Proteolytic degradation of mucus in the colon

DAVID A. HUTTON, ADRIAN ALLEN, WILLIAM J. CUNLIFFE and JEFFREY P. PEARSON
Department of Physiological Sciences, Medical School University, Framlington Place, Newcastle Upon Tyne NE2 4HH, U.K.

A continuous adherent layer of insoluble mucus gel is found on the colonic mucosal surface, mean thickness 150 ± 110 μm in the rat (Sakata & Engelhardt, 1981). Such a mucus layer will provide a protective barrier against harmful agents in the lumen and mechanical damage from motile forces of digestion. The protective gel layer is formed by polymeric glycoproteins which are fragmented by proteases to dissolve the mucous layer (mucolysis) (Allen et al., 1984; Bell et al., 1985). Recent studies of proteolytic activity in stools (using non-mucous protein substrates) showed that inflammatory bowel disease patients had significantly higher levels of activity compared to non-symptomatic controls (Corfield et al., 1986). If such proteolytic activity reflects excessive mucolytic activity (mucous degrading) then this may be an important factor in disruption of the colonic mucosal barrier in inflammatory bowel disease. Here we demonstrate mucolytic activity in human faecal extracts from non-inflammatory bowel disease patients.

Glycoproteins from pig and human colonic mucous and pig gastric mucous were extracted in proteinase inhibitors [1.0 mM-phenylmethyl sulphonl fluoride (PMSF), 50 mM-i-iodoacetamide, 100 mM-α-aminohexanoic acid, 5 mM-benzamidine HCl, 1.0 mg l⁻¹ soybean trypsin inhibitor and 10 mM-Na₂ EDTA in 0.5 M-Tris/HCl pH 8.0] and purified by two successive equilibrium centrifugation steps in 3.5 M-CaCl₂. Purified glycoproteins were largely excluded from Sepharose CL-2B and were fragmented into smaller species, included on Sepharose CL-2B, by reduction (0.2 mM-mercaptoethanol 48 h, 4°C) and exhaustive proteolysis (1:100 (w/w) papain: glycoprotein, 48 h, 60°C). This fragmentation was reflected by a substantial drop in solution viscosity (approximately 10-fold on in vivo digestion of pig colonic mucous glycoprotein at 4 mg ml⁻¹).

Human faecal extracts (from non-inflammatory bowel disease patients) were obtained by suspending samples of stool in 4 vol. of 15 mM phosphate buffer pH 7.5, containing 50 mM-NaCl and centrifuging at 10,000 g for 15 min at 4°C. Protease activity was measured by a sensitive trinitrobenzene sulphonic acid assay for formation of new peptide N-terminals at pH 7.5 and 37°C using succinyl albumin or purified mucous glycoprotein as substrate (Hutton et al., 1986). Protopolytic action on succinyl albumin by the extracts was inhibited by soybean trypsin inhibitor and PMSF (1 mM) but not by iodoacetamide (50 mM) or Na₂ EDTA (10 mM) suggesting that the activity was due to a trypsin-like serine-dependent protease. Subsequently, levels of faecal protease activity (against succinyl albumin) were quantified as equivalents of porcine pancreatic trypsin (Sigma) activity by weight. On this basis, faecal protease activity was completely inhibited by soybean trypsin inhibitor [1:1 (w/w) inhibitor: faecal protease activity] and the synthetic polycarlyate Carbomer 934P [1000:1 (w/w) polycarlyate: faecal protease activity]. PMSF [100:1 (w/w)] inhibited activity by ~60%. Mucolytic activity of the faecal extracts was assayed by monitoring the fall in specific viscosity of solutions of purified mucous glycoproteins, as a function of time, on incubation with faecal extract at pH 7.5 and 37°C. Mucolysis was also followed by measuring the increase in free N-terminals and the amount of glycoprotein included in Sepharose CL-2B. In this manner, extracts were shown to proteolytically digest purified pig gastric and colonic mucous glycoproteins. For example, incubation of purified pig colonic mucous glycoprotein with extract [0.1% enzyme: glycoprotein (w/w)] induced a rapid fall in the specific viscosity of the glycoprotein in the first 15 min of digestion (74.5% of the fall in specific viscosity after complete digestion) followed by a slower fall over the subsequent 48 h. This fall in viscosity was accompanied by an increase in the proportion of glycoprotein included on Sepharose CL-2B this being 35% at time 0, and 77% and 89% after 15 min and 24 h, respectively. Decrease in viscosity was also mirrored by an increase in the number of free N-terminals and a 50% drop in specific viscosity corresponded to 3.1 × 10⁻² mol of peptide bonds cleaved.

The synthetic polycarlylate Carbomer 934P [1200:1 (w/w) polycarlyate: faecal protease activity] inhibited mucolysis by 45%.

The polycarlylate Carbomer 934P, in addition to inhibiting degradation of mucous, was shown to have a potential role in strengthening the protective mucous barrier. Carbomer 934P synergistically increased the viscosity of purified undegraded colonic mucous glycoproteins from both man and pig. Thus, viscosity of glycoprotein/carbomer mixtures (GC) was substantially greater than the sum of the individual viscosities (G + C). This synergistic increase in viscosity was approximately 460% at concentrations of glycoprotein and carbomer of 4 mg ml⁻¹ and 2 mg ml⁻¹, respectively.

These studies demonstrate endogenous mucolytic activity, by proteases, exists in the lumen of the colon in vivo. Such mucolysis may be an important factor in disruption of the colonic mucous barrier in disease.


Received 1 April 1987