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THE INFLUENCE OF SIDE-CHAINS ON THE DILUTE-SOLUTION PROPERTIES OF THREE STRUCTURALLY RELATED, BACTERIAL ANIONIC POLYSACCHARIDES

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ABSTRACT

Viscometric, calorimetric, and chiroptical data collected by using dilute aqueous solutions of the extracellular polysaccharides [Me_4N^+ salt form] from *Pseudomonas elodea* (S-60), *Alcaligenes* (ATCC 31555) (S-130), and *Alcaligenes* (ATCC 31961) (S-194) over a range of ionic strength (Me_4NCl concentrations) and temperature are reported. The data demonstrate that increasing the ionic strength of dilute solutions of the nonbranched S-60 at 25° promotes a distinctly co-operative, conformational transition over a narrow range of added salt concentration. Moreover, over the range of ionic strength examined, S-60 also undergoes a thermally reversible, conformational transition which exhibits no hysteresis: the temperatures at the midpoint of the sigmoidal transitions (T_M) increase moderately with increasing ionic strength. This conformational ordering has been monitored under conditions where S-60 does not gel. However, because both higher concentrations of S-60 at the same ionic strength, and higher ionic strengths at the same S-60 concentration, lead to gel formation, it is likely that the conformational ordering we have observed is the one involved in the gelation of S-60. The data also indicate that the two different modes of branching along the S-60 backbone present in S-130 (α-L-rhamnose and α-L-mannose linked to O-3 of the D-glucose after the D-glucuronic acid in the tetrasaccharide repeating-unit) and S-194 [β-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl units linked to O-6 of the D-glucosyl residue before the D-glucosyluronic acid] mediate against conformational ordering. This is consistent with the fact that S-60 forms firm aqueous gels, whereas S-130 and S-194 are non-gel-forming, but give stable, highly viscous solutions. In addition, the measurements indicate that the carboxyl group in S-30 is in a screened, molecular environment. It is suggested that this screening could arise from hydrogen bonding involving the L-rhamnose–L-mannose side-chains.

INTRODUCTION

The extracellular polysaccharides from *Pseudomonas elodea, Alcaligenes* (ATCC 31555), and *Alcaligenes* (ATCC 31961) provide an interesting opportunity to investigate polysaccharide structure–function relationships, as they have similar, but distinct, chemical repeating-units. *Pseudomonas elodea* polysaccharide has the tetrasaccharide repeating-unit shown in Fig. 1 (top). Both of the *Alcaligenes* polysaccharides possess regular branching along the same repeating-unit (see Fig. 1). In the case of *Alcaligenes* (ATCC 31555) polysaccharide, every D-glucosyl residue after the D-glucosyluronic acid is substituted at O-3 by either an α-L-rhamnopyranosyl or an α-L-mannopyranosyl residue, in the ratio[1] of ~2:1. In contrast, the

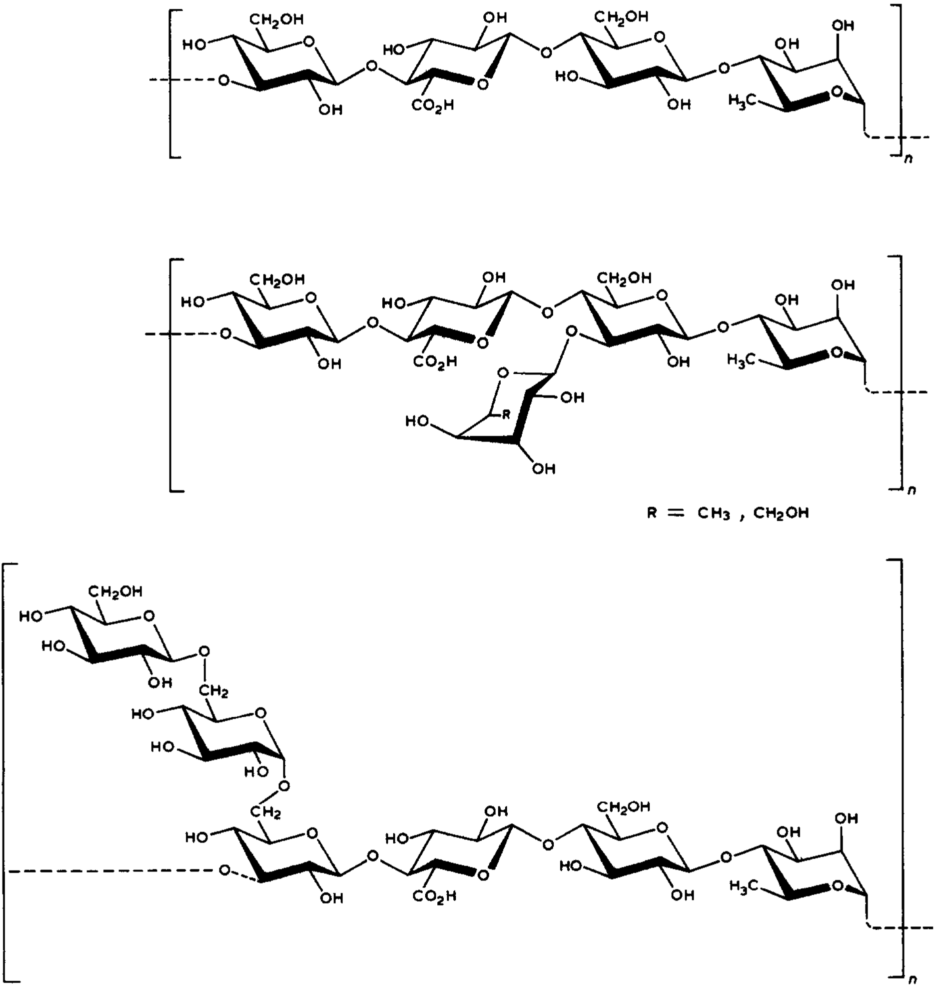

Fig 1 Repeating-unit structure of the polysaccharides from *Pseudomonas elodea* (S-60, top), *Alcaligenes* (ATCC 31555) (S-130; middle), and *Alcaligenes* (ATCC 31961) (S-194, bottom).

Alcaligenes (ATCC 31961) polysaccharide is substituted at O-6 of every D-glucosyl residue before the uronic acid by β-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl disaccharide side-chains[2].

All three polysaccharides in the native state are acetylated. In the case of *Pseudomonas elodea* polysaccharide, the acetyl groups are believed[3] to be on O-6 of one of the D-glucosyl residues. The position of *O*-acetylation in the *Alcaligenes* polysaccharides is not known. In this study, we have used commercially produced samples of these polysaccharide (supplied by Kelco Co.), which we have subsequently purified. The polysaccharide sample from *Pseudomonas elodea* had been chemically deacetylated before receipt by us, and will be referred to as S-60. The polysaccharide sample from *Alcaligenes* (ATCC 31555) contains approximately one *O*-acetyl group for every two repeating units, and will be referred to as S-130. The polysaccharide sample from *Alcaligenes* (ATCC 31961) contains an *O*-acetyl group for every repeating unit, and will be referred to as S-194.

The occurrence of sugar side-chains in the S-60 structure has a profound effect on the rheological properties. Thus, whereas S-60, at low concentration, forms firm, brittle, aqueous gels whose strength and thermal stability are influenced by the type and concentration of cations present, both S-130 and S-194 give highly viscous aqueous solutions having high degree of thermal, pH, and salt stability[4]. We have already reported[5] a preliminary comparison of the dilute-solution properties of S-60 and S-130. We now present a detailed comparison of the dilute-solution properties of all three polysaccharides, with the aim of investigating the ability of the fundamental tetrasaccharide repeating-unit to undergo conformational ordering, and how the sugar side-chains affect the physical properties of S-130 and S-194. In order to avoid possible complications arising from polymer aggregation, we have chosen to use purified polysaccharide samples quantitatively exchanged into the Me_4N^+ forms, so that stable aqueous solutions exhibiting little aggregation at polymer concentrations of <0.1% (w/v) could be obtained.

MATERIALS AND METHODS

The samples of S-60, S-130, and S-194 were kindly donated by Kelco Division of Merck & Co. (San Diego, California). S-60 and S-194 were received as white powders, and S-130 was a pale-yellow powder. The sample of S-60 was purified by the procedure of Carroll *et al.*[6]. The sample of S-130 was mixed with water, and the mixture filtered repeatedly through sintered glass. The polysaccharide was then precipitated from the filtrate with 2-propanol, dissolved in water, and the solution dialyzed overnight against NaCl. It was finally dialyzed exhaustively against double-distilled water, until the absence of an excess of salts was indicated by conductivity measurements. Because they were so viscous, solutions of S-194 were initially subjected to sonication for 1 h. S-194 was then dissolved in concentrated Me_4NCl solution, exhaustively dialyzed against Me_4NCl and, finally, against double-distilled water until the absence of an excess of salts was indicated by conductivity measurements. A number of batches of S-194 were prepared that differed slightly in molecular weight and viscosity. To ensure the preparation of the pure Me_4N^+ salt form, the solutions of all three polysaccharides were finally passed through a column of an ion-exchange resin (Merck; strongly acid cation-exchanger) in the Me_4N^+ form. Samples of the Me_4N^+ salts of S-60, S-130, and S-194 were finally obtained as solids by freeze-drying. The purified samples of S-60 and S-130 used in this investigation had properties very similar to those of the ones used in a previous study[5].

The *O*-acetyl contents of the purified materials were estimated from the ^{1}H-n.m.r. spectra, and these indicated, for S-60, one acetyl group present for every ~4 repeating units; for S-130, one acetyl group present for ~2 repeating units; and, for S-194, an acetyl group for every repeating unit. The equivalent weights of the three polysaccharides were determined by potentiometric titration with standard NaOH solutions, the polysaccharides having been previously transformed into the H^+ form by ion-exchange. The experimental equivalent weights for S-60 (720), S-130 (840), and S-194 (1068) in the Na^+ form are in good agreement with the structures shown in Fig. 1 when the *O*-acetyl contents are taken into account.

Viscosity measurement for the Me_4N^+ salts of S-60, S-130, and S-194 were performed in the temperature range 20–45° using a Schott–Geraete automatic viscometer equipped with a water thermostat. A range of ionic strengths was investigated; these strengths were controlled by varying the level of Me_4NCl added. Optical activity measurements were performed with a Perkin–Elmer 241 polarimeter, using a 10-cm path-length; the temperature was controlled by means of a Lauda circulating-water bath. The values of optical activity were calculated after taking account of the measured moisture content of the polysaccharide samples, and therefore differ slightly from those reported earlier[5]. Circular-dichroism spectra were obtained with a Jasco (model J-500A) dichrograph. Isothermal calorimetric mixing and protonation experiments were performed with an LKB twin-cell bath microcalorimeter at 25°. Measured heats were corrected for dilution effects. Differential scanning calorimetry experiments were made by using a Bio-DSC Microcalorimeter "Setaram" The ability of the three polysaccharides to form chiral complexes with Methylene Blue was assessed by mixing aqueous solutions of each polysaccharide (1.2 mequiv./dm^3) with Methylene Blue at a ratio of polysaccharide to dye of 20:1. The polysaccharide–dye complexes were subsequently examined by recording the circular dichroism spectra in the range of 450–700 nm.

RESULTS AND DISCUSSION

(a) Viscosity measurements

The results of the viscosity measurements carried out on aqueous solutions of S-60 for different Me_4NCl concentrations, both at 25 and at 45° are reported in Fig. 2. The notable difference in the ionic-strength dependence of S-60 viscosity at these two temperatures is clearly demonstrated by our data, and concisely evidenced by the insert of Fig. 2, in which the values of the Huggins' constant, *K*, are plotted

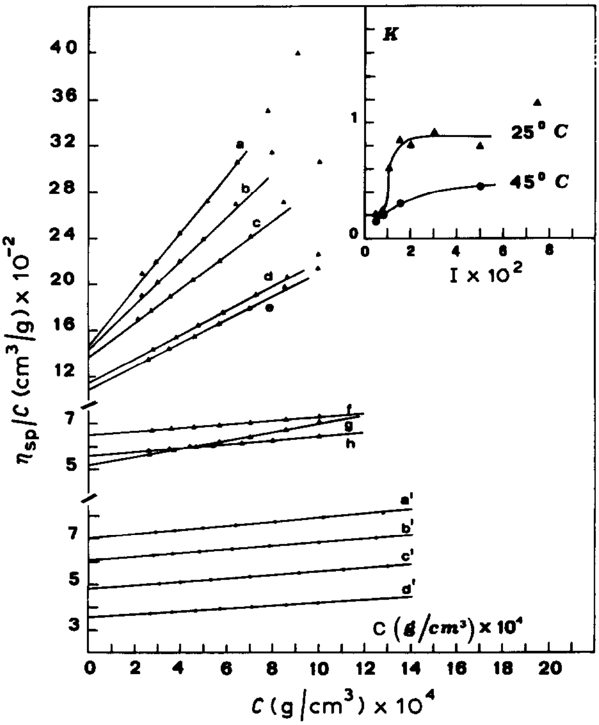

Fig 2 Reduced specific viscosity of aqueous S-60 at different Me_4NCl concentrations, at 25 and 45° [At 25°, the I values (M × 10²) are a, 7.5, b, 3.0, c, 5.0; d, 2.0; e, 1.5, f, 0.5, g, 0.1; and h, 0.75. At 45°, the I values (M × 10²) are a', 0.5; b', 0.75, c', 1.5; and d', 5.0. In the insert dependence of Huggins' constant, K, on ionic strength.]

against the ionic-strength, I. From Fig. 2, it is also noticed that, at 25° and for polymer concentrations >0.08% (w/v), the reduced specific viscosity points deviate from linearity, thus suggesting the onset of aggregation-phenomena. The data at 45° are typical of disordered polyelectrolyte behavior whereas those at 25° are complex and indicative of conformational ordering and aggregation processes. By extrapolation of the data from Fig. 2, the values of intrinsic viscosity, $[\eta]$, plotted in Fig. 3a, have been obtained. At 25°, the intrinsic viscosity of S-60 initially decreases, and then, for ionic strengths in the range 0.01–0.03M, steadily increases by a factor of ~2.5, and finally, at higher Me_4NCl concentrations, reaches a plateau. In contrast, at 45°, the intrinsic viscosity decreases regularly with increase in ionic strength, in a manner typical of disordered polyelectrolytes (see Fig. 3a).

In addition, Fig. 3b indicates that the reduced specific viscosity of S-60 (polymer concentration: 0.05% w/v in 0.03M Me_4NCl) changes in a discontinuous manner with temperature, between 25 and 45°. The transition is completely reversible, and no hysteresis is observed. The viscosity data of Figs. 2 and 3 give clear indication of the occurrence of a salt-induced, thermally reversible, conformational transition of S-60 chains. This transition would bring the polysaccharide into

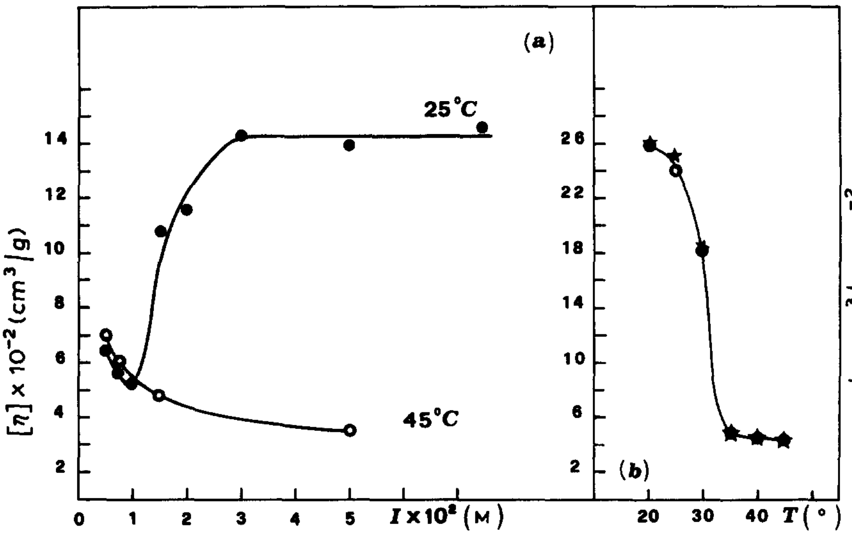

Fig 3 (a) Dependence of S-60 intrinsic viscosity on ionic-strength, I, (Me_4NCl), at 25 and 45° (b) Dependence on temperature of the reduced specific viscosity of S-60 (polymer conc : 0 05% w/v) in 0.03M Me_4NCl [Key: ★, heating; O, cooling.]

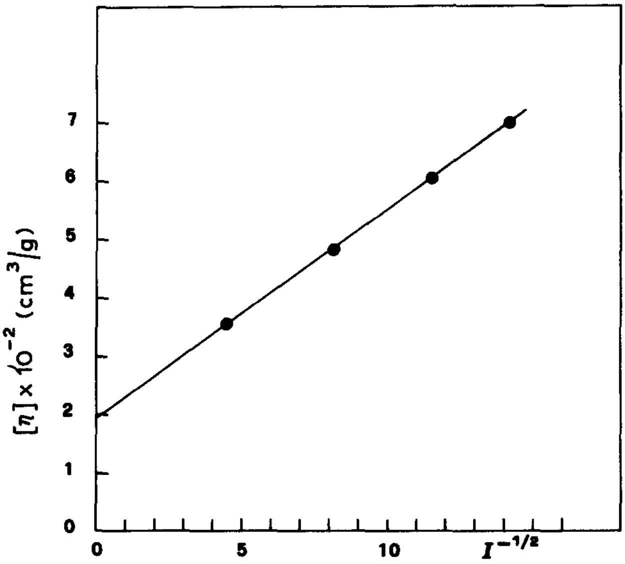

Fig. 4 Variation of S-60 intrinsic viscosity at 45° with $I^{-1/2}$

an ordered, elongated conformation from an initial, relatively disordered, state. The hydrodynamic properties of S-60 chains in the latter state would be similar at 25° (for I <0.01M) and at 45°, as suggested by the data of Fig. 3a.

In this context, it is interesting that the $[\eta]$ values for S-60 at 45° are linearly related to $I^{-1/2}$ (see Fig. 4), as had already been found for a number of polyelectro-

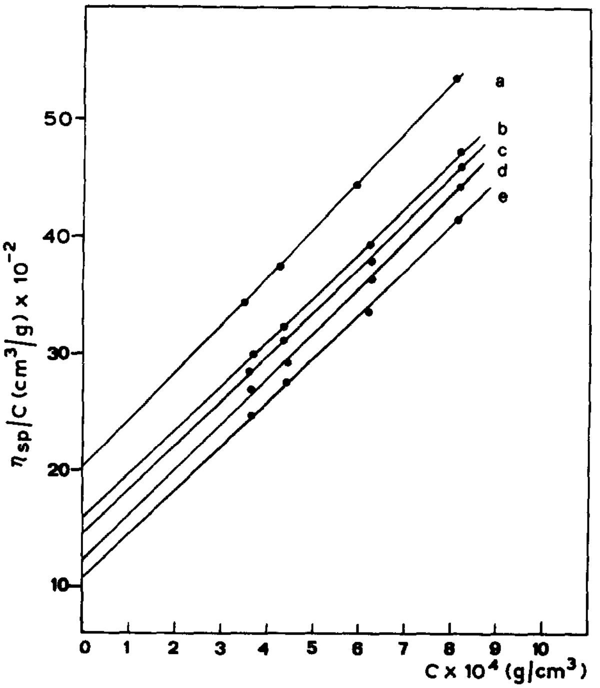

Fig 5 Reduced specific viscosity at 25° of aqueous S-194 at different Me_4NCl concentrations. [The I values (M × 10^2) are: a, 0.5, b, 1.0, c, 1.5, d, 5.0; and e, 15.0.]

lytes, including ionic polysaccharides[7]. Data from Fig. 4, lead, for S-60, to an estimated value of 0.1 for the “rigidity” parameter B in the notation of Smidsrød and Haug[7]. This B value falls approximately in between the corresponding values reported for *O*-(carboxymethyl)amylose and for *O*-(carboxymethyl)cellulose.

In contrast, the branched polysaccharides S-130 and S-194 exhibit quite different viscosity properties. Thus, Fig. 5 indicates that, at 25°, S-194 shows dependence of viscosity on ionic strength typical of a disordered polyelectrolyte.

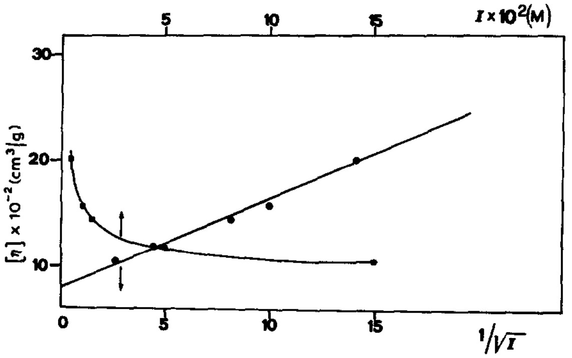

Fig. 6 Dependence of S-194 intrinsic viscosity on ionic strength, I, (Me_4NCl) at 25°. [The slope of the linear plot leads to an estimate of the value for the stiffness parameter B (see text) of 0.05.]

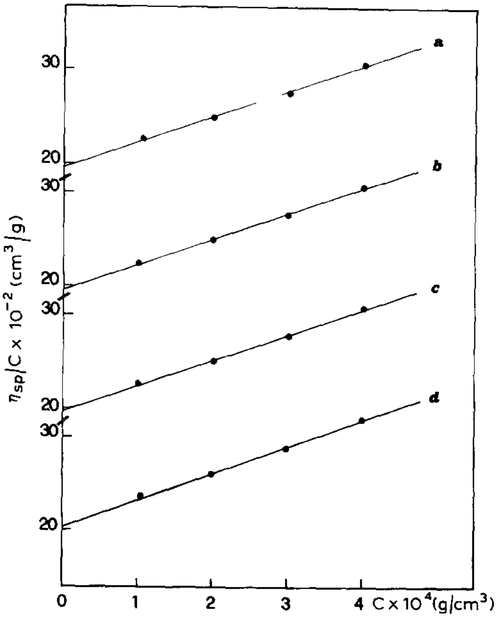

Fig 7 Reduced specific viscosity at 25° of aqueous S-130 at different Me_4NCl concentrations [The *I* values (M × 10^2) are a, 0 8, b, 0 40, c, 6 0, and d, 15 0]

Extrapolation of these viscosity data results in the intrinsic viscosity values plotted in Fig. 6. Although S-194 is clearly much more viscous than S-60, the ionic strength dependence of intrinsic viscosity of S-194 at 25° is closely similar to that of S-60 at 45°, emphasizing that it possesses typical, disordered polyelectrolyte properties.

Polysaccharide S-130 is also much more viscous than S-60. The viscosity properties of this polysaccharide differ significantly from those of both S-60 and S-194, in that there is virtually no dependence on ionic strength within the range 0.8–150 × 10^{-2}M Me_4NCl (see Fig. 7). Indeed, S-130 exhibits viscosity properties expected for a disordered neutral polysaccharide, despite its having a significant content of D-glucuronic acid. Thus, when the viscosity data are extrapolated to obtain intrinsic viscosities, no variation is observed with change in ionic strength.

As would be expected for disordered polymers, very little variation in reduced specific viscosity with temperature is observed for S-194 and S-130 (see Fig. 8). It should be pointed out that S-60 also shows this virtual temperature-independent behavior for reduced specific viscosity at ionic strengths where it adopts a disordered conformation.

Thus, viscosity measurements clearly indicate an ionic strength- and temperature-dependent, conformational ordering for S-60. The two different modes of branching of the S-60 backbone present in S-194 and S-130 appear to prevent this conformational ordering. S-194, with a disaccharide side-chain attached to O-6 of the D-glucosyl residue prior to the D-glucosyluronic acid residue in the main chain, exhibits viscosity properties typical of a disordered polyelectrolyte. S-130, with a

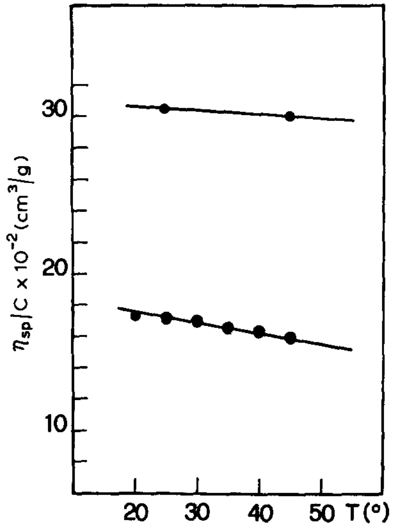

Fig 8 Dependence on temperature of the reduced specific viscosity of S-130 (polymer concentration 0 04 w/v in 60mM Me_4NCl) and S-194 (polymer concentration 0.04 w/v in 15mM Me_4NCl) [Key ○, heating, ●, cooling]

monosaccharide residue attached to O-3 of the D-glucosyl residue after the D-glucuronic acid residue in the main chain, exhibits viscosity properties typical of a disordered neutral polysaccharide. This indicates that the L-rhamnose or L-mannose stubs, or both, of S-130 in some way screen the carboxyl group in this polysaccharide.

(b) Chiroptical measurements

As previously reported[5], the optical activity at 302 nm and the circular dichroism in the region 190–250 nm of S-60 (Me_4N^+ form) change sharply at 25° on increasing Me_4NCl concentration in the range 0.01–0.03M salt. This parallels the viscometric measurements illustrated in Figs. 2 and 3. Moreover, the variation of the optical activity of S-60 (at 302 nm) as a function of temperature for different Me_4NCl concentrations is shown in Fig. 9. These data clearly indicate that (*1*) S-60 in the presence of salt undergoes a temperature-dependent conformational change which is perfectly reversible and which exhibits no hysteresis, and (*2*) the temperatures at the midpoint of the sigmoidal optical activity–temperature plots (T_M) increase moderately with increasing Me_4NCl concentration. For all of the Me_4NCl concentrations examined, apart from the highest (0.25M), the optical activity levels off at the lower temperatures. In contrast, for 0.25M Me_4NCl, the optical activity rises slightly at the lower temperatures. The reason for this different behavior at high salt levels is not known. However, it is not related to gel formation, because, at the polymer concentration used (0.08% w/v), gelation only occurs at Me_4NCl concentration >0.9M.

Plotting the T_M values in the form $1/T_M$ against $-\log I$ (see Fig. 10) gives a

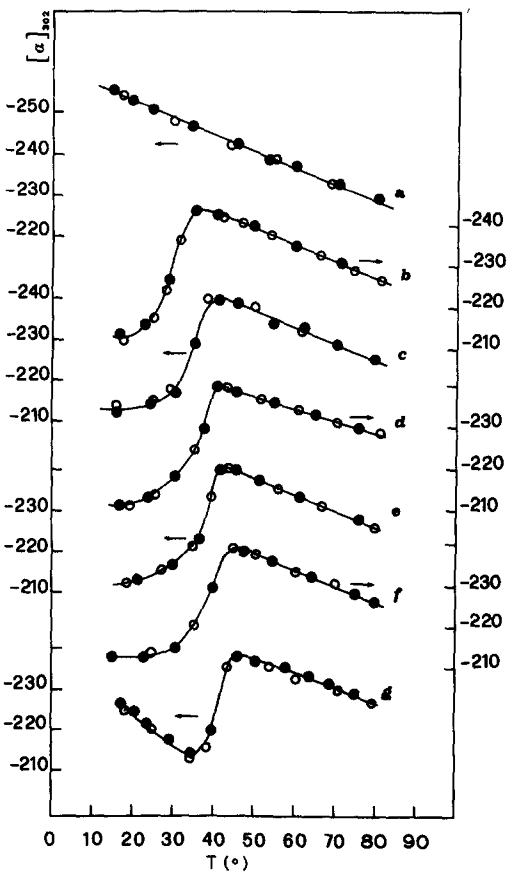

Fig 9 Optical activity at 302 nm of S-60 for different Me_4NCl concentrations as a function of temperature. [Salt concentration (M × 10^2). a, 0; b, 3 0, c, 7.5, d, 9.21, e, 12.0; f, 15 0; and g, 25 0 Key ○, heating; ●, cooling Polymer concentration· 1.2 mequiv./dm^3]

straight-line relationship. This is in apparent agreement with theoretical predictions for cooperative conformational changes of linear polyelectrolytes in solutions[8]. It is noteworthy that the solid points in Fig. 10 correspond to the T_M/log I result obtained from the specific viscosity data of Fig. 3b. Fig. 10 may be considered to be the conformational-state diagram for S-60 in aqueous Me_4NCl: in region I, disordered chains preponderate whereas region II would represent the stability domain of the ordered conformation.

It should be noted that the T_M temperature interval spanned by these data is less than half of the minimum interval found for ι- and κ-carrageenan[9–11] and xanthan[12] under similar ionic-strength conditions. This would indicate that, compared with these other polysaccharides, increasing the ionic strength has a relatively small effect on the thermal stability of the S-60 ordered conformational state in dilute Me_4NCl.

In contrast with these results for S-60, neither of the branched variants, S-130

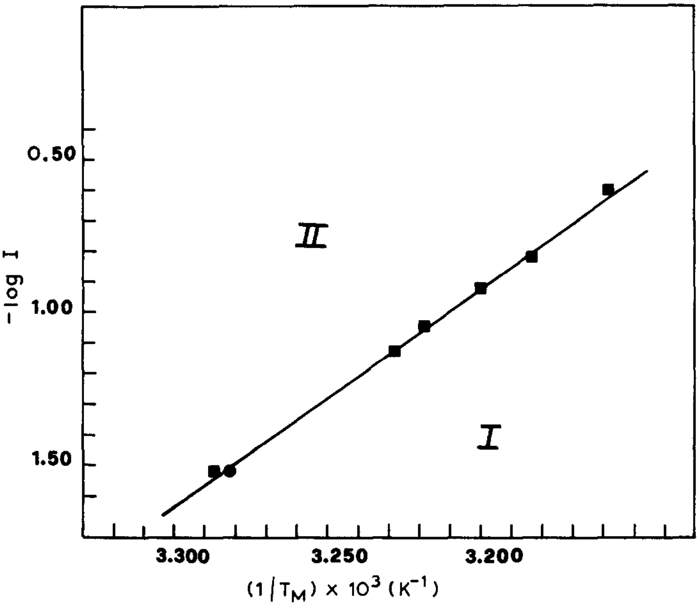

Fig 10. Correlation between T_M, the temperatures at the midpoints of the plots of Fig 9, and ionic strength, *I*

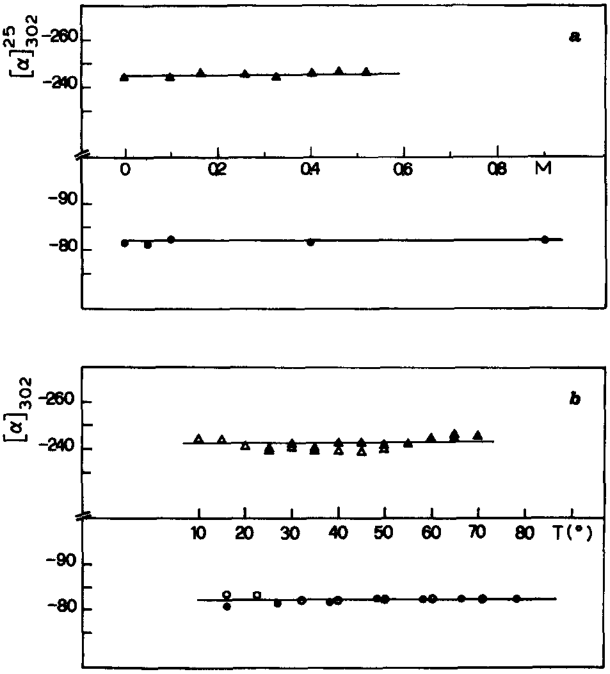

Fig 11 (a) Optical activity at 302 nm and 25° as a function of increasing concentration of Me_4NCl for S-130 (▲, polymer conc. 1.4 mequiv./dm^3). (b) Optical activity at 302 nm for S-130 (polymer conc. 1 2 mequiv /dm^3) and S-194 (polymer conc. 1.4 mequiv./dm^3) as a function of temperature. S-130 ▲, heating, △, cooling S-194. ●, heating; ○, cooling.

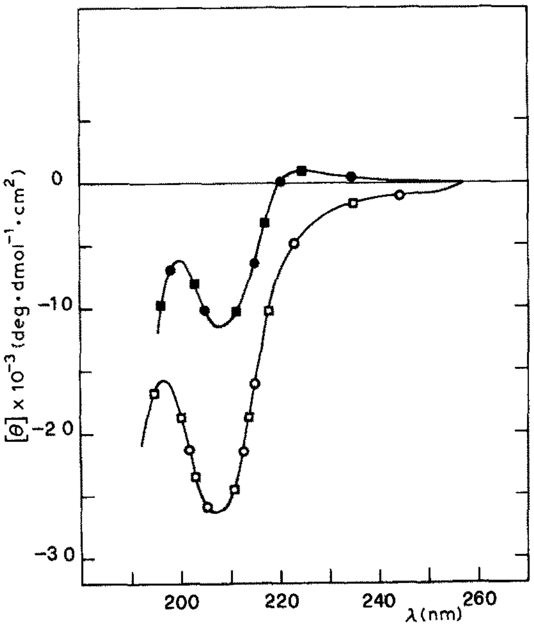

Fig. 12. Circular dichroism spectra of solutions of S-130 (polymer concentration, 1.2 mequiv./dm^3) in water (■) and 70mM Me_4NCl (●), and of S-194 (polymer concentration, 1 4 mequiv./dm^3) in water (□) and 900mM Me_4NCl (○)

and S-194, exhibits unusual chiroptical properties. Thus, both polymers give optical activity measurements at 302 nm which are independent of Me_4NCl concentration, up to a level 10 times the concentration of Me_4NCl required to effect the large optical activity transition for S-60 (see Fig. 11a). Similarly, both polymers give circular dichroism spectra in the range 190–250 nm which are invariant with Me_4NCl concentration (see Fig. 12). In addition, neither polymer exhibits temperature-dependent changes in optical activity in the presence of added Me_4NCl (see Fig. 11b). The absence of any sharp changes in optical activity for S-130 and S-194 would be expected for disordered polysaccharides having no ability to adopt an ordered conformation in solution, and is in agreement with the viscosity measurements already reported.

(c) Calorimetric measurements

Isothermal, microcalorimetric data for the mixing at 25° of solutions of S-60, S-130, and S-194 with aqueous Me_4NCl are shown in Fig. 13. These data further discriminate between S-60 and the two branched variants. The trend of the enthalpy of mixing (ΔH_{mix}) for S-60 is consistent[13,14] with the onset, at R ~5, of a conformational transition, this exothermic process being superimposed on the endothermic interaction between monovalent counterions and the polymer. The onset of this trend in ΔH_{mix} occurs at the same Me_4NCl concentration as do the viscometric and chiroptical transitions already reported for S-60, further confirming that this phenomenon is associated with a conformational-ordering process.

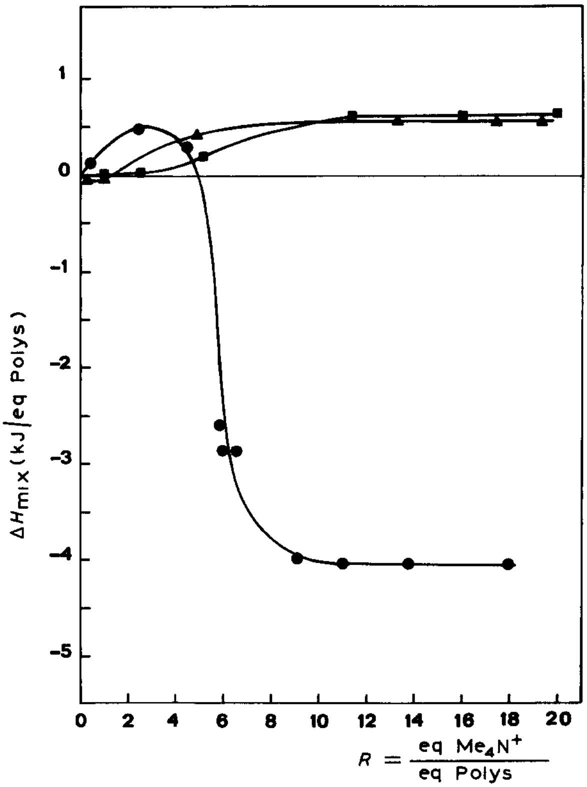

Fig. 13 Enthalpy of mixing (25°) of solution of S-60 (●, polymer concentration, 2.2 mequiv./dm^3), S-130 (▲, polymer concentration, 1 5 mequiv /dm^3), and (■, polymer concentration, 1 5 mequiv./dm^3) with Me_4NCl, as a function of the ratio (R) between the concentration of cations in equiv./dm^3 and the polymer concentration in the same units.

In contrast, both S-194 and S-130 exhibit trends in ΔH_{mix} with increasing Me_4NCl concentration typical of disordered polyelectrolytes which do not conformationally order, in that only the endothermic interactions between the monovalent cations and the polymer are observed (see Fig. 13). It is significant that this endothermic interaction is smaller for S-130 than for S-194. Furthermore, it would also appear that the endothermic-interaction component for S-60 is larger than in the case of S-130. This is consistent with the concept that the carboxyl group in S-130 is more screened than that in S-194 and S-60, as is indicated by the viscosimetric measurements (see Fig. 7). This lower endothermic interaction for S-130 is not the result of the polymer's being in an ordered conformation throughout the entire course of the isothermal mixing experiment, because, in such a case (*e.g.*, depyruvated xanthan), no change in ΔH would be observed[14].

The isothermal, microcalorimetric data for the mixing, at 25°, of S-60 solutions with aqueous Me_4NCl are also reported in Fig. 14a in differential form. This calorimetric plot can be formally divided into three main regions: for (*1*) salt

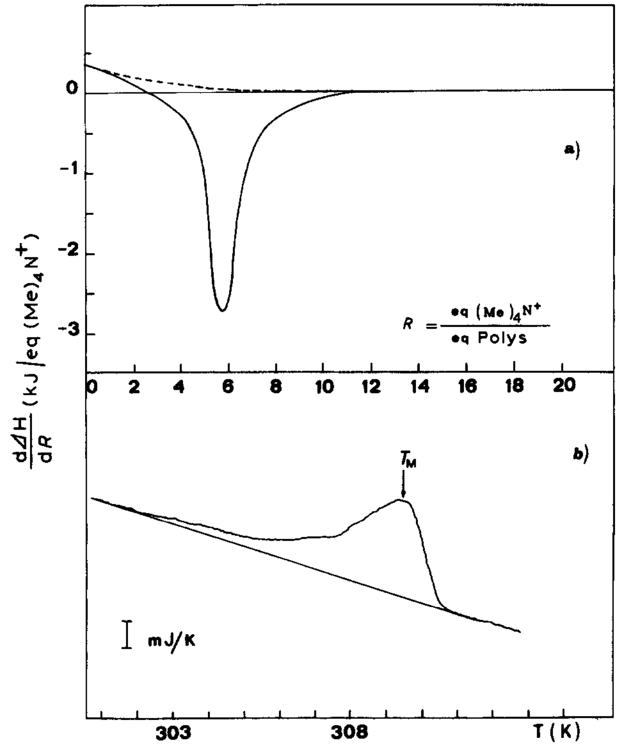

Fig 14 (a) Differential heat of mixing S-60 with Me_4NCl solutions at 25° Isothermal calorimetry data. Polymer concentration 2 2 mequiv./dm^3. (b) Differential scanning calorimetry of S-60 in 70mM Me_4NCl (thermogram recorded on cooling) Polymer conc 2 mequiv./dm^3

concentrations lower than ~8mM, in which a slightly endothermic mixing prevails; (*2*) salt concentrations in the approximate range of 8–25mM, in which a distinctly exothermic process takes place; (*3*) Me_4NCl concentrations greater than ~30mM, for which mixing produces very little excess heat, if any. The conformational change of S-60, an exothermic process, would take place essentially in the middle region (in agreement with the viscometric and chiroptical measurements). The first region, which might obviously extend farther (dotted line in Fig. 14a) were it not for the polysaccharide conformational transition, reflects the moderately endothermic polyelectrolyte–simple salt mixing-process. In the third region, mixing of Me_4NCl with S-60 in the ordered conformational state is characterized by a practically zero heat-effect. From the area of the (isothermal) peak of Fig. 14a the enthalpy of the S-60 transition, $-\Delta_M H$, is estimated as ~ −5 kJ per mol of repeating unit (*i.e.*, per equivalent of polymer) at 25°.

Differential scanning calorimetry (d.s.c.) measurements made using dilute solutions of S-60 (0.1% w/v) in 0.07M Me_4NCl led to an average determination (4 thermograms) for $-\Delta_M H$ close to −4 kJ per equivalent of polysaccharides at ~37°. A typical d.s.c. thermogram obtained on cooling is reproduced in Fig. 14b. The two values for $\Delta_M H$ may be considered to be in reasonable agreement, in view of their relative uncertainty (estimated as ±0.5 kJ per equivalent of polysaccharide) and of possible heat-capacity effects.

Isothermal, microcalorimetric investigations of the protonation of solutions of S-60, S-130, and S-194 at 25° were also performed. The data obtained (see Fig. 15) again discriminated between S-60 and the two branched variants. The trend of the enthalpy of protonation (ΔH_p) for S-60 is consistent with the onset, at around degree of protonation 0.5, of a conformational transition whose exothermic change is superimposed on the normal endothermic reaction between protons and the polyelectrolyte. By analogy with the exothermic ΔH_{mix} of S-60 with Me_4NCl, the onset of this exothermic ΔH_p for S-60 should be accompanied by rheological and chiroptical changes. This is indeed the case. Thus, at degree of protonation 0.6 and higher, S-60 solutions form gels, and give major changes in circular dichroism spectra in the region 190–250 nm reminiscent of the change in circular dichroism spectra accompanying the conformational ordering induced by added Me_4NCl at pH 7 (see Fig. 16).

In contrast, both S-130 and S-194 show endothermic trends of ΔH_p with protonation that is typical of disordered, random-coil polyelectrolytes (see Fig. 15). It is worth noting that all three polysaccharides show zero ΔH_p at the early stages

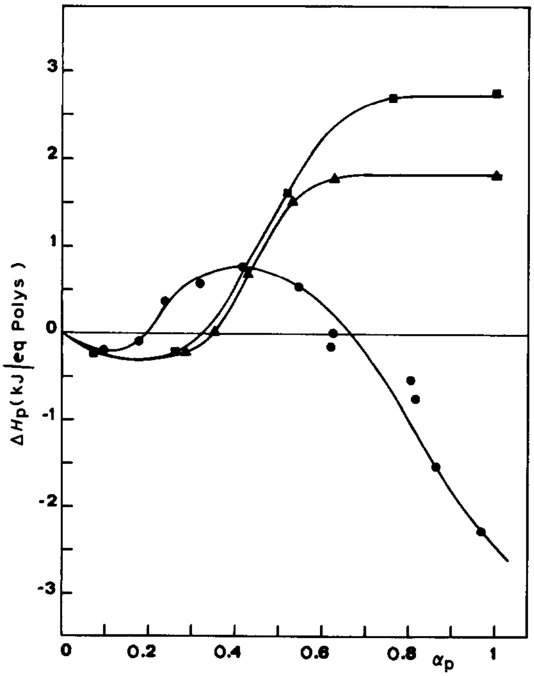

Fig. 15. Enthalpy of protonation (HCl) at 25° of solutions of S-60, (●, polymer concentration, 2 2 mequiv /dm^3), S-130 (▲, polymer concentration, 1.2 mequiv./dm^3), and S-194 (■, polymer concentration, 1.3 mequiv./dm^3), as a function of the ratio (R) between the concentration of protons in equiv /dm^3 and the polymer concentration in the same units

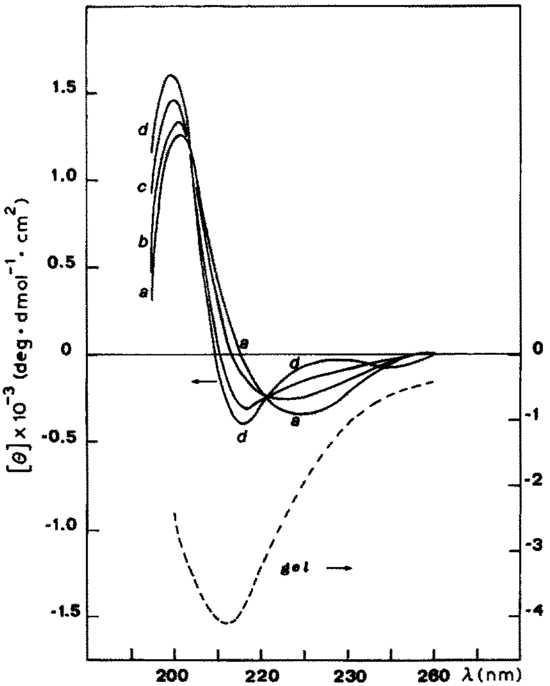

Fig. 16. Circular dichroism spectra (25°) of S-60 (polymer concentration, 1 2 mequiv./dm^3) at different degrees of protonation a, α_p = 0 48, b, α_p = 0.35; c, α_p = 0 23, and d, α_p = 0, and in an excess of acid chloride in the gel state (---). (Polymer conc , 5 mequiv /dm^3)

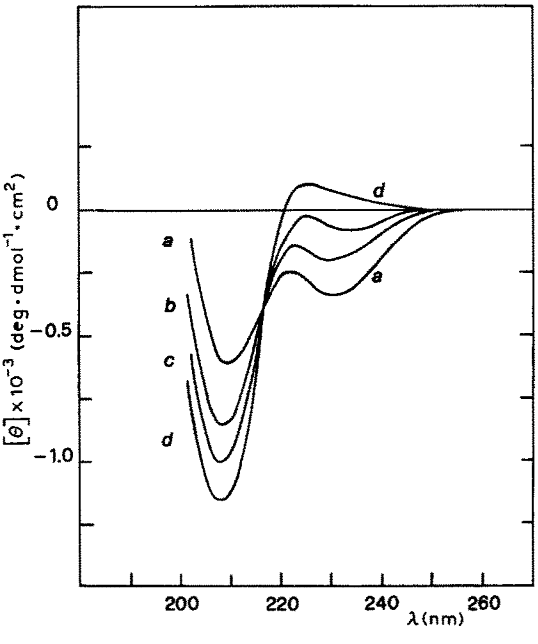

Fig. 17. Circular dichroism spectra (25°) of S-130 (polymer concentration, 1 2 mequiv./dm^3) at different degrees of protonation. a, α_p = 0.76; b, α_p = 0.5; c, α_p = 0.4; and d, α_p = 0

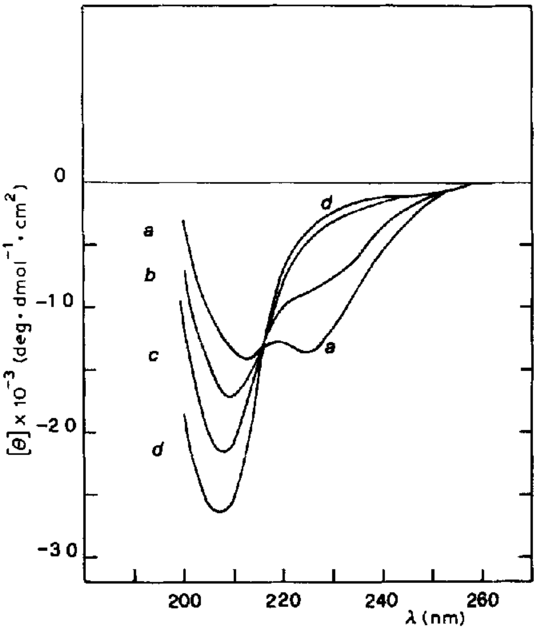

Fig 18 Circular dichroism spectra (25°) of S-194 (polymer concentration, 1 4 mequiv /dm^3) at different degrees of protonation. a, α_p = 1 0; b, α_p = 0 97, c, α_p = 0.59; and d, α_p = 0.

of protonation. For other carboxylic acid-containing polysaccharides, of both higher and comparable charge density to those of these three polysaccharides, endothermic changes in ΔH_p occur immediately on the commencement of protonation[15–17]. This unusual behavior of S-60, S-130, and S-194 at low degrees of protonation could be related to the carboxyl environment being more screened than is normal for polysaccharides. Viscosity measurements indicate that the carboxyl function of S-130 is particularly screened, compared with those of S-60 and S-194. It is significant that S-130 has a lower plateau value for ΔH_p than does S-194; this is consistent with a high degree of carboxyl-group screening in S-130. Because protonation of S-130 and S-194 is not accompanied by conformational ordering, circular dichroism spectra at different pH values should indicate only a simple equilibrium between ionized and un-ionized carboxyl chromophores, with no symptoms of spectral perturbations traceable to a conformational change. This is, indeed, what is observed (see Figs. 17 and 18).

(d) Methylene Blue binding measurements

The circular dichroism spectra for the complexes of the three polysaccharides with Methylene Blue are illustrated in Fig. 19, and indicate that S-60 interacts strongly with the dye to give a chiral complex. In contrast, S-194 interacts only weakly, and S-130 shows virtually no evidence for the formation of a chiral complex. This is consistent with only S-60 (of the three polysaccharides) being capable of

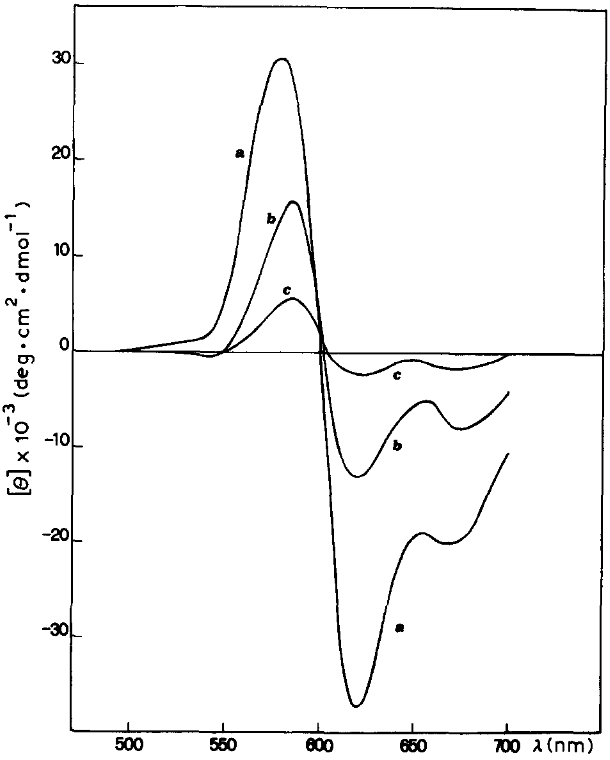

Fig. 19 Circular dichroism spectra (25°) of Methylene Blue in the presence of (a) S-60, (b) S-194, and (c) S-130. In all cases, the polymer concentration was 1 2 mequiv./dm^3, and the polymer to dye ratio was 20:1

conformational ordering in solution, and provides further evidence that the carboxylate group in S-130 is more screened than those in the other two polysaccharides, so inhibiting access and binding by the bulky Methylene Blue species.

CONCLUSIONS

The experiments outlined herein demonstrate that increasing the ionic strength (Me_4NCl) in dilute aqueous solutions of S-60 (in the Me_4N^+ form) at 25° promotes a distinctly cooperative conformational transition of the polysaccharide over a narrow range of added salt concentration. The process would bring the chains of S-60 from a relatively disordered initial state to a lower energy, helical state. Moreover, over the range of ionic strengths studied, this conformational transition is thermally reversible with no hysteresis.

From the data presented here, it is not possible to determine whether the conformational ordering of S-60 which we have observed in dilute nongelling solutions is single helical, or involves co-axial association of more than one polysaccharide chain. We believe, however, that because both higher concentrations of polysaccharide at the same ionic strength and higher ionic strengths at the same polysaccharide concentration lead to gel formation, the conformational ordering we have monitored is the one involved in the gelation of S-60. Preliminary X-ray diffraction studies have been reported[6] for S-60. Successful determination of the ordered conformation of S-60 in the condensed state could give some insight into the nature of the conformational ordering which occurs in the solution and gel states.

For carrageenan[18] and xanthan[19], it has been possible to use stopped-flow polarimetry employing salt-jumps to investigate the kinetics of the order–disorder process, and so distinguish between single-helical and double-helical models of the ordered conformations. The studies presented here indicate that the temperature at which the order–disorder transition for S-60 occurs is sensitive to changes in ionic strength, and indicate that, with a suitable choice of conditions, stopped-flow polarimetry could be applicable to the distinction between single- and multiple-helical models for the ordered conformation.

The experiments reported here also indicate that the different modes of branching along the S-60 backbone that are present in S-130 and S-194 mediate against conformational ordering under the concentration and salt conditions we have investigated. This is consistent with the fact that S-60 forms firm aqueous gels, whereas S-130 and S-194 are non-gel-forming, but give stable, highly viscous solutions. Calorimetric measurements of protonation give unusual trends for ΔH_p with degree of protonation for S-60, S-130, and S-194, in that the endothermic change occurs after a lag phase. We interpret this as indicating that the carboxyl groups in S-60, S-130, and S-194 are screened more than is normal for uronic acid-containing polysaccharides. Indeed, model building indicates that, for all three polysaccharides, hydrogen bonding is possible between O-2 of the D-glucosyl residue before the D-glucuronic acid and the carboxyl function, which could be responsible for the screening of the carboxyl group.

S-130 shows this unusual trend in ΔH_p with degree of protonation to a greater extent than do the other two polysaccharides, and, in addition, viscometry and calorimetric measurements on mixing indicate that the carboxyl group in S-130 is screened to a much greater extent than are those in S-60 and S-194. This is in qualitative agreement with the dye-binding data of Fig. 19. Preliminary vacuum-u.v., circular dichroism examination of S-60 and S-130 by Stevens[20] indicated a significantly stronger band at 180 nm for S-130, consistent with the carboxyl group of S-130 being more restricted. Similarly, model building and preliminary conformational analysis calculations indicated that, in S-130, the carboxyl group additionally appears capable of hydrogen bonding with O-3 of the L-rhamnose or L-mannose side-chain[21]. Such a complex hydrogen-bonding pattern could contribute to the screening of the carboxyl function, which is particularly apparent for S-130. For example, the hydrogen bonding would be favored by protonation, and could account for the unusually low ΔH_p for S-130.

This possible double involvement of the carboxyl group in hydrogen bonding for S-130 would be expected to stiffen the already stiff β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc cellulosic-like trisaccharide segment, which is flanked by rather flexible α-L-rhamnopyranosyl linkages, and thus influence the ordered conformation adopted in the condensed state. Preliminary X-ray fibre diffraction results for S-130 indicated an ordered conformation in the condensed state different from that of S-60, although the ordered conformations of both polysaccharides remain to be determined[22].

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