STRUCTURAL STUDIES ON GASTRIC MUCOPROTEINS: LOWERING OF MOLECULAR WEIGHT AFTER REDUCTION WITH 2-MERCAPTOETHANOL.

by

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Summary

The lowering of viscosity of mucus by thiol reagents has been investigated at the molecular level. Gel filtration of a water-soluble extract from pig surface gastric mucosal cells yields two major mucoprotein fractions, A and B, characteristic of the macromolecular components of gastric mucus. Molecular weights have been measured by combination of sedimentation and diffusion data and by sedimentation equilibrium. Mercaptoethanol causes a 4-fold drop in molecular weights from 1.9×10^6 to 0.52×10^6 (Fraction A) and from 1.1×10^5 to 0.28×10^5 (Fraction B). It is concluded that the marked drop in viscosity of gastric mucous secretion is associated with a reduction in molecular weight of its mucoprotein components.

Certain pulmonary conditions, such as cystic fibrosis and chronic bronchitis, have frequently been treated clinically with mild reducing agents in order to disperse the characteristic heavy secretion of mucus (Sheffner, 1963). The breakdown of structure in such secretions is illustrated by the 75% reduction in specific viscosity of emulsified bronchitic mucus following treatment with N-acetyl cysteine (Galzigna and Reggiani, 1965). This reagent had already been shown by Sheffner (1963) to be capable, by virtue of its thiol group, of reducing by 31% the reduced specific viscosity of commercial pig mucin - a pepsin digestion product of pig stomach mucosa. Dunstone (1970) obtained soluble products from ovarian cyst mucus by reducing with sulphite. In the light of such facts and as part of a study of the biosynthesis and structure of stomach mucoproteins, it was clearly of

interest to study the effects of reducing agents on their molecular structure. In this communication a water soluble mucoprotein fraction prepared from surface mucosal cells of pig stomach without prior enzymic treatment has been studied. This mucoprotein fraction has been shown to possess blood group activity and to be chemically similar to other epithelial mucins (Snary and Allen, 1969). In this communication we show that the reduction in viscosity of this material after treatment with mercaptoethanol is associated with a decrease in molecular weight.

Methods:

Materials. The water-soluble mucoprotein fraction was prepared from surface mucosal cells obtained from the cardiac region of pig stomach. The preparation was dialysed against distilled water for three days, then centrifuged at 5,000 g for 20 minutes. The supernatant was freeze-dried prior to use. Freeze-drying was shown to have no detectable influence upon the sedimentation properties of the extract. Concentrations were related to dry weights of the mucoprotein.

Viscosity. Viscosity was measured at 25° using viscometers constructed according to the pattern of Schachman (1957) and having a flow time for water of 220 secs.

Sedimentation coefficients. Sedimentation velocity runs were performed at 25°, using a Beckman Model E analytical ultracentrifuge. Sedimentation coefficients were calculated from radial displacement of the maximum refraction gradient and were corrected for buffer viscosity and density. Linear relations were obtained when values of $1/s$ were plotted against the mean plateau-region concentrations. The latter were obtained from Schlieren peak areas which, together with areas used for calculating the optical recovery in sedimentation runs, were corrected for radial dilution.

Diffusion coefficients. Height-area diffusion coefficients were calculated from the spreading of boundaries formed between solution and solvent in a double sector, synthetic boundary cell in the analytical

ultracentrifuge. Runs were carried out at 25° and at 2,600 rpm, and the diffusion coefficients were corrected for solvent viscosity. Zero time corrections, Δt, were always measured and values of $D\Delta t$ never exceeded 7×10^{-5} cm^2. Values of 1/D were plotted against $C_o/2$ to give $D^o_{25,w}$.

Molecular weights. Values of $s^o_{25,w}$ and $D^o_{25,w}$ were combined to give average molecular weights making use of the Svedberg equation and a value of $\bar{v} = 0.64$ ml g^{-1} obtained from a comparison of published values of a range of mucoproteins. Sedimentation equilibrium experiments were performed using the multichannel cell of Yphantis (1960) and solution-handling techniques of Pain (1963).

Buffers. Sedimentation, diffusion and sedimentation equilibrium runs were made in the following buffers:

0.18 M-KCl, 0.02 M-K acetate brought to pH 5.5 with acetic acid, 0.02 % Na azide

$$(\eta_{r,25} = 1.017;\ \rho_{25} = 1.011 \text{ g ml}^{-1})$$

0.,8 M-KCl, 0.02 M-Na veronal brought to pH 8.5 with HCl, 0.02% Na azide, 0.2 M-2-mercaptoethanol. ($\eta_{r,25} = 1.037$; $\rho_{25} = 1.013$ g ml^{-1}). Solutions were dialysed for 48 hours before examination.

Results and Discussion:

The water-soluble extract of surface scrapings of pig gastric mucosa is highly viscous and readily forms a gel at concentrations above 5 mg ml^{-1}. Table 1 shows that at a concentration of 2.1 mg ml^{-1} and in the presence of 0.2 M mercaptoethanol, it undergoes a reduction in specific viscosity ($\eta_{sp} = \eta_r - 1$) of 75%. This is similar to the large reduction quoted above for whole bronchial mucus. The mucoproteins of this extract can be separated by gel filtration (Snary and Allen, 1969) to give three fractions, A, B and C, characterised by $S^o_{25,w}$ values of 18.7, 4.9 and 3.9 respectively. The two faster sedimenting fractions A and B, comprising the major part of the water-soluble extract, each show a marked drop in viscosity with mercaptoethanol (Table 1). These viscosity values, measured at finite

TABLE 1

The Effect of Mercaptoethanol on the Viscosity of Mucus Extracts

Mucoprotein Fraction	0.2 M-Mercaptoethanol	Specific Viscosity
Water-soluble extract	-	0.34_8 at 2.1 mg ml^{-1}
Water-soluble extract	+	0.08_8 at 2.1 mg ml^{-1}
Fraction A	-	0.45_7 at 2.9 mg ml^{-1}
Fraction A	+	0.13_3 at 2.9 mg ml^{-1}
Fraction B	-	0.10_4 at 2.1 mg ml^{-1}
Fraction B	+	0.06_2 at 2.1 mg ml^{-1}

Buffer used in all these experiments was 0.18 M-KCl, 0.02 M-Na veronal brought to pH 8.5 with HCl, 0.02% azide.

concentration and not corrected for possible shear dependence, indicate nevertheless a marked change in structure of the soluble mucoprotein macromolecules.

The results of ultracentrifugal characterisation of fractions A and B are detailed in Table 2, together with the changes in the properties of A and B on treatment with mercaptoethanol. The concentration dependence of S is shown in Fig 1. Diffusion coefficients are virtually independent of concentration over the concentration range measured (1-4 mg/ml). Reduction was necessarily carried out at pH 8.5 in contrast to pH 5.5 used for the controls, but no changes in the specific viscosity of the water-soluble mucoprotein fraction were observed in the absence of mercaptoethanol between pH 5.5 and pH 8.5. The decision to calculate the molecular weights from sedimentation and static diffusion coefficients is dictated by the nature of the material. Both fractions are shown to be markedly polydisperse by comparison of static with dynamic diffusion

TABLE 2

The Effects of Mercaptoethanol on the Molecular Weights of Mucoproteins

Mucoprotein Fraction	0.2 M-Mercaptoethanol	$S^{o}_{25,w} \times 10^{13}$ sec^{-1}	$D^{o}_{25,w} \times 10^{7}$ cm^{2} sec^{-1}	$\bar{M}_{S,D}$	$\bar{M}_{W}$
A	-	18.7	0.69	1.85×10^{6}	
A	+	12.0	1.59	519,000	
B	-	4.9	3.05	110,000	128,000
B	+	3.5	8.62	27,700	35,700

Buffers are detailed under Methods

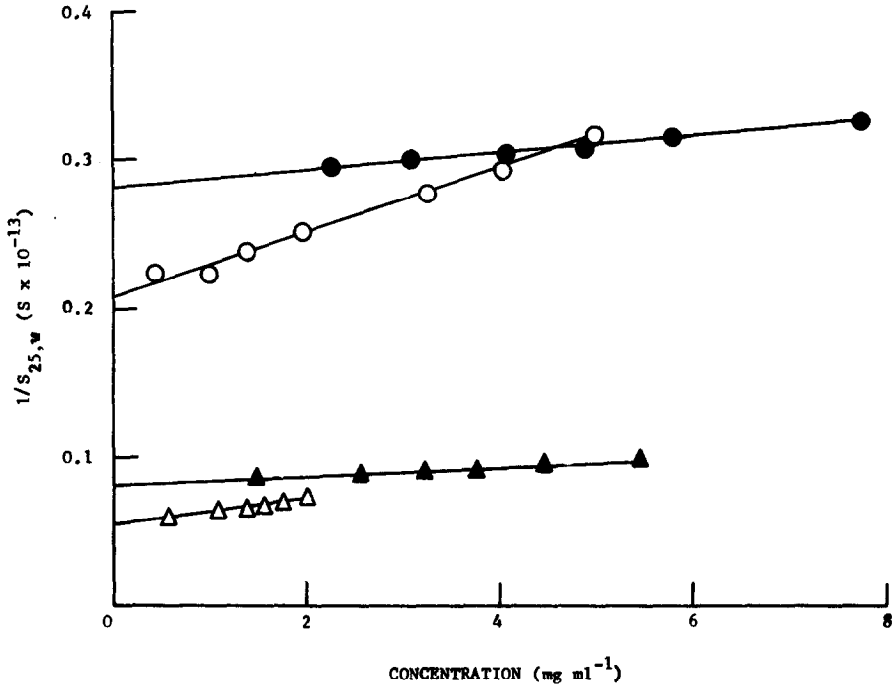

Fig. 1. The effect of mercaptoethanol on mucoprotein fractions of pig stomach mucus. Plots of $1/S_{25,w}$ versus mean plateau-region concentration.

- △ - Fraction A.
- ▲ - Fraction A in the presence of 0.2 M-mercaptoethanol.
- ○ - Fraction B.
- ● - Fraction B in the presence of 0.2 M-mercaptoethanol.

coefficients (Creeth and Pain, 1967). These mucoprotein fractions possess a broad spread of sedimentation coefficients, as do the blood group specific mucoproteins studied by Creeth and Knight (1967). The sedimentation coefficients are characteristic of about 90% of fraction B and about 65% of fraction A, as shown by optical recovery during the sedimentation velocity runs, while the diffusion coefficients refer to the whole of the material. It is possible to obtain fraction B free of detectable amounts of fraction A but the reverse is not true, samples of A usually containing about 20% of B. Diffusion coefficients for fraction A were therefore calculated

taking into account the known concentration and diffusion coefficient of fraction B according to the relation for the height-area average diffusion coefficient $D_A = \left(\frac{\sum c_i D_i^{-\frac{1}{2}}}{\sum c_i} \right)^{-2}$ (Creeth and Pain, 1967).

The molecular weights for both fractions are reduced by the action of mercaptoethanol to approximately one quarter of the respective values in the water-soluble extract. These values are confirmed for fraction B by values of $\bar{M}_w$ obtained from short-column equilibrium runs. It seems most reasonable to postulate that the reducing agent is breaking disulphide bonds between polypeptide chains.

The mucoproteins from the water-soluble material obtained from surface mucosal cells *in vitro* (Snary and Allen, 1969) have similar immunological and chemical properties to the mucous secretion of these cells *in vivo* (Szulman, 1960). When the surface mucosal cells of pig stomach mucosa are incubated in vitro with radioactive amino acids or glucose the mucoprotein components of the water-soluble extract and the insoluble residue are the only radioactively-labelled macromolecules produced by the cells. Further, enzymic digestion and solvent extraction studies point to the mucoproteins of the water-insoluble material being very similar to the water-soluble mucoproteins whose properties are studied here (Snary and Allen, 1970). It is reasonable therefore to postulate that the macromolecular components in this study are representative of the macromolecular components of the mucous secretion, albeit probably in a different state of covalent or non-covalent polymerisation. The marked change in the rheological properties of mucus *in vivo* caused by thiol reagents is thus considered to be consequent primarily on a molecular disaggregation of its mucoprotein constituents. Further work is in progress to characterise the bonds split by mercaptoethanol and the nature of the disaggregating process.

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