Biochem. J. (1974) **141**, 641–646
Printed in Great Britain

Conformational Changes in Gastric Mucoproteins Induced by Caesium Chloride and Guanidinium Chloride

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(*Received* 19 *December* 1973)

1. Caesium chloride and guanidinium chloride were shown to cause conformational changes in the high-molecular-weight mucoprotein A of water-soluble gastric mucus with no change in molecular weight. 2. Increasing concentrations of CsCl decrease the viscosity of the mucoprotein bringing about a transition which is essentially complete in 0.1 M-CsCl. The shear-dependence of viscosity of the mucoprotein is abolished by low concentrations of CsCl. The normally highly expanded molecule becomes contracted in CsCl to a molecule having the same symmetry but a smaller volume and decreased solvation, in keeping with an increased sedimentation coefficient (18.7S → 33S). 3. This contracted form does not revert to the native conformation on removal of the CsCl. 4. A mechanism is discussed in terms of the effect of the Cs^+ and Cl^- ions on water structure and the water–mucoprotein interaction. 5. Guanidinium chloride causes the CsCl-treated material to expand, in keeping with a decrease in $s^0_{25,w}$ (33S → 26S). This is analogous to the known unfolding effect of guanidinium chloride on proteins and suggests that guanidinium chloride solubilizes groups involved in stabilizing the contracted structure. Removal of the guanidinium chloride results in a limited aggregation of four mucoprotein molecules. 6. These results show that caution must be exercised before interpreting the physical properties of mucoproteins which have been treated with CsCl and/or guanidinium chloride.

An effective way of removing non-covalently bound protein from protein–polysaccharide complexes is to use equilibrium centrifugation in a density gradient of CsCl (Creeth & Denborough, 1970). In a case where specific interaction between glycoprotein and mucopolysaccharide molecules was suspected their separation was achieved by adding guanidinium chloride to the gradient (Hascall & Sajdera, 1969). Water-soluble mucus from pig gastric mucosa has been freed from non-covalently bound protein by using a CsCl gradient (Starkey *et al.*, 1972), and the two purified mucoprotein components have been characterized chemically (Starkey *et al.*, 1974). Preliminary investigation of the mucoproteins showed anomalous sedimentation behaviour compared with that of the native material (Snary *et al.*, 1972). To understand what effects CsCl and guanidinium chloride have on the native material we have characterized the interactions, conformations and transitions of the mucoproteins.

The conclusion of the present paper is that CsCl and guanidinium chloride bring about dramatic changes in the conformation of the mucoprotein without decreasing the molecular weight. These conformational changes are associated with complete loss of those rheological properties of the native mucoprotein which have been shown to be responsible for the formation of the mucus gel (Allen & Snary, 1972). The physical properties of such molecules after contact with CsCl and/or guanidinium chloride must therefore be interpreted with great care.

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Methods

Preparation of mucoproteins

Water-soluble mucus was prepared by the method of Snary & Allen (1971) from the superficial scrapings of the mucosa of the cardiac region of pig stomach, without enzymic digestion. Fractionation of the water-soluble mucus, by using gel filtration on a column (85cm × 2.5cm) of Sepharose 4B with elution by 0.2M-NaCl, gave mucoprotein fraction A in the excluded volume and mucoprotein B/C in the included volume. The water-soluble gastric mucus was also fractionated by density equilibrium centrifugation in CsCl (initial density 1.43g/ml) (Creeth & Denborough 1970). After centrifugation for 48h the carbohydrate-containing mucoproteins were collected from the bottom of the tube free from the contaminating protein at the top of the tube (Starkey *et al.*, 1974). The

mucoprotein from the gradient was further fractionated by gel filtration on Sepharose 4B into mucoprotein A and mucoprotein B/C. All fractions were dialysed against six changes of water over 48h and freeze-dried. Freeze-drying has been shown to have no effect on the sedimentation properties of these molecules (Snary & Allen, 1971).

Physical measurements

Viscosity, sedimentation and diffusion measurements were carried out as previously described (Snary *et al.*, 1971). Values of $M_{s,D}$ (molecular weight determined by the combination of sedimentation and diffusion coefficients) were calculated by using the Svedberg equation, and optical recoveries were determined as described previously (Snary *et al.*, 1971). Molecular weights were also measured by the equilibrium technique of Yphantis (1960). The following buffers were used: 0.18M-KCl–0.02% (w/v) sodium azide–0.02M-potassium acetate adjusted to pH5.5 with acetic acid ($\eta_{rel.,25} = 1.017$, $\rho_{25} = 1.011$ g/ml); 4M-guanidinium chloride–0.018M-KCl–0.002M-potassium acetate adjusted to pH5.5 with acetic acid–0.02% (w/v) sodium azide ($\eta_{rel.,25} = 1.273$, $\rho_{25} = 1.098$ g/ml). Buffers used for the effect of CsCl on the viscosity of the water-soluble mucus were based on 0.018M-KCl–0.002M-potassium acetate adjusted to pH5.5 with acetic acid–0.02% (w/v) sodium azide, with the appropriate concentration of CsCl added. All solutions were dialysed against the required buffer for 48h before experimentation.

Results and Discussion

Conformational changes in the mucoprotein caused by CsCl

Gel filtration on Sepharose 4B separates the water-soluble extract from pig gastric mucus into mucoprotein A and mucoprotein B/C with $s^0_{25,w}$ values in 0.2M-KCl of 18.7S and 4.3S respectively. After equilibrium density-gradient centrifugation of the extract in CsCl, removal of the CsCl by dialysis and subsequent separation on Sepharose 4B in 0.2M-NaCl, the same two mucoproteins had $s^0_{25,w}$ values of 33S and 5.7S (Fig. 1, Table 1). Both mucoproteins sediment with single schlieren peaks, with polydispersity visible at concentrations below which boundary sharpening was evident. The diffusion coefficients, $D_{25,w}$, were independent of mucoprotein concentration c up to $c/2 = 4$ g/l, and, combined with values of $s^0_{25,w}$, yield average molecular weights that are not appreciably different from those of the material before CsCl fractionation (Table 1). Mucoprotein A without CsCl treatment always contains a small amount of mucoprotein B/C, but not after CsCl fractionation. The molecular weight of

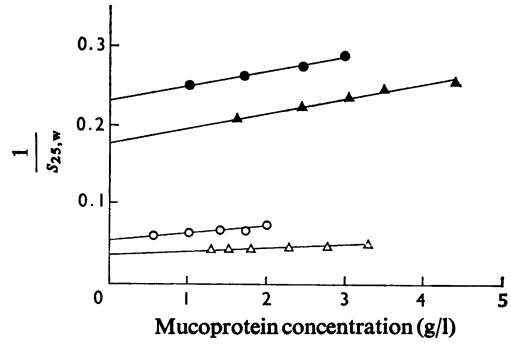

Fig. 1. *Effect of CsCl on the variation of sedimentation coefficient with concentration for mucoprotein A*

For details see the text. Mucoprotein A before treatment (○); mucoprotein A after CsCl treatment (△); mucoprotein B/C before CsCl treatment (●); mucoprotein B/C after CsCl treatment (▲). All samples were examined in 0.2M-KCl buffer, pH5.5.

Table 1. *Effect of CsCl on the flow properties of the mucoproteins of the water-soluble gastric mucus*

For details see the text. All measurements were made in 0.2M-KCl buffer, pH5.5.

	Mucoprotein A		Mucoprotein B/C	
Physical property	Before CsCl	After CsCl	Before CsCl	After CsCl
$s^0_{25,w}$ (S)	18.7	33	4.3	5.7
K_s (ml·g^{-1})	260	120	120	85
$10^7 \times D^0_{25,w}$ (cm^2·s^{-1})	0.69	0.94	3.05	3.38
$10^{-6} \times$ Mol.wt.	1.9	2.3	0.11	0.11
$[\eta]$ (ml·g^{-1})	320	160		
$K_s/[\eta]$	0.81	0.75		
f/f_0	4.45*	3.27		

* The value of f/f_0 for mucoprotein A in 0.2M-KCl in Snary *et al.* (1971) is in error.

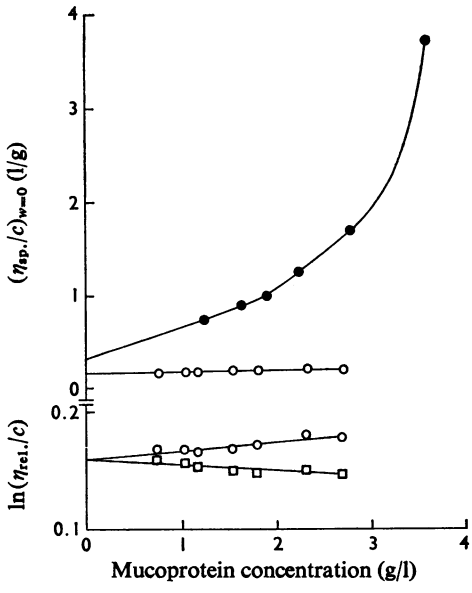

Fig. 2. *Variation of viscosity with concentration for mucoprotein A, prepared by gel filtration of the water-soluble mucus* (●) *or prepared by gel filtration of the mucoprotein purified by CsCl-density-gradient centrifugation* (○, □)

For details see the text. $(\eta_{sp.}/c)_{w=0}$ (○, ●); $\ln\eta_{rel.}/c$ (□). All solutions were measured in 0.2M-KCl buffer, pH5.5; all points refer to zero shear.

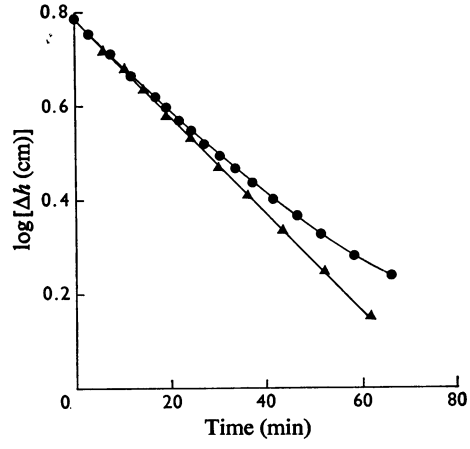

Fig. 3. *Effect of CsCl on the shear dependence of viscosity for mucoprotein A prepared by gel filtration of the water-soluble mucus* (●) *or gel filtration after CsCl-density-gradient centrifugation* (▲)

Δh is the hydrostatic pressure difference in the variable shear viscometer and is proportional to the shear stress. Deviation from linearity of this plot represents non-Newtonian behaviour.

1.9×10^6 for the former is therefore necessarily approximate (Snary & Allen, 1971).

The considerable increase in sedimentation coefficient and the decrease in value of the frictional ratio, f/f_0 (Table 1), indicates a decrease in asymmetry and/or a contraction of the mucoprotein after treatment with CsCl. The intrinsic viscosity (Fig. 2, Table 1) and the value of $K_s[1/s = 1/s^0\,(1+K_sc)]$ were used to calculate values of $K_s/[\eta]$, which Creeth & Knight (1965) have shown to be a measure of asymmetry. The constancy of these values (Table 1) shows that there is little or no change in asymmetry of the molecule on treatment with CsCl. The considerable increase in $s^0_{25,w}$ and the decrease in intrinsic viscosity on treatment with CsCl are interpreted therefore in terms of contraction of the mucoprotein A. The increase in $s^0_{25,w}$ of the low-molecular-weight mucoprotein B/C probably indicates a similar contraction, since its behaviour parallels that of mucoprotein A under a variety of experimental conditions (Allen & Snary, 1972; Snary *et al.*, 1970, 1971). Since all these physical measurements on the CsCl-treated mucoprotein were made in 0.2M-KCl, it follows that the conformational changes brought about by CsCl are irreversible.

Assuming that the change in flow properties is due solely to a change in volume, the order of the contraction can be indicated by calculating the 'effective hydrodynamic volume', V_e, from the relationship:

$$V_e = \left(\frac{f}{6\pi\,\eta_0}\right)^3 \cdot \frac{4\pi N}{3\bar{M}}$$

where f is the frictional coefficient calculated from the diffusion coefficient, η_0 is the viscosity of the solvent in poise, N is Avogardo's number and $\bar{M}$ is the molecular weight. CsCl causes the value of V_e for mucoprotein A to alter from $169\,ml\cdot g^{-1}$ before treatment to $67\,ml\cdot g^{-1}$ after treatment, on the assumption that no change in symmetry takes place.

Notable in the viscous behaviour is the lack of shear dependence for mucoprotein A after treatment with CsCl, together with the almost complete loss of concentration dependence compared with the untreated molecule (Figs. 2 and 3). In 0.2M-KCl there is a steep non-linear concentration dependence of viscosity with a Huggins constant, $k \approx 3$ as $c \to 0$ ($\eta_{sp.}/c = [\eta]+k[\eta]^2c$) and the viscosity rises asymptotically to very high or infinite values at a mucoprotein concentration of 4g/l. In CsCl the viscous behaviour changes to a concentration dependence which is linear, at least up to 3g/l, with a Huggins constant of 0.27. This indicates a considerable decrease in the intermolecular interactions which normally lead to gel formation by the native molecules.

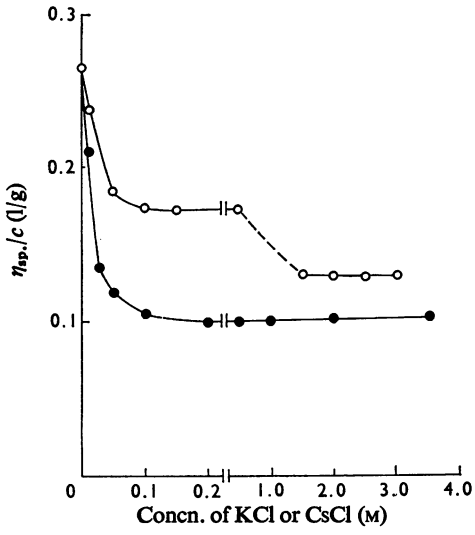

Fig. 4. *Viscosity of water-soluble mucus as a function of salt concentration*

For details see the text. KCl (○); CsCl (●). Mucoprotein concentration, 3.1 g/l.

It is possible that the groups responsible for intermolecular interaction in the native molecule are now involved in intramolecular interactions, thus stabilizing the CsCl-induced conformation and contributing to the lack of reversibility.

The isolated CsCl-treated mucoproteins described in this paper had been separated from non-covalently bound mucoprotein by density-gradient centrifugation (Starkey *et al.*, 1974). After treatment with CsCl, the mucoprotein contains 13.6% protein, so that 18.5% of the protein previously associated with mucoprotein A was non-covalently bound. This formed 2.5% by weight of the total mucoprotein complex.

The possibility must be considered that this protein, non-covalently bound to mucoprotein A before CsCl purification, is in some way determining the conformation of the mucoprotein and that the effect of CsCl is solely to separate the two, allowing the mucoprotein to take up a more compact conformation. This would imply that the highly contracted conformation is the thermodynamically more stable state under physiological conditions. This is unlikely, since this form has lost its biological function of gel formation. Further, the non-covalently bound protein is heterogeneous (Starkey *et al.*, 1974) and therefore less likely to contribute structural specificity. Stronger evidence against this possibility comes from the dependence of the conformational change on CsCl concentration (Fig. 4). The qualitative difference of the two salt effects in terms of a final conformational product and the low concentration at which CsCl begins to exert its effect, render it unlikely that its effect is primarily to separate protein from mucoprotein with a subsequent conformational change of the mucoprotein. On the basis of these findings and of those quoted earlier, it is concluded that CsCl is perturbing the mucoprotein conformation directly.

Comparison of the CsCl-induced conformational changes in the mucoprotein with those induced by KCl

Previous work has shown that KCl decreases the viscosity of the water-soluble mucus by producing conformational changes in the mucoprotein (Snary *et al.*, 1971). The decreased specific viscosity was measured at a single concentration and at a fixed shear rate for the water-soluble mucus as a function of CsCl concentration for comparison with the results obtained as a function of KCl concentration (Fig. 4). The water-soluble mucus contains a mixture of mucoproteins A and B/C, the high viscosity being very largely contributed by the former (Snary *et al.*, 1970). The viscosity falls rapidly from that at zero salt concentration, and at concentrations of CsCl higher than 0.1 M-CsCl no further change takes place.

In contrast, below 0.1 M-KCl there is a reversible increase in viscosity and this has been attributed to a classical polyelectrolyte effect. Between 0.1 M- and 0.5 M-KCl there is a plateau region where no change in flow properties takes place (Fig. 4). A conformational change, only partially reversible, takes place between 0.5 M- and 1.5 M-KCl, above which concentration the mucoprotein behaves as a less expanded but more asymmetric molecule. The single transition in CsCl occurs at low ionic strength and, by analogy with the behaviour in KCl, most likely combines a reversible charge-shielding effect with a non-reversible change owing to other causes. The broad plateau region in the presence of KCl shows that the polyelectrolyte effect is of limited extent even if Cs^+ were more effective in charge shielding than K^+ on a molar basis. The transition in CsCl is, however, distinct from the transition at high concentrations of KCl in terms of both the direction and extent of changes in sedimentation coefficient, intrinsic viscosity and the parameter $K_s/[\eta]$ (Snary *et al.*, 1971). It is also distinct from the transition at low KCl concentration in extent and, at least in part, in not being reversible. In terms of hydrodynamic models, the native mucoprotein in 0.2 M-KCl, pH 5.5, behaves as an expanded, approximately spherical molecule (Snary *et al.*, 1971). Above 1.5 M-KCl it contracts mainly about one axis to become a prolate ellipsoid or, as a possible alternative, contracts generally, leaving flexible arms which will have the same effect on the flow properties as asymmetry. From the sedimentation coefficients the model is seen as contracting

further in CsCl to become more symmetrical, or again, contracting in such a way as to avoid partial flexibility.

Role of solvent in determining the mucoprotein conformation

Since the mucoprotein contains 86% carbohydrate (Starkey *et al.*, 1974), it is reasonable to look for an explanation of the very large conformational changes observed at least partially in terms of the effect of salts on the sugar moieties. It is possible to account for the contraction and decrease in solvation of the mucoprotein by two different types of mechanism, one based on solvent environment changes and the other on specific ion interactions.

It has been suggested that the KCl-induced transition is a solubility phenomenon (Snary *et al.*, 1971). Sugar residues have generally a decreased solubility in water when simple salts are added. The increased activity of the sugar moiety in water would be expected to lead to exclusion of solvent, increased intramolecular contacts and a contraction of the molecule. Fluidity, entropy and dielectric relaxation measurements on salt solutions have been interpreted in terms of a structure-breaking effect on water of certain of the alkali-metal and halide ions. Whatever the significance of the absolute values, the order of effects places Cs^+ as a stronger structure breaker than K^+ (for reviews, see Jencks, 1969; Robinson & Stokes, 1970). Frank & Evans (1945) have calculated the 'structure-breaking entropy' contributed by the effect of ions on the water beyond the close, 'irrotationally' bound water immediately adjacent to the ion. These values amount to +90 and +120 $J \cdot °C^{-1} \cdot mol^{-1}$ for KCl and CsCl respectively. It has been proposed that the solvation of a sugar residue depends on the compatibility of the solute with the water lattice (Tait *et al.*, 1972; Franks *et al.*, 1973) and that this solvation is decreased when the lattice structure is disrupted thermally. Such a mechanism is compatible with either of the two main groups of models for water structure, the 'flickering cluster' model involving ice-like structures (e.g. Frank & Evans, 1945), and the 'continuum' model, a continuously hydrogen-bonded network with a continuous distribution of bond energies and geometries (e.g. Pople, 1951). Disruption of the 'lattice' or, in terms of Pople's (1951) model, increased departure from the ideal tetrahedral angles between water molecules, induced by the introduction of ions into the solvent, would decrease the solvation of the sugar residues and lead to the observed contraction of the molecule.

On the basis of the values of the 'structure-breaking entropy', Cs^+ would be expected to be more effective than K^+ in affecting the mucoprotein conformation and this is borne out by the experimental results. The degree of relative effectiveness of CsCl on a molar basis may be due to the fact that the degree of disordering will increase more slowly as higher concentrations of salt are reached since, in 1 M salt, no water molecule is, on average, more than two or three molecules away from an ion (Robinson & Stokes, 1970).

A second type of mechanism involving specific ion interactions might be suggested by the relatively low concentration at which CsCl brings about the dramatic change in mucoprotein conformation. Alkali-metal ions are known to be chelated by ether oxygen atoms such as exist in the glycosidic bonds of the polysaccharide moiety. It is, however, difficult to envisage a sufficiently critical steric assembly of groups for chelation to take place, given the current idea of flexibility of structure for mucoproteins. The difference between K^+ and Cs^+ in this context would demand a high degree of specificity for chelation.

Conformational changes in the mucoprotein caused by guanidinium chloride

The effect of guanidinium chloride on the mucoprotein is also considerable. When mucoprotein A, prepared by density-gradient centrifugation in CsCl, is dialysed against 4M-guanidinium chloride, the sedimentation coefficient, $s^0_{25,w}$, is lowered from 33S to 26S, and the concentration dependence is markedly increased from $K_s = 120$ to 590ml/g (Table 2, Fig. 5). The weight-average molecular

Table 2. *Effect of guanidinium chloride on the physical properties of mucoprotein A prepared by density equilibrium centrifugation in CsCl*

Mucoprotein	$s^0_{25,w}$ (S)	K_s(ml·g^{-1})	$10^7 \times D^0_{25,w}$ (cm^2·s^{-1})	10^{-6}×Mol.wt.
A*	33	120	0.94	2.3‡
A+guanidinium chloride†	26	590	—	2.7§
A after guanidinium chloride*	52	260	0.44	8.2‡

* Examined in 0.18M-KCl–0.02M-potassium acetate, pH5.5.
† Examined in 4M-guanidinium chloride–0.018M-KCl–0.002M-potassium acetate, pH5.5.
‡ Molecular weight from $s^0_{25,w}$ and $D^0_{25,w}$.
§ Molecular weight by sedimentation equilibrium.

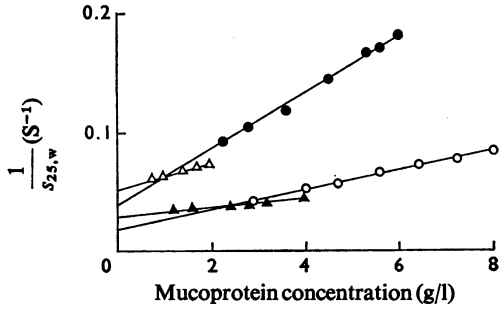

Fig. 5. *Effect of guanidinium chloride on the sedimentation behaviour of mucoprotein A purified by CsCl-density-gradient centrifugation*

For details see the text. Mucoprotein A (gel-filtered) in 0.2M-KCl (△); mucoprotein A (CsCl-purified) in 0.2M-KCl (▲); mucoprotein A (CsCl-purified) taken from 4M-guanidinium chloride and measured at 0.2M-KCl (○); mucoprotein A (CsCl-purified) in 4M-guanidinium chloride (●).

weight, $\bar{M}_w$, measured by short-column sedimentation equilibrium, is only slightly higher than for the native or CsCl-treated mucoprotein. Sedimentation equilibrium has been found to give slightly higher values for $\bar{M}_w$ than for $\bar{M}_{s,D}$ with these mucoproteins (Snary *et al.*, 1970). After removal of the guanidinium chloride and sedimentation in 0.2M-KCl, pH5.5, the sedimentation coefficient had risen to $s^0_{25,w}$ = 52S. Diffusion measurements gave an average molecular weight of 8.2×10^6. This aggregation is apparently not completely random, since the mucoprotein still sediments within a single schlieren peak with high optical recovery.

The decrease in $s^0_{25,w}$ in guanidinium chloride and the aggregation that occurs when the guanidinium chloride is removed is reminiscent of the behaviour of proteins in guanidinium chloride where the aggregated state, reached via the unfolded state, is frequently more stable than the native monomolecular state. For this reason and in keeping with the known solubilizing effect of guanidinium chloride on protein side chains and the peptide backbone (Tanford, 1970), it is suggested that at least part of the protein moiety of the mucoprotein becomes more flexible in guanidinium chloride. This does not rule out a contribution from the effect of guanidinium chloride on the sugar moieties.

Conclusions

The principal conclusion to be drawn from these results is that low concentrations of CsCl exert a dramatic effect on the conformation of mucoprotein from gastric mucus. Transfer into 0.2M-KCl leaves the mucoprotein irreversibly fixed in a conformation very different from that of the native molecule before CsCl treatment. Unless this mucoprotein is structurally unique, it is clear that the technique of equilibrium density-gradient sedimentation in CsCl and in CsCl–guanidinium chloride mixtures as a means of purifying protein–polysaccharide polymers should be used with discretion if conformational studies on the purified product are envisaged. The conclusion of immediate structural interest is that neither CsCl nor guanidinium chloride lowers the molecular weight, thus adding further evidence to the picture of mucoprotein A as a single covalently linked polymer.

We thank the Medical Research Council for their financial support.

References

Allen, A. & Snary, D. (1972) *Gut* **14**, 666–672
Creeth, J. M. & Denborough, M. A. (1970) *Biochem. J.* **117**, 879–891
Creeth, J. M. & Knight, C. G. (1965) *Biochim. Biophys. Acta* **102**, 549–558
Frank, H. S. & Evans, M. W. (1945) *J. Chem. Phys.* **13**, 507–532
Franks, F., Reid, D. S. & Suggett, A. (1973) *J. Solution Chem.* **2**, 99–113
Hascall, V. C. & Sajdera, S. W. (1969) *J. Biol. Chem.* **244**, 2384–2396
Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology*, chapter 7, McGraw-Hill, London
Pople, J. A. (1951) *Proc. Roy. Soc. Ser. A* **205**, 163–178
Robinson, R. A. & Stokes, R. H. (1970) *Electrolyte Solutions*, chapter 1, Butterworths, London
Snary, D. & Allen, A. (1971) *Biochem. J.* **123**, 845–853
Snary, D., Allen, A. & Pain, R. H. (1970) *Biochem. Biophys. Res. Commun.* **40**, 844–851
Snary, D., Allen, A. & Pain, R. H. (1971) *Eur. J. Biochem.* **24**, 183–189
Snary, D., Allen, A. & Pain, R. H. (1972) *Biochem. J.* **128**, 123–124P
Starkey, B. J., Snary, D. & Allen, A. (1972) *Biochem. J.* **128**, 123P
Starkey, B. J., Snary, D. & Allen, A. (1974) *Biochem. J.* **141**, 633–639
Tait, M. J., Suggett, A., Franks, F., Ablett, S. & Quickenden, P. A. (1972) *J. Solution Chem.* **1**, 131–151
Tanford, C. (1970) *Advan. Protein Chem.* **24**, 1–95
Yphantis, D. A. (1960) *Ann. N.Y. Acad. Sci.* **88**, 586–601