ELSEVIER

PII: S0032-3861(97)00079-7

Polymer Vol. 38 No. 21, pp. 5289–5295, 1997

0032-3861/97/$17.00 + 0.00

About the native and renatured conformation of xanthan exopolysaccharide

I. Capron*†, G. Brigandt and G. Muller*‡

**UMR 6522 du CNRS Polymères, Biopolymères et Membranes, Université de Rouen, 76 821 Mont-Saint-Aignan cedex, France and †Systems-Bio-Industries, Laboratoire de Recherches, Baupte 50500, Carentan, France*

(Received 13 January 1997)

Low-angle laser light scattering (LALLS), size exclusion chromatography coupled with multi-angle light scattering (s.e.c./MALLS), low shear intrinsic viscosity and circular dichroism measurements were performed for elucidating the size and conformation changes associated with the temperature-induced denaturation and renaturation of native xanthan in different salt conditions (0.01 M and 0.1 M). Upon heating, at temperatures above the order–disorder transition temperature (T_m) the denaturation of the native ordered conformation occurs with a reduction of M_w by a factor of roughly two therefore indicating a double strand conformation for the native form. The molecular weight has been found invariant after renaturation on cooling that favours the hypothesis that the restoration of the ordered form of xanthan takes place through the same molecule. The most probable conformation for the renatured form of xanthan is that of an antiparallel double stranded structure consisting of one such chain folded as a hairpin loop.

(Keywords: size exclusion chromatography; laser light scattering; xanthan)

INTRODUCTION

The exopolysaccharide xanthan is largely used in the food industry as a thickening agent, but it is also a widely used structurant in all areas of technology. Much work has been published since its discovery 30 years ago[1], regarding the structure of the molecule and conformational changes essentially induced by varying temperature and salinity.

The primary structure of xanthan has been clearly established[2] and consists of a linear β-1,4-linked D-glucose chain substituted on every second glucose residues by charged trisaccharide side chains. The inner mannose residue is normally acetylated at C(6) and every side chain carried one carboxyl group from the central β-D glucuronic acid residue. The terminal mannose has a variable degree of pyruvate substitution, which is thought to be dependent on both the bacterial strain and fermentation conditions.

The secondary structure is known to undergo an order (helix) to disorder (coil) transition[3–9], but structural details of the ordered conformation remain controversial and arguments for two different models are given in literature. Most authors suggest the stabilization of the ordered helix by noncovalent side chain/main chain interactions involving hydrogen bondings, but the question of whether the xanthan molecule is a single or a double strand in aqueous solutions is still being debated. A study of the literature shows that both of them seem to coexist, depending on the treatment imposed to the native molecule after the fermentation step including post-fermentation treatment and purification notably.

‡To whom correspondence should be addressed

The ordered molecule exists in solution as a semi-rigid helix stabilized by hydrogen bonds and can undergo a conformational transition to a disordered flexible coil either by decreasing salinity or by increasing temperature above the characteristic 'melting' temperature (T_m). This transition has been monitored by various techniques including optical rotation[3,10], circular dichroism[4,23], light scattering[5,7,11–14], viscometry[15,16], viscoelasticity[17,18], electron microscopy[19], calorimetry[20], electric birefringence[21], n.m.r.[22] or neutron scattering[24]. Literature data reported experimental evidence for a double[8,12–15,19–21] and/or a single[4–7,9,23] stranded structure.

Below T_m, the polymer exists in a rigid ordered conformation responsible for the remarkable rheological properties of the molecule, whereas above T_m a flexible disordered conformation with a lower viscosity predominates.

There is substantial evidence suggesting that the ordered form is, at least for some xanthan samples, double stranded. This raises the possibility that the order to disorder transition may be associated with complete or partial disruption of the double-stranded form in favour of an intermolecular dissociation process occurring with change in molecular weight. Other results are consistent with a strictly intramolecular process without molecular weight change.

Whereas the order–disorder transition has been widely studied, denatured[7,8,25] and renatured[7,26,27] states showed comparatively little interest in the literature. This is surprising as generally the fermentation broths are thermally treated. This treatment should induce an order–disorder conformation change in the polymer and on subsequent cooling the renatured ordered structure should differ from that of the native xanthan.

The main objective of this work was to investigate the solution properties of xanthan, both in dilute and semi-dilute concentration range in relation to the temperature induced denaturation/renaturation process.

EXPERIMENTAL

Xanthan solutions

The xanthan sample was supplied by Systems-Bio-Industries as a powder (CX12 lot 41752PO). It is issued from a fermentation broth which has been thermally treated, then intensively purified by filtration, precipitated in isopropanol and finally dried and ground.

The dry powder was dispersed into water at a concentration of $1\,g\,l^{-1}$, gently stirred and kept in a refrigerator overnight, 400 ppm NaN_3 (antimicrobial agent) was added when solution had to be kept more than a few days. The stock solution was then diluted and salts were added to obtain the desired compositions in salt (0.01 M and 0.1 M in NaCl). Dilute solution was finally clarified through 0.45 μm Millipore filters. We observed no significant difference in concentration after filtration. A humidity content of 10 wt% was found and was taken into account in all calculations.

The optically transparent solution showed u.v. absorption in the vicinity of 260 nm indicating the presence of proteins. The extent of pyruvate substitution was found near 0.5 by colorimetry and potentiometry measurements. The characteristics of the aqueous solution of the xanthan sample, under conditions where an ordered conformation prevails, are given in *Table 1*.

Table 1 Xanthan solution characteristics at 25°C in NaCl 0.1 and 0.01 M (ordered conformation)

Salinity	M_w ($g\,mol^{-1}$)	A_2 ($ml\,mol\,g^{-2}$)	$[\eta]$ ($ml\,g^{-1}$	k'	c^* (ppm)
0.1 M	5.3×10^6	5.1×10^{-4}	7150	0.6	210
0.01 M	5.2×10^6	1.4×10^{-3}	7400	0.6	–

Light scattering measurements

Light scattering measurements were carried out using both a low-angle (LALLS) and a multi-angle laser light scattering (MALLS) photometers with He–Ne illumination ($\lambda = 632.8$ nm). The dn/dc value was measured with a Brice–Phenix differential refractometer at room temperature in 0.1 M NaCl. The value of dn/dc of $0.143\,ml\,g^{-1}$ has been used to analyse all light scattering data inasmuch as no significant dependence on both temperature and NaCl concentration has been reported in the literature[28].

A KMX-6 low-angle light scattering photometer (LCD/Milton Roy) was used for the measurement of the scattered light intensity at a low forward angle (6–7°) and at a concentration lower than 200 ppm. The weight average molecular weight M_w and the second virial coefficient A_2 were computed from scattered light intensities using a plot of the excess Rayleigh ratio as a function of concentration.

A multi-angle light scattering photometer DAWN-F (WYATT Technology Corp., Santa Barbara, CA) was used in series with the size exclusion chromatography (s.e.c.) and refractive index (RI) (Shimadzu RID-6A) detection. A set of TOYO SODA TSK G4000PW and G6000PW columns were used with an aqueous mobile phase of 0.1 M $LiNO_3$ and a Shodex precolumn was used as a packing guar column. The light-scattering intensity was measured at 15 angles in the range 14–160°.

As discussed in a previous paper[29], the molecular weights, M_w and radius of gyration, R_g, were calculated

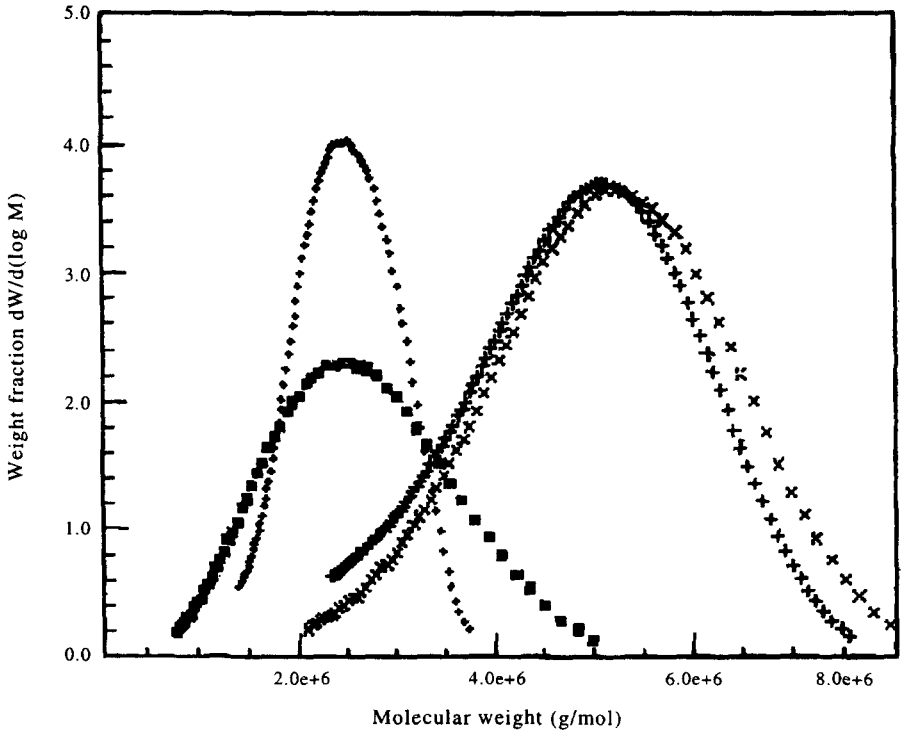

Figure 1 Evolution of the differential molecular weight distribution of xanthan solution between 25 and 60°C in $LiNO_3$ 0.01 M. +, 25°C; ×, 40°C; +, 50°C; ■, 60°C

across all increments of chromatograms using the Debye plot of $R(\theta)/K_c$ vs. $\sin^2\theta/2$ with a fourth-order fit.

Other physical measurements

Low-shear rate viscosity measurements were carried out using a Contraves Low-Shear LS 30 viscometer in the range 0.277–0.945 s^{-1} corresponding in any cases to the Newtonian plateau equipped with a water thermostat.

Circular dichroism was determined with a DC III Jobin–Yvon (France) dichrograph in the range 195–260 nm with a 1 mm cell. Molar ellipticity was deduced with a molecular weight of 970 g mol^{-1} for the repeat unit of the xanthan molecule in accordance with the DS_{pyr} of 0.5. Both apparatus and cell were thermostated at the required temperature.

RESULTS

Denatured xanthan molecule

Xanthan could exist under disordered conformation at high temperature and/or at low salinity and/or in cadoxen[12]. A variety of techniques shows that the order–disorder conformational change (denaturation) of the xanthan molecule in 0.01 M NaCl occurs at a temperature near 50°C[23].

In consequence solution properties of xanthan were studied in 0.01 M NaCl in a temperature range 25–60°C in order to get information on both ordered ($T < 50$°C) and denatured ($T > 50$°C) xanthan molecules.

The changes in molecular weight, M_w, and intrinsic viscosity, $[\eta]$, associated with the denaturation process were followed with the s.e.c./MALLS and low shear equipment operating at 25, 40, 50 and 60°C respectively (*Figures 1* and *2*).

The results of light scattering and viscosity experiments are presented in *Table 2*. The M_w remains essentially constant over the temperature range 25–40°C then suddenly decreases at $T \cong 50$°C and finally at 60°C the M_w is about half of the original value. This variation in M_w strongly suggests that the temperature induced denaturation occurs by way of an intermolecular process implying a dissociation of the native ordered double-strand into disordered single chains. The molecular weight variation is associated with conformational changes as illustrated by the decrease in molecular size (R_g) and the corresponding decrease in the solution viscosity.

It is interesting to note that, whereas the dissociation is largely initiated over the temperature range 40–50°C as indicated by the observed M_w decrease, both R_g and $[\eta]$ practically did not change. This is an indication that the xanthan molecule is in an intermediary state where it still exists under an extended conformation. Probably the side chains that represent about 65% of the molecular weight play a major role on the conformation of the denatured molecule. In this intermediary state the denatured flexible molecule is extended and is quite totally dissociated. This agrees with the previously reported two state process of denaturation[31].

At this intermediary temperature a thermodynamically stable state exists where the side chains can be oriented toward the periphery, as shown for a single strand using molecular modelling as the theoretical general tendency[32]. This extended form is consistent with the lower Huggins constant ($k' = 0.45$) indicating the absence of associations at 50°C.

Circular dichroism measurements were performed for evidencing the changes in molar ellipticity at a wave length of 210 nm that is attributed to the acetate chromophore[4] of xanthan in pure water ($c = 4\,g\,l^{-1}$) over the temperature range 25–60°C. The results are reported in *Figure 3* together with the corresponding

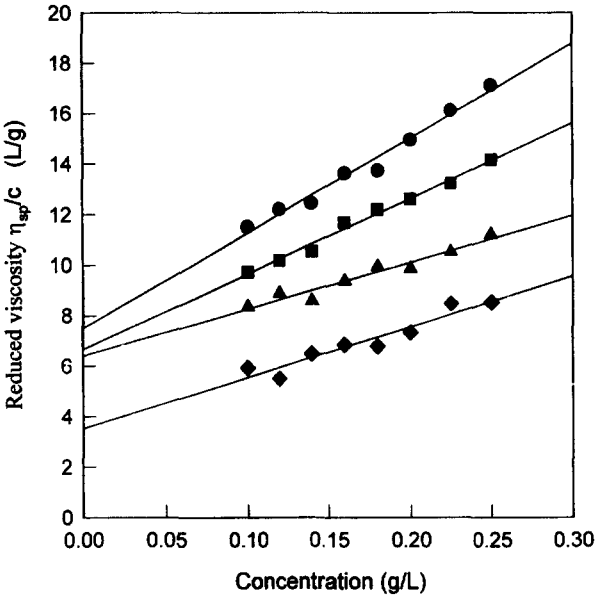

Figure 2 Reduced viscosity η_{sp}/c vs. polymer concentration for xanthan sample in NaCl 0.01 M between 25 and 60°C. ●, 25°C; ■, 40°C; ▲, 50°C; ◆, 60°C

Table 2 Molecular weight, radius of gyration, intrinsic viscosity and Huggins constant of xanthan in 0.01 M NaCl between 25°C and 60°C

Temperature (°C)	M_w (g mol^{-1})	R_g (nm)	$[\eta]$ (ml g^{-1})	k'
25	4.2×10^6	155	7400	0.6
40	4.3×10^6	155	6660	0.6
50	2.8×10^6	152	6410	0.45
60	2.2×10^6	125	3530	1.6

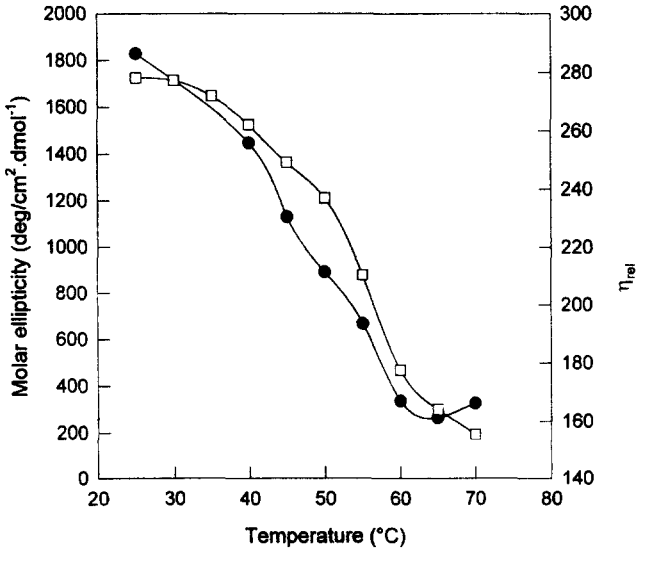

Figure 3 Evolution of molar ellipticity and relative viscosity in NaCl 0.01 M vs. temperature in NaCl 0.01 M (polymer concentration 2 g l^{-1}). ●, Dichroic curve; □, viscosity curve

relative viscosity ($c = 2\,g\,l^{-1}$) variation. Increasing the temperature causes a progressive decrease in the chain rigidity with a larger decrease over the temperature range ($T > 50°C$) of the denaturation.

On recooling to 25°C no hysteresis was observed and the c.d. spectrum was well superimposed on that recorded on heating (*Figure 4*).

This behaviour was analysed by some authors as evidence of an intramolecular process involving a one-step mechanism. This is not in agreement with the above reported light scattering data that showed that denaturation and dissociation occur simultaneously ('intermolecular process'). As suggested by Beiso *et al.*[21] this contradiction is only apparent and more probably indicates that the circular dichroism is not sensitive enough to observe real differences in such a local organization.

Renatured xanthan molecule

The renatured state of the molecule was obtained by heating solutions of xanthan ($1\,g\,l^{-1}$) in 0.1 M NaCl at three different temperatures near and above the transition temperature, T_m (90°C, 110°C and 140°C), and then allowing them to cool to room temperature. The samples to be heated were prepared in glass tubes (8 ml of xanthan at $1\,g\,l^{-1}$); the tubes were immersed in an oil bath, thermostated at the required temperature for 30 min and finally cooled in an ice-bath.

After cooling the samples were diluted at $0.2\,g\,l^{-1}$ in 0.1 M NaCl and the dilute solutions were characterized at room temperature by LALLS and low shear viscosity.

Figures 5 and *6* illustrate the dependence of the molecular weight and the intrinsic viscosity measured at 25°C on the duration of the thermal treatment at 90, 110 and 140°C respectively. After 5 min at 90°C, a temperature near T_m, the M_w has decreased from the original value reaching a constant value of $4 \times 10^6\,g\,mol^{-1}$ that remains constant whereas in the same time no change in viscosity is measured. Probably the M_w decrease could be the consequence of the disruption of aggregates the concentration of which is too low to affect the viscosity but sufficiently high to perturb low-angle light scattering data. The value of $4 \times 10^6\,g\,mol^{-1}$ is attributed to the molecular weight of the isolated xanthan molecule under ordered conformation.

After 20 min at 110°C, a temperature above T_m, we observe a strong and continuous decrease in M_w and $[\eta]$, both of them reaching values lower than the original ones that remain constant after 20 min heating. Finally the molecular weight reaches a value of roughly 50% of the molecular weight of the isolated ordered xanthan molecule. This indicates that the isolated xanthan molecule was a double-stranded helix that denatures and dissociates after 20 min heating into two single strands the reordered conformation of which is probably different from the initial one as indicated by the lower viscosities.

After 5 min heating at 140°C both M_w and $[\eta]$ are sharply decreased and reach values identical to those obtained after 20 min heating at 110°C. After a longer time, both M_w and $[\eta]$ are continuously decreased as a consequence of a degradative process. This was expected as it is well known that the disordered conformation is

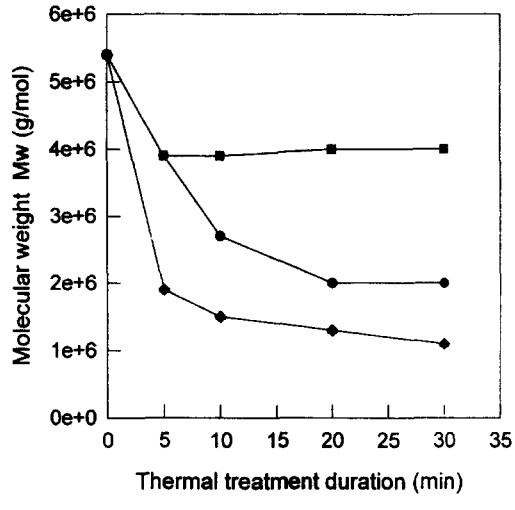

Figure 5 Molecular weight measured at 25°C vs. temperature and heating time at $1\,g\,l^{-1}$ in 0.1 M NaCl. ■, 90°C; ●, 110°C; ◆, 140°C

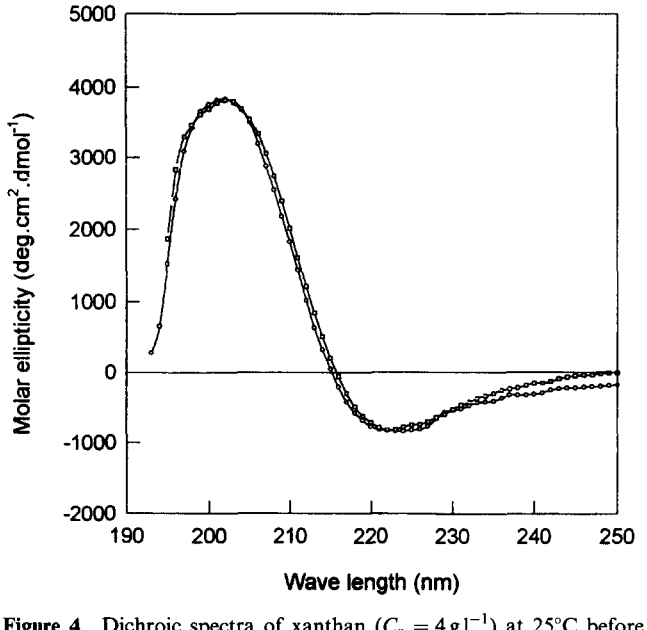

Figure 4 Dichroic spectra of xanthan ($C_p = 4\,g\,l^{-1}$) at 25°C before and after thermal treatment at 70°C in 0.01 M NaCl

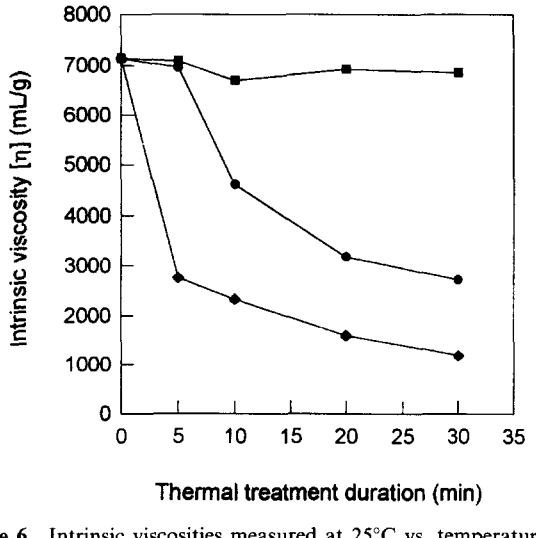

Figure 6 Intrinsic viscosities measured at 25°C vs. temperature and heating time at $1\,g\,l^{-1}$ in 0.1 M NaCl. ■, 90°C; ●, 110°C; ◆, 140°C

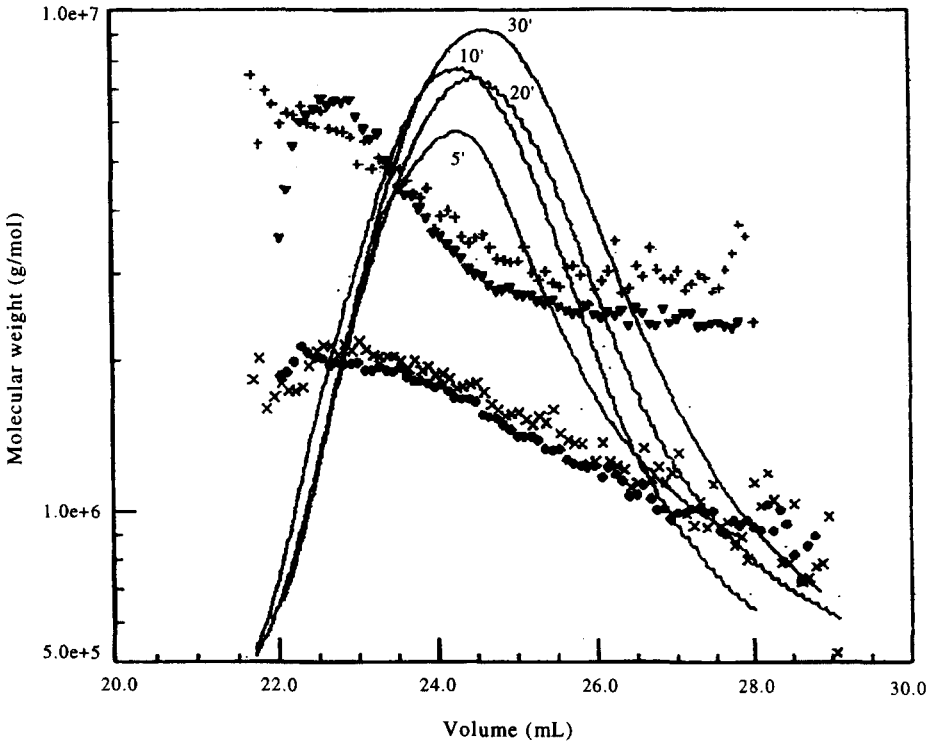

Figure 7 Molecular weight distribution and RI profile *versus* elution volume of xanthan in 0.1 M NaCl heated between 5 and 30 min at 110°C in 0.01 M $LiNO_3$. ▽, 5 min; +, 10 min; ×, 20 min; ●, 30 min

much more temperature sensitive than the ordered one[33]. Moreover 140°C is a critical temperature for the glycosidic linkages.

The above reported data have been confirmed by on-line s.e.c./MALLS analysis of xanthan 0.1 M NaCl solutions that have been heated at 110°C for a period of time between 5 and 30 min. After being heated the samples were immediately cooled and then injected at 25°C in the s.e.c./MALLS equipment. Before injection the cooled solutions were diluted to 200 ppm in 0.1 M $LiNO_3$. Elution profiles are reported in *Figure 7*. The data clearly show that the molecular weight is roughly halved after being heated at 110°C for 20 and 30 min. This totally agrees with the above reported data. Moreover it appears that the whole MWD is divided by a factor two that means that dissociation does not concern some xanthan molecules but the whole set of biopolymer molecules. As previously found M_w is around 4×10^6 g mol^{-1} for the native ordered xanthan and about 2×10^6 g mol^{-1} for the renatured form.

Similar results have been obtained on heating lower M_w sonicated xanthan molecules except that dissociation has occurred earlier. After 10 min at 110°C the M_w was 6.3×10^5 g mol^{-1} against 1.3×10^6 for the untreated sample.

These light scattering data give arguments in favour of a double-strand for the unheated xanthan molecule. On heating, this conformation is maintained as far as the temperature is lower than the salt dependent denaturation temperature T_m. Only after heating above T_m denaturation and dissociation of the double strand could occur.

As all measurements were done just after thermal treatment, the question we asked was whether the reassociation of xanthan molecules was a slow process compared to the dissociation and therefore needed more time. We have verified that once the dissociation of the native molecule has occurred, no reassociation happened as evidenced by the fact that the M_w of a sample stored for one month in the refrigerator was identical to that measured immediately at the end of the thermal treatment.

Table 3 Conformation parameters of ordered xanthan molecule

Treatment	Mark–Houwink relation	Molecular organization
Native sample	$[\eta] = 5.3 \times 10^{-3} M_w^{1.07}$	Native double helix
Commercial sample CD12	$[\eta] = 1.6 \times 10^{-3} M_w^{1.00}$	Aggregated double helix
CX12 heated 30 min at 110°C	$[\eta] = 1.5 \times 10^{-4} M_w^{1.15}$	Renatured monofilament

Conformation of xanthan molecules

Useful information concerning the conformational characteristics of polymers in solution are provided from the $[\eta]$ and/or R_g dependence on molecular weight. For obtaining the Mark–Houwink coefficients samples of lower M_w were obtained by sonicating a solution of xanthan (1 g l^{-1}, 0.1 M NaCl) before and after thermal treatment at 110°C. The Mark–Houwink coefficients were determined from log–log plots of $[\eta]$ vs. M_w for a native sample (a powdered xanthan precipitated from a native fermentation broth) and a commercial sample (CX12) before and after being treated for 30 min at 110°C. They are reported in *Table 3*.

Irrespective of the treatment after fermentation the values of the Mark–Houwick exponents are consistent with the fact that xanthan under ordered and/or reordered state has a 'rod like' conformation in agreement with data reported in the literature[12,33,34]. The slightly higher value of the exponent ($a = 1.15$) after complete dissociation of the native structure indicates

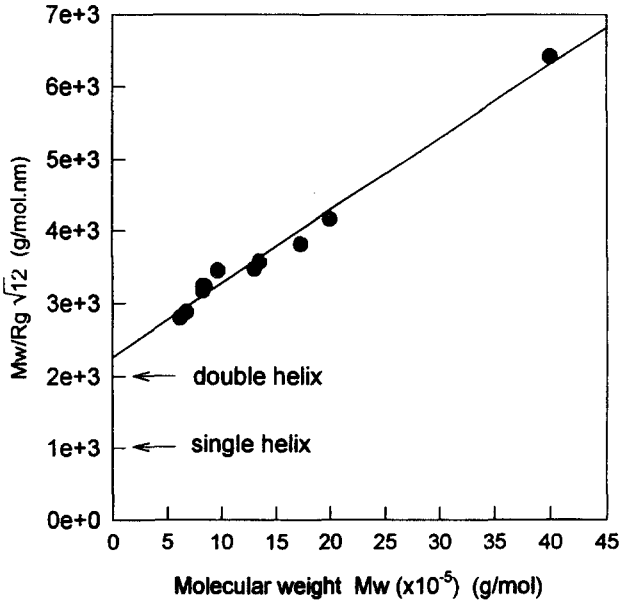

Figure 8 Strandedness of the native unheated xanthan molecule

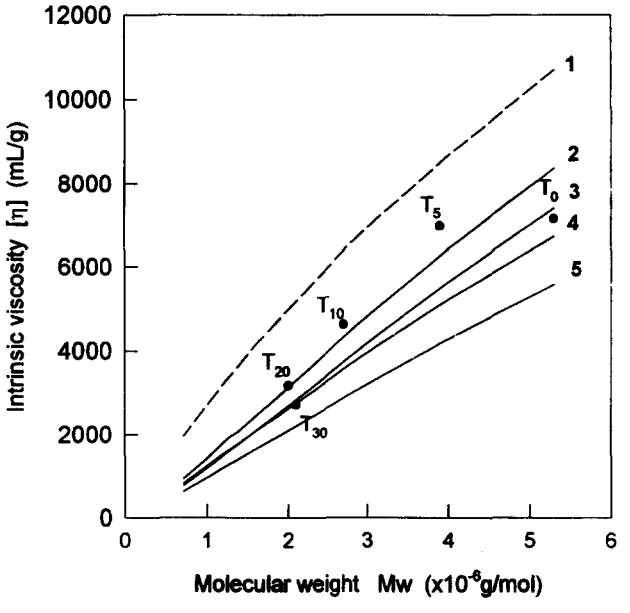

Figure 9 Experimental molecular weight dependence of $[\eta]$ (measured at 25°C) for a commercial xanthan in 0.1 M NaCl after heat treatment (110°C, 30 min) superimposed on theoretical curves for a worm-like chain deduced from the theory of Yamakawa–Fujii. 1, Single strand model with $d = 2.0$ nm and $q = 50$ nm; 2, double strand model with $d = 3.8$ nm and $q = 120$ nm; 3, double strand model with $d = 2.0$ nm and $q = 120$ nm; 4, double strand model with $d = 3.8$ nm and $q = 96$ nm; 5, double strand model with $d = 2.0$ nm and $q = 96$ nm

that renatured single strands have a more rigid conformation of the chain as was reported by Milas *et al.*[35].

Indications upon the average strandedness of the xanthan molecule can be deduced from the experimentally determined light scattering parameters (M_w and R_g).

The pentasaccharide repeat unit of the xanthan molecule has a length of about 1 nm and a mass of near 1000 D therefore the linear mass density (mass per unit length) will be close to 1000 and 2000 D nm^{-1} for the single and double strand respectively.

The high molecular weight native xanthan is a semi-rigid polymer having a large persistence length

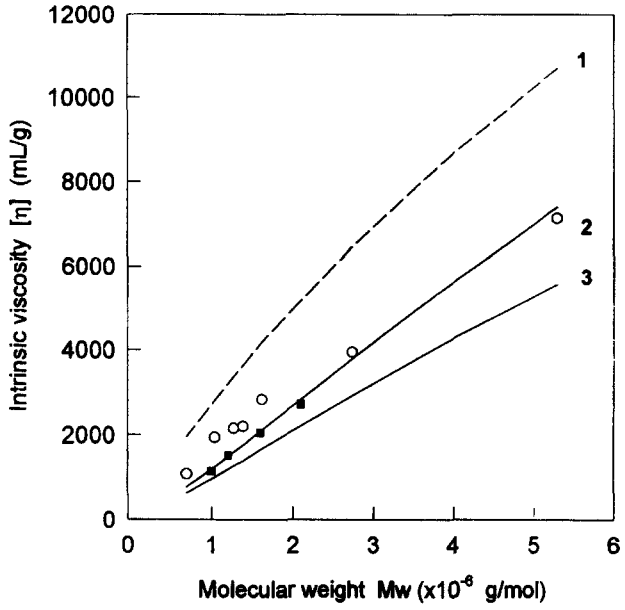

Figure 10 Experimental molecular weight dependence of $[\eta]$ for sonicated fractions of a xanthan sample in 0.1 M NaCl heated 30 min at 110°C (measurements at 25°C) superimposed on theoretical curves for a worm-like chain deduced from the theory of Yamakawa–Fujii. 1, Single strand model with $d = 2.0$ nm and $q = 50$ nm; 2, single strand model with $d = 2.0$ nm and $q = 120$ nm; 3, single strand model with $d = 2.0$ nm and $q = 96$ nm. O, Unheated sample; ■, sample heated 30 min at 110°C

($q \cong 100$–150 nm)[36] but such that q is smaller than the contour length L_c of the molecule. The behaviour of the xanthan molecule changes with decreasing molecular weight from a semi-rigid to a rigid rod and lower M_w xanthan molecules can be considered as strictly rigid rod when $L_c \leq q$. In that case the contour length of the molecule can be approximated from $L_w = R_g\sqrt{12}$ and M_L could be deduced from the limit for $M \to 0$ of the ratio between the experimentally determined M_w and R_{gw}. The data are reported in *Figure 8*. A value of M_L near 2000 nm^{-1} has been found which indicates an average strandedness of two for the native unheated xanthan.

Correlation between measured intrinsic viscosities and measured molecular weights are shown in *Figures 9* and *10* and compared with theoretical values using a worm-like chain model. Data in *Figure 9* are concerned with the treatment of CX12 at 110°C for various periods (5–30 min) whereas in *Figure 10* the data are concerned with xanthan samples using the Yamakawa–Fujii model[37] for monostranded molecules (with $M_L = 1000$ nm^{-1}, $q = 50$ nm and $d = 2$ nm) (dotted lines) and for double stranded molecules (with $M_L = 2000$ nm^{-1} and various values of diameters and persistence lengths) (solid lines). It is clear that the calculated curve assuming a single strand does not match the experimental data whereas both the native ordered conformation (before heating) and the renatured conformation are rather well described by a double stranded conformation with the same M_L. After 30 min at 110°C, complete splitting of the double native helix has been shown. This result can be interpreted in terms of helix reformation through the same molecule by folding of the single strand according to a hairpin-shaped structure.

CONCLUSIONS

The results described in this paper indicate that the

change in solution properties (size and conformation) of the xanthan molecules are associated with the temperature-induced order–disorder transition. On heating xanthan samples to temperatures above the transition temperature T_m and then cooling to room temperature useful information on the native ordered, disordered and ordered renatured xanthan molecule were obtained. Further evidence that the native rigid molecule exists as a double-strand helix that can dissociate into two flexible coils on heating above T_m can be deduced from the viscosity and light scattering data. Moreover, on cooling a thermally-treated xanthan molecule to room temperature, it is shown that renaturation occurs according to an intramolecular process (no molecular weight changes). The most probable conformation for this renatured form is that of a hairpin-like structure. This agrees with previously reported electron microscopy observation[19] and electric birefringence data[21]. This also agrees with the atomic force microscopy observation we will report in a following paper.

ACKNOWLEDGEMENT

The authors are indebted to Systems-Bio-Industries for financial support.

REFERENCES

1. Jeanes, A., Pittsley, J. E. and Senti, F., *J. Applied Polym. Sci.*, 1961, **5**, 519.
2. Jansson, P. E., Kenne, L. and Lindberg, B., *Carbohydr. Res.*, 1975, **45**, 275.
3. Holthwarth, G., *Biochemistry*, 1976, **15**, 4333.
4. Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D. and Darke, A., *J. Mol. Biol.*, 1977, **110**, 1.
5. Norton, I. T., Goodall, D. M., Frangou, S. A., Morris, E. R. and Rees, D. A., *J. Mol. Biol.*, 1984, **175**, 371.
6. Muller, G., Anrhourrache, M., Lecourtier, J. and Chauveteau, G., *Int. J. Biol. Macromol.*, 1986, **8**, 167.
7. Milas, M. and Rinaudo, M., *Carbohydr. Res.*, 1986, **158**, 191.
8. Liu, W., Sato, T., Norisuye, T. and Fugita, H., *Carbohydr. Res.*, 1987, **160**, 267.
9. Haache, L. S., Washington, G. E. and Brant, D. A., *Macromolecules*, 1987, **20**, 2179.
10. Morris, V. J., Frankin, D. and I'anson, K., *Carbohydrate Research*, 1983, **121**, 13.
11. Coviello, T., Kajiwara, K., Burchard, W., Dentini, M. and Crescenzi, V., *Macromolecules*, 1986, **19**, 2826.
12. Sato, T., Kojita, S., Noritsuye, T. and Fugita, H., *Polym. J.*, 1984, **16**(5), 423.
13. Paradossi, G. and Brant, D. A., *Macromolecules*, 1982, **15**, 874.
14. Gamini, A. and Mandel, M., *Biopolymers*, 1994, **34**, 783.
15. Sato, T., Noritsuye, T. and Fugita, H., *Macromolecules*, 1984, **17**, 2696.
16. Kierulf, C. and Sutherland, I. W., *Carbohydr. Polym.*, 1988, **9**, 185.
17. Rochefort, W. E. and Middleman, S., *J. Rheol.*, 1987, **31**(4), 337.
18. Ross-Murphy, S. B., Morris, V. J. and Morris, E., *Faraday Symp. Chem.*, 1983, **18**, 115.
19. Stokke, B. T., Smidsrod, O. and Elgaeter, A., *Biopolym.*, 1989, **28**, 617.
20. Kitamura, S., Takeo, K., Kuge, T. and Stokke, B. T., *Biopolym.*, 1991, **31**, 1243.
21. Besio, G. J., Leavesley, I. M. and Robert, K., *J. Appl. Polym. Sci.*, 1987, **33**, 825.
22. Gamini, A., de Bleijser, J. and Leyte, C., *Carbohydr. Res.*, 1991, **220**, 33.
23. Milas, M. and Rinaudo, M., *Carbohyd. Res.*, 1979, **76**, 189.
24. Milas, M., Rinaudo, M., Duplessix, R., Borsali, R. and Lindner, P., *Macromolecules*, 1995, **28**(9), 3119.
25. Lecourtier, J., Chauveteau, G. and Muller, G., *Int. J. Biol. Macromol.*, 1986, **8**, 306.
26. Holthwarth, G. and Prestridge, E. B., *Science*, 1977, **197**, 757.
27. Kawakami, K., Okabe, Y. and Norisuye, T., *Carbohydr. Polym.*, 1991, **14**, 189.
28. Haache, L. S., Washington, G. E. and Brant, D. A., *Macromolecules*, 1987, **20**, 2179.
29. Capron, I., Grisel, M. and Muller, G., *J. Polymer Analysis and Characterization*, 1995, **2**, 9.
30. Wyatt, P. J., *Analytica Chimica Acta*, 1993, **272**, 1.
31. Muller, G. and Lecourtier, J., *Carbohydr. Polym.*, 1988, **9**, 213.
32. Perez, S. and Vergelati, C., *Int. J. Biol. Macromol.*, 1987, **9**, 211.
33. Lund, T., Lecourtier, J. and Muller, G., *Polymer Degradation and Stability*, 1990, **27**, 211.
34. Muller, G., Lecourtier, J., Chauveteau, G. and Allain, C., *Makromol. Chem., Rapid Commun.*, 1984, **5**, 203.
35. Callet, F., Milas, M. and Tinland, B., in *Gums and Stabilisers for the Food Industry*, Vol. 4, ed. G. O. Phillips, D. J. Wedlock and P. A. Williams. Pergamon Press, Oxford 1998, pp. 203–210.
36. Sho, T., Sato, T. and Norisuye, T., *Biophys. Chem.*, 1986, **25**, 307.
37. Yamakawa, H. and Fujii, M., *Macromolecules*, 1974, **7**(1), 128.