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Studies on the Chemical Composition and Antitumor Activity of the Acid Polysaccharide from Alga *Sargassum fusiforme*

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Abstract – An acid polysaccharide (SFP), was extracted from alga *Sargassum fusiforme* in hot water, was purified by ion exchange chromatography on DEAE-cellulose. The PC, chemical analysis, electrophoresis and IR of SFP indicated that it was a kind of alginate with a mol. wt. of 13,000 and a molar ratio of mannuronic acid to guluronic acid 2.75. Pharmacological tests showed that SFP could prolong the survival duration of mice suffering from ascitic Sarcoma 180 with a rate of life prolongation of 63.44%.

Key words – Alga, *Sargassum fusiforme*, acid polysaccharide, alginate, antitumor.

Introduction

The alga *Sargassum fusiforme*, a brown alga of *Sargassaceae Sargassum* C. AGARDH., is distributed mainly along the coast of Fujian, Guangdong and Zhejiang province in China. It was used as a kind of Chinese traditional medicine for thousands of years in China, and was recorded as "a salty alga can moisten, let out heat and draw water, therefore it can remove tumor and tuberculosis." in the Pen Tsao Kang Mu. Although the antitumor activity of *Sargassum fusiforme* polysaccharide was reported[a,b], the chemical composition and the relationship between the composition and antitumor activity of the *Sargassum fusiforme* polysaccharide have not been reported in recent years. The chemical composition and antitumor activity of polysaccharide from *Sargassum fusiforme* were studied in this paper.

Experimental

Materials – The alga *Sargassum fusiforme*, an artificial cultured product, was collected and dried in the sun at the coast of Dongtou county, Zhejiang province, China, in October, 1994.

Extraction and purification of polysaccharide – The dried alga (100 g) was milled and extracted with hot water (2×1 L) at room temperature. The crude extract (300 ml) was added slowly with ethanol (900 ml), and the solution was laid at 4℃ for 24 h and then filtered and centrifuged to collect precipitate (12 g). The procedure was repeated twice. The precipitate collected was dissolved in distilled water (120 ml), dialysed in 30 volumes of water, and freeze-dried to get crude polysaccharide (7.5 g).

The crude polysaccharide (5 g) was chromatographed on DEAE-cellulose column, using a gradient of 0~2 M NaCl as eluant. Eluates were monitored by UV absorbance at 270 nm with phenol-sulfuric acid reaction. The fractions containing the polysaccharide were pooled, concentrated, desalted and lyophilized, and pure acidic polysaccharide (SFP) (2 g) was obtained.

Chemical composition analysis – The composition of unit sugar of SFP was determin-

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ed by paper chromatography (PC) of the hydrolyzed solution of SFP which was hydrolyzed with 80% sulfuric acid. The PC of SFP was developed by *n*-butylalcohol, acetic acid and water (4:1:5) and colored with aniline and phthalic acid. The molar ratio of mannuronic acid to guluronic acid (M/G) was determined by chemical method reference to that of Ji Minghou[c]. The SFP (50 mg) was hydrolyzed with 80% sulfuric acid and neutralized with calcium carbonate. The hydrolyzed solution was concentrated and subjected to Dowex 1×8 (200~400 mesh) column chromatography, eluting with gradient of 0~2 mol/L acetic acid. The eluted fractions were collected and detected by phenol-sulfuric acid reaction. The amounts of M and G in the fractions were determined by the method of phenol-sulfuric acid and compared with the curves of standard M and G. The cellulose acetate membrane electrophoresis of SFP was performed on DYY-III 8 electrophoresis instrument (China) in 0.1% 1 M pyridine-acetic acid buffer (pH 3.5) at 100 v, 25℃ for 20 min. Chromogenic reaction was carried by spraying toluidine-blue solution on the cellulose acetate membrane at the end of electrophoresis. Ubbelohde viscosimeter was used to detect the molecular weight of SFP. The IR spectra of SFP was recorded on a Nicolet Model 510 FT-IR spectrometer.

Assay of antitumor activity – Sarcoma 180 ascites cells solution was taken out from H_{22} ascites male mice under bacteria-free condition, then washed and diluted to obtain suspensible cells solution (5×10^6/ml). The suspensible cells solution was implanted into the abdominal cavity of mice (0.2 ml each, Kunming strain) that were divided into two groups randomly next day (10 mice/group). One group was injected with the aqueous solution of SFP, ip, 75 mg/Kg, daily for 10 days, and the other group with 0.3 ml normal saline in the same method. The survival durations of mice were measured with the life prolongation rate.

Result and Discussion

The PC of SFP appeared as two brown-yellow spots of which Rf values were 0.24 and 0.28 respectively corresponding to that of standard guluronic acid and mannuronic acid. And the ratio of M/G in SFP resulted from the chemical analysis was 2.75, which was higher than that of the alginate from *Laminaria joponica* 2.01[d]. The result of electrophoresis showed that SFP was a acidic polysaccharide of which blue spot on the cellulose acetate membrane colored by toluidine-blue migrated from cathode to anode. The molecular weight of SFP was about 13,000 by the method of viscometry.

The feature absorption peaks of IR for SFP were as follows. a: 3429 cm^{-1}, the stretch vibration of O-H, existed in the hydrogen bond of molecules; b: 2930 cm^{-1}, the stretch vibration of -CH; c: 1614cm^{-1}, the asymmetric stretch vibration of $-COO^-$; d: 1417 cm^{-1}, the symmetric stretch vibration of $-COO^-$ and the stretch vibration of C-O within -COOH; e: 1255 cm^{-1}, the change angle vibration of C-H; f: 1035 cm^{-1}, the stretch vibration of C-O within C-O-H;. g: 821 cm^{-1}, the feature absorption of mannuronic acid; h: 790 cm^{-1}, the feature absorption of guluronic acid. The IR spectra of SFP was similar to that of the alginate from a brown alga *Laminaria joponica* which was used as raw materials for production of alginate in China [Fig. 1]. It was found in the IR spectra of SFP that the intensity of the peaks of mannuronic acid at 821 cm^{-1} and guluronic acid at 790 cm^{-1} were different from that of the alginate from alga *Laminaria joponica*. The peak of mannuronic acid of SFP was stronger, and the peak of guluronic acid weaker than that of alginate from *Laminaria joponica*. It indicated that the rate of M/G in SFP was higher than that of the alginate from *Laminaria joponica*. This result corresponded with that of chemical analysis. In addition, there was an asymmetry ring stretch vibration at 940 cm^{-1} for the al-

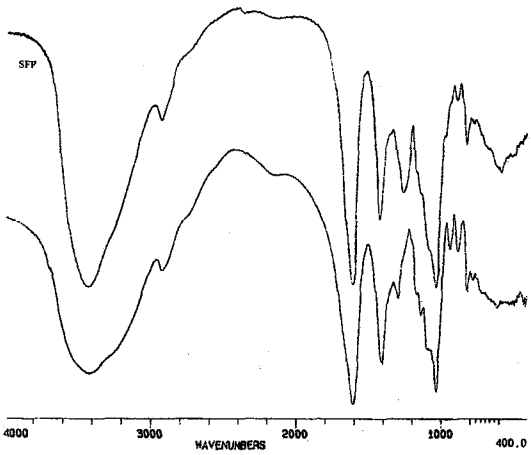

Fig. 1. The IR spectra of SFP and alginate from *Laminaria joponica.*

ginate from *Laminaria joponica,* but it disappeared in SFP. It suggested that the properties for substitution groups, hydroxy and carboxyl groups, on the pyran ring of SFP were different from that of the alginate from *Laminaria joponica.*

The effects of SFP on the survival duration of ascitic Sarcoma 180 mice were assayed for 10d. It was found that SFP could obviously prolong the survival duration of mice suffering from ascitic Sarcoma 180. The rate of life prolongation of SFP group was 63.44% (Table 1).

The chemical composition of the polysaccharide from *Sargassum fusiforme* was studied by the first time. We found the polysaccharide from *Sargassum fusiforme* was a kind of alginate composed of mannuronic acid and guluronic acid. The M/G ratio of SFP was higher than that of common alginates in China[d], for example, the alginate from *Laminaria joponica.* The properties of the substitution groups on pyran rings of SFP were different from that of common alginates. It could be deduced that the crosslinked structures between molecules in SFP were different from that of the latter. These chemical structure characteristics of SFP were similar to that of

Table 1. Effect of SFP on survival duration of mice suffering from ascitic Sarcoma 180

Group	Dosage $mg \cdot Kg^{-1}$	No. of mice	Average of life (days) ($\bar{x} \pm SD$)	Rate of life prolongation (%)
Control	-	10	14.5±2.99	
SFP	75	10	23.7±8.35*	63.44

*$p<0.05$.

the polysaccharide from *Sargassum fulvellum* reported by Michio, F., but the antitumor activity of SFP was lower than that of the latter[e]. Both of the results from Michio, F. and our experiment showed that the antitumor activities of the polysaccharide from *Sargassum fulvellum* and *Sargassum fusiforme* were associated with higher M/G ratio. And modes of linkages of pyran rings between polysaccharide chains were also associated with the antitumor activities. The relationship between structure and antitumour activity of SFP is under investigation.

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