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Alkali modification of carrageenans — II. The cyclization of model compounds containing non-sulfated β-D-galactose units

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The alkaline cyclization of model compounds containing non-sulfated β-D-galactose units is about 3·6–7·6 times faster than that of the original lambda carrageenan, suggesting an influence of the 2-sulfate of the β-D-galactose units on the cyclization rate of the α-D-galactopyranoyl 2,6-disulfated residues.

INTRODUCTION

Lambda carrageenans are cyclized in alkaline medium producing 3,6-anhydrogalactose derivatives slower than those of the kappa family. This was attributed, at least in part, to the fact that the 2-sulfate groups located on the β-D-galactose units could interact with the hydroxyl on C3 of the α-D-galactose residue shielding it from polarization or ionization and thus precluding the cyclization (Ciancia *et al.*, 1993).

The autohydrolysis of a lambda carrageenan from *Gigartina skottsbergii* produced, after 35 h reaction, the splitting of traces of 3,6-anhydrogalactosidic linkages together with the hydrolysis of major amounts of the above-mentioned 2-sulfate of the β-D-galactose units. Further reaction split galactosidic linkages producing low molecular weight fragments without that sulfate group (Noseda & Cerezo, 1993).

We report the kinetics of the alkaline cyclization of degraded lambda carrageenans after 26 and 35 h of autohydrolysis, and of two oligosaccharides with non-sulfated β-D-galactose units produced by total autohydrolysis of the same carrageenan.

EXPERIMENTAL

The isolation of the lambda carrageenan from the tetrasporic phase of *Gigartina skottsbergii* and its fractionation to produce lambda carrageenan $1T_2$, as well as its autohydrolysis were described elsewhere (Matulewicz *et al.*, 1989; Noseda & Cerezo, 1993).

The alkaline treatment was carried out with 1 M sodium hydroxide at 80°C as previously reported (Ciancia *et al.*, 1993). Samples were taken at regular intervals and the reaction was stopped by cooling in an ice bath, the solutions 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 half-lives were determined.

RESULTS

The alkaline treatment was carried out with degraded lambda carrageenans after 26 h ($T_{2(26)}$) and 35 h ($T_{2(35)}$), respectively, of autohydrolysis. The same treatment was carried out also with α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose (T_{10}) and with β-D-galactopyranosyl-(1 → 4)-α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose (T_9). Their structure was demonstrated by ^{13}C NMR spectroscopy, optical rotation, sulfate determination and FAB-MS (Noseda & Cerezo, unpublished results).

The cyclization reaction follows a pseudo first-order kinetics as determined by the plot of $\ln(A_0 - A_\infty/A_t - A_\infty)$ as a function of time, where A is the absorbance determined by the resorcinol test (Fig. 1). Table 1 shows the rate contents and half-lives of this reaction for $T_{2(26)}$, $T_{2(35)}$, T_9 and T_{10}.

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Table 1. Rate constants and half-lives of the cyclization reaction in model compounds containing non-sulfated β-D-galactose units

Sample	K ($\times 10^4$ s^{-1})	$T_{1/2}$ (min)
$1C_3$[a]	26·0	4·5
$1T_2$[a]	0·7	170·0
$T_{2(26)}$	4·2	27·7
$T_{2(35)}$	5·3	21·7
$T_{(9)}$[b]	3·9	29·5
$T_{(10)}$[c]	2·5	47·2

[a]The rate constants and half-lives of a partially cyclized mu/nu carrageenan ($1C_3$) (Ciancia *et al.*, 1993) and of the original lambda carrageenan ($1T_2$) (Noseda & Cerezo, 1993), obtained from the cystocarpic and tetrasporic phases, respectively, of *Gigartina skottsbergii* are given for comparison.
[b]β-D-galactopyranosyl-(1 → 4)-α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose.
[c]α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose.

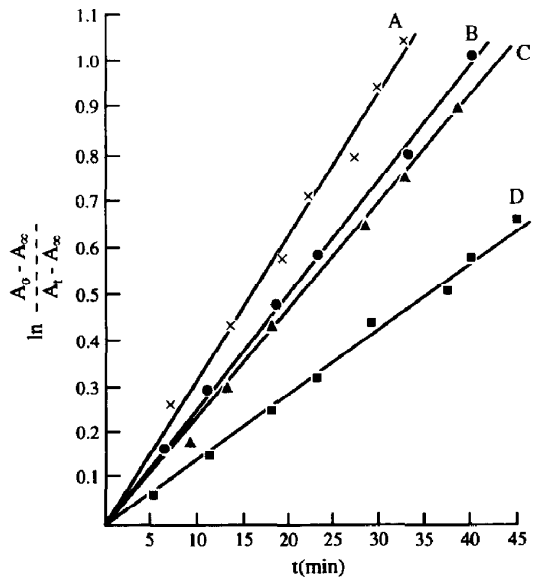

Fig. 1. Determination of the rate constant of the cyclization reaction for model compounds containing non-sulfated β-D-galactose units: A: $T_{2(35h)}$; B: $T_{2(26h)}$; C: $T_{(9)}$; and D: $T_{(10)}$.

DISCUSSION

Degraded carrageenans were obtained by autohydrolysis of traces of 3,6-anhydrogalactosidic linkages present in the original lambda carrageenan (Noseda & Cerezo, 1993). They differ from the starting product in the lack of 2-sulfate on the β-D-galactose unit (65 and 82%, respectively). Neither hydrolysis of the 2-sulfate nor that of the glycosidic linkage of the α-unit was detected by ^{13}C NMR spectroscopy (Noseda & Cerezo, 1993). The α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose (T_{10}) and β-D-galactopyranosyl-(1 → 4)-α-D-galactopyranosyl 2,6-disulfate-(1 → 3)-D-galactose (T_9) resulted from the total autohydrolysis of the starting lambda carrageenan (Noseda & Cerezo, unpublished results).

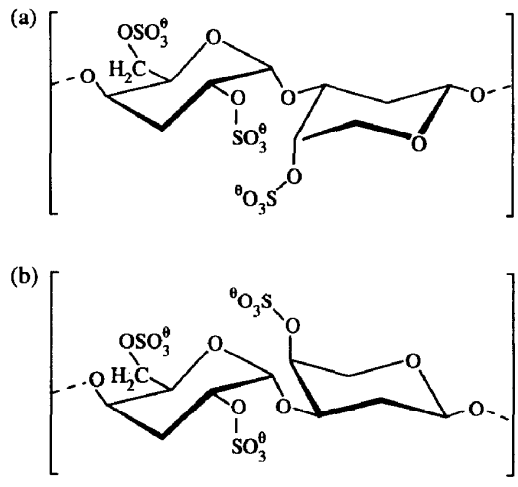

Fig. 2. Mu/nu repeating disaccharide showing the possible interaction between the 4-sulfate of the β-D-galactose unit with the (a) 2-sulfate or the (b) 6-sulfate of the α-D-galactose residue.

The cyclization reactions have similar rate constants (K 2·5–5·3 × 10^4 s^{-1}) in all four cases and these rate constants were about 3·6–7·6 times higher than the cyclization rate constant of the original lambda carrageenan (K 0·7 × 10^4 s^{-1}), showing the influence of the 2-sulfate of the β-D-galactose units on the cyclization rate of α-D-galactopyranosyl 2,6-disulfate residue as previously suggested. When compared with the rate constant of the cyclization reaction in a partially cyclized mu/nu carrageenan (K 26·0 × 10^4 s^{-1}) (Ciancia *et al.*, 1993), the model compound reacts 5–10 times slower, suggesting a rate accelerating influence in the mu/nu and kappa/iota carrageenans not previously suspected (Table 1). Examination of molecular models (Fig. 2) of the disaccharide repeating units of a mu/nu carrageenan suggests that the interaction of the 4-sulfate group in the β-D- unit is either with the 2-sulfate (Fig. 2a) or with the 6-sulfate (Fig. 2b) groups in the α-unit, thus destabilizing the $^4C_1$ conformation of the latter residue.

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