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COMPARATIVE ANALYSIS OF THE BEHAVIOR OF GELLAN GUM (S-60) AND WELAN GUM (S-130) IN DILUTE AQUEOUS SOLUTION*

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ABSTRACT

Optical rotation, circular dichroism, and microcalorimetric data clearly and consistently show that gellan gum, S-60 (Me_4N^+ form), undergoes in water at 25° a rather sharp conformational transition upon increasing the concentration of added Me_4NCl. Similar data show that S-60 behaves anomalously upon addition of Ca^{2+} ions with, eventually, formation of aggregates and/or gels. The Me_4NCl-induced conformational change of S-60 is thermally reversible with no hysteresis. In contrast, with welan gum, S-130 (Me_4N^+ form), no evidence could be found for a dependence of chain conformation on the main external variables considered. Comparison of the circular-dichroism spectra of the two polysaccharides suggests that S-130 in water might be present in a stiff conformation similar to that assumed by S-60 in aqueous Me_4NCl.

INTRODUCTION

Two structurally similar microbial polysaccharides, gellan gum (S-60) and welan gum (S-130), have recently attracted attention for their useful solution properties that have commercial applications[1].

The repeating units of the two polymers (Fig. 1) are quite similar, but S-60 lacks a glycosyl side-group[2]. Both polysaccharides in the native state contain *O*-acetyl groups, approximately one group for every repeating unit (S-60) or every two repeating units (S-130). The position of the *O*-acetyl residues has not yet been precisely ascertained, although for native S-60, ^{13}C-n.m.r. suggests acetylation at C-6 in one of the glucose rings[3], whereas the primary hydroxyl groups of S-130 are not acetylated[2].

Another structural feature to be clarified regards the distribution of terminal

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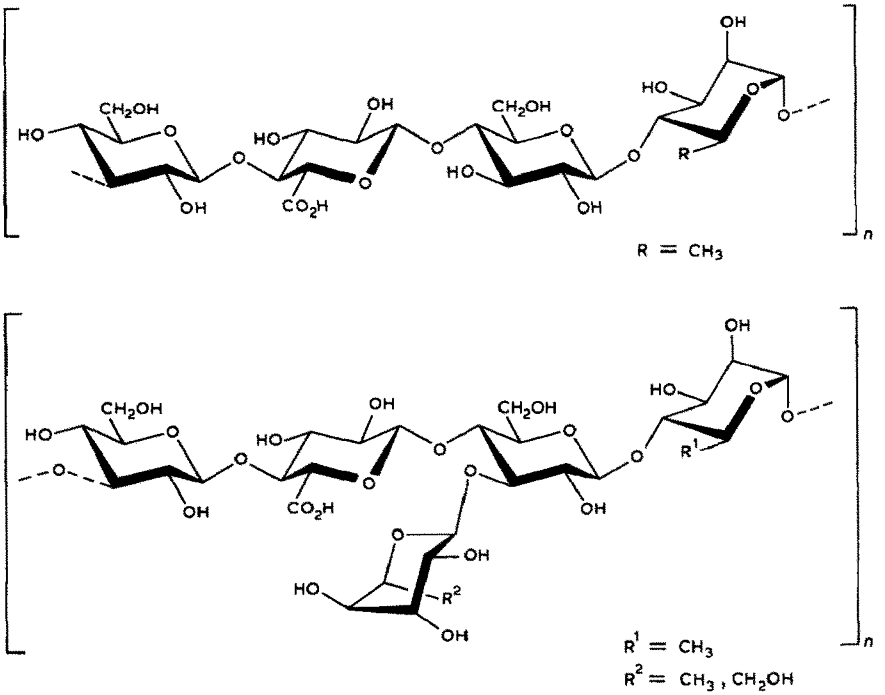

Fig. 1. Repeating-unit structure of gellan (S-60; upper) and welan gum (S-130; lower).

α-L-rhamnopyranosyl and α-L-mannopyranosyl residues present in the approximate molar ratio 2:1 along the chains of S-130 (Fig. 1).

In terms of properties in aqueous solution of particular relevance for technological applications, S-60 and S-130 exhibit marked differences. Notably, S-60 readily forms gels whose strength and thermal stability are influenced by the type and concentration of cation present in solution and by the degree of acetylation of the polymer[1].

S-130, which forms only weak gels, is primarily promoted[1] for its excellent stability towards pH changes and shear, as well as for retention of viscosity at temperatures up to 150°.

On the other hand, the two polysaccharides share the tendency to form chain aggregates, even in dilute solution in water[4]. This drawback, indeed common to different degrees for many polysaccharides, makes it difficult to obtain for S-60 and S-130 the accurate physicochemical characterization needed for a better understanding of the relations between structure and solution behavior in water. As a partial resolution of this impasse, we have found that purified polysaccharide samples quantitatively exchanged into the Me_4N^+ forms do yield stable aqueous solutions with very little aggregation at polymer concentrations less than ~0.1% w/v. In this manner, a comparative analysis of certain aspects of the dilute-solution behavior of the two polymers could be performed.

We report here preliminary data on the conformational states of deacetylated S-60 and of S-130 (in their (Me_4N^+, forms) in aqueous solution as determined from chirooptical and microcalorimetric experiments. Our results indicate profound differences in solution behavior between these two closely related polysaccharides.

RESULTS AND DISCUSSION

Some results with S-130 are displayed in Figs. 2 and 3. These data show that the optical activity of S-130 at 302 nm is practically invariant with increasing concentration of the added salts Me_4NCl and $Ca(ClO_4)_2$, as well as with increasing temperature over the range 10–80°. Similarly, the circular-dichroism spectrum of S-130 remains almost unchanged on passing from water to 0.07M Me_4NClO_4 at 25°. This behavior, rather uncommon among ionic polysaccharides, suggests that the chains of S-130 are quite stiff and would resist almost unperturbed any attempts to change their conformation in dilute aqueous media. In contrast, the conformational state of S-60 appears to be a sensitive function of ionic strength (Me_4NCl), of the nature of added counterions (Ca^{2+}), and of temperature.

The data on optical activity recorded in Fig. 4 indicate that addition of

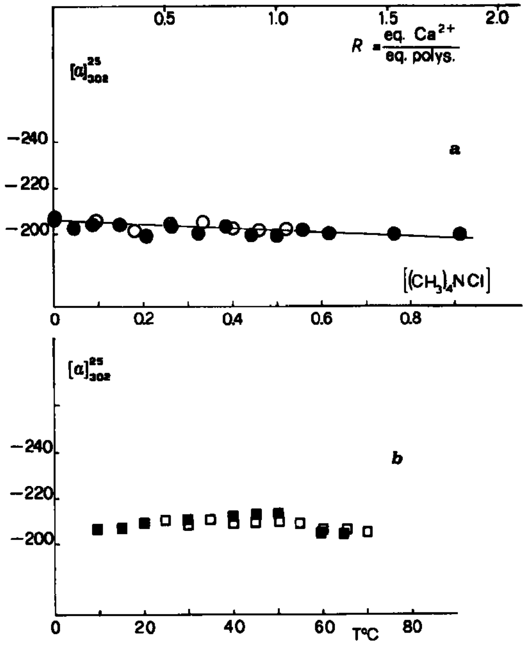

Fig. 2. (*a*) Optical activity of S-130 solutions as a function of increasing concentration of salts: (Me_4NCl, ○, lower scale; and $Ca(ClO_4)_2$, ●, upper scale). *R* is the ratio of $Ca(ClO_4)_2$ concentration, in equivalents per dm^3, to the polymer concentration in the same units. Polymer concentration = 0.8 mequiv./dm^3. (*b*) Optical activity of solutions of S-130 in water as a function of temperature. Polymer concentration = 0.8 mequiv./dm^3. Data are obtained both by raising (□) and decreasing (■) the temperature.

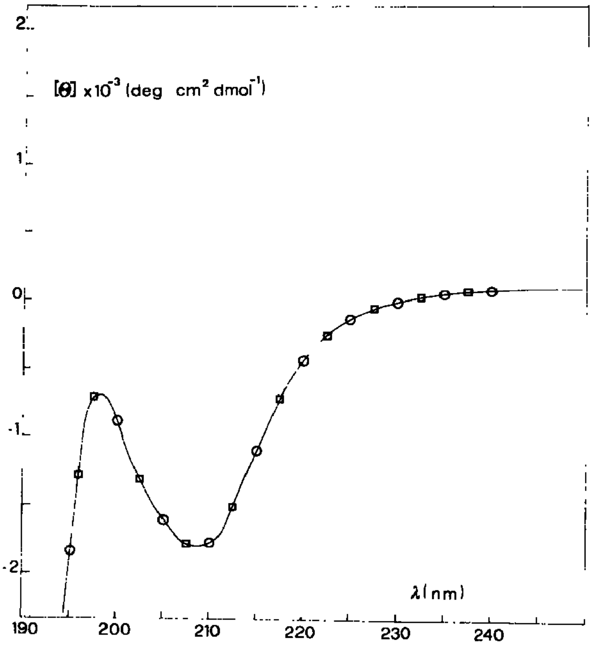

Fig. 3. Circular-dichroism spectra of solutions of S-130 in water (□), and 70mM Me_4NClO_4 (O). Polymer concentration = 0.6 mequiv./dm^3.

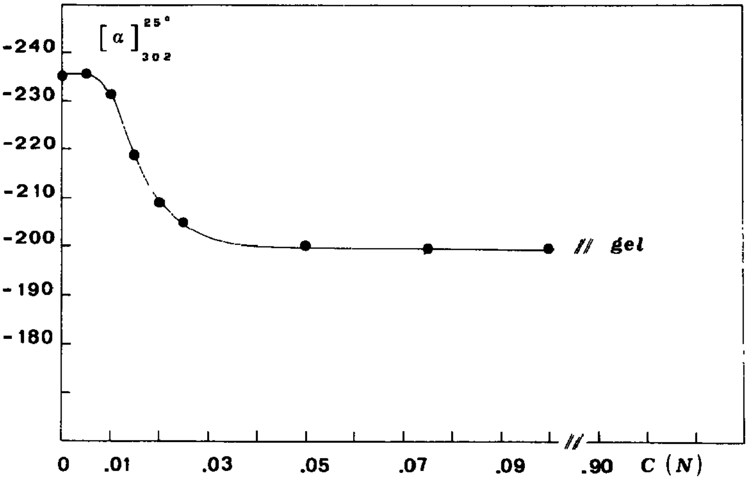

Fig. 4. Optical activity of S-60 solutions as a function of Me_4NCl concentration. Polymer concentration = 1.2 mequiv./dm^3.

Me_4NCl to a dilute aqueous solution of S-60 promotes a conformational change of the polysaccharide chains. The salt-stabilized conformation may then be reversibly melted (at constant ionic strength) by varying the temperature (Fig. 5). Addition of Ca^{2+} ions has similar consequences, as shown in Fig. 6. In this case, however,

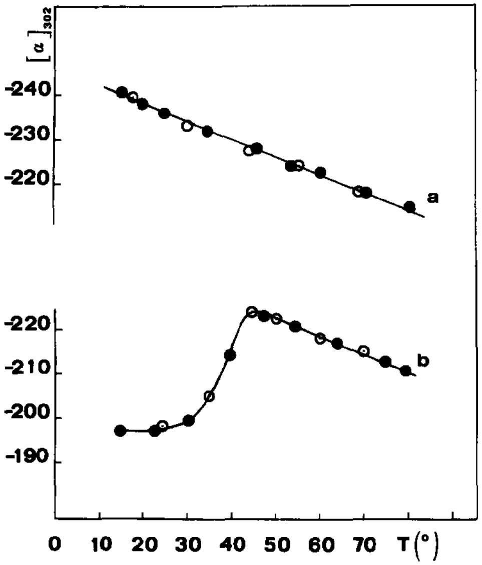

Fig. 5. Dependence of optical activity of S-60 on temperature. (*a*) Upper: solution in water; (*b*) lower: 75mM solution in Me_4NCl (○ heating, ● cooling). Polymer concentration = 1.2 mequiv./dm^3.

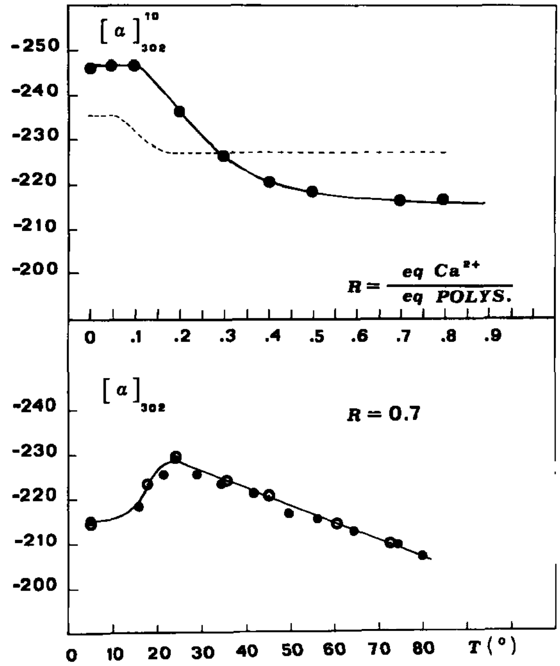

Fig. 6. Upper: Optical activity at 10° of S-60 as a function of increasing concentration of Ca^{2+} ions. Polymer concentration = 1.2 mequiv./dm^3. *R* is the ratio of $Ca(ClO_4)_2$ concentration in equivalents per dm^3 to the polymer concentration in the same units. The dotted line denotes data obtained at 25°. Lower: Thermal denaturation of S-60 polymer in the presence of $Ca(ClO_4)_2$ at *R* = 0.7. Polymer concentration = 1.2mM.

because of the need to keep the concentration of Ca^{2+} low enough to avoid gelation, the conformational change had to be monitored over a lower temperature-range (10–25°).

The enthalpy of mixing data (25°) illustrated in Fig. 7 appear to corroborate the interpretation of the optical activity results already mentioned. In particular we conclude, based upon experience accumulated in the microcalorimetric study of a variety of polysaccharide polyelectrolytes[5], that the trend of the ΔH_{mix} data with increasing Me_4NCl concentration typically reflects the onset, at around $R = 5$, of a conformational transition in S-60, an exothermic process superimposed on the endothermic interaction between monovalent counterions and the polymer. ($R = 5$ corresponds to Me_4NCl = 11mM, the polymer concentration being 2.2 mequiv./dm^3).

Addition of $Ca(ClO_4)_2$ also leads to a ΔH_{mix} *vs* R plot (Fig. 7) indicative of a conformational change in the polymer. In this case, however, considering the optical-activity data and the fact that microgels could be detected visually for R ~0.8, it is difficult to assess to what extent the possible conformational transition on one hand, and the aggregation and incipient gelation of the polysaccharide on the other, contribute to the observed trend of the calorimetric data.

Passing to the circular-dichroism (c.d.) data for S-60 (Fig. 8), it is evident that the c.d. spectrum of this polysaccharide sensitively monitors the Me_4N^+-induced conformational transition, in complete agreement with observations made

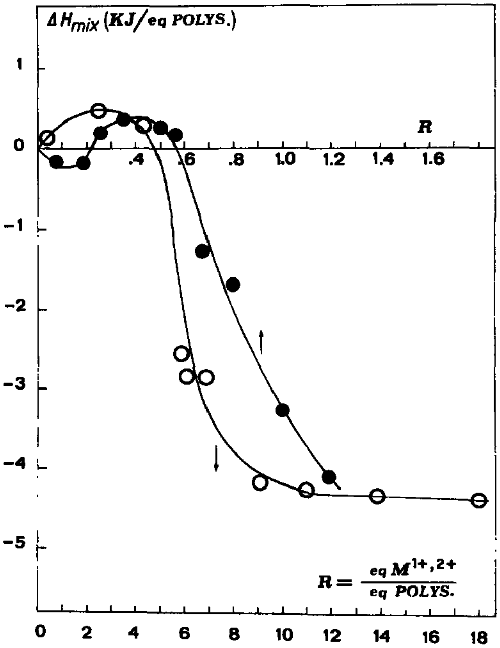

Fig. 7. Enthalpy of mixing (25°) of solutions of S-60 with Me_4NCl (O, lower scale) and $Ca(ClO_4)_2$ (●, upper scale) as a function of the ratio (R) between the concentration of cations in equiv./dm^3 and the polymer concentration in the same units. Polymer concentration = 22 mequiv./dm^3.

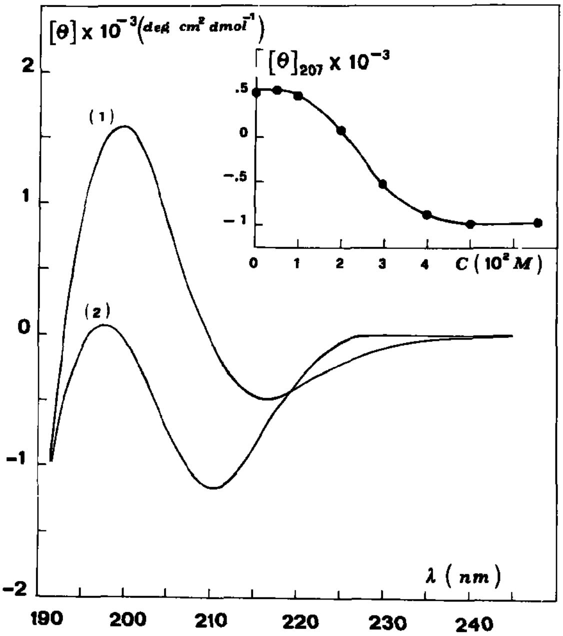

Fig. 8. Circular-dichroism spectrum of S-60 at different concentrations of Me_4NClO_4 at 25°: (*1*) H_2O; (*2*) 66mM Me_4NClO_4. Polymer concentration = 1.2 mequiv./dm^3. In the insert, the ellipticity of S-60 at 207 nm is plotted against concentration of Me_4NClO_4.

by other experimental approaches. Clearly, the mechanism and nature of such transitions cannot be ascertained on the basis of our data, and more experiments are necessary to elucidate these important aspects. We can at present make the reasonable assumption that Me_4N^+ ions would stabilize one of the *more ordered*, allowed, conformational states of gellan chains.

In addition, by comparison with the c.d. spectrum in Fig. 3, the final conformation of S-60 in Me_4NClO_4 at 25° gives results spectroscopically similar to those of S-130 chains either in water or in Me_4NClO_4.

As a consequence of the foregoing discussions, it would appear that S-130 has an ordered, particularly stable conformation in aqueous solution. More data are necessary to substantiate this finding and to speculate on the possible origins of the phenomenon. At this stage, based on structural information available, it seems logical to invoke specific side group–main chain interactions as being mainly responsible for the behavior of S-130 in aqueous media.

EXPERIMENTAL

Materials. — Samples of S-60 and S-130 were kindly donated by Kelco Merck Co. (San Diego, California). S-60 was purified by the procedure reported in the literature[6]. S-130 was precipitated with cetyltrimethylammonium bromide solution, redissolved in concentrated Me_4NCl solution, and then exhaustively dialyzed against Me_4NCl and, eventually, double-distilled water. The absence of excess salts was determined by means of conductivity measurements. To avoid the presence of counterions other than tetramethylammonium, the solutions of polysaccharide (S-60 and S-130, respectively) were also passed through an ion-exchange resin (Merck; strongly acid cation-exchanger) in the Me_4N^+ form. The polysaccharides were finally obtained as solids by freeze-drying.

Equivalent weights of the polysaccharides were determined by potentiometric titrations with standard NaOH solutions. The polysaccharides were previously transformed into the H^+ form by ion-exchange. The experimental equivalent weights for S-130 (840) and S-60 (720), in the Na^+ form, are in good agreement with the theoretical values calculated according to the proposed structures, taking into account the *O*-acetyl contents estimated from ^{1}H-n.m.r. spectra. For S-60, one acetyl group is present every ~4 repeating-units, whereas S-130 has ~50% of the residues bearing an *O*-acetyl group. Viscosity measurements carried out in 15mM Me_4NCl solution and 25° yielded the following intrinsic-viscosity values: S-60, 1090 cm^3/g; S-130, 1900 cm^3/g.

Measurements. — Optical activity measurements were performed with a Perkin Elmer 241 polarimeter (10-cm pathlength). The temperature was controlled by means of a Lauda circulating water bath. Circular-dichroism spectra were obtained with a Jasco (model J-500A) dichrograph. Calorimetric experiments were performed with an LKB twin-cell batch microcalorimeter at 25°. Measured heats were always corrected for dilution effects. Viscosity measurements were performed at 25° on a Schott Geräte automatic viscometer equipped with a water thermostat. The capillary viscometer utilized is characterized by an efflux time of ~200 s for water at 25°.

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