Phytochemistry, Vol. 21, No. 12, pp. 2835–2843, 1982.
Printed in Great Britain.

0031-9422/82/122835–09$03.00/0

BIOPHYSICAL PROPERTIES OF *OPUNTIA FICUS-INDICA* MUCILAGE

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(*Revised received* 24 *March* 1981)

Key Word Index—*Opuntia ficus-indica*; Cactaceae; mucilage; gelation; circular dichroism; light scattering; sedimentation; viscosity.

Abstract—The purified mucilage from *Opuntia ficus-indica* is a high MW polysaccharide which behaves as a polyelectrolyte. Viscosity of its solution is dependent on the Ca^{2+} ion concentration and on pH, being greatest at alkaline pH. The sedimentation coefficient was dependent on concentration. The molecule had an estimated axial ratio of 256 in water, and this was reduced at low pH and in the presence of high concentrations of Ca^{2+}. The molecule was studied with light scattering and CD techniques and its UV spectrum was recorded. All these parameters were influenced by pH and by ion concentration. The gelation properties also changed with pH and with Ca^{2+} giving dense gels in its presence and loose ones in its absence. The results are interpreted in terms of changes in conformation of the molecule, changes in Ca^{2+} binding and degree of ionization of the molecule. An attempt is made to relate the molecular properties to the physiological function of the mucilage in the calcium and water economy of the plant.

INTRODUCTION

Polysaccharides capable of forming gels in water are common throughout the plant kingdom. Some of them such as the pectins in higher plants [1–12] and the carrageenans and agarose in algae [13–30], algal [4, 5, 11, 12, 28–35] and bacterial [36, 37] alginates and xanthan [38–44] have been investigated in great detail and a fair amount is known about their biochemistry and biophysical properties. In contrast, the compounds often referred to as mucilages have been much less studied. The mucilages are generally hetero-polysaccharides, with a varying uronic acid content [45]. They are produced in specialized cells in the plant [46] or in the outer layers of cells in seed coats or the root cap (see ref. [47] for references). We have described the site of formation [46] and chemical properties of the mucilage of *Opuntia ficus-indica* (L.) Mill. [47], and also followed the development of the mucilage-producing cells [48, 49] and their relation to cells containing calcium oxalate [50]. Here we describe some of the biophysical properties of this mucilage. It is hoped that these will contribute to an understanding of its physiological role in the plant, which is still quite uncertain.

RESULTS

The viscosity of *Opuntia* mucilage was measured under various conditions (Figs. 1–6). Viscosity strongly depended on ion concentration, indicating that the molecule is a polyelectrolyte [51]. High values of viscosity are obtained in water and these are drastically reduced in the presence of Ca^{2+} up to 10 mM, after which a constant value is obtained (Figs. 1 and 4). In the presence of a buffer the viscosity is also reduced by the presence of Ca^{2+} ions, but the changes are more moderate than in water (Figs. 2, 5 and 6). Substitution of Na^+ for Ca^{2+} in the presence of buffer results in a similar pattern of reduction of the intrinsic viscosity with increasing ion concentration. The values of viscosity at equal molar concentration of Na^+ and Ca^{2+} were slightly higher in the presence of Na^+ (Fig. 5).

The effect of different pHs in the presence of 100 mM calcium chloride is shown in Figs. 3 and 6. Viscosity was constant between pH 5.0 and 7.0 but rose sharply in the alkaline region. In the absence of Ca^{2+} viscosity remains constant in the alkaline region but declines sharply between pH 7.0 and 1.9. Viscosity values are somewhat higher than in the absence of Ca^{2+} (Fig. 6).

The sedimentation coefficients of the mucilage were strongly dependent on mucilage concentration (Fig. 7). The apparent MW calculated for $S = 21.3 \times 10^{-13}$ sec and $D = 0.31 \times 10^{-7}$ cm^2/sec gave MW values of 4.3×10^6 for mucilage dissolved either in 20 mM Tris-HCl, pH 7.4 in the presence of 10 mM Na^+ or 10 mM Ca^{2+}. When the Ca^{2+} concentration was raised to 100 mM the intrinsic S value decreased to 18×10^{-13} sec. In water the bulk of the mucilage precipitated as a gel in the centrifuge cells and could be detected at the edge of the Schlieren pattern. This might be due to molecular aggregation if the original material was polydispersed. A residual lower MW fraction was then detected in the solution with $S = 8.4 \times 10^{-13}$ sec. However cleavage of the molecule due to shear cannot be ruled out.

Since both viscosity, η, and sedimentation coefficient, S, are dependent on shape, we deter-

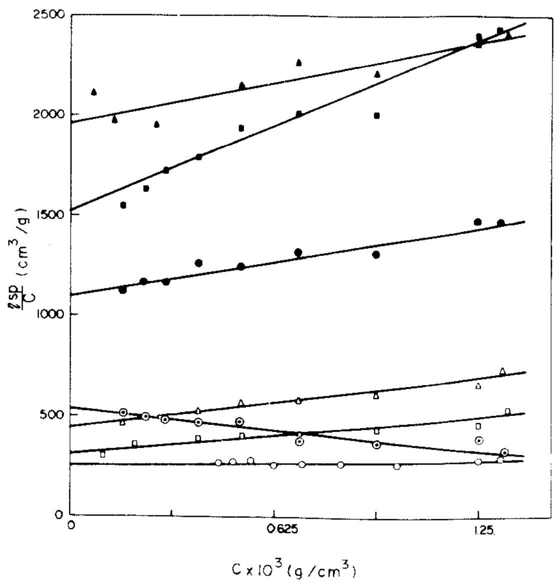

Fig. 1. Dependence of reduced viscosity (η_{sp}/c) of mucilage dissolved in H_2O in the presence of different molar concentrations of $CaCl_2$ on mucilage concentration. η_{sp}, Specific viscosity; η_{sp}/c, reduced viscosity; H_2O (▲), $CaCl_2$: 0.01 mM (■), 0.1 mM (●), 1 mM (△), 10 mM (□), 100 mM (◉) and 1000 mM(○).

mined the axial ratio of the mucilage molecule, assuming it to be shaped like a prolate ellipsoid, since in the electron microscope the molecules appeared to be clearly elongated. The axial ratio was calculated on the basis of an assumed partial specific volume of 0.6. This is the volume normally assumed for polysaccharides and the reputed variations in this volume are small (*ca* 5%). The axial ratio (p) changed greatly depending on experimental conditions. It was 256 in water (Fig. 8) and dropped to *ca* 80 at low pH in the presence of 100 mM Ca^{2+} and above (Table 1). At a constant pH of 7.4 there was little effect of either the Ca^{2+} or Na^+ ion concentration, although there was some decrease with increasing concentration. A decrease of the apparent MW was observed from 4.3×10^6 to 1.77×10^6 (calculated from S and $[\eta]$) or to 1.56×10^6 (calculated from light scattering) in the presence of 10 and 100 mM calcium chlorate at pH 7.4, respectively. This decrease was more or less continuous for Ca^{2+}, but only began above 10 mM for Na^+ (Fig. 8). The pH had a clear effect on the molecular shape and this effect was dependent on the ion present (Table 1). In the absence of Ca^{2+} the molecule 'shrank' as the pH was reduced from 7.4 to 1.9. In the presence of Ca^{2+} such an effect was only noticed between pH 10.0 and 7.0, below which it no longer changed.

The dimensions of the molecule, in its most compact form, i.e. the smallest axial ratio (pH 7.4, 100 mM calcium chloride) were calculated from light

Table 1. Dependence of axial ratio of mucilage molecule on pH in the absence or presence of 100 mM calcium chloride

	Axial ratio (p)	
pH	No Ca^{2+}	+100 mM $CaCl_2$
1.9	78.5	78.5
3.3	89	82
3.9	83	84
5.0	101	82.5
5.8	106	82.6
7.4	118	83
9.3	118	93
9.8	118	100
10.4		108

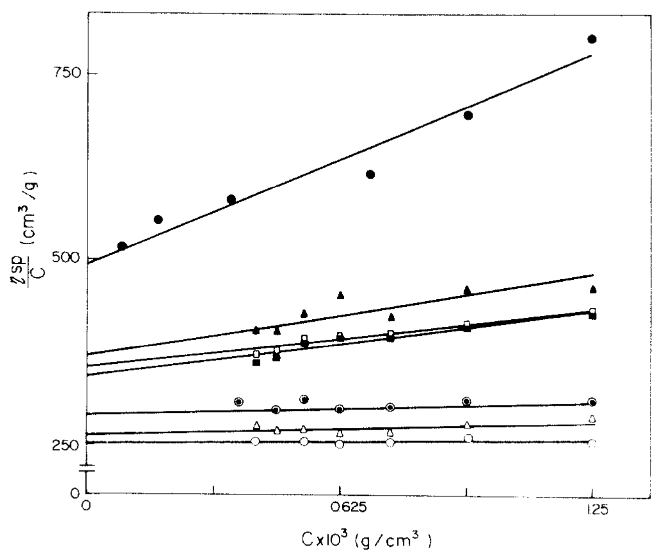

Fig. 2. Changes of reduced viscosity (η_{sp}/c) of mucilage dissolved in Tris–HCl, pH 7.4, buffer with various $CaCl_2$ concentrations. Tris–HCl (●) $CaCl_2$: 0.01 mM (▲), 0.1 mM (□), 1 mM (■), 10 mM (◉), 100 mM (△) and 1000 mM (○).

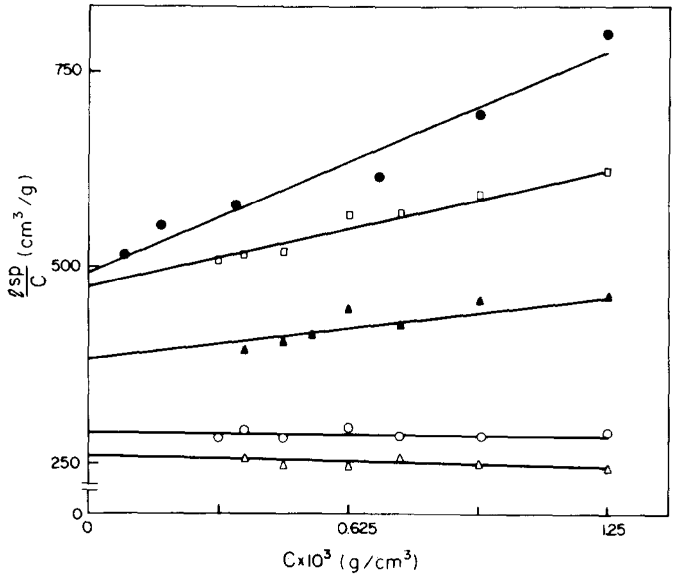

Fig. 3. Changes of reduced viscosity (η_{sp}/c) of mucilage with mucilage concentration, at different pH values. pH 9.8 (●), pH 7.4 (□), pH 5.8 (▲), pH 3.3 (○) and pH 1.9 (△).

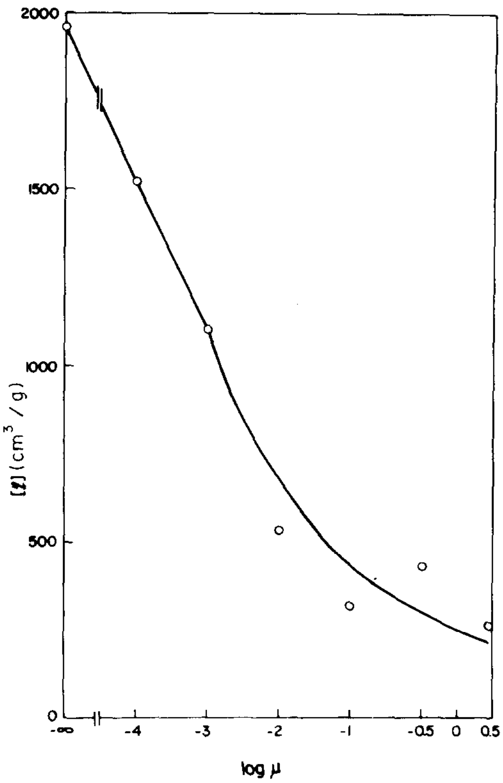

Fig. 4. Changes of intrinsic viscosity [η] of mucilage dissolved in H_2O in the presence of $CaCl_2$ with ionic strengths (μ). ($\mu = 1/2\Sigma m_i z_i^2$; m_i, molal concentration; z_i, ionic charge.)

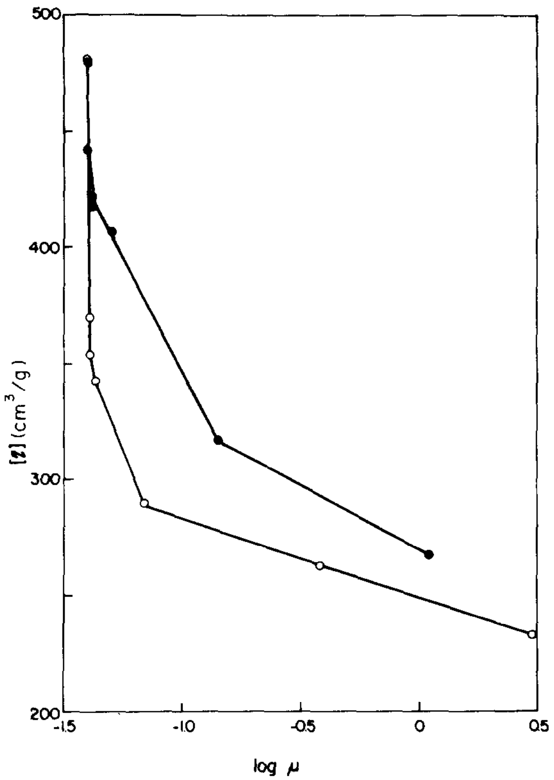

Fig. 5. Changes of intrinsic viscosity [η] of mucilage at pH 7.4 with ionic strength due to $CaCl_2$ (○) or NaCl (●).

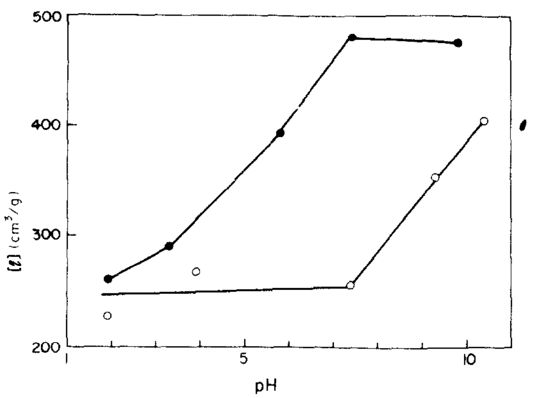

Fig. 6. Change of intrinsic viscosities [η] of mucilage with pH values in the presence (○) or absence (●) of 100 mM $CaCl_2$.

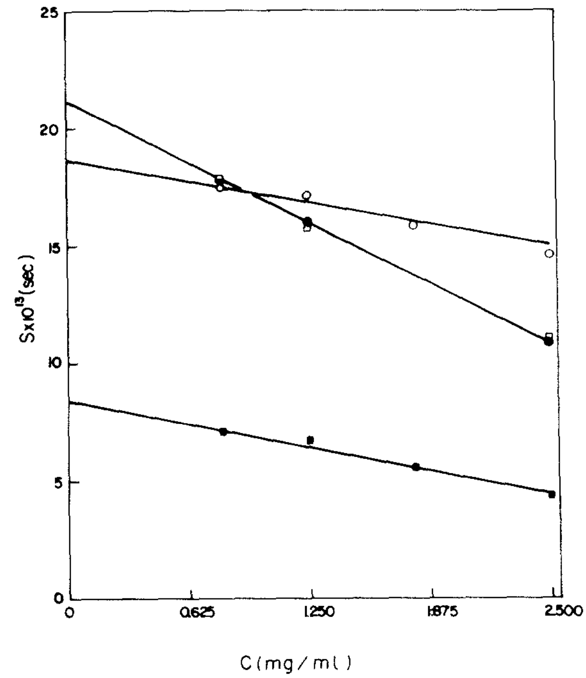

Fig. 7. Dependence of sedimentation coefficient (S) on mucilage concentration in H_2O (■) or in the presence of 10 mM $CaCl_2$ (●), 100 mM $CaCl_2$ (○) or 10 mM NaCl (□).

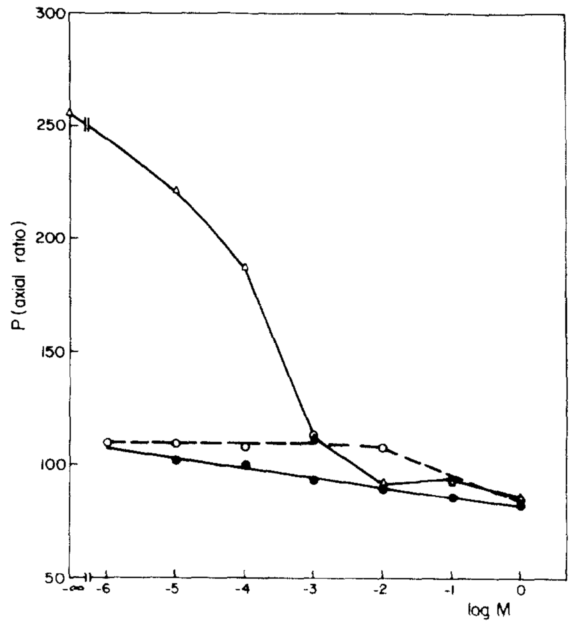

Fig. 8. Dependence of the axial ratio (p) of mucilage on ionic concentration in H_2O in the absence of $CaCl_2$ (△) or in Tris–HCl buffer 20 mM, pH 7.4, in the presence of $CaCl_2$ (●) or NaCl (○).

Table 2. Increase in weight of dried mucilage blocks when imbibed in the presence of different cations

	% increase in wt (10^{-3})	Electrolyte	Valence
1	13.5	H_2O	0
2	2.8	NaCl	1
3	1.5	$CaCl_2$	2
4	2.1	$LaCl_3$	3

scattering data (Fig. 9). The radius of gyration (R_g) was found to be *ca* 850 Å from which a length (L) of *ca* 2945 Å was calculated assuming a shape of a long rod. Assuming a shape of a prolate ellipsoid of semi axes a and $b (a > b)$ and an axial ratio of 80 gave dimensions of *ca* 1900 and *ca* 24 Å, respectively.

Gelation properties are often an indication of interactions between molecules. We therefore studied the gelation of the mucilage and its dependence on pH and Ca^{2+} ions by following swelling in the presence of mono-, di- or trivalent cations (Table 2). Minimal swelling was observed in the presence of Ca^{2+}. The trivalent La^{3+} had about the same effect, while Na^+ also reduced swelling greatly. Swelling decreased with decreasing pH in the absence of Ca^{2+}, but slightly increased in the presence of Ca^{2+} (Table 3).

The UV spectrum of the mucilage in the presence and absence of different ions shows no peak in any of the experimental conditions, but two shoulders can be seen at 250 and 280 nm. The CD spectrum of the mucilage has a positive band with a peak at 195 nm, when it is dissolved in water (Fig. 10). However, in 10 mM sodium chloride, calcium chloride, magnesium chloride or copper sulphate at pH 7.4 or in aqueous solution, a negative peak is observed at *ca* 220 nm. The intensity of the CD bands is high in water or in the presence of Ca^{2+}. In the presence of either Na^+ or Mg^{2+} it becomes progressively less. Ca^{2+} which usually intensifies the signal of chromophores neither increased the intensity nor shifted the peak to a longer wavelength. The difference in effect of Ca^{2+} and Mg^{2+} point to a special affinity of the molecule for Ca^{2+} ions [52, 53].

The ion concentration affects the intensity of the CD peaks at 220 nm in a complex fashion (Fig. 11). For all the cations the peak height was lowest in value when the ion concentration was 10 mM,

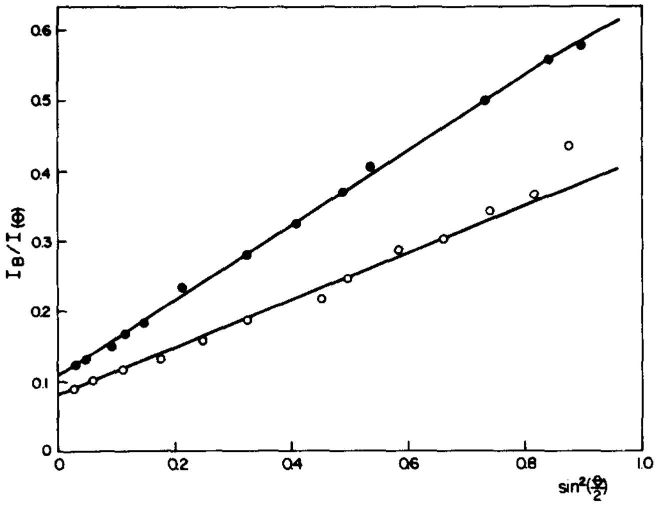

Fig. 9. Light scattering data for mucilage at two concentrations 1 mg/ml (○) and 0.52 mg/ml (●) dissolved in 10 mM Tris–HCl, pH 7.4, buffer in the presence of 100 mM $CaCl_2$.

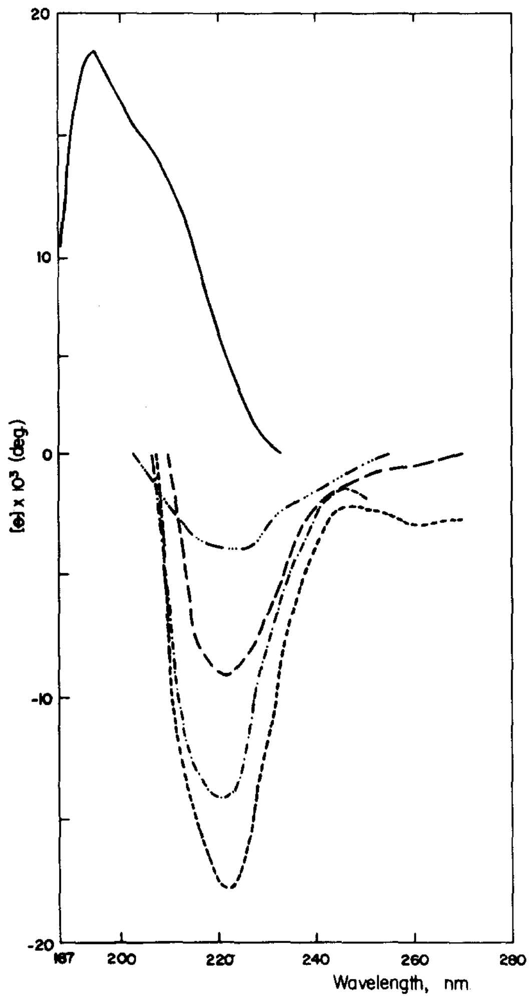

Fig. 10. CD spectra of mucilage dissolved in H_2O without counter ion or dissolved in 10 mM Tris–HCl buffer pH 7.4, in the presence of Cu (-···-···), Na(-·-·), Mg (——) or Ca (----).

Table 3. Change in weight ΔW (percentage) of inbibed mucilage with pH, in the presence or absence of 40 mM calcium chloride

	$\Delta W (\% \times 10^{-3})$	
pH	No Ca^{2+}	+40 mM $CaCl_2$
1.9	3.95	3.80
3.3	4.10	3.35
5.2	4.75	3.05
7.4	4.80	2.68
9.2	5.35	2.55
10.5	5.60	2.00

decreasing as the ion concentration increased from 0.01 to 10 mM. A further increase in ion concentration resulted in an increase in band intensity. Peak height increases with increasing pH up to 7.4 in the absence of Ca^{2+} (Fig. 12) while the presence of Ca^{2+} intensifies the band and in addition induces a decrease at pH above 7.4.

DISCUSSION

The physiological role of plant mucilages is still unclear; general functions have been attributed to them, most of which are related to water economy especially in succulents and plants of the arid and semiarid zones [54]. Mucilage is synthesized in specialized cells which die and become mucilage containers [46]. The properties and development of the mucilage cells in *Opuntia ficus-indica*, and the calcium oxalate idioblasts in the same tissue, showed good stereological correlation [50]. In order to understand the physiological functioning of the mucilage both in the water and calcium economy of the plant we examined the molecular behaviour of purified mucilage [47] in different conditions.

The mucilage contains 10–12% galacturonic acid

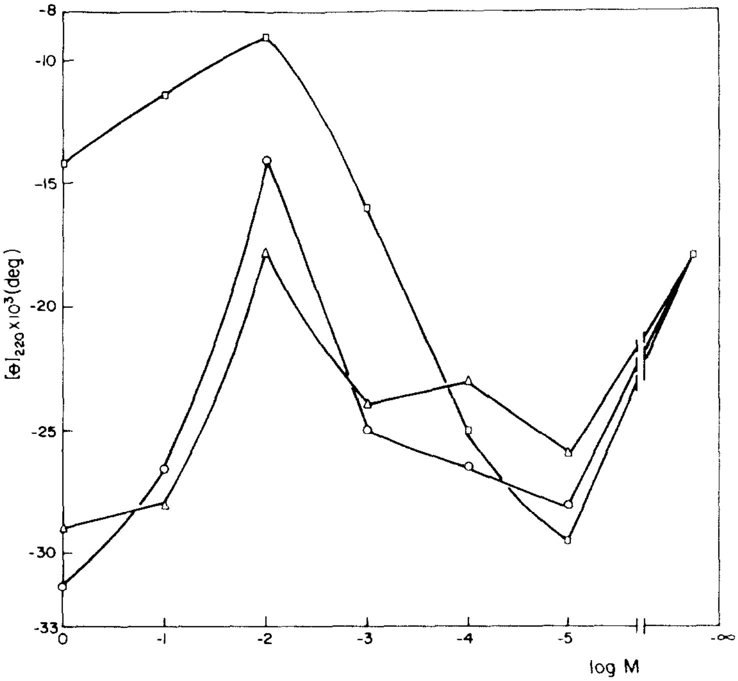

Fig. 11. Dependence of the intensity of the CD peaks (220 nm) of mucilage dissolved in Tris–HCl buffer, pH 7.4, on ionic concentration of $MgCl_2$ (□), $CaCl_2$ (△) and NaCl (○).

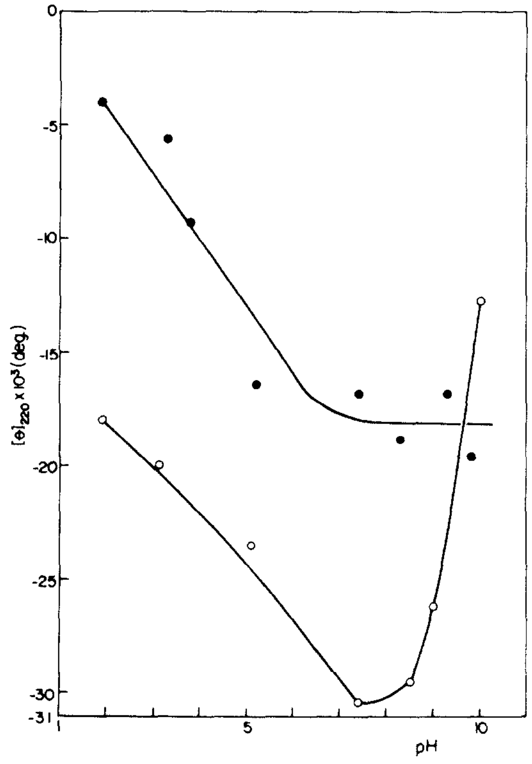

Fig. 12. Dependence of the intensity of the CD peaks (at 220 nm) of mucilage on pH in the presence (○) or absence (●) of 100 mM $CaCl_2$. (pH values were obtained using 20 mM buffers, see Experimental.)

and appears in the cells as a calcium salt [50]. During purification it is decalcified [47]. All experiments were carried out using the free acid form. The fact that the mucilage is a negatively charged polyelectrolyte is clearly seen from the viscosimetric studies in which a very strong dependence on concentration is seen (Figs. 1–6). The high viscosity in water, without a counter ion, can be attributed to the fact that the negative charges are exposed causing strong intermolecular repulsion resulting in expansion of the molecules and apparently inducing rigidity. Addition of Ca^{2+} ions neutralizes the negative charges, reducing both rigidity and repulsion. Replacing water by buffer as the solvent at a physiological pH causes a general reduction in viscosity due to moderation of the electrostatic effect. Nevertheless an effect of the cation concentration is noted. This is presumably due to effects on molecular shape and conformation.

Increasing the pH in the acid region (Fig. 6) causes an increase in viscosity since the carboxyls of the mucilage become ionized, being fully ionized above pH 7.0. Addition of Ca^{2+} prevents this increase in the acid region probably due to binding to the carboxyl groups preventing their ionization. Above pH 8.0 viscosity increases in the presence of Ca^{2+} perhaps due to a change from intra- to intermolecular binding of the Ca^{2+}. Sedimentation studies (Fig. 7) show that the solutions studied are molecular dispersed and that no intermolecular binding exists. This can be deduced from the fact that the same values of S and MW are obtained in the presence of Ca^{2+} and Na^+ at pH 7.4. Increasing the concentration of Ca^{2+} reduces the value of S as well as of MW indicating that its effect is intramolecular. An increase in S is expected for a water solution of mucilage due to the expansion of the molecule. The decrease in these values is due to the fact that a gel is formed and the Schlieren patterns are related to a lighter fraction of molecular fragments resulting from the breakdown of extremely long molecules under the high forces (g) caused by the ultracentrifuge.

Both pH and Ca^{2+} affect the hydrodynamic properties of the mucilage molecules as deduced from their axial ratios. In dilute solutions these effects are on a monomolecular basis. Ca^{2+} probably affects both the dimensions and rigidity of the molecule (Fig. 7). The strong repulsion due to electrostatic charges both in water or at high pH (Fig. 8 and Table 1) indicates either the ability to form dense and strong cross-linked gels in the presence of Ca^{2+} (Tables 1 and 3) or to form loose gels, in the absence of Ca^{2+}, which contain large amounts of water. This also suggests two mechanisms of gelation [8–10]. Buffering the environment probably causes a reduction in the electrostatic forces but the effect of Ca^{2+} is maintained (Fig. 8 and Table 1).

The molecule is shortest at pH 7.4 in the presence of 100 mM Ca^{2+}. The length of the molecule under these conditions is 2940 Å. Light scattering measurements were carried on the mucilage under these conditions to avoid absorbance phenomena. If the R_g increases as the axial ratio increases it can be assumed that in its fully expanded state in water (in the absence of Ca^{2+}) the molecule is three times as long as in the contracted form. Direct observations with the electron microscope of both spread and negatively stained preparations, and frozen-hydrated and freeze dried dilute solutions [Trachtenberg, S., unpublished] of the mucilage confirm the calculations based on hydrodynamic properties. These very long molecules must be very flexible in conformation.

Conformational changes as a function of the above mentioned conditions were studied by means of CD spectroscopy, following the $n \to \pi^*$ band of the carboxylic groups [55, 56]. Helical structures in polysaccharides are a well known phenomenon [57, 58]. Although Ca^{2+} induces the highest degree of asymmetry, the pattern of the CD band is the same for Na^+. The intensity of the CD band does not change very much between water and Ca^{2+}. These results indicate an order of asymmetric structure whose organization changes with pH and the counter ion. This might be due to helical structures and preliminary results with the electron microscope indicate such structures. However other interpretations are also possible. Following the influences of cation concentrations on the CD peaks (Fig. 11) reveals three zones. This might indicate that the mucilage binds the different cations differently and that there are differences in the 'strength' of binding, in some cases a buffering effect being noted, in others Ca^{2+} chelation. The effect of the pH (Fig. 12) is in good correlation with the viscometric results (Figs. 5 and 6). Both CD and viscosity in water and absence of Ca^{2+} increase in the acid region of the pH range and are constant in the alkaline range. Changes in the general hydrodynamic shape of the molecule are presumably the result of conformational changes in the molecule, such as the degree of its folding.

Large amounts of calcium oxalate are present in the Cactaceae and wide fluctuations in the pH of the vacuole of succulents occur due to formation and consumption of organic acids which is characteristic of CAM plants. Thus it is not unreasonable to expect that calcium is released at times from the oxalate, which might require binding at some other site. A strong relation exists between pH and calcium concentration on the molecular behaviour of the mucilage yet a reciprocal relation exists between the relation of water and Ca^{2+} with the mucilage. The interaction with Ca^{2+} causes the formation of smaller hydrodynamic volumes in solution and stronger gels of lower water contents, whereas the absence of calcium increases the hydrodynamic volumes of the molecules and forms gels, possibly via another gelation mechanism, of high water content and lower mechanical strength. The small volumetric fraction (3%) occupied by mucilage cells [48] makes it more likely that these cells are involved more in the Ca^{2+} economy of the plant than in its water economy, *ca* 20% of the insoluble Ca^{2+} present in the tissue can be bound to the mucilage present there [50].

EXPERIMENTAL

Mucilage was purified from *O. ficus-indica* plants as previously described [47]. Triple distilled H_2O and analytical reagents were used throughout. The buffers (20 mM) used were: KCl–HCl for pH 1–2; NaPi–citrate for pH 3–5; NaPi buffer for pH 6; Tris–HCl for pH 7.4–10 and glycine–NaOH buffer for pH 10.5.

Viscosimetry. Carried out in an Ubbelohde type viscosimeter (H_2O time 96 sec) at 30° ± 0.01. Values of viscosity are given as intrinsic viscosity

$$[\eta] = \lim_{c \to 0} \left(\frac{t}{t_0} \times \frac{\rho}{\rho_0} - 1\right) c^{-1}$$

where t and t_0 and ρ and ρ_0 are the flow times and densities of mucilage soln and solvent, respectively. For studies of the effect of ionic strength, the mucilage was dialysed against the salt soln and dilutions then made with the dialysate.

Molecular shape and dimensions. Followed by estimating changes in the axial ratio (ρ). The axial ratio was estimated from intrinsic viscosity, $[\eta]$ and partial specific vol., $\bar{v}(\bar{v} = 0.6$ ml/g) [59, 60]:

$$\frac{[\eta]}{\bar{v}} = \frac{14}{15} + \frac{p^2}{15 \ln 2p - 1.5} + \frac{p^2}{5 \ln 2p - 0.5}.$$

Values of $\Lambda(p)$ as a function of p are given in ref. [61]. The molecule was assumed to be a prolate ($p > 1$) ellipsoid of revolution in all experimental conditions.

Light scattering was measured with a Malvern photon correlator [62] using green laser light ($\lambda_0 = 5145$ Å) and C_6H_6 as a standard. I_B/I_θ (where I_B and I_θ are the intensities of C_6H_6 and mucilage at angle θ respectively) was plotted against $\sin^2(\theta/2)$ at mucilage concentrations between 0.2 and 1 mg/ml, dissolved in 20 mM Tris–HCl, pH 7.4, containing 100 mM $CaCl_2$ and for θ 0–180°. The radius of gyration (R_g) was calculated from

$$R_g = \left(\frac{4\pi n_0}{(\lambda_0)^{1/3}}\right)^{-1} \times \left(\frac{\text{limiting slope}}{\text{limiting intercept}}\right)^{1/2}.$$

The actual dimensions of the molecule were calculated for a prolate ellipsoid of revolution with semi axes a and b as $R_g = \left(\frac{a^2+2b^2}{5}\right)^{1/2}$ or for a long rod of length L as $R_g = \frac{L}{\sqrt{12}}$. MW was calculated from light scattering data (100 mM $CaCl_2$, pH 7.4) according to:

$$\mathrm{MW} = \left(\frac{4\pi n_b^2}{\lambda_0^4 \mathrm{N_A R_B}}\right) \times \left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)^2 + \left(c\,\frac{I_B(90)}{\Delta I(\theta)\sin\theta}\right)$$

where n_B is the index of refraction of C_6H_6, N_A is Avogadro's number, R_B is Rayleigh's ratio for C_6H_6 and (dn/dc) the refraction index increment for dextran.

Circular dichroism. Recorded at 20° on a Cary 61 CD spectrophotometer using cells with 1 cm path length. Absorption spectra in the UV range were recorded on a Cary 15 spectrophotometer.

Sedimentation and diffusion experiments. Carried out in a Beckman model E analytical ultracentrifuge equipped with Schlieren optics. MW was determined from sedimentation (S) and diffusion (D) coefficients according to:

$$\mathrm{MW} = \frac{\mathrm{RT}S}{D(1-\bar{v}\rho)}$$

or from (S) and (η] [63] (100 mM, pH 7.4) according to:

$$\mathrm{MW} = \frac{S[\eta]^{1/3}\mathrm{N_A}\eta_0}{2.5\times10^6(1-\bar{v}\rho)}.$$

Gelation experiments. Acid mucilage was dissolved in H_2O coagulated in EtOH and sedimented in centrifuge tubes in the form of small discs which were freeze dried. These discs were imbibed under different conditions of pH and ionic valency until equilibrium was reached.

Acknowledgements—Our thanks are due to Mrs. Y. Silpan for assistance in the CD determination, Mr. P. Yanai for running the sedimentation analysis, Dr. Z. Kam for help with the light scattering measurements and to Professors G. Blauer and W. Stein for helpful discussions.

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