Carbohydrate Research, 123 (1983) 173–178
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

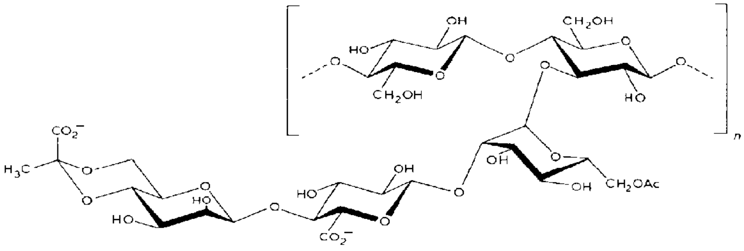
Note

Thermally induced conformational transition of xanthan polyelectrolyte

SERGIO PAOLETTI, ATTILIO CESÀRO, AND FRANCO DELBEN
Istituto di Chimica, Università di Trieste, Trieste (Italy)

(Received July 28th, 1982; accepted for publication, May 19th, 1983)

Xanthan, the polysaccharide elaborated by *Xanthomonas campestris*, has a pentasaccharide repeating-unit (**1**) and is a (1→4)-β-D-glucan having a trisaccharide side-chain attached on alternate D-glucosyl residues[1–3]. Xanthan solutions exhibit a distinctive, non-linear behavior in several physicochemical properties when heated in the range 0–100°. These changes can be reconciled with a thermally induced, cooperative transition. Two models have been proposed to describe the secondary (and the tertiary) structure of xanthan. Model 1 assumes that the fundamental conformations in the ordered and in the disordered states are a single-stranded helix, stabilised by alignment and packing of the side chains along the polymer backbone, and a disordered, stretched, worm-like chain, respectively[4]. Support for this model mainly derives from the first-order kinetics of the disorder–order transition. Model 2 postulates the transition to a single-stranded, disordered chain from a multistranded helix on the basis of electron microscopy[5], sedimentation, viscosity[6], and light-scattering data[7–11].



1

0008-6215/83/$ 03.00

To widen the range of theoretical and experimental approaches to the problem of the existence of a dimeric structure, we have studied the polyelectrolytic properties of xanthan. Manning's counterion condensation theory has provided a tool for describing several properties of polyelectrolytes in terms of the structural parameter, ξ, which is unequivocally defined as $e^2/DkTb$, where e is the value of the elementary charge, D is the dielectric constant of the medium, k is the Boltzmann constant, T is the Kelvin temperature, and b is the distance between the projections of the fixed charges of the polyelectrolyte on its contour axis. In the case of monovalent ions, for all structural values of $\xi > 1$, a defined amount of counterions will "condense" from the solution into the domain of the polymer chain so as to reduce the "effective" value of ξ to unity. For water, $\xi = 7.14\, b^{-1}$ (with b expressed in Å), and is practically independent of T. For thermally induced, conformational transitions between states i and f of a polyelectrolyte, characterised by a set of ξ_i and ξ_f (*i.e.*, b_i and b_f) values, Manning's theory predicts[12] a simple relationship between the values of the "melting" temperatures (T_M, the temperature of the transition midpoint) and the logarithm of the ionic strength, I:

$$\frac{d(\log I)}{d(1/T_M)} = -\frac{\Delta H^{M}_{\text{equiv}}}{2.288}\left(\xi_f^{-1} - \xi_i^{-1}\right)^{-1}, \qquad (1)$$

where $\Delta H^{M}_{\text{equiv}}$ is the value of the enthalpy of transition [in cal.(mol of charge)$^{-1}$, *i.e.*, cal.equivalent^{-1}] determined calorimetrically. $\Delta H^{M}_{\text{equiv}}$ is supposed to be essentially due to non-ionic contributions and largely independent of I.

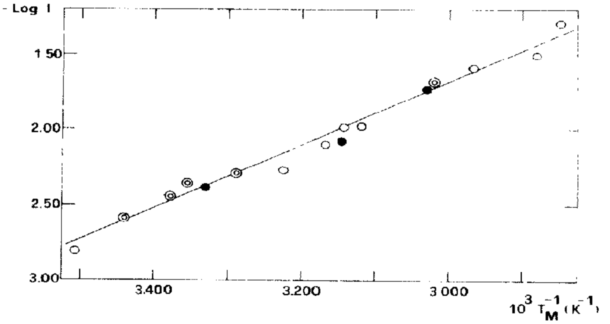


Fig. 1. Variation of the logarithm of the total ionic strength[14] (I) with the inverse of the temperature of transition midpoint (melting) (T_M) for aqueous solutions of xanthan. Open circles are values taken from ref. 15; the T_M values were determined by optical rotation, circular dichroism, or birefringence by polarising microscopy. Filled circles are values obtained on sample B (see Table I), the T_M values were determined from the sigmoidal increase in viscosity[1,20], measured with a conventional Ubbelohde capillary-viscometer.

This relation has been successfully applied to the transition processes of DNA, polynucleotides[13], and ionic polysaccharides (carrageenans)[14]. In the last instance, the approach has been proved to be of great utility, especially in discriminating between models that implied, for the same conformation, b values differing by a factor of two.

An accurate determination of the T_M values of xanthan solutions as a function of the ionic strength has been published by Milas and Rinaudo[15]. Their optical data are recorded in Fig. 1 together with our viscometric results. The slope of the linear plot (−2090 ±70 K) is the l.h.s. term of equation *1*, which enables the value of ΔH^{M}_{equiv} for a given pair of values of ξ_i and ξ_f to be determined.

X-Ray fiber-diffraction data[16,17] indicate that xanthan has a 5-fold screw symmetry and a pitch of 47 Å, with the possibility for single chains to be built in parallel or antiparallel 5_1 double-helices. From this structural work, the only information relevant to polyelectrolytic aspects is the axial translation per cellobiose unit, which is 9.4 Å. Using the values of the degree of pyruvate substitution of the samples of the present work (0.9 and 0.6 for samples A and B, respectively), the calculated values of b for the single chain are 4.95 and 5.88 Å, respectively.

For model 2, a dimeric ordered structure of xanthan is assumed, which doubles the above values of the linear charge density. In this case, the calculated values of b are 2.47 and 2.94 Å for samples A and B, respectively.

For the "disordered" conformation, a fully stretched cellulosic backbone can be reasonably assumed; the values of b for the two samples A and B then become 5.42 and 6.44 Å, respectively.

In Table I, the data pertaining to the two "melting" processes are summarised. The corresponding values of ΔH^{M}_{equiv}, calculated by using Equation *1*, are reported in column 7. These ΔH values can be converted to give the enthalpy changes per repeating unit, ΔH^{M}_{mol}, which are independent of pyruvate substitution (see column 8 in Table I), according to the implicit hypothesis that the presence of

TABLE I

CALCULATED VALUES OF THE ENTHALPY OF TRANSITION OF XANTHAN FOR DIFFERENT INITIAL CONFORMATIONS (FROM EQUATION *1*)

Process	*Sample*[a]	*Initial (i) state*	ξ_i	*Final (f) state*	ξ_f	ΔH^{M}_{equiv} *(kcal.equiv.$^{-1}$)*	ΔH^{M}_{mol} *[kcal.(repeating unit)$^{-1}$]*
1	A	Single 5-fold helix	1.442	Stretched coil	1.316	0.32 ±0.01	0.60 ±0.02
1	B	Single 5-fold helix	1.214	Stretched coil	1.108	0.38 ±0.01	
2	A	(5-fold helix)$_2$	2.884	Stretched coil	1.316	1.98 ±0.06	3.75 ±0.12
2	B	(5-fold helix)$_2$	2.429	Stretched coil	1.108	2.35 ±0.08	

[a] Sample A was a gift from Professor V. Crescenzi; sample B was a gift from Professor D. A. Brant (Keltrol lot No. KTL 24607, Kelco; similar to that used in ref. 11). The degrees of pyruvate substitution (^{1}H-n.m.r. spectroscopy) were 0.9[18] and 0.6[11], respectively. The molecular weights of the repeating unit (sodium salt form) were accordingly assumed to be 1061.6 and 1034.0, respectively.

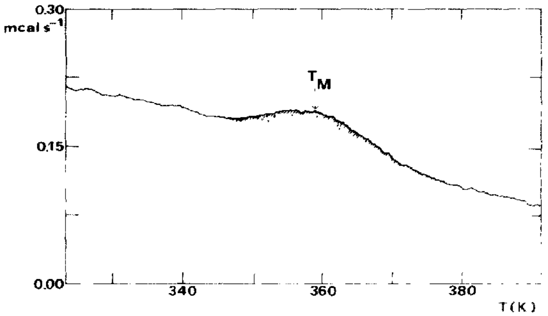


Fig. 2. Differential scanning calorimetry (d.s.c.) thermogram of a 2.33% (w/w) solution of xanthan (sample A) in 9.4mM aqueous NaCl; sample weight, 64.2 mg; scan rate, 10.0 deg.min^{-1}; T_M = 359.0 K; Q = 2.37 cal.(g polym)$^{-1}$. The thermogram was recorded with a Perkin–Elmer DSC 2C Model, equipped with a Thermal Analysis Data Station (TADS).

the pyruvate groups affects only the electrostatic part of the total free-energy.

Experimental values of the enthalpy of transition have been determined by differential scanning calorimetry (d.s.c.) of solutions in 10mM NaCl at three polymer concentrations in the range 1.45–2.89% (w/w). Three different amounts of solution (from 30 to 80 mg) were used routinely for each polymer concentration. The experiments were normally carried out at a scan rate of 10 K.min^{-1}, with occasional confirmatory runs at 2.5, 5, and 20 K.min^{-1}. Many samples were analysed twice or more after storage overnight at 4° in order to check the reversibility of the transition. A representative example of a thermogram showing a rather broad, but well defined, endothermic peak is shown in Fig. 2. The experimental heat of transition, determined under these various conditions, was randomly dependent on scan rate, concentration of polysaccharide, and amount of xanthan solution. The average value of the enthalpy of transition is 2.47 ±0.37 kcal.(repeating unit)$^{-1}$ for sample A (4 measurements; ±s.e.) and 2.66 ±0.13 kcal.(repeating unit)$^{-1}$ for sample B (32 measurements; ±s.e.), respectively. They have to be compared with the ΔH^{M}_{mol} values calculated for the two processes outlined above (see Table I).

The predicted ΔH^{M} values for process 1 (an *exclusively* intramolecular conformational transition) clearly fall short of the experimental result, and no speculation can reconcile this discrepancy. On the other hand, the agreement between the experimental ΔH^{M} value and that predicted by process 2 also appears not to be fully satisfactory. However, should the fraction of the ordered, initial state be <1, the agreement between prediction and experiment improves for process 2, and the disagreement for process 1 increases. The presence of an additional equilibrium between the dimeric and the monomeric forms of the 5-fold helix cannot be excluded for a given fraction of the total ordered form. (To say whether such monomers and dimers are isolated molecular species or parts of branched structures is beyond the limitations of the polyelectrolyte theory.) The experimental ΔH^{M}_{mol} would then be an average of the two entries of the last column of Table I, depending on the position of equilibrium. The occurrence and extent of the described phenomena cannot be assessed at present. However, the reported polyelectrolytic features of the thermal transition of xanthan seem to exclude the possibility that, at low temperature, the single 5-fold helix is the only ordered conformation present.

This conclusion is apparently contradicted by the kinetic study of Norton *et al.*[4] and by scattering data of Rees[19]. Indeed, the correct interpretation[4] that "the rate-limiting process in conformational ordering of disordered chains is helix nucleation" by no means precludes "further association into larger molecular assemblies". Rees[19] has presented the changes with temperature in the optical rotation and in the Rayleigh ratio measured by LALLS. This latter parameter can be considered approximately proportional to the inverse of molecular weight. A distinctive halving of the molecular weight occurred upon increasing the temperature, with a trend which parallelled the change in the optical rotation but displaced to slightly lower temperatures.

Rinaudo and Milas[7] and Paradossi and Brant[11] reported linear mass-density ($\bar{M}_w/L$) data from classical light-scattering measurements. The former authors found $\bar{M}_W/L$ values which varied, with the ionic strength, from 1320 to 2020 dalton/nm in the range mM to M of I. For the hypothetical single-stranded conformation, the same authors gave 934 dalton/nm. Paradossi and Brant [11] worked on a series of fractions of xanthan polysaccharide in 0.1M NaCl. From the angular dependence of the scattering intensity of samples of different $\bar{M}_W$, they obtained convergent evidence to provide the value of ~2000 dalton/nm for $\bar{M}_W/L$, which corresponds to that of a double-stranded helical chain.

In our opinion, the following behavior of xanthan in aqueous solution can be tentatively proposed. At high temperature and low ionic strength, a disordered, single-stranded chain preponderates. Upon cooling and/or increasing I, an *intramolecular* "freeze-out" of the linkage conformations occurs, probably involving the side chains. Chain ordering is thereafter followed by chain *dimerisation*. The dimeric helical conformation could therefore be the preponderant ordered form at equilibrium, at low temperatures and moderate-to-high ionic strength. However, none of the above results indicates whether a side-by-side or an intertwined dimerisation-process occurs.

ACKNOWLEDGMENTS

The authors thank Ing. O. Pera (Perkin–Elmer Italiana S.p.A.) for access to the d.s.c. apparatus, Professor V. Crescenzi for the sample of xanthan, and the Italian CNR (Progetto Finalizzato Chimica Fine e Secondaria) for financial support.

REFERENCES

1 A. Jeanes, J. E. Pittsley, and F. R. Senti, *J. Appl. Polym. Sci.*, 5 (1961) 519–526
2 J. H. Sloneker and A. Jeanes, *Can. J. Chem.*, 40 (1962) 2066–2071
3 P.-E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282
4 I. T. Norton, D. M. Goodall, E. M. Morris, and D. A. Rees, *J. Chem. Soc., Chem. Commun.*, (1980), 545–547.
5 G. Holzwarth and E. G. Prestridge, *Science*, 197 (1977) 757–759
6 G. Holzwarth, *Carbohydr. Res.*, 66 (1978) 173–186
7 M. Rinaudo and M. Milas, *Biopolymers*, 17 (1978) 2663–2678
8 J. G. Southwick, M. E. McDonnell, A. M. Jamieson, and J. Blackwell, *Macromolecules*, 12 (1979) 305–311.
9 J. G. Southwick, H. Lee, A. M. Jamieson, and J. Blackwell, *Carbohydr. Res.*, 84 (1980) 287–295
10 J. G. Southwick, A. M. Jamieson, and J. Blackwell, *Carbohydr. Res.*, 99 (1982) 117–127
11 G. Paradossi and D. A. Brant, *Macromolecules*, 15 (1982) 874–879
12 G. S. Manning, *Biopolymers*, 11 (1972) 937–949
13 M. T. Record, C. P. Woodbury, and T. M. Lohman, *Biopolymers*, 15 (1976) 893–915
14 S. Paoletti, O. Smidsrød, and H. Grasdalen, *Biopolymers*, submitted for publication
15 M. Milas and M. Rinaudo, *Carbohydr. Res.*, 76 (1979) 189–196
16 R. Moorhouse, M. D. Walkinshaw, and S. Arnott in P. A. Sandford and A. Laskin (Eds.), *Extracellular Microbial Polysaccharides, ACS Symp. Ser.*, 45 (1977) 90–102; R. Moorhouse, M. D. Walkinshaw, W. T. Winter, and S. Arnott, in J. C. Arthur (Ed.), *Cellulose Chemistry and Technology, ACS Symp. Ser.*, 48 (1977) 133–152
17 K. Okuyama, S. Arnott, R. Moorhouse, M. D. Walkinshaw, E. D. T. Atkins, and Ch. Wolf-Ullish, in A. D. French and K. H. Gardner (Eds.), *Fibre Diffraction Methods, ACS Symp. Ser.*, 141 (1980) 411–427
18 M. Dentini, personal communication
19 D. A. Rees, *Pure Appl. Chem.*, 53 (1981) 1–14
20 E. R. Morris, D. A. Rees, G. Young, M. D. Walkinshaw, and A. Darke, *J. Mol. Biol.*, 110 (1977) 1–16