Heterogeneity of rat goblet-cell mucin before and after reduction

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Goblet-cell mucin of rat small intestine was purified from mucosal scrapings by using centrifugation, Sepharose 4B and Sepharose 2B chromatography. The mucin was applied in low concentrations (1 µg/track) to slab gels containing 0.5% agarose/2% (w/v) polyacrylamide, and bands were detected after electrophoresis by silver stain or by fluorography of ³H-labelled mucin. Before reduction the mucin contained three distinct components: a polymeric species at the top of the gel and two large glycoproteins of higher mobility. After reduction, the polymer disappeared, the two glycoproteins remained unchanged, and two glycopeptide bands of higher mobility appeared. In addition, a non-glycosylated, heavily stained peptide of mol.wt. 118 000 was detected. The individual mucin components were partially separated on Sepharose 2B, 0.2 M-NaCl/1% sodium dodecyl sulphate being used as eluant. Individual amino acid and carbohydrate analyses suggested that the glycosylated components, despite their differences in size, had identical profiles. The 118 000-mol.wt. peptide had a very different amino acid profile, with much less serine, threonine and proline. Glycine and aspartic and glutamic acids comprised 34% of the total amino acids. Thus the 'native' mucin is a heterogeneous structure containing at least two non-covalently associated glycoproteins plus polymeric material. The latter is stabilized by disulphide bonds and consists of several glycopeptides of different size as well as a 'link' peptide of mol.wt. 118 000.

Mucus glycoproteins (mucins) are polydisperse molecules of high molecular weight ($\sim 2 \times 10^6$). Although secreted from different sites (salivary glands, respiratory, gastrointestinal and reproductive tracts), they serve common basic functions as a lubricant and protective layer for the underlying epithelial cells. There is considerable controversy and confusion about the extent of structural heterogeneity in 'native' mucins. Most investigators consider that true heterogeneity does not exist, but that mucins exhibit polydispersity due to a natural variation in the number, linkage and composition of sugars within mucin oligosaccharides (Gibbons, 1963; Allen, 1978). In consequence, mucins produce broad, but unimodal, sedimentation-velocity profiles (Scawen & Allen, 1977), a broad range of molecular-weight estimates (Mantle *et al.*, 1981), very little entry and 'smearing' in agarose or acrylamide gels (Forstner *et al.*, 1973; Holden *et al.*, 1971; Hatcher *et al.*, 1977) and a tendency to elicit more than one antibody when mucins are used as antigens

(Gold *et al.*, 1981; Bhushana Rao *et al.*, 1973; Jabbal *et al.*, 1976).

With thiol reduction, many mucins undergo depolymerization (e.g., Holden *et al.*, 1971; Hatcher *et al.*, 1977; Creeth *et al.*, 1977; Roberts, 1978; Pearson *et al.*, 1980; Mantle *et al.*, 1981), liberating smaller components or subunits with molecular weights ranging from 250 000 to 600 000 (Roberts, 1978; Meyer, 1977; Allen, 1978; Mantle *et al.*, 1981) that nevertheless continue to exhibit considerable polydispersity of size and density (Mantle *et al.*, 1981). During reduction of some mucins, non-glycosylated peptides are released (Pearson *et al.*, 1981; Creeth *et al.*, 1977; Mantle *et al.*, 1981) and it is assumed therefore that these peptides account for the linking of mucin monomers within the native polymeric mucin.

Although this general scheme is consistent with data obtained by ultracentrifugation and gel-exclusion chromatography, its validity is clouded by the strong tendency of mucins to self-aggregate, even at low concentrations (Gibbons, 1972), the difficulty in establishing the ratio of monomeric species within

Abbreviation used: SDS, sodium dodecyl sulphate.

the average mucin polymer, and the relative insensitivity of these methods for separating molecules of closely related size.

In an attempt to improve resolution and characterization of the components of native and reduced mucins, we have examined the behaviour of a model 'native' mucin from rat intestinal goblet cells in a composite SDS/agarose/polyacrylamide-gel system. The outstanding sensitivity of the silver-staining technique and fluorography have been combined to permit resolution at very low mucin concentrations. Our results indicate that the native mucin consists of a mixture of at least two discrete monomeric species plus a disulphide-bond dependent polymer. The polymer was degraded after reduction and produced at least two smaller glycopeptides and a non-glycosylated 'link' peptide having mol.wt. 118000. Partial separation of mucin components was achieved on Sepharose 2B columns by using elution with 1% SDS in 0.2M-NaCl. Analyses of electrophoretic mobility, amino acid and carbohydrate profiles of each component suggests that the glycoprotein constituents of the mucin are all very similar in composition despite their heterogeneity of size. The 'link' peptide clearly differed, however, from the core protein of the rest of the mucin components, and thus appears to be a distinctly different peptide.

Materials and methods

Rat goblet-cell mucin was prepared as described earlier (Forstner *et al.*, 1973) with some modifications. Briefly, rat intestinal mucosal scrapings were homogenized in 5mM-EDTA, pH 7, containing 2mM-phenylmethanesulphonyl fluoride, centrifuged at 30000g for 30min, and the supernatant applied to a Sepharose 4B column (140cm × 4.2cm). The void-volume peak was pooled, dialysed against water, concentrated and incubated for 24h at room temperature with 250 units of ribonuclease A (type X-A) and 250 units of deoxyribonuclease I (type I) (Sigma, St. Louis, MO, U.S.A.) per mg of mucin protein in 0.01M-Na₂HPO₄/NaH₂PO₄/0.01M-MgCl₂/0.02% NaN₃, pH 7.4. The mixture was then rechromatographed on a Sepharose CL 2B (110cm × 2.2cm) column. The void-volume peak containing the mucin was pooled and concentrated.

Electrophoresis of mucin on agarose/polyacrylamide composite gels

Gels of 0.5% agarose and 2% polyacrylamide were prepared by the method of Peacock & Dingman (1968), with the following modifications: the stock buffer consisted of 181.7g of Trizma base, 98.2g of boric acid and 10g of SDS in a final volume of 1 litre, pH 8.6. The bisacrylamide/

acrylamide ratio was 1:37. After electrophoresis the gel was stained with the silver-stain method of Switzer *et al.* (1979).

Radioactive labelling of mucin

A modification of the method of Gahmberg & Hakomori (1973), for labelling erythrocyte membranes, was used. Mucin (containing 200µg of protein) was dissolved in 0.1M-Na₂HPO₄/NaH₂PO₄, pH 5, and treated with 1 unit of neuraminidase for 1h at 37°C. After incubation, the pH of the solution was adjusted to pH 7.4 with 0.1M-NaOH, and NaCl was added to a final concentration of 0.15M. Galactose oxidase (50 units) was added, together with 1% toluene, and the mixture incubated at room temperature for 24h. Another 50 units of the enzyme was then added and the incubation continued for an additional 24h. To the oxidized mucin was added 10mCi of freshly prepared NaB³H₄ in 0.1M-Na₂HPO₄/NaH₂PO₄ in 0.15M NaCl, pH 7.4. After 30min at room temperature with occasional shaking, 1mg of non-radioactive NaBH₄ was added to complete the reduction process. The whole mixture was then applied to a column (1cm × 20cm) of Sepharose CL 6B to separate the enzymes, NaBH₄ and free radioactivity from the labelled mucin, which eluted in the void volume.

Fluorography of ³H-labelled mucin on slab gels

The labelled mucin was electrophoresed as described above, and stained with silver stain. The gels were then soaked in En³Hance (New England Nuclear Corp., Boston, MA, U.S.A.) for 1h, followed by water for another hour, as described in the New England Nuclear Corp. product booklet. The gel was dried on a Bio-Rad drier (model 224) for 1h. A Kodak X-Omat R film was pressed firmly to the gel and incubated at -70°C for approx. 16h.

Reduction of the mucin

Disulphide bonds were reduced by incubating mucin in a water bath at 100°C for 5min in 2.5% (v/v) 2-mercaptoethanol/1% SDS, pH 8.6. In experiments involving subsequent gel-filtration chromatography, the mucin was alkylated after reduction by incubating it in the dark overnight at 4°C with 0.4M-iodoacetamide. The mixture was then dialysed exhaustively against water and concentrated before applying to a Sepharose CL 2B column.

Gel-filtration chromatography

In all experiments involving fractionation of the reduced mucin, samples were chromatographed on a long, narrow (1.2cm × 100cm) Sepharose CL 2B column. The column was eluted with 0.2M-NaCl with or without 1% SDS. Column eluate was monitored for A₂₈₀ and by the periodic acid/Schiff assay method of Mantle & Allen (1978). For

analytical purposes SDS was removed by ion-pair extraction by the method of Henderson *et al.* (1979).

Sugar analyses

Carbohydrates were analysed in a Varian Aerograph series 2100 g.l.c. analyser by the method of Zanetta *et al.* (1972). Deoxyribose was assayed by the fluorimetric method of Hinegardner (1971).

Amino acid analysis

Analyses were performed on a Durrum model D-500 analyser after hydrolysis of the samples in 5.5 M constant-boiling HCl at 110°C for 16 h.

Results

Composition of purified goblet-cell mucin

Tables 1 and 2 show the carbohydrate and amino acid compositions respectively of native purified rat goblet-cell mucin. The uniquely high content of threonine, serine and proline is typical of purified epithelial mucins, including many isolated from the gastrointestinal tract of several species (Schrager & Oates, 1971; Forstner *et al.*, 1973; Scawen & Allen, 1977; Jabbal *et al.*, 1976). No mannose, uronic acid, ribose or deoxyribose were detected, indicating that the preparation was free of serum glycoproteins, mucopolysaccharides and nucleic acids. Previous studies indicated that the mucin was free of lipid, that carbohydrate made up 85% of the mucin by weight, and the average mol.wt. was 2×10^6 (Forstner *et al.*, 1973).

Electrophoresis of mucin

In preliminary experiments the mucin was electrophoresed on composite agarose (0.5%)/polyacrylamide (2%) gels (10–50 µg of protein per track), since it was reported previously (Peacock & Dingman, 1968) that such a gel is suitable for studies of nucleic acids having a molecular weight as high as 10^8 . As monitored by staining with Coomassie Blue and periodic acid/Schiff reagent, however, the mucin sample did not enter, except for a faint smear 0.2–0.3 mm inside the top of the gel (results not shown). There were no other protein or glycoprotein bands detected, indicating freedom from contaminating proteins or smaller glycoproteins. To avoid concentration-dependent visco-elastic properties of the mucin (Gibbons, 1972; Holden *et al.*, 1971), we considered that better electrophoretic separation might be obtained by decreasing the concentration of the applied sample. To detect bands we employed the silver stain for protein which has been reported by Switzer *et al.* (1979) to be 100 times more sensitive than Coomassie Blue. Fig. 1 shows the pattern obtained with electrophoresis of 1 µg of mucin (protein) on SDS-containing composite gels. Intact, unreduced mucin (track a) showed a broad

Table 1. Carbohydrate analyses of native and reduced rat intestinal mucin

Analyses were performed on a Varian Aerograph series 2100 using the trifluoroacetic method of Zanetta *et al.* (1972). Each value is the mean for two separate analyses, results of which were almost identical. Peaks 1, 2 and 3 were obtained after chromatography of reduced/alkylated mucin on Sepharose 2B as described in Fig. 6.

Sugar	Content (mol/100 mol)			
	Native mucin	Reduced mucin		
		Peak 1	Peak 2	Peak 3
Fuc	14.1	16.0	15.7	15.2
Gal	27.2	28.8	29.4	29.5
GlcNAc	21.8	23.0	22.7	21.0
GalNAc	20.9	18.9	19.8	22.3
AcNeu	16.0	13.3	12.4	12.0

Table 2. Amino acid analyses of native and reduced rat intestinal mucin

Analyses were performed on a Durrum analyser model D-500, after hydrolysis of the samples in 5.5 M-constant-boiling HCl at 110°C for 16 h. Cysteine and tryptophan were not estimated. Values are the means for four separate analyses of each preparation. Peaks 1, 2, 3, 4 were obtained after chromatography of reduced/alkylated mucin on Sepharose 2B as described in Fig. 6.

Amino acid	Content (mol/100 mol)				
	Native mucin	Reduced mucin			
		Peak 1	Peak 2	Peak 3	Peak 4
Asp	4.8	2.8	2.5	4.0	11.5
Thr	30.7	39.3	39.5	32.8	8.5
Ser	13.6	17.6	16.2	14.8	8.8
Glu	6.7	4.4	4.6	5.6	13.5
Pro	10.8	17.6	19.0	15.6	6.5
Gly	7.3	4.6	4.5	8.0	9.3
Ala	3.5	2.0	2.0	2.7	6.3
Val	2.5	1.6	2.0	2.0	4.2
Met	0.5	0.4	0.3	0.5	0.9
Ile	3.9	2.1	1.8	2.2	4.4
Leu	5.1	2.0	2.2	4.4	8.5
Tyr	2.7	0.8	0.8	1.5	2.6
Phe	3.0	0.9	0.6	0.8	4.8
His	2.3	1.1	0.9	1.5	3.0
Lys	1.5	1.8	1.5	1.8	3.8
Arg	1.1	1.0	1.6	1.8	3.4

band, a₁, on top of the gel, followed by two other bands, a₂ and a₃, of higher mobility. Continuous with a₃ there was a faint smear. Further down the gel there was usually a very faint band, a₆ (mol.wt.

approx. 200 000), although it was never clear on photographs. Because this band was so faint, no further investigations on it were carried out.

As expected, the resolution of the bands depended upon the concentration of applied sample (Fig. 2) with the best resolution being achieved with 1–2 μ g

of mucin (protein). Boiling for 2–5 min and/or pretreatment of the mucin with 1% SDS did not alter the pattern of the bands, indicating that no significant degradation or dissociation of the mucin had been induced. Taken altogether these results indicated that the 'native' mucin consists of at least three molecular-weight species of glycoprotein separable on gels.

We then examined the electrophoretic profile of rat goblet-cell mucin after reduction with mercaptoethanol. In previous work we noted that 0.01 M-dithiothreitol (37°C, 1–4 h) had little effect on human (Forstner *et al.*, 1979) or rat (Forstner *et al.*, 1973) intestinal mucin. In the present study, however, we used harsher conditions (100°C for 5 min in 2.5% mercaptoethanol and 1% SDS, pH 8.6). The reduced mucin (track b in Fig. 1) showed a loss of most of the heavily stained band designated a_1 in the intact mucin. The other two heavily stained bands (b_2 and b_3) appeared to be equivalent to a_2 and a_3 of the unreduced mucin, although both had a slightly increased mobility. These bands apparently represent glycoproteins that are not polymerized by a thiol-dependent mechanism in the native mucin. Other changes with reduction included the appearance of two minor bands, b_4 and b_5 , and a heavily stained broad band b_7 , of much greater mobility, having no counterpart in the unreduced mucin. By using a series of molecular-weight standards ranging from 14 300 to 200 000 (Bio-Rad Laboratories), the molecular weight of band b_7 was judged to be 118 000. The other bands in track b had apparent molecular weights of more than 200 000.

To discover whether the bands were actually glycoprotein in nature, we labelled the native mucin radioactively with ^3H on its terminal galactose and *N*-acetylgalactosamine residues, and then repeated electrophoresis before and after thiol reduction. Fluorography of the unreduced mucin (Fig. 3, track a) showed that the position of the radioactive bands corresponded to those seen in Fig. 1, track a. After reduction (Fig. 3, track b) the resulting bands corresponded to those seen in Fig. 1, track b, except for the absence of b_7 (the 118 000-mol.wt.

Fig. 1. Electrophoresis of rat intestinal mucin on 0.5% agarose/2% acrylamide composite slab gels with detection by silver stain

Samples of approx. 1 μ g of mucin protein/track were applied after heating at 100°C for 5 min in 1% SDS, pH 8.6, without 2-mercaptoethanol (a), and with 2.5% 2-mercaptoethanol (b). The gel was electrophoresed for 4 h at 25 mA. —0— represents the origin and the numbers bearing a kDa suffix show the positions of the molecular-mass markers.

Fig. 2. Effect of sample concentration on the electrophoresis of rat intestinal mucin on 0.5% agarose/2% acrylamide composite slab gels

Duplicate samples of the amounts (μ g of mucin protein applied) shown on the Figure were electrophoresed after heating at 100°C for 3 min in 1% SDS, pH 8.6. The gel was run at 25 mA for 5 h.

Fig. 3. Electrophoresis of ^3H -labelled rat intestinal mucin on 0.5% agarose/2% acrylamide composite gels with detection by fluorography

Samples of approx. $1\text{ }\mu\text{g}$ of mucin protein/track were applied after heating at 100°C for 4 min in 1% SDS, pH 8.6, without mercaptoethanol (a), and with 2.5% 2-mercaptoethanol (b). The gel was run for 4 h at 25 mA. —0— represents the origin. Details of the radioactive labelling of the mucin are given in the Materials and methods section.

band) and the minor band, b_7 . Since band b_3 was not very prominent even with silver stain (Fig. 1), it seemed possible (and was confirmed later) that its concentration was so low that fluorography was simply not sensitive enough to detect the sugars in it. In contrast, b_7 was heavily stained with silver (Fig. 1) and therefore failure to define it by fluorography suggests that it was mainly protein.

Sephacrose 2B column chromatography of mucin before and after reduction

To understand the basis of the observed heterogeneity, we attempted to isolate the different mucin components for separate analysis. For these experiments, another mucin preparation was used which was identical in every way with the former preparation, except that after reduction it had a somewhat more pronounced b_3 band on gel electrophoresis. The mucin (1 mg of protein) was applied to a long narrow ($1.2\text{ cm} \times 100\text{ cm}$) Sepharose CL 2B column, before and after reduction/alkylation, and eluted with 0.2 M-NaCl . The profile of unreduced mucin (Fig. 4) shows that all of the sample eluted as an excluded peak, with a tailing minor fraction partially included. After reduction and alkylation four peaks were detected, three of which contained glycoprotein components as judged by periodate/Schiff reactivity (Fig. 5).

Samples of each of the four peaks were electrophoresed on composite gels. Tracings of the gel patterns (Fig. 5) demonstrate that separation of mucin components on Sepharose 2B was incomplete. The most surprising finding was the presence of the b_7 118000-mol.wt. peptide, together with the

Fig. 4. Gel filtration on Sepharose CL 2B of rat intestinal goblet-cell mucin (untreated) as detected by periodic acid/Schiff stain for sugar (●) and A_{280} (O). V_0 and V_t are the excluded and total volumes respectively.

large glycoproteins (b_2 , b_3 , b_4) in the excluded and partly included volumes of the Sepharose 2B column. Apparently strong non-covalent interaction between the glycoproteins and the b_7 peptide prevented the latter from entering the gel pores of the Sepharose 2B column.

Sepharose 2B chromatography of reduced/alkylated mucin was therefore repeated with 1% SDS in the elution salt. Again four peaks were detected. (A periodate/Schiff profile was not done because of the presence of SDS, which interferes with the assay). Much better separation of mucin components was achieved, and in particular SDS caused b_7 to be separated from the glycoprotein contents of peaks 1 and 2. Peak 4 consisted exclusively of b_7 , whereas peak 3 was almost solely b_3 , although slightly contaminated by b_7 . Peak 2 was highly enriched in component b_3 , but also contained some b_2 and b_4 components. Peak 1 was enriched in b_2 but contained minor traces of b_3 and b_1 . As yet we have not been able to achieve total separation of the two main glycoprotein components b_2 and b_3 .

Analysis of Sepharose 2B fractions

After concentration of each peak and removal of SDS (see the Materials and methods section) carbohydrate and amino acid analyses were performed. The sugar profile (Table 1) of the 'native' mucin and peaks 1, 2 and 3 were virtually identical. The data of peak 3 confirmed that band b_3 was glycoprotein in nature. However, as expected from the fluorography data of Fig. 3, the fourth peak did not contain sugar, and thus b_7 is a non-glycosylated peptide released from the native mucin by thiol reduction.

Results of the amino acid analyses are presented in Table 2. The profile of peak 4 was dramatically different from the other peaks. The threonine, serine

Fig. 5. Gel filtration on Sepharose CL 2B of reduced and alkylated rat goblet-cell mucin detected by periodic acid/Schiff staining (●) and A_{280} (○)

Tracings above each peak are given for gel electrophoresis on composite agarose/acrylamide gels stained with silver stain.

and proline contents were only about one-third those of the other peaks, and contents of the acidic amino acids, as well as alanine, valine, leucine, isoleucine, tyrosine and phenylalanine, more than doubled. Clearly, peak 4 represented a different peptide to that of the glycoproteins in peaks 1 and 2. The profile of peak 3 reflected its slight contamination by b_7 . Peak 2 was identical with peak 1, both having serine, threonine and proline altogether comprising about 75% of the total amino acids. Thus, despite their differences in size, the large glycoproteins, b_2 and b_3 (and the minor glycopeptides b_4 and b_5), appear to have the same peptide core and carbohydrate profile.

Discussion

The present study was performed on rat goblet-cell mucin isolated from small-intestinal scrapings. Previous work had shown this material to exhibit no cross-reactivity against rat serum proteins, secretory immunoglobulin A, or brush-border-membrane glycoproteins (Forstner *et al.*, 1977). Chemical analyses and composite agarose/polyacrylamide gels in the present study also indicated no evidence of contamination. Band ultracentrifugation profiles indicated a single but polydisperse peak of sedimentation coefficient 16 S (Jabbal *et al.*, 1975). Thus, by several criteria, the 'native' mucin appears to be highly purified.

When the native mucin was applied in low

concentrations (1 to 2 μ g of protein) to composite SDS/agarose/polyacrylamide gels, it displayed evidence of at least three molecular-weight species: a glycoprotein polymer (a_1) that was susceptible to thiol reduction and dissociation, and two large glycoproteins (a_2 and a_3) that resisted a change in size with reduction. In addition to these components, the 'native' mucin contained a non-glycosylated peptide (b_7) and two minor glycopeptides (b_4 and b_5), all of which appeared after thiol reduction, and were therefore likely released from the polymer a_1 .

The two large glycoproteins appear to be intimately involved with the polymeric species, since they were initially present in the void volume of Sepharose 2B along with the polymer. After dissociation of the polymer by thiol reduction, the two glycoproteins did not change in size but entered the Sepharose 2B matrix, particularly when SDS was included in the eluting solution. Thus the polymer of the 'native' mucin exhibits strong non-covalent interactions with these glycoproteins. It may be that molecular entanglement is a more important non-covalent force than specific chemical interactions in the unreduced form.

We cannot rigorously exclude the possibility that the non-polymerized species (a_2 and a_3) were produced by spontaneous reduction of the polymer during the preparation of the mucin. If so, however, the glycosylated peptide (b_7) ought to have been released as well. As shown in Figs. 5 and 6, some of the non-glycosylated peptide remains bound to the glycoprotein species unless removed by dissociating

Fig. 6. Gel filtration on Sepharose CL 2B of reduced and alkylated rat goblet-cell mucin detected by A_{280} (●). The column was eluted with 1% SDS in 0.2M-NaCl and 0.02% NaN_3 . Tracings are given for gel electrophoresis on composite agarose/acrylamide, stained with silver stain.

agents, and therefore one might have expected to find evidence of it on electrophoresis of the 'native' mucin in SDS. The fact that no free peptide was found within the heterogeneous 'native' mucin suggests therefore that polymerized and non-polymerized species exist together *in vivo*. Two other possibilities for the presence of a_2 and a_3 in the native mucin are: (1) that they are precursors of the polymer not yet incorporated into it during biosynthesis, or (2) that they represent glycoproteins released during purification by the action of tissue proteinases on 'naked' peptide regions of the polymer. The latter seems unlikely in view of the care taken during preparation (including the addition of phenylmethanesulphonyl fluoride) to avoid degradation by proteinases. The important point from our findings, however, is that 'native' high-molecular-weight mucins, which are often assumed to be single species by ultracentrifugal and chromatographic criteria (Pearson *et al.*, 1980; Mantle *et al.*, 1981), may be far more complex. If discriminating methods of separation are used, size heterogeneity of components may turn out to be a common feature of mucin structure.

The b_7 peptide was dramatically different in its amino acid composition from the glycoprotein and glycopeptide components, yet closely resembled a peptide of mol.wt. 70 000 reported by Pearson *et al.* (1981) to be released from pig gastric mucin by reduction. Mantle *et al.* (1981) also reported the existence of a 90 000-mol.wt. protein released after thiol reduction from pig intestinal mucin. Further, Creeth *et al.* (1977) have calculated, on the basis of

density-gradient ultracentrifugation, that a 100 000-mol.wt. peptide enriched in acidic amino acids must have been released after thiol-reduction of bronchial mucin. By using bovine cervical mucin, Roberts (1978) postulated that two differently sized glycopeptides (mol.wt. > 250 000) were normally bridged by a 'naked' peptide region enriched in cysteine residues. In all of these cases, the non-glycosylated peptide has been assumed to serve as a 'thiol-linkage' peptide stabilizing the polymerization of mucin glycopeptide subunits. The same stabilizing function seems to exist for the b_7 peptide produced from rat mucin in the present study, since its removal from the polymer is accompanied by dissociation and release of smaller glycopeptide components.

After Sepharose 2B chromatography, the separated glycoprotein components of rat mucin (peaks 1, 2 and 3) had a similar carbohydrate composition. Amino acid profiles were also similar, although peak 3 reflected its mixture of b_3 and b_7 . These results do not suggest that the differently sized glycosylated species differ in composition, although the number of oligosaccharide chains and their overall charge characteristics still need not be identical. It is possible, for example, that differences in sialic acid, and fucose content, and in the peptide backbone of individual glycopeptides could exist, as shown previously for human colonic mucin (Gold *et al.*, 1981). This possibility can only be assessed when the individual mucin components are purified and subjected to separation techniques based on ionic charge rather than molecular size.

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