

Conformation

On the Existence of Two Different Secondary Structures for the Xanthan in Aqueous Solutions

M. Milas and M. Rinaudo

Centre de Recherches sur les Macromolécules Végétales, C.N.R.S.*, B.P. 68,
38402 Saint Martin d'Hères cedex, France

Summary

This paper gives experimental evidence for two different ordered conformations in solution for the xanthan molecules. Their hydrodynamic characteristics are quite different and their existence is controlled by experimental conditions.

Introduction

Different recent investigations on xanthan gum in solution support the existence of a single helical conformation (1, 2). Nevertheless some authors find results consistent with a double stranded helix (3-7).

On the other hand, Sandford et al (8) show that there is an increase of viscosity when the native polymer is heated, depending on the external salt concentration and the pyruvic acid content. To explain these results it is proposed by Seeger (9) that there exist single-helical molecules in addition to double helical threads, which are composed of several xanthan molecules. G. Southwick et al. suggest that aggregation of rod-like chains occurs for xanthan in salt solution (10). These authors think that this aggregation is perhaps the explanation of the different results found in the literature.

In this paper a series of experiments is discussed which seems to indicate that two different conformations really exist in solution depending on the experimental conditions.

* Laboratoire Propre du C.N.R.S., associé à l'Université Scientifique et Médicale de Grenoble, France

Experimental partPolymer samples

A native xanthan broth was used without preheating. The degrees of pyruvate and acetate substitutions (measured by ^1H n.m.r. spectroscopy (11)) are 0.4 and 0.75 respectively, expressed as average number of substituents per side chain. The broth was purified first by filtration through Millipore filters (0.45 μm) or by centrifugation (20 000 g, 4 hours) to eliminate the cell debris ; secondly it was ultrafiltered on Diaflo ultrafiltration membranes (Amicon XM 100) with NaCl 0.1 N as solvent to avoid the collapse of the ordered conformation. The ultrafiltration can be replaced by precipitation of the xanthan by ethanol (final ratio ethanol/water = 50 % v/v) in the presence of NaCl (100 g/l) ; NaCl was then entirely removed by washing the precipitate with ethanol-water mixtures (from 50 to 95 % v/v). In some cases, the ultrafiltered solution was diluted with NaCl $5 \cdot 10^{-3}$ N to give a NaCl concentration around 10^{-2} N and heated at 80° C (over the conformational change temperature, T_M , at 55° C) before precipitation.

These different treatments lead to precipitates of xanthan in one of the two ordered conformations suggested. The ultrafiltration and precipitation treatments eliminated proteins and enabled control of the counterions (Na form). Weight average molecular weights, M_w , and the radius of gyration were determined using a FICA 5 000, scattering angles from 30° to 150°. Values of M_w were also measured at low angle (6°) in static measurement and by gel permeation chromatography with a Chromatix KMX 6 photometer (12). The refractive index increment dn/dc measured with a BRICE-PHOENIX differential refractometer at $\lambda = 546$ nm is equal to 0.155. The viscosities were measured in the low shear rate limit by using a Contraves low shear viscometer LS 30 ; for temperatures higher than 35° C the solutions and the viscometer were heated at the required temperature before pouring the solution into the viscometer. The specific optical rotation $[\alpha]$ was obtained at $\lambda = 300$ nm in a Spectropol 1b from FICA.

Results and discussion

Different solutions were prepared as given in Table I. After preparation all the solutions were at a constant polymer concentration

TABLE I : Preparation of the solutions*

Preparation	Conformational state	Remarks
I : Native broth filtered and ultra-filtered in NaCl 0.1 N ; then dilute at 0° C to NaCl 10^{-2} N	A	Ordered conformation preserved
II : Na-xanthan after precipitation is dissolved in water ; then NaCl is added to 10^{-2} N	B	Conformation disordered in water before the addition of salt
III : Na-xanthan after precipitation is dissolved directly in NaCl 10^{-2} N	A	Ordered conformation preserved
IV : Na-xanthan precipitated in NaCl 10^{-2} N after preheating 80° C is dissolved in water ; then NaCl is added to 10^{-2} N.	B	Disordered conformation before precipitation and during dissolution in water
V : Na-xanthan precipitated in NaCl 10^{-2} N after preheating 80° C is dissolved directly in NaCl 10^{-2} N	B	Disordered conformation before precipitation

* The final concentrations of the solutions are all : NaCl 10^{-2} N and xanthan 0.66 g/l.

(0.66 g/l) and at the same NaCl concentration, 10^{-2} N ; under these conditions, at ambient temperature an ordered conformation exists (figure 1). The xanthan in solutions I and III did not undergo a conformational change from the native state before measurement.

The relative viscosity in the low shear rate limit of the solutions I and III is 35 ± 2 but is 146 ± 6 for the other solutions (II, IV, V). The molecular weight of the xanthan is found to be constant and equal to $6.1 \pm 0.5 \times 10^6$. The radius of gyration for samples I and III is $2\ 940 \pm 150$ A and is $3\ 800 \pm 300$ A for the other samples. The radii of gyration are obtained by using the FICA data (scattering angles from 30° to 150°) and the Chromatix point (scattering angle 6°) to extrapolate to zero angle.

The intrinsic viscosity at a rate of shear $\dot{\gamma} \rightarrow 0$ is 6 100 ml/g for the solutions I and III and 10 500 ml/g for the other solutions.

All these results seem to indicate that two well defined secondary structures (A and B) exists. In the native form, the conformation of the xanthan (called A) seems more compact than after the first order-disorder-order cycle. This cycle can be carried out either with temperature or ionic strength like Seeger (9). But, unlike the result of Seeger, the molecular weight is found to be constant ; this excludes the dissociation of double helical threads.

In the figure 2, the relative viscosity during the order-disorder-order cycle is given for the two conformations and may be related to the variation of conformation monitored by optical rotation (figure 1). One starts either with the xanthan A (samples I and III) or B (samples II, IV, V) ; the results are compared with measurements in 10^{-1} N NaCl in which the conformational transition temperature (T_M) is never reached in our experiments.

The relative viscosity of the B form decrease greatly with temperature compared to the A form (compare curves a and b with curves c and d). The conformational transition at T_M leads to a decrease of the relative viscosity for the B form (curve b) but a small increase for A (curve c). All these variations are reversible except when we start from

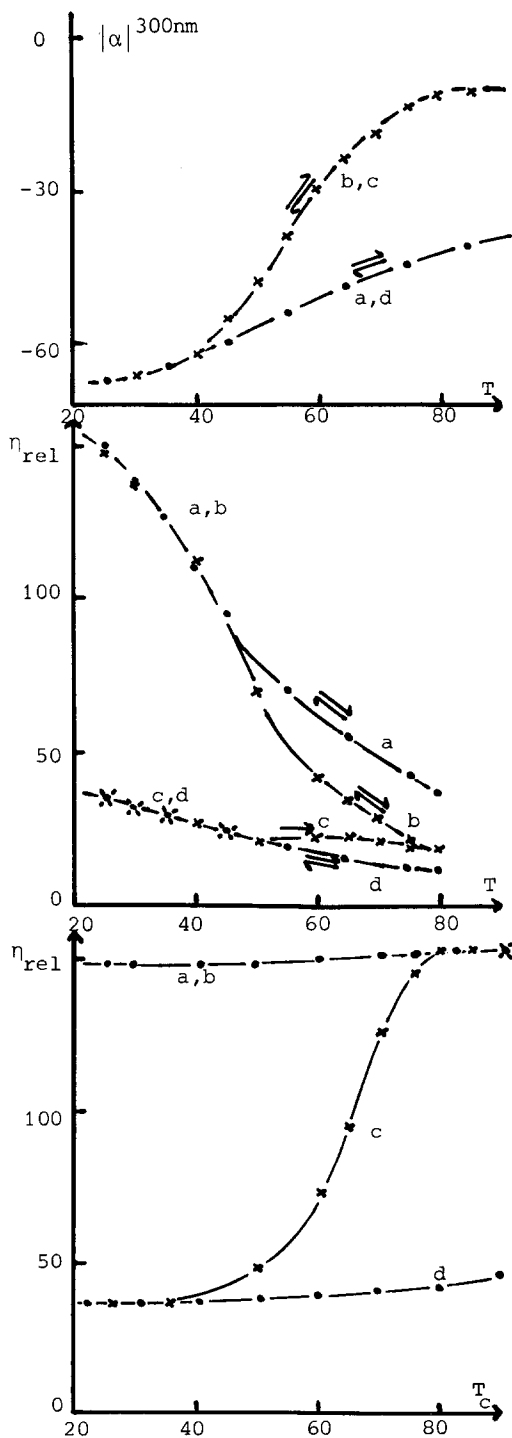


Figure I : Specific optical rotation of xanthan solutions versus temperature ($^{\circ}$ C). a : form B in NaCl 10^{-1} N ; b : form B in NaCl 10^{-2} N ; c : form A in NaCl 10^{-2} N ; d : form A in NaCl 10^{-1} N (Polymer concentration 0.66 g/l ; wavelength 300 nm).

Figure II : Relative viscosity in the lower Newtonian region for the different xanthan solutions (see Fig I) as a function of temperature ($^{\circ}$ C).

Figure III : Relative viscosity in the lower Newtonian region for the different xanthan solutions (see Fig I) at $T = 25^{\circ}$ C after preheating at different temperatures T_c .

the A form in NaCl 10^{-2} N (curve c) ; in this case the B form is obtained when the temperature decreases (curve b).

The relative viscosities at 25° C of forms A and B in 10^{-1} and 10^{-2} N following preheating to different temperatures T_c are indicated on figure 3. The variation of the viscosity is interpreted by reference to the optical rotation measurement at the corresponding temperatures. The optical rotation versus temperature curve does not depend on the starting form A or B and it is reversible with temperature. For the A form, the viscosity increases (curve c) in accordance with the fraction of polymer in the disordered conformation estimated from $[\alpha]$ in NaCl 10^{-2} N (this fraction is transformed into the B form on cooling).

For the A form in NaCl 10^{-1} N the relative viscosity remains constant and equal to 35 (curve d) ; on the contrary, that of the B form in NaCl 10^{-2} and 10^{-1} N remains constant but equal to 145 (curves a and b). From B in NaCl 10^{-2} N, the A form is restored when a large amount of NaCl is added to the disordered conformation at a temperature over 80° C i.e. when the conformational change is induced by salt addition at this temperature. The relative viscosity of the solution was found to be 40 at 25° C and all the characteristics of form A were observed in the polymer recovered by precipitation.

In contradiction to our results, SATO et al (7) show that after heating 15 min in water at 95° C and addition of NaCl at 20° C, the viscosity of the solution is never the same that after direct dissolution of xanthan in NaCl at the same concentration. They explain this result by a dimer dissociation in water at 95° C like observed in cadoxen.

From these different experimental results, it seems clear that two different ordered conformations with the same T_M exist for xanthan in dilute aqueous solution. Moreover, it is possible to control the passage from one to the other secondary structure by using particular experimental conditions which depend at least on the salt and polymer concentrations, and on the temperature compared to the conformational transition temperature T_M (reflecting the ordering of side chains).

But no more can be inferred about the structure of these two ordered conformations by this work except that we have no change in the molecular weight. So we suggest a single chain process in agreement with the recent theoretical predictions by D. Gagnaire et al (13). These authors predict from conformational analysis for xanthan only single chain conformation with three extended helical structures with very similar pitch (with axial advance per dimeric unit $h = 9,09$ A) and another much more compact in which $h = 3,96$ A. Further experiments are in progress to confirm and describe these structures.

Conclusion

The increase of the viscosity reported when native xanthan broth is heated, before isolation, suggests the existence of two different secondary molecular structures showing different hydrodynamic behaviour. In this paper, experiments are described which support this hypothesis. It is possible that these secondary structures correspond to some of those recently predicted by D. Gagnaire et al. It is also demonstrated that the relative stability of the two secondary structures can be controlled by the experimental conditions. In addition, it is shown that the conformation which exists at the time of precipitation is maintained when the polymer is redissolved in a solvent in which the conformational melting temperature T_M is higher than the dissolution temperature.

Acknowledgments. The authors thank Shell Research Ltd for the sample of xanthan broth and Dr Clarke-Sturman for helpful discussions.

References

1. I.T. NORTON, D.M. GOODALL, E.D. MORRIS and D.A. REES, J.C.S. Chem. Comm. 545 (1980).
2. G. MULLER, J. LECOURTIER, G. CHAUVETEAU and C. ALLAIN, Makromol. Chem. Rapid Commun. 5, 203-208 (1984).
3. G. HOLZWARTH and E.B. PRESTRIDGE, Science 197, 757 (1977).
4. S. PAOLETTI, A. CESARO and F. DELBEN, Carbohydr. Res. 123, 173 (1983).
5. G. PARADOSSI and D.A. BRANT, Macromolecules 15, 854 (1982).

6. T. SATO, T. NORISUYE and H. FUJITA, *Polymer J.*, 16 341 (1984).
7. T. SATO, S. KOJIMA, T. NORISUYE and H. FUJITA, *Polymer J.* 16 423 (1984).
8. P.A. SANDFORD, J.E. PITTSLEY, C.A. KNUSTON, P.R. WATSON, M.C. CADMUS and A. JEANES in *Extracellular Microbial Polysaccharides*, P.A. Sandford and A. Laskin (Eds) A.C.S. Symposium Series 45, 192-210 (1977).
9. B. SEEGER, *Die Nahrung* 25 655-656 (1981).
10. J.G. SOUTHWICK, A.M. JAMIESON and J. BLACKWELL, *Carbohydr. Res.*, 99, 117-127 (1982).
11. M. RINAUDO, M. MILAS, F. LAMBERT and M. VINCENDON, *Macromolecules* 16, 816 (1983).
12. F. LAMBERT, M. MILAS and M. RINAUDO, *Polymer Bull*, 7, 185-189 (1982).
13. D. GAGNAIRE, S. PEREZ, V. TRAN and C. VERGELATI, Poster n° 24, "International workshop on Plant Polysaccharides", Nantes, Juillet 1984.

Accepted October 22, 1984