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# Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*

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**Abstract**

A sulfated polysaccharide from the green alga *Monostroma latissimum* was extracted in hot water and purified by ion-exchange and size-exclusion chromatography. Five sulfated polysaccharide fragments with different molecular weights were prepared from the sulfated polysaccharide by $H_2O_2$ degradation. The molecular weights of the parent sulfated polysaccharide and its fragments were 725.4, 216.4, 123.7, 61.9, 26.0 and 10.6 kDa, respectively. These sulfated polysaccharide preparations have high contents of rhamnose. Anticoagulant activities of the parent sulfated polysaccharide and its fragments were investigated by studying the activated partial thromboplastin time (APTT), thrombin time (TT) and prothrombin time (PT) using human plasma. The six sulfated polysaccharide preparations did not affect PT even at the concentration at which APTT and TT were prolonged. The sulfated polysaccharides fragments with molecular weights of 216.4–61.9 kDa had similar anticoagulant activities as the parent sulfated polysaccharide. A decrease in the molecular size of the sulfated polysaccharide fragments dramatically reduced their anticoagulant activities. The results indicated that molecular size had an important effect on the anticoagulant activity of the sulfated polysaccharide obtained from *M. latissimum*, and an even longer chain was necessary to achieve thrombin inhibition.


*Keywords:* *Monostroma latissimum*; Sulfated polysaccharide; Molecular weight; Activated partial thromboplastin time; Thrombin time

## 1. Introduction

The green seaweed *Monostroma latissimum* has been used as fundamental source of human food and drug in traditional Chinese medicine for centuries. It grows in the brackish water area in the upper part of the intertidal zone in the warm waters. One particularly interesting feature of the seaweeds is their richness in polysaccharides, which are useful in topical skin treatments and may have some pharmaceutical applications related to healing of muscle tissue. Sulfated polysaccharides from Monostromaceae also exhibit many biological activities such as anticoagulant, antiviral, antiherpetic and antioxidant activities (Lee, Hayashi, Hayashi, Sankawa, & Maeda, 1999; Lee, Hayashi, Maeda, & Hayashi, 2004; Maeda, Uehara, Harada, Sekiguchi, & Hiraoka, 1991; Wu & Pan, 2004). Especially, polysaccharides from Monostromaceae show potent anticoagulant activity. Maeda et al. (1991) discovered that the active polysaccharide extracts from *Monostroma nitidum* yield a sixfold higher activity than that of heparin. Hayakawa et al. (2000) found that two different sulfated polysaccharides from *M. nitidum* and *M. latissimum* had more potent effect on the inhibition of thrombin than heparin or dermatan sulfate. Studies on the structures of the polysaccharides from Monostromaceae have been limited. Some works showed that the polysaccharides from *M. latissimum* mainly consist of $1 \rightarrow 2$ and $1 \rightarrow 3$ linked rhamnose in a ratio of 3:2 and sulfate was mainly at the C-3 or C-4 position of the $1 \rightarrow 2$ linked rhamnose residues (Lee, Yamagaki, Maeda, & Nakanishi, 1998). Harada and

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Maeda (1998) obtained a polysaccharide with similar structure in *M. nitidum*.

Previous studies indicated that molecular weight distributions of polysaccharides had influence on their biological activities (Chen & Wang, 1997; Zhou, Sheng, Yao, & Wang, 2006; Zhou et al., 2004; Zhao et al., 2006). Anticoagulant activity largely depends on the molecular size of the polysaccharides and also relate to monosaccharide composition, sulfate content and position (Shanmugam & Mody, 2000). Jurd, Rogers, Blunden, and McLellan (1995) reported that the sulfated proteoglycan with high molecular weight and sulfated polysaccharides with low molecular weight from the same species have strong anticoagulant activity. Nishino, Aizu, and Nagumo (1991) found that heparin cofactor II-mediated antithrombin activity of fucan sulfate is dependent on both its sulfate content and molecular weight. The low molecular weight fucoidan FF7/3 (50 kDa) combines potent anticoagulant and fibrinolytic properties with only minor platelet activating effects (Durig et al., 1997). The inhibitory effects of fucans on both coagulation and cell proliferation were dependent on their sulfation degree and molecular weight (Ferial, Mosstafa, Corinne, & Catherine, 2000).

The relationship between the structure and activity of sulfated polysaccharide from *M. latissimum* has not been fully characterized. The anticoagulant activities of the sulfated polysaccharides with different molecular weights have not been reported. In this study, a sulfated polysaccharide was isolated from *M. latissimum* and it has high anticoagulant activity. Five sulfated polysaccharide fragments with low molecular weight were prepared from the sulfated polysaccharides by oxidative degradation. The chemical characteristics and anticoagulant activities of the six sulfated polysaccharide preparations from *M. latissimum* were investigated. Relationship between the molecular size of the sulfated polysaccharides and the anticoagulant activities is discussed.

## 2. Experimental

### 2.1. Materials

*Monostroma latissimum* was collected on the coast of Zhejiang province, China. The raw material was thoroughly washed with tap water. The sample was air dried, then kept in plastic bags at room temperature in a dry environment. APTT assay regent (ellagic acid + bovine phospholipids reagent), PT assay regent (rabit thromboplastin) were from Shanghai Sun Co. (China). TT assay reagent (bovine thrombin) was from Dade Behring Inc. (USA).

### 2.2. Isolation and purification of the sulfated polysaccharide from Monostroma latissimum

Dried algae were dipped into 20 volumes of distilled water and kept at room temperature for 2 h, then homogenized and 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 8,000) against distilled water for three successive days. The retained fraction was recovered, concentrated under reduced pressure, and precipitated by addition of fourfold 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 extraction was fractionated by a Q Sepharose Fast Flow column with a linear gradient of 0–3 mol/L NaCl. The eluate was determined by the phenol–sulfuric acid method. The fraction containing the most abundant hexose was further purified by a Sephacryl S-400/HR column with 0.2 mol/L sodium acetate buffer. The major fractions were pooled, concentrated, desalted and freeze-dried. A purified sulfated polysaccharide was obtained and named as P.

### 2.3. Depolymerization of the sulfated polysaccharide by $H_2O_2$ hydrolysis

Polysaccharide P was dissolved in distilled water. The solution (1%, w/v) was then sealed in a two-necked bottle and degraded by $H_2O_2$ separately in four different conditions. The reaction times, temperatures and $H_2O_2$ concentrations in the depolymerization process are shown in Table 1. After degradation the reaction was terminated by catalase, and cooled to room temperature followed by lyophilization. The resulting solution was fractionated by a Sephacryl S-400/HR column. Five fractions were obtained and named as P1, P2, P3, P4 and P5, respectively.

### 2.4. Composition analysis

Total sugar content was estimated by the phenol–sulfuric acid analysis using rhamnose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Sulfate 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 standard (Bitter & Muir, 1962). The composition of neutral monosaccharide was measured by gas chromatography 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. The acid was

Table 1
Conditions for $H_2O_2$ degradation of the sulfated polysaccharide from *Monostroma latissimum*

| Fractions | Temperature (°C) | $H_2O_2$ concentration (%) | Time (h) |
|---|---|---|---|
| P1 | 30 | 2.5 | 2 |
| P2, P3 and P4 | 50 | 1.5 | 5 |
| P5 | 50 | 2.5 | 7 |