the total volume (V_t) of the column. The SM column

Table 3. Distribution of purified IM and SM on Sepharose CL 2B

IM and SM (1 h) samples were purified by CsCl-density-gradient ultracentrifugation as described in the Materials and methods section and and then subjected to Sepharose CL 2B column (0.9 cm × 55 cm) chromatography. Carbohydrate was calculated by g.l.c. analyses, mucin by radioimmunoassay, and protein by amino acid analyses of mucin fractions. V_0, V_{pi} and V_t refer to void (5–12 ml), partially included (13–20 ml) and total (21–31 ml) volumes respectively.

	Distribution (%)					
	IM			SM		
Component	V_0	V_{pi}	V_t	V_0	V_{pi}	V_t
Carbohydrate (%)	65	29	6	9	60	31
Mucin (%)	89	11	Trace	2.6	70	27.4
Carbohydrate/protein ratio (w/w) ...	3.2	4.7	0.5	2.8	7.2	1.9

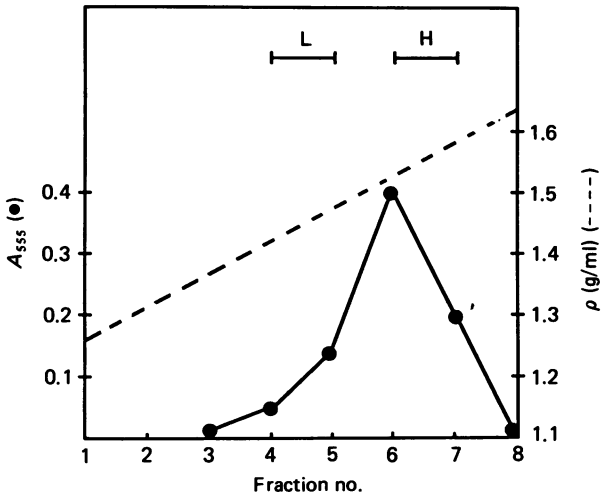

Fig. 4. CsCl-density-gradient ultracentrifugation of SM

Secretions from intestinal loops were mixed with CsCl to a density of 1.4 g/ml and subjected to CsCl-density-gradient ultracentrifugation. Fractions 4–7 (ρ = 1.35–1.55 g/ml) from the first run were subjected to a second CsCl-density-gradient ultracentrifugation. The resulting gradient (shown as the broken line above) was divided into eight equal parts and assayed for carbohydrate by PAS assay (A_{555}). L and H represent the light (ρ = 1.38 g/ml) and heavy (ρ = 1.50 g/ml) density fractions respectively.

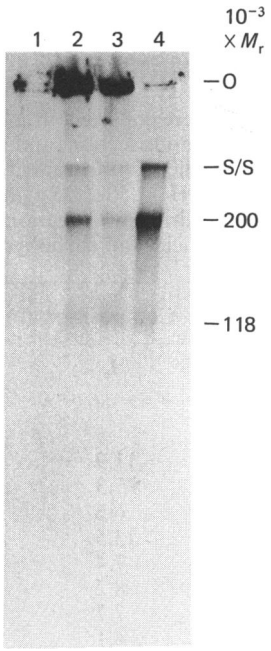

Fig. 5. Western blot of IM, SM and heavy (H) and light (L) density species of SM, using the antibody to IM

Aliquots (~ 3 μg of protein) of IM (track 1), SM (track 2), H species (ρ = 1.5 g/ml, track 3) and L species (ρ = 1.38 g/ml, track 4) of SM were subjected to SDS/7.5%-(w/v)-PAGE under non-reducing conditions, followed by Western blot. O represents the origin of the gel and S/S represents the interface between the stack and separating gels.

fractions were then subjected to gel electrophoresis and immunoblotting (Fig. 3) in an attempt to identify a fraction highly enriched in the 200000-M_r band. The V_0 fraction contained mainly high-M_r components, although a small amount of the 200000-M_r band was also observed. The V_{pi} fraction, which contained most of the mucin (Table 3) appeared to have high-M_r and 200000-M_r bands. The V_t fraction was enriched in the 200000-M_r band, but still was not free of higher-M_r material. Thus CL-2B chromatography was not effective in separating the 200000-M_r band in SM from higher-M_r mucin components. Amino acid and carbohydrate analyses of each column fraction (results not shown) indicated that the V_t was enriched in the 'minor' amino acids and had a lower carbohydrate/protein ratio (Table 3) than the other two fractions. We therefore considered that the 200000-M_r component would be less dense than the rest of the mucin and could best be separated by density-gradient ultracentrifugation. Accordingly, during the purification of SM by d.g.u. (second centrifugation step), we collected a light (L) species (ρ = 1.38 g/ml) and a heavy (H) species (ρ = 1.50 g/ml) (Fig. 4). SDS/PAGE of the H and L species (as well as intact SM and IM) was performed and gels were subjected to Western blots (Fig. 5). As expected, IM (track 1) barely penetrated the stacking gel, SM (track 2) entered the stacking gel somewhat more readily, and gave a band at 200000 M_r as well as a faint band at 118000 M_r. There was also a faint band seen at the interface between the stacking and separating gels which was not investigated further. The heavy (H) species (track 3) of SM was found mainly in the stacking gel. In contrast, when an equivalent concentration (3 μg of protein) of the light (L) species was applied, most of it appeared at the 200000-M_r position, with a smaller amount at the interface. Thus the L species was enriched in the 200000-M_r component.

Compositional analyses of the L and H species of secreted mucin showed striking differences (Table 4). The L species was much less glycosylated, had a much higher proportion of 'minor' amino acids and a much lower proportion of the 'major' mucin amino acids. The L species also contained more mannose and GlcNAc with less fucose, galactose and GalNAc. The most notable feature of the species, however, was that it resembled in composition (particularly in amino acid profile) the 118000-M_r 'link' glycopeptide isolated from reduced native (IM) mucin (Fahim *et al.*, 1983; Table 5).

The similarity of the L species and 118000-M_r 'link' glycopeptide was further tested immunologically by SDS/PAGE and Western blots of SM, H and L species using an affinity-purified antibody monospecific for the 118000-M_r component of IM (Fahim *et al.*, 1987). Fig. 6(*a*) shows that, before reduction of SM and the H species, immunoreactivity appeared in the stacking gel and at positions corresponding to 200000 and 118000 M_r. For the L species the only bands visible were at 200000 and 118000 M_r. After reduction (Fig. 6*b*), the only immunoreactive band in all three samples was at a position corresponding to 118000 M_r. Thus the 200000- and 118000-M_r components are antigenically similar, and, with reduction, the 200000-M_r component appears to generate the 118000-M_r component.

The 200000- was separated from the 118000-M_r component in the L species by elution in the V_0 of Bio-Gel P-100 columns. The amino acid and carbohydrate profiles of the 200000-M_r component were compared with those of the 118000-M_r 'link' glycopeptide of

Table 4. Amino acid (*a*) and carbohydrate (*b*) compositions of heavy (H; $\rho = 1.50$ g/ml) and light (L; $\rho = 1.38$ g/ml) density species of SM

Intestinal secretions were collected after 4 h as described in the Materials and methods section, and the mucin purified by CsCl-density-gradient ultracentrifugation. The L (1.38–1.40 g/ml) and H (1.50–1.55 g/ml) density species from the second ultracentrifugation were analysed separately for amino acid and carbohydrate.

Amino acid or sugar	Composition (mol/100 mol)	
	L	H
(*a*) Amino acid		
Asp	11.9	8.8
Thr	7.3	21.4
Ser	6.6	11.8
Glu	13.5	9.8
Pro	7.4	13.0
Gly	8.2	7.1
Ala	7.7	5.0
Val	4.1	4.5
Met	1.3	1.3
Ile	2.8	2.8
Leu	8.4	5.3
Tyr	3.1	0.9
Phe	4.7	1.7
His	2.9	1.9
Lys	6.4	3.7
Arg	3.7	1.0
(*b*) Sugar		
Fucose	8.0	14.0
Galactose	17.4	24.3
GlcNAc	24.7	20.0
GalNAc	18.7	26.4
Sialic acid	15.4	12.4
Mannose	15.8	2.9
Carbohydrate/ protein ratio (w/w) ...	0.7	4.6

Table 5. Amino acid (*a*) and carbohydrate (*b*) analyses of the 200000- and the 118000-M_r components

The 200000-M_r component was purified from the L density species of SM by Bio-Gel P-100 chromatography. The 118000-M_r component was purified from IM as described in the Materials and methods section. N.D., not determined.

Amino acid or sugar	Composition (mol/100 mol)	
	200000 M_r	118000 M_r
(*a*) Amino acid		
Asp	11.2	11.0
Thr	9.2	8.2
Ser	10.1	8.5
Glu	12.3	13.0
Pro	6.1	6.3
Gly	11.0	9.0
Ala	7.0	6.0
Val	5.4	4.0
½-Cys	N.D.	4.1
Met	1.6	0.8
Ile	3.8	4.2
Leu	7.4	8.2
Tyr	2.0	2.5
Phe	3.6	4.6
His	1.8	2.8
Lys	4.3	3.6
Arg	3.2	3.2
(*b*) Sugar		
Fucose	10.3	10.1
Galactose	25.2	21.6
GlcNAc	26.8	30.2
GalNAc	21.3	22.1
Sialic acid	8.1	6.0
Mannose	8.3	10.0
Carbohydrate/ protein ratio (w/w) ...	0.8	0.9

intracellular mucin (Table 5). The similarity in composition of the two was striking, including the relative proportions of threonine, serine and proline (approx. 24% total for both), aspartic acid, glutamic acid, glycine and alanine (approx. 41% for both) and carbohydrate, including mannose (8–10%). Thus the purified 200000- and 118000-M_r glycopeptides were virtually identical in composition. Our results indicate that the secreted 200000-M_r component is a disulphide-linked dimer of the 118000-M_r 'link' glycopeptide.

DISCUSSION

Our electrophoretic compositional and chromatographic analyses revealed that SM differed in many respects from IM. Even though IM is likely to contain a small amount of SM, which remains adherent to the mucosa before purification of IM (Forstner *et al.*, 1981), the two mucins were nevertheless quite different. SM was more included on CL-2B columns and entered gels more readily during electrophoresis, producing a smear rather than discrete bands near the origin. These features indicated that SM was smaller than IM and suggested partial depolymerization of SM (Allen, 1981). The most striking difference, however, was the appearance in SM, but not in IM, of a prominent glycopeptide band having an apparent M_r of about 200000. This glycopeptide could be separated from the heavier, more-glycosylated, mucin components by density-gradient ultracentrifugation. Compositional analyses were virtually identical with those of the 118000-M_r 'link' glycopeptide of IM. The close relationship between the two glycopeptides was confirmed by Western blots, which demonstrated that the 200000-M_r band reacted with an antibody previously shown to be specific for the 118000-M_r 'link' glycopeptide of IM (Fahim *et al.*, 1987). After thiol reduction, the 200000-M_r band gave rise to the 118000-M_r band on gel electrophoresis. Thus the 200000-M_r band appears to be an S–S-bonded dimer of the 118000-M_r component.

The mechanism by which the 200000-M_r component appears in SM is not known, but at least three possible explanations can be considered. Shortly after secretion of IM, the putative 'link' glycopeptide of 118000-M_r may be liberated from mucin by luminal proteinases, and then undergo spontaneously disulphide-mediated dimerization to form a 200000-M_r component. Earlier experiments showed that brief exposure of purified IM to trypsin or Pronase could in fact release a component of

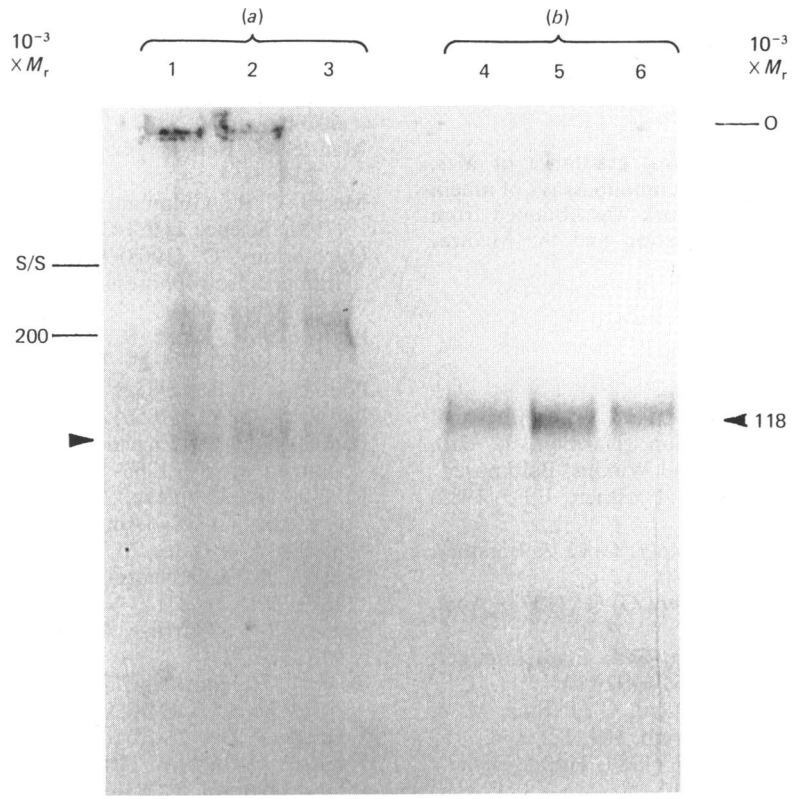

Fig. 6. Western blot of H and L density species of SM, using an antibody specific for the 118000-M_r component of IM

Secreted mucin (ρ = 1.35–1.55 g/ml, tracks 1 and 4), H species (track 2 and 5) and the L species (track 3 and 6) were subjected to SDS/PAGE and Western blot before (*a*) and after (*b*) thiol reduction. O represents the origin of the gel, S/S represents the interface between the stack and separating gels, and the arrowhead on the left (with no value) points to the position of a band in secreted mucin (non-reduced) having a mobility corresponding to an M_r of approx. 118000.

M_r 118000 (Fahim *et al.*, 1987). However, no evidence of spontaneous conversion into a component of M_r 200000 was noted in these experiments or in the present study.

A second possibility is that the 200000-M_r component forms at an early stage of mucin biosynthesis as a dimer of the 118000-M_r component, and some of it is secreted independently from goblet cells before it can be further attached through disulphide bonds to the larger mucin glycopeptides. Since no information is available concerning the biosynthetic events responsible for the mucin 'link' component, this explanation awaits further experiments for validation.

A third possibility, but one that would not account for all the changes in SM (see below) is that the 200000-M_r dimer is synthesized and fully incorporated into the mucin within goblet cells, but during secretion some of the 200000-M_r glycopeptide is liberated from the mucin by the selective action of proteinases. This hypothesis implies that at each end of the 200000-M_r glycopeptide, near terminal disulphide bridges, there are peptide bonds highly sensitive to proteolytic cleavage, such that the 200000-M_r glycopeptide is released in a relatively intact state. The diffuseness of the 200000-M_r band on gels suggests considerable heterogeneity in the size of the liberated product, possibly indicating that several cleavage sites are involved.

Even if IM releases a 200000-M_r component during or after secretion, this alone does not explain fully why the overall composition of purified SM was different from that of IM (Tables 1 and 2). The amino acid and carbohydrate differences that we observed suggest that there is an increased proportion of the 200000-M_r component in SM relative to the proportion of 118000-M_r 'link' glycopeptide in IM. This implies that SM is not fully representative of mucin contained within goblet cells and makes more attractive the notion of independent secretion of the 200000-M_r glycopeptide. In addition, however, intestinal goblet cells and their products are extremely heterogeneous (Podolsky, 1985*a*,*b*, Podolsky *et al.*, 1986), and therefore it is also possible that a discrete subpopulation of goblet cells or storage granules (Specian & Neutra, 1984) enriched in mucin containing the 200000-M_r component is selected for secretion under resting conditions.

Even at the earliest time (1 h) that we were able to examine mucin in these studies, SM gave evidence of being smaller than IM. This finding serves to focus attention on the possibility that goblet-cell mucin normally remains as a polymeric gel or in a 'protective' form for only a short period after secretion. Generation of the 200000-M_r glycopeptide by proteolysis of the mucin or by other mechanisms appears to occur rapidly, and may represent the earliest stage of mucin degradation and solubilization. Thus the susceptibility of the 'link' region of mucin to enzymic cleavage or to independent

secretion may govern the extent to which secreted mucin offers protection of the mucosal surface against potentially injurious luminal agents or micro-organisms.

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