

Monomer composition and sequence of sodium alginate extracted at pilot plant scale from three commercially important seaweeds from Mexico

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Abstract The marine waters of the Baja California peninsula (Mexico) are a rich source of brown seaweeds with a great potential for exploitation. For that reason, *Sargassum sinicola*, *Eisenia arborea*, and *Macrocystis pyrifera* collected from different locations were subjected to extraction of sodium alginate using a pilot-plant scale process developed in our facilities. The composition and sequence parameters of the recovered alginate were studied by infrared and nuclear magnetic resonance spectroscopy. The spectral analysis of the products revealed that sodium alginate from *S. sinicola* contains a greater proportion of guluronate monomers (64%) than that from *E. arborea* (48%), and *M. pyrifera* (38%). Computation of the frequencies of diads and triads indicated that the alginate from *S. sinicola* was constructed by intercalated guluronate-blocks of 14 residues in length. In contrast, the length of the G-block in the alginates from *E. arborea* and *M. pyrifera* were 7 and 4 residues, respectively. The results show that *S. sinicola*, *E. arborea*, and *M. pyrifera* are sources of sodium alginate with different mannuronate/guluronate ratios, as well as a varied building-block length. In consequence, aqueous dispersions of sodium alginate from the three studied species are expected to exhibit different physical properties.

Keywords *Eisenia arborea* · Guluronic · *Macrocystis pyrifera* · Mannuronic · *Sargassum sinicola* · Uronates

Abbreviations

Alg-Ea	Sodium alginate from <i>Eisenia arborea</i>
Alg-Mp	Sodium alginate from <i>Macrocystis pyrifera</i>
Alg-Ss	Sodium alginate from <i>Sargassum sinicola</i>
FTIR	Fourier transformed infra-red spectroscopy
G	α -L-guluronic acid
M	β -D-mannuronic acid
$N_{G>1}$	Average G-block length
$^1\text{H-NMR}$	Nuclear magnetic resonance spectroscopy of protons

Introduction

Alginates are a family of linear polysaccharides occurring as a structural component of the cell wall in marine brown algae and bacteria. The monomers in alginate, β -D-mannuronic acid (M) and its C-5 epimer α -L-guluronic acid (G), can be arranged in varying proportions and sequence in a chain bound by 1→4 linkages (Haug 1964, p. 6). The M and G residues are organized in blocks of consecutive M (M blocks), consecutive G (G blocks), or alternating M and G residues (MG blocks). The gelling characteristics of alginates are greatly influenced by the monomer composition and sequence (Gacesa et al. 1983). The composition of the alginates is a very important issue, because of the extensive range of industrial applications of alginates, which in turn are related to their physical properties.

The seaweeds belonging to the order Laminariales [*Macrocystis pyrifera* (Linnaeus) (Agardh 1820); *Eisenia arborea* (Areschoug 1876)] and Fucales [*Sargassum sinicola* (Setchell and Gardner 1924)] are the most abundant in the seas of the Baja California peninsula, Mexico, making them highly suitable for industrial exploitation as a source of

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alginate (Pacheco-Ruiz et al. 1998; Hernández-Carmona 2000, p. 3). Of those three brown seaweeds species, the giant kelp *M. pyrifera* has been harvested in Baja California since the 1950s. In 1989, more than 44,000 tons of fresh *M. pyrifera* were sent for alginate extraction to the CP Kelco plant (San Diego, CA). That amount was estimated to account for only one-half of the total estimated harvestable biomass of *M. pyrifera* (Hernández-Carmona 2000, p. 3). A total biomass of around 150,000 tons of *S. sinicola* was estimated from beds in the coast of the Gulf of California (Pacheco-Ruiz et al. 1998). Along the west side of Baja California, adjacent to and in the *M. pyrifera* beds, *E. arborea* grows abundantly (unpublished field observation), but the harvestable stock of this seaweed has not yet been determined. The algal resources mentioned might account for more than 200,000 tons of fresh algae harvestable annually. With this in mind, our research center has for many years invested considerable effort dedicated to the optimization of techniques for extraction of alginates. The monomer composition and sequence in alginates from *M. pyrifera* has been previously reported (Stokke et al. 1991; Smidsrød and Draget 1996). However, no information was found in the literature concerning the composition of alginates from *E. arborea*, and *S. sinicola*. In this manuscript, we report the detailed composition and sequence parameters of uronic acids in the alginates from *M. pyrifera*, *E. arborea*, and *S. sinicola* following the extraction procedures optimized by our research group. In this way, we hope to evaluate potential uses for alginates when a specific composition is required, as this is known to vary widely from species to species.

Materials and methods

Algae

For this study, algae were collected from different localities of the Baja California peninsula (Mexico) between summer 2002 and autumn 2003 by scuba diving. The gathered seaweeds were sun dried, and milled. The sodium alginate extraction of 10 kg dry *M. pyrifera* was conducted at pilot plant scale as described in previous papers (Hernández-Carmona et al. 1998, 1999, 2002; McHugh et al. 2001). Alginates from *S. sinicola* (20 kg) and *E. arborea* (10 kg) were extracted using the same method, but the process was optimized for each one. The alginates studied here are referred to as: Alg-Mp (sodium alginate from *M. pyrifera*), Alg-Ss (sodium alginate from *S. sinicola*) and Alg-Ea (sodium alginate from *E. arborea*).

Spectroscopy

Infrared (Fourier transformed infra-red spectroscopy; FTIR) spectra were acquired in transmission mode from thin films.

The films were prepared by drying 2 mL of a 1% aqueous solution (w/v) of sodium alginate at 50°C in polystyrene molds. The FTIR spectra were recorded with a Perkin Elmer Paragon 500 FTIR spectrometer. All spectra were acquired in the spectral range of 4,000–400 cm⁻¹ at a resolution of 4 cm⁻¹.

The sequence parameters of alginates were determined by ¹H-nuclear magnetic resonance (¹H-NMR) spectroscopy at the Norwegian Biopolymer Laboratory of the Norwegian University of Science and Technology. Spectra were recorded at 300.13 MHz with a Jeol FX-300 FT-NMR spectrometer, a sweep width of 3,591.9 Hz, and an acquisition time of 4.561 s. The data were processed with a line broadening of 0.3 Hz. 3-(Trimethylsilyl)-propane-sulfonate was used as an internal standard. The frequencies of monads (G, M), diads (GG, GM, MM) and triads (GGG, GGM, MGM) were determined by integration of peaks at δ5.05, 4.75, 4.72, 4.70, 4.67, and 4.45 ppm (Grasdalen et al. 1979; Davis et al. 2003).

Results and discussion

The FTIR spectra showed absorption bands or shoulders that were readily assigned to sodium alginate at 3390, 2930, 1615, 1410, 1320, 1300, 1170, 1150, 1125, 1090, 1035, 950, 900, 890, 815 and 780 cm⁻¹. A detailed comparison of the fingerprint region of the FTIR spectra revealed differences in the relative intensity of several bands, shown in Table 1 as the absorbance ratio relative to the carboxylate band at 1615 cm⁻¹. In particular, the greater relative intensity of bands at 1125 and 1090 cm⁻¹ for Alg-Ss, together with absorption at 1320 and 1000 cm⁻¹ and a lack of absorption at 1300, 1170, 1050 and 890 cm⁻¹, was a clear indication that Alg-Ss is composed of a higher proportion of guluronate monomers than the other alginates examined here. In contrast, Alg-Mp showed a typical set of bands of a high mannuronate alginate at 1300, 1250, 1170, 1050, 890, and 820 cm⁻¹. The FTIR trace of Alg-Ea was clearly a combination of that of Alg-Ss and Alg-Mp, suggesting that Alg-Ea is composed of similar amounts of both M and G monomers. In a semi-quantitative approach, FTIR spectroscopy revealed the content of guluronic residues in the analyzed alginates to be Alg-Mp < Alg-Ea < Alg-Ss. These findings were confirmed by computation of the frequencies of monads, diads, and triads (Table 2). The M/G molar ratio of the alginates was determined to be 0.56, 1.08, and 1.63 for Alg-Ss, Alg-Ea, and Alg-Mp, respectively. Of these three samples, Alg-Ss was highest in guluronic monomer content (64%), followed by Alg-Ea (48%), then Alg-Mp (38%). The homopolymeric G-blocks were 14, 7 and 4 G-residues in length for Alg-Ss, Alg-Ea, and Alg-Mp, respectively. All FTIR and ¹H-NMR data were

Table 1 Assignment and absorbance ratio of infrared (Fourier transformed infra-red spectroscopy; FTIR) spectrum bands relative to the carboxylate band at 1615 cm⁻¹ from sodium alginates extracted from *Macrocystis pyrifera* (Alg-Mp), *Eisenia arborea* (Alg-Ea) and *Sargassum sinicola* (Alg-Ss)

Band (cm ⁻¹)	Assignment ^a	Absorbance ratio ^b		
		Alg-Mp	Alg-Ea	Alg-Ss
1410	COO ⁻ stretching	0.707	0.558	0.478
1320	C—O stretching	NO ^c	NM ^d	0.017
1300	C—O stretching	0.125	0.054	NO ^c
1170	C—O stretching	0.059	0.023	NM ^d
	C—C stretching			
	C—C—C bending			
1150	Unknown	0.049	0.012	NO ^c
1125	C—O stretching	0.065	0.106	0.171
	C—C stretching			
1090	C—O stretching	0.064	0.206	0.238
	C—O—C stretching			
1000	Unknown	NO ^c	NO ^c	0.063
950	C—O stretching	0.134	0.113	0.086
	C—C—H stretching			
900	Unknown	NO ^c	0.001	0.036
890	C—C stretching	0.105	0.014	NM ^d
	C—C—H stretching			
	C—O bending			
815	Unknown	0.162	0.090	0.059

^a According to Sartori et al. (1997)

^b Non-dimensional magnitude, calculated by dividing the absorbance of a selected band by the absorbance of the carboxylate band at 1615 cm⁻¹

^c Not observed

^d Not measurable

consistent with previously published works (Mackie 1971; Grasdalen et al. 1979; Sartori et al. 1997; Davis et al. 2003).

The determination of the monomer composition and sequence of Alg-Ss, Alg-Ea, and Alg-Mp revealed great differences between them. Such variations are important,

because they determine the physical properties of these alginates. In general, alginates having a low M/G ratio (such as Alg-Ss) would give strong, brittle gels, making them suitable for cell encapsulation for biomedical or environmental applications, whereas alginates having a

Table 2 Compositional data and sequence parameters of sodium alginates extracted from *M. pyrifera* (Alg-Mp), *E. arborea* (Alg-Ea) and *S. sinicola* (Alg-Ss). M β-D-Mannuronic acid monomer, G α-L-guluronic acid monomer

	Frequency ^a		
	Alg-Mp	Alg-Ea	Alg-Ss
G	0.38	0.48	0.64
M	0.62	0.52	0.36
GG	0.18	0.33	0.54
MM	0.42	0.37	0.25
MG=GM	0.20	0.15	0.11
GGG	0.13	0.27	0.50
GGM=MGG	0.05	0.05	0.04
MGM	0.18	0.12	0.08
	Length of G-monoblocks and M/G ratio		
	Alg-Mp	Alg-Ea	Alg-Ss
N _{G>1} ^b	4	7	14
M/G ratio	1.63	1.08	0.56

^a Expressed as molar fraction, calculated according to Grasdalen et al. (1979)

^b Expressed as number of consecutive G residues

high M/G ratio (such as Alg-Ea and Alg-Mp) would form more elastic gels, which may be desirable for food, cosmetic or pharmaceutical products.

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