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Note

The effect of the content of D-mannuronic acid and L-guluronic acid blocks in alginates on antitumor activity

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We have previously reported that the alginate from *Sargassum fulvellum* showed antitumor activity against various murine tumors, such as Sarcoma-180 (solid and ascitic type) and Ehrlich ascites carcinoma tumors as allogeneic ones, and IMC carcinoma as a syngeneic one[1,2]. Smidsrød and associates have reported that alginate is a linear glycuronan having (1→4)-linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) units arranged in a nonregular, blockwise pattern along the chain[3–5], and also that biofunctional properties strongly correlate with both the composition (M/G ratio) and the MG sequence of the polyuronide[6]. These results suggested that the chemical properties of alginate may also correlate with the development of the antitumor activity. Thus, in order to clarify the relationship between the chemical properties and the antitumor activity of alginate, we have examined the effect of the content of D-mannuronic acid and L-guluronic acid blocks in alginates on their antitumor activity.

The alginates purified from Ek, Ln, La, Sf, Mm, St, and Sk were tentatively named as Ek-AlgNa, Ln-AlgNa, La-AlgNa, Sf-AlgNa, Mm-AlgNa, St-AlgNa, and Sk-Algna, respectively. Among them, five kinds of alginate (Ek-, Ln-, La-, Sf-, and Mm-AlgNa) were found to be very similar in molecular weight (mol.wt. range, 2.25–2.52 × 10^5, Table I), whereas St- (mol.wt. 0.69 × 10^5) and Sk-AlgNa (mol.wt. 1.48 ×

TABLE I

Molecular weight, ratio of MM-to-GG block, and antitumor activity of alginates

Alginate	*Mol.wt. (× 10^5)*	*Ratio of MM-to-GG block*	*Antitumor activity (ILS %) for dose (mg kg^{-1})*		
			50	*25*	*6.3*
Ek-AlgNa	2.36	3.01	162	154	77
Ln-AlgNa	2.52	2.36	117	58	50
La-AlgNa	2.25	2.20	31	46	38
Sf-AlgNa	2.26	1.16	38	8	−15
Mm-AlgNa	2.34	0.73	67	20	0

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10^5) had very different molecular weights. Therefore, St- and Sk-AlgNa were not used for the present study, as an explanation of the relationship between M and G content and the antitumor activity of the alginates requires all the alginates used to have almost the same molecular weight.

In the spectral regions of C-1 of the M and G residues of the ^{13}C-n.m.r. spectra of Ek-AlgNa (D-mannuronic acid-rich) and Mm-AlgNa (L-guluronic acid-rich) (see Fig. 1), the peaks of triad MGM, GGM, GMM, MMM, GGG, MGG, GMG, and MMG were assigned to the chemical shifts at δ 102.2, 102.3, 102.7, 102.8, 103.2, 103.3, 103.8, and 103.9, respectively. The block compositions (diad frequencies) were calculated from the proportions of the peak heights of the signals by the method of Grasdalen *et al.*[6], *i.e.*, $F_{MM} = F_{MMM} + F_{GMM}$, $F_{MG} = F_{MMG} + F_{GMG}$, and the corresponding expressions for G units are given by interchanging M and G (see Table II). The ratio of MM-to-GG block was found to vary considerably from alginate to alginate (from 0.73 to 3.01). The total content of MM and GG blocks was more than two third of the total block content of MM, MG, GM, and GG blocks. Among the alginates tested, Mm-AlgNa had the highest content of GG block, indicating that the alginate was the only L-guluronic acid rich one. On the other hand, the M/G ratios were also different from each other, except for those of Ln- and La-AlgNa. The other alginates were D-mannuronic acid-rich ones, and especially Ek-AlgNa had the highest content of MM blocks among them.

Both Ek- and Ln-AlgNa showed high antitumor activity against Sarcoma-180 in all the doses tested (see Table III). However, Sf- and Mm-AlgNa showed no activity at doses of 6.3 and 25 mg/kg, except for the dose of 50 mg/kg.

As shown in Table I, the alginates showed higher antitumor activity as the MM block content increased. The correlation coefficients of linear relationship between the

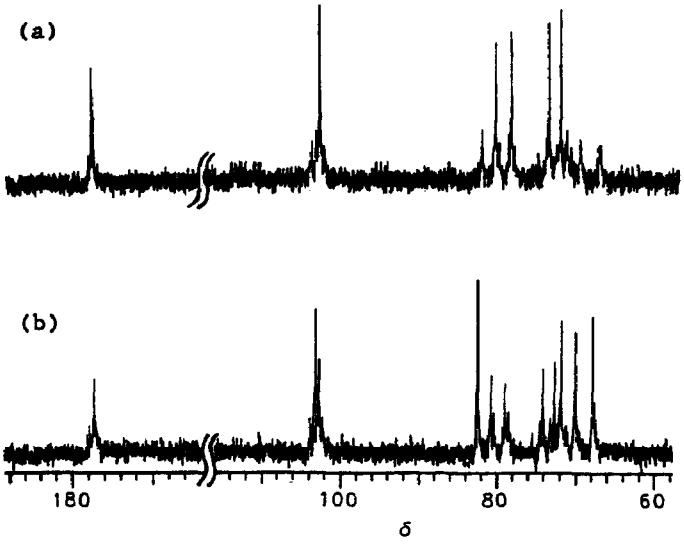


Fig. 1. ^{13}C-N.m.r. spectra (100 MHz) at 90° of a D_2O solution (22 mg mL^{-1}) of (a) Ek-AlgNa and (b) Mm-AlgNa.

TABLE II

Block compositions of alginates

Alginate	*Block content (%)*				*Ratio of MM to GG*	*Ratio of M to G*
	MM	*MG*	*GM*	*GG*		
Ek-AlgNa	53.7	14.7	13.7	17.8	3.01	2.12
Ln-AlgNa	45.6	19.3	15.9	19.3	2.36	1.71
La-AlgNa	47.9	15.2	15.1	21.7	2.20	1.71
Sf-AlgNa	34.5	15.9	19.9	29.7	1.16	1.10
Mm-AlgNa	32.5	11.6	11.7	44.3	0.73	0.78

TABLE III

Antitumor activity of alginates against Sarcoma-180 ascites tumor cells[a]

Alginate	*Dose (mg/kg/day)*	*MSD*	*ILS (%)*	*60-Day survivors*
None		13	0	0/7
Ek-AlgNa	50	34	162	2/7
	25	33	154	0/7
	6.3	23	77	0/7
La-AlgNa	50	17	31	0/7
	25	19	46	1/7
	6.3	18	38	1/7
Sf-AlgNa	50	18	38	0/7
	25	14	8	0/7
	6.3	11	−15	0/7
None		12	0	0/7
Ln-AlgNa	50	26	117	1/7
	25	19	58	1/7
	6.3	18	50	0/7
None		15	0	0/7
Mm-AlgNa	50	25	67	1/7
	25	18	20	1/7
	6.3	15	0	0/7

[a] S-180 cells (1×10^5) were inoculated into ICR mice at day 1,2,3,4,5,7,8,9, and 10; for further details see the Experimental section.

ratio of MM-to-GG block of alginates and their antitumor activity (ILS) at doses of 6.3, 25, and 50 mg kg^{-1} were found to be 0.86, 0.86, and 0.95, respectively. These results indicated that the higher content of MM block in alginate may correlate with the higher antitumor activity, because the molecular weights of the alginates tested were very similar. The conformation of D-mannuronan is extremely different from that of L-guluronan, the former being twisted, whereas the latter being buckled[7] and forming a so-called "egg-box model"[8] in the presence of Ca^{2+}. Therefore, the development of

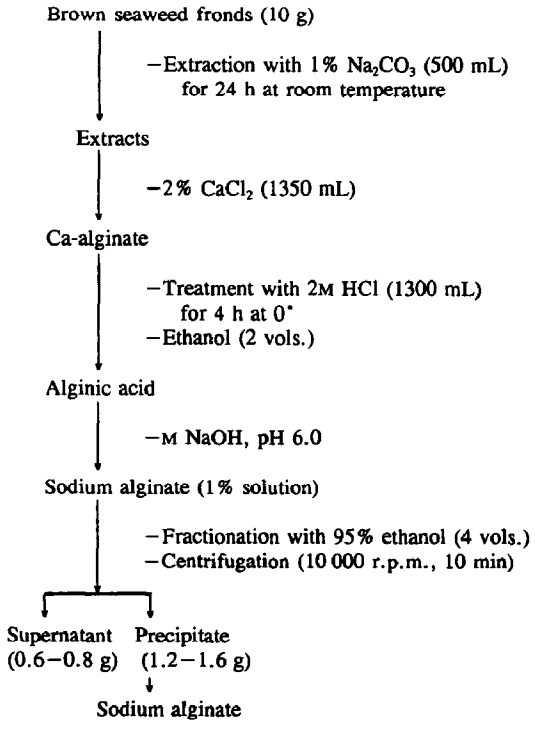
antitumor activity in alginates may be affected by their conformation, which is related to the content of MM or GG block.

EXPERIMENTAL

Materials. — The brown seaweeds used in the present study were as follows: *Ecklonia kurome* (Ek), *Sargassum fulvellum* (Sf), *Myagropsis myagroides* (Mm), and *Sargassum tortile* (St). These were collected at a seashore of the Japan Sea in December 1981 (Ek and Sf) and 1982 (Mm and St); *Laminaria angsutata* var. *longissima* (La) was collected at a seashore of Nemuro in May 1982, and *Sargassum kjellmanianium* (Sk) at a foreshore of the Inland Sea of Japan in April 1980; and *Lessonia nigrescens* (Ln) was imported from Chile in 1982.

Preparative methods of alginates. — Alginates were prepared from the fronds of the aforementioned seaweeds according to the method of Drummond *et al.*[9] as shown in Scheme 1. Electrophoresis[1] in 0.1M HCl and 0.1M zinc acetate showed a single band for each alginate.

Estimation of molecular weight. — The molecular weight of each alginate was estimated from its degree of polymerization, which was calculated from the intrinsic viscosity by use of the formula of Donnan and Rose[10], d.p./$[\eta]$ = 58. Viscosity was



Scheme 1. Procedure for the preparation of alginate from brown seaweeds.

determined for a 0.1M NaCl solution (0.1–0.5 mg/mL) of the alginate with an Ostwald-type viscosimeter at 25.00 ±0.02°.

^{13}C-N.m.r. estimation of the monomeric and block compositions of D-*mannuronic (M) and* L-*guluronic acid (G) units in alginates.* — Monomeric and block compositions of M and G were estimated from the ^{13}C-n.m.r. spectra of slightly depolymerized alginates by the method of Grasdalen *et al.*[6]. Depolymerized alginates were prepared by mild hydrolysis with HCl (pH 3.0, 100°, 30 min). The depolymerized sample was dissolved in D_2O (20–25 mg/mL), and the ^{13}C-n.m.r. spectrum was recorded with a Varian XL-400 FT (100 MHz) spectrometer at 90° using a 45° pulse, a pulse-repetition time of 2 s, 27 000–43 000 scans, and sodium 4,4-dimethyl-4-silapentanesulfonate (Aldrich Chem. Co.) as an internal standard.

Assay of antitumor activity. — Antitumor activity of the alginates was assayed against a murine tumor of Sarcoma-180 (ascitic type). Sarcoma-180 ascites cells (1×10^5 cells) were inoculated intraperitoneally (i.p.) into five week-old male ICR mice. Each test sample, dissolved in physiological saline solution, was injected i.p. in a dose of 6.3, 25, and 50 mg kg^{-1} day^{-1} at 24 h and on the days described in Table III after the tumor implantation. Antitumor activity was evaluated by the increase in life span (ILS), ILS = $(T/C - 1) \times 100\%$, where T is the median survival days (MSD) of the treated group and C the MSD of the control group. Survival of mice was scored 60 days after implantation of tumors, and mice remaining alive after this period were considered cured.

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