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Alkaline modification of carrageenans. Part III. Use of mild alkaline media and high ionic strengths[1]

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The cyclization reaction (formation of 3,6-anhydro-α-D-galactose and 3,6-anhydro-α-D-galactose 2-sulfate units from α-D-galactose 6-sulfate and 2,6-disulfate residues, respectively) of carrageenans follows a pseudo-first-order kinetics. This reaction can be carried out, at reasonable rates, in sodium hydroxide solutions less concentrated than those usually employed or by using a milder base such as sodium carbonate. Rate constants were also determined at a fixed sodium hydroxide concentration but using different ionic strengths. No specific salt effects were observed at a given sodium hydroxide concentration and ionic strength. © 1997 Elsevier Science Ltd

INTRODUCTION

Alkaline modification of carrageenans is produced through the cyclization of the α-D-galactose 6-sulfate and 2,6-disulfate units to the corresponding 3,6-anhydro derivatives in strong alkaline media. In a previous paper (Ciancia *et al.*, 1993b) it was shown that the cyclization reaction follows a pseudo-first-order kinetics and the change of the rate constant with strong sodium hydroxide concentrations and temperature was investigated. Considering the ease of the reaction for carrageenans of the kappa family and that the strong alkaline medium usually used produces some depolymerization, it was suggested that this treatment could be carried out under milder conditions to obtain less degraded products of higher gel strengths.

Here we report the use of sodium carbonate and also of lower concentrations of sodium hydroxide and high ionic strengths for the cyclization reaction.

EXPERIMENTAL

Materials

Cystocarpic plants of *Gigartina skottsbergii* were collected in Bahía Camarones (Provincia de Chubut, Argentina) and sorted by the Instituto Nacional Patagónico (CONICET; Puerto Madryn, Chubut).

Extraction and fractionation

The extraction and fractionation of the carrageenan (1C) and the analysis of the mu/nu-carrageenan ($1C_3$) have been described elsewhere (Matulewicz *et al.*, 1989).

Alkaline treatment

All the reactions were carried out at 80°C. The general procedure for the treatment in 0.02–0.5 M sodium hydroxide and 0.5–1.0 M sodium carbonate was carried out as described before (Ciancia *et al.*, 1993b).

For 1.5 and 2.0 M sodium hydroxide as well as for 1.5 M sodium carbonate final concentrations, the sample (30 mg) was dissolved in water (11.5 ml) and an equal volume of 3-4 M sodium hydroxide or 3 M sodium carbonate was added. For the reaction carried out at 2.4 M sodium carbonate final concentration, the sample (30 mg) was dissolved in water (4.6 ml) and 3 M sodium carbonate (17.7 ml) was added.

When sodium chloride or sodium sulfate were added to modify the ionic strength, carrageenan $1C_3$ was dissolved in a solution of the desired final concentration of the corresponding salt and the alkaline solution was also prepared in this way.

The standard deviation was determined for the rate constants and half-lives, and an average of 5–10% was estimated.

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RESULTS

The alkaline treatments were carried out on the same partially cyclized mu/nu-carrageenan ($1C_3$) used previously (Ciancia *et al.*, 1993b). It contained (mol%) 7.3% of α-D-galactose 6-sulfate and 21.2% of α-D-galactose 2,6-disulfate units as determined by the resorcinol method before and after periodate oxidation (Rees, 1961); average values of 5 and 10% were estimated for the former and latter residues by ^{1}H- and ^{13}C-NMR spectroscopy (Ciancia *et al.*, 1993a). Tables 1 and 2 show the pseudo-first-order rate constants (k_1) at different sodium hydroxide (0.02–2.0 M) and sodium carbonate (0.5–2.4 M) concentrations, respectively; these pseudo-first-order rate constants were determined from the corresponding plot of ($\ln A_0 - A_\infty / A_t - A_\infty$) as a function of time, where A is the absorbance assayed by the resorcinol method (Yaphe, 1960). To know whether the cyclization reactions of the α-D-galactose 6-sulfate and 2,6-disulfate units followed pseudo-first-order kinetics with different rate constants, attempts were made to describe the plot of ($\ln A_0 - A_\infty / A_t - A_\infty$) as a function of time by two straight lines. Ambiguous results were obtained and only for three of the 11 graphs was this achieved. In those cases k_1' and k_1'' values of the same order of magnitude were calculated (Tables 1 and 2). Thus, the system was treated as two homocompetive reactions with only one observed total rate constant (Lee, 1953). From the plot of the pseudo-first-order rate constant as a function of the sodium hydroxide concentration in the range 0.5–2.0 M, the real second-order rate constant was calculated ($k_2 = 2.3 \times 10^{-3}$ l mol^{-1} s^{-1}; for sodium carbonate (range 1.0–2.4 M) a value of $k_2 = 6.4 \times 10^{-3}$ l mol^{-1} s^{-1} was obtained. At the lower concentrations of sodium hydroxide (0.02 M) and sodium carbonate (0.5 M; 10 mM hydroxyl concentration) both reactions gave the same k_1 (0.05×10^{-4} s^{-1} and became very slow ($t_{1/2} \sim 37$–38 h).

Table 1. Cyclization reaction of $1C_3$ at different sodium hydroxide concentrations

NaOH (M)	I	pH	k_1 ($\times 10^4$ s^{-1})	$t_{1/2}$ (min)
0.02	0.02	12.3	0.05	2300
0.05	0.05	12.7	0.46	250
0.1[a]	0.1	13.0	1.3	88
0.5	0.5	13.7	8.3	14
1.0	1.0	14.0	26	4.5
1.5[b]	1.5	14.2	35	3.3
2.0	2.0	14.3	45	2.5

[a] For 0.1 M NaOH the plot of ($\ln A_0 - A_\infty / A_t - A_\infty$) as a function of time was satisfactorily described by two straight lines giving k_1' and k_1'' of 13×10^{-5} and 7.6×10^{-6} s^{-1}, respectively.

[b] For 1.5 M NaOH values for k_1' and k_1'' of 2.0×10^{-3} and 1.6×10^{-3} s^{-1} were obtained.

It is noteworthy that, while in 0.02 M sodium hydroxide (pH 12.3) the k_1 for the reaction was 0.05×10^{-4} s^{-1}, in 1.5 M sodium carbonate (also pH 12.3; equivalent to 18 mM hydroxyl concentration) a k_1 of 1×10^{-4} s^{-1} was determined, showing the influence of the ionic strength (0.02 and 4.5 for the sodium hydroxide and sodium carbonate solutions, respectively). This influence is also shown by the fact that the same rate constant (1.3–1.4×10^{-4} s^{-1} was obtained when the reaction was carried out with 0.1 M sodium hydroxide and 2.4 M sodium carbonate (22 mM hydroxyl concentration).

Table 3 shows the rate constant at different ionic strengths using a fixed and low sodium hydroxide concentration (0.05 M). A linear relationship was found between the pseudo-first-order rate constant and the ionic strength at sodium chloride concentrations in the range 0–2 *I*. At higher ionic strengths (2.5–5) the linear relationship was maintained but the slope changed.

Table 4 indicates the rate constant of the cyclization reaction at different sodium hydroxide concentrations but at a fixed ionic strength ($I = 1$) using sodium chloride or sodium sulfate as salts. For each of the sodium hydroxide concentrations, similar rate constants were obtained independent of the salt used, suggesting that there were no specific salt effects.

DISCUSSION

The cyclization reaction can be carried out, at reasonable rates, in sodium hydroxide solutions less concentrated than those usually employed (Table 1). A milder base, sodium carbonate, can also be used (Table 2) with the advantage that the higher ionic strength, at a similar hydroxyl concentration, accelerates the reaction (Tables 1 and 2). At pHs 12.0–12.3 (Tables 1 and 2) the cyclization reaction slows down and almost stops.

In a previous paper (Ciancia *et al.*, 1993b) it was suggested that the cyclization of α-D-galactose 2,6-

Table 2. Cyclization reaction of $1C_3$ at different sodium carbonate concentrations

Na_2CO_3 (M)	I	HO^- (mM)	pH	k_1 ($\times 10^4$ s^{-1})	$t_{1/2}$ (min)
0.5	1.5	10	12.0	0.05	2200
1.0	3.0	14	12.2	0.9	130
1.5	4.5	18	12.3	1.0	110
2.4[a]	7.0	22	12.4	1.4	84

[a] For 2.4 M NaOH the plot of ($\ln A_0 - A_\infty / A_t - A_\infty$) as a function of time was satisfactorily described by two straight lines giving k_1' and k_1'' of 10×10^{-5} and 7.5×10^{-5} s^{-1}, respectively.

Table 3. Cyclization reaction for $1C_3$ in 0.05 M NaOH at different ionic strengths[a]

I	NaCl (M)	k_1 ($\times 10^4$ s^{-1})	$t_{1/2}$ (min)
0.05	—	0.46	250
1	0.95	1.4	80
2	1.95	2.4	48
3	2.95	2.8	44
4	3.95	3.1	37
5[b]	4.95	3.8	30

[a] The pH of the solution was 12.7.

[b] It was not possible to measure the 3,6-anhydrogalactose content from 35 min onwards due to gel formation.

disulfate units to the corresponding 3,6-anhydrogalactose 2-sulfate residues involved a first step in which, after the ionization of the hydroxyl group on C-3, there was a repulsion between the net negative charges on the equatorial groups on C-2 and C-3 producing the destabilization of the $^4C_1$ conformation of these units. In the partially cyclized mu/nu-carrageenan $1C_3$ there are major amounts of α-D-galactose 2,6-disulfate units, but there are also considerable quantities of α-D-galactose 6-sulfate residues which also cyclize but do not have a substituent with a permanent negative charge on C-2. Thus, for these latter units the negative charge is created by ionization of the more acidic C-2 hydroxyl group (Haines, 1976). The ionization of the hydroxyl on C-3 would then be much more difficult, as is the ionization of the hydroxyl group on C-3 of the α-D-galactose 2,6-disulfate residues, due to the shielding effect of the net negative charge. This second ionization is, possibly, the rate determining step in agreement with the strong influence of the ionic strength. The ambiguous results obtained, when attempts were made to differentiate the pseudo-first-order rate constants for the cyclization of the α-D-galactose 6-sulfate and 2,6-disulfate units, could be understood considering that both rate constants are of the same order of magnitude.

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Table 4. Cyclization reaction for $1C_3$ at different NaOH concentrations, keeping the ionic strength equal to 1 by adding NaCl or Na_2SO_4

NaOH (M)	NaCl (M)	Na_2SO_4 (M)	pH	k_1 ($\times 10^4$ s^{-1})	$t_{1/2}$ (min)
0.5	—	0.17	13.7	14	8.4
0.5	0.5	—	13.7	14	8.5
0.1	—	0.3	13.0	3.6	32
0.1	0.9	—	13.0	3.8	31
0.05	—	0.317	12.7	1.1	103
0.05	0.95	—	12.7	1.4	80