

## 정렬된 *iota*-와 *kappa*-Carrageenan 사슬의 X-ray 회절과 분자구조의 상변화와의 연관성

이 임 식\*

중국과학원 화학연구소  
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### X-ray Diffraction Patterns from Oriented Samples of *iota*- and *kappa*- Carrageenans and Implications for Conformational Transition

Imshik Lee\*

*Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100080, Peoples Rep. of China*

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**요약:** 정확히 180° 위상차를 갖는 이중나선 구조로부터의 변화 또는 이중나선 구조의 각사슬의 부동한 구조변환을 보여주는 새로운 X-ray 회절모양을 관찰하였다. *Iota*-carrageenan 용액속에 NaI 함유량을 증가시키에 따라 새로 출현된 첫번째와 다섯번째 layer line의 모양은 더욱 선명해졌으나 여섯번째 layer line의 모양은 점점 약화되어졌다. 또 X-ray 회절 모양에 의하면 Cl<sup>-</sup>와 Br<sup>-</sup> 이온의 역할도 용액속에서 I<sup>-</sup> 이온이 *iota*-carrageenan에 주는 역할과 같음을 보여 주었다. 이러한 관계는 분자구조의 변환이 용액속에서 counteranion과 countercation의 수용해 에너지의 차이에 의존함을 보여주는 듯하다. 더우기 섬유상태를 유지한 상태에서 투입확산과 추출확산 방법을 적용하여 분자구조의 가역적 변화를 X-ray 회절실험을 통해 관찰할 수 있었다. 가역적 구조변화의 출현은 *iota*-carrageenan의 분자구조가 이중나선 구조임을 보여주는 좋은 증거가 된다. 이러한 결과들을 통해 *iota*-carrageenan 분자구조의 상변화는 나선 수준에서 유연성 사슬로부터 견고성 사슬구조로의 변화임을 유추할 수 있으며, gelation은 side-by-side stacking임을 시사해 주고 있다.

**ABSTRACT:** New additional X-ray diffraction layers were observed from KI induced *iota*-carrageenan, being interpreted: 1) as a screw displacement from exact half-staggered double-helix. 2) as a difference between the two chains resulting conformational perturbation. As NaI wt. ratio in *iota*-carrageenan solution increases, the 1<sup>st</sup> and the 5<sup>th</sup> layer line get stronger, but the 6th layer line becomes weaker. According to X-ray diffraction observation, Cl<sup>-</sup> or Br<sup>-</sup> ions with a counterpart metallic cation in aqueous solution give almost the same result as I<sup>-</sup> ions. It seems that the structural changes depend on the relative difference of hydration energy between counteranions and countercations in solution. Upon the addition of NaI and KI in fiber state reversible change was detected by X-ray diffraction with performing diffusion-in and -out techniques. Reversible conformation change supports a double-helix model for *iota*-carrageenans. Therefore, the structural changes of *iota*-carrageenan implicate to a conformational transition from a flexible molecule to a stiff molecule showing no topological constraint and gelation can be considered in helical level by side-by-side stacking.

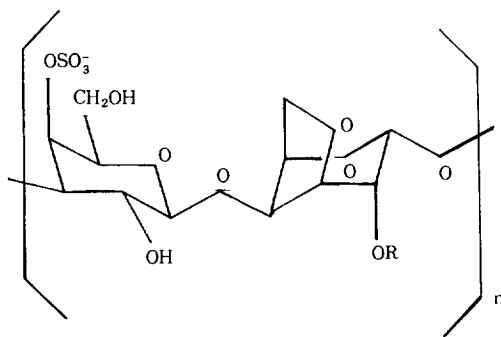
**Keywords:** X-ray diffraction, double-helix, conformational transition, diffusion techniques, relative hydration, gelation.

\*Present address : Dept. of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong

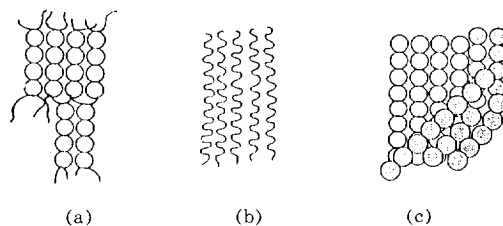
## INTRODUCTION

Carrageenans are a family of sulphated D-galactoses which are extracted from certain marine red algae, *Euheuma cottonii*, *Euheuma spinosum* and *Gigatina acicularis*, and characterized by alternating (1e→3e)- and (1e→4e)-linked structures (Fig. 1). They are widely used and studied because of their ability to act as thickening agents or to form aqueous gels. Carrageenan is polyelectrolytes with ionisable sulphate appendages: *iota*-carrageenan being the more heavily sulphated than *kappa*-carrageenan. Electrostatic interactions of the sulphated polyanions with hydrated counterions have a remark influence on the conformational transitions and gelation behavior of carrageenans.<sup>1-4</sup> In addition a dramatic cation specific effect on the conformational (coil-helix) transition has been noticed.<sup>4,6,7</sup> Iodine-NMR line broadening accompanying the disordered-ordered transition of tetramethylammonium chloride (TMACl) associated *kappa*-carrageenan upon addition of tetramethylammonium iodide (TMAI) was interpreted as resulting from specific site binding of iodine to single-helical chains.<sup>2,8</sup> But it is not clear to what extent the specific binding of ions is sensitive to the conformation of the carrageenan molecule. Partial dehydration cations also create different spin relaxation to indicate the cation-specific effects or their binding in carrageenan systems.<sup>1,9-11</sup>

Models of proposed-gelation mechanisms are displayed in Fig. 2. A cross-linked and double helical model<sup>12,13</sup> is associated with the presence of a few modified units about 10%, called "kinks", as illustrated in Fig. 2a. Such a mechanism presents enormous topological problems to form double helical gelation. The (short) segments can form double helices during gelation of single coils, and the helical chains can aggre-



**Figure 1.** Idealized disaccharide repeating structure for *iota*- (for R=SO<sub>3</sub><sup>-</sup>) and *kappa*-carrageenans (for R=H).



**Figure 2.** Schematic diagram of the conformational transition in carrageenan: (a) the domain model,<sup>17</sup> (b) the single chain model<sup>14</sup> and (c) flexible to stiff double helix model.<sup>28,29</sup>

gate under the specific influence of K<sup>+</sup>, for instant, but long chain cannot for topological reason. In the present of iodine, the conformational change in *kappa*-carrageenan occurs without any observable change in molecular weight.<sup>8,14</sup> So Smidsrod *et al.*<sup>14</sup> proposed that aggregation results from the cross-linking of single-helix (Fig. 2b) by the specific anion mediator I<sup>-</sup>. But Atkins<sup>15</sup> pointed out that the gelation mechanism is always a double helix both solution and solid state.<sup>16</sup> So Fig. 2c presents new proposal based on present work and reviewed results.

X-ray (fibre) diffraction pattern does not show a clear distinction between a pattern of single helical chain and that of the exactly half-staggered double helical chain with the same chemical polarity intertwines around a common

axis.<sup>17-19</sup> Therefore, strong arguments occur in favor of double helical structures for *kappa*- and *iota*-carrageenan. Scanning tunneling microscopic (STM) images of *kappa*- and *iota*-carrageenan<sup>20</sup> have presented two intertwining chains with helical pitches of 2.50 nm and 2.60 nm, respectively.

In the present paper author attempted to break a half-staggered structure of *iota*-carrageenan by controlling ionic environment, and described the characteristics of the gelation mechanism and the structures of *iota*-carrageenan. According to the new observations from X-ray diffraction, a model for a transition from flexible double helices to stiff was ordered on upon gelation. Finally, cation selective effects in the gelation of *iota*- and *kappa*-carrageenan were attempted to classify as relative hydration.

## EXPERIMENTAL

**Preparation of Oriented Fibers.** Solution of *iota*- and *kappa*-carrageenan with molecular weights  $4-8 \times 10^2$  (Sigma Chemical Company Ltd.) was prepared by dispersion appropriated amount of powdered materials in distilled water. For the preparation of fibers, the solution was poured onto a glass slide and allowed to dry. Strips were cut from this dried material and stretched under conditions of controlled humidity.<sup>21</sup>

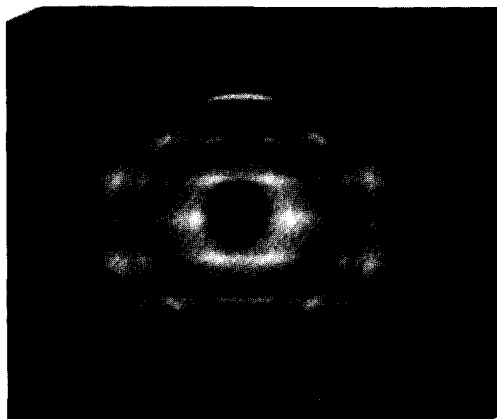
**Diffusion Techniques.** Diffusion techniques were developed for investigating the structural changes of fibrous biopolymers with keeping fibrous state in the presence of different ion environments.

The diffusion-out method is applied to diffuse out counterions from samples that contain salt, whereas the diffuse-in method to diffuse counterions into the solid (fibrous) samples. For

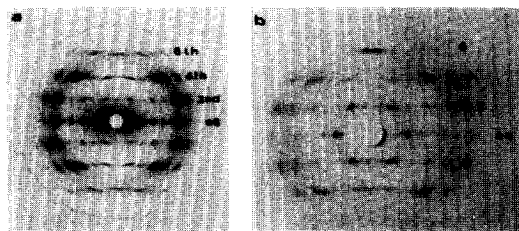
diffusion-out an oriented and salted sample was dipped into a poor solvent (a mixture solvent of 30% distilled water and 70% ethanol) under constant load to preserve the fiber orientation. The diffusion-in procedure is similar to dialysis except that solvent was used with a mixture of 40% saturated electrolyte aqueous solution (saturated alkali halide solution) and 60% ethanol. After diffusion-in or-out processes, the samples were dried before exposure to X-ray for removing mostly remained solvent on the surface of the sample. The co-solvent ethanol could be considered not to perturb the polymer conformation as the samples used for X-ray diffraction still had a high relative humidity.

**X-ray Diffraction.** X-ray diffraction photographs were obtained on Phillips and Elliot rotating anode generators using pinhole collimator and flat-plate camera. The camera was filled with helium and water vapor. The humidity was controlled by bubbling the helium through a saturated salt solution. The specimens were also dusted with finely powdered calcite, characteristic spacing, 0.3035 nm, in order to calibrate the diffraction patterns. Most of the X-ray photographs were obtained at a high relative humidity (RH), about 90-98%, to reduce possible disturbances of polymer conformation by ethanol and to stabilize it. Normally, the X-ray photographs taken at high RH are clearer and higher orientation than at low RH. Some photographs of dry samples were taken with the camera evacuated to a pressure of  $10^{-2}$  torr, but the crystallization of sample was poor.

**New X-ray Diffraction Evidence from *iota*-Carrageenan.** Fig. 3 displays a typical X-ray diffraction pattern of *iota*-carrageenan. The reflections index on a trigonal unit cell with dimensions  $a = b = 1.37$  nm,  $c$  (fiber axis) = 1.33 nm and  $\gamma = 120^\circ$ , which are similar to those reported by Arnott *et al.*<sup>19</sup> Their interpretation was that



**Figure 3.** Typical X-ray diffraction patterns from *iota*-carrageenan.



**Figure 4.** Fibre diffraction photographs of a highly oriented *iota*-carrageenan; (a) TMACl induced structure with calcite ring for calibration and (b) dialyzed TMAI induced structure.

*iota*-carrageenan had isomorphous crystal structures, with two right-handed, half-staggered threefold helix with a pitch of 2.66 nm running statistically up-ward or down-ward.

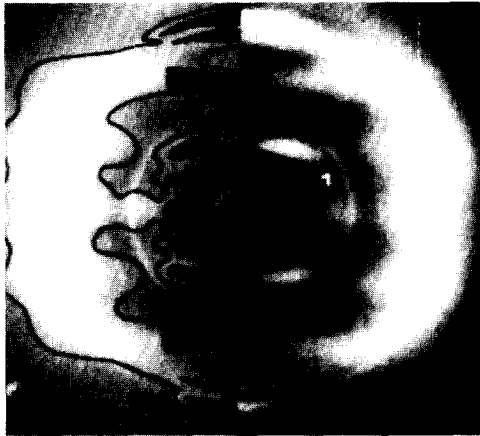
A fiber diffraction photograph obtained from the TMACl associated *iota*-carrageenan is highly oriented without containing the odd layer reflections (Fig. 4a). A meridional reflection appears at the 6<sup>th</sup> layer line presenting three-fold symmetry along the helical axis. In order to get the information of chain packing, the equatorial reflections were indexed in Table 1. The first equatorial reflection appears at 2.39 nm which is related to superlattice. This first equatorial reflection been reconfirmed using synchrotron ra-

**Table 1.** The Equatorial Reflections of Figure 4a (Unit Cell  $a=2.44$  nm,  $b=1.50$  nm,  $c$  (chain axis)=1.33 nm and  $\gamma=101^\circ$ )

hkl	calculated d (hkl)	observed d (hkl)
100	2.40	2.40 (-0.066)
010	1.46	1.46 (-0.025)
200	1.20	1.13 ( $\pm 0.026$ )
110	1.15	
210	0.85	0.87( $\pm 0.012$ )
300	0.80	0.75( $\pm 0.014$ )
020	0.73	
120	0.67	
310	0.65	0.63( $\pm 0.016$ )
400	0.60	
220	0.58	0.55( $\pm 0.013$ )
320	0.49	
030	0.49	0.47( $\pm 0.017$ )
500	0.48	
130	0.46	
230	0.42	0.42

diation from the SRS at Daresbury. A superlattice was determined as a unit cell  $a=2.44$  nm,  $b=1.50$  nm,  $c$  (fiber axis)=1.29 nm, and  $\gamma=101^\circ$  aligning two double helical chains with alternating translation. Alternated chain alignment produces a better crystallinity. The organic counteraction  $TMA^+$  seems to drive a chain alternation. A similar improvement of the chain orientation was obtained from the dialyzed sample of TMAI-associated *iota*-carrageenan (Fig. 4b). It is expected that the orientation improvement is associated not with counteranion but with counter cation  $TMA^+$ . It was expected that  $I^-$  should remain in the dialyzed sample if iodine ion has a binding on *iota*-carrageenan. However, a highly ordered diffraction pattern (Fig. 4b) was obtained from the sample. This feature does not support a strong  $I^-$  binding on carrageenan.

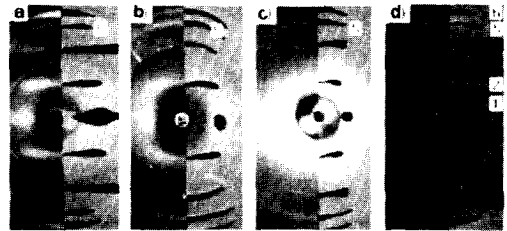
In presence of  $I^-$  ions the new diffraction spot at the 1<sup>st</sup> and/or 5<sup>th</sup> layer line (Fig. 5) appears, but there is no remarkable reflection on the 3<sup>rd</sup> layer line. The new diffraction, the 1<sup>st</sup> layer line



**Figure 5.** New diffraction pattern from KI-induced *iota*-carrageenan at a KI wt./*iota*-carrageenan wt. ratio of 0.1.

and the additional 5<sup>th</sup> layer line, is important to represent a displacement from the half-staggered, double helical, structure in the *iota*-carrageenan model. Two possible explanations can be considered: 1) one chain “differs” from other caused by some perturbation of its conformation, or 2) one chain to move relative to the other is by a screw operation, e.g. a coupled rotation and translation. Since in present case the transitional component is ruled out on experimental grounds, the effect of rotation only is to alternatively reduce and enlarge the grooves between chains in a manner similar to the minor and major grooves in DNA.

A different interaction between ionic environment and carrageenan has been reported in the presence of I<sup>-</sup> ions.<sup>8,11,22</sup> Anions with reduced water binding captivity may influence the hydrophilic interactions of the chains.<sup>23</sup> It is expected that a less hydrated anions produce an electrostatic repulsion, and the polymer chains will be less oriented which is in agreement with a diffuse X-ray diffraction pattern. Such a pattern indicates low crystallinity, poor lateral association and weak polymer-polymer interaction is

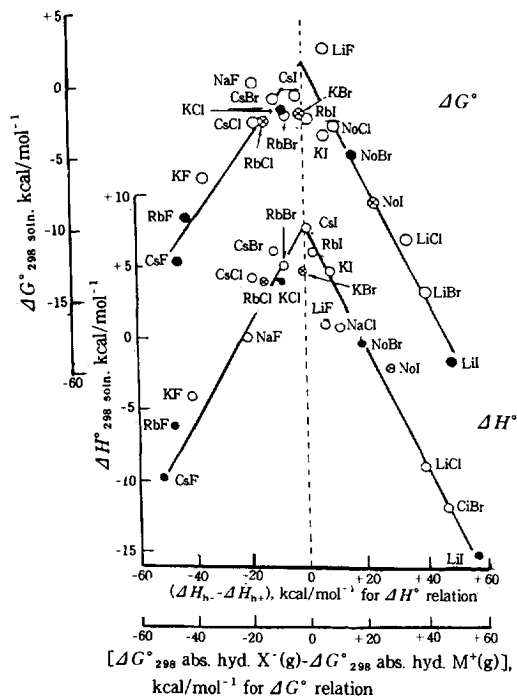


**Figure 6.** New diffraction patterns from RbI- (a), LiI- (b), NaCl- (c) and NaBr- (d) induced structure of *iota*-carrageenan.

associated with an inability to gel. In carrageenan, molecular association is consistent with crystallization of segment of neighboring helices. So the effects of anions on the ordered conformation could be attributed to the repulsive interactions between polyanion and anion resulting in a lower crystallinity of carrageenan.

**Classification of Metallic Salts.** Each disaccharide of carrageenan has charged sulphate groups as illustrated in Fig. 1 and the chains interact selectively with Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Rb<sup>+</sup> and Cs<sup>+</sup> cations and Cl<sup>-</sup>, Br<sup>-</sup> and I<sup>-</sup> anions from metallic salts.<sup>19,24-26</sup> The ionic environment thus influences to the degree of orientation. As judged from the X-ray patterns recorded in the presence of different salts, the degree of orientation is linked with the ability to form gels.<sup>26</sup>

The 1<sup>st</sup> and/or the 5<sup>th</sup> layer lines were obtained not only in the presence of I<sup>-</sup> but also Cl<sup>-</sup> and Br<sup>-</sup>. Fig. 6 illustrates that a new X-ray diffraction pattern appears not only from the RbI and LiI-induced structure of *iota*-carrageenans but also from the NaCl and NaBr-induced one. Metallic salts which produce the 1<sup>st</sup> and/or 5<sup>th</sup> layer lines, NaBr, NaCl, KI, LiI and RbI, are found on the right hand side of the classification in Fig. 7, based on Fajans' evaluation of the heat of hydration of alkali halide ions.<sup>27</sup> This indicates that H(h<sup>-</sup>)-H(h<sup>+</sup>)>0 and means that the fully hydrated cation (h<sup>+</sup>) has a higher activity than the partially hydrated an-



**Figure 7.** Fajans' relationship for alkali halides (After Fajan, see Conway, 1977).<sup>26</sup>

ion (h-) in water. The hydration effect seems to be strongly correlation with a short-range repulsive force preventing adhesive contact. This view has been approved by the Zimmer and Luck.<sup>28</sup> When anions have more strong influence to the conformation of biopolymers, anions are hydrogen bond breakers with a high polarity.

At this stage, it is necessary to consider the relative hydration mechanism of the polyion, cation and anion. A conformational transition, such as the stiff-flexible transition, can be considered to result from two opposing tendencies: 1) from states of high internal energies to states of low internal energies and 2) from states of low entropy to states of high entropy. The stable state which reflects both tendencies is that with the lowest free energy ( $\Delta G$ ).

Shape and size affect the packing process in the final states and polarity determines the na-

ture and strength of interactions between polyion and anions and cations in aqueous solution and between the ions and water. The polar groups have strong electrostatic interactions. Non-polar groups could also attract or repel each other by van der Waals forces, albeit more weakly, but at short distances repulsive forces dominate.

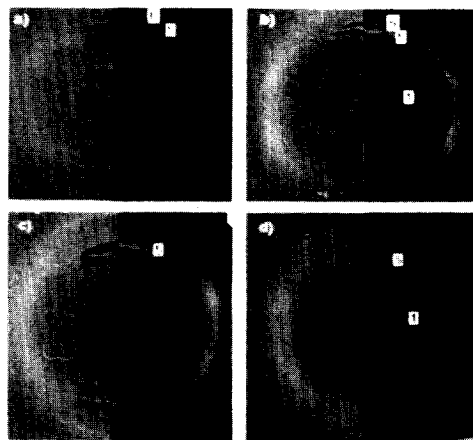
In the case of carrageenan hydration increases in the order  $\kappa < \iota < \lambda$ -carrageenan, mainly due to an increase in an electrostriction of sulphate group.

To understand the conformational transition of carrageenan one can put  $\Delta G = G(\text{flexible}) - G(\text{stiff})$ , the difference between the free energies of the flexible and stiff states. For  $\Delta G > 0$ , the stiff molecule is stable, for  $\Delta G < 0$ , the flexible one is preferred and for  $\Delta G = 0$  the two are in equilibrium. The temperature at  $\Delta G = 0$  is the transition temperature or mid-temperature ( $T_m$ ). The free energy change  $\Delta G$  will be considered as the sum of two terms,  $\Delta G = \Delta G_o + \Delta G_e$ , where  $\Delta G_o$  represents all non-electrostatic interactions and  $\Delta G_e$  all electrostatic interaction. The latter can be put as  $\Delta G_e = \Delta G_{pp} + \Delta G_{p-} + \Delta G_{p+} + \Delta G_{+-}$ , i.e. as the sum of the electrostatic interaction between polyions ( $\Delta G_{pp}$ ), polyions and cations ( $\Delta G_{p+}$ ), polyions and anions ( $\Delta G_{p-}$ ) and counteranions and countercations ( $\Delta G_{+-}$ ).  $G_{p+}$  and  $\Delta G_{p-}$  are effected on polymer conformation by hydration screening of both countercations and counteranions. Previously hydration effect of polyion was mostly treated in terms of only one kind of counterion. The degree of hydration of both the countercations and the counteranions has, however, an influence on polyelectrolytes. As mentioned earlier, the water content of the fibers, as measured by the relative humidity, affects the polymer conformation also by influencing the relative hydration of ions and ionic groups.

According to Schildkraut's representation,<sup>23</sup> the mid-temperature  $T_m = (\Delta H_o + \Delta G_e) / \Delta S_o$ , where  $\Delta H_o$  is the non-electrostatic energy change and  $\Delta S_o$  the corresponding entropy change. This temperature decreases with increasing repulsive energy. If  $\Delta G_{p-}$  increases, for instance,  $T_m$  will decrease in accordance with the trends in the right hand side of the Fajans' classification in Fig. 6.  $\Delta G_e$  should be also proportional to the charge density, expressed in terms of the identical polymer concentration and to the metallic salt concentration to the conformational transition ( $T_m$ ). For further work, we discussing the relative electrolyte effect,  $\Delta G_{p-}$  and  $\Delta G_{p+}$ , using the relative ion activity  $a_r = (a^+ - a^-) / (a^+ + a^-)$  with the activity of countercation ( $a^+$ ) and that of counteranion ( $a^-$ ) theoretically.

Previous studies have tackled the polyions-cations interactions or the polyions-anions interactions separately. Although it is well known that individual hydration of ions contributes to the conformation of carrageenans, the hydration of salts cannot be represented accurately in terms of individual cation or anion because of the combination of different types of ion-solvent, ion-polyion, and polyion-solvent interactions, viz. dipolar, quadropolar, hydrophobic and hydrophilic interactions that mut arise in any real situation of polyelectrolyte and electrolytes in a polar solvent.

Fajans' classification indicates that when  $H(h-) - H(h+)$  is positive, the partially hydrated anions have lower activity than the fully hydrated cations in aqueous solution. The reduced activity of anions seems to prevent association of the ordered conformation of carrageenan. The strong repulsion interactions,  $\Delta G_{p-}$ , in *iota*-carrageenan modify the interactions between sulphate groups and partially hydrated anions and induce destabilizing sec-

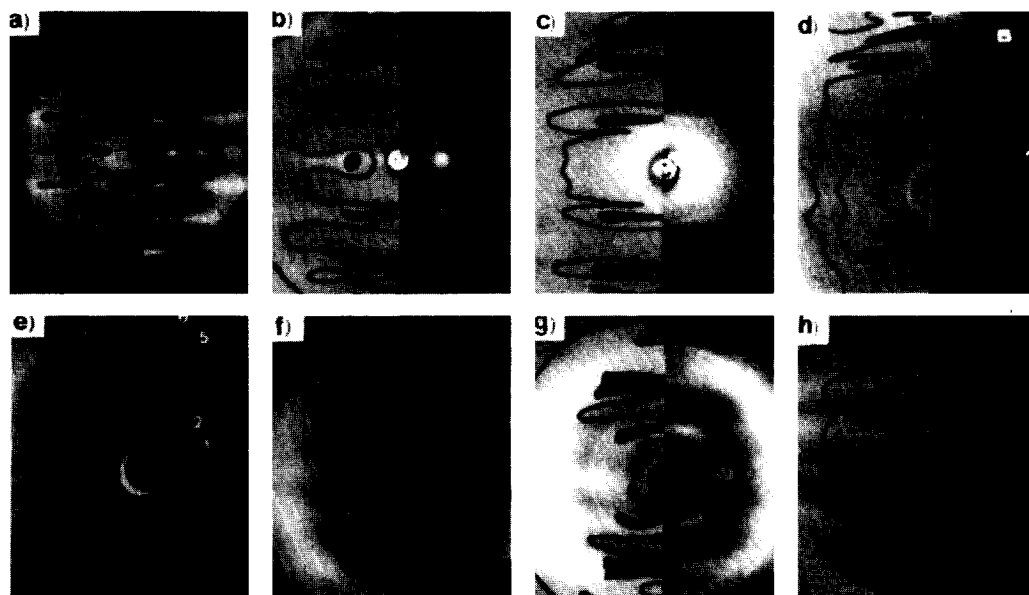


**Figure 8.** Changes in X-ray diffraction pattern of *iota*-carrageenan at different NaI wt. ratios: (a) 0.1, (b) 0.4, (c) 0.8 and (d) = (c) but tilted 14°. The 1<sup>st</sup> and the 5<sup>th</sup> layer lines become stronger with increasing salt concentration, whereas the 6<sup>th</sup> layer line becomes weaker and finally disappears. The diffused patterns are due to a poor orientation of samples resulting from the effect of NaI solutions.

dary and tertiary conformations. Hence for  $H(h-) - H(h+) < 0$ , cation binding is associated with ordered conformation of carrageenan.

**Reversible Structure Changes of *iota*-Carrageenan.** Fig. 8 shows the changes in the X-ray diffraction pattern with increasing a concentration ratio (NaI wt./*iota*-carrageenan wt.) from 0.1 to 0.8. As the NaI concentration increased the 1<sup>st</sup> and the 5<sup>th</sup> layer lines get stronger, whereas the 6<sup>th</sup> layer line becomes weaker. The reflections on the most of the layer lines become diffuse, indicating poor lateral crystallinity. Finally, the 6<sup>th</sup> layer line disappears (Fig. 8d) and a tilted (14°) fiber diffraction photograph has no reflection on the 6<sup>th</sup> layer line. A disappearance of the 6<sup>th</sup> layer line is consistent with a screw displacement in the double stranded chain with a 30° rotation and 0.22 nm translation along the common helical axis.

Similar conformational changes were observed in different preparations obtained by diffusion-



**Figure 9.** Reversible changes in the X-ray diffraction patterns of *iota*-carrageenan in the solid state sample after diffusion-in (a-d) and -out(e-h). Diffusion-in was performed in the mixture of 40% KI saturated solution and 60% ethanol. (a) : pattern recorded after diffusion-in for 33 hrs at room temperature, (b) : after 10 hrs at room temperature and 18 hrs at 45°, (c) : after 2 days at 45° and (d) : after 2 days at 45° and 2 hrs in magnetic stirring. Diffusion-out was done in 30% distilled water and 70% ethanol using the KI-induced (0.5 wt. ratio) *iota*-carrageenan. (e) : KI-induced structure of *iota*-carrageenan, (f) : after diffusion-out for 2 days and 4 hrs magnetic stirring of sample (e) at room temperature, (g) : after diffusion-out for 63 hrs and 10 hrs magnetic stirring of sample (e) at 45° and (h) : after diffusion-out for 4 days and 20 hrs magnetic stirring for 20 hrs of sample (e) at room temperature.

in and diffusion-out techniques. Both techniques aim at preserving the solid state during diffusion of cations or anions. Fig. 9 was obtained by reversible diffusion-out and diffusion-in on the condensed fibers. After 33 hrs of diffusion-in at room temperature, the KI diffused structure of *iota*-carrageenan (Fig. 9a) gives the 5<sup>th</sup> layer line. The pattern of fully KI-diffused *iota*-carrageenan (Fig. 9c) displays the 1<sup>st</sup> and 5<sup>th</sup> layer lines with diffuse reflections. After subsequent diffusion-out the 1<sup>st</sup> and 5<sup>th</sup> layer lines characteristic of the KI-induced structure of *iota*-carrageenan progressively disappears (Fig. 9e-h). Fig. 9e corresponds to 0.5 (wt. ratio) KI associated *iota*-carrageenan. Fig. 9f, obtained after diffusion-out for 2 days, including 4 hrs magnetic stirring at room temperature, illus-

trates that the even layer lines become stronger and the odd ones weaker. Finally, in Fig. 9h the odd diffraction is absent. This should be compared with Fig. 4b which illustrates that the diffraction pattern of a dialyzed TMAI-associated *iota*-carrageenan, which has been dialyzed in solution before making a dried sample, also does not have the odd layer lines but the clear Bragg reflections on even layer lines, indicating a high degree of polymer orientation. The reversibility of the process suggests that the anions are not strongly bounded to specific sites of carrageenan.

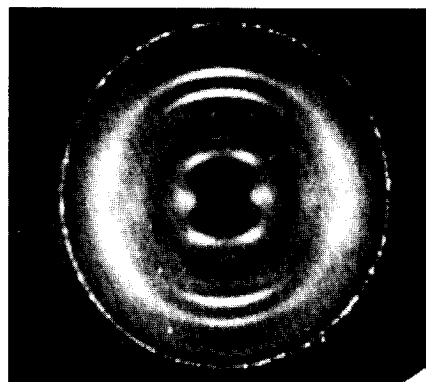
The observation of reversible conformational changes in *iota*-carrageenan supports the double helical gelation model. The osmometry and light-scattering experiments showed that the



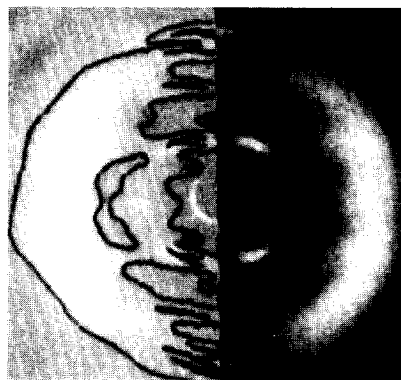
formation of the ordered conformation was not associated with any increase in molecular weight but the higher intrinsic viscosity suggested a stiffening of the chains.<sup>8,14</sup> Therefore, the ordered-disordered conformation transition must be intramolecule without any change in molecular weight. The primary mechanism for gelation is a change in the solvent properties rather than ion binding which occurs in the aggregated state below the sol/gel transition temperature. X-ray diffraction patterns are consistent in the double helical gelation mechanism (Fig. 2c), which involves the transformations of a flexible double helix to stiffen double helix. The two types of interactions between carrageenan and salt can also consider here: the stabilizing salt association interaction originating from the salts on the left hand side  $H(h^-)-H(h^+) < 0$  in Fajans' classification and the destabilizing salt dissociation interaction by the salts on the right side  $H(h^-)-H(h^+) > 0$  of this classification.

**X-ray Diffraction Pattern of the Mixture of *iota*- and *kappa*-Carrageenan.** Diffraction pattern of the KI-induced kappa-carrageenan contains a meridional reflection at 0.83 nm on the 3<sup>rd</sup> layer line (Fig. 10). This result may relate with NMR result<sup>22</sup> that the spectra of the KF- and KCl-induced structures of kappa-carrageenan are considerably different from that of the KI-induced structures.

Synergistic interactions which are exhibited by certain polysaccharide mixtures and enhance their ability to gel have been studied with kappa-carrageenan.<sup>29</sup> The mixture xanthan or algal polysaccharide helix and the glucomannan backbone showed intermolecular binding and helped to establish the stereochemical basis of such binding. No synergistic intermolecular interaction was, however, found in *iota*- and *kappa*-carrageenan except the electrostatic interaction caused by two



**Figure 10.** X-ray diffraction pattern of the KI-induced structure of kappa-carrageenan after 1 year annealing at room temperature.



**Figure 11.** X-ray diffraction pattern of a mixture of *iota*- and *kappa*-carrageenans.

polyanions. Fig. 11 illustrates the relative weakness, but not absence, of the 1<sup>st</sup> odd layer line in X-ray diffraction pattern, similar to anion effect in *iota*-carrageenan. The reflections of *iota*- and *kappa*-carrageenan overlap on the 1<sup>st</sup> even layer.

**Conclusions.** X-ray diffraction patterns of the destroyed half-staggered structure of *iota*-carrageenan indicate conformational transition. This could be due 1) to a displacement of one chain relative to other or 2) to a "difference" between the two chains resulting from a perturbation of the conformation. New X-ray diffraction patterns suggest an interpretation of the gela-

tion mechanism and of the structure of carrageenans.

The reversible structural changes not only in aqueous solution but also in solid fibrous state suggest that the best model of the mechanism of gelation is the one in Fig. 2c. Diffused diffraction patterns in the presence of right hand side metallic salts in Fajans' classification indicates poor lateral association and is consistent with an inability to gel. In terms of conformational changes the alkali halides fall in two classes. Those with  $H(h^-) - H(h^+) < 0$  stabilize the conformation, whereas those with  $H(h^-) - H(h^+) > 0$  destabilize it. The counteraction-mediated mechanism occurs with  $H(h^-) - H(h^+) < 0$  of salts. The way to consider both contercations and counteranions relatively is a new approach method to interpret the relations in solution.

The diffusion-in and -out techniques which we developed for sample preparation allow to examine the structural changes of carrageenans in fibrous state. The results support models with a double-helical half-staggered structure. The observation of the 1<sup>st</sup> and 5<sup>th</sup> layer lines of *iota*-carrageenan is in agreement with a double helical structure.

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