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Progress report

The structure and function of gastric mucus

A complete understanding of the biology of gastrointestinal mucus requires a knowledge of its molecular components, their structure and behaviour. With this knowledge it is possible to understand the molecular interactions involved in the viscous and gelling properties of the mucus which are responsible for its protective and lubricating functions[1, 2]. This report is centred on our own work on gastric mucus which, because of the difficulties of obtaining large amounts of human material, has been principally on mucus from the pig stomach[3, 4] which physiologically is closely related to that of man[5].

It is firmly established that mucoproteins are the principal constituents of mucus[6, 7, 8] but a prerequisite to studying their structure and function is to isolate them free from the numerous other gastrointestinal secretions[9]. The methods used for isolating the components of mucoproteins can be divided into three broad categories: isolation from gastric washouts[9, 10, 11]; proteolytic digestion of the gastrointestinal mucosa and its attendant mucus liberating in a soluble form the mucoproteins which are partly resistant to enzyme attack[12, 13, 14]; and thirdly, isolation of the mucus from mucosal scrapings of mucosa previously washed to remove most of the gut contents and non-mucus secretions[15, 3]. The advantage of using mucosal scrapings, in contrast to the first two methods, is that it minimizes the problem of enzymic breakdown of the mucoproteins and certainly gastric mucoproteins isolated by enzymic digestion, unlike those isolated from mucosal scrapings without enzymic digestion, do not form gels. Further, an added advantage of using scrapings is that it provides a good system for studying the biosynthesis of mucus *in vitro* by the mucosal cells of, for example, sheep colonic mucus[16], pig gastric mucus,[4] and also human gastric mucus[17].

The non-dialysable fraction of the aqueous extract of mucosal scrapings from the cardiac region of the pig gastric mucosa can be separated by centrifugation into a water-insoluble mucus gel (80% by weight) and a viscous but soluble mucus (20% by weight)[4]. To understand gel formation it is first necessary to study the water-soluble mucus to elucidate the three-dimensional structure and to discover the ways in which these molecules can interact to form a gel. However, as explained later, there is good evidence that the structures of the water-soluble mucus and the water-insoluble mucus gel are very similar. The water-soluble mucus can be separated by gel filtration into high and low molecular weight mucoprotein components, A and B respectively (Table I). These two mucoproteins comprise about 85% by weight of the water-soluble mucus, the remaining material being principally protein contaminant. Apart from their different molecular weights,

Mucoprotein Component	*Reduced Specific Viscosity at 2 mg/ml (ml g^{-1})*	*Molecular Weight*[2]
A in 0·2M KCl	1 050	2×10^6
A in 0·2M mercaptoethanol	120	5×10^5
A in 2·5M KCl	430	2×10^6
A in 0·2M CsCl	180	2×10^6
B in 0·2M KCl	89	$1{\cdot}1 \times 10^5$

Table I *The molecular weight and viscosity of the mucoproteins from the water-soluble mucus of pig stomach*

[1]Viscosities are corrected to zero shear where applicable.
[2]Molecular weights are calculated from $S^\circ_{25,w}$ and $D^\circ_{25,w}$ values.

the two mucoprotein components A and B are identical in chemical analysis, blood group A and H activity,[3] and in the structural changes they undergo in solvents such as 0·2M mercaptoethanol[18] and 1·5M KCl[19]. On the biosynthetic side, the gastric mucosal cells incubated *in vitro* for two and a half hours will incorporate added radioactive glucose and threonine solely into the carbohydrate and protein respectively of the mucoprotein in the mucus. Only the mucoprotein components become radioactively labelled in these experiments and, what is more important, the amount and the pattern of the radioactivity incorporated are the same for the two mucoprotein components A and B. This identity in the biosynthetic pattern of the two mucoproteins, together with the analytical data, indicates that the low molecular weight mucoprotein B (molecular weight $1{\cdot}1 \times 10^5$) is a repeating unit of the high molecular weight mucoprotein A (molecular weight 2×10^6). It would follow that A is a discrete assembly of about 18 mucoprotein B units since no molecules of intermediate molecular weight range are found. However, it is characteristic of these mucoproteins that, unlike enzymes, they have no single unique molecular size, but form a family of different size molecules centred around a mean value. These variations in molecular weight are probably due to incompleteness of the carbohydrate chains and/or different numbers of these chains attached to the proteins, although a small variation either way in the number of repeating mucoprotein B units in mucoprotein A cannot be ruled out. As can be seen from Table I, it is the polymerized high molecular weight mucoprotein A that accounts for the major part of the viscosity of the water-soluble mucus.

For chemical analysis it is necessary to remove protein, which although not covalently bound, is entangled with the mucoprotein molecules and consequently separates with them on gel filtration. The non-covalently bound protein, which represents about 5% of the total mucoprotein (Table II),

	Mucoprotein Isolated by Gel Filtration[2]	*Mucoprotein after Removal of Protein by Caesium Chloride Treatment*[2]	*Mucoprotein Isolated from Pepsin-digested Mucosa (blood group substance)*[12]
Hexose	28	26	28
Hexosamine	29	28	32
Fucose	11	12	12
Sialic acid	0·9	—	—
Protein	22	17	13

Table II *Chemical composition of pig gastric mucus*

[1]Uronic acids, ribose, phosphate, and sulphate are absent.
[2]These two fractions each consist of high and low molecular weight mucoprotein components of identical analysis. Details of analysis see Snary and Allen[3].

can be removed by equilibrium density gradient centrifugation in a caesium chloride gradient. In this method the caesium chloride gradient separates the more dense mucoproteins ($\rho \approx 1{\cdot}6$ g/ml) at the bottom of the tube from the less dense protein ($\rho \approx 1{\cdot}3$ g/ml) at the top of the tube[20, 21]. The mucoproteins A and B, after removal of this non-covalently bound protein, have a carbohydrate analysis closely similar to the classical blood group substance mucoproteins isolated by proteolytic digestion of the pig gastric mucosa[12] or extracted from ovarian cysts[22]. The amino acid analysis of mucoproteins A and B are identical[21] and, in particular, they are characterized by a high content of serine, threonine, and proline, which together account for 42% of the total amino acids present. A high content of proline, serine, and threonine is also characteristic of the blood group substance mucoproteins where threonine and serine are involved in the linkage of the carbohydrate side chains to the protein backbone[23]. However, the purified mucoproteins A and B do have a significantly higher protein content than the latter (Table II) in keeping with their not having been degraded by proteolytic enzymes. The absence of sulphate in the analysis means that the water-soluble mucus contains none of the sulphated mucosubstances that have been reported from gastric preparations[24, 14]. Also, the low protein content indicates there is no relation of the mucoproteins of the water-soluble mucus to the intrinsic factor[25] and serum glycoproteins[9] reported in gastric secretions.

In the water-soluble mucus, besides mucoprotein A being composed of repeating units of mucoprotein B, both A and B consist of four mucoprotein subunits joined by disulphide bridges between cysteine residues[18] (Fig. 1). These intermolecular sulphur-sulphur linkages can be split by

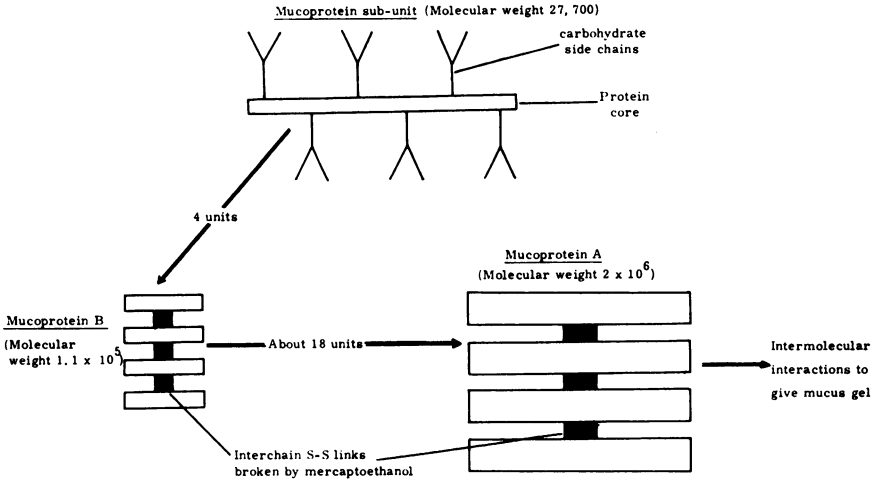

Fig. 1 *Proposed structure of gastric mucus*

reducing agents such as N-acetylcysteine and mercaptoethanol with a corresponding drop in viscosity. Thus the viscosity of the water-soluble gastric mucus is reduced by 75% in the presence of mercaptoethanol and there is a corresponding four-fold decrease in the molecular weights of mucoproteins A and B (Table I). This effect explains the action of drugs such as N-acetyl cysteine which have been used in the clinical treatment of pulmonary condi-

tions like cystic fibrosis and chronic bronchitis to disperse the characteristic heavy secretion of mucus[26].

From the above studies on the water-soluble mucus a picture can be obtained of the highly polymeric structure of the mucoproteins which is necessary for its viscous properties. The structure of the mucoprotein subunit, which has a molecular weight of 27 700, is presumably the same as that of other mucoproteins from mucous secretions[6]. These mucoproteins, such as submaxillary mucins and ovarian cyst blood group substances, have a central protein core with carbohydrate side chains attached giving a 'bottle brush' structure (Fig. 1). In blood group mucoproteins from pig stomach it has been proposed that each carbohydrate side chain consists of about 15 sugar residues[23]. If this is about the size of the sugar chains in the gastric mucoproteins then from the analysis there would be one of these large carbohydrate chains for every three or four amino acids on the peptide chain—a very tightly packed structure. Four of these mucoprotein units (molecular weight 27 700) are joined by disulphide bridges to form the low molecular weight mucoprotein B (molecular weight $1{\cdot}1 \times 10^5$) of the water-soluble mucus. Mucoprotein B in turn is joined up to form the high molecular weight mucoprotein A (molecular weight 2×10^6) in such a way that this mucoprotein A is also composed of four subunits joined by disulphide linkages (Fig. 1). It is unlikely that mucoprotein B is polymerized by non-covalent interactions to give the high molecular weight mucoprotein since reagents expected to disrupt these linkages such as high concentrations of KCl, CsCl, or guanidinium hydrochloride, do not alter the molecular weight or the ratio of the amounts of these two mucoproteins[19, 27] Treatment of the high molecular weight mucoprotein with proteolytic enzymes like trypsin also causes a reduction of 75% in the viscosity and recent results show that a mucoprotein of a quarter of the molecular weight of the parent mucoprotein is produced (Starkey and Allen, unpublished work) rather than the low molecular weight mucoprotein component. These results, as with mercaptoethanol, show that mucoprotein A can be split into subunits possessing a quarter of the molecular weight but does not throw any light on the exact relationship between the mucoproteins A and B in the water-soluble mucus. This relationship remains to be elucidated.

The structure of the water-insoluble mucus gel can now be viewed in the light of what is known about the structure of the component macromolecules. There are two methods for solubilizing the macromolecular components of the insoluble mucus gel so that they can be studied. The first involves the use of compounds that disrupt the tertiary structure of these molecules such as urea, salt, and deoxycholate, or that break the mucoprotein into subunits such as mercaptoethanol. Such solvents, while solubilizing 70-80% of the water-insoluble mucus gel, do not solubilize it completely, even when it is successively treated with different solvents[3]. The second approach is to use proteolytic enzymes, and pronase, for example, will completely solubilize the mucus gel. Furthermore, pronase digestion of either the water-soluble mucus or the water-insoluble mucus gel gives the same mucoprotein products as judged by chemical analysis and physical properties[4]. This means that the carbohydrate side chains and the protein core of the mucoproteins in the water-soluble mucus are very closely similar to those in the water-insoluble mucus, although these experiments do not eliminate differences in the protein part, especially that which was hydrolysed.

The high molecular weight mucoprotein A which, as previously stated accounts for the viscosity of the water-soluble mucus, will gel reversibly at concentrations above 5 mg per ml. When treated with mercaptoethanol and trypsin, which bring about a lowering of the viscosity and which break the mucoprotein A into subunits, this mucoprotein will not gel. Thus, the high molecular weight mucoprotein with intact disulphide bridges is necessary for the gel formation which enables the mucus to function *in vivo*. Further, the degree of hydration of the mucoprotein is important. High concentrations of KCl (above 1·5M) or CsCl (above 0·2M) dehydrate the mucoproteins and viscosity and ultracentrifugation studies of this effect give information about the three-dimensional structure of these molecules[19, 27]. In isotonic KCl the three-dimensional structure of mucoprotein A is that of an expanded, highly hydrated and spherical molecule, the viscosity of which, corrected to zero shear, increases exponentially with concentration above a mucoprotein concentration of 2 mg per ml until gel formation occurs above 5 mg per ml. When mucoprotein A is dehydrated by high KCl concentrations or CsCl there is a collapse of this expanded three-dimensional structure which is accompanied by the loss of the exponential rise of viscosity with concentration and gel-forming ability. Furthermore, the large shear dependence of the viscosity of mucoprotein A, which varies continuously with concentration, suggests that the reversible gelling of the water-soluble mucus does not involve a cooperative effect as in the case of the double helix formation in the carrageenan gels[28] and may involve just entanglement of these large molecules[29]. The water-insoluble mucus is obviously different in some way from the gel formed by the water-soluble mucus since only the latter gels reversibly. Since the solvents deoxycholate and urea will dissolve 70-80% of the water-insoluble mucus, at least this percentage of the mucus gel probably involves non-covalent bonding. It may be that the reason for the water-insolubility of the mucus gel is that it is just more concentrated and more entangled than the gel formed from the water-soluble mucus *in vitro*. However, while it is evident that integrity of the large molecular weight mucoprotein A, with its highly hydrated and expanded tertiary structure, is a prerequisite for the formation of the mucus gel, a further difference in molecular structure between this water-soluble mucoprotein and the water-insoluble gel cannot be ruled out.

The nature of this mucoprotein complex raises some interesting questions as to its biosynthesis. The biosynthesis of the basic mucoprotein structure of these mucous secretions is well documented for a variety of tissues[30] including the gastric mucosa[17, 31]. The protein core is formed on the ribosomes of the cell followed by the sequential addition of individual nucleotide sugars to build up the branched carbohydrate chains. The enzymes which add the sugar residues are very specific for the nature and position of the sugar in the branched chain, and are located in the membranes of the endoplasmic reticulum and Golgi apparatus. Inhibitors of protein biosynthesis such as puromycin inhibit the formation of the protein core and consequently subsequent addition of the sugar moieties[32]. Drugs like salicylate, which also inhibit mucus biosynthesis, have a particularly selective effect on the amino sugar incorporation[17]. What is unknown is where and how the polymerization of the mucoproteins found in the gastric mucus occurs. The disulphide crosslinks may be formed before the molecule leaves the vicinity of the membrane-bound ribosome and before most of the sugar

is attached or, alternatively, relatively low molecular weight mucoprotein units may be polymerized while the mucoprotein is in the secretory vesicles[33] or even when it has reached the outside surface of the mucosal cell.

A final question is, how universal is the structure that we have found for pig gastric mucus, namely, that of large molecular weight, gel-forming mucoprotein molecules built up of smaller mucoprotein units and joined by interchain disulphide links'? The other model for these mucoproteins from mucus secretions is that of an expanded, single chain 'bottle brush' mucoprotein structure and comes principally from work on ovine and bovine submaxillary mucins[6]. A number of mucous secretions, including porcine and canine submaxillary mucins, canine tracheal mucin, and bovine cervical mucin, have been shown to be broken down into small molecular weight products by disulphide bond-splitting reagents[34, 35] This strongly suggests other mucins may have a subunit structure similar to that of the pig gastric mucus. It is interesting that neither ovine nor bovine submaxillary mucins appear to be affected by mercaptoethanol[35]. Also, the urinary glycoprotein of Tamm and Horsfall has been shown to polymerize into larger molecular weight units but in contrast to gastric mucus these glycoproteins contain 75% protein[6]. The analysis of human gastric mucus is closely similar to that of pig gastric mucus[36] but demonstration of a similar three-dimensional structure has yet to come.

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