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The presence of polysaccharide in normal human gastric mucus

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1. Polysaccharide material was found in the proteolysis glycopolypeptide fraction from normal human gastric mucus. 2. The polysaccharide was identified by carbohydrate and amino acid analyses, by elemental analysis and from its behaviour on density-gradient ultracentrifugation. 3. The polysaccharide is polydisperse with a weight-average molecular mass of 300000 Da. 4. Over 85% of the polysaccharide consists of galactose, and this represents 26% of all the galactose present in the fractions after β-elimination with reduction of the glycopolypeptide material.

Mucus is a complex secretion containing a range of different substances (see, e.g., Clamp, 1978). These include inorganic material, proteins arising by transudation from plasma and by specific secretion and a large-molecular-mass glycoprotein (mucus glycoprotein). However, no-one, as far as we know, has previously reported the presence of polysaccharide material in normal human gastric mucus.

Experimental

Glycoconjugates

Healthy volunteers were used in this study. Subjects were requested to fast for at least 13h before, and not to swallow during the study. An orogastric tube was passed and its position verified. The gastric contents were aspirated over 15min and discarded. The aspiration was then continued for a further 60min, each 15min collection being neutralized with saturated $NaHCO_3$ solution.

The pooled neutralized aspirate was exhaustively dialysed against distilled water and freeze-dried. The dried material was subjected to gel-permeation chromatography on Sepharose 2B followed by proteolytic digestion with Pronase as described by Clamp *et al.* (1981). The digested material was dialysed and freeze-dried, and then subjected to gel-permeation chromatography on a column (94cm × 2.4cm internal diam.) of Sephadex G-150 in 0.15M-NaCl containing 0.5% (w/v) sodium dodecyl sulphate. The carbohydrate-containing peaks were identified (Dubois *et al.*, 1956), and the excluded fraction (glycopolypeptide) was dialysed and freeze-dried. The glycopolypeptide material was then subjected to the β-elimination procedure with reduction (Carlson, 1968). After this procedure, the reaction mixture was adjusted to pH5.0 by the batchwise addition of Dowex-50W (X2; −400 mesh; H^+ form), and after removal of the resin by filtration the solution was freeze-dried. Boric acid was removed as methyl borate by the addition of methanol followed by flash-evaporation. The dried material was dissolved in water and applied to a column (148cm × 1.4cm internal diam.) of Sephadex G-25. The carbohydrate-containing peaks were identified, and the excluded material was applied to a column (148cm × 1.4cm internal diam.) of Sephadex G-50. The material excluded from this column (polysaccharide fraction) was dialysed and freeze-dried.

Analyses

Carbohydrate analyses were performed by g.l.c. on the trimethylsilyl ethers of the methyl glycosides (Clamp, 1977), with arabinitol and mannitol as internal standards.

Ultracentrifugation studies

The glycopeptide and the polysaccharide were subjected to density-gradient-equilibrium experiments in the Beckman model E analytical ultracentrifuge with CsCl at initial densities of 1.53g/ml and 1.65g/ml.

Determinations of molecular mass were made by sedimentation-equilibrium experiments (Creeth *et al.*, 1974).

Results and discussion

The proteolysis glycopolypeptide (see Gibbons, 1978) is a convenient final material from which to prepare the major oligosaccharide units of mucus

glycoprotein. It is a relatively defined material with a characteristic molecular mass and carbohydrate and amino acid composition. When the proteolysis glycopolypeptide from normal human gastric mucus was subjected to the standard conditions for alkaline β-elimination with reduction (Carlson, 1968), we consistently found that a significant amount of the carbohydrate remained associated with a large-molecular-mass fraction. The use of more vigorous conditions did not significantly decrease the size of this fraction, but caused non-specific degradation, as shown by the presence of glycitols other than *N*-acetylgalactosaminitol (Fraser, 1974).

When the large-molecular-mass fraction was studied in more detail, it was found to have properties characteristic of a polysaccharide (Table 1). Thus 85% of this material consisted of just one monosaccharide, namely galactose, with smaller amounts of fucose, glucose, *N*-acetylglucosamine and *N*-acetylgalactosamine. Amino acids constituted less than 1% of the weight, with small amounts of threonine, serine, glutamic acid, proline, glycine and alanine. The elemental analysis corresponds to $C_6H_{11}O_5$, which is in good agreement with a galactose-containing polysaccharide. Both the glycopolypeptide and the polysaccharide were subjected to a density-gradient-equilibrium experiment in the analytical ultracentrifuge. The glycopolypeptide was examined in a CsCl density gradient at an initial value (1.53 g/ml) suitable for banding mucus glycopolypeptides from, for example, ovarian-cyst glycoproteins. The glycopolypeptide gave a broad zone of mean buoyant density 1.50 g/ml, as expected. However, a much denser component was also present, visible as a steep rise in the gradient at the base. The polysaccharide was examined at an initial density of 1.65 g/ml, as suggested by the behaviour of the first sample. A well-defined nearly symmetrical zone was found, with a buoyant-density value of 1.65 g/ml, which is characteristic of polysaccharide material but less than the value for nucleic acids (>1.7 g/ml). A slight depression was seen at the meniscus, indicating the presence of a trace amount of lower-density material, which may be part of the broad distribution of the polysaccharide but could be the presence of some remaining glycopolypeptide. To clarify this, the glycopolypeptide was examined at the higher density and the polysaccharide at the lower. The glycopolypeptide material showed a small but well-defined band of density 1.66 g/ml, strongly suggesting that the dense component in this material is the polysaccharide. However, no trace of glycopolypeptide was visible at the expected density in the experiment on the polysaccharide. Thus the glycopolypeptide material contains approx. 30% of polysaccharide, whereas the polysaccharide material is essentially a single component. A sedimentation-equilibrium experiment on the polysaccharide gave a pattern indicating considerable polydispersity of molecular mass, in common with many polysaccharides, but with a mean weight-average molecular mass of 300000 Da. By contrast, human gastric mucus glycoprotein has a molecular mass of 2000000 Da and the proteolysis glycopolypeptide a mass of 500000 Da (Pearson *et al.*, 1980).

The presence of significant amounts of polysaccharide material in normal human gastric mucus may be biochemically and clinically important. Great care was therefore taken to ensure that co-operative volunteers were used who could be relied on to fast for at least 12 h before collection of gastric secretions. The first gastric aspirate was discarded, and the remaining collection was supervised by an experienced clinic nurse or a medical registrar to ensure that the subject did not swallow during the procedure.

The evidence for polysaccharide material in gastric mucus is overwhelming. The survival of large-molecular-mass material after exhaustive proteolysis and the alkali-catalysed elimination reaction, the carbohydrate and amino analyses, the elemental composition and the behaviour on density-gradient ultracentrifugation are all consistent with a polysaccharide. For a molecular mass of 300000 Da, the polysaccharide would contain over 1500 galactose residues, with much smaller amounts of other monosaccharides. Thus the polysaccharide is about 200 times larger than the average-sized oligosaccharide unit in gastric-mucus glycoprotein.

The galactose present in the polysaccharide material represents 26% of the total galactose

Table 1. *Properties of polysaccharide material from gastric mucus*

	Carbohydrate content	
Monosaccharide	(nmol/mg)	(g/100 g of total carbohydrate)
Fucose	90	1.93
Galactose	3557	84.65
Glucose	228	5.43
N-Acetylglucosamine	268	7.99
N-Acetylgalactosamine, *N*-Acetylgalactosaminitol, Glucuronic acid	Trace amounts	

Amino acid	Amino acid content (g/100 g of polysaccharide)
Threonine	0.2
Serine, glutamic acid, proline, glycine, alanine	Trace amounts

Elemental analysis (g/100 g): C, 41.19; H, 6.13; N, 0.45; O, 44.05; S, 0.83; H_2O, 5.87; residue, 3.78

Buoyant density in CsCl: 1.65 g/ml

Weight-average molecular mass: 300000 Da

present in all the fractions after the β-elimination with reduction procedure. Thus the polysaccharide is a significant component of mucus and would be expected to play an important part in the overall properties of that secretion.

A number of questions remain. It is not known at present whether some or all of the polysaccharide is covalently linked to mucus glycoprotein when the latter is first synthesized. Alternative possibilities are that the polysaccharide is always free in mucus secretions or that it is originally a membrane component projecting into the mucus and interacting with glycoprotein. Nor do we know whether polysaccharide is present at other levels of the gastrointestinal tract or in the mucus secretions of other systems.

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