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Dissociation of Dimerized Xanthan in Aqueous Solution

Kikuji Kawakami, Yuko Okabe & Takashi Norisuye

Department of Macromolecular Science, Osaka University, Toyonaka, Osaka 560,
Japan

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ABSTRACT

Aqueous solutions of low-molecular-weight sodium xanthan with a degree of pyruvation of about 1/3 were heated at 95°C for different time periods t_d *between 2·5 and 15 h, mixed with aqueous sodium chloride to an NaCl concentration of 0·01 or 0·1* M *and subjected to viscosity, optical rotation and sedimentation equilibrium measurements at 25°C. The measured specific rotations showed that, upon addition of aqueous NaCl to 0·1* M*, Na xanthan disordered in water at 95°C became ordered to a conformation locally similar to that of each chain in the double helix of the polysaccharide in 0·1* M *aqueous NaCl at 25°C. However, both viscosity and molecular weight decreased considerably below the values for the double-helical dimer as* t_d *increased. No substantial degradation of the sample was found to occur within* $t_d = 9$ *h. It was concluded from data analysis that at least 70% of the dimers dissociated into monomers when pure water solutions were heated at 95°C for 9 h.*

INTRODUCTION

Xanthan, an ionic polysaccharide produced by the bacterium *Xanthomonas campestris*, undergoes a thermally induced order–disorder conformation change in aqueous salt solution of low ionic strength (Holzwarth, 1976; Morris *et al.*, 1977; Rees, 1981). This conformation change usually detected by optical rotation is not always reversible (Holzwarth, 1976; Jamieson *et al.*, 1983; Hacche *et al.*, 1987; Liu *et al.*, 1987) and should involve a certain kinetic process. Previous studies (Milas & Rinaudo, 1979; Hacche *et al.*, 1987; Liu *et al.*, 1987)

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showed that it is not accompanied by any discernible change in weight-average molecular weight M_w up to 80°C at which virtually no residues in the ordered conformation state appear to remain. This finding could be taken for granted if the ordered conformation were a single helix as proposed earlier (Morris *et al.*, 1977). However, it is a double helix consisting of paired chains, as was demonstrated by our previous work (Sato *et al.*, 1984*a, b, c*) with extensively fractionated sodium xanthan samples (degree of pyruvation $DS_{pyr} \sim 1/3$) covering a wide range of M_w. Thus, the order–disorder change has to be considered a process of partial melting of the double helix at least for samples 'purified' by extensive fractionation.

In our recent paper, Liu and Norisuye (1988) showed that the temperature dependence of the statistical radius for Na xanthan samples with different M_w in 0·01 M aqueous NaCl is almost quantitatively explained by a model in which the double helix melts from both ends but is not allowed to separate into single chains. This strict restriction on the dimerized form was consistent with the above finding on M_w, but there remained a question of how to reconcile the dimer model with the notion that, statistically, some single separated chains ought to be present in a seemingly completely disordered state. Liu and Norisuye speculated that dissociation of the xanthan dimer should occur much later than the conformational change.

In the present work, we performed viscosity, optical rotation and sedimentation equilibrium experiments on Na xanthan ($DS_{pyr} \sim 1/3$) to check this hypothesis. Since the disordered conformation is more predominant at a higher temperature and a lower ionic strength, pure water at 95°C was chosen as the initial solvent, but measurements were made at 25°C after pure water solutions heated at 95°C for different time periods had been brought to 0·1 M NaCl solutions by the addition of aqueous NaCl.

Near the end of the present study, reference was made to a paper by Muller and Lecourtier (1988), who concluded from low-angle light-scattering measurements that the xanthan dimer completely dissociated into monomers when 0·01 or 0·1 M aqueous NaCl solutions of a highly pyruvated xanthan with $M_w = 4{\cdot}8 \times 10^6$ were kept at 110°C. However, this conclusion does not seem definite because the observed halving of M_w could be due to the sample's degradation at such a high temperature. In this connection, we note that Hatakenaka *et al.* (1987) showed Na xanthan with a viscosity-average molecular weight of $5{\cdot}8 \times 10^5$ degraded in aqueous NaCl at 80°C regardless of NaCl concentration (in the range 0·005–0·1 M) though they did not preclude the possibility of slow dimer separation.

EXPERIMENTAL

Sample and preparation of solutions

A very low-molecular-weight xanthan sample designated X13-3-4 was used for the present experiment as it was anticipated that this would show a smaller degree of degradation than that encountered by Hatakenaka *et al.* (1987). This sample was one of the middle fractions extracted previously (Liu *et al.*, 1987) by repeated fractional precipitation from an extensively sonicated, purified sample supplied by Kelco. It was converted to the Na salt form as described elsewhere (Sato *et al.*, 1984*a*). The viscosity-average molecular weights M_v of the Na salt sample in 0·01 M aqueous NaCl and tris(ethylene diamine) cadmium dihydroxide (cadoxen), a single-coil solvent for xanthan (Sato *et al.*, 1984*a*), at 25°C were $9{\cdot}3 \times 10^4$ and $4{\cdot}7 \times 10^4$, respectively. The ratio of the former to the latter is equal to about two, thus, indicating that our sample maintains the dimerized form in the aqueous salt at 25°C. The cadoxen solutions kept at about 15°C were stable at least within one day after preparation; when they were kept at 25°C for one day, a decrease in intrinsic viscosity $[\eta]$ was observed, but this was no more than 2%.

Aqueous solutions of the sample with different polymer mass concentrations c were exposed to 95°C ($\pm 0{\cdot}1$) for a given time period t_d after the containers had been sealed completely. They were cooled slowly to room temperature and mixed with 0·2 M aqueous NaCl under stirring to obtain a series of 0·1 M NaCl solutions. In the following presentation, the solutions thus prepared are referred to as heat-treated 0·1 M NaCl solutions of a given t_d value. Those prepared similarly but without exposure to 95°C are indicated by $t_d = 0$; the measured specific rotation suggested that Na xanthan in water at 25°C is only partially disordered and contains a considerable amount of double-helical residues, unlike the polymer in water at 95°C.

The addition of aqueous NaCl in the above procedure may cause disordered xanthan (for nonzero t_d) to form aggregates, but the extent of aggregation should become less as the concentration in the original water solution is lowered (Sato *et al.*, 1984*b*). To minimize aggregation effects on M_w and the viscosity at infinite dilution, we made no dilution of heat-treated 0·1 M NaCl solutions with the solvent (0·1 M NaCl).

Estimation of the Extent of Degradation

Since both degradation and dimer separation (if it occurs) lead to decreases in viscosity and M_w with t_d, it is crucial to avoid or minimize a sample's degradation for our purpose mentioned in the Introduction. We estimated the extent of degradation of sample X13-3-4 in pure water at 95°C according to the viscosity method of Hatakenaka *et al.* (1987) described below.

From heat-treated 0·1 M NaCl solutions for different t_d, xanthan was recovered by reprecipitation into a large quantity of 95% aqueous acetone followed by washing with 95% aqueous and anhydrous acetone two to three times. The recovered samples were dried *in vacuo* for four days. They were dissolved in cadoxen, and their intrinsic viscosities at 25°C were determined, using a conventional capillary viscometer of the Ubbelohde type. It should be noted that $[\eta]$ so determined is independent of whether the species of disordered xanthan in water at 95°C was a dimer or a monomer, thereby reflecting only chain degradation in water at 95°C.

The results obtained are shown in Fig. 1, where $[\eta]_0$ denotes the value of $[\eta]$ for the original sample X13-3-4 in cadoxen. The ratio $[\eta]/[\eta]_0$ stays at about unity up to $t_d \sim 9$ h and appears to decrease slightly for $t_d > 9$ h. Thus, we may conclude that no appreciable degradation of sample X13-3-4 takes place for t_d up to 9 h in pure water at 95°C.

Sedimentation equilibrium

Weight-average molecular weights of xanthan solutes in heat-treated 0·1 M NaCl solutions for $t_d = 2{\cdot}5$, 7, 9 and 15 h were determined at 25°C by sedimentation equilibrium in a Beckman Model E ultracentrifuge. A filled-Epon 30-mm double-sector cell was used. The liquid column was adjusted to 1·5–2·0 mm in most experiments, and the rotor speed was chosen in order to make the product of M_{app} and λ — see eqns (2) and (4) — about unity.

The data were analysed according to the equation (Fujita, 1975):

$$M_{\text{app}}^{-1} = M_w^{-1} + 2A_2\bar{c} + \ldots \qquad (1)$$

where

$$M_{\text{app}} = (c_b - c_a)/\lambda c_0 \qquad (2)$$

$$\bar{c} = (c_a + c_b)/2 \qquad (3)$$

with

$$\lambda = (\partial\rho/\partial c)_\mu (r_b^2 - r_a^2)\,\omega^2/2RT \qquad (4)$$

In these equations, A_2 is the sedimentation second virial coefficient, c_a and c_b are the equilibrium polymer mass concentrations at the liquid meniscus and the cell bottom, respectively, c_0 is the initial concentration, r_a and r_b are the radial distances from the center of rotation to the meniscus and the cell bottom, respectively, ω is the angular velocity of the rotor, ρ is the density of the solution, R is the gas constant and T is the absolute temperature; the subscript μ attached to $(\partial\rho/\partial c)$ signifies the condition that the chemical potentials of all diffusible components in the solution are held constant.

To see the effect of NaCl concentration on M_w, heat-treated 0·01 M NaCl solutions of sample X13-3-4 for $t_d = 7$ and 9 h were prepared and investigated by ultracentrifugation. Further, z-average molecular weights M_z were estimated for both 0·01 and 0·1 M NaCl solutions by use of the relation (Norisuye *et al.*, 1980):

$$Q = (M_w/M_z)(1 + 2A_2\bar{c} + \ldots) \tag{5}$$

where

$$Q = \frac{(c_b - c_a)^2}{c_0(r_b^2 - r_a^2)[c_b(\partial \ln c/\partial r^2)_{r=r_b} - c_a(\partial \ln c/\partial r^2)_{r=r_a}]} \tag{6}$$

Specific refractive index increments $(\partial n/\partial c)_\mu$ at the 546-nm wavelength and $(\partial\rho/\partial c)_\mu$ for heat-treated 0·01 and 0·1 M NaCl solutions dialysed at 25°C were determined by a modified Schulz–Cantow-type differential refractometer and a bicapillary pycnometer with a capacity of about 30 cm^3. Use was made of Na salt samples with $M_w = 80 \times 10^4$ and $M_v = 77 \times 10^4$ (in 0·01 M aqueous NaCl at 25°C). The dialysis was

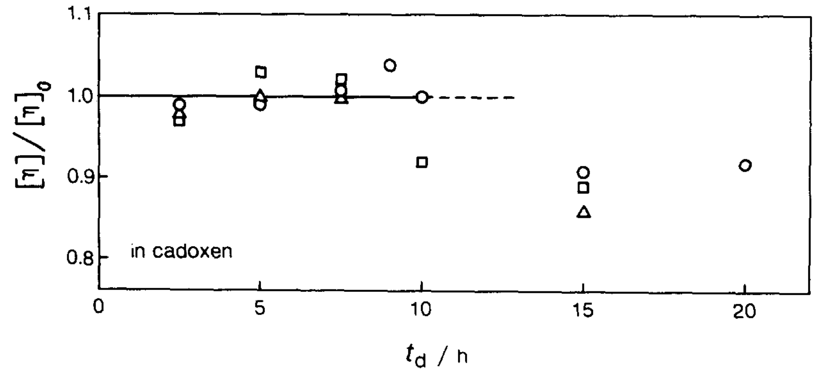

Fig. 1. Dependence of $[\eta]$ (in cadoxen at 25°C) on t_d for samples recovered from preheated water solutions (95°C) of Na xanthan sample X13-3-4. $[\eta]_0$, $[\eta]$ of the original sample in cadoxen at 25°C; different symbols, different c ($1{\cdot}2 \times 10^{-3}$–$6{\cdot}6 \times 10^{-3}$ g cm^{-3}) in water solutions.

effected using Visking gel-cellophane membranes and the dialyser described elsewhere (Sato *et al.*, 1983). The results of $(\partial n/\partial c)_\mu$ for heat-treated 0·1 M NaCl solutions at 25°C were 0·140 and 0·137 $cm^3\ g^{-1}$ for $t_d = 2{\cdot}5$ and 9 h, respectively, and those for 0·01 M NaCl solutions were 0·140 and 0·139 $cm^3\ g^{-1}$ for $t_d = 7$ and 9 h, respectively. The values for 0·1 M NaCl solutions of $t_d = 7$ and 15 h were estimated by interpolating or extrapolating the data for $t_d = 2{\cdot}5$ and 9 h. The measured $(\partial \rho/\partial c)_\mu$ values at 25°C were 0·405 $cm^3\ g^{-1}$ (±2%) irrespective of NaCl concentration and t_d.

Polarimetry

Sample X13-3-4 (about 2 g) was almost exhausted in viscosity and sedimentation equilibrium experiments, and another Na salt sample X12-2-3 with $M_w = 34 \times 10^4$ in 0·01 M aqueous NaCl at 25°C (Liu & Norisuye, 1988) was used for polarimetry. Specific rotations $[\alpha]_{300}$ at the 300-nm wavelength for its heat-treated 0·1 M NaCl solutions were determined at 25°C as a function of t_d on a JASCO ORD/UV-5 spectropolarimeter with 10-cm cylindrical quartz cells. Data were also taken for a pure water solution at 95°C, but their accuracy was not too high since bubble formation was inevitable near the boiling temperature. In all the measurements, c was adjusted below $3 \times 10^{-3}\ g\ cm^{-3}$.

RESULTS

Viscosity

Figure 2 illustrates changes in $(\ln \eta_r)/c$ with time that occurred at 25°C after water solutions of sample X13-3-4 preheated at 95°C for 9 h had been mixed with 0·2 M aqueous NaCl to 0·1 M. Here, η_r denotes the relative viscosity, and the time on the abscissa should not be confused with t_d. All the curves are almost horizontal; their slight declines suggest that aggregates are formed in excess upon addition of aqueous NaCl but these excess aggregates gradually dissociate. Similar time dependence of $(\ln \eta_r)/c$ was observed for solutions with different values of t_d.

The values of $(\ln \eta_r)/c$ at a fixed time of 4 h (chosen just for illustration) were read from the curves in Fig. 2 and similar ones obtained for other values of t_d. They are plotted against c in Fig. 3, in which the data obtained for sample X13-3-4 in 0·1 M aqueous NaCl and cadoxen at 25°C are also shown for comparison. The plots for $t_d = 2{\cdot}5$, 7 and 9 h have positive slopes, showing the presence of aggregates in heat-treated

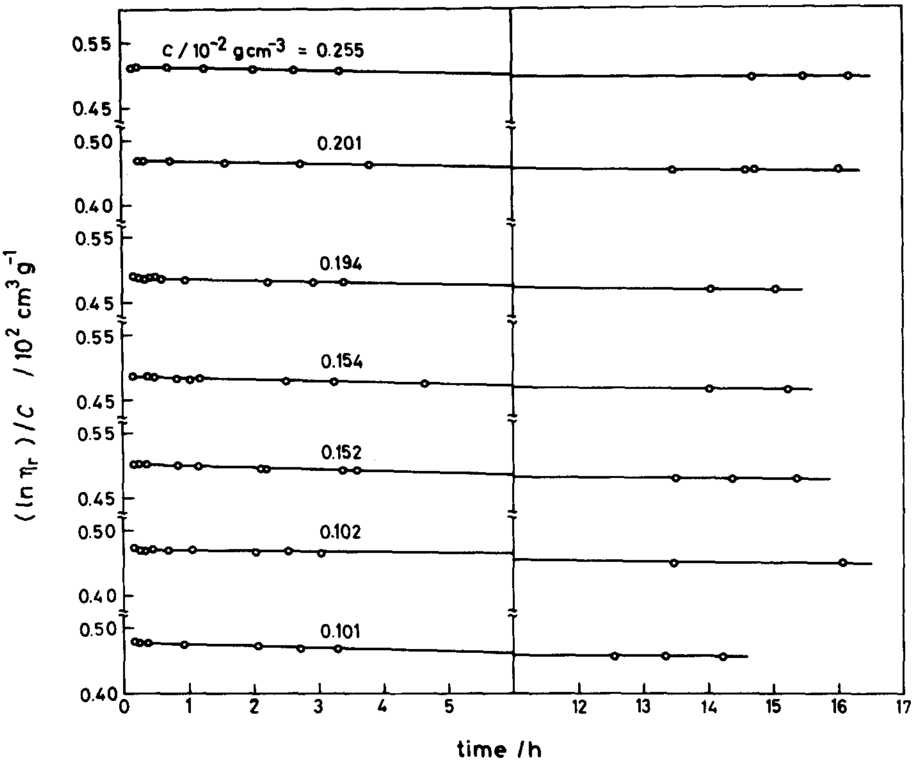

Fig. 2. Changes in viscosity at 25°C with time following dilution of 9-h preheated water solutions of Na xanthan sample X13-3-4 with 0·2 M aqueous NaCl to 0·1 M.

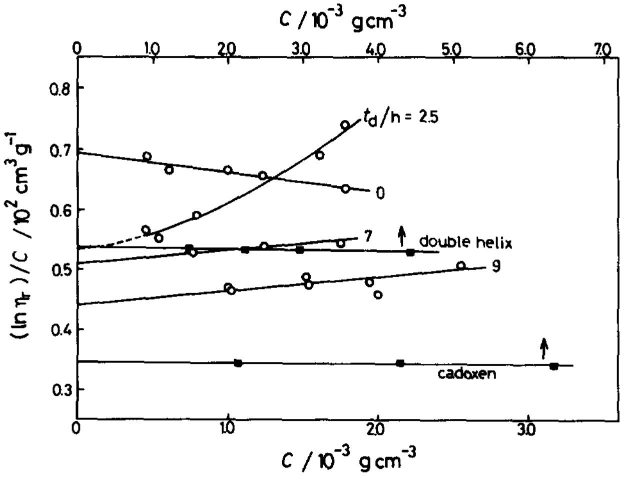

Fig. 3. Concentration dependence of viscosity for heat-treated 0·1 M NaCl solutions of Na xanthan sample X13-3-4 and that for the same sample in 0·1 M aqueous NaCl (double helix) and cadoxen, all at 25°C. Filled squares, upper *c* scale.

0·1 M NaCl solutions of finite concentration. The negative slope for $t_d = 0$ (i.e., for solutions without heat treatment) cannot be taken as indicating the absence of aggregates, since the $(\ln \eta_r)/c$ values for it are much larger than those for the rigid double helix in 0·1 M aqueous NaCl at any concentration. The implication of the negative slope is not self-evident, however. We tentatively interpret it as follows: At a lower concentration, the double-helical dimers in water at 25°C are broken to a larger extent because of a lower total ionic strength, and dimers with less double-helical residues (i.e., with more unpaired residues) are more liable to self-associate upon addition of NaCl. What is more significant in Fig. 3 is the finding that $(\ln \eta_r)/c$ at a fixed low concentration decreases as t_d increases. This viscosity decrease is not due to chain degradation (see Fig. 1).

Optical rotation behavior

The $[\alpha]_{300}$ versus t_d curves A and B in Fig. 4 refer to pure water solutions at 95°C and heat-treated 0·1 M NaCl solutions at 25°C, respectively. The former is much higher than the latter at any t_d examined. The value of $[\alpha]_{300}$ for A at $t_d = 0$ (in water at 25°C) was -60×10 deg cm^2 g^{-1}. The decrease from this value to that for B at $t_d = 0$ shows a salt-induced conformation change at the fixed temperature of 25°C, whereas that from A to B at a fixed positive t_d involves effects of both salt and temperature on conformation. At small t_d the curve B is close to the arrow actually representing $[\alpha]_{300}$ for the double helix in 0·1 M aqueous NaCl at 25°C. This closeness implies that Na xanthan disordered in water at 95°C for a short time period is renatured to a large extent with respect to the local conformation when aqueous NaCl is added to 0·1 M. We note that $[\alpha]_{300}$ for B did not appreciably vary with time at least within 1 day after the preparation of solutions (cf. Fig. 2).

The gradual rise of the curve B with t_d in Fig. 4 appears to show that the local conformation of Na xanthan in the solution depends on t_d, but this seems unlikely because the curve A also arises in parallel. According to Rinaudo *et al.* (1983), pyruvate groups attached to the terminal mannose residues in the trisaccharide side chains of xanthan are cleaved when aqueous xanthan is exposed to 90°C for 2 days. If the rise of the curve B is due not to a change in local conformation but to cleavage of pyruvate groups, heat-treated 0·1 M NaCl solutions of pyruvate-free xanthan should have $[\alpha]_{300}$ independent of t_d. To check this, we prepared a pyruvate-free xanthan from sample X12-2-3 by the method of Holzwarth and Ogeltree (1979) (about 90% of the groups are removed), and determined $[\alpha]_{300}$ for its heat-treated 0·1 M NaCl solutions.

The results represented by the line C in Fig. 4 show that no substantial change in $[\alpha]_{300}$ occurs with t_d. The curve B appears to approach this horizontal line at large t_d. Thus it seems reasonable to conclude that the local conformation of Na xanthan in heat-treated 0·1 M NaCl solutions does not change with t_d.*

Molecular weights

Figure 5 shows M_{app}^{-1} plotted against $\bar{c}$ for heat-treated 0·01 and 0·1 M NaCl solutions of sample X13-3-4 and for the double-helical dimer in 0·1 M aqueous NaCl at 25°C. All the plots for heat-treated 0·1 M NaCl solutions have small negative slopes, which confirm the finding from viscometry that, at finite concentration, aggregates are present in the solutions. On the other hand, the slopes of the plots for heat-treated 0·01 M NaCl solutions are positive, suggesting either diminished aggregate formation or enhanced electrostatic repulsions between charged groups in aggregates at the lower salt concentration, or both.

The values obtained for M_w are presented in Table 1, together with that of M_v for the monomer in cadoxen. It can be seen that for either 0·01 M or 0·1 M NaCl, M_w decreases with increasing t_d. This demonstrates that xanthan dimers in water at 95°C dissociate successively to monomers as the time elapses, since no appreciable degradation of our sample takes place within $t_d = 9$ h. It should be noted, however, that M_w decrease with the change in t_d from 9 to 15 h for 0·1 M NaCl solutions is attributable primarily to degradation (see Fig. 1). Another remark pertinent here is the effect of cleaved pyruvate groups on M_w. Even if all the groups in our sample were lost, M_w should only be lowered by 3%. This correction is even unnecessary, since the use of $(\partial\rho/\partial c)_\mu$ and $(\partial n/\partial c)_\mu$ for ultracentrifugal analysis (low-speed experiments) gives M_w of the original sample with pyruvate groups, provided that c refers to the mass of the original sample and the membrane used for dialysis is permeable to cleaved pyruvate groups.

Figure 6 shows the plots of Q versus $\bar{c}$ for the same solutions as those in Fig. 5. The values of M_z and M_z/M_w estimated are presented in the fourth and fifth columns of Table 1. The ratios M_z/M_w for heat-treated solutions are not very different from that for the dimer in 0·1 M aqueous NaCl. For $t_d = 15$ h (when partial degradation will occur), the ratio

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*The cleavage of pyruvate groups was found to contribute towards increasing the viscosity of Na xanthan in cadoxen at 25°C. However, its effect on $[\eta]$ was at most 2–3% for $t_d \lesssim 9$ h when estimated from the measured $[\eta]$ for the pyruvate-free xanthan sample in cadoxen and the optical rotation data (curves B and C in Fig. 4). Hence the conclusion of negligible degradation, drawn from the $[\eta]$ data in Fig. 1, does not alter.

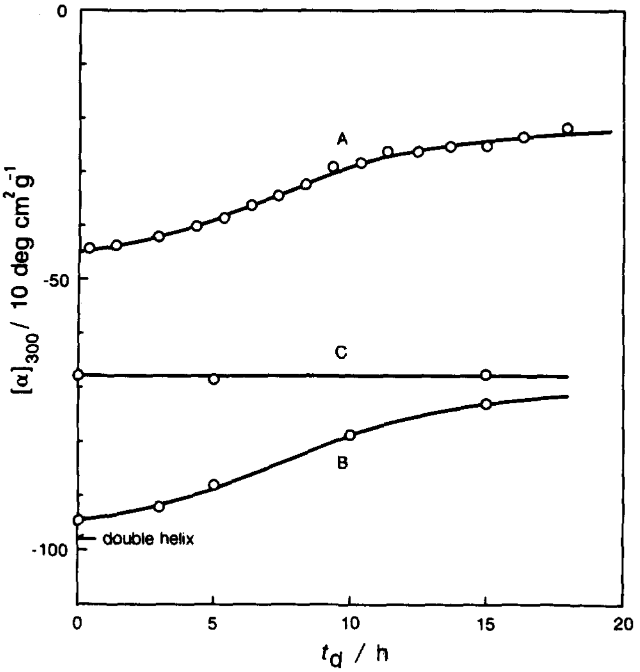

Fig. 4. Dependence of $[\alpha]_{300}$ on t_d for Na xanthan sample X12-2-3. A, water solutions at 95°C; B, heat-treated 0·1 M NaCl solutions at 25°C; C, heat-treated 0·1 M NaCl solutions of pyruvate-free xanthan at 25°C; arrow, value for the double helix in 0·1 M aqueous NaCl at 25°C.

appears to be even smaller than that for the dimer. Taken literally, this is indicative of more probable degradation of the longer chains.

DISCUSSION

The molecular weight data for heat-treated NaCl solutions in Table 1 show that an appreciable fraction of the dimers of our Na xanthan dissociate to monomers in water at 95°C. Since upon addition of NaCl to a preheated water solution of finite concentration, aggregates are formed from separated monomers and dimers remaining in the water solution, the resulting heat-treated NaCl solution should contain monomers, dimers, trimers and so on. However, if the water solution is very dilute, the formation of higher aggregates is less probable, and the values of M_w and M_z obtained by extrapolating M_{app}^{-1} and Q for a series of independently prepared solutions (see the Experimental section) to $\bar{c} = 0$ may not

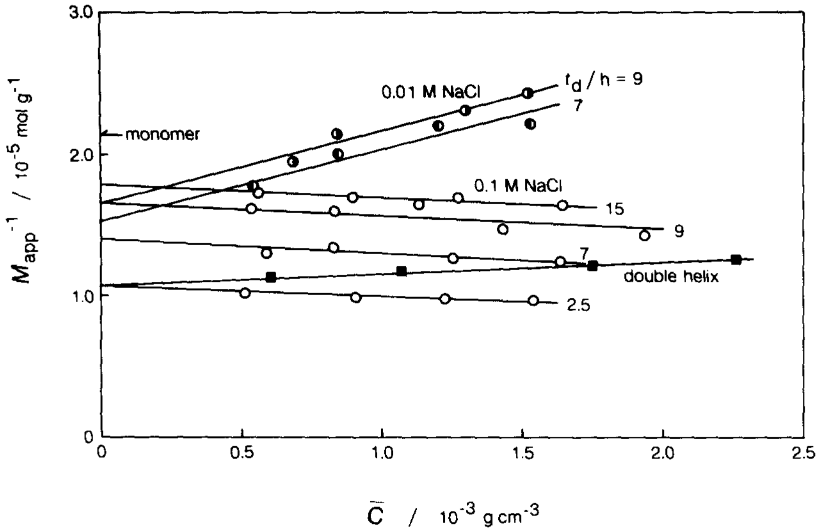

Fig. 5. Plots of M_{app}^{-1} versus $\bar{c}$ for heat-treated 0·01 (◑, ◐) and 0·1 (○) M NaCl solutions of Na xanthan sample X13-3-4 and for the double-helical dimer in 0·1 M aqueous NaCl (■), all at 25°C. The arrow points to the M_v value for the monomer in cadoxen.

TABLE 1
Results from Sedimentation Equilibrium Measurements on Heat-treated NaCl Solutions of Na Xanthan Sample X13-3-4 at 25°C

NaCl conc./M	*t_d/h*	*$M_w \times 10^{-4}$*	*$M_z \times 10^{-4}$*[a]	*M_z/M_w*[b]
0·10	(dimer)	9·4	11·0	1·20
0·10	2·5	9·4	12·0	1·25
0·10	7·0	7·1	9·2	1·30
0·01	7·0	6·5	8·5	1·30
0·10	9·0	6·0	7·8	1·30
0·01	9·0	6·0	7·8	1·30
0·10	15·0	5·5	6·3	1·15
cadoxen	(monomr)	4·7[c]	—	—

[a] Calculated from M_w and M_z/M_w.

[b] The graphically estimated accuracy is about ±0·1 for t_d = 2·5 h and ±0·05 for the others.

[c] Viscosity-average molecular weight.

contain large contributions from aggregates higher than dimers. In fact, the M_z/M_w values for heat-treated NaCl solutions in Table 1 are fairly close to that for the dimer.

On the basis of the above considerations, we assume that heat-treated NaCl solutions contain only three species — monomers (species 1), dimers (species 2) and trimers (species 3) — all heterogeneous in molecular weight, at infinite dilution. The measured M_w and M_z are then expressed in terms of the weight fraction f_i of i-mers ($i = 1, 2, 3$) as

$$M_w = (f_1 + 2f_2 + 3f_3)(M_{w2}/2) \tag{7}$$

$$M_z = [(f_1 + 4f_2 + 9f_3)/(f_1 + 2f_2 + 3f_3)](M_{z2}/2) \tag{8}$$

with

$$f_1 + f_2 + f_3 = 1 \tag{9}$$

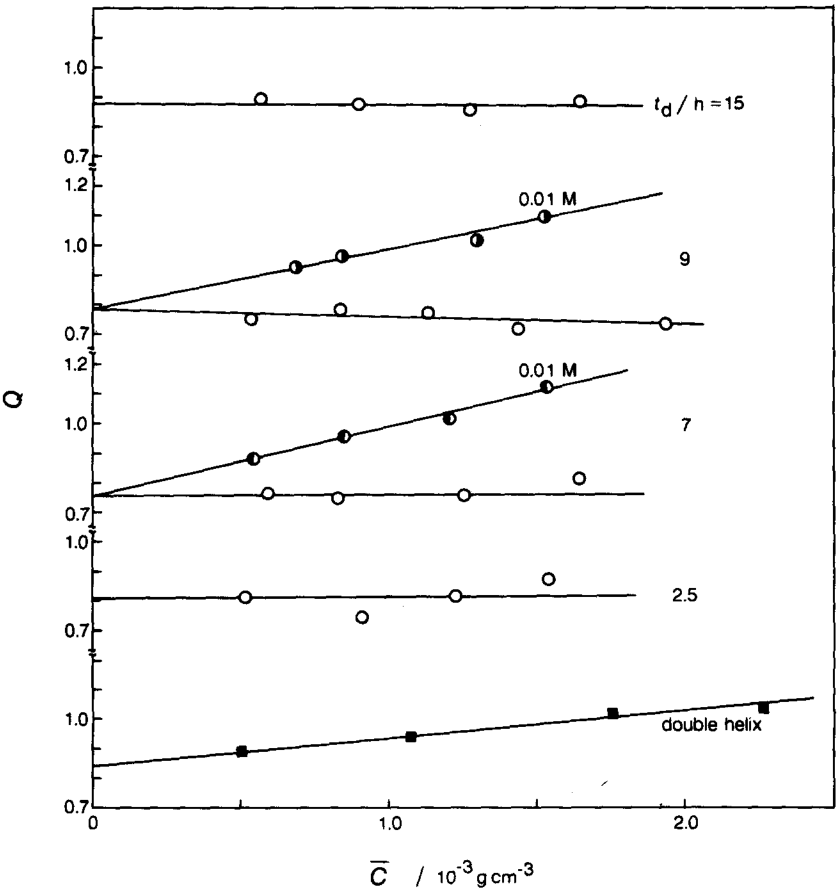

Fig. 6. Plots of Q versus $\bar{c}$ for the same solutions as in Fig. 6.

provided that $iM_{w1} = M_{wi}$ and $iM_{z1} = M_{zi}$. Here, M_{wi} and M_{zi} are the weight-average and z-average molecular weights of i-mers. Equations (7) and (8) combined with eqn (9) allow us to evaluate f_1, f_2 and f_3 in heat-treated NaCl solutions from the M_w and M_z data for them and the M_{w2} and M_{z2} values for the dimer in Table 1.

The results obtained are summarized in Table 2. It can be seen that monomers and dimers are predominant in the solutions of $t_d = 7$ and 9 h (at the limit of zero c) and that f_1 increases up to about 0·7 as t_d increases. The f_i values for $t_d = 2{\cdot}5$ h are not accurate, but may be taken to indicate the presence of large aggregates in the solution; the above assumption that all the large aggregates are trimers may not be adequate for this solution. The agreements of both $(\ln \eta_r)/c$ at $c = 0$ and M_w for $t_d = 2{\cdot}5$ h with those for the double-helical dimer, observed in Fig. 3 and Table 1, are most likely to be due to the compensation of the two opposite contributions (relative to the dimers) from the monomers and the higher aggregates present. Though the f_3 values at any t_d in Table 2 are not accurate, it would be reasonable to consider that the formation of high aggregates is continuously diminished as the monomer fraction in water increases.

The specific rotations for heat-treated 0·1 M NaCl solutions (the curve B) in Fig. 4 may be considered to be independent of t_d and hence of f_1 if the effect of cleaved pyruvate groups is taken into account. Since at small t_d they are close to the value for the double helix, it follows that regardless of t_d, both monomers and dimers in water at 95°C become ordered to a conformation locally similar to that of each chain in the double helix when aqueous NaCl is added to 0·1 M; the same should be true for the higher aggregates formed. This salt-induced ordering may be explained as due to the alignment of trisaccharide side chains with the main chain through hydrogen bonding (Morris *et al.*, 1977; Kitagawa *et al.*, 1985). Though locally ordered, the monomers are much more flexible than the

TABLE 2

Monomer, Dimer and Trimer Fractions in Heat-treated NaCl Solutions of Na Xanthan Sample X13-3-4 at 25°C

t_d/h	*NaCl conc./M*	f_1	f_2	f_3
2·5	0·10	0·20 (±0·20)	0·60 (±0·40)	0·20 (±0·2)
7·0	0·01	0·65 (±0·05)	0·35 (±0·05)	≤0·10
7·0	0·10	0·55 (±0·05)	0·45 (±0·05)	<0·10
9·0	0·01	0·75 (±0·05)	0·25 (±0·05)	<0·05
9·0	0·10	0·75 (±0·05)	0·25 (±0·05)	<0·05

intact double helix and have smaller average dimensions than do the locally ordered dimers and higher aggregates. Thus $(\ln \eta_r)/c$ for heat-treated 0·1 M NaCl solutions at $c = 0$ should decrease with an increase in f_1 or t_d. This conforms with what we have observed in Fig. 3.

CONCLUSIONS

The present study has demonstrated that double-helical dimers of Na xanthan dissociate to monomers in aqueous solution at 95°C. This substantiates the conjecture of Liu and Norisuye (1988) mentioned in the Introduction and lends support to their dimer model for the thermally induced order–disorder conformation change of the polysaccharide.

The monomer fractions given in Table 2 appear to indicate that the dissociation in water at 95°C proceeds very slowly. An example of slow dissociation is known for the triple helix of schizophyllan (Sato *et al.*, 1981). However, our approach, which is not directly concerned with aqueous xanthan at 95°C, allows no quantitative discussion on the rate of dimer dissociation. In heat-treated NaCl solutions at 25°C, dimers are reformed as aggregates from separated monomers. Further, dimer reformation may be specifically favorable in the presence of added NaCl (Stokke *et al.*, 1989), though the viscosity data in Fig. 3 do not show intact double helices to be reformed. Thus, under our experimental conditions, the dissociation of dimers is offset by their reformation to a considerable extent depending on t_d and, eventually, no further conversion from dimers to monomers may become experimentally observable, as the molecular weight data for $t_d = 15$ h in Table 1 suggest. In any event, the estimated monomer fractions, which are concerned with the monomers that remain in aqueous NaCl without dimerization or aggregation, should be considerably smaller than the actual values in water at 95°C. We therefore conclude that at least 70% of the original helical dimers dissociate into monomers when water solutions of our Na xanthan sample are heated at 95°C for 9 h.

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