Kinetic Evidence for Intramolecular Conformational Ordering of the Extracellular Polysaccharide (Xanthan) from *Xanthomonas campestris*

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Summary The salt (K^+) induced disorder–order transition of xanthan has been monitored by stopped-flow polarimetry, and shows first-order kinetics, indicating that the conformational ordering is intra- rather than intermolecular.

THE extracellular bacterial polysaccharide (xanthan) from the plant pathogen *Xanthomonas compestris* shows[1–4] a thermally induced order–disorder transition in aqueous solution, which occurs at higher temperatures with increasing ionic strength, and may be characterised by the temperature course of optical rotation. It has been suggested[2] that the ordered conformation has a biological role in host–pathogen recognition, and it is also believed to be important for the technologically valuable solution properties of xanthan.[3,5] The nature of the ordered structure, however, remains uncertain since *X*-ray diffraction studies on oriented fibres have not yet given a firm conclusion.[6] Two alternative models can be proposed,[6] each of which has found some support from other evidence: a single stranded intramolecular structure[2,3] in which the sidechains align and pack along the cellulose backbone (see Figure 1); and a double helical conformation.[7,8] In the present work we have measured the dynamics of the disorder–order transition by a polarimetric stopped-flow technique,[10] using a rapid

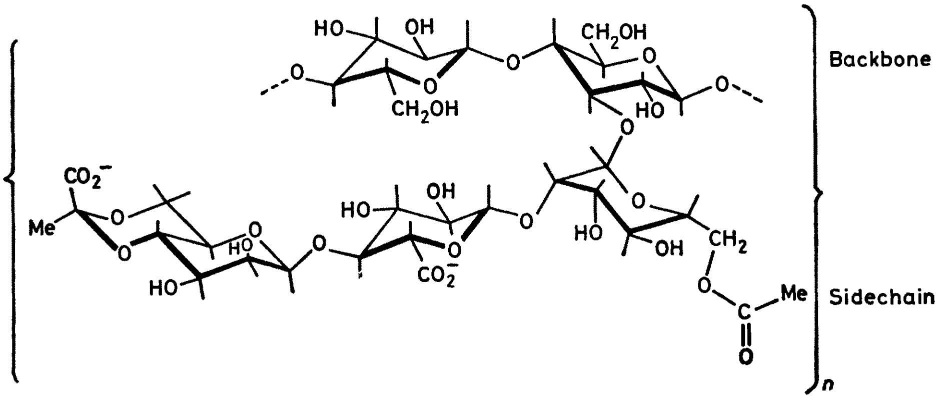

FIGURE 1. Pentasaccharide repeating structure of xanthan.[9] The sample used in this work was a commercial material (Keltrol) from Kelco, identical to that in previous studies.[1–3] Terminal pyruvate acetal substituents are present on *ca.* 60% of the trisaccharide side-chains, as judged from high resolution ^{1}H n.m.r. spectroscopy.[2] Solutions were prepared by autoclaving (10 min; 125 °C) and filtering (0·45 μ; Millipore).

increase in ionic strength (salt jump) to induce the transition to the ordered conformation.

Solutions of xanthan in deionised water and solutions of KCl were simultaneously discharged from separate syringes through a high speed mixer, to give typical final concentrations in the flow-through polarimeter cell of 0·25% *w/v* xanthan and 0·5 M KCl. On cessation of flow, the rate of optical rotation change from the equilibrium 'salt-free' value to the final value for the ordered conformation (see Figure 2) was measured and analysed in terms of the reactions (1) and (2), using the first-order and second-order

$$\text{coil} \xrightarrow{k_1} \text{helix} \qquad (1)$$

$$2\,\text{coil} \xrightarrow{k_2} \text{double helix} \qquad (2)$$

rate equations (3) and (4) respectively. In both equations

$$\ln[a_0/(a_0 - x)] = k_1 t \qquad (3)$$

$$1/(a_0 - x) - 1/a_0 = k_2 t \qquad (4)$$

a_0 is the total pentasaccharide residue concentration (based on a mean repeat unit molecular weight of 1000) and x is the residue concentration in the helix form at time t. At all temperatures our results are in good agreement with the first-order reaction scheme (equation 3), as illustrated in Figure 3, while data plotted according to equation (4) give a curve rather than the linear relationship expected if second-order kinetics were obeyed.

When the xanthan solution is completely disordered before the addition of salt, the first-order rate constant (k_1) is essentially constant at *ca.* (0·26 ± 0·04) s^{-1} and independent of xanthan concentration and temperature, which is consistent with an intramolecular process. At lower temperatures, where initially the solution is partially ordered, k_1 increases sharply (Figure 2), reaching a final steady value of *ca.* (9·6 ± 0·5) s^{-1} at and below the transition midpoint (T_m). Our interpretation of these results is that the rate-limiting process in conformational ordering of disordered chains is helix nucleation, while for chains which are already partially ordered the rate of further ordering is determined by the (more rapid) propagation process.

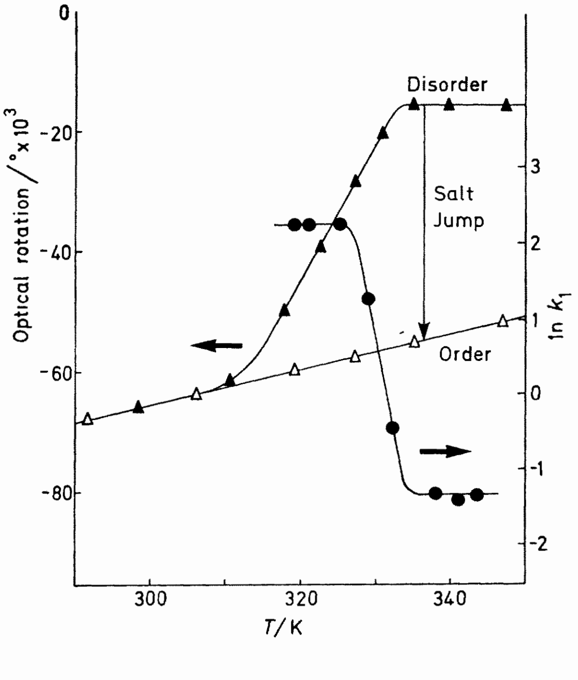

FIGURE 2. Equilibrium optical rotation of xanthan (0·5% w/v) in distilled water (▲) and in 0·5 M KCl (△), measured at 365 nm on a Perkin-Elmer 141 MC polarimeter (10 cm pathlength). The temperature dependence of the first-order rate constant, k_1 (●), for the salt-induced transition from total or partial disorder (▲) to the ordered conformation (△) is as shown. The vertical arrow at 338·2 K indicates the particular transition illustrated in Figure 3.

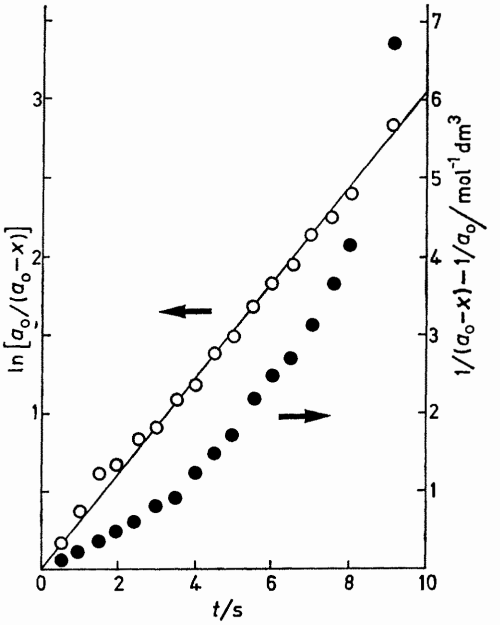

FIGURE 3. Dynamics of the xanthan disorder–order transition (338·2 K; 0·5% w/v xanthan; 0·5 M KCl) analysed in terms of first-order (○) and second-order (●) kinetics.

Under no conditions have we seen any evidence of second order kinetics, such as those observed for DNA[11] and for carrageenan,[12] where chain pairing has been demonstrated.[13] A likely model would therefore be a single stranded helix stabilised by interactions between side-chains and backbone,[6] although from the present evidence alone we cannot exclude the possibility of an antiparallel, two-stranded intramolecular structure formed by chain folding. The proposed model does not, of course, preclude further association into larger molecular assemblies, as has been suggested from hydrodynamic[8] and electron microscopy[7] studies. In particular, a side-by-side dimerisation such as occurs in the sol–gel transitions of alginate[14] and pectin[15] could account for the reported value of mass per unit length in solution,[8] which is approximately twice that calculated for isolated xanthan chains.

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