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The Effect of Alkali Treatment on the Chemical Heterogeneity and Physical Properties of Some Carrageenans

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Alkali treatment markedly influences the weight distribution of carrageenan fractions with respect to their solubility in potassium chloride solutions. All samples investigated originally contained molecules of widely different solubilities; in most cases, only two distinct fractions were present after the treatment, one precipitating below 0.15 M potassium chloride and one soluble at any potassium chloride concentration up to 1.5 M. Both the chemical and the physical properties of these two fractions varied among the algae.

Results are presented to demonstrate that the main effect of the alkali treatment is on the fraction originally precipitated at intermediate potassium chloride concentrations, leading to a higher content of 3,6-anhydro-D-galactose, a higher gel strength, and a lower solubility. The content of 3,6-anhydro-D-galactose cannot, however, be the only factor controlling the solubility level.

The results obtained are discussed with view of the present knowledge about the chemical structure of the *Chondrus* carrageenan.

The same technique was also applied to furcellaran, phyllophoran, and agar.

In a previous publication [1] the heterogeneity of carrageenan samples prepared from three different species, *Chondrus crispus*, *Gigartina stellata*, and *Gigartina skottsbergii*, was investigated by means of fractional precipitation with potassium chloride. The fractionation obtained was sufficiently sharp to allow the conclusion that the carrageenans could not consist of only two components ("$\varkappa$"- and "λ"-carrageenan), but rather of a series of molecules of different chemical composition. The distribution of molecules of different composition was found to be remarkably different for the three carrageenans. A fractionation according to the classical scheme [2] (precipitation at 0.125 M potassium chloride) was shown to give two chemically heterogeneous fractions,

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an observation that may explain the results obtained by Black *et al.*[3] — that $\varkappa$- and λ-preparations of different carrageenans were of different chemical composition. A close correlation was found to exist within each carrageenan sample between the content of 3,6-anhyro-D-galactose (3,6-AG) of the molecules and the potassium chloride concentration at which they became insoluble. The molecules with the highest 3,6-AG content had the lowest solubility and the highest gel strength and gave the main contribution to the gel strength of the unfractionated sample.

It is well known among producers of carrageenan [4] that the gel strength may be increased considerably by treating the carrageenan with alkali. Rees [5,6] has shown that the 3,6-AG content of λ-preparations increased by alkaline treatment with a concomitant release of sulfate from the 6-position of the 1,4-linked galactose unit. Stanley [4] reported that neither the $\varkappa$- nor the λ-preparation gave any increase in milk gel strength upon alkali treatment, whereas an unfractionated carrageenan sample gave a considerable increase. Data obtained by combined physical and chemical investigations are lacking in the literature, and it is therefore uncertain which fraction of the carrageenan is responsible for the change in gelling properties effected by the alkali treatment. The main purpose of this paper is to obtain information on the heterogeneity of carrageenans prepared from different raw materials by using fractional precipitation with potassium chloride, and to study the effect of the alkaline treatment on different carrageenans and carrageenan fractions.

RESULTS

Carrageenan prepared from *Gigartina stellata* was treated with aqueous potassium hydroxide solutions of different concentrations. The reaction was carried out at temperatures between 60 and 100° and the gel strength in milk determined on samples removed at intervals. According to Rees,[7] a small amount of potassium borohydride was used during the reaction to avoid excessive degradation. The gel strength increased towards a maximum and then decreased sharply upon further treatment. A temperature of 100° and a potassium hydroxide concentration of 1 M was found to be suitable conditions for routine treatments. The results of an experiment carried out under these conditions are given in Fig. 1. The reaction was followed by determining the milk gel strength, the intrinsic viscosity, and the 3,6-AG content. Fig. 1 shows that the 3,6-AG content increased markedly during the first hour of treatment and rapidly approached a limit value. The intrinsic viscosity decreased steadily, the gel strength reaching a maximum value after 1 hour. All subsequent alkali treatments were therefore carried out for one hour at 100° in 1 M potassium hydroxide.

The three carrageenans prepared from *Chondrus crispus*, *Gigartina stellata*, and *Gigartina skottsbergii*, used in the previous investigation,[1] were treated with alkali. Analytical data, obtained before and after the treatment, are given in Table 1. The treatment led to a higher 3,6 AG content, a lower intrinsic viscosity, and a slightly lower sulfate content for all three samples. The most pronounced effect was the increase in gel strength. It should be noted that the

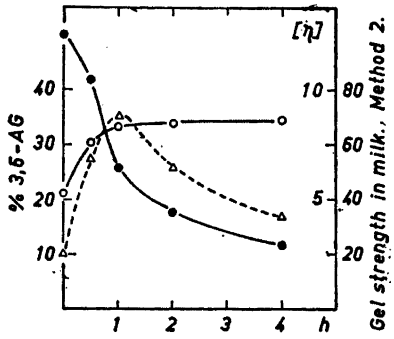

Fig. 1. The influence of alkaline treatment on the 3,6-AG content, the intrinsic viscosity, and the gel strength in milk, *Gigartina skottsbergii*. Treatment in 1 N KOH at 100°C.
○, 3,6-AG; ●, [η]; △, Gel strength in milk.

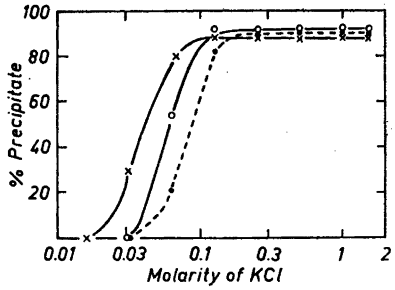

Fig. 2. Precipitation curves for alkaline treated carrageenan samples (1 h, 100°C, 1 N KOH).
×, *Chondrus crispus;* ○, *Gigartina stellata;* ●, *Gigartina skottsbergii.*

gel strengths did not reach the same value for the three samples, but ranked them in the same order as before the treatment.

Fractional precipitation with potassium chloride was carried out for the alkali treated samples and the results are given in Fig. 2. The precipitation curves, giving the amount of insoluble carbohydrate as a function of the potassium chloride concentration, demonstrate that the precipitation occurs in a rather narrow range of potassium chloride concentrations below 0.125 M. A small fraction of the material is soluble at this molarity, and at any concentration up to 1.5 M. The amount and composition of this soluble material

Table 1. Chemical and physical properties of carrageenan samples before and after alkali treatment (1 h, 100°, 1 N KOH).

	Chondrus crispus	*Gigartina stellata*	*Gigartina skottsbergii*
Before treatment:			
% 3,6-AG	23.5	23.5	20.9
% SO_3Na	32.2	33.7	34
Gel strength in milk[a]	280	60	20
[η], (100 ml/g)	8.7	9.2	8.4
After treatment:			
% 3,6-AG	31.5	30.9	30
% SO_3Na	32.0	33.1	33.5
Gel strength in milk[a]	980	630	280
[η]	6.6	6.6	7.0

[a] Method 1.

is given in Table 2. For comparison, the data obtained with the corresponding fractions isolated from untreated samples are included in the table. In contrast to the other two algae, the fraction from *Chondrus*, soluble in 1.5 M potassium chloride after treatment, has a considerably higher 3,6-AG content than the corresponding fraction isolated before treatment. The amount of soluble material has been reduced by approximately 10 % by the treatment in the carrageenan from *Chondrus*, by approximately 50 % for the two other carrageenans.

Table 2. Yield and 3,6-AG content of fraction soluble in 1.5 M KCl obtained before and after alkali treatment of whole carrageenan.

	Before treatment		After treatment	
	Yield	% 3,6-AG	Yield	% 3,6-AG
Chondrus crispus	12	5	11	19
Gigartina stellata	8	7.5	4	9.5
Gigartina skottsbergii	18	12	10	13

The differential distribution curves given in Fig. 3 (calculated from the curves of Fig. 2, *cf.* Ref. 1) demonstrate a considerable effect of the alkaline treatment on the weight distribution of material of different solubility. The amount of material precipitating between two potassium chloride concentrations is seen in the diagrams as the area under the curves between the two concentrations in question. The total area corresponds to the amount rendered insoluble at the highest potassium chloride concentration. This is approximately 90 % for the three carrageenans. The curves demonstrate that the alkali treatment has decreased the solubility level only of the fractions, which, before treatment, precipitated at intermediate and high potassium chloride concentrations. The solubility of the fractions precipitated at the lowest concentrations before treatment has remained unchanged. The material was therefore markedly more homogeneous with respect to solubility in potassium chloride after the alkali treatment.

In the previous investigation we divided the carrageenan into three fractions by precipitation with potassium chloride. Essentially the same procedure was now repeated for the three untreated carrageenans. Three different fractions were prepared: Fraction 1 was the precipitate at 0.0625 M potassium chloride, Fraction 2 the precipitate between 0.0625 M and 1.5 M, and Fraction 3 the part soluble in 1.5 M. The recovery in the fractionation procedure was between 84 and 90 %. The yield (calculated as percent of total recovery), the 3,6-AG content, and the gel strength for each fraction is given in Table 3. All data are in close accordance with those obtained in the previous experiment, and the yields also compare favourably with those expected from the differential distribution curves.

The above fractions were then treated with alkali, according to the standard procedure and the gel strength, and the 3,6-AG content, determined. The results are given in Table 3 to allow comparison with the corresponding

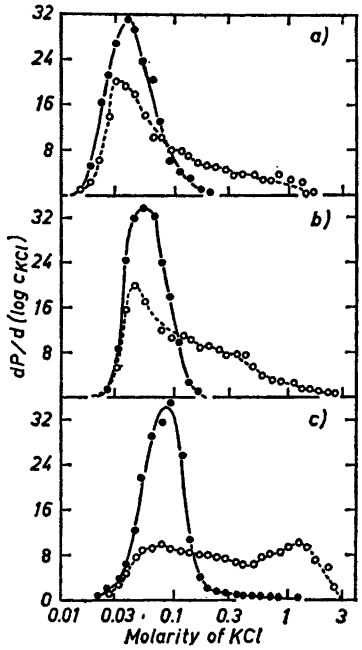

Fig. 3. Differential distribution of material with different solubility before and after alkaline treatment (1 h, 100°C, 1 N KOH). - - - - bejore treatment; ——— after treatment. a) *Chondrus crispus;* b) *Gigartina stellata;* c) *Gigartina skottsbergii.*

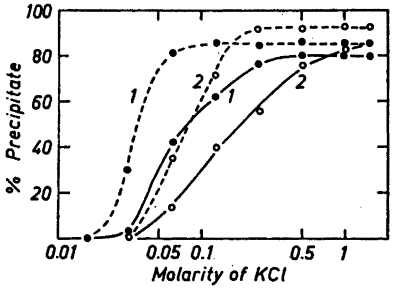

Fig. 4. Precipitation curves. 1, *Chondrus crispus*, east coast of Canada. 2, *Gigartina stellata*, east coast of Canada. ————, original sample; - - - - - -, after alkali treatment.

data before the treatment. The 3,6-AG content increased in all fractions, but considerably more in fractions 2 and 3 than in Fraction 1. The significant difference in 3,6-AG content between the original fractions 1 and 2 thus disappeared as a result of the alkali treatment. This also applied to the gel strengths of the same fractions. These observations give further support to the assumption above that the carrageenans are more homogeneous after the treatment than before.

A comparison of the alkali-treated Fraction 3 with the fractions soluble in 1.5 M KCl, and obtained from the same carrageenan treated with alkali prior to fractionation (Table 2), reveal that, except for *Chondrus crispus*, the former fractions are considerably higher in 3,6-AG. As the yield is also higher, this indicated a chemical heterogeneity in the alkali-treated fractions 3. The treated fractions were therefore fractionated at 1.5 M potassium chloride, and the results are given in Table 4. The 3,6-AG content of the material soluble in 1.5 M potassium chloride is now in close accordance with the fraction previously obtained (Table 2). The yield, on the other hand, is somewhat lower than expected. A preferential loss of the most soluble material may very likely be the explanation of this difference. Table 4 also shows that fraction

Table 3. Fractionation of carrageenan (5 g) and alkaline treatment of the fractions.

Fraction No.	Weight, g	Before treatment: % of recovery	Before treatment: Theoretical amount[a]	Before treatment: % 3,6-AG	Before treatment: Milk gel strength[b]	After treatment: % 3,6-AG	After treatment: Milk gel strength[b]
Chondrus crispus, unfractionated				23.5	60	31.5	150
1	2.21	50.9	48	29.2	145	34.0	175
2	1.60	36.6	39	25.0	20	33.0	120
3	0.53	12.2	13	4.9	0	22.0	0
Recovery: 4.34 g = 87 %							
Gigartina stellata, unfractionated				23.5	30	30.9	90
1	1.19	28.3	30	28.4	61	32.4	95
2	2.51	59.6	62	25.0	19	32.9	85
3	0.51	12.1	8	10.1	0	26.5	35
Recovery: 4.21 g = 84 %							
Gigartina skottsbergii unfractionated				20.9	15	30.0	60
1	0.52	11.7	14	29.4	70	33.3	80
2	3.12	69.9	68	20.6	8	33.4	60
3	0.82	18.4	18	10.1	0	30.1	54
Recovery: 4.46 g = 89.4 %							

[a] These figures are taken from the precipitation curves in the preceding paper.[1]
[b] Method 2.

3 contains a certain amount of material which, on alkali treatment, obtains a 3,6-AG content and a solubility in potassium chloride similar to those of the treated fractions 1 and 2. This material must be the cause of the high gel strengths of the treated fractions 3 from *Gigartina stellata* and *Gigartina skottsbergii*.

Table 4. Fractionation of the alkali-treated Fraction 3 at 1.5 M potassium chloride.

Algae	Insoluble			Soluble		
	% of fraction	% of total	% 3,6-AG	% of fraction	% of total	% 3,6-AG
Chondrus crispus	19	2.3	22.8	81	9.9	20.2
Gigartina stellata	78	9.5	30.5	22	2.6	7.6
Gigartina skottsbergii	75	13.8	33.0	25	4.6	12.3

The sharp fractionation obtained with potassium chloride, together with the characteristic change in solubility level occurring for part of the carrageenan during the alkaline treatment, suggested this technique as a valuable tool for characterizing carrageenans of different origin. The method was applied to carrageenans isolated from *Chondrus crispus* and a variety of *Gigartina* species and the results are collected in Table 5. The gel strength in milk, the intrinsic viscosity, and the content of 3,6-AG and sulfate was determined before and after the standard alkali treatment. Precipitation curves were obtained (Figs. 4, 5, and 6) and the 3,6-AG content determined in the two fractions insoluble in 0.125 ($I_{0.125}$) and soluble in 1.5 M potassium chloride ($S_{1.5}$), respectively. The yields of these, and of the intermediate fractions, are given in Table 5. The results show that the samples investigated differ widely in chemical composition and physical properties. Among the whole, untreated carrageenans there seem to be no correlation between the 3,6-AG content and the gel

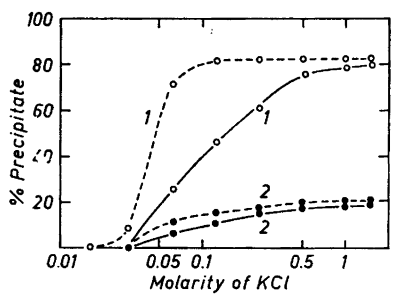

Fig. 5. Precipitation curves. 1, *Gigartina stiriata*, South Africa; 2, *Gigartina acicularis*, Portugal. ————, original sample; - - - - -, after alkali treatment.

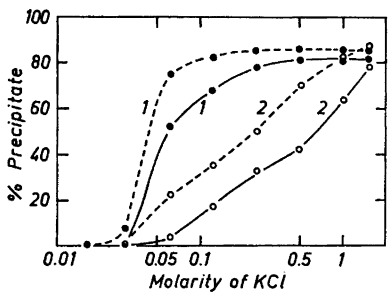

Fig. 6. Precipitation curves. 1, *Gigartina tenella*, Korea; 2, *Gigartina pistillata*, Portugal. ————, original sample; - - - - -, after alkali treatment.

Table 5. Chemical and physical properties of whole carrageenan samples before and after alkali treatment.

Algae	Milk gel strength[a]	$[\eta]$ 100 ml/g	3,6-AG %	SO_3Na %	3,6-AG in fractions		Amount of fractions, %		
					$I_{0.125}$	$S_{1.5}$	I_{0125}	Inter-mediate	$S_{1.5}$
C. crispus, Stackh. sunbleached, Canada.									
Untreated	185	8.3	19.3	29.0	31.5	4.4	62	18	20
Treated	830	4.9	28.5	29.0	36.5	18.5	85	0	15
G. stellata (Stackh.) Batt. Canada.									
Untreated	60	11.3	24.0	33.0	26.2	7.1	39	46	15
Treated	180	5.5	30.2	32.7	33.2	6.7	73	19	8
G. Stiriata (Turn) J.Ag., South Africa.									
Untreated	110	12.8	23.0	37.3	30.8	2.5	46	34	20
Treated	850	6.6	33.2	35.3	37.4	19.0	82	1	17
G. acicularis (Wulf.) Lamour., Portugal.									
Untreated	0	11.7	5.9	40.2	18.6	3.2	11	8	81
Treated	20	7.0	14.6	36.3	25.2	13.0	16	5	79
G. pistillata (Gmel.) Stackh., Portugal.									
Untreated	25	14.2	9.5	40.4	14.1	2.5	17	61	22
Treated	85	4.0	15.6	39.8	24.6	5.1	35	52	13
G. tenella Harvey, Korea.									
Untreated	135	6.0	24.2	28.4	32.0	9.8	68	14	18
Treated	475	4.2	30.8	27.6	34.0	16.2	83	3	14

[a] Method 1.

strength. A good correlation does, however, exist between the gel strength and the solubility in potassium chloride.

For all the carrageenans the effect of the alkali treatment was to increase the 3,6-AG content and the gel strength, and consequently, to decrease the solubility in potassium chloride. The samples of the highest gel strength before the treatment also have the highest gel strength after the treatment, which is in keeping with the results of Table 1.

Table 6. Chemical and physical properties of red algae extracts before and after an alkali-treatment.

Extract	Milk gel strength[a]	$[\eta]$ 100 ml / g	3,6-AG %	SO_3Na %	3,6-AG in fractions	
					$I_{0.125}$	$S_{1.5}$
Phyllophora nervosa (D.C.) Grev. commercial, USSR.						
Untreated	40	3.6	27.5	31.0	34.5	4.4
Treated	145	2.8	31.3	28.6	36.5	4.0
Agar, Difco						
Untreated			38.5		42.0	31.0
Treated			42.0		42.5	38.3
Furcellaria fastigiata (L.) Lamour. commercial, Denmark.					$I_{0.03}$	$S_{0.125}$
Untreated	500		33.0	18.6	35.5	9.5
Treated	> 1000		35.6	18.4	37.0	12.5

[a] Method 1.

The precipitation technique may be used for polysaccharides from red algae not belonging to the carrageenan group. The results obtained with commercial preparations of furcellaran, phyllophoran, and agar are given in Table 6 and Fig. 7. The precipitation procedure had to be slightly modified for these compounds. To avoid gelling, the concentration had to be kept considerably lower than 0.25 %. The furcellaran sample was extremely sensitive to potassium ions and had to be dialysed before the precipitation experiment. For the agar it was found particularly important to equilibrate the solution overnight at 20° before use. Table 6 shows that all three samples contain material capable of forming 3,6-AG upon alkali treatment. The furcellaran sample had a higher gel strength and 3,6-AG content, and a markedly lower sulfate content than any of the untreated carrageenans. The 3,6-AG content of the phyllophoran is slightly higher than the highest found for carrageenan. The sulfate content, the milk gel strength, and the solubility in potassium chloride do not differ very much from that found for the carrageenan from *Gigartina stellata*. The agar sample had a limited solubility in cold water

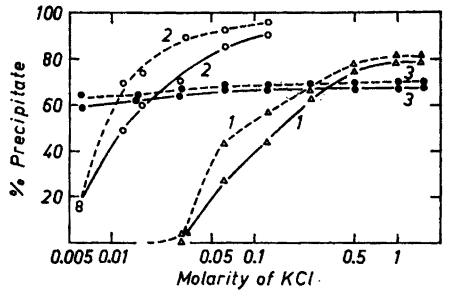

Fig. 7. Precipitation curves. 1, Phyllophoran, USSR, 0.1 %; 2, Furcellaran, Denmark, 0.1 % 3, Agar, Difco, 0.05 %. ————, original sample; - - - - -, after alkali treatment.

and the solubility was influenced only to a small extent by the addition of potassium chloride. The 3,6-AG content of the soluble fraction increased upon alkaline treatment without altering the solubility in potassium chloride. An agarose sample (commercial preparation from Litex, Denmark) was tested by precipitation with potassium chloride. The amount of precipitate was approximately 80 %, regardless of potassium chloride concentration.

DISCUSSION

In our previous publication [1] we showed that the solubility of carrageenan molecules in potassium chloride solutions was closely correlated to the 3,6-AG content of the molecules. The influence of co-precipitation and of variation in the intrinsic viscosity was negligible, and a precipitation curve was found to be useful as a characterization of the distribution of the carrageenan molecules with respect to their 3,6-AG content. We tentatively distinguished between three fractions: a) the fraction soluble at all KCl concentrations ("λ"-fraction), b) the fraction precipitated with KCl at concentrations above 0.125 M KCl ("intermediate" fraction), and c) the fraction precipitated at KCl concentrations below 0.125 M ("$\varkappa$"-fraction). All three fractions were heterogeneous with respect to chemical composition. The $\varkappa$-fraction, defined in this way, is in agreement with the original definition of Smith and Cook;[2] while the other two fractions are not.

The results described above (Figs. 2, 4, 5, and 6) show that the precipitation curves of the carrageenan samples changed significantly when the samples were treated with alkali. As previously reported, the gel strength of the samples also increased markedly (Tables 1 and 5). These observations clearly demonstrate that the physical properties of the carrageenan samples were changed by the alkali treatment. Three carrageenan samples, prepared from *Chondrus crispus*, *Gigartina stellata*, and *Gigartina skottsbergii*, were examined in detail. The distribution curves (Fig. 3) obtained after alkali treatment, compared to the corresponding curves obtained before the treatment, indicate that the main effect of the alkali treatment is to transform the "intermediate" fraction into a fraction which precipitates at KCl concentrations below 0.125 M, *i.e.* similar or identical to the $\varkappa$-fraction. In order to confirm this, the fractions obtained by KCl precipitation of the original carrageenan samples were

treated separately with alkali. The results show (Table 3) that the main effect was on the "intermediate" fraction, leading to a marked increase of the 3,6-AG content and gel strength of this material. The difference between this fraction and the $\varkappa$-fraction after the treatment is negligible compared to the differences between the untreated fractions.

The results, therefore, indicate that the narrow-distribution curves of the alkali-treated carrageenans really represent a very narrow distribution of 3,6-AG contents. It should be noted that the material not precipitated by KCl is not included in the distribution curves. The significant differences in physical properties among the carrageenans also persisted after the alkali treatment, even though the 3,6-AG contents were very similar.

In our previous publication the marked physical difference between samples with similar 3,6-AG content were attributed to a different distribution of the 3,6-AG units among the macromolecules. The explanation holds good for untreated carrageenan samples. Owing to the narrow 3,6-AG distribution obtained, the same explanation cannot be valid after the alkali treatment. The results therefore strongly suggest that chemical differences, other than different 3,6-AG contents, occur among the KCl insoluble fractions of the alkali-treated carrageenans. This may be differences in the position and distribution of the sulfate half-ester groups, in the repeatability of 1,3- and 1,4-linkages, and possibly in degree of branching. Structural investigations of such alkali-treated samples may yield valuable information about the correlation between chemical structure and gel strength. Assuming the formation of 3,6-AG to take place by the accepted alkali-catalysed elimination of sulfate half-ester, a decrease of the sulfate content should occur simultaneously; an increase in the 3,6-AG content from 20 to 30 % corresponding to a decrease in sulfate from 30 to 27 %. By alkali treatment of λ-carrageenan Rees [5] observed the release of 1.2 moles of sulfate for each mole of 3,6-AG formed. Stanley,[4] on the other hand, was unable to detect any loss of sulfate by the alkali treatment. The data obtained during this investigation indicated only a slightly lower sulfate after the treatment, *i.e.* an apparent loss of sulfate somewhat lower than that expected for a stoichiometric elimination reaction. Together with the comparatively low accuracy of the analytical method employed, this prevents any conclusion concerning the apparent discrepancy met with in the cited publications.

The chemical composition of the KCl soluble fraction after alkali treatment also varies considerably from one sample to another. The *Chondrus crispus* sample contains (after alkali treatment) a relatively high proportion of KCl soluble material with a high 3,6-AG content. This is in contrast to the other two samples, which contain a smaller amount of KCl soluble material with a lower 3,6-AG content.

Structural investigations of carrageenan components of *Chondrus crispus* have been carried out by Rees and coworkers.[5,7,9] According to their results, the λ-component of *Chondrus crispus* carrageenan has a very low 3,6-AG content before alkali treatment, and one which increases after treatment to a high value (13.8 and 30 % reported for two different preparations). The solubility properties, however, have not been reported in any detail; but, according to the fractionation procedures used, it has a relatively high solu-

bility in KCl solutions both before and after alkali treatment. It is therefore reasonable to assume that our KCl soluble component is at least closely related to the λ-carrageenan investigated by Rees. According to Rees, a distinctive feature of this compound is the occurrence of a sulfate half-ester group at position 2 in the 4-linked galactose unit, *i.e.* in the 3,6-AG unit after alkali-treatment. The effect of the 3,6-AG units on the solubility of the polymer was discussed by Painter.[10] He suggested that the less hydrophilic character of the 3,6-AG units decreases the solubility of the polymer. It should then be expected that a highly hydrophilic substituent like a sulfate group on the 3,6-AG units should exert a marked effect on the solubility of the polymer. The high solubility of the alkali-treated "λ"-fraction from *Chondrus crispus* carrageenan, in spite of its high 3,6-AG content, therefore, agrees closely with Rees' structural formula.

In addition to the two classical components, λ- and ϰ-carrageenan, Rees also found a third component. This component, as originally described by Rees, had a very low content of 3,6-AG, and was, by alkali treatment, transformed into ϰ-carrageenan. In a recent review article,[8] however, Rees indicated a very large variation in the chemical composition of the "third component". If we assume that "the third component" may occur in native carrageenan at different stages in the transformation to ϰ-carrageenan, this component may correspond to our "intermediate fraction".

Our results give no indication of any sharply defined fractions in the KCl insoluble part of the three carrageenans investigated, but are consistent with a continuous distribution of molecules with compositions varying from a low 3,6-AG content to a content corresponding to that reported by Rees for ϰ-carrageenan.

Even if no sharp distinction exists between the ϰ-fraction and the intermediate fraction, it may, from a practical point of view, be convenient to distinguish between them. In native carrageenans the gel strength is almost solely due to the ϰ-fraction. The intermediate fraction is, however, potentially a gel forming agent, in that an alkali treatment transforms it into material with approximately the same gel-forming ability of the ϰ-fraction from the same species.

The carrageenans isolated from the different species vary over a very wide range. Native carrageenans rich in ϰ-carrageenan, and containing a relatively small amount of intermediate fraction, have been isolated from *Chondrus crispus* and *Gigartina tenella*. This is also to some extent the case for the sample isolated from *Gigartina stiriata*. All these three carrageenans have a relatively high gel-strength, both before and after alkali-treatment. They are also characterized by a λ-fraction with high 3,6-AG content after alkali treatment. *Gigartina stellata* and *Gigartina skottsbergii*, on the other hand, are typical representatives of algae giving carrageenans with a high content of intermediate fraction.

Gigartina pistillata and *Gigartina acicularis* are clearly distinguished from the rest of the species investigated. Both of them give carrageenans containing a very small amount of ϰ-fraction, and samples from the two species would not be distinguished by the usual fractionation procedure (0.125 M KCl). Commercially the two species are usually not separated, but are sold under the

designation *Gigartina acicularis, pistillata*. The results given in Table 5, however, reveal that the two species give very different carrageenans. For *Gigartina pistillata* both the solubility in potassium chloride and the gel strength of the intermediate fraction is altered by the alkali treatment; but the distribution after treatment is not of the narrow type obtained for the other carrageenans. The carrageenan from *Gigartina acicularis*, on the other hand, contains mainly a fraction which remains soluble after the alkali treatment and the gel strength is negligible both of native and alkali-treated samples. It should be emphasized that the results reported above are necessarily not representative of the different species, since large variation within the same species have previously been reported.[3]

With some modifications, the precipitation technique may also be used for polysaccharides from other red algae than those yielding carrageenan. The precipitation curves for phyllophoran and furcellaran indicate that both these samples contain material with different solubilities in KCl and that the solubility is decreased by the alkali treatment. The furcellaran sample has a solubility in KCl solutions lower than any of the carrageenans investigated. The phyllophoran sample, on the other hand, cannot be distinguished from the carrageenans by its solubility in KCl solution. The agar sample behaves very differently from the other samples investigated and shows a remarkable lack of salt sensitivity — a characteristic of neutral polysaccharides.[11] It is well known that agar contains two components: agarose and agaropectin. Hence it seems reasonable to assume that the observed fractionation with potassium chloride has given a separation between these two compounds.

EXPERIMENTAL

The preparation of the carrageenan samples was carried out essentially as described previously.[1] When repeated extractions at 80° failed to remove more carbohydrate, the algae were extracted two times for half an hour at 120°. The extracts were combined before precipitation.

The alkali treatment was carried out using 1 % carrageenan solution containing 0.1 % potassium borohydride and 1 M potassium hydroxide. The solution was heated on a boiling water bath for one hour and then rapidly cooled. The reaction mixture was placed in an ice bath and neutralized with 5 M hydrochloric acid with vigorous stirring and continuous pH recording. The carrageenan was precipitated with equal amounts of ethanol and washed with 70 % ethanol until salt-free, washed with ethanol and ether, and dried over night at 40°. Even with the above precautions, the neutralization step some times resulted in severe acid degradation and, when small amounts of carrageenan were prepared, the neutralization step was omitted, the carrageenan being washed with 70 % ethanol until neutral.

The carrageenan samples were fractionated in essentially the same way as described previously.[1] Fraction 1 was the precipitate at 0.125 M potassium chloride, Fraction 2 the precipitate between 0.125 and 1.5 M, and Fraction 3 the soluble part at 1.5 M potassium chloride.

Precipitation curves, 3,6-AG contents, intrinsic viscosity, and gel strength in milk were determined as described before.[1] The sulfate content was determined in the following way: 50 mg carrageenan was dissolved in 2 ml of water and dialysed three times against distilled water to remove traces of salts. The solution was washed onto a column of cation exchange resin in acid form, washed out with distilled water, and titrated with 0.01 M sodium hydroxide.

REFERENCES

1. Pernas, A. J., Smidsrød, O., Larsen, B. and Haug, A. *Acta Chem. Scand.* **21** (1967) 98.
2. Smith, D. B. and Cook, W. H. *Arch. Biochem. Biophys.* **45** (1953) 323.
3. Black, W. A. P., Blakemore, W. R., Colquhoun, J. A. and Dewar, E. T. *J. Sci. Food Agr.* **16** (1965) 573.
4. U.S. Patent No. 3.094.517 (1963).
5. Rees, D. A. *J. Chem. Soc.* **1963** 1821.
6. Dolan, T. C. S. and Rees, D. A. *J. Chem. Soc.* **1965** 3534.
7. Rees, D. A. *J. Chem. Soc.* **1961** 5168.
8. Rees, D. A. *Ann. Rept. Chem. Soc.* **62** (1965) 469 with references to most chemical work on carrageenan.
9. Anderson, N. S. and Rees, D. A. *Proc. 5th Intern. Seaweed Symposium*, Halifax 1965, Pergamon, London 1967, p. 243.
10. Painter, T. J. *Proc. 5th Intern. Seaweed Symposium*, Halifax 1965, Pergamon, London 1967, p. 305.
11. Smidsrød, O. and Haug, A. *J. Polymer Sci.* **C 16** (1967) 1587.

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