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The influence of the conformational state of κ- and ι-carrageenan on the rate of acid hydrolysis

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Abstract

κ- And ι-carrageenan were hydrolysed in 0.1 M HCl in the presence of LiI (κ-) or LiCl (ι-). The rate constant for the hydrolysis determined from the decrease in the specific viscosity $[k' = \Delta(c/\eta_{sp})/\Delta t]$ increased by a factor 200 and 10 for κ- and ι-carrageenan, respectively, when passing above the conformational transition temperature (T_m). The rate constant determined from the increase in the number of reducing end-groups (k) increased by a factor 10 for κ-carrageenan, whereas no increase was observed for ι-carrageenan. The activation energy (E_a) for κ-carrageenan increased from 120 to 190 kJ/mol upon conformational ordering, whereas for ι-carrageenan the activation energy was 135 kJ/mol in both conformational states. The activation energies were virtually independent of the ionic strength. The results indicate that the stability properties of κ- and ι-carrageenan as reflected by viscosity or molecular weight decay is determined by differences in the *nature* of the ordered and disordered conformation, respectively, rather than differences in the hydrolytic stability of glycosidic linkages. The results are best described in terms of a multiple-stranded structure of the ordered conformations of both κ- and ι-carrageenan.

Keywords: κ- and ι-carrageenan; Polysaccharide conformation; Rates of acid hydrolysis of polysaccharides

1. Introduction

Several polysaccharides can exist in different conformations in solution depending on ionic strength, temperature, etc. The transition from one conformation to another, which is usually a cooperative process, can easily be detected by monitoring changes in for

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Table 1
Examples of polysaccharides with order/disorder transitions

Polysaccharide	Conditions promoting ordering
κ-Carrageenan [1]	I^--ions, high I, low T
ι-Carrageenan [2]	high I, low T
Agarose [7]	low T
Gellan [8]	high I, low T
Xanthan [9]	high I, low T, low pH
Scleroglucan/schizophyllan [10]	$T < 135$ °C, $< 90\%$ Me_2SO, pH < 13
Curdlan [11]	$T < 135$ °C, $< 90\%$ Me_2SO
Amylose [12]	Me_2SO

instance optical rotation [1,2], heat capacity [3,4], or by NMR [5,6]. However, the physical nature, for instance helical form or strandedness, associated with different conformations is in many cases a matter of debate. Examples of polysaccharides with order/disorder transitions are listed in Table 1.

The conformational or physical state of polysaccharides may have a strong influence on the stability towards degradation [13]. This is known for cellulose; where the crystalline cellulose shows strong resistance towards acid hydrolysis as compared to amorphous cellulose [14]. It has further been shown that xanthan in its ordered, double-stranded conformation shows a particularly high stability towards acid hydrolysis and free radical induced depolymerisation, whereas xanthan in its disordered, single-stranded conformation is degraded more rapidly [15,16]. These differences in stability can be due to a higher rate of cleavage of glycosidic linkages in the disordered states. Moreover, the formation of multiple-stranded ordered structures stabilised by non-covalent inter-chain bonds may increase the stability as they can tolerate cleavages of glycosidic linkages without any pronounced changes in the physical structure and hence, in measured physical properties such as molecular weight or intrinsic viscosity [13,17,18]. This type of stabilisation seems to be the dominating factor governing the stability of double-stranded xanthan [15,16] and double-stranded DNA [19].

Depolymerisation studies can be used as an independent approach to study the nature of conformational states in polymers. In our previous work [13] the influence of the conformation on the depolymerisation rate for a series of different polysaccharides was investigated by viscosity measurements. The degradation of single-stranded polysaccharides showed the expected linear relationship between the inverse of the specific viscosity ($1/\eta_{sp}$) and the depolymerisation time, whereas double-stranded xanthan and triple-stranded scleroglucan went through an initial period with apparently slow degradation, followed by a regime with increasing degradation rate. In this regime the decrease in the molecular weight followed the power law; $\overline{M}_w \sim \overline{M}_{w,0} \cdot t^{-\nu}$, and the exponent, ν, was predicted in a Monte Carlo analysis (1.66 for double-stranded and 2.3 for triple-stranded polymers) [20], although the experimental data could not verify these values in all cases.

In this work we investigate the hydrolytic stability of κ- and ι-carrageenan in their ordered and disordered conformations, respectively. The carrageenans are a family of

sulfated, linear galactans extracted from marine red algae, and have a broad range of applications as gelling-agents (κ- and ι-carrageenan) and viscosifiers (λ-carrageenan). The typical uses are in fruit-, milk- and pet food products, toothpastes, emulsions, and milk imitations [21]. κ- and ι-carrageenan are built up by alternating residues of 3-linked β-D-galactose (G), substituted with a sulfate hemiester in the 4-position, and 4-linked 3,6-anhydro-α-D-galactose (A). In addition the A-unit is sulfated in the 2-position in ι-carrageenan. Each of the two polymers can exist in two different conformations: random coils (disordered states) and single- or double-stranded helices (ordered states), in addition to the gel states promoted by high polymer concentration and ionic strength. It is convenient to induce the different conformational states in solution by varying the ionic strength, temperature, or salt type [1,2], although it should be noted that the conformational transitions, and in particular the disorder–order transition, are often subject to pronounced hysteresis [2] as well as aggregation or gelation. The carrageenans in their disordered states are known to be very unstable even in weak acid, due to the very labile anhydro-galactose linkages [22]. In spite of this, κ- and ι-carrageenan in the gel- and ordered states, e.g. in fruit jams, appear to be very resistant towards acid hydrolysis, possibly due to interstrand stabilisation forces. In contrast to xanthan, which contains carboxylate groups, the conformational states of the carrageenans are in practical use (pH ≥ 1) independent of pH because of the low pK_a-values of the $-SO_3^-$-groups.

There has been some dispute about the nature of the ordered conformations of both κ- and ι-carrageenan, particularly whether they are single- or double-stranded. Many authors have concluded that the carrageenans are double-stranded in the ordered state based on light scattering data [23,24], stopped flow calorimetry [25,26], NMR [27] and X-ray diffraction [28]. Other reports favour single-stranded ordered conformations of both carrageenans [1,3,6,29,30].

The purpose of the present study is first to clarify to which extent the rate of degradation, measured as the decrease in η_{sp}, depends on the conformation. A second objective is to find out whether the changes in stability are caused primarily by differences in the rate of hydrolysis of glycosidic linkages, or by other factors such as multiple-strandedness. A final objective is to obtain information about the nature of the conformation of the carrageenans by degradation studies.

The degradation process may be characterised by the decrease in the molecular-weight or viscosity [13,15] or by the increase in the number of reducing end-groups [17,18], the latter giving directly the rate of cleavage of glycosidic linkages. Together these results may give information about the nature of the ordered state in the molecules.

A nonspecific (random) depolymerisation of a single-stranded polymer should obey the following equations [31]:

$$\frac{1}{\bar{x}_{n,t}} = \frac{1}{\bar{x}_{n,0}} + kt, \tag{1a}$$

and:

$$\frac{1}{\bar{x}_{w,t}} = \frac{1}{\bar{x}_{w,0}} + \frac{kt}{2}, \tag{1b}$$

where $\bar{x}_{n,t}$ and $\bar{x}_{n,0}$ and $\bar{x}_{w,t}$ and $\bar{x}_{w,0}$ are number- and weight-average degrees of polymerisation at times t and 0, respectively, and k is the rate constant for bond cleavage. As $\bar{x}$ is proportional to the molecular weight, eqs (1a) and (1b) indicate that the inverse of the molecular weight should increase linearly with the depolymerisation time. This has previously been demonstrated for alginate [32,33], hyaluronate [34], and other single-stranded polysaccharides [13]. The relationship between the molecular weight and the intrinsic viscosity $[\eta]$ is usually given by the Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = K \cdot M^a. \tag{2}$$

At low concentrations of polymer $[\eta] \approx \eta_{sp}/c$, and combination of eqs (1b) and (2) yields:

$$\left(\frac{c}{\eta_{sp,t}}\right)^{(1/a)} = \left(\frac{c}{\eta_{sp,0}}\right)^{(1/a)} + k't, \tag{3}$$

where $k' = k/2M_0K^{1/a}$, k is the rate of bond cleavage, M_0 is the monomer molecular weight, K and a are MHS-parameters, and c is the polymer concentration. The decrease in η_{sp} can easily be monitored by performing the degradation in a capillary viscometer, and a plot of $(c/\eta_{sp})^{(1/a)}$ versus the degradation time for a random depolymerisation of a single-stranded polymer should be linear. In the case of multiple-stranded polymers the rate of bond cleavage will not be proportional to the observed degradation rate constant with respect to viscosity decay ($k \neq k' \cdot 2M_0K^{1/a}$), as only 'effective' chain breaks will be observed. A plot according to eq (3) will deviate from linearity with an upward curvature for multiple-stranded polymers [13,15,19].

The formation of reducing end-groups can be described as follows:

$$A \rightarrow B, \tag{4}$$

where A is the glycosidic linkages available for cleavage and B is reducing end-groups. The reaction is first order with respect to A [eq (5)]:

$$\frac{d[B]}{dt} = -\frac{d[A]}{dt} = k \cdot [A] = k \cdot ([A]_0 - [B]), \tag{5}$$

where $[A]_0$ is the initial concentration of glycosidic linkages and k is the *pseudo* first order rate constant for cleavages of glycosidic linkages. Integration and assuming $[B] = 0$ at $t = 0$ yields:

$$\ln\{1 - ([B]/[A]_0)\} = \ln(1 - \alpha) = -k \cdot t, \tag{6}$$

where α ($= [B]/[A]_0$) is the fraction of cleaved glycosidic linkages, i.e. the degree of chain scission. The k-value obtained is a 'true' rate constant, as all chain breaks are analysed independently of whether or not they lead to fragmentation (in the case of multiple-stranded polymers).

In single-stranded polysaccharides, the cleavage of glycosidic linkages always leads to two independent fragments (Fig. 1A), and the decay in $[\eta]$ or $\bar{M}_w$ is uniquely determined by k according to eq (3). For multiple-stranded polysaccharides a different situation occurs (Fig. 1B). Because of inter-chain stabilisation forces, linkages may

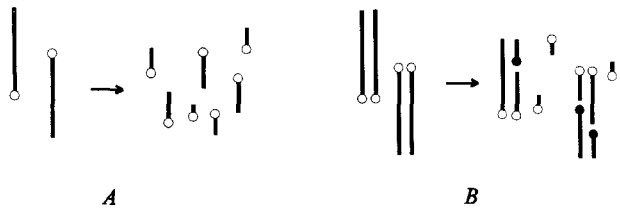

Fig. 1. Random degradation of single-stranded polymers (A) lead to one reducing end (○) per molecule, whereas the double-stranded polymers (B) can in addition contain several internal reducing ends (●) detected by reducing end-group analysis. In the latter case, the changes in the physical properties, such as intrinsic viscosity, are small, when the fraction of cleaved glycosidic linkages is very low.

either be cleaved without further fragmentation, or with the formation of metastable structures stabilised by overlapping 'sticky-ends' and the release of short single-stranded fragments, as shown for double-stranded xanthan [35]. This happens primarily in the initial phase when the degree of chain scission is low. Consequently, the initial decay in molecular-weight and viscosity will in this case be very small, compared to that of single-stranded molecules [13,17,18].

In the present study we investigate the degradation by acid hydrolysis of κ- and ι-carrageenan as a function of the degree of ordered conformation. Both the rate constant in terms of the viscosity decay (k') [eq (4)] and the rate constant determined from the increase in the number of reducing end-groups (k) [eq (6)], are determined. The melting temperature of the ordered states for the two carrageenans at a given ionic strength, are determined by optical rotation measurements. The temperature dependence of k is analysed by Arrhenius-plots, which yields the activation energies (E_a). The chemical compositions of the two carrageenan samples are determined by ^{1}H NMR experiments. Salt is added as LiI and LiCl to induce the ordered conformations below the transition temperature for κ- and ι-carrageenan, respectively. It is known that I^--ions will stabilise the ordered conformation of κ-carrageenan without further aggregation [1]. For ι-carrageenan no anion specificity in the disorder–order transition has been found [36] and LiCl is used.

2. Experimental

Samples.—κ-Carrageenan from *Eucheuma cottonii* was obtained from Sigma (lot No. 120H0502). ι-Carrageenan from *Eucheuma spinosa* was obtained from Sigma (lot No. 27F0373). Both samples were dissolved (4 mg/mL) in MQ-water and stirred overnight. The samples where then filtered through a 0.8 μm membrane filter (Millipore-AA) and dialysed extensively against 0.1 M LiCl and then MQ-water to remove excess salt. The samples were finally freeze-dried. Prior to use, freeze-dried carrageenan was dissolved in MQ-water (ca. 4 mg/mL) and filtered as described above. The concentrations were determined by the phenol–sulfuric acid method [37].

^{1}H NMR studies.—The chemical compositions of the carrageenan samples were determined by ^{1}H NMR experiments. The carrageenans (2 mg/mL) were slightly

depolymerised in HCl (0.01 M) at 100 °C for 5 min, cooled, neutralised by adding 0.01 M LiOH, dialysed against MQ-water and freeze-dried. Samples were dissolved (10 mg/mL) in D_2O, freeze-dried and redissolved (10 mg/mL) in D_2O and ^1H NMR spectra were obtained at 90 °C with a Bruker Avance DPX300 spectrophotometer.

Optical rotation experiments.—Optical rotation was measured at 365 nm in a Perkin-Elmer (type 241) polarimeter of the carrageenan-samples with added salt and HCl (described below). The 10 cm cell was thermostated by a circulating-water bath (Haake D8-G). A computer was used for automatic data acquisition and temperature control. Data were calculated as the specific rotation, corrected for minor changes in sample volume at high temperatures.

Viscosity measurements.—Carrageenan (2 mg/mL (κ) or 0.48 mg/mL (ι)), LiI (0.2 M) for κ- and LiCl (0.05 M) for ι-carrageenan and HCl (0.1 M) were mixed in a capillary viscometer (Schott Geräte Ubbelohde, type 531 01/0a) and placed in a thermostated water bath. The degradations were performed in a temperature interval from 20 to 80 °C, and 2 or 3 degradations were performed at each temperature. The flow-through times were measured continuously throughout the degradation with an AVS-310 (Schott Geräte) control unit, and a computer was used for automatic data acquisition. The accuracy and reproducibility of the measurements at very low relative viscosities have previously been demonstrated [13].

Reducing end-groups measurements.—Carrageenan (2 mg/mL), LiI (0.2 or 0.1 M) for κ- and LiCl (0.2 or 0.05 M) for ι-carrageenan and HCl (0.1 M) were mixed in acid-washed glass-flasks and placed in a thermostated water bath. The degradations were performed at constant temperature ranging from 30 to 75 °C. 10 samples were taken during each degradation, neutralised with LiOH and dialysed against MQ-water. After dialysis the concentrations were again determined by the phenol–sulfuric acid method, and the number of reducing end-groups were determined by the Nelson–Somogyi method [38] using D-galactose for the standard curve. The initial concentration of glycosidic linkages available for cleavage, $[A]_0$, was assumed to be the polysaccharide concentration divided by the molecular weight of the repeating disaccharide units in the carrageenans, assuming that hydrolysis occurs predominately at the 3,6-anhydrogalactose linkage [22]. The pseudo-first-order rate constant (k) was determined according to eq (6), i.e. from the initial slope in plots of $\ln(1-\alpha)$ versus the degradation time.

3. Results and discussion

Sample characterisation (NMR).—The chemical composition of the two commercial carrageenan samples was determined by ^1H NMR [39]. The assigned peaks in the spectra (Fig. 2) indicate the presence of 10% ι-segments (H-1, A-unit) in the κ-carrageenan (A) and 6% κ-segments (H-1, A-unit) in the ι-carrageenan (B), respectively. The appearance of about 6–10% impurities in the carrageenan samples is in accordance with analyses reported on similar samples [30,40,41].

Conformational properties.—The conformational properties of the carrageenans prevailing under the conditions where the degradation experiments took place were assessed by monitoring the optical rotation. Fig. 3 shows the temperature dependence of

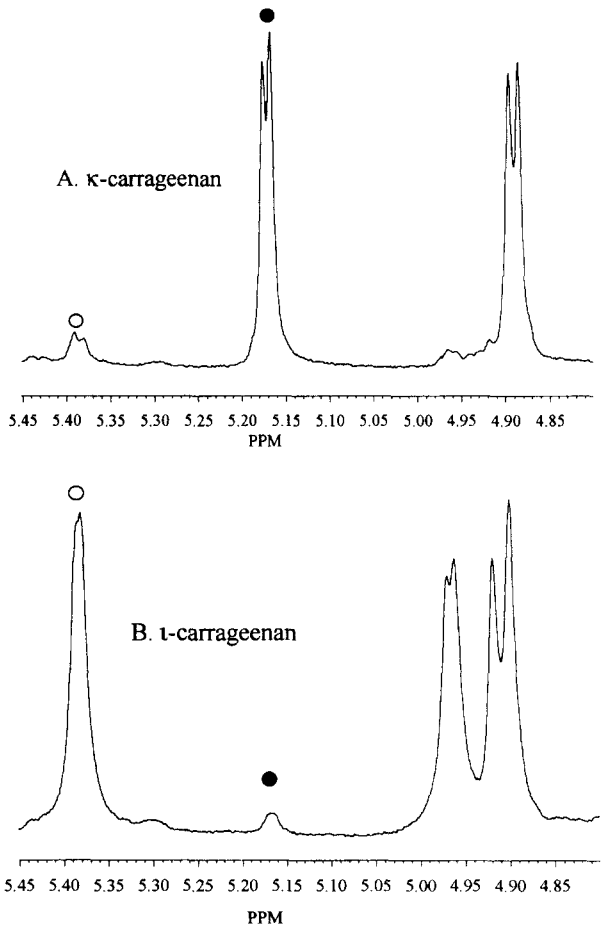

Fig. 2. ^{1}H-NMR spectra of slightly depolymerised κ- (A) and ι-carrageenan (B) in D_2O at 90 °C. The assigned peaks (● and ○) arise from the H-1 in the A-units of κ- and ι-carrageenan, respectively. The composition in the samples were determined by comparing the area below these peaks (● = κ, ○ = ι) and the total area below the peaks of H-1 in the A-units (● + ○).

the specific optical rotation of κ-carrageenan in 0.2 M LiI containing 0.1 M HCl. The corresponding data for ι-carrageenan in 0.05 M LiCl containing 0.1 M HCl are shown in Fig. 4. In both cases the characteristic order–disorder transition is observed [1,2] with midpoints (T_m) of 53 and 40 °C, respectively. Similar heat curves were obtained in the presence and absence of 0.1 M HCl (data not shown), when comparison was made at constant ionic strength assuming full dissociation of HCl. The cooling curves in the absence of HCl follow the heat curves with a tendency of hysteresis in the ι-sample, but no hysteresis in the κ-sample. Only the heat curves are shown for samples containing HCl, as the carrageenans were rapidly degraded above T_m and $[\alpha]_{365}$ remained low upon cooling.

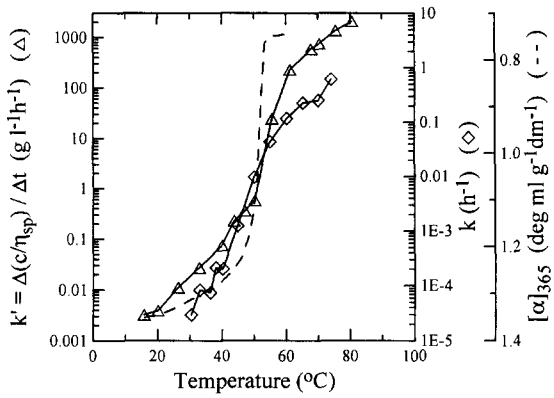

Fig. 3. The initial degradation rate constants k' (△) and k (◇), for the acid hydrolysis of κ-carrageenan and the specific optical rotation (- - -) ($[\alpha]_{365}$) in 0.1 M HCl and 0.2 M LiI as a function of temperature. The data points (△) are averages of 2–3 degradation experiments.

Degradation monitored by viscosity measurements.—Acid hydrolysis in 0.1 M HCl was performed on carrageenan-samples at different temperatures ranging from 15 to 80 °C, and thereby in different conformational states as shown above. The initial degradation rate constants (k') were determined from plots of $(c/\eta_{sp})^{1/a}$ against time [eq (3)]. Examples of such plots are given in reference [13]. In the case of acid hydrolysis the deviation of the very first measuring point from the general trend (due to long efflux times in the capillary viscometer) was negligible in all cases. The results are shown in Fig. 3 (κ) and Fig. 4 (ι) together with the corresponding optical rotation data.

For κ-carrageenan (Fig. 3) log k' seems to increase linearly with temperature in the region below T_m. At the transition temperature a sudden increase in k' (about 200-fold)

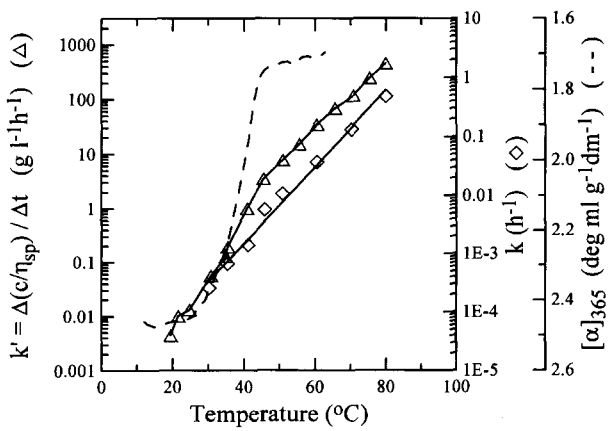

Fig. 4. The initial degradation rate constants k' (△) and k (◇) for the acid hydrolysis of ι-carrageenan and the specific optical rotation (- - -) ($[\alpha]_{365}$) in 0.1 M HCl and 0.05 M LiCl as a function of temperature. The data points (△) are averages of 2–3 degradation experiments.

can be observed, whereas above T_m the increase in log k' again appears to be linear. Similar data are observed for ι-carrageenan (Fig. 4), although the increase in k' occurring at T_m is clearly less pronounced in this case (about 10-fold). These data support the earlier observations [13] on the enhanced stability occurring upon ordering of the carrageenan-molecules.

Degradation monitored by reducing end-groups measurements.—The next step was to determine to which extent the increase in k' during the transition was due to an increase in the rate of cleavage of glycosidic linkages. The rate constants (k) obtained from analysis of reducing end-groups, are also shown in Figs. 3 and 4 for κ- and ι-carrageenan, respectively.

For κ-carrageenan log k seems to increase linearly with T when $T < T_m$ (Fig. 3). A 10-fold increase in k occurs during the transition. Above T_m, the carrageenan-molecules are fully disordered, and log k again enters a linear range.

ι-Carrageenan show a markedly different behaviour than that observed for κ-carrageenan. In this case the increase in log k appears to be linear over the entire temperature range, and no increase in k can be ascribed to the conformational transition.

A comparison of the stabilisation at T_m as monitored by the viscosity decay (k') and increase in the rate of formation of reducing end-groups (k) can give an indication on the strandedness of the carrageenan ordered conformations. The ratio k'/k increases with a factor 20 when going from the ordered to the disordered state for κ-carrageenan. For ι-carrageenan the ratio was 10. These observations clearly demonstrate that the considerable increase in stability, measured as k', upon conformational ordering of the carrageenan-molecules (Figs. 3 and 4) cannot be ascribed to the slightly lower rate of cleavage of glycosidic linkages in the ordered conformation. We therefore suggest that inter-chain stabilisation forces may give a substantial contribution to the enhanced stability with respect to the loss of viscosity. Hence, these results are best described in terms of a multiple-stranded structure of the ordered conformations of both κ- and ι-carrageenan.

The rate constants (k) are shown in Arrhenius-plots in Fig. 5. The solvent conditions were the same as in the previous (viscosity) experiments. In addition, data were also obtained at decreased (κ) and elevated (ι) ionic strengths. Optical rotation curves are included in the figures. The relatively large error of estimating the rate constant (k) at low temperatures (Fig. 5), is mainly due to the low content of reducing end-groups ($\alpha < 0.03$). The estimated errors are the standard deviations in the slopes obtained from plots of $\ln(1-\alpha)$ versus time.

The data for κ-carrageenan in 0.2 and 0.1 M LiI are shown in Fig. 5 (left). By comparing the two plots it may seem that there is a general increase in the rate constant by lowering the ionic strength. The increase is 1.5 times in the disordered state and 8 times in the ordered state. This can be explained by a reduction of the local, high, H^+-concentration in the proximity of the negatively charged carrageenan-molecules upon increasing the counter-ion concentration. In addition, the activity of H^+-ions will decrease slightly with increasing ionic strength [31].

Corresponding data for ι-carrageenan in 0.2 and 0.05 M LiCl are shown in Fig. 5 (right). The rate constant increases with a factor 2 by lowering the ionic strength from 0.2 to 0.05 M, both in the ordered and disordered conformations. This is in contrast to

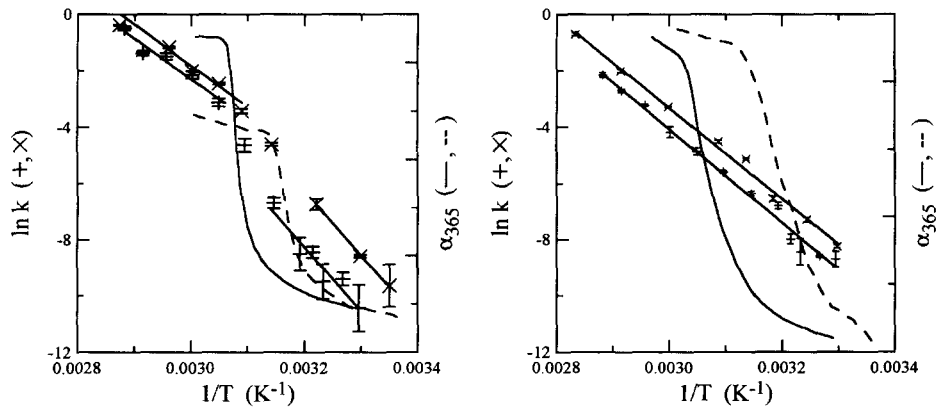

Fig. 5. Arrhenius-plots of the initial degradation rate constant (k) for the acid hydrolysis (0.1 M HCl) of (left) κ-carrageenan in 0.2 M (+) and 0.1 M (×) LiI and the respective optical rotation curves (————, ---) (α_{365}), and (right) ι-carrageenan in 0.2 M (+) and 0.05 M (×) LiCl and the respective optical rotation curves (————, ---) (α_{365}).

the behaviour of κ-carrageenan, where the ordered state showed a larger increase (8-fold) than the disordered state (1.5-fold). This difference between the two polymers may possibly be associated with differences in the charge density. The lateral distance between the charges along the polymer chain in ι-carrageenan is below the critical distance for Manning-condensation ($b_{\text{critical}} \approx 7$ Å at 25 °C) [42], and the effective charge density will therefore not increase upon ordering even if chain dimerisation occurs. In contrast, the lateral distance between the SO_3^--groups on the κ-carrageenan chains in the disordered state is 2 times larger than for ι-carrageenan and larger than b_{critical}. Upon ordering of the κ-carrageenan molecules the charge density along the polymer chains may increase partly due to the inclusion of I^--ions within the helix [5,27] and partly due to the coil–helix transition and possibly dimerisation of the helices. Polymer chains with a high charge density will generally be more influenced by changes in the ionic strength with respect to the concentration of counter-ions near the chains [31]. For κ-carrageenan this results in a higher local concentration of protons and hence, an increase in the rate of hydrolysis.

The rate constants for the acid hydrolysis of κ-carrageenan in the disordered state are about 5–6 times higher than for ι-carrageenan at the same conditions. This can be explained by the stabilisation of the 3,6-anhydrogalactose linkage in ι-carrageenan by the sulfate groups in 2-position. The sulfate-group is more electronegative than the corresponding OH group in κ-carrageenan, and the electron density at the O-atom in the glycosidic linkage is lowered, thereby decreasing its reactivity towards H^+ [43]. In addition, the sulfate group may be a steric hindrance in the formation of the carbocation in the half-chair conformation in the acid hydrolysis (activated complex).

Determination of activation energies (E_a) from reducing end-group measurements.—According to the Arrhenius-equation the activation energies (E_a) for the acid hydrolysis can be calculated from the slope of the linear regions in plots of ln k versus $1/T$ below and above T_m in Fig. 5. The pre-exponential factor (A) can be

Table 2
Activation energies (kJ/mol), E_a, and pre-exponential factor, A, determined from the Arrhenius-equation for the acid hydrolysis of κ- and ι-carrageenan

Solvent (+0.1 M HCl)	κ-carrageenan		ι-carrageenan	
	0.2 M LiI	0.1 M LiI	0.2 M LiCl	0.05 M LiCl
$E_{a,disorder}$ (kJ/mol)	120	120	140	130
$E_{a,order}$ (kJ/mol)	190	190		
$A_{disorder}$ (h^{-1})	$4.9 \cdot 10^{17}$	$1.9 \cdot 10^{18}$	$8.4 \cdot 10^{19}$	$3.9 \cdot 10^{19}$
A_{order} (h^{-1})	$1.1 \cdot 10^{28}$	$7.0 \cdot 10^{28}$		

calculated from the intercept of these linear regions with the ordinate. The results of these calculations are given in Table 2.

According to Table 2, the activation energies appear to be virtually independent of the ionic strength. There is, however, a large difference between $E_{a,disorder}$ and $E_{a,order}$ for κ-carrageenan, while for ι-carrageenan there is no observable difference. The activation energy for the acid hydrolysis of ι-carrageenan is somewhat higher than for κ-carrageenan in the disordered state, but still much lower than for κ-carrageenan in the ordered state.

Activation energies of 100–120 kJ/mol for the acid hydrolysis of κ-carrageenan in the disordered conformation have been reported previously [44,45]. No activation energies have been reported for the acid hydrolysis of κ-carrageenan in its iodide-induced ordered conformation. The relatively high $E_{a,order}$ observed in this work may be related to the nature of the ordered state in κ-carrageenan. It has been proposed that the κ-carrageenan molecules are arranged in double helices with the sulfate groups pointing out into the solvent, and that the interior surface of the helix is hydrophobic and can bind 2 iodide molecules per helical turn [27]. Because of the higher surface charge on ι-carrageenan molecules and also a much narrower interior of the helices, ι-carrageenan will not be able to bind iodide, and an ordered conformation cannot be induced by the same mechanism as in κ-carrageenan [27]. The binding of iodide to the helices of κ-carrageenan is energetically favourable, as reflected in a higher T_m than for e.g. chloride [1]. This may stabilise the monomer units in their preferred ring-conformations and make any conformational changes towards the activated state more difficult. Thus, the activation energy for the acid hydrolysis will most probably increase in the iodide-induced ordered state.

In the disordered state, the steric hindrance of the sulfate group at the 2-position in the 3,6-anhydrogalactose is assumed to lead to the higher activation energy observed for ι-carrageenan than for κ-carrageenan.

The pre-exponential factor (Table 2) is proportional to the entropy of formation of the activated complex, e.g. activation entropy ($\Delta S^{\neq}$). For κ-carrageenan in the disordered state and ι-carrageenan the calculated activation entropies are not significantly different. The activation entropy for the acid hydrolysis of κ-carrageenan in its ordered state, on the other hand, is considerably higher, and this is probably due to the influence of I^--ions bound to the ordered molecules of κ-carrageenan.

4. Conclusions

The results indicate that the stability properties of κ- and ι-carrageenan as reflected by viscosity or molecular weight decay is determined by differences in the *nature* of the ordered and disordered conformation, respectively, rather than differences in the hydrolytic stability of glycosidic linkages. The results are best described in terms of a multiple-stranded structure of the ordered conformations of both κ- and ι-carrageenan, although this is in contrast to conclusions regarding strandedness reached earlier in our laboratory [1,3,5,6]. This work shows that degradation studies used as an independent approach to the study of conformation in solution can give supplementing information on the strandedness of the polysaccharide molecules.

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