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Alkali modification of carrageenans. Part IV. Porphyrans as model compounds

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Abstract

The rate of alkaline cyclization of porphyran is in the same order as those of carrageenan model compounds containing non-sulfated β-D-galactose units, showing that the cyclization of the α-galactosyl units does not depend on the β-D-adjacent sugar residues when they carry no sulfate groups. It also suggests that there is no influence of the α-D-galactose 2-sulfate on the cyclization rate, in spite of its change from the equatorial to the axial position during the conversion of the $^4C_1$ to $^1C_4$ chair conformation.

Keywords: Non-sulfated β-D-galactose units; α-Galactosyl units; α-D-Galactose 2-sulfate

1. Introduction

The α-D-galactose 6- and 2,6-disulfate units in carrageenans of the kappa and lambda families cyclized in alkaline medium producing 3,6-anhydrogalactose derivatives, with very different rates (Ciancia, Noseda, Matulewicz & Cerezo, 1993) and this was attributed to the influence of sulfate groups in different positions of the β-D-galactose unit (Noseda & Cerezo, 1995).

On the other hand, the rates of cyclization of both 6-sulfate and 2,6-disulfate α-D-galactose residues in partially cyclized kappa/iota and mu/nu carrageenans could not be differentiated although, in the latter unit, the bulky 2-sulfate group changes from equatorial to axial position during the change of conformation ($^4C_1 \rightarrow {}^1C_4$) of the α-D-galactose residue prior to the formation of the 3,6-anhydro ring (Ciancia et al., 1993), and this was attributed to the experimental difficulties in the measurement of high rates of cyclization and to the lack of 'pure' samples of partially cyclized kappa, iota, mu or nu carrageenans (Ciancia et al., 1993).

Porphyrans constitute a family of agaroids extracted from red seaweeds of the genus *Porphyra*, composed by alternating 3-linked β-D-galactose units and 4-linked α-L-galactose residues or its derivatives, which contain sulfate groups only on the C-6 of the α-L-residues (Morrice, McLean, Long & Williamson, 1983; Peat, Turvey & Rees, 1961). Thus, it is a good natural model polysaccharide to study the cyclization rate of that unit, free from the presence of other sulfates and in a diasteromeric surrounding different from that of the samples previously studied.

2. Experimental

Samples of *Porphyra columbina* (gametophytic phase) were collected in Comodoro Rivadavia (Provincia de Chubut).

Extraction: The milled seaweed was extracted with water (1.5% (w/v)) at room temperature, with mechanical stirring, for 15 h. The residue was removed by centrifugation and the supernatant was poured into ethanol (two volumes) where part of the polysaccharide precipitated. Further addition of ethanol (one volume) to 75% ethanol concentration yielded the remaining polysaccharide (PC75). The fraction was purified by redissolution in water, dialysis, centrifugation and lyophilization. The monosaccharide composition was determined, after reductive hydrolysis (Stevenson & Furneaux, 1991) by GC and GC-MS.

Methylation analysis was carried out by the method of Ciucanu and Kerek (1984) on the triethylammonium salts of the porphyrans (Stortz & Cerezo, 1993) using methyl iodide and deuterated methyl iodide. The permethylated polysaccharides were hydrolyzed following

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Table 1
Yields, analysis and monosaccharide compositions of fraction PC75, $PC75m_1$ and $PC75m_3$

	$[\alpha]_D$ (°)	Monosaccharide composition (mol %)		
		Gal (%)	6-*O*-Me-Gal (%)	3,6-AnGal (%)
PC75	−12.6	38.6	38.7	22.7
$PC75m_1$	−27.3	15.2	32.5	52.3
$PC75m_3$	−45.5			
PC1[a]	−55.4	59.3	15.5	25.2
PC2	−61.1	62.2	17.9	19.8
PC3	−69.0	65.0	16.3	18.7
PC3m	–	37.5	16.2	46.3
PC4	−65.3	66.0	17.8	16.3
BR1[b]	−14.0	57.8	28.3	13.9
BF2	−75.0	53.5	25.7	20.8

[a] PC1–PC4 fractions from Brasch, Chang, Chuah & Melton (1981).
[b] BF fractions from Villarroel and Zanlungo (1981).

the reductive hydrolysis procedure (Stevenson & Furneaux, 1991) and analyzed by GC and GC-MS as alditol acetates.

GC analysis were carried out with a HP-5890 gas chromatograph equipped with a flame ionization detector (FID), using a fused silica capillary column (30 m × 0.25 mm) coated with DB-225. Chromatography was run isothermally at 210°C. Both injector and FID temperature were at 250°C. Nitrogen was used as carrier gas at a flow rate of 1 ml/min and a split ratio of 100:1. GC-MS analysis was performed using a Varian 3300 chromatograph and a Finnigan Mat ITD spectrometer. Helium was the carrier gas (1 ml/min).

For NMR analysis the lyophilized sample was dissolved in D_2O (20–40 mg). The NMR spectra of the solutions were recorded at 70°C using a Bruker Avance DRX400 NMR spectrometer. NMR spectra were obtained using a multinuclear inverse detection, 5 mm probe. The polysaccharide samples were analyzed by ^{13}C NMR spectroscopy and also by DEPT (DEPT135).

Fourier transform infrared (FTIR) spectra of polysaccharide films were recorded on a Perkin–Elmer Series 2000 FTIR spectrometer in transmittance mode (eight scans, collected at a resolution of 4 cm^{-1}).

Optical rotation of aqueous solution of the polysaccharide samples (0.2%), were measured at 20°C, using a 10-cm cell and the sodium D line (589.3 nm) with a Rudolph Autopol III automatic polarimeter.

The alkaline cyclization was carried out as previously described (Ciancia et al., 1993). Briefly, the sample reduced with sodium borohydride was heated, in 1 M sodium hydroxide, at 60, 70, 80 and 90°C. Samples were taken at intervals, the reaction stopped and the solution neutralized with 1 M hydrochloric acid and the 3,6-anhydrogalactose content was determined by the resorcinol method (Yaphe, 1960). From the results, the rate constants and the half-life were determined at each temperature.

3. Results

The seaweed was extracted with water at room temperature and the extract was precipitated with ethanol (two volumes), and further with another volume of ethanol. This fraction (PC75), precipitated between 66% and 75% ethanol concentration, that was obtained with 3.3% yield contained 15.6% of sulfate (as SO_3Na) and only galactose, 6-*O*-methyl galactose and 3,6-anhydrogalactose as monosaccharide constituents, was used in the kinetic studies. It was submitted to alkaline treatment giving $PC75m_1$ with 84.0% yield. ^{13}C NMR spectrum showed that the

Table 2
Anomeric, substituted and unsubstituted C-6 and methyl groups absorptions (from Morrice et al., 1983) at the ^{13}C NMR spectra of PC75 and its cyclic derivatives

Absorptions	Attributed to	3(4)-linked to	1-linked to	$PC75m_3$	$PC75m_1$	PC75
C-1 carbon atoms						
103.2	(6 Me)βD-Gal[a]	αL-6S	αL-6S			++
103.1	(6 Me)βD-Gal[a]	AnGal	αL-6S		tr.[b]	+
102.0	(6 Me)βD-Gal[a]	AnGal	AnGal	+++	+++	++
100.9	αL-6S	(6 Me)βD-Gal	βD-Gal			++
100.7	αL-6S	(6 Me)βD-Gal	6 MeβD-Gal		tr.	++
98.0	AnGal	(6 Me)βD-Gal	βD-Gal			+
97.9	AnGal	(6 Me)βD-Gal	6 MeβD-Gal	+++	+++	+
6-carbon atom						
61.2	C-6, βD-Gal	αL-6S	AnGal			++
61.0	C-6, βD-Gal	AnGal	AnGal	++	++	+
60.8	C-6, βD-Gal	αL-6S	αL-6S			+
Methyl carbon atom						
58.7	C-6-*O*Me	AnGal	AnGal	+	+	
58.6	C-6-*O*Me	αL-6S	AnGal			+

[a] Units in parentheses suggest alternative assignations.
[b] tr. = trace.

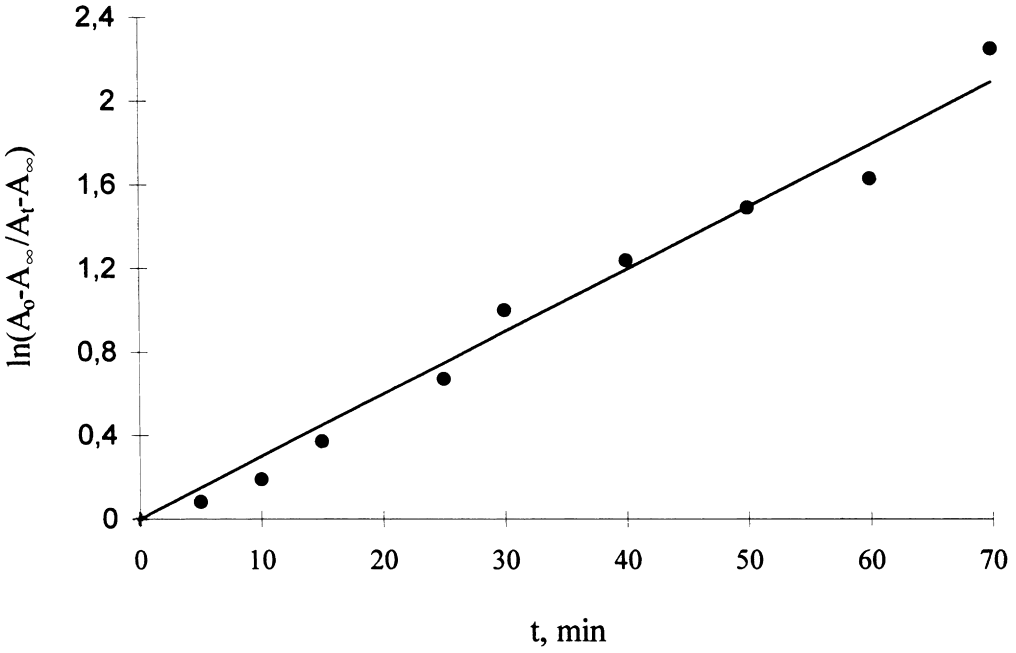

Fig. 1. Determination of the rate constant of the cyclization reaction for porphyran PC75, at 80°C.

1 h-alkaline treatment did not produce the complete cyclization of the α-L-galactose 6-sulfate units, so the original fraction was treated in the above conditions for 3 h, with total cyclization (PC75m_3). Monosaccharide compositions and rotations of the porphyran fraction and its cyclic derivatives are given in Table 1, together with similar data from porphyrans previously reported.

Methylation analysis of PC75m_1, using deuterated methyl iodide indicate the presence of 51.6% of 2-*O*-methyl-3,6-anhydrogalactose together with 2,4,6-tri-*O*-methylgalactose (47.3%). GC-MS of this last compound showed that the parent galactoses were 3-linked galactose and 3-linked 6-*O*-methylgalactose in a molar ratio 3:7. Small amounts of 2,3-di-*O*-methylgalactose (1.7%) compatible with the slightly incomplete cyclization and 3,6-anhydrogalactose (1%), were also detected. The FT-IR spectrum of PC75 showed absorption bands at 913.6 and 817.8 cm^{-1} corresponding to the 3,6-anhydro cycle and to a sulfate group linked to a primary alcohol, respectively. This last band disappears in the spectrum of PC75m_1 while the former was considerably increased. The anomeric absorption peaks, those of substituted and unsubstituted C-6 and of the methyl groups of the ^{13}C NMR spectra of PC75 and of its cyclic derivatives are given in Table 2.

The cyclization reaction of PC75 follows, as the previous ones (Ciancia et al., 1993), a pseudo first-order kinetics as determined by the plot $\ln(A_0 - A_\infty/A_t - A_\infty)$ as a function of time, where A is the absorbance determined by the resorcinol method (Fig. 1). Table 3 shows the rate constants and half-lives of this reaction for PC75 in 1 M sodium hydroxide at different temperatures, compared to those of mu/nu-carrageenans, lambda-carrageenans (Ciancia et al., 1993), two degraded lambda-carrageenans and two oligosaccharides (Noseda & Cerezo, 1995). These last two types of compounds contain non-sulfated β-D-galactose units.

Table 3
Cyclization reactions for porphyran PC75 in 1 M NaOH, at different temperatures

Temp. (°C)	Rate constant k ($\times 10^4$ s^{-1})							$t_{1/2}$ (min)						
	$1C_3$[a]	PC75	$T_{2(35)}$[b]	$T_{2(26)}$	T_9	T_{10}	$1T_2$	$1C_3$	PC75	$T_{2(35)}$	$T_{2(26)}$	T_9	T_{10}	$1T_2$
60	4.8	1.0	–	–	–	–	0.14	24.0	139	–	–	–	–	850
70	13.0	1.5	–	–	–	–	0.35	9.0	77	–	–	–	–	320
80	26.0	4.9	5.3[c]	4.2	3.9	2.5	0.67	4.5	23	21.7	27.7	29.5	47.2	170
90	59.0	11.0	–	–	–	–	1.11	2.0	10.5	–	–	–	–	130

[a] $1C_3$: Partially cyclized mu/nu carrageenan and $1T_2$: Lambda carrageenan (Ciancia et al., 1993).

[b] $T_{2(35)}$ and $T_{2(26)}$ are fragments of a partially degraded lambda carrageenan which differs from the original product in the lack of the 2-sulfate group on the β-D-galactose unit. T_9 is the trisaccharide β-D-galactopyranosyl (1 → 4) α-D-galactopyranosyl 2,6-disulfate (1 → 3) galactose and T_{10} is the disaccharide α-D-galactopyranosyl 2,6-disulfate (1 → 3) D-galactose (Noseda & Cerezo, 1995).

[c] These constants were determined only at 80°C.

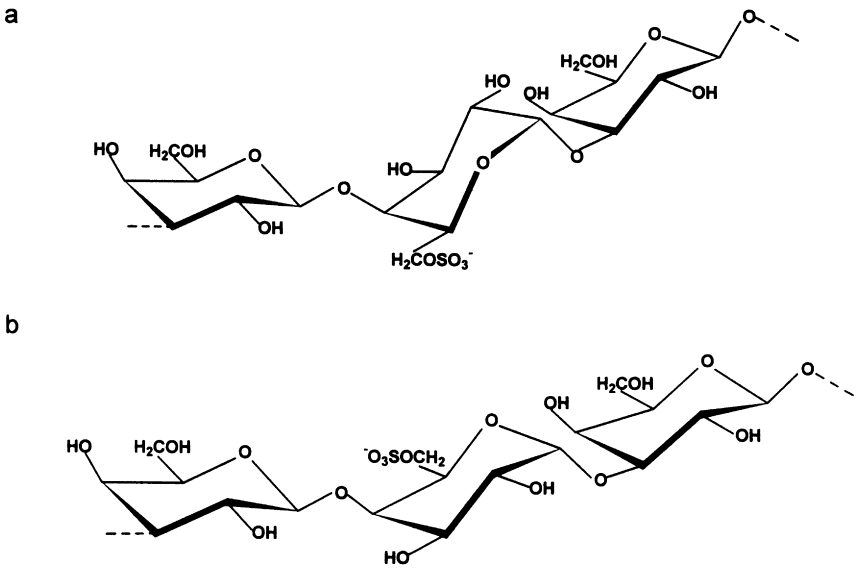

Fig. 2. The diasteromeric environments of α-L-galactose 6-sulfate and of α-D-galactose 6-sulfate in: (a) agaroids and (b) carrageenans.

4. Discussion

Porphyrans are a family of polysaccharides produced by red seaweeds of the genera *Porphyra* and *Bangia*. They are composed of alternating 3-linked β-D-galactose plus 6-*O*-methyl-β-D-galactose units and 4-linked α-L-galactose, α-L-galactose 6-sulfate and 3,6-anhydro-α-L-galactose residues (Brasch et al., 1981). As the D:L galactose molar ratio is about 1 due to the alternating structure of the polysaccharide, the dispersion of the structures in the family is reduced to the different percentages of 6-*O*-methoxyl or 6-*O*-sulfate groups in the D- and L-forms. With these structures porphyrans are good model compounds to study the kinetics of the conversion of the α-L-galactose 6-sulfate units into 3,6-anhydro-α-L-galactose residues, without the influence of other sulfates in the same unit or in the β-D-adjacent ones, and in a different diasteromeric surrounding (Fig. 2).

Porphyran fractions from *Porphyra columbina*, extracted with boiling water, have been studied (Brasch et al., 1981; Villarroel & Zanlungo, 1981). Table 1 gives the monosaccharide composition and the rotation of those porphyrans together with similar data of PC75 fraction extracted from the same seaweed at room temperature and fractionated by ethanol precipitation. Table 1 also shows the monosaccharide compositions and the rotations of some alkali-treated derivatives. The low rotation of PC75 (−12.6°) is noteworthy. To the best of our knowledge the only porphyran reported with similar rotation (−14.0°) was isolated from a Chilean sample of *P. columbina* by extraction with water and fractionation with cetyltrimethylammonium bromide (Villarroel & Zanlungo, 1981). It was suggested that this value was due to the low proportion of 3,6-anhydro-α-L-galactosyl residues (Villarroel & Zanlungo, 1981). The sequence of increasing values (−12.6, −27.3 and −45.5°) with the amount of cyclization of PC75 is in agreement with the above hypothesis and suggests that the rotation increases with the regularity of the repetitive sequence in the polysaccharide backbone.

Methylation analysis, ^{13}C NMR and FT-IR spectroscopy of PC75 and its alkaline-treated derivatives showed that the fraction extracted with water at room temperature and precipitated at ethanol concentrations between 66% and 75% has the usual structure of porphyrans with 70% of the β-D-units methoxylated at C-6 and all the α-L-galactopyranosyl residues sulfated also in the C-6 position.

The cyclization reaction of PC75 follows, as the previous ones (Ciancia et al., 1993), a pseudo first-order kinetics (Fig. 1). Table 3 shows the rate constants and half-live of this reaction for PC75 in 1 M sodium hydroxide at different temperatures compared to that of mu/nu- and lambda-carrageenans (Ciancia et al., 1993), degraded lambda-carrageenans without 2-sulfate on the β-D-unit, and di and trisaccharides (Noseda & Cerezo, 1995) in the same conditions. The cyclization of the α-L-galactopyranosyl 6-sulfate units to 3,6-anhydro-α-L-galactose residues in the porphyran PC75 has a rate constant intermediate between those of the reactions in a partially cyclized mu/nu-carrageenan and in a lambda-carrageenan, in agreement with the accelerating effect of the 4-sulfate (Ciancia et al., 1993), or the retarding effect of the 2-sulfate groups (Noseda & Cerezo, 1995), on the β-D-units. The rate constant determined at 80°C is in the same range as that of the degraded lambda-carrageenans without sulfates in the β-D-residues or those of the oligosaccharides resulting from the total autohydrolysis (Noseda & Cerezo, 1995) of a lambda-carrageenan that also fulfil these conditions. The diasteromeric and structural differences between the previous compounds and the porphyran suggested that the rate of cyclization of the α-unit does not depend on the β-D-adjacent sugar residues, at

least when they carry no sulfates, but only on the position of its sulfate substituents. The fact that similar rate constants were obtained for the cyclization of the α-L-6-sulfate unit in the porphyran and the α-D-2,6-disulfate residue in the degraded lambda-carrageenan or in the oligosaccharides suggests that there was no influence of the α-D-2-sulfate group on the cyclization rate, in spite of its change from the equatorial to the axial position during the conversion of the $^4C_1$ to $^1C_4$ chair conformation. This could be explained by the low 1,3-axial repulsion of the new axial group further relieved by the distortion of the chair caused by the 3,6-anhydro bridge (Eliel, Allinger, Angyal & Morrison, 1981).

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