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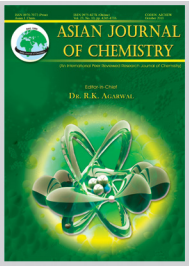

Fucoidan from Vietnam *Sargassum swartzii*: Isolation, Characterization and Complexation with Bovine Serum Albumin

THANH THI THU THUY[1,*], TRAN THI THANH VAN[1], YASUNAGA HIDEKAZU[2] and URAKAWA HIROSHI[2]

[1]Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam
[2]Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

*Corresponding author: Fax: +84 4 38361283; Tel: +84 4 37563788; E-mail: thuyttt@ich.vast.ac.vn

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Many brown seaweeds of *Sargassum* genus such as *S. polycystum, S. mcclurei, S. oligocystum, S. denticarpum, S. swatzii* are available in Nha-Trang bay of Vietnam. The structural characteristics of polysaccharides extracted from Vietnam's brown seaweed have not been fully established up to now. In this work, a fucoidan was extracted from brown seaweed *Sargassum swartzii* harvested at Nha-trang bay of Vietnam, the yield was 0.68 % (w/w) based on the dry seaweed. The uronic acid and sulfate content were obtained 6.3 % and 24.7 % of dried weight, respectively. The molar ratio of sugar residues was estimated to be fucose:galactose:mannose:xylose:glucose = 3.6:3.2:1.9:1:0.3 by gas chromatography. By using static and dynamic light scattering methods, conformation of fucoidan and its complex with bovine serum albumin at different pH environment were observed. The results showed that overall conformation of fucoidan is rigid rod. At pH value of 7.4, interaction between fucoidan and bovine serum albumin was very weak and conformation of native albumin was maintained. At pH value of 4.0 the interaction becomes strong, this is an electrostatic interaction occurred between sulfate groups of fucoidan and binding site in bovine serum albumin and a complex between fucoidan and bovine serum albumin forms very quickly and the complex has a spherical structure.

Key Words: *Sargassum swartzii*, Fucoidan, Structure, Light scattering, Complex, Bovine serum albumin.

INTRODUCTION

Fucoidans are sulfated polysaccharides derived from marine brown seaweed. They were reported to exhibit a wide range of physiological and biological activities such as anti-inflammatory, antiviral, anticoagulant, antitumor, antiangiogenesis activities[1-4]. Due to these activities fucoidans have potential applications in medicine and thus these polysaccharides, their production, structure and properties have been intensively investigated.

Fucoidans essentially contain fucose and sulfate groups along with galactose, xylose, mannose and uronic acids. This structural complexity, chemical composition of fucoidans may vary depending on the algae source, place of cultivation, method of extraction, *etc.*[5,6]. Thus, each new fucoidan has unique compound with unique structural characteristics which may display varied bioactivity and would be a potentially new drug.

In biochemical reaction, molecular assemblies concerned with such biopolymer as proteins, polysaccharides and nucleic acid plays an important role on the appearance of biofunctionalities. In particular, complexes of protein with polysaccharides are fundamental constituents of the tissues of living bodies. Many studies have been carried out on this type of complexes, mostly using bovine serum albumin (BSA) as the sample[7-9]. Serum albumin consists of a globular protein synthesized by liver in mammals and has many physiological functions. The most outstanding function of albumin is that they serve as a depot protein and a transport protein for numerous endogenous and exogenous compounds. Moreover, it plays an important role in the interaction between biomedical polymer surface and biocomponents.

Vietnam has a coastline of about 3200 km with the climate varying from subtropical in the northern to tropical in the southern part of the country, suitable for different seaweed species to grow. Among alga diversity, the brown seaweed *Sargassum* genus is the largest natural seaweed resource of Vietnam, including about 50 species. *Sargassum* genus has considerable potential for use in remediation schemes and is enriched in polysaccharides. However, the structural characteristics and biological activities of polysaccharide in general and fucoidan in particular extracted from Vietnam's brown seaweed *Sargassum* species have not been fully established up to now. In this paper, first we report isolation procedure, chemical characterization of fucoidan from brown seaweed *Sargassum swartzii* harvested in Nha Trang bay, Khanh Hoa

TABLE-1
CHEMICAL COMPONENTS OF STUDIED FUCOIDAN

Sugar component (% mol)					Uronic acid (%) dry weight	Sulfate content (%) dry weight
Fucose	Galactose	Mannose	Xylose	Glucose		
3.6	3.2	1.9	1	0.3	6.3	24.7

province of Vietnam. Second, light scattering methods including both dynamic and static measurements were applied to elucidate the conformational characteristics of the fucoidan chain and fucoidan-albumin complexes.

EXPERIMENTAL

Seaweed collection: Brown seaweed *Sargassum Swarzii* was harvested from the coastal area of Nha-Trang bay, Vietnam. The collected seaweed was washed with tap water in order to remove salt, epiphytes and sand attached to the surface of the sample and then dried by air in a shade. The dried seaweed was crushed and ground into a powder form and passed through a 40-mesh sieve and stored at room temperature.

Fucoidan extraction: 100 g of dried seaweed was treated at room temperature with a 4:2:1 MeOH-$CHCl_3$-H_2O mixture to remove coloured matter, filtered and vacuum dried to yield 72 g of defatted algal biomass. This material was extracted with 2 % aqueous $CaCl_2$ solution (400 mL × 3) under mechanical stirring at 85 ºC (each for 8 h). An aqueous hexadecyltrimethylammonium bromide solution (10 %, 80 mL) was added to the combined extracts. The precipitate formed was centrifuged, washed with water, stirred with 20 % ethanolic NaI solution (5 × 150 mL) for 2-3 days at room temperature, washed with ethanol and dissolved in water. The solution was dialyzed, concentrated and recovered fucoidan as sodium by freeze-drying. The yield of fucoidan obtained is 0.68 % calculated based on the weight of dried seaweed.

Purification of extracted fucoidan: The fucoidan sample was purified by dialysis and subsequent percolation of respective aqueous solution (C_p = 2 %) through an ion exchange resin (IR120). The resulted acidic aqueous solution were neutralized with aqueous solution of NaOH and then lyophilized to give pure fucoidan as sodium form. Pure fucoidan sample was used for all experiments

Bovine serum albumin (fragment V, fatty acid free) was purchased from Sigma and used without further purification. Buffers with pH values of 7.4 and 4.0 were purchased from Wako and Fluka respectively.

Chemical characterization

Neutral monosaccharide: Alditol acetate derivative was prepared[10] by hydrolysis of fucoidan sample in 2 M CF_3COOH (TFA), 8h at 100 ºC and analyzed by gas chromatography on a 17AAFW Shimadzu equipped with a FID detector (GC-FID).

Uronic acid content was determined by the carbazole method[11] using D-gluconic acid as a standard. Interference from hexoses in this assay was determined by use of controls containing the same ratio of component sugars as found in fucoidan. Differences in the absorption characteristics of products derived from uronic acid and hexoses were used to determine the final uronic acid content.

Sulfate content was estimated using gelatin/$BaCl_2$ method[12] after hydrolysis of fucoidan in 2 M CF_3COOH as above.

IR spectra: IR spectra were recorded on a FT-IR Bruker spectrometer.

Light scattering experiment: Light scattering measurements were performed with ALV spectrometer at 25 ºC with a He-Ne laser (λ_o = 633 nm), within an angular range from 35º to 130º and at 5º stepping. The refractive index increment dn/dc was estimated at λ_o = 633 nm with a Brice-Phoenix differential refractometor. To obtain dust-free solutions, the fucoidan and albumin aqueous solutions were filtered several times through 0.22 mm MF filters. Meanwhile, 0.45 mm MF filters (Millipore) was used to filter the aqueous solutions of fucoidan-albumin complex.

Sample preparation

For structural studies of fucoidan: NaCl solution 0.1 M was used as solvent for all light scattering measurements. Aqueous solution of fucoidan (3 mg/mL) was prepared as a stock solution.

For structural studies of bovine serum albumin-fucoidan complex: Ionic strength of all buffers is adjusted to 0.1 M by NaCl. Those buffers were used as solvents for all light scattering measurements of fucoidan-albumin system.

The stock solutions of fucoidan (final concentration of 1 mg/mL at pH 7.4 and 0.0001 mg/mL at pH 4.0) and bovine serum albumin (final concentration of 10 mg/mL at pH 7.4 and 0.001 mg/mL at pH 4.0) were prepared. All samples were obtained by diluting the stock solution by adding appropriate buffer.

RESULTS AND DISCUSSION

Structural characterization of fucoidan: The results of chemical analysis were shown in Table-1

IR spectrum: Major absorption bands were observed in IR spectrum at 3433 cm^{-1} (O-H stretching), 1151-1048 cm^{-1} (hemiacetal stretching) and 1227 cm^{-1} (S = O stretching). An absorption peak at around 1633 cm^{-1} indicated the presence of uronic acid. Absorption at 841 cm^{-1} was suggested to be due to sulfate groups at the axial C-4 position of fucopyranose residues. The position of sulfate groups is important to the biological activities of sulfated polysaccharides.

Light scattering: Results of light scattering measurements of fucoidan in 0.1 M NaCl solution at 25 ºC were shown in Fig. 1. Convenient Zimm plots of static and dynamic light scattering yield weight-average molecular weight (Mw), radius of gyration (Rg), second virial coefficient (A2) and hydrodynamic radius (Rh) as presented in Table-2. Structural parameter (ρ) is defined as a ratio of Rg and Rh. It is used as indicator for the shape of a solute molecule[13].

The ρ value obtained at 2.4 indicated a rigid rod-like structure of the fucoidan. Meanwhile, positive of second virial coefficient A2 observed by static light scattering explained a high density of sulfate groups at fucoidan chain as elucidated from chemical analysis.

TABLE-2
MOLECULAR CHARACTERISTICS OF THE FUCOIDAN IN 0.1 M NaCl EVALUATED FROM STATIC AND DYNAMIC LIGHT SCATTERING AT 25 ºC

C_{NaCl} (M)	dn/dc (mL g^{-1})	Mw × 10^{-3} (g mol^{-1})	A_2 × 10^3 (mol mL g^{-1})	R_g (nm)	R_h (nm)	ρ = Rg/Rh
0.1	0.119	124	1.42	51.0	21.5	2.4

TABLE-3
LIGHT SCATTERING RESULTS OF BOVINE SERUM ALBUMIN AND BOVINE SERUM ALBUMIN-FUCOIDAN COMPLEX AT SEVERAL pH

pH	Solvent	Sample	dn/dc (mLg^{-1})	Mw ×10^{-3} (g mol^{-1})	R_g (nm)	R_h (nm)	$\rho=R_g/R_h$
	Water	BSA	0.170	66.0	26.8	3.6	7.4
7.4	Buffer	BSA	0.180	125.4	24.6	5.9	4.2
	Buffer + Fuc.	BSA + Fuc.	0.160	140.4	29.2	6.3	4.3
4.0	Buffer	BSA	0.164	186.5	20.9	6.5	3.2
	Buffer + Fuc.	BSA + Fuc.	0.140	211.8	54.0	57.1	0.95

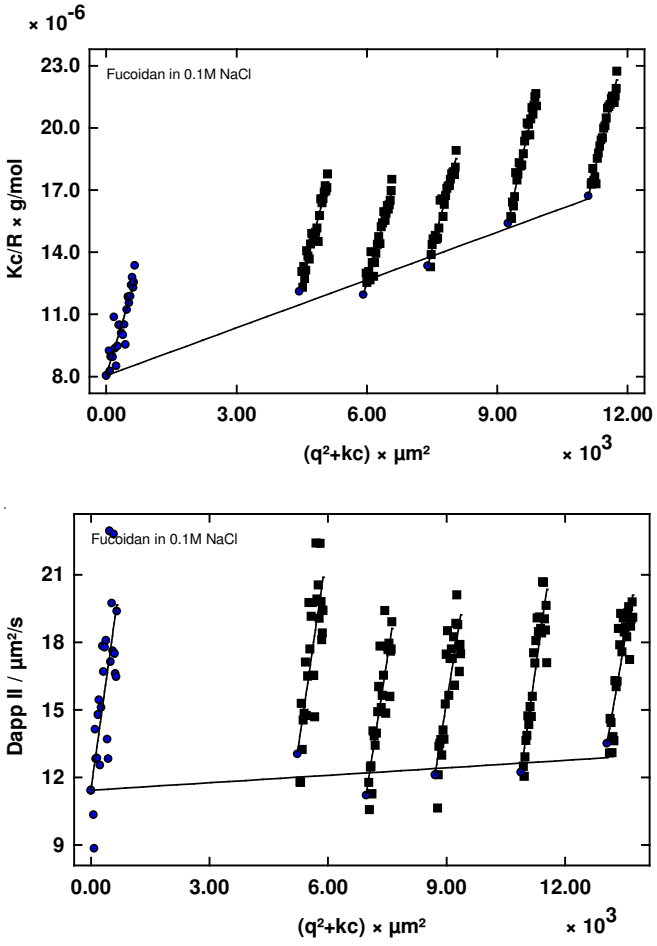

Fig. 1. Zimm plots for static (a) and dynamic (b) light scattering from fucoidan in 0.1 M NaCl solution

Many studies showed that biological activity of fucoidan may be have some relation with sulfate content and position, molecular weight and sugar composition[1,14]. Nishino *et al.*[15] found that a higher content of fucose and sulfate groups coincided with higher anticoagulant activities in sulfated polysaccharide fractions from *E. kurome*. They also showed that anticoagulation activity of fucans was positively correlated with sulfate content and that only fucans with a sulfate-total sugar residue ratio greater than one possessed significant activity[16,17]. Higher molecular weight fucans (*e.g.*, 27 and 58 kDa) showed greater anticoagulant activity than lower molecular weight ones (-10 kDa)[18]. The characterization of our fucoidan from *Sargassum swartzii* by different analytical methods showed that it is a high-molecular weight, highly sulfated heteropolysaccharide, its sulfate groups located at 4 position of fucopyranose residue. Our expectation that this fucoidan may have potent biological activities like anticoagulant, antioxidant or antitumor activities and this expectation is under investigation.

Structural characterization of bovine serum albumin-fucoidan complex: Bovine serum albumin has a molecular weight of 66.411 g mol (calculated from amino acid composition) and consists of 538 amino acids in a single polypeptide chain. Bovine serum albumin has an isoelectric point of about 4.7[19]. For bovine serum albumin-polysaccharide systems, they are both negative charged above the isoelectric point of the protein and oppositely charged below the isoelectric point. The purpose of this work is to observe the interaction and conformational changing of bovine serum albumin in complexation with fucoidan in aqueous solution at two pH values of 7.4 and 4.0, using light scattering method. In light scattering measurements, buffer-fucoidan-albumin system was looked upon as a two component system. This meant the fucoidan-buffer was regarded as solvent for bovine serum albumin.

The results of light scattering measurements are shown in Table-3.

At pH value of 7.4, the measured molecular weigh of albumin 125.4 k. It almost doubles that labelled by the chemical supplier (66.3 k) and our measurement results in distilled water (66.0 k). This was likely explained by dimerization tendency of albumin, which contains 17 disulfide bridges and one free SH group, being able to form covalent linked dimers[19]. In addition, the quaternary structure or ordered self-association state of a protein can be influenced by solution properties such as the pH and the ionic strength. At pH 7.4 and ionic strength of 0.1 M, the bovine serum albumin is consistent with dimeric quaternary structure.

The net charge of both albumin and fucoidan is negative at pH 7.4. This indicated a repulsive force between them. The molecular parameters and ρ value of bovine serum albumin and bovine serum albumin-fucoidan complex obtained from light scattering measurements were almost the same. This could be caused by weak electrostatic interaction between fucoidan and albumin. The ρ values of both albumin and bovine serum albumin-fucoidan complex are very high, more than 2, such high ρ values are characteristics of extended rigid rod structures[14]. It indicates that conformation of bovine serum

albumin was not changed and still kept its extended rigid structure by adding fucoidan at pH 7.4.

The result obtained from the light scattering measurement shows that the ρ value of albumin decreased from 4.2 at pH 7.4 to 3.2 at pH 4.0. This could provide an evidence of pH dependence of albumin conformation in solution (Table-3).

At pH value of 4.0, albumin has positive charge. Therefore, it can interact with fucoidan to form polyelectrolyte complex. The albumin forms trimer indicated by molecular weight value (Table-3). At pH 4.0, the fucoidan-albumin complex is formed quickly at very low concentrations of both albumin and fucoidan. Bovine serum albumin-fucoidan complex is found to possess a small ρ value of 0.95, suggesting a soft sphere structure. By adding fucoidan, the conformation of albumin is changed from extended rigid structure to a spherical structure as the solution becomes acidic.

Other authors reported that bovine serum albumin-SDS and bovine serum albumin-dextran complexes have Rh values of 5.87 nm and 7.5-49.8 nm, respectively[7,20]. Present result showed that, radius of the bovine serum albumin-fucoidan complex grew rapidly to 57.1 nm and up to more than 200 nm as complexes association occurred with the fucoidan. The complex was also formed at low concentrations, indicating fucoidan interacts strongly with bovine serum albumin. This is an electrostatic interaction occurred between sulfate groups of fucoidan and binding site in bovine serum albumin.

Conclusion

Fucoidan can be extracted simply from brown seaweed *Sargassum swartzii*. This fucoidan consists of fucose, galactose, mannose, xylose, glucose in the molar ratio of 3.6:3:2:1.9:1:0.3. Fucoidan extracted from *Sargassum swartzii* has rod-like structure in NaCl solution. At pH value of 4.0, intermolecular chain associations were easily formed between bovine serum albumin and fucoidan solution by electrostatic interactions. The rod-like fucoidan, when conforming a complex with albumin, results in a complex with spherical structure.

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