Dynamics of the Salt-induced Random Coil to Helix Transition in Segmented ι-Carrageenan

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Summary The coil to double helix transition of segmented ι-carrageenan was induced by 0·1 mol dm^{-3} NaCl and observed by a polarimetric stopped-flow technique; activation parameters for helix nucleation are strikingly different from those observed in polynucleotide double helix formation.

EXTENSIVE investigations of the transitions from coil to double helix forms of poly- and oligo-nucleotides have been carried out, using both equilibrium and dynamic techniques.[1,2] In this communication we report the first example of a study of the dynamics of the coil to helix transition of a polysaccharide.

ι-Carrageenan is an alternating polysaccharide which is transformed from coil to double helix on reducing the temperature or increasing the salt concentration. Structurally regular segments (Figure 1) were prepared from the native material, using Smith degradation followed by treatment with alkaline borohydride.[3]

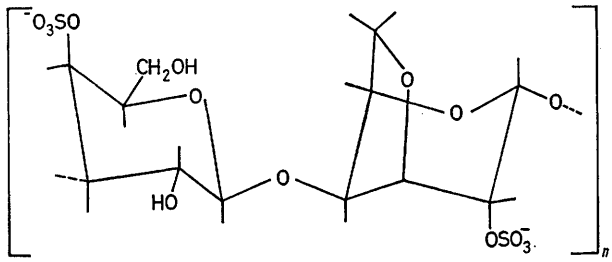

FIGURE 1. Segmented ι-carrageenan. *n ca.* 100 for our sample.

We have studied the dynamics of the salt-induced transition of segmented ι-carrageenan, using a stopped-flow polarimeter.[4] Out-gassed and filtered aqueous solutions of the polymer, with disaccharide residue concentration *ca.* 10^{-2} mol dm^{-3} assayed by polarimetry, were mixed with equal volumes of 0·2 mol dm^{-3} NaCl. In all cases, transition amplitudes were found to be in excellent agreement with values calculated from reactant and product rotations.

Between 5 and 10 runs were analysed at each of 8 temperatures in the range 291—305 K, using the rate equation (1)[5] for the kinetic scheme, 2 coil $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ helix, where a_0 is the total

$$\frac{x_e}{a_0^2 - x_e^2} \ln \frac{x_e (a_0^2 - x x_e)}{a_0^2 (x_e - x)} = k_1 t \qquad (\ )$$

disaccharide residue concentration, and x and x_e are disaccharide residue concentrations in the helix form at time t and at equilibrium, respectively. Values of x_e/a_0 were found from analysis of optical rotation curves as previously described.[6]

The fit with equation (1) accords with the conclusion from equilibrium studies that the ordering process is due to a two state, all-or-none transition from the single coil to the double helix.[6,7]

The generalized model of Crothers, Davidson, and Kallenbach for such a transition[8] requires the use of equation (2) to find the activation enthalpy for helix nucleation, ΔH^*.

$$\mathrm{d} \ln[k_1/(1-K)]/\mathrm{d}T = \Delta H^*/RT^2 \qquad (2)$$

K is the equilibrium constant for the helix-to-coil transition of an elementary unit of the chain. It was calculated using equation (3),[8] where ΔH_f was determined calori-

$$K^{-1} = \exp[-\Delta H_f (T_m - T)/RTT_m] \qquad (3)$$

metrically[6] per pair of disaccharide residues. The integrated form of equation (2) was used to plot our data (see Figure 2). Rate constants and activation parameters for segmented ι-carrageenan and poly A+poly U[9] of similar $\overline{M}_w$ are summarised in the Table.

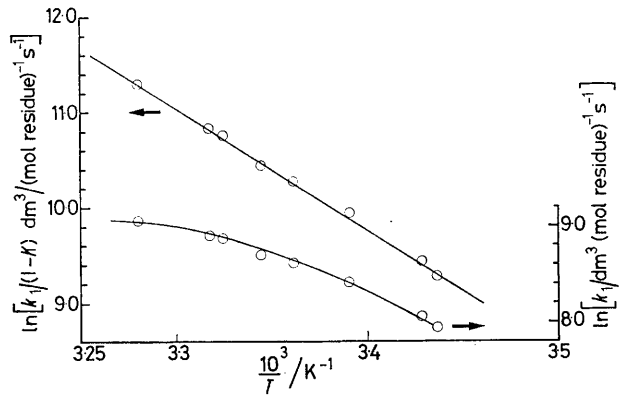

FIGURE 2. Activation energy plots for second order rate constants for helix formation, k_1, and helix nucleation $k_1/(1 - K)$.

TABLE. Kinetic parameters for transitions from coil to double helix at 298 K in 0·1 mol dm^{-3} NaCl for segmented ι-carrageenan and poly A + poly U

	Segmented ι-carrageenan	poly A + poly U
k_1/dm^3 (mol residue)$^{-1}$ s^{-1}	$5{\cdot}6 \times 10^3$	$9{\cdot}8 \times 10^4$
$[k_1/(1 - K)]$/dm^3 (mol residue)$^{-1}$ s^{-1}	$3{\cdot}0 \times 10^4$	$1{\cdot}5 \times 10^5$
T_m/K	314	331
ΔH_f/kJ (mol residue pair)$^{-1}$	10[a]	25
ΔH^*/kJ mol^{-1}	105 ± 2	27
ΔS^*/J (mol residue)$^{-1}$ K^{-1}	184 ± 5	−66

[a] See ref.6.

The most striking contrast between the kinetics of polysaccharide and polynucleotide double-helix nucleation lies in the activation parameters; typically $\Delta H^* < 30$ kJ mol^{-1} and $\Delta S^* < -50$ J K^{-1} (mol residue)$^{-1}$ for oligonucleotides[2] and polynucleotides,[8–10] while for segmented ι-carrageenan, $\Delta H^* = 105$ kJ mol^{-1} and $\Delta S^* = 184$ J K^{-1} (mol residue)$^{-1}$. This implies that there are substantial differences between the molecular mechanisms for nucleation in the two classes of biopolymers. In particular, the change of sign of ΔS^* may indicate the greater importance of solvation changes of site-bound ions and/or the chain itself in the polysaccharide case.

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