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Note

Determination of the diadic composition of alginate by means of circular dichroism: a fast and accurate improved method

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Abstract

Circular dichroism (CD) is presented as a reliable and sensitive method of determining the diadic frequency composition of alginate (F_{GG}, F_{MM} and F_{GM+MG}). The availability of samples, very largely or even completely conforming to the limiting structures of polymannuronate $(MM)_n$, polyguluronate $(GG)_n$ and polyalternating MG $(MG)_n$, respectively, allowed the limiting CD spectra for each alginate diad to be obtained. These showed very different CD behaviour, thus pointing out the crucial importance of the neighbouring residue in chiroptical properties. Using an iterative best-fit procedure, the diadic composition of commercial alginates could be obtained from their respective CD spectra by means of a linear combination of the spectra of the three limiting diads. The results were found in excellent agreement with the composition parameters obtained by ^{1}H NMR spectroscopy. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Alginate; Circular dichroism; Diads; Sugar composition

1. Discussion

Alginate is a polysaccharide whose occurrence in nature is mainly limited to the marine brown algae (Phaeophyta),[1] although microbial alginates as exocellular polymeric material are also produced by *Azotobacter vinelandi*[2] and several species of *Pseudomonas*.[3] In molecular terms, alginate is a family of unbranched binary copolymers of (1→4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) of widely varying composition and sequential structure. The well-established biocompatibility has allowed alginate to be used industrially and in medical and biotechnological applications.[4] In particular, the ability to form gels in the presence of calcium ions has given alginate increasing importance in the field of encapsulation.[5] The high selectivity of binding of alginate towards calcium ions, which accounts for its capacity to form ionotropic gels, is determined by the composition. Furthermore, parameters such as the stability, strength and porosity of the obtained gels are influenced by the diadic frequencies F_{GG}, F_{GM+MG} and F_{MM} of alginate.[6,7] Many efforts have been therefore devoted to the structural characterisation of alginates.[8,9] In the present work, circular dichroism (CD) is assessed as a rapid, non-destructive and reliable method for determining the diadic composition of alginate, obtaining higher accuracy than previously reported.

Three alginate samples with a controlled composition were produced, and characterised by means of ^{1}H NMR spectroscopy,[8,9] in the laboratory of Professor G. Skjåk-Bræk, (Institute of Biotechnology, NTNU, Trondheim, Norway). A homopolymeric mannuronan ($F_M = 1$) was produced from an epimerase-negative mutant of *Pseudomonas aeruginosa* as described by Ertesvåg and Skjåk-Bræk.[10] G-enriched alginate ($F_G = 0.92$, $F_{GG} = 0.86$) was prepared from *Laminaria hyperborea* outer cortex after fractionation,[11] while the

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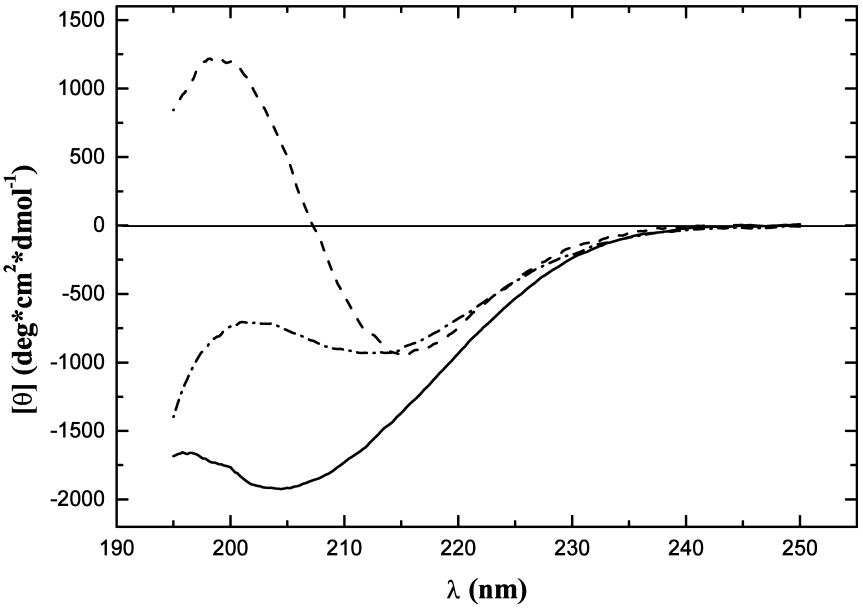

Fig. 1. CD spectra of diads present on alginate: (—) GG, (- -) MM, (-●-) MG + GM.

polyalternating MG ($F_G = 0.47$, $F_{GG} = 0$)‡ was produced by in vitro epimerisation of mannuronan with the recombinant mannuronan C-5 epimerase AlgE4.[12]

A solution of the sodium salt of each sample, at a concentration of 4×10^{-3} monomol/L, was prepared in deionized water and the pH was adjusted to 7. Spectra were recorded at 25 °C with a JASCO J-700 spectropolarimeter in the wavelength (λ) range of 195–250 nm with the following setup: bandwidth, 1 nm; time constant, 2 s; scan rate, 20 nm/min. Four spectra corrected for background were averaged for each sample. The validity of the Lambert–Beer law was tested for a number of wavelengths in the concentration range from 2×10^{-3} to 5×10^{-3} monomol/L (i.e., from 0.43 to 1.08 g/L). The molar ellipticity of pure diads in all the range considered, namely $[\theta]_{MM}(\lambda)$, $[\theta]_{MG+GM}(\lambda)$ and $[\theta]_{GG}(\lambda)$, was calculated from the CD spectra of polymannuronate, polyalternating MG and G-enriched alginate, respectively. However, it is important to stress that the following relation (Eq. (1)) always holds for any alginate sample.

$$[\theta]^i_{rec}(\lambda) = F^i_{GG} \times [\theta]_{GG}(\lambda) + F^i_{MM} \times [\theta]_{MM}(\lambda) + F^i_{MG+GM} \times [\theta]_{MG+GM}(\lambda) \quad (1)$$

‡ F_G and F_{GG} fractions suffice to determine the diadic composition of any alginate sample, since the following relations always hold:

$F_M = 1 - F_G$

$F_{MG+GM} = 2(F_G - F_{GG})$

$F_{MM} = 1 - F_{GG} - 2(F_G - F_{GG}) = 1 + F_{GG} - 2F_G$

Therefore, considering the CD spectrum ($[\theta]_{rec}(\lambda)$) of three alginate samples ($i = 1, 2, 3$) characterised by different (but known) composition, Eq. (1) leads to a system of three equations in which the values $[\theta]_{MM}(\lambda)$, $[\theta]_{MG+GM}(\lambda)$ and $[\theta]_{GG}(\lambda)$ denote the three unknowns to be determined at each wavelength. However, the availability of homo- (or very nearly such) polymers allows a high simplification of the three equation-system to be obtained. In fact, although Eq. (1) still holds for polymannuronate, the absence of both GG and alternating diads leads to a system with one-unknown; this enables $[\theta]_{MM}(\lambda)$ to be determined directly. In the case of polyalternating MG, only MM and alternating diads are present and, considering that $[\theta]_{MM}(\lambda)$ has already been determined, Eq. (1) is again reduced to a one-unknown (namely $[\theta]_{MG+GM}(\lambda)$) equation. Finally, when G-enriched alginate is considered, although all the three diads are present, only the term $[\theta]_{GG}(\lambda)$ has to be determined.

The CD spectra of the (ideally assumed as) pure GG ($[\theta]_{GG}(\lambda)$), MM ($[\theta]_{MM}(\lambda)$) and (sum of) alternating ($[\theta]_{MG+GM}(\lambda)$) diads in the range 195–250 nm are shown in Fig. 1. It should be emphasised that the three diads showed highly different CD behaviour depending upon: (i) the local ring geometry around the carboxyl chromophore;[13,14] and; (ii) the effect of the neighbouring group. In particular, while the MG and GG diads are negative throughout the considered range, the MM diad becomes positive below 210 nm. It is noteworthy that, due to the availability of a polymer of a strictly alternating structure such as the present polyalternating MG, for the first time to our best knowledge, the molar

ellipticity of the alternating diad is determined with high accuracy.[§]

Once $[\theta]_{MM}(\lambda)$, $[\theta]_{MG+GM}(\lambda)$ and $[\theta]_{GG}(\lambda)$ have been determined, Eq. (2), a slightly modified version of Eq. (1), can be used to analyse alginate samples of unknown composition. In this Eq. (2), P_1, P_2 and P_3 (accounting for the composition in terms of GG, MM and (sum of) alternating diads, respectively) represent the unknown values to be determined at f ranging from 2 to all the wavelengths values considered

$$[\theta]_{rec}(\lambda)^f = P_1 \times [\theta]_{GG}(\lambda)^f + P_2 \times [\theta]_{MM}(\lambda)^f + P_3 \times [\theta]_{MG+GM}(\lambda)^f \quad (2)$$

where $P_3 = (1 - P_1 - P_2)$ and $[\theta]_{rec}(\lambda)^f$ is the recorded spectrum of alginate at each wavelength.

Commercial samples of sodium alginate isolated from *L. hyperborea* (LF 10/60 with $F_G = 0.69$, $F_{GG} = 0.56$ and LV G with $F_G = 0.7$, $F_{GG} = 0.58$) and from *Macrocystis pyrifera* ($F_G = 0.42$, $F_{GG} = 0.20$) were provided by Protan A/S (Norway). The CD spectrum of each sample was recorded and then analysed with a best fit of Eq. (2) in the wavelength range considered. In Fig. 2(A–C), a comparison between the recorded spectrum and the best fit obtained with Eq. (2) for *L. hyperborea*, (LF10/60 and LV G) and *M. pyrifera* alginate samples is reported. It should be emphasised that a high quality of fit is reached in the entire range 195–250 nm and the differences between the two curves are barely detectable. The results reported in Table 1a, showed good agreement when compared to those obtained from ^{1}H NMR spectroscopy. Furthermore, a higher accuracy in the determination of the diadic composition of alginate, especially for alginate from *L. hyperborea* source, was achieved compared to previous results[13] (Table 1b).

Although an excellent fit of the composition parameters can be obtained using molar ellipticity values for all available wavelengths, a simplified but more straightforward method can be followed. It uses a system of two equations in two unknowns (e.g., F_{GG}

[§] The molar ellipticity values in wavelength steps of 1 nm in the range 195–250 nm for the three limiting compositions are available in electronic format upon request to the authors.

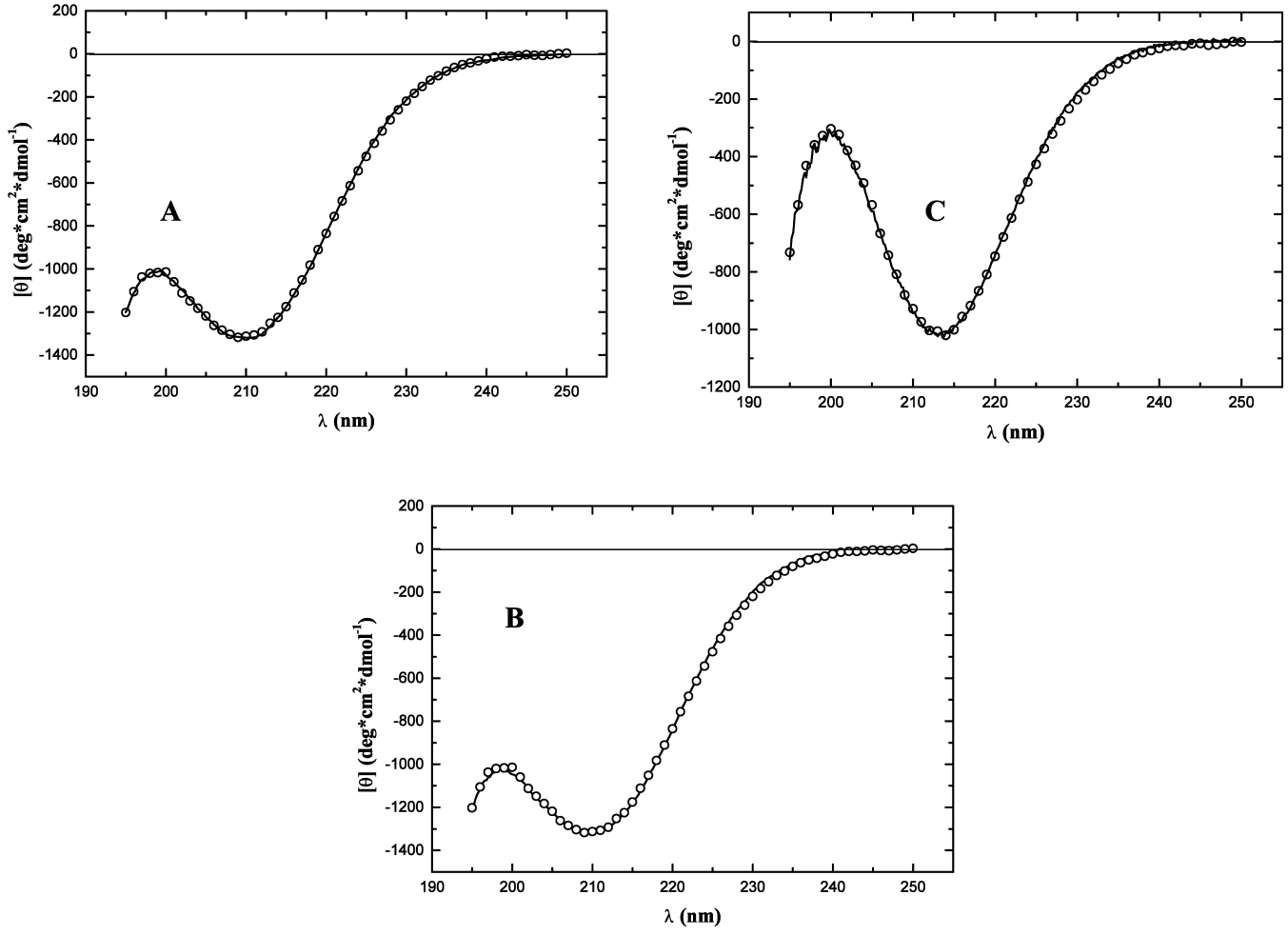

Fig. 2. Comparison between the recorded CD spectra (—) and the best fit obtained (○○○) for alginate from *L. hyperborea* (A) LF10/60 and (B) LV G and *M. pyrifera* (C).

Table 1
Diadic composition obtained for different alginate samples

Sample	Composition	NMR	CD
(a) Present work			
	F_{GG}	0.56	0.57
L. hyperborea	F_{MM}	0.18	0.16
LF 10/60	F_{MG+GM}	0.26	0.27
	F_{GG}	0.58	0.57
L. hyperborea	F_{MM}	0.18	0.17
LV G	F_{MG+GM}	0.24	0.26
	F_{GG}	0.20	0.19
M. pyrifera	F_{MM}	0.37	0.37
	F_{MG+GM}	0.43	0.44
(b) Ref. 13			
	F_{GG}	0.586	0.642
L. hyperborea	F_{MM}	0.187	0.220
Stipe	F_{MG+GM}	0.227	0.138
	F_{GG}	0.177	0.185
M. pyrifera	F_{MM}	0.406	0.365
	F_{MG+GM}	0.417	0.450

and F_{MM}, with $F_{MG+GM} = 1 - F_{GG} - F_{MM}$), with two sets of three coefficients (e.g., the $[\theta]$ values of limiting compositions) for two different values of λ (i.e., $f = 2$ in Eq. (2)). The values of $[\theta]_{MM}(\lambda)$, $[\theta]_{MG+GM}(\lambda)$ and $[\theta]_{GG}(\lambda)$ at two different wavelengths, namely 198 and 207 nm, are: $[\theta]_{MM}(198) = 1214.6$; $[\theta]_{MM}(207) = 38.16$; $[\theta]_{MG+GM}(198) = -879.8$; $[\theta]_{MG+GM}(207) = -837.7$; $[\theta]_{GG}(198) = -1713.9$; $[\theta]_{GG}(207) = -1867.2$ (values in deg cm^2/dmol). Since alginate from *L. hyperborea* is one of the most commonly used in biotechnological applications, it was decided to test the method on samples from this source. A good evaluation of the diadic composition is achieved: in the present case, $F_{GG} = 0.56$, $F_{MM} = 0.17$ and $F_{MG+GM} = 0.27$ and $F_{GG} = 0.57$, $F_{MM} = 0.19$ and $F_{MG+GM} = 0.25$ for LF10/60 and LV G, respectively, have been obtained. Trivially, from the relationships $F_G = F_{GG} + 1/2F_{MG+GM}$ and $F_M = F_{MM} + 1/2F_{MG+GM}$, one can determine the less informative values F_G and F_M giving the monomer composition. In the present case, the values calculated from the two-wavelength molar ellipticities coincide to 0.005 or less with those from NMR, for both *L. hyperborea* samples. Using the method of Ref. 13 the disagreement is about 0.011.

In conclusion, although Eq. (1) suffices to determine the limiting CD spectrum of each diad present on alginate when three alginate samples with different and known composition are available, this equation system has been largely simplified by using polymers of limiting composition such as polymannuronate and polyalternating MG. A very high accuracy in the determination of the diadic composition of alginate has then been achieved.

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