# CONFORMATIONAL TRANSITION OF KAPPA-CARRAGEENAN

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CENTRE DE RECHERCHE SUR LES MACROMOLECULES VEGETALES
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The calorimetry of kappa carrageenan using differential scanning calorimetry and calorimetry at constant temperature is presented. Both sets of experimental results confirmed the formation of a helical dimer in the absence of gel formation. When gel is formed, the formation of a helical dimer is the major effect, the contribution of gelation represents only 20% of the enthalpy recorded.

Many polysaccharides are used in foods, pharmaceuticals and industrial materials for the gels or viscous solutions they form [1]. These physical properties are often governed by the ordered conformation of these polysaccharides. Kappa-carrageenan, a galactan sulfate with alternating $\alpha(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linkages, is one such polymer and has been extensively studied by Rees and coworkers [2–3]. This polysaccharide adopts an ordered conformation when the temperature T of the solution is lowered or when the ionic concentration C_T of the solution is increased:

$$T \searrow \quad C_T \nearrow$$
$$\text{coil} \rightleftharpoons \text{ordered conformation}$$
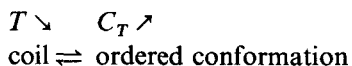
This ordered conformation has been described as a single helix [4], a double helix [5], a helical dimer [6–7], or a mixture of single helices and helical dimers [8].

Experimental

A Calvet calorimeter (calorimètre basse température, Setaram, Lyon, France) was used with standard cells (12 cm³) for scanning calorimetry. At constant temperature (25–30°), gold-plated brass reference and sample cells were used. These were composed of two reservoirs: an upper reservoir (3 cm³) containing the salt solution (1.5 cm³), and a lower reservoir (8 cm³) containing the polymer solution (5 cm³). The upper reservoir was connected by a small, air-filled tube (inside

diameter 1 mm) to a calibrated peristaltic pump (Gilson). The salt solution was aspirated in the upper reservoir before each experiment and, after temperature equilibration in the cells (1–2 h), the salt solution was pushed by the pump to the lower reservoir. Continuous stirring was applied in the lower reservoir by the vertical motion of a disk in inox. The frequency of the motion was 5–20 seconds.

Results and discussion

The dependence on the temperature and the ionic concentration of the solution has been well documented for the conformational transition of kappa-carrageenan [3, 6–7]. The two molecular states random coil and helical conformation occur in separate domains in a diagram plotting the inverse of temperature (T_M^{-1}) vs. the log of ionic concentration (log C_T) of the solution. According to this diagram [6, 7], it is possible to work under two different conditions in calorimetry. In isoionic calorimetry, the ionic concentration of the solution remains constant while the temperature is varied to induce the conformational transition of the polymer. In contrast, in isothermal calorimetry the transition is induced by varying the ionic concentration of the solution at a constant temperature.

For a polyelectrolyte which undergoes a conformational transition, the theory of Manning [9–10] predicts a linear relationship between the ionic concentration C_T and the temperature T_m^{-1} at the mid point of the transition, the charge parameters λ_{coil} and $\lambda_{helical}$ of both states being considered. According to the different charge parameters, the following equations arise:

if $\quad\quad\quad \lambda_c, \lambda_h < 1, \Delta H = -\dfrac{R}{2}(\lambda_h - \lambda_c)\,\mathrm{d}\ln C_T/\mathrm{d}\,(1/T_m)$ \hfill (1)

if $\quad\quad\quad \lambda_c < 1 < \lambda_h, \Delta H = -\dfrac{R}{2}(2 - \lambda_c - \lambda_n^{-1})\,\mathrm{d}\ln C_T/\mathrm{d}\,(1/T_m)$ \hfill (2)

if $\quad\quad\quad 1 < \lambda_c < \lambda_h, \Delta H = -\dfrac{R}{2}(\lambda_c^{-1} - \lambda_n^{-1})\,\mathrm{d}\ln C_T/\mathrm{d}\,(1/T_m)$ \hfill (3)

λ is defined [9–10] as $\lambda = e^2/K\,TD\,b$, where e is the elementary charge, D the dielectric constant, K the Boltzman constant, T the absolute temperature and b the distance between two adjacent charged groups.

Taking the b value used in the literature [6] and the experimental slope [11] $\mathrm{d}\ln C_T/\mathrm{d}(1/T_m)$, we can predict two values for ΔH: 24 and 5 kJ/mol of disaccharide for the transition from a helical dimer or a single helix, respectively, to a coil.

1) Isoionic calorimetry

This technique is based on the variation of the temperature of the solution; under such conditions the sensitivity of the calorimeter limits the experiments to high concentrations of polymer (Table 1). Nevertheless, the comparison of these values shows that the energy recorded arises mainly from the conformational change of the polymer and to a minor extent from the intermolecular crosslinking process. In fact the energy observed is increased by about 4 kJ in the presence of gelation. Such values for high concentrations of polymer cannot be compared, as done in the literature [8], with those derived from Manning's theory, that are calculated for very low concentrations. Fortunately, the theory [9] correlates the activity coefficients [7] of the counterions with the charge parameters:

$$\ln \gamma = -\frac{1}{2}\lambda \qquad \lambda < 1 \tag{4}$$

$$\ln \gamma = -0.5 - \ln \lambda \quad \lambda > 1 \tag{5}$$

For a helical dimer, using Eq. (2) we can write

$$\Delta H = -\frac{R}{2}(2 + 2\ln \gamma_c - e^{\ln \gamma_h + 0.5})\, d \ln C_T/(1/T_m) \tag{6}$$

γ_c and γ_n being the activity coefficients of the polymer in the coil state and in the helical conformation, respectively.

From Eq. (6) it is possible to predict a ΔH from the activity coefficient of the polymer in two different states: coil or ordered conformation. However, for high concentrations of polymer the activity coefficients (Fig. 1) and enthalpies disagree
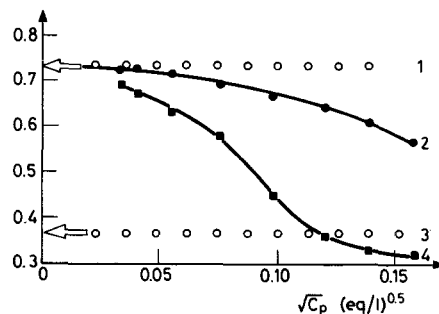

Fig. 1 Concentration dependence of activity coefficient of kappa-carrageenan at 15°C. 1. Theoretical activity coefficient of the coil; 2. apparent activity coefficient of the coil; 3. theoretical activity coefficient of the helical dimer; 4. apparent activity coefficient of the helical form

Table 1 Calorimetric data, for kappa-carrageenan potassium form in presence or in absence of potassium nitrate, recorded at 4 deg/h

Polymer concentration, $g \cdot L^{-1}$	Salt concentration eq. L^{-1}	Physical states	$-\Delta H$, kJ/mol dissacharide
4	0	solution	12.1
8	0	solution	12.8
6.8	0.033	gel	15.0
13.3	0.067	gel	15.5
20	0.100	gel	16.9

with the theoretical values for the ΔH calculated according to the difference of the counterion activities between the helical and coil states of the polymer. It is therefore wiser to use the apparent activity coefficient (γ_{ap}) determined experimentally in order to predict an apparent enthalpy ΔH_{ap}:

$$\Delta H_{ap} = -\frac{R}{2}(2 + 2 \ln \gamma_{ap,c} - e^{\ln \gamma_{ap,h} + 0.5}) \, d \ln C_T / d(1/T_m) \qquad (7)$$

The direct use of Eqs (6) or (2) predicts ΔH for very dilute solutions and it is impossible to obtain experimental values for such concentrations by means of scanning calorimetry. However, through Eq. (7) we can calculate from the experimental activity coefficient γ_{ap} observed for high concentrations of polymer an approximation of the ΔH_{ap} for the same range of polymer concentrations. This approach permitted the calculation of ΔH_{ap} values of 13.0 and 12.0 kJ at polymer concentration of 4 and 8 $g \cdot 1^{-1}$, respectively, which are in agreement with those obtained experimentally (Table 1). This method allows a closer evaluation of the values derived from Manning's theory and those obtained experimentally. However, it is evident that work at very low concentrations permits a better use of the theoretical Eqs (1), (2), or (3).

2) Isothermal calorimetry

The experimental conditions which use a fixed temperature increase the sensitivity of the calorimeter. These permit the study of dilute solutions. With this technique we obtained results (Table 2) very close to the theoretical value of 24 kJ predicted for a helical dimer. Two ranges of concentrations can be considered. In the first case, for concentrations from 1 to 0.17 g/l, the ΔH value increases up to the theoretical value. This increase is probably a result of the dependence of the activity

coefficient on the concentration [11]. In the second case, for concentrations from 1 to 5 g/l, the ΔH value also increases, but this effect may be attributed to the enthalpy generated by the gelation, as previously observed (Table 1). At these latter

Table 2 Enthalpy change of kappa-carrageenan potassium form mixed with potassium chloride at 25°C. The values are corrected for the enthalpic contribution due to the mixing polymer-salt according to the ref. [12]

Finaly polymer concentration, $g \cdot l^{-1}$	Final salt $eq \cdot l^{-1}$	$-\Delta H$, kJ/mol disaccharide
0.17	0.087	23.5
0.32	—	21.8
0.60	—	20.3
0.65	—	20.6
1.29	—	20.7
2.56	—	22.7
4.94	—	23.3

concentrations, a gel appeared during the mixing of the salt solution to the polymer, preventing the use of stirring.

If no stirring was applied during the experiment, the graph representing enthalpy vs. time was a curve with two steps. These steps disappear with stirring and may represent the formation of isolated helical dimers and aggregates of helical dimers, as previously described [6, 13], or may be due to different rates of diffusion of the salt solution before and after gel formation.

Conclusion

Calorimetry is a very powerful tool for the conformation determination of kappa-carrageenan. The agreement of the experimental values with the theory confirmed the existence of coil⇌helical dimer transition in dilute solution. The use of dilute solutions is essential for two basic reasons:

1) The theory of Manning is applicable only in this range of concentrations.

2) We need to work in the absence of gel formation in order to observe only the conformational transition.

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Zusammenfassung — Es wird über die Kalorimetrie von Kappacarrageen mittels DSC und Kalorimetrie bei konstanter Temperatur berichtet. Die experimentellen Resultate bestätigen beim Ausbleiben der Gelbildung, daß ein helixartiges Dimer gebildet wird. Auch bei Gelbildung steuert die Bildung des helixartigen Dimers den Hauptanteil zum thermischen Effekt bei, der Beitrag der Gelierung stellt nur etwa 20% der registrierten Enthalpie dar.

Резюме — Методами дифференциальной сканирующей калориметрии и калориметрии при постоянной температуре проведены калориметрические измерения каппа-каррагина. Оба метода подтверждают образование димера со спиральной структурой в случае отсутствия гелеобразования. В случае уже имеющегося геля, главным процессом является образование спирального димера, а вклад гелеобразования при высокой концентрации составляет только 20% всей энтальпии.