# Seed Mucilages as Examples of Polysaccharide Denaturation

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*Summary* Soluble polysaccharide particles from mustard mucilage are similar to globular proteins in having "internal crystallinity," and in showing denaturation and dissociation behaviour.

UNLIKE the proteins and nucleic acids, polysaccharides are not normally considered to have special conformational properties crucial to chemical behaviour and biological functions. However, most methods of extraction must disrupt natural associations and the conditions are usually harsher than would be used for other biopolymers—for example to obtain proteins with their native conformations intact. We now report a polysaccharide system in which specific interactions seem to exist. This is additional to the carrageenan coil $\rightleftharpoons$ double helix transition which was reported from this Laboratory[1] and which provides a different type of example of denaturation and renaturation of native polysaccharide tertiary structure.

Plant mucilages have been known for many years which contain a glucan, supposedly cellulose, solubilized in some way by complex acidic polysaccharides.[2] There are reports[3] that they contain microfibrillar material morphologically similar to native cellulose. We have investigated several such mucilages and, as an example, describe the product of cold water extraction of mustard seeds (*Brassica Sinapis alba*). It turns out that this *soluble* material does indeed contain cellulose in the crystalline condition: oriented fibres and films gave the *X*-ray diffraction photographs and i.r.–deuteriation behaviour[4] of cellulose IB and, after regeneration from cuprammonium solution, the i.r.–deuteriation behaviour changed to the highly characteristic[5] cellulose II pattern. Electron microscopy with negative staining showed long filaments which corresponded in width (37 Å) to the apparent diameter which is typical[6] of native cellulose. In view of the well known impossibility of forming the cellulose I lattice *in vitro*, and the evidence given below, these microfibrils must have been present in solution. The ultracentrifuge showed a sharp peak which, in salt solution, corresponded to a sedimentation coefficient of 25s—slower, for example, than tobacco mosaic virus. Free polysaccharide molecules were also present, as shown by broad peaks at higher force fields. The component which corresponded to the sharp peak, which we call the "mucilage particle," was purified by preparative ultracentrifugation in a density gradient and isolated by freeze-drying or precipitation with ethanol. It was freely soluble in water and the diffraction and spectroscopic properties were similar to the unfractionated material, though better defined. Hydrolysis and paper chromatography showed, in addition to large amounts of glucose from the cellulose, a complex mixture of the typical sugars of plant cell walls, especially the components of the so-called pectic substances: galacturonic acid, rhamnose, galactose, arabinose, and xylose. Acid or alkaline digestion, which presumably degraded noncellulosic polysaccharides, gave insoluble cellulose I in a yield of approximately 50% by weight.

We conclude that crystalline bundles of cellulose chains are solubilized by association with, perhaps encapsulation by, other polysaccharides. This would also explain why we have found cellulose in the particles to be resistant to cellulase action, and the particles to be mobile on boundary electrophoresis. Biologically, we consider that they represent units of cell wall structure which, for special biological reasons in a freak situation,[7] are assembled without the covalent and noncovalent cohesion which normally exists to make harsh conditions necessary for extraction. The characteristics of the particles in solution show some similarities to globular proteins:

(i) Despite their solubility, they have internal crystallinity *cf.* myoblobin and other soluble proteins with high content of $\alpha$-helix or $\beta$-structure.

(ii) When the solution is boiled coagulation occurs to give a gelatinous precipitate with chemical composition (sugars released on hydrolysis) indistinguishable from untreated particles *cf.* protein denaturation on boiling an egg.

(iii) 'Denaturation' is facilitated by certain reagents, sometimes to the extent that the process occurs in the cold, which include familiar denaturants in protein chemistry: urea, sodium laurylsulphate, potassium thiocyanate.

(iv) The precipitate from (iii) differs from (ii) in giving much lower proportions of galacturonic acid, galactose, and rhamnose on total hydrolysis. This means that partial *dissociation* of the particle has occurred, as well as denaturation, under conditions unlikely to have broken covalent bonds. Within the soluble particle, therefore, there would

seem to be noncovalent cohesion between two or more chains *cf.* haemoglobin.

In classical language, these mucilage solutions would be said to contain "colloidally dispersed" cellulose—a term which, as proteins and nucleic acids have taught us, is a poor substitute for a molecular description. Although the similarities between mucilage particles and globular proteins are superficial, they suggest that important advances may come when we begin to think of these and other polysaccharides as biopolymers with scope for specific cohesion.

We thank Mr. David Wright, Mr. Robert Hart, and Dr. Ian Steele for experiments, Sir Edmund Hirst for his interest, and Professor Robert Brown, Dr. C. T. Greenwood, Mr. A. J. Tulett, and Mr. G. A. Wilson, for access to the ultracentrifuge, boundary electrophoresis apparatus, and electron microscopes, respectively. We are also grateful to H. P. Bulmer Limited, Marine Colloids, Inc., and the Pioneering Research Committee of the Institute of Paper Chemistry for research studentships, and the S.R.C. for a grant to purchase the preparative ultracentrifuge.

(*Received, June 4th,* 1969; *Com.* 785.)

[1] A. A. McKinnon, D. A. Rees, and F. B. Williamson, *Chem. Comm.*, 1969, 701.
[2] F. Smith and R. Montgomery, "The Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959; G. O. Aspinall, *Adv. Carbohydrate Chem.*, 1969, **24**, in the press; J. M. Tyler, *J. Chem. Soc.*, 1965, 5288, 5300.
[3] K. Mühlethaler, *Exp. Cell. Res.*, 1950, **1**, 341; P. A. Roelofsen, "The Plant Cell Wall," Gebruder Borntraeger, Berlin, 1959.
[4] H. J. Marrinan and J. Mann, *J. Polymer Sci.*, 1956, **21**, 301.
[5] J. Mann and H. J. Marrinan, *Trans. Faraday Soc.*, 1956, **52**, 481.
[6] A. Frey-Wyssling and K. Mühlethaler, "Ultrastructural Plant Cytology," Elsevier, Amsterdam, 1965.
[7] A. Tschirche, "Angewandte Pflanzenanatomie," Urban and Schwarzenberg, Vienna 1889, Volume I.