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Preliminary studies on the chemical characterization and antihyperlipidemic activity of polysaccharide from the brown alga *Sargassum fusiforme*

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Abstract

A polysaccharide (SFP) extracted from the brown alga *Sargassum fusiforme* (Harv.) Setch. was purified by chromatography on DEAE-Sephadex A50 and Sephadex G-100. Studies using paper chromatography (PC), electrophoresis and infrared spectroscopy (IR) indicated that SFP was a kind of alginate with a molecular weight of 16 000 and a molar ratio of mannuronic acid (M) to guluronic acid (G) of 2.75. Pharmacological experiments showed that SFP could markedly decrease the content of total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-C) in the serum of experimental hyperlipidemic rats, and significantly increase the level of high density lipoprotein-cholesterol (HDL-C).

Introduction

China has a rich flora of brown algae. However at present, mainly members of the *Laminariaceae* (kelps) have been commercially exploited. Members of Sargassaceae are of lesser importance. *Sargassum fusiforme* (Harv.) Setch. is distributed along the coasts of Guangdong, Fujian, and Zhejiang provinces in southeastern China. It has been part of the cornucopiae of the traditional Chinese medicine for thousands of years. In the classic Ming Dynasty script, the Pen Tsao Kang Mu, it was recorded as "a salty alga [that] can moisten, let out heat and draw water, therefore it can remove tumor and tuberculosis". Although many investigations have discovered that the bioactivity of seaweed polysaccharide was closely associated with its chemical composition or structure, the relationship between the chemical characterization and the antihyperlipidemic activity of the polysaccharide from *S. fusiforme* has not been reported. The present paper is an attempt to fill this gap.

Materials and methods

Polysaccharide preparation

Individuals of the cultured alga *Sargassum fusiforme* were collected and dried in the sun at the coast of Dongtou county, Zhejiang province, eastern China, in November 1996. The dried seaweed was milled and extracted with water at 80 °C for 10 h. The crude extract was added slowly with 3 volumes of ethanol, and the solution was laid at 4 °C for 24 h. Filtration and centrifugation were conducted to collect precipitate. The precipitate collected was dissolved in distilled water, dialyzed in 30 volumes of water with VISKING dialyze membrane, and freeze-dried successively to get crude polysaccharide. Solution of the crude polysaccharide in water was applied to a column 26 × 100 cm containing DEAE-Sephadex A50 (Pharmacia Co.), and chromatographed using a gradient of 0~2 M NaCl as eluant. The eluates were monitored by UV absorbance at 270 nm. The fractions containing the polysaccharide were purified further by chromatography on Sephadex G-100 (Pharmacia Co.). The major fractions were pooled, concentrated, desalted and lyophilized to obtain polysaccharide (SFP).

Analysis of the polysaccharide

The constituent sugar of SFP was determined by paper chromatography (PC) of the hydrolyzed solution of SFP. Hydrolysis was conducted with 80% sulfuric acid at 20 °C for 18 h. The PC of SFP was developed on XinHua chromatographic paper in the solvent system n-butylalcohol, pyridine, and water (4:1:5). Spots of reducing sugars were visualized by spraying with aniline hydrogen phthalate reagent and subsequently heated at 105 °C for 5 min.

The molar ratio of mannuronic acid to guluronic acid (M/G) was determined by chemical method (Ji et al., 1981). SFP 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 eluted with gradient of 0~2 M acetic acid. The amounts of M and G in the fractions were determined by phenol-sulfuric acid reaction and compared with the curves of standard M (Sigma Co.) and G (Sigma Co.). 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 °C for 20 min. Chromogenic reaction was carried by spraying toluidine-blue solution on the cellulose acetate membrane at the end of electrophoresis.

The viscosity of SFP was determined by Ubbelohde viscometer with 1% SFP in 0.1 M NaCl at 25 °C (Haug et al., 1962; Moore, 1975), and the molecular weight of SFP was calculated from the intrinsic viscosity $[\eta]$ by Mark formula (Donnan & Rose, 1950; Ji, 1997).

The Infrared (IR) spectroscopy of SFP was recorded on a Nicolect model 510 FT-IR spectrometer (Mackie, 1971).

Assay of antihyperlipidemic activity

Twenty rats (Wistar strain, weight of body is about 200 ± 20 g) were fed with high fat diet for 30 days to obtain hyperlipidemic rats model. These rats were then divided into two groups randomly (10 rats/group): Hyperlipidemia group and SFP group. Hyperlipidemia group was continuously fed with the high fat diet. SFP group was given SFP by gastrointestinal injection at a daily dose (200 mg kg^{-1}) when raised with the high fat diet. Moreover, a control group (10 rats/group) raised with the basal diet was established. The ingredients of the diets are given in Table 1. The diet and water were supplied *ad libitum* during the experiment. The test was conducted for 15 days. Blood samples were taken from the tail region of the rat 12 h after the SFP gastrointestinal injection. The serum obtained by centrifugation was used to determine the level of total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) by enzyme methods. Results were expressed as mean ± SD and differences between groups were compared statistically using Student *t* test.

Table 1. Composition of basal diet and high fat diet for rats used in the experiment

Basal diet ingredients	%	High fat diet ingredients	%
Wheat powder	39.2	Basal diet	82.5
Rice	10.5	Cholesterol	2.0
Bean cake powder	15.1	Cholate	0.5
Corn meal	15.1	Egg yolk powder	5.0
Fish meal	15.1	Lard	10.0
Yeast powder	3.8		
Fish-liver oil	0.7		
Sale	0.5		

Results and discussions

Chromatography of polysaccharides from *Sargassum fusiforme* on DEAE-Sephadex A50 (100 × 2.6 cm) is shown in Figure 1. Fraction I was concentrated and further eluted on Sephadex G-100 (100 × 2.6 cm) column with water (Fig. 2). The major fractions were pooled, concentrated *in vacuo* and desalted on a Sephadex G-25 column (100 × 2.6 cm) with water. The eluate was concentrated and freeze-dried to obtain SFP.

The paper chromatogram (PC) of SFP appeared as two brown-yellow spots of which R_f values were 0.24 and 0.28, respectively, corresponding to that of standard guluronic acid and mannuronic acid. The molar ratio of mannuronic acid to guluronic acid (M/G) in SFP resulted from the chemical analysis was 2.75, which was higher than that of the alginate from most of the brown seaweeds, such as, *Laminaria japonica* Aresch. (2.26), *Sargassum pallidum* (Turn.) Ag. (1.26) and *Sargassum hemiphyllum*(Turn.) Ag. (1.06) (Ji & Wang, 1984). The result of electrophoresis showed

Table 2. Mean (± SD) levels of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) (10^{-3}M) in control, hyperlipidemia and SFP rats ($n = 10$)

Group	TC	TG	HDL-C	LDL-C
Control	1.51 ± 0.14	0.67 ± 0.13	1.26 ± 0.08	0.96 ± 0.15
Hyperlipidemia	5.24 ± 1.20$^{\Delta\Delta}$	1.29 ± 0.25$^{\Delta\Delta}$	0.63 ± 0.07$^{\Delta\Delta}$	2.50 ± 0.23$^{\Delta\Delta}$
SFP	2.12 ± 0.14**	0.68 ± 0.14*	1.09 ± 0.06*	0.80 ± 0.19**

Student's *t*-test, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with hyperlipidemia. $^{\Delta\Delta}$ $p < 0.01$ compared with control.

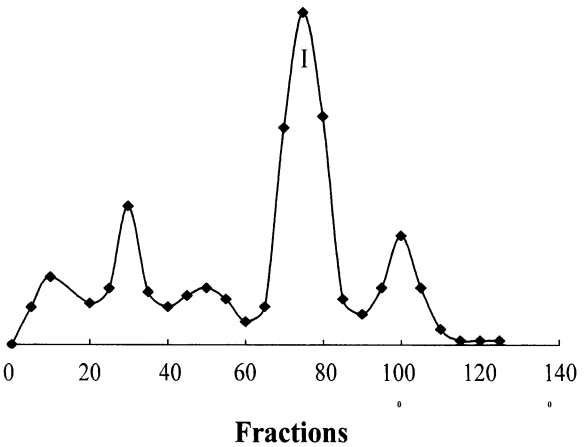

Figure 1. Chromatogram of polysaccharides from *Sargassum fusiforme* on DEAE-Sephadex A50 (100 × 2.6 cm).

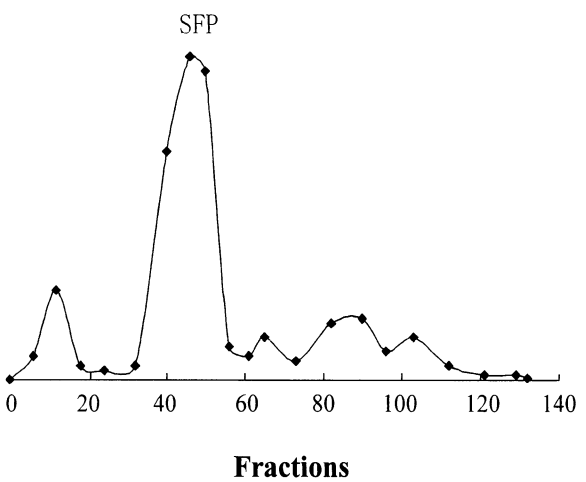

Figure 2. Chromatogram of fraction I on Sephadex G-100 (100 × 2.6 cm).

that SFP was an acidic polysaccharide which appeared as a blue spot colored by toluidine-blue on the cellulose acetate membrane. The blue spot migrated from cathode to anode. Using the method of viscometry, the molecular weight of SFP was estimated to be about 16 000.

The absorption peaks of IR for SFP were as follows: 3435 cm^{-1}, the stretch vibration of O-H, existed in the hydrogen bond of the molecules; 2930 cm^{-1}, the stretch vibration of −CH; 1614 cm^{-1}, the asymmetric stretch vibration of $-COO^{-}$; 1417 cm^{-1}, the symmetric stretch vibration of $-COO^{-}$ and the stretch vibration of C-O within −COOH; 1260 cm^{-1}, the stretch vibration of S=O; 1040 cm^{-1}, the stretch vibration of C-O and change angle vibration of O-H; 821 cm^{-1}, the feature absorption of mannuronic acid; 790 cm^{-1}, the feature absorption of guluronic acid. The spectrum of SFP was similar to that of the alginate from *Laminaria japonica* which was used as raw material for alginate production in China. It was found from the IR spectrum of SFP that the intensity of the peaks of mannuronic acid at 821 cm^{-1} and guluronic acid at 790 cm^{-1} was different from that of the alginate from *L. japonica*. The peak of mannuronic acid of SFP was stronger, and the peak of guluronic acid was weaker. It indicated that the M/G ratio in SFP was higher than that of the alginate from *L. japonica*. In addition, there was an asymmetry ring stretch vibration at 940 cm^{-1} for the alginate from *L. japonica,* but this is not present in SFP. It suggested that the properties for substitution groups, hydroxyl and carboxyl groups on the pyran rings of SFP were different from those of the alginate from *L. japonica*.

Table 2 shows that TC, TG, LDL-C levels in serum of the hyperlipidemic rats were significantly higher than those of the control and SFP groups ($p < 0.01$). HDL-C levels in serum of the hyperlipidemic rats was significantly lower than those of the other groups ($p < 0.01$). This indicates that SFP could obviously decrease the content of TC, TG and LDL-C in serum of experimental hyperlipidemic rats, and also significantly increase the level of serum HDL-C (Table 2). The decrease in TC level was 59.54%, TG 47.28%, LDL-C 68%, and the increase in HDL-C level was 42.2%. We deduce from the above findings that SFP has strong antihyperlipidemic activity.

The chemical composition of the polysaccharide from *Sargassum fusiforme* was studied for the first time. We found that it was a kind of acidic polysaccharide-alginate composed of mannuronic acid and guluronic acid with a M/G ratio higher than that of common alginate in China, for example, the alginate from *Laminaria japonica*. Individual seaweed species characteristically differ from each other in their relative proportions (M/G ratio) of these two constituent sugar acids, as well as in their sequencing with the polymer chain structural elements that have a bearing on their physical properties. Also, for a given species, its polysaccharide composition varies considerably depending upon the season, age, and the part of the plant used for extraction. The properties of the substitution groups on pyran rings in the SFP polysaccharide were different from those of common alginate. It could be deduced that the cross-link structures between molecules in SFP were different from those of the latter. These chemical structure characteristics of SFP were similar to those of the polysaccharide from *Sargassum fulvellum* (Turn.) C. Ag. reported by Michio et al. (1984). Though the research showed that the antitumour activities of the polysaccharides from *S. fulvellum* could be associated with its higher M/G ratio, the relation between antihyperlipidemic activity of polysaccharide from *S. fusiforme* and its M/G ratio could not be confirmed by our experiment. We think that the modes of linkages of pyran rings between polysaccharide chains could also be associated with the antihyperlipidemic activities. Further studies on the relationship between chemical structure and antihyperlipidemic activity of the polysaccharide from *S. fusiforme* are currently being undertaken.

References

Donnan F. G. & R. C. Rose, 1950. Osmotic pressure, molecular weight, and viscosity of sodium alginate. Can. J. Res. 28B: 105–113.

Haug A., & O. Smidsrod, 1962. Determination of intrinsic viscosity of alginate. Acta Chem. Scand. 16: 1569–1578.

Ji, M. H., 1997. Chemical composition and structure of algin. In Ji, M. H. (ed.), Seaweed Chemistry. Science Press, Beijing: 231–250.

Ji, M. H. & Y. J. Wang, 1984. Studies on the M:G ratios in alginate. Hydrobiologia 116: 554–556.

Ji, M. H., C. Wenda & H. Lijun, 1981. Determination of uronic acid composition of algin. Oceanol. Limnol. Sin. 12: 533–539.

Mackie, W., 1971. Semi-quantitative estimation of the composition of alginates by infrared spectroscopy. Carbohyd. Res. 20: 413–415.

Michio, F., I. Noriko, Y. Ichiro & N. Terukazu, 1984. Purification and chemical and physical characterization of an antitumour polysaccharide from the brown seaweed *Sargassum fulvellum*. Carbohyd. Res. 125: 97–106.

Moore, W. R., 1975. Viscosity of dilute polymer solutions. Progress in Polymer Science 1: 52–89.

Xu, S. Q., R. L. Bian & X. Chen, 1982. Pharmacology Experiment Methods. Renmin Hygiene Press, Beijing.

Zheng, N. Y., Y. X. Zhang & X. Fan, 1992. Studies on the compositions and sequential structures of uronate residues in alginates from Chinese brown algae *Laminaria* and *Sargassum*. Oceanol. Limnol. Sin. 23: 445–452.