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# Heparinoid-active two sulfated polysaccharides isolated from marine green algae *Monostroma nitidum*

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ABSTRACT

Two sulfated polysaccharides WF1 and WF3 were isolated from marine green algae *Monostroma nitidum*, and their structural characteristics were determined. Anticoagulant activities of WF1 and WF3 were evaluated by assays of the activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), antithrombin and anticoagulation factor Xa activities. The results showed that WF1 and WF3 had similar high contents of rhamnose, whereas their sulfate contents, sulfation positions, molecular sizes and linkage patterns of rhamnose residues were different. The bioassay results demonstrated that WF1 and WF3 had high anticoagulant activities, and were potent thrombin inhibitors mediated by heparin cofactor II, especially WF3. They also hastened thrombin and coagulation factor Xa inhibition by potentiating antithrombin III, but at a lower effectiveness. The differences of anticoagulant activities between WF1 and WF3 were directly due to their structural features discrepancy.



## 1. Introduction

Naturally occurring or synthetic polysaccharides having similar biological activities to those of heparin are referred to as heparinoids. The polysaccharides isolated from marine algae are known to be one abundant source of heparinoids. Various anticoagulant-active polysaccharides, especially from marine red and brown algae, have been isolated and characterized (McLellan & Jurd, 1992). They contain a variety of sulfated galactans and sulfated fucans, and exhibit high anticoagulant activity. However, there are fewer reports of anticoagulant-active polysaccharides from marine green algae than those from brown and red algae. Jurd, Rogers, Blunden, and McLellan (1995) reported that the anticoagulant-active polysaccharides isolated from *Codium fragile ssp. atlanticum* were xyloarabinogalactans. Matsubara, Matsuura, Hori, and Miyazawa (2000) found anticoagulant activity in the extract of *Codium pugniforms* and isolated a highly sulfated galactoarabinoglucan. A sulfated galactan with anticoagulant activity was also isolated from green algae *Codium cylindricum* (Matsubara et al., 2001). Mao et al. (2006) discovered that the sulfated polysaccharide from *Ulva conglobata* showed high anticoagulant activity, and was mainly consisted of rhamnose with variable content of glucose and fucose. The anticoagulant polysaccharide from *Monostroma nitidum* yielded a 6-fold higher activity than that of heparin (Maeda et al., 1991). Hayakawa et al. (2000) found that two different sulfated polysaccharides from *Monostroma* species had more potent effect on the inhibition of thrombin than heparin or dermatan sulfate. Zhang et al. (2008) reported that a sulfated polysaccharide and its fragments from *M. latissimum* had high anticoagulant activities. With today's interest in new renewable sources of chemicals and polymers, the marine green algae represent potential source to be explored. Further work on the polysaccharides isolated from various marine green algae will aid in the development of new drugs and health foods.

The green seaweed *M. nitidum* grows in the warm water and is cultivated as edible algae. In this study, two sulfated polysaccharides WF1 and WF3 were isolated from marine green algae *M. nitidum*, and their chemical characteristics and anticoagulant activities were investigated.

## 2. Experimental

### 2.1. Materials

*Monostroma nitidum* was collected on the coast of Zhejiang Province, China. The raw material was thoroughly washed with tap water. The sample was air dried and kept in plastic bags at room temperature in a dry environment. APTT assay reagent (ellagic acid + bovine phospholipids reagent) and PT assay reagent (rabbit thromboplastin) were from Shanghai Sun (China). TT assay reagent (bovine thrombin) was from Dade Behring (USA). Thrombin, coagulation factor Xa, antithrombin III, heparin cofactor II and thrombin generation colorimetric substrate were purchased

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from Calbiochem (Germany). Coagulation factor Xa colorimetric substrate was from Sigma (USA). Standard heparin was purchased from Sigma (USA). All other reagents used were analytical grade.

### 2.2. Isolation and purification of the sulfated polysaccharide from M. nitidum

Dried algae were dipped into 20 volumes of distilled water and kept at room temperature for 2 h. The algae were then homogenized and the solution was refluxed at 100 °C for 2 h. After cooling, the supernatant was separated from the algae residues by centrifugation. The supernatants were concentrated under reduced pressure, dialyzed in cellulose membrane tubing (molecular weight cut off 8000) against distilled water for three successive days. The retained fraction was recovered, concentrated under reduced pressure, and precipitated by addition of 4-fold volume of 95% (v/v) ethanol and washed twice with absolute ethanol, followed by drying at 40 °C to obtain a crude polysaccharide. The crude extract was fractionated by a Q Sepharose Fast Flow column with distilled water, 1.0 mol/L NaCl and 3.0 mol/L NaCl. Total sugar content of the eluate was determined by the phenol-sulfuric acid method. The fractions eluted with 1.0 mol/L NaCl and 3.0 mol/L NaCl were, respectively, further purified by a Sephacryl S-400/HR column with 0.2 mol/L sodium acetate. The major fractions were pooled, concentrated, desalted and freeze-dried. Two sulfated polysaccharides were obtained and named as WF1 and WF3, respectively.

### 2.3. Composition analysis

Total sugar content was estimated by the phenol-sulfuric acid assay using rhamnose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Sulfate ester content was determined after hydrolysis with 1 mol/L HCl according to the methods of Therho and Hartiala (1971). Uronic acid content was determined by the carbazole-sulfuric acid method using glucuronic acid as the standard (Bitter & Muir, 1962). Optical rotation value was measured at 20 °C with digital polarimeter at 589 nm.

The composition of neutral monosaccharide was determined by gas chromatography (GC) after converting them into acetylated aldononitrile derivatives. Briefly, 10 mg of polysaccharide was hydrolyzed in a sealed glass tube with 2 mol/L trifluoroacetic acid (TFA) at 105 °C for 10 h. The hydrolysate was evaporated to dryness. TFA was removed under reduced pressure by repeated co-evaporations with methanol. The hydrolysates were then converted into alditol acetates according to conventional procedures. After adding 10 mg of hydroxylammonium and 3 mg of inositol (as internal reference), the mixture was dissolved in 0.5 ml of pyridine and incubated at 90 °C for 30 min. The mixture was cooled to room temperature. Acetic anhydride (0.5 ml) was then added to the mixture and the solution was incubated at 90 °C for another 30 min. The following neutral monosaccharides were used as references: rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose. GC was performed on an HP5890II instrument.

### 2.4. Measurement of molecular weight

Molecular weight was determined by high performance gel permeation chromatography. The column (A Shodex OHpak SB-804 HQ column, Japan) was maintained at 35 °C and the mobile phase was 0.1 mol/L $Na_2SO_4$ at a flow rate of 0.5 ml/min. The samples were dissolved in 0.1 mol/L $Na_2SO_4$ to reach a final concentration of 0.5% (w/v) and the sample solution was filtered through 0.45 μm filter membrane before injection (20 μl). Detection was at 35 °C with a refractive index detector (Agilent 1100 Series). Column calibration was performed with standard dextrans ($M_w$: 5.9, 22.8, 47.3, 112, 212, 404 and 788 kDa, respectively, purchased from Fluka). Calculation of the molecular weights of samples was carried out using the Angilent GPC software (USA).

### 2.5. Desulfation of the sulfated polysaccharide

The sulfated polysaccharide (30 mg) was dissolved in water and passed through an ion-exchange column (731 resin, $H^+$ form), which was eluted with distilled water. The combined effluent and washes were neutralized with pyridine to pH 7.0 and then lyophilized to give a white powdered pyridium salt. The salt was dissolved in 10 ml of dimethyl sulfoxide (DMSO) containing 10% (v/v) of anhydrous methanol and 1% pyridine, and then the solution was then shaken at 100 °C for 4 h. After the reaction was completed, the reaction mixture was diluted with an equal volume of water and adjusted to pH 9.0–9.5 by adding 1 mol/L sodium hydroxide. The solution was then dialyzed and the non-dialyzable portion was lyophilized. Decreased amounts of sulfate ester were calculated from sulfate ester analysis. Desulfation was confirmed by disappearance of sulfate ester peaks in its IR spectrum.

### 2.6. Methylation analysis

Each sample was treated according to the method of Hakomori (1964) with some modification. Each sample (2 mg) was dissolved in DMSO (2 ml) and anhydrous NaH (100–200 mg) were then added. The mixture was stirred at room temperature for 1.5 h. $CH_3I$ then was added to the mixture and stirred for a further 1.5 h. After the reaction was terminated with addition of water, the residue was extracted with $CHCl_3$. The extract was washed with distilled water and evaporated to dryness. The completion of methylation was confirmed by IR spectroscopy as the disappearance of OH bands. Methylated samples were hydrolyzed with 2 mol/L trifluoroacetic acid at 105 °C for 6 h. The methylated products were converted into their corresponding alditols by reduction with $NaBH_4$ and acetylated. The products were analyzed by gas chromatography–mass spectrometric (GC–MS) on DB 225 using a temperature gradient: first 100–240 °C with a rate of 5 °C/min; then keeping at 240 °C for 15 min. The peaks on the chromatogram were identified from their retention times. GC–MS was performed on an HP6890II instrument.

### 2.7. IR spectroscopy analysis

For IR spectroscopy, samples were mixed with KBr, grounded, and pressed into a 1 mm pellet. IR spectra of polysaccharides were recorded on a Nicolet Nexus 470 spectrometer.

### 2.8. Anticoagulant activities of the sulfated polysaccharides

All coagulation assays were performed with a coagulometer. Activated partial thromboplastin time (APTT) clotting assay was carried out by the method of Mourâno et al. (1996). Human plasma samples (90 μl) were mixed with 10 μl of a solution of different amounts of polysaccharide in 0.9% NaCl and incubated at 37 °C for 60 s before addition of 100 μl of pre-warmed APTT assay reagent and incubation at 37 °C for 2 min. Pre-warmed calcium chloride (100 μl, 0.25 mol/L) was then added and the APTT was recorded as the time for clot formation in a coagulometer.

Prothrombin time (PT) assay was as follows. Citrated normal human plasma (90 μl) was mixed with 10 μl of a solution of polysaccharide and incubated at 37 °C for 1 min. Then, 200 μl of PT assay reagent pre-incubated at 37 °C for 10 min was added and clotting time was recorded.

Thrombin time (TT) assay was performed as follows. Citrated normal human plasma (90 μl) was mixed with 10 μl of a solution of polysaccharide and incubated at 37 °C for 60 s. Then, 200 μl of

TT assay reagent pre-warmed to 37 °C was added and clotting time was recorded.

### 2.9. Inhibition of thrombin or coagulation factor Xa by antithrombin III and heparin cofactor II in the presence of the sulfated polysaccharide

These assays were performed according to the method of Colliec et al. (1991). Human thrombin or coagulation factor Xa and inhibitors (heparin cofactor II or antithrombin III) were incubated with or without the sulfated polysaccharide in 200 μl of 50 mmol/L Tris–HCl (pH 7.4), 7.5 mmol/L EDTA, 0.15 mol/L NaCl at 37 °C. After 5 min incubation, 150 μl of Tris–HCl buffer containing 1.5 mmol/L chromogenic substrate S-2238 for thrombin or S-2222 for coagulation factor Xa was added, and the residual thrombin or coagulation factor Xa activity was determined by measuring the change in the absorbance at 405 nm. The rate of change of absorbance was proportional to the thrombin or coagulation factor Xa activity remaining in the incubation. No inhibition occurred in control experiments in which thrombin or coagulation factor Xa was incubated with antithrombin III or heparin cofactor II in the absence of the sulfated polysaccharide.

### 2.10. Statistics

All bioassay results were expressed as means ± standard deviation (*SD*). All analyses were carried out with GraphPad Instat 3.0 for Windows (GraphPad Software, San Diego, CA, USA).

## 3. Results and discussion

### 3.1. Chemical compositions of the sulfated polysaccharides

The crude polysaccharide was extracted from *M. nitidum* in hot water, and then was purified by a combination of Q Sepharose Fast Flow column and Sephacryl S-400/HR column. The fractions eluted with 1.0 mol/L NaCl and 3.0 mol/L NaCl contained abundant total sugar. The polysaccharide fractions were, respectively, pooled, dialyzed and were further fractionated on a Sephacryl S-400/HR column based on molecular mass. By the process, two sulfated polysaccharides were obtained and named as WF1 and WF3, respectively.

The chemical compositions of WF1 and WF3 were shown in Table 1. The two polysaccharides were found to contain mainly rhamnose with small amounts of xylose and glucose. They were similar in terms of uronic acid content (7.92–6.76%) and protein contents (0.66–0.99%). The sulfate contents of WF1 and WF3 were high, especially WF3 (34.4%). On the other hand, WF1 and WF3 showed optical rotation $[\alpha]_D$ −37.2° and −64.8°, respectively. The result suggested that WF1 and WF3 were homogeneous in terms of its electrical charge. Molecular weight of WF1 (870 kDa) was notably higher than that of WF3 (70 kDa), as determined by high performance gel permeation chromatography.

**Table 1**
Chemical compositions of WF1 and WF3 isolated from *Monostroma nitidum*

| Component | Polysaccharide | |
|---|---|---|
| | WF1 | WF3 |
| Sulfate ester content (%) | 28.2 | 34.4 |
| Protein content (%) | 0.99 | 0.66 |
| Uronic acid content (%) | 7.92 | 6.76 |
| Molecular weight (kDa) | 870 | 70 |
| Optical rotation | −37.2° | −64.8° |
| *Neutral monosaccharide content (mol %)* | | |
| Rhamnose | 79.4 | 78.2 |
| Xylose | 5.2 | 14.7 |
| Glucose | 10.1 | 3.7 |
| Galactose | 5.3 | nd[a] |
| Mannose | nd[a] | 3.4 |
| Arabinose | nd[a] | nd[a] |

[a] Below detection limit (0.001).

### 3.2. Methylation analysis

After successive permethylation of polysaccharide materials using the Hakomori method, completely methylated products were obtained. The completion of methylation was confirmed by IR spectroscopy as the disappearance of OH bands. Partially *O*-methylated rhamnitol acetates were detected from the hydrolysate of permethylated polysaccharide from the GC–MS analysis. Larger amounts of 1,2,5-Tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol, which originated from a 1,2-linked L-rhamnose residue (Table 2), was found in the desulfated WF1 than WF1. Reduced amounts 1,2,3,4,5-Penta-*O*-acetyl-L-rhamnitol were detected. The results showed that the sulfate substitutions were at C-3 and C-4 of 1,2-linked L-rhamnose residues. 1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol was also detected, indicating the presence of a 1,4-linked L-rhamnose residues. The two kinds of rhamnosyl linkages may constitute the consecutive repeating units of WF1. A peak of 1,3,5-Tri-*O*-acetyl-2,4-di-*O*-methyl-L-rhamnitol, which originated from a 1,3-linked L-rhamnose residue, appeared most abundantly in the desulfated WF3 than WF3. 1,2,5-Tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol was also detected, indicating the presence of a 1,2-linked L-rhamnose residues. The results showed that WF3 was composed of 1,3- and 1,2-linked L-rhamnose residues. Moreover, reduced amounts 1,2,3,4,5-Penta-*O*-acetyl-L-rhamnitol were detected. Thus, the sulfate substitutions were deduced to be at C-2 and C-4 of 1,3-linked L-rhamnose residues or to be at C-3 and C-4 of 1,2-linked L-rhamnose residues.

### 3.3. IR spectroscopy

IR spectra of WF1 and WF3 showed the signals at 3419–3444 $cm^{-1}$ were from stretching vibration of O–H, 2940–2963 $cm^{-1}$ were due to stretch vibration of C–H, and 1059–1061 $cm^{-1}$ were from stretching vibration of C–O (Fig. 1). In addition, signals at 1643–1660 $cm^{-1}$, asymmetric stretch vibration of $COO^-$ of uronic acids; 1434–1455 $cm^{-1}$, symmetric stretch vibration of $COO^-$ and stretch vibration of C–O within COOH. IR spectra showed several bands corresponding to sulfate ester: the peaks at 865–857 $cm^{-1}$ and 1255–1260 $cm^{-1}$ derived from the bending vibration of C–O–S of sulfate in axial position and stretching vibration of S–O of sulfate, respectively.

### 3.4. Anticoagulant activities of the sulfated polysaccharides

Anticoagulant activities based on APTT and TT assays with WF1 and WF3 were listed in Figs. 2 and 3. Their anticoagulant activities were compared with that of heparin, a classical anticoagulant. APTT and TT were effectively prolonged by WF1 and WF3. The signals for clotting time of WF1 and WF3 became excessively saturated of high concentration level (15 and 100 μg/ml for APTT and 50 and 200 μg/ml for TT), at 200 s and 120 s. The anticoagulant activities of WF1 and WF3 were weaker than that of heparin, and high concentrations were required to obtain the same effect as with heparin. Generally, 12 blood coagulant factors are sequentially combined with coagulation process, which include the intrinsic and/or common pathway and extrinsic pathway. The prolongation of APTT indicates inhibition of the intrinsic and/or common pathway. TT assay is helpful to investigate the sample effect in thrombin accelerated clot formation in platelet poor plasma, whereas prolongation of TT suggests inhibition of thrombin activ-

**Table 2**
Methylation analysis of WF1, WF3 and their desulfated polysaccharides isolated from *Monostroma nitidum*

| Retention time (min) | Methylation product | Molar ratio | | | | Deduced linkage pattern |
|---|---|---|---|---|---|---|
| | | WF1 | Desulfated WF1 | WF3 | Desulfated WF3 | |
| 21.09 | 1,2,5-Tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol | 1.08 | 1.80 | 2.0 | 4.84 | →2)Rha(1→ |
| 21.30 | 1,4,5-Tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol | 1 | 1 | 1.65 | 0.06 | →4)Rha(1→ |
| 24.39 | 1,2,3,4,5-Penta-*O*-acetyl-L-rhamnitol | 12.59 | 1.48 | 19.67 | 0.62 | →2,3,4)Rha(1→ |
| 21.48 | 1,3,5-Tri-*O*-acetyl-2,4-di-*O*-methyl-L-rhamnitol | | | 1.51 | 8.53 | →3)Rha(1→ |
| 17.99 | 1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol | | | 1 | 1 | Rha(1→ |

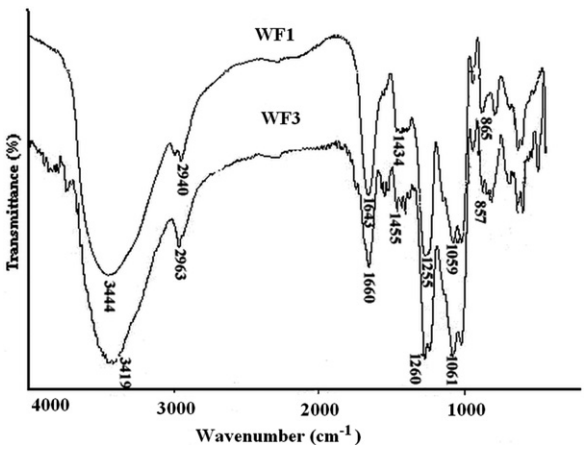


**Fig. 1.** IR spectra of WF1 and WF3 isolated from *Monostroma nitidum*.

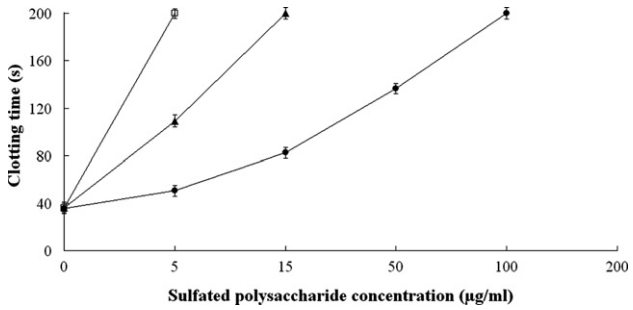


**Fig. 2.** Analysis of the anticoagulant activity by APTT on WF1 and WF3 isolated from *Monostroma nitidum*. Results representative of four separated experiments. WF1 (●), WF3 (▲), Heparin (□).

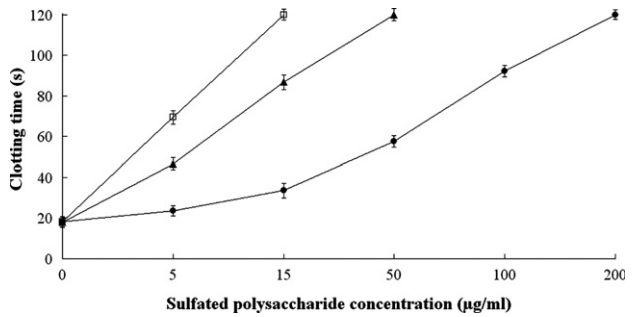


**Fig. 3.** Analysis of anticoagulant activity by TT on WF1 and WF3 isolated from *Monostroma nitidum*. Results representative of four separated experiments. WF1 (●), WF3 (▲), Heparin (□).

ity or fibrin polymerization. Thus, WF1 and WF3 inhibited both the intrinsic and/or common pathways of coagulation and thrombin activity or conversion of fibrinogen to fibrin. The effects of WF1 and WF3 on PT were significantly different from that of heparin. Lack of prolongation effect of WF3 and WF1 on PT was discovered (data not shown). The results suggested that WF1 and WF3 did not inhibit extrinsic pathway of coagulation. The differences between the anticoagulant activities of WF1 and WF3 were pronounced. WF3 had higher anticoagulant activity than WF1 at the same concentration in the APTT assay. The similar result was observed in the TT assay. The differences of anticoagulant activities between WF1 and WF3 may be directly due to their structural features variation.

Zhang et al. (2008) discovered that the molecular size had an important effect on anticoagulant activity of the sulfated polysaccharide isolated from *M. latissimum*. In this study, the molecular size of WF1 was significantly higher than that of WF3. The interaction of WF1 with coagulation inhibitors and their target proteases could be influenced by the high molecular size. Shanmugam and Mody (2000) reported that anticoagulant activity largely depended on the molecular size of the polysaccharides, its sulfate content and position. Melo, Pereira, Fogue, and Mourão (2004) also revealed that the nature of the sulfation position markedly modified the anticoagulant activity of sulfated galactan. In the study, WF3 had higher sulfate content, and showed notably higher anticoagulant activity than WF1 in APTT and TT assay. Moreover, the differences of sulfation positions in the two sulfated polysaccharides may influence their anticoagulant activities. Furthermore, the linkage patterns of rhamnose residues in WF1 and WF3 were essentially different. From these results, we suggested that the difference in anticoagulant activity between WF1 and WF3 was not merely a consequence of molecular size, but an increased charge in density, sulfation positions and the linkage pattern of rhamnose residues may also play an essential effect on the anticoagulant activity.

### *3.5. Inhibition of thrombin or coagulation factor Xa by antithrombin III and heparin cofactor II in the presence of the sulfated polysaccharide*

To elucidate the inhibitory mechanism of WF1 and WF3, further investigations on their inhibition of coagulation factors were carried out with the amidolytic anti-factor thrombin and coagulation factor Xa assays in the presence and in the absence of antithrombin III or heparin cofactor II. Normally, antithrombin III inhibits all intrinsic pathway coagulation enzymes, and heparin cofactor II is a serine protease inhibitor and selectively inhibits thrombin. In the absence of antithrombin III or heparin cofactor II, the amidolytic activity of thrombin was weakly inhibited by WF1 and WF3 in a dose-dependent manner (Figs. 4 and 5). The effects of WF1 and WF3 on heparin cofactor II-dependent thrombin inhibition showed that WF1 and WF3 had a considerable effect on the amidolytic activity of thrombin in the presence of heparin cofactor II, and the ability of WF3 and WF1 to stimulate inhibition of thrombin by heparin cofactor II was stronger than that of heparin, especially WF3. However, WF1 and WF3 had a weak antithrombin III-dependent thrombin inhibition. In the absence of antithrombin III or heparin cofactor II, the amidolytic activity of coagulation factor Xa was not inhibited by WF1 and WF3 in a dose-dependent manner (Fig. 6). WF1 and WF3 had a weak effect on the amidolytic activity of

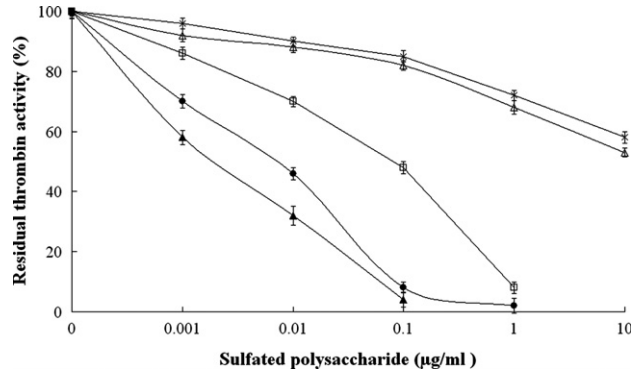


**Fig. 4.** Effect of WF1 and WF3 on inhibition of thrombin activity in the absence and presence of heparin cofactor II. Thrombin was incubated with the sulfated polysaccharide in the absence (WF1, ×; WF3, △) and in the presence (WF1, ●; WF3, ▲) of heparin cofactor II. Thrombin was incubated with heparin in the presence of heparin cofactor II (□).

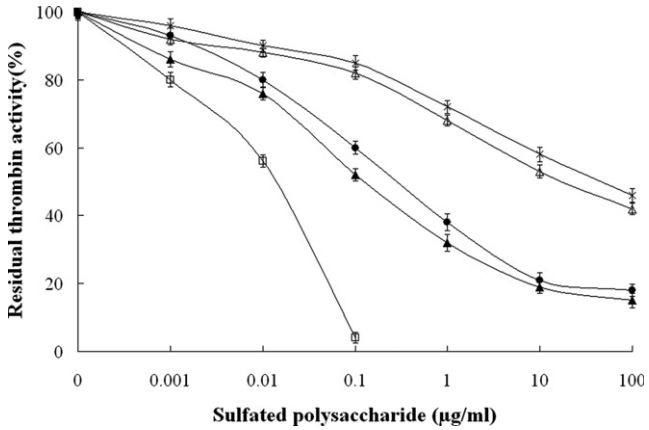


**Fig. 5.** Effect of WF1 and WF3 on inhibition of thrombin activity in the absence and presence of antithrombin III. Thrombin was incubated with the sulfated polysaccharide in the absence (WF1, ×; WF3, △) and in the presence (WF1, ●; WF3, ▲) of antithrombin III. Thrombin was incubated with heparin in the presence of antithrombin III (□).

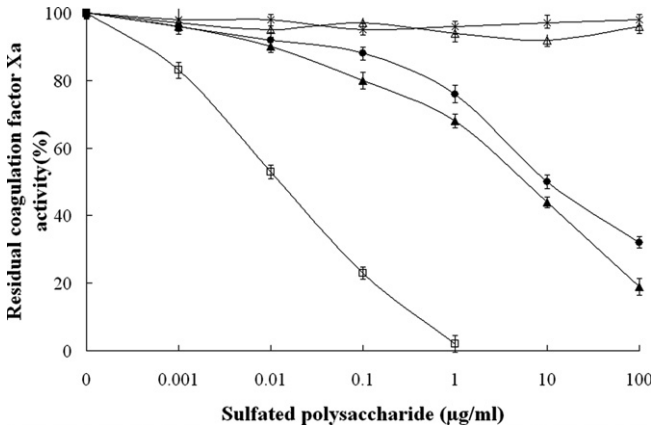


**Fig. 6.** Effect of WF1 and WF3 on inhibition of coagulation factor Xa activity in the absence and presence of antithrombin III. Coagulation factor Xa was incubated with the sulfated polysaccharide in the absence (WF1, ×; WF3, △) and in the presence (WF1, ●; WF3, ▲) of antithrombin III. Coagulation factor Xa was incubated with heparin in the presence of antithrombin III (□).

coagulation factor Xa in the presence of antithrombin III. Additionally, none of WF1 and WF3 had heparin cofactor II-dependent coagulation factor Xa inhibition (data not shown). These results suggested that WF1 and WF3 had a strong antithrombin activity, but had a weak anticoagulation factor Xa. The powerful antithrombin activity was mediated mainly by heparin cofactor II, whereas the weak anticoagulation factor Xa was mediated by antithrombin III.

Hayakawa et al. (2000) reported that the ability of rhamnan sulfates from *Monostroma* species to stimulate inhibition of thrombin mediated by heparin cofactor II was stronger than heparin. Compared with rhamnan sulfates, WF1 and WF3 exhibited some differences for the thrombin inhibition in the presence of heparin cofactor II. The differences of thrombin inhibition among them may be attributed to their structural features variation. Nishino, Aizu and Nagumo (1991) found that the heparin cofactor II-mediated antithrombin activity of fucan sulfate was dependent on both its sulfate content and molecular weight. Antithrombin III–mediated anticoagulant activity of the sulfated polysaccharides from marine organisms have been studied in detail by Melo et al. (2004). The investigation suggested that structural requirements for the interaction of sulfated galactans with coagulation inhibitors and their target proteases were not merely a consequence of their charge density, and the structural basis of this interaction was complex, and depended on the distribution of sulfate groups and on monosaccharide compositions. Ferial, Mosstafa, Corinne and Catherine (2000) reported that the inhibitory effects of fucans on both coagulation and cell proliferation were dependent on not only molecular weight but also sulfation degree. Pereira, Mulloy and Mourão (1999) discovered that branched complex fucans from brown algae directly inhibited thrombin activity, whereas linearly linked fucans from echinoderms inhibited thrombin activity by potentiating antithrombin III and heparin cofactor II, and do not have direct inhibitory effect on thrombin activity. The paradigm of heparin–antithrombin interaction could not be extended to other sulfated polysaccharides, and each type of polysaccharide may form a particular complex with the plasma inhibitor and the target protease (Melo et al., 2004). The structural studies on the sulfated polysaccharides isolated from marine green algae *M. nitidum* will indubitably play an indispensable role in the understanding of the anticoagulant activity.

## 4. Conclusion

WF1 and WF3 isolated from marine green algae *M. nitidum* had high anticoagulant activities, and specially interfered with the coagulation cascade at several stages. The anticoagulant property of WF1 and WF3 was mainly attributed to powerful potentiation thrombin by heparin cofactor II. Complex relationships existed between the structure and anticoagulant property of the sulfated polysaccharides.

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