The structure of secreted mucins isolated from the adherent mucus gel: comparison with the gene products.

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In the colon there is a continuous, insoluble adherent mucus gel covering the surface mucosa and forming a protective barrier. The gel forming properties of the mucus barrier are dependent on the component mucins. Mucin subunits consisting of a central protein core with glycosylated and non-glycosylated regions are joined together by disulphide bridges (between the non-glycosylated regions) to form polymers.[1] Following exhaustive proteolysis only the glycosylated region of the subunit remains intact. The polymeric structure of mucin is essential for it's gel forming and viscous properties.[2]

At present 7 mucin genes have been partially characterised, 6 of which are secreted mucins (MUC 2-7).[3,4] Sequencing has essentially been confined to the major glycosylated region of the mucin core which consists of tandemly repeated amino acid sequences. For each MUC gene the number of tandem repeats varies as does the number and composition of amino acids characteristic of these tandem repeats. Although all the tandem repeats are rich in threonine and to a lesser extent serine, the proportion of threonine to serine varies. MUC 3,4,5,6 & 7 have thr:ser ratios of 1.4:1, 1.3:1, 2:1, 1.8:1 & 1.4:1 respectively, whereas serine is absent from MUC2. At least three of these genes, MUC 2,3 &4, have been shown to be expressed in the colon.

MUC 2 (which is expressed in the colon) is the best characterised mucin gene, it's sequence being essentially complete apart from the amino terminal.[5,6] It comprises ~4700 aa and has two glycosylated repetitive regions of different molecular size; a 23 aa tandem repeat region of ~2300aa which is very rich in thr, 60% of the amino acids, and a 347 aa base pair repeat region containing 49% thr and 10% ser. Cys rich regions are located both at the carboxyl and amino ends of the molecule.

This study set out to investigate the structure of human and pig colonic mucin isolated from the secreted adherent mucus gel and to reconcile these structures with the identified gene products.

Glycoprotein from pig and human colonic mucus was extracted in proteinase inhibitors and purified by equilibrium centrifugation in 3.5M CsCl followed by gel filtration on Sepharose CL-2B. Reduced glycoprotein was produced by reduction with mercaptoethanol, 24 hr, 20°C; proteolytically digested mucin by digestion with papain 0.08mg mg^{-1} mucin, 60°C, 48 hr. The molecular weight of mucin was determined by sedimentation equilibrium, using a Beckman Model E analytical ultracentrifuge, and multi-angle light scattering. Dilute solution viscosity measurements were made using a Couette rotating viscometer at 25 °C. Thiol analysis was by the method of Mantle *et al.* (1990)[6] Purified mucin was reduced with 0.33M $NaBH_4$, thiol groups labelled with 4,4 dithyopyridine. The number of total and free thiols were calculated using the molar absorption coefficient. Amino acid analysis was by the method of Carlton and Morgan (1988).[7] Samples hydrolysed for 24 hr at 110°C were derivatised with 9 fluorenylmethylchloroformate and analysed by reverse phase HPLC.

Purified mucin glycoproteins were largely excluded (60%) from Sepharose CL-2B. Reduction of mucin produced a broad single peak which was also mainly excluded. Digestion produced a smaller species included on Sepharose CL-2B (Kav,0.56).

The weight average molecular weight of polymeric and digested mucin was determined by two methods, sedimentation equilibrium and light scattering. Measurements from both methods were in close agreement. The M_r for polymeric and digested mucin were determined to be 5.7×10^6 and 5.5×10^5 respectively. The papain digested mucin was a single sized polydisperse species. The weight average molecular weight of reduced mucin determined by light scattering was 3.3×10^6. This size distribution was confirmed by intrinsic viscosity studies. Polymeric, reduced and digested mucin having intrinsic viscosity values of 0.27, 0.10 and 0.02 mg ml^{-1} respectively.

Polymeric colonic mucin contained 35 ±3.1 nmol mg^{-1} total thiols including 7.0 nmol mg^{-1} free thiol groups. Reduced mucin contained 23±4.5 nmol mg^{-1} total thiols including 17 nmol mg^{-1} free thiol groups. The majority of thiols in polymeric colonic mucin ~28 nmol mg^{-1} are involved in disulphide bridge formation. This together with the large number present and the decrease in molecular size on reduction of the isolated mucin is consistent with their involvement in polymerisation. The presence of ~3 nmol mg^{-1} disulphide bridges after reduction suggests the presence of intra-molecular disulphide bridges possibly involved in a globular protein structure and presumeably inacessible to the reducing agent. Proteolysis removed all thiols suggesting that they are confined to the non-glycosylated region of the protein core acessible to the proteolytic enzyme.

Both human and pig colonic mucin contained a high proportion of ser, thr and pro, a total of 39% and 40% by weight of total protein content respectively. Digestion of pig colonic mucin resulted in loss of ~30% of protein by weight from 17% to 12% of the total molecule. Thr and ser were conserved which is characteristic of the glycosylated regions of other mucins.[1] The ratio of thr:ser in papain digested human and pig mucin was ~1:1.

From these results pig colonic mucin isolated in the presence of proteolytic inhibitors has a molecular weight of 5.7×10^6 and contains ~14 nmol mg^{-1} disulphide bridges (ie 84 disulphide bridges per polymer). Each polymer consists of at least 2 subunits M_r 3.3×10^6 linked by disulphide bridges. Each subunit contains 4-6 similar size regions of M_r 5.5×10^5. The glycosylated region of both human and pig mucin is rich in thr, ser and pro with a thr:ser ratio of ~1:1. Proteolysis removes all the non-glycosylated part of the mucin to leave the heavily glycosylated protected protein core which presumeably represents the tandem repeat segment of the mucin. Therefore it is interesting to compare the amino acid analysis of the digested mucins with the published sequences of tandem repeats of the MUC genes. The amino acid analysis for both human and pig glycosylated region has a much higher ser content relative to thr than would be expected for the tandem repeat region of MUC 2&3, expressed in the human colon. The thr:ser ratio is most compatible with that of the tandem repeat region of MUC 4 which has a thr:ser ratio 1.2:1, however the total amino acid composistion of human digested mucin is different to that of tttthe tandem repeat region of MUC 4. Further the size of the glycosylated region and the presence of a single species does not equate with MUC 2.

This analysis described here for the secreted colonic mucin is not compatible with the products of the mucin genes so far sequenced being the major component of the secreted adherent colonic mucus gel.

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