bridges and 51 free thiol groups. Amino acid analysis of the polymeric mucin showed it to be rich in ser, thr and pro, 51%, which is characteristic of other mucins (1). Digested mucin having the highest content 55% (16% ser, 28% thr, 11% pro). These results suggest that colonic polymeric mucin consists of subunits, each containing 4–6 glycosylated regions rich in thr, pro and ser, which are linked together by disulphide bridges. This suggests that the best characterised human colonic mucin gene MUC 2 (2) is unlikely to be the major gene expressed in the colon.

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S20.8

Evidence for Two Difference Subunits in Respiratory Mucins

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Our previous studies of respiratory mucins identified a number of different families of mucins at the carbohydrate level (1 and 2). Are these families different glycoforms of a single core protein or different core proteins? To address this question, a respiratory mucin preparation was treated after reduction with ^{14}C-iodoacetamide to introduce a radiolabel onto the cysteine residues. Agarose electrophoresis and rate-zonal centrifugation indicated the preparation contained two mucin subunits present in equimolar amounts. The two subunits, after deglycosylation, showed no reactivity with monoclonal antibodies to MUC 1, 2 and 3, although the MUC 1 protein is a minor component of the preparation. The two subunits showed a differential reactivity with a monoclonal antibody AM3 (whose epitope is the carbohydrate structure sialyl-Lewis X) and one of the subunits could be selectively immunoprecipitated with this antibody. Preliminary investigation of the two subunits suggests they have a similar amino acid composition and similar ^{14}C-tryptic peptide maps (revealed by chromatography on a C2/C18 reverse phase column) but have different gross carbohydrate compositions and buoyant densities. Thus, it appears the two molecules may be different glycoforms of the same gene product (MUC ?). Immunoprecipitation of the intact parent mucins with AM3 gave no selective precipitation (i.e. all the molecules precipitated) indicating both subunits are in the same mucin molecule or are present in two different mucins that are in association.

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S20.9

An Insoluble Mucin Complex from Rat Small Intestine — Characterization of Two Different Glycosylated Domains

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The highly glycosylated domains of rat small intestinal mucins were isolated and separated into two populations (A and B) by gel chromatography on Sephacryl S-500. The values of M_r were 650 kDa and 335 kDa respectively, and the relative yields suggest that the two glycopeptides occur in equimolar proportions. Electron microscopy revealed relatively homogenous populations of linear structures with a weight average length of 230 nm (A) and 110 nm (B). The oligosaccharides (approx. 80%) were released and separated into neutral, sialic acid- and sulphate-containing species and GC/MS showed that the patterns of neutral and sialic acid-containing glycans were very similar in the two glycopeptides. Both contain a significant amount of single GalNAc residues, the average oligosaccharide is 3–4 sugar residues long and the largest species observed are heptasaccharides. The major neutral oligosaccharide is Fuc1-2Gal1-3GalNAcol and the major sialic acid-containing one GlcNAc1-6(NeuGc1-Gal1-3) GalNAcol. Sialic acid is present as both *N*-acetyl- and *N*-glycoloyl-neuraminic acid.

To solubilize the 'native' mucins containing A and B, mucosal scrapings from rat small intestine were subjected to gentle stirring in guanidinium chloride, urea, salts and detergents. None of the extractants were successful, whereas exposure to dithiothreitol or high-speed homogenization accomplished complete solubilization. Repeated extractions with guanidinium chloride left a residue that was a relatively pure, insoluble complex of mucus glycoproteins accounting for approximately 80%. Reduction of disulphide bonds afforded complete solubilization and most of the 'subunits' chromatographed with the void volume of Sephacryl S-500. Trypsin digestion yielded smaller fragments corresponding in size to A and B.

We conclude that the major portion of rat small intestinal mucins occurs as an insoluble glycoprotein complex composed of 'subunits' joined by disulphide bonds. The 'subunits' contain equimolar proportions of two highly glycosylated domains with different lengths but substituted with very similar oligosaccharides.

S20.10

Identification of Three Different Populations of Mucus Glycoproteins from Pig Gastric Mucosa

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Mucus glycoproteins (mucins) constitute the matrix of the viscoelastic, highly hydrated gel that protects the gastric mucosa from mechanical stress as well as from erosion by pepsin and hydrochloric acid. There is histochemical evidence that two different mucins, arranged in alternating laminated arrays, contribute to the surface mucus layer (Ota, H. and Katsuyama, T., *Histochem. J.*, **24**, 86–92, 1991). In order to study the mucins, mucosal scrapings from the cardiac, corpus and pylorus regions of pig gastric mucosa were solubilized in