Carbohydrate Research, 76 (1979) 189–196

CONFORMATIONAL INVESTIGATION ON THE BACTERIAL POLYSACCHARIDE XANTHAN

MICHEL MILAS AND MARGUERITE RINAUDO
Centre de Recherches sur les Macromolécules Végétales (CNRS) 53 X, 38041 Grenoble Cédex (France)
(Received September 28th, 1978; accepted for publication in revised form, December 22nd, 1978)

ABSTRACT

The conformation of xanthan has been investigated as a function of temperature, ionic strength, and polymer concentration. A reversible transition induced by temperature is demonstrated; the melting temperature (T_M) is directly correlated to the total ionic-strength and is independent of the polymer concentration. Measurements of circular dichroism show that the polysaccharide exists in a combination of only two characteristic conformations (random and ordered), regardless of the temperature and the concentrations of salt and polymer. Hydrodynamic measurements show that the hydrodynamic volume of both conformations is almost constant over the range of temperature investigated. The mechanism proposed by Morris for melting is confirmed, and a multichain process is excluded. The birefringence stability of the concentrated solutions is discussed.

INTRODUCTION

The chemical structure of the bacterial polysaccharide (xanthan) produced by *Xanthomonas campestris* has been established by Lindberg *et al.*[1] (see Fig. 1). The backbone is a chain of (1→4)-β-D-glucose residues substituted by short, lateral chains linked to alternate monomeric residues of the main chain. The conformation of xanthan has been investigated previously by Jeanes *et al.*[2], Holzwarth[3] and Morris

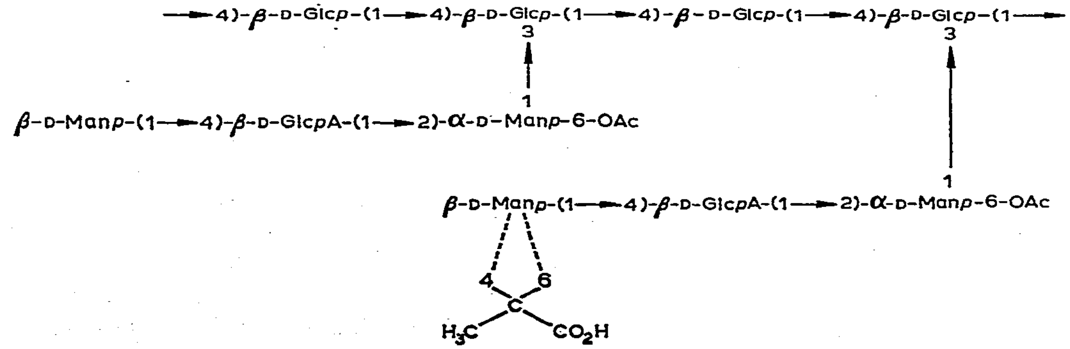

Fig. 1. Chemical structure of xanthan[1].

et al.[4], basically by optical rotatory methods. Holzwarth[3] has shown that there exists a secondary structure whose melting temperature varies with the ionic strength of the solution and with the valence of the counterions. In the solid state, Moorhouse *et al.*[5] proposed a single, helical conformation for the main chain; in solution, the change of the optical rotatory power indicates that the ordered conformation is helical. In addition, Holzwarth has proposed a multichain, helical structure for xanthan in solution[6]. Our purpose was to establish conclusively, from conformational investigations, the origin of the observed stability of the viscosity of xanthan in presence of external salt[7].

EXPERIMENTAL

Materials. — Xanthan was supplied by the Institut Français du Pétrole and was further purified; it was centrifuged and isolated in the sodium salt-form as previously described[7]. The solutions were prepared by direct dissolution in pure water; when it was necessary to control the ionic strength, external salt was added to this aqueous solution. The concentration of xanthan was varied from 0.05 to 30 g/L and the pH of solution lay between 6.5 and 7 in each experiment.

Measurements. — The specific rotation was determined at 300 nm with a Fica Spectropol 1 spectropolarimeter equipped with a 10-mm, thermostated cell. The circular dichroism (c.d.) was measured with a JOBIN-YVON dichograph III in the range 200–300 nm with a 1-mm cell.

The weight-average molecular weight was determined with a Fica photogoniodiffusometer model 50; the angular range of 30–150° was investigated. The increment of refractive index was earlier determined on a Brice Phoenix differential refractometer and found to be 0.160 in aqueous salt solutions (sodium chloride, 1–100mM). Experiments were performed at different temperatures and salt concentrations.

Viscometric measurements were made with an automatic viscometer (Viscomatic Fica), having a capillary of 0.5-mm diameter; the shear stress at the wall in the capillary was ~30 dyn/cm^2.

RESULTS AND DISCUSSION

The weight-average molecular weight of the purified sample of xanthan (sodium salt form) was found to be 3.6×10^6; its ionic content was 1.56×10^{-3} equiv. per g (or 641 g/equiv.). Experiments were performed on dilute solutions having concentrations <2.5 g/L and also on concentrated solutions.

Dilute solutions. — From the large increase of $[\alpha]$ at low temperature in presence of external salt, it is generally assumed that there is an ordering of the polysaccharide conformation, at least in parts of the molecule[4]. Fig. 2 shows the dependence of $[\alpha]_{300}$ with temperature for different solvents. From these experimental results, the melting temperature (T_M) corresponding to half-transition is plotted as a function of the ionic strength; to express the total ionic strength (μ_{tot}), the concentra-

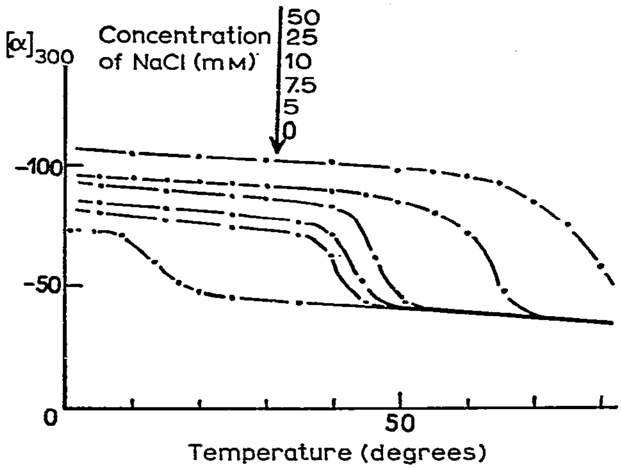

Fig. 2. Specific rotation, $[\alpha]_{300}$, as a function of temperature for different concentrations of salt (C_p = 0.4 g/L) and in water (C_p = 1.5 g/L).

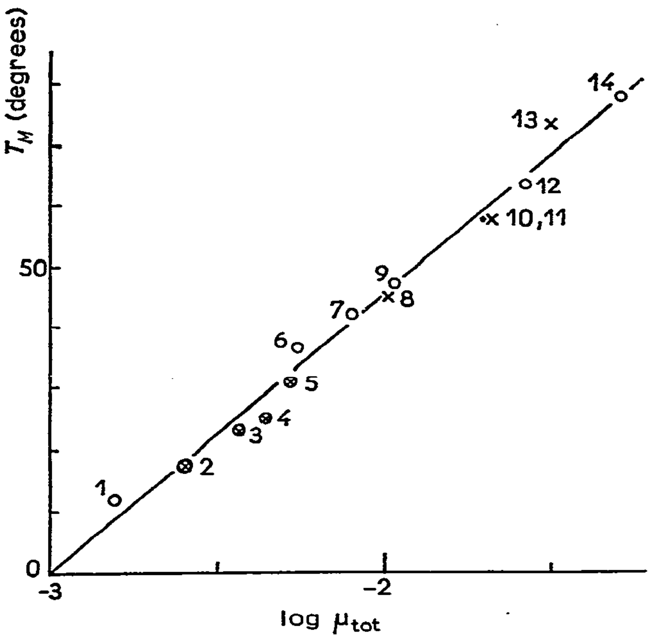

Fig. 3. Variation of the melting temperature (T_M) with the logarithm of the total ionic strength. For experimental conditions, see Table I. ○, T_M value of ordered structure by optical rotation; ●, T_M value of ordered structure by c.d.; ×, T_M value of birefringence by polarizing microscopy.

tion of the osmotically free counterions is taken into account (Table I). Fig. 3 shows T_M to vary linearly with log μ_{tot}, as found for other biopolymers that adopt helical structures in solution[8], and also for xanthan[3]. Furthermore, no hysteresis was ever observed in the dependence of $[\alpha]$on T, and T_M is thus precisely determined.

TABLE I

MELTING TEMPERATURES OBTAIN BY DIFFERENT TECHNIQUES AS A FUNCTION OF THE TOTAL IONIC-STRENGTH[a] ($\mu_{tot} = \phi c_p' + c_s$)

Expt. no.	c_p *(g/L)*[b]	C_s *equiv.*, L^{-1} × 10^2	μ_{tot} *equiv.* L^{-1} × 10^2	T_M *(degrees)*	*Techniques*
1	1.5	0	0.155	12	[c]
2	2.5	0	0.257	17.5	[c,d]
3	3.5	0	0.359	23	[c,d]
4	4.25	0	0.437	25	[c,d]
5	5	0	0.514	31	[c,d]
6	0.4	0.5	0.541	37	[c,d]
7	0.4	0.75	0.791	42.5	[c]
8	10	0	1.03	45	[d]
9	0.4	1	1.041	47.5	[c]
10	20	0	2.06	58	[e]
11	20	0	2.06	58	[d]
12	0.4	2.5	2.54	64	[c]
13	30	0	3.1	74	[d]
14	0.4	5	5.04	78	[c]

[a] ϕ is the activity coefficient of the counterions in the absence of salt, and is taken as 0.65 (ref. 6). [b] The C_p' and C_s values are the concentrations (in equiv./L) of xanthan and sodium chloride. [c] optical rotation. [d] polarizing microscope for birefringent solutions. [e] circular dichroism.

The slope of the curve corresponding to 44° is much higher than that for DNA, for which the slope is 19°. The secondary structure of xanthan is less stable than that of DNA but is quite similar to that of carrageenan[10]. The equivalence of free counterions and external salts is shown by the fact that the values of T_M, as determined in salt-free solutions as a function of polymer concentration, are on the same curve of T_M versus log μ_{tot}. Consequently, the value of T_M is independent of the polymer concentration, after considering the polymers own contribution to the ionic strength. The concentration-independence of transition temperature is in agreement with the interpretation of Morris *et al.*[4], who concluded that the ordered form is a single-chain species that could have the same conformation as in the solid state.

The c.d. spectra are depicted as a function of polymer concentration, temperature, and ionic strength. Series of spectra are obtained depending on these three parameters, that are always located between two limit spectra, experimentally obtained for extreme conditions chosen from o.r.d. measurements, that correspond to ordered and random conformations, respectively (Fig. 4a). This result means that, as soon as the $[\alpha]_{300}$ value reaches a limit when the temperature, ionic strength, or concentration are varied, the c.d. spectra are always the same. The ranges investigated were 25–70° for temperature, up to 10^{-1}M for ionic strength, and polymer concentration up to 20 g/L. This conclusion has not been drawn before, but appears very important for conformational investigations on xanthan. The ordered structure is characterized by a maximum in ellipticity at $\lambda = 200$ nm and a minimum at $\lambda = 220$ nm. The random structure presents a maximum at $\lambda = 197$ nm and a minimum at $\lambda = 216$ nm.

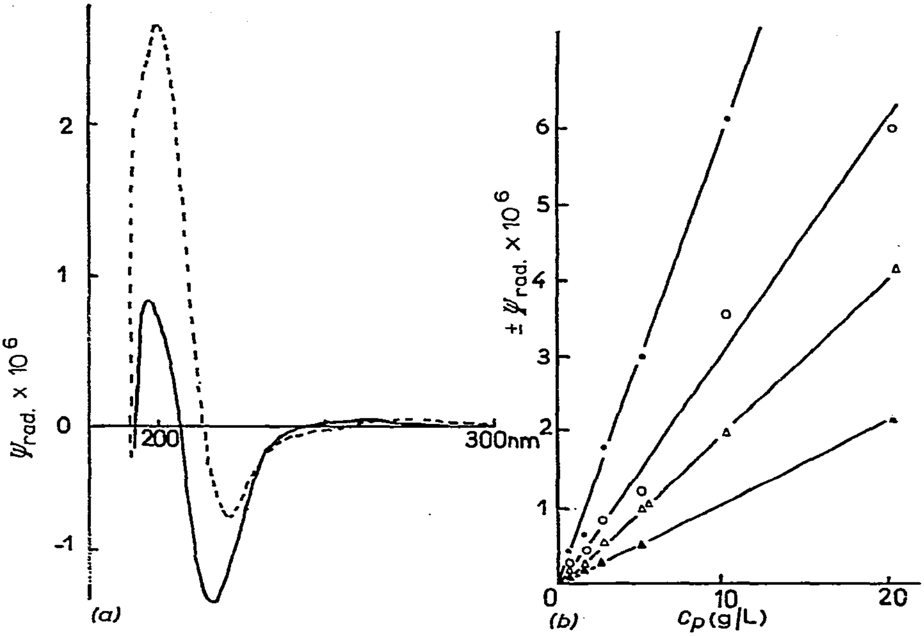

Fig. 4. (*a*) Circular-dichroism spectra of xanthan at 25°. —— $C_p = 5$ g/L, $C_s = 0$, random structure; ----- $C_p = 5$ g/L, $C_s = 0.1$M sodium chloride, ordered structure. (*b*) Concentration dependence of maximum and minimum ellipticities for both structures: ○, $\lambda = 197$; △, $\lambda = 216$ nm; random structure ($C_s = 0$, $t = 60°$); ●, $\lambda = 200$; ▲, $\lambda = 220$ nm; ordered structure ($C_s = 0.1$M, $t = 25°$).

Fig. 4b also shows that the specific ellipticity is quite independent of polymer concentration up to 20 g/L for the two limit-spectra; to control the conformation, the experimental conditions were chosen as $C_S = 10^{-1}$M sodium chloride, $T = 25°$ for the ordered structure, and as a salt-free solution, $T° = 60°$, for the random one. The independence of the spectra with polymer concentration confirms a monomolecular order.

From the dependence of ellipticity with temperature at a given wavelength in the range 220–240 nm (attributed by Morris *et al.*[4] to the acetate chromophore), we found a melting temperature in good agreement with the previously recorded values; the isodichroic points indicate an equilibrium between the two limit spectra. The chromophores implied are that for $>C=0$, as discussed by Morris *et al.*[4], and it is concluded that the melting corresponds to spreading of the lateral chains. The intrinsic viscosity, $[\eta]$, was determined as a function of the temperature in the range of T_M (Fig. 5). A small increase of $[\eta]$ was observed, which corresponds to the T_M value determined by optical rotation. The increase of 10% in the reduced viscosity is independent of the concentration of xanthan.

As the molecular weight and radius of gyration obtained by light scattering, the sedimentation constant[7], and the potentiometric behaviour are independent of temperature, such multichain structures as proposed by Holzwarth[6] must be ex-

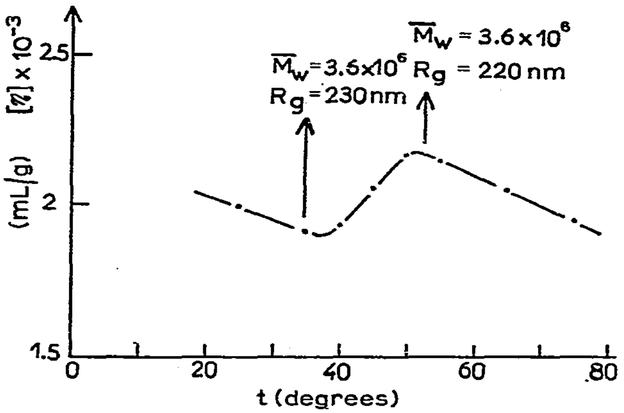

Fig. 5. Intrinsic viscosity as a function of temperature. C_s = 10 mM sodium chloride.

cluded. As with solutions of DNA, it may be necessary to investigate lower angles in light-scattering experiments to reach definitive conclusions[11].

The foregoing results suggest that there is no dramatic modification of the morphology of the macromolecule, and it must be recalled that the increase of axial ratio between helical and linear conformations in the model of Moorhouse *et al.*[5] is a maximum of 10%. Furthermore, the spreading of the lateral chains interacting with the backbone at low temperature may increase the swelling and hydration of the chain.

To interpret this set of results, we propose that the conformational transition observed corresponds to a helix–coil transition of the backbone with simultaneous release of the lateral chains, followed by progressive decrease of the rigidity of the (1→4)-β-D-glucan chain as the temperature rises above T_M.

Concentrated solutions. — In water and salt solutions, xanthan is birefringent, as observed by polarizing microscopy. Such general factors as rate of shear, concentration, presence of external salt, heat, pH, and content of pyruvic acid affect this birefringence[12,13]. In water solution, the birefringence is established for concentrations of xanthan >2.5 g/L when the solution has been stored for a few h at low temperature (10 h at 4° for example). The decrease of birefringence with increasing temperature has been tested as a function of the polymer concentration. These melting temperatures are given in Fig. 3 and in Table I; they correspond quite well with the values of T_M determined from optical rotatory experiments on dilute solutions. The process is perfectly reversible. The birefringence may arise from partial orientation of the highly asymmetric particles when the helical structure exists[16].

In salt solution, the birefringence appears when the polymer exceeds 5 g/L; it disappears at a temperature equal to the T_M value of the secondary structure established with dilute solutions. The birefringence of the concentrated solutions appears to correspond to an orientation of the rigid molecules in the absence or presence of external salt; it is reversible and is established at the same time as the secondary, monomolecular structure.

It must be pointed that an electrostatic, lateral organization exists in salt-free, concentrated solutions of xanthan as previously demonstrated for other polyelectrolytes[14,15]. This order is disrupted by the addition of external salt, but the birefringence is not, indicating an ordered, apparently secondary, structure. Secondary, weak, intermolecular forces align the rod-like molecules[16]. Liquid-crystal structures have been demonstrated previously, for example with more-concentrated solutions of poly(glutamic acid), sodium salt[17]. The mechanism of organization of xanthan in aqueous solutions cannot be discussed further without new experimental data; toward this aim, neutron-scattering and Cotton Mouton experiments are in progress in our laboratory.

CONCLUSIONS

The conformational stability of xanthan in dilute aqueous solutions has been investigated. The melting temperature (T_M) is directly correlated to the total ionic strength of the solution and is independent of the polymer concentration, if the free osmotic counterions are taken into account.

From specific rotation and c.d. spectra recorded as a function of the temperature, the same T_M value is deduced; the chromophores involved are those of the lateral side-chains. As a consequence, the T_M value corresponds to the spreading of the lateral chains according to thermal energy and to electrostatic screening.

From the dependence of intrinsic viscosity with temperature, which strictly paralleled the $[\alpha]$, T dependence, it is shown that an increase of 10% in viscosity corresponds to a conformational transition; there is no dramatic change in the conformation. In fact, Moorhouse[5] has shown that the axial ratio of the molecule change is 10% at most from the extended to the helical conformation; the spreading of the lateral side-chains may also increase the swelling of the molecule. The large decrease of viscosity for $T > T_M$ value may be attributed to a continuous decrease in the rigidity of the chain. These results evidently involve a one-step mechanism, with a breakdown of the helical conformation of the main chain and release of the lateral chains. The highly asymmetric conformation corresponding to the ordering conditions, and its independence of increasing ionic strength, allow interpretation of the original stability of the viscosity observed for dilute solutions, whatever may have been the counterions and the ionic strength[7]. In concentrated solutions, in the absence or presence of external salts, the observed birefringence indicates an orientation of the molecules. This birefringence disappears at a temperature corresponding exactly to the T_M value obtained for an identical, total ionic-strength on dilute solutions; the stiffness of the ordered molecule is evidently necessary for birefringence. The nature of the secondary, intermolecular forces involved has to be further investigated. It seems very important to understand the mechanism of organization of rigid, anisotropic molecules in solution as a prerequisite for interprating the rheological properties of the xanthan solutions; for example, this ordered structure should explain the yield stress observed over a critical concentration-range by Whitcomb *et al.*[18].

REFERENCES

1 P. E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
2 A. Jeanes, J. E. Pittsley, and F. R. Senti, *J. Appl. Polym. Sci.*, 5 (1961) 519–526.
3 G. Holzwarth, *Biochemistry*, 15 (1976) 4333–4339.
4 E. R. Morris, D. A. Rees, G. Young, M. D. Walkinshaw, and A. Darke, *J. Mol. Biol.*, 110 (1977) 1–16; E. R. Morris, *A.C.S. Symp. Ser.*, 45 (1977) 81–89.
5 R. Moorhouse, M. D. Walkinshaw, and S. Arnott, *A.C.S. Symp. Ser.*, 45 (1977) 90–102.
6 G. Holzwarth and E. B. Prestridge, *Science*, 197 (1977) 757–759.
7 M. Rinaudo and M. Milas, *Biopolymers*, 17 (1978) 2663–2678.
8 G. S. Manning, *Q. Rev. Biophys.*, 11 (1978) 179–246.
9 A. Heyraud, Thesis, Univ. Grenoble, 1978.
10 A. Karimian, Thesis, Univ. Grenoble, 1978.
11 A. I. Krasna, *J. Colloid Interface Sci.*, 39 (1972) 632–646.
12 J. E. Pittsley, J. H. Sloneker, and A. Jeanes, *Abstr. Pap. Am. Chem. Soc. Meet.*, 160 (1970) CARB-21.
13 P. A. Sandford, J. E. Pittsley, C. A. Knutson, P. R. Watson, N. C. Cadmus, and A. Jeanes, *A.C.S. Symp. Ser.*, 45 (1977) 192–210.
14 G. Maret, J. Torbet, E. Senechal, A. Domard, M. Rinaudo, and M. Milas, *Int. Symp. Dynamic Properties Polyionic Systems*, Kyoto, Japan, Sept. 9–10, 1978.
15 M. Rinaudo and A. Domard, *Polym. Lett.*, 15 (1977) 411–415.
16 P. J. Flory, *Proc. R. Soc. London, Ser. A*, 234 (1956) 50–73.
17 E. Iizuka, Y. Kondo, and Y. Ukai, *Polym. J.*, 9 (1977) 135–144.
18 P. J. Whitcomb, B. J. Ek, and C. W. Macosko, *A.C.S. Symp. Ser.*, 45 (1977) 160–173.