

Kinetic Evidence Consistent with Double Helix Formation in κ -Carrageenan Solutions

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Summary The dynamics of the salt (K^+) induced disorder-order transition of κ -carrageenan have been studied by a polarimetric stopped-flow technique; formation of the

ordered state is a second-order process, which supports the proposal that the aggregates of ordered polysaccharide chains contain double helical entities.

κ -CARRAGEENAN is an abundant polysaccharide in certain genera of marine red algae (*Rhodophyceae*), and is based¹ on a disaccharide repeating unit (see Figure 1). X-Ray

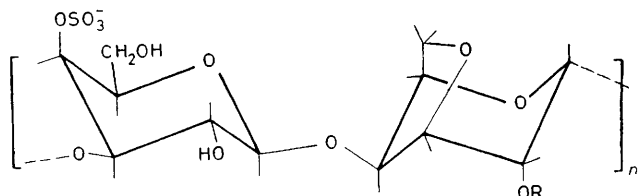


FIGURE 1. Idealised carrageenan disaccharide repeating structure. κ : R = H; ι : R = SO₃⁻.

fibre diffraction studies of the closely related, but more highly sulphated ι -carrageenan led to the derivation of a double helix conformation,² which was confirmed by linked-atom refinement against the measured diffraction intensities.³ Diffraction photographs² for κ -carrageenan, however, were of much poorer quality, and do not conclusively rule out alternative models such as nested single helices.⁴ Under conditions of extensive hydration, both ι -⁵ and κ -⁶ carrageenan undergo thermally induced order-disorder transitions (which may, under appropriate ionic conditions, be accompanied by a gel-sol transition), and are conveniently monitored by optical rotation. Many measurements on the ι -system, including molecular weight changes,⁷ dimension changes,⁸ heat changes,⁹ the sign and magnitude of optical rotation shift,¹⁰ and more recently

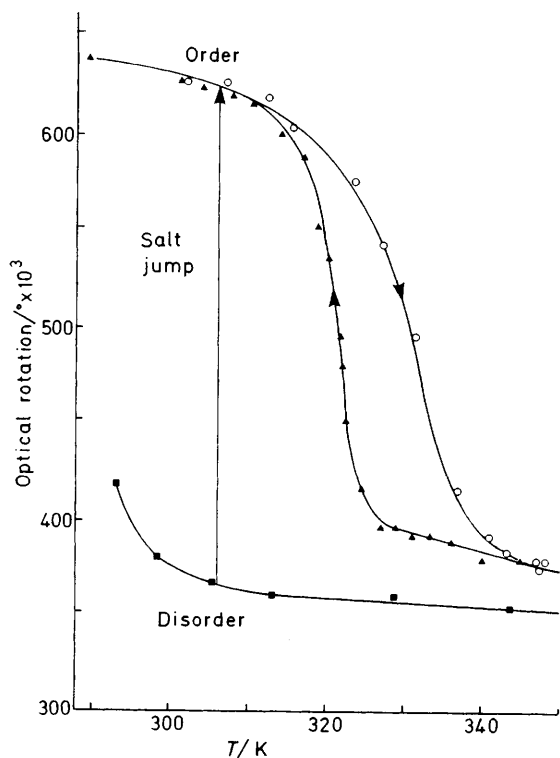


FIGURE 2. Temperature dependence of κ -carrageenan optical rotation (pathlength = 1 dm; carrageenan concentration = 0.05% w/w), without added salt (■), and in the presence of 0.1 M KCl; heating curve (▲); cooling curve (○).

the kinetics,¹¹ have given evidence to support a two-state all-or-none coil to double helix mechanism.⁹ Analogous studies of κ -carrageenan, however, are complicated by extensive helix-helix aggregation¹² so that, for example, it is meaningless to interpret changes in particle weight or dimensions in terms of conformational entities. We now show that the kinetics of conformational ordering can be separated from the aggregation step, and that the results are consistent with the double helix proposal.

The dynamics of the salt (K⁺) induced conformational transition of κ -carrageenan were studied at 308 K, using a stopped-flow polarimetric technique as described previously.^{11,13} κ -Carrageenan was a commercial sample (GS 350) from Pierrefitte-Auby. Concentrations in the stopped-flow cell were: carrageenan, 0.10%, w/w (2.4×10^{-3} mol dm⁻³ disaccharide residue concentration); KCl, 0.1 mol dm⁻³. Equilibrium optical rotation behaviour of the κ -carrageenan solution used in the kinetic studies is shown in Figure 2, in the presence and absence of KCl. The observed hysteresis between the true thermodynamic equilibrium curve obtained on heating and the metastable values observed on cooling⁸ has previously been ascribed^{8,14} to stabilisation of the ordered conformation by aggregation. The salt jump was made at a temperature well removed from the hysteresis region, and the expected amplitude of optical rotation change (Figure 2) was in good agreement with that found kinetically.

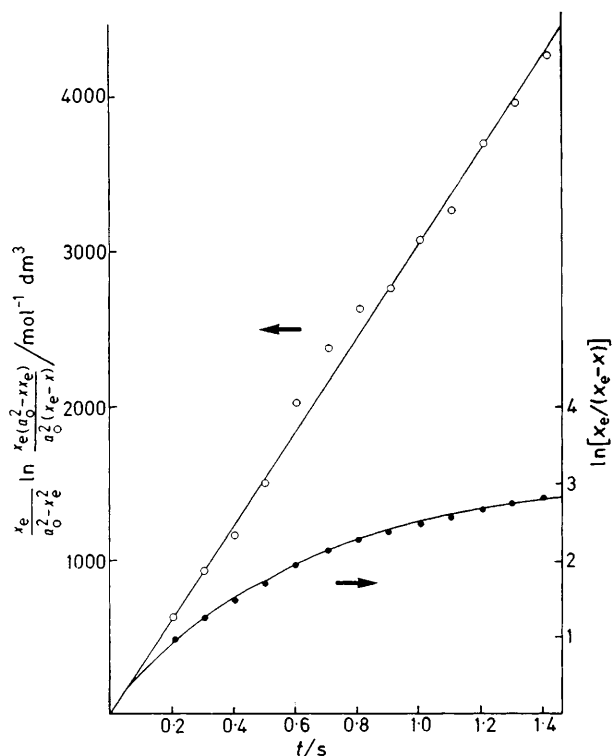


FIGURE 3. Comparison of first-order (●) and second-order (○) analyses for the kinetics of κ -carrageenan disorder-order behaviour (308 K; 0.10% w/w carrageenan; 0.1 mol dm⁻³ KCl).

In Figure 3 we show analysis of our kinetic data (averaged over 5 runs), in terms of two reaction schemes:

coil $\xrightleftharpoons[k_b]{k_f}$ single helix, or 2 coil $\xrightleftharpoons[k_b]{k_f}$ double helix, using, respectively, the rate equations (1) and (2).¹⁵ a_0 is the total

$$\ln[x_e/(x_e - x)] = (k_f + k_b) t \quad (1)$$

$$\frac{x_e}{a_0^2 - x_e^2} \ln \frac{x_e(a_0^2 - xx_e)}{a_0^2(x_e - x)} = k_f t \quad (2)$$

disaccharide residue concentration, and x and x_e are disaccharide residue concentrations in the helix form at time t and at equilibrium, respectively. Data plotted according

to the first order treatment (1) show marked departure from linearity, but our results are in good agreement with kinetic scheme (2), giving a second-order forward rate constant $k_f = (3.03 \pm 0.08) \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. We therefore conclude that the disorder-order transition of κ -carrageenan follows second order kinetics, indicating that the primary process on the path to aggregates of conformationally ordered polysaccharide chains is a dimerisation step, for which a reasonable model would be double helix formation.

We thank the S.R.C. for a research studentship (C.A.S.E. award to I.T.N.).

(Received, 17th July 1979; Com. 762.)

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