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Evidence for a single stranded xanthan chain by electron microscopy

M. Milas[1], M. Rinaudo[1]*, B. Tinland[1], and G. de Murcia[2]

[1]Centre de Recherches sur les Macromolécules Végétales, Laboratoire Propre du C.N.R.S. associé à l'Université J. Fourier, B. P. 53, F-38042 Grenoble Cedex, France

[2]Institut de Biologie Moléculaire et Cellulaire, 15, rue René Descartes, F-67084 Strasbourg Cedex, France

SUMMARY

Electron microscopy of xanthan adsorbed to positively charged carbon coated grids indicates that this technique can yield reliable informations about the structure of the molecule. The results presented in the present work suggest that the xanthan exists only as single stranded wormlike molecules in clean dilute solutions. In contrast the electron micrographs obtained with native and renatured xanthans adsorbed to uncharged supports indicate side by side associations. A possible role of the acetyl substituent content on this behavior is suggested.

INTRODUCTION

Working for a long time on xanthan gum properties in dilute and semi-dilute solutions, it is well established that the rheological and hydrodynamic properties depend on the samples and conditions used. Several laboratories, including our own, have used electron microscopy of dilute solutions of xanthan for understanding this behavior. We have proved that, depending on the origin of the xanthan and salt concentrations, different aggregates can be visualized by electron microscopy (1). HOLZWARTH and PRESTRIDGE showed evidence for multistranded molecules (2) and concluded that xanthan is a stiff rigid rod like molecule. Recently, STOKKE et al (3,4) analyzed electron micrographs obtained from different xanthan samples and determined the contour length distribution of the molecules and the persistence length q. The molecules have molecular diameters of about 4 nm and 2 nm and persistence lengths (q) of 150 nm for double stranded and 60 nm for single stranded molecules respectively. They concluded that single and double stranded structures exist in different yields depending on the ionic strength of the solution. These conclusions are not consistent with the properties observed using other experimental approaches. For example, from a thermodynamic analysis we concluded that xanthan is a single chain molecule (5) and the persistence length cannot be accurately determined from hydrodynamic behavior (6).

In this paper different techniques for obtention of electron micrographs of xanthan molecules on carbon coated grids are described and the results are compared with those of STOKKE et al, using a different procedure (4).

* To whom offprint requests should be sent

EXPERIMENTAL

Xanthan

Xanthan from a broth (kindly provided by Shell Research, Ltd) was diluted with 0.01 M NaCl solution up to a concentration of 1 g/L. This solution was filtered through millipore filters with pore diameters of 3µm to 0.22µm ; 100 ml of this solution were subjected to ultrafiltration using an Amicon X M 300 membrane. 4 Liters of 0.4 g/L NaCl and 0.2 g/L NaN_3 solution were used as washing solution. The recovered solution was filtered again, through a 0.22µm Millipore filter. This solution was diluted with either suitable NaCl solutions or water in order to reach a xanthan concentration of 70 µg/L. In this way, the xanthan could be prepared in its native, renatured and denatured conformations depending on the conditions and the solvent used (7). The xanthan weight average molecular weight in native and renatured conformation is equal to 7×10^6, the acetyl and pyruvate contents, expressed as an average number per side chain are equal to 0.7 and 0.4 respectively. The intrinsic viscosities $[\eta]$ are equal to 7100 and 10 600 ml/g for the native and renatured conformations respectively.

Electron microscopy

a) Adsorption of the specimen to amylamine-coated carbon films

Carbon-coated grids (400 mesh) were positively charged by glow discharge (150 V per cm) in the presence of amylamine vapor for 30 sec according to the procedure of DUBOCHET (8,9). The vacuum was maintained at 10^{-1} Torr during this operation. After 5 min adsorption, the specimens were stained with uranyl acetate [2 % (wt/vol) in H_2O] and air dried. Finally the grids were rotary shadowed with carbon/platinum at an angle of 7° in an Edwards evaporator equiped with an electron gun (EVM 052 Balzers). The thickness of the metal deposition was monitored on a quartz thin crystal monitor (QSG 201 D, Balzers). The grids were examined in a Hitachi H600 electron microscope equiped with a video system (CV 152 Sofretec). The magnification was calibrated using a carbon-grating replica (Fullman, Schenectady, N.Y.).

b) Cytochrome C spreading

The procedure described by DAVIS et al was used (10). The hyperphase contained 50 % formamide, the hypophase was distilled water. The spread specimens were then processed for shadowing as described above.

RESULTS AND DISCUSSION

Using the amylamine technique, which charges the grid positively, the xanthan conformations appears as a wormlike chain with an apparent thickness of 3 to 4 nm ; only few aggregates are visible. (Figure 1).
With the cytochrome C technique, the native xanthan appears as a branched polymer with an uniform apparent thickness of about 8 nm without correction for Pt deposited.(Figure 2a). In contrast, the xanthan looks like as a linear polymer with the same thickness but much stiff when the same technique is applied to the renatured xanthan conformation (Figure 2b). Similar results were obtained by STOKKE (11) with our xanthan samples using his technique which involves spraying glycerol-xanthan solutions of native and renatured xanthans on freshly cleaved mica. (4). Given that the M_w in these two conformations is the same and that the conformational transition is an intramolecular process, with only a slight change in the linear charge density (7), the behavior observed in the electron micrographs can be only explained by

side by side interactions induced by the sample preparation.In reference to STOKKE et al (3,4) and after correction for the replica thickness the lower thickness found when charged grids are used is compatible with a single stranded molecule for the xanthan in this condition. The estimated persistence lengths (q) from Figure 1 are approximately equal to about 40 to 60 nm, values very close to the experimental ones for single stranded xanthan (7). In contrast, the results obtained in this work by the other method with the same xanthan solutions or by STOKKE et al (3,4,11) show side by side interactions which look like double stranded molecules or higher degree of aggregation.It is possible that these interactions appear during the drying stage. Consequently a possible advantage of using charged grids is the avoidance of contraction and aggregation of the molecules. This technique seems to be well suited for studying the actual state of the xanthan molecules in very dilute solutions no matter what solvent is used (H_2O, NaCl 0.01 M). Using a xanthan provided by Dr. I.W. SUTHERLAND, (strain PX 061), STOKKE et al (3) found that in 2 mM ammonium acetate, this sample appeared only as single stranded chains, but as the ionic strength was increased it appeared as aggregated single strands. We have characterized this sample (Table I) (kindly provided by Dr. SUTHERLAND, University of Scotland). We have found that in the native conformation it exhibits a low viscous conformation while the renatured conformation is more viscous.LECOURTIER et al (12) explained this increase in viscosity by a transition from a compact to an extended double helix. They claim that the dissociation of the extended double helix can appear only for very high pyruvate content and in ionic strengths below 10^{-4} M. In the work of STOKKE et al (3) the xanthan used (strain PX 061) had no pyruvate (Table I) and it was dissolved directly in 2 mM ammonium acetate at pH 7 without any heating. Under these conditions, the xanthan should exist as a double stranded molecule and not as a single strand as pointed from electron microscopy (3). Clearly, the results of LECOURTIER et al (12) and STOKKE et al (3) are conflicting. Furthermore, we have been unable to observe the dissociation of the double helix suggested by LECOURTIER et al (12), using similar sample and conditions (13). Similarly , we showed that the behavior of xanthan in cadoxen described by SATO et al (14,15) can be only explained by a degradation of xanthan molecules in this solvent and not to a dissociation of the double helix in this solvent (16). These considerations, together with the result presented in the present work and elsewhere (5,7) lead us to suggest that xanthan ordered conformations exist only as single stranded molecules in clean dilute solutions. In the work of STOKKE et al (3), the peculiar behavior of the xanthan from strain PX061 is perhaps only due to the exceptionally high content of acetyl substituent, which may inhibit in low ionic content the aggregation by side by side interactions during the drying stage. It is well known for example that acetyl groups prevented gelation in K54 or gellan bacterial polysaccharides (17).

CONCLUSION

We have shown that xanthan molecules may appear as aggregates or single stranded molecules by electron microscopy depending on the samples and the spreading technique used. The technique using a positively charged carbon coated grid is well suited for obtaining electron micrographs of xanthan. With this technique, xanthan molecules

TABLE I : CHARACTERISTICS OF XANTHAN FROM THE STRAIN P X 061

	$\overline{M}_w$ x10^{-6}	[η] (ml/g)	η_{rel} (a)	Acetyl (b)	Pyruvate (b)
Native conformation	2.2	1800	4.2	1.5	0
Renatured conformation	2.2	3000	10.7	1.5	0

(a) xanthan concentration 1 g/L in 0.01 M NaCL at 25°C
(b) expressed as an average number per side chain, measured by ^{1}H n.m.r.

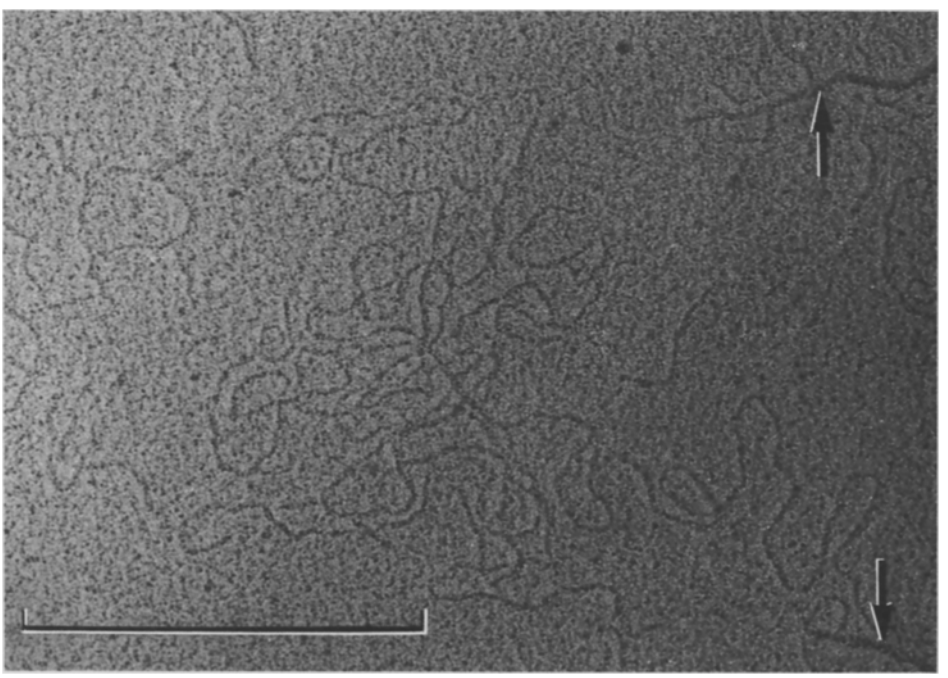

Figure 1 : Representative electron micrographs of xanthan using the positively charged grid technique. Native xanthan in 0.01 M NaCl. Bar = 400 nm. (⟶ aggregated molecules).

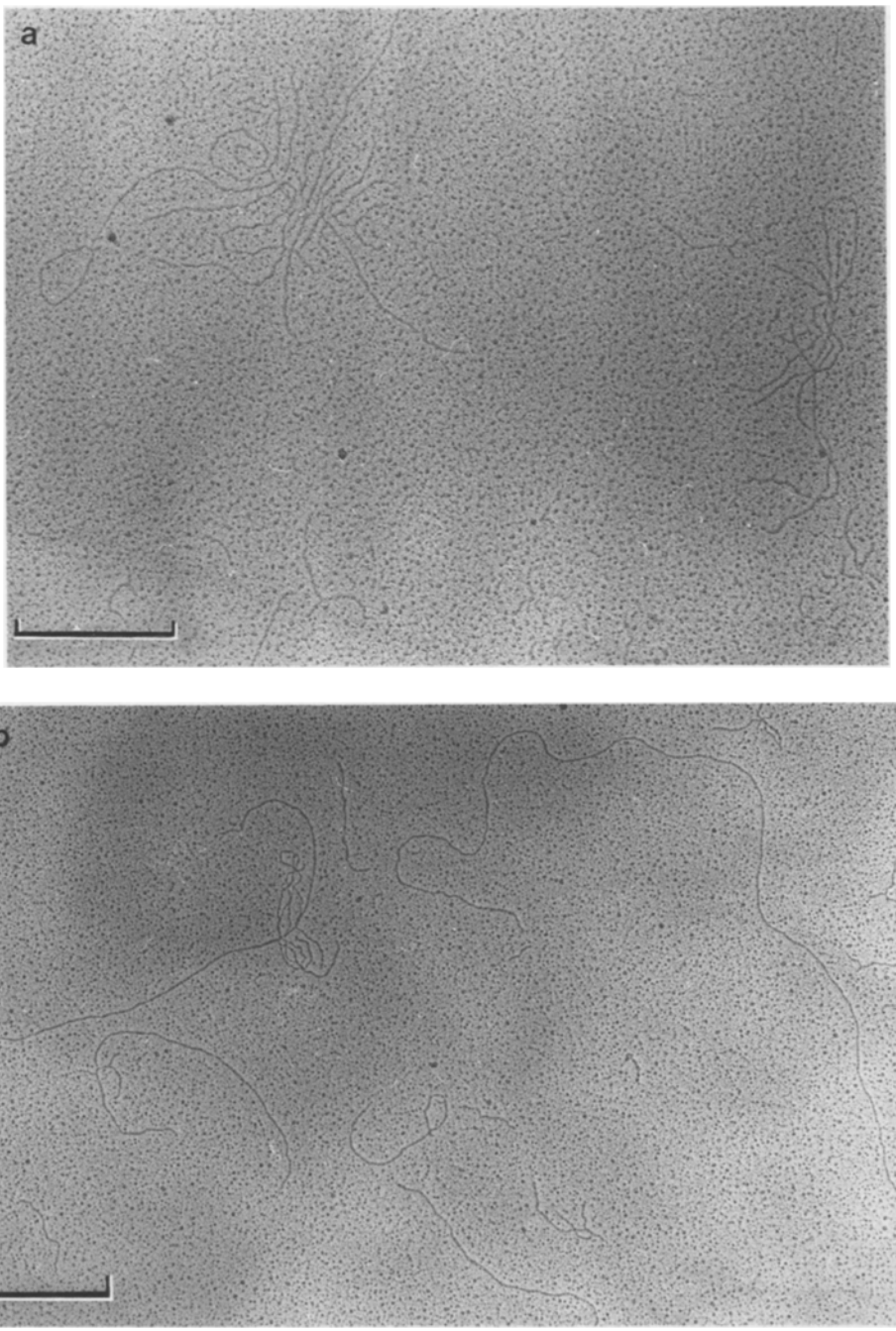

Figure 2 : Representative electron micrographs of xanthan using the cytochrome C technique. Native xanthan in 0.01M NaCl (a). Renatured xanthan in 0.01 M NaCl (b). Bar = 400 nm.

appear as wormlike chains with a smaller diameter compared to the linear or branched shapes obtained with other techniques, where side by side associations are probably created during the drying stage. From these observations and other work, we conclude that xanthan is a single stranded chain in solution.
Given the low contrast for the single chain molecules, it is very difficult to obtain the distribution of the contour lengths. The pictures we have obtained using charged grids have been directly related to the quality of the xanthan solution i.e. the fraction of aggregated polymer in relation with lack of filtrability. This technique may be useful for testing the quality of polymer samples, for instance after post fermentation treatments.

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