STRUCTURAL CHARACTERISTICS AND ANTICOAGULANT ACTIVITY OF SULFATE POLYSACCHARIDE FROM THE BROWN ALGA *SARGASSUM ALIQUALIUM*

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Received: 15 June 2016; Accepted for publication: 24 October 2016

ABSTRACT

Four fractions of sulfate polysaccharide extracted from the brown alga *Sargassum aliqualium* (Fucales, sargassaceae) were fractionated by using anion – exchange chromatography. Their structural characteristics were determined by desulfation, methylation and NMR spectroscopic methods. The results showed that four sulfate polysaccharides have branched structure, their chemical compositions are galactose, fucopyranose, fucofuranose, mannose, some other monosaccharides and sulfate groups in different positions. The sulfate polysaccharides from *Sargassum aliqualium* showed anticoagulant activity and higher sulfate content fraction has higher activity.

Keywords: sulfate polysaccharide, *Sargassum aliqualium*, structure, anticoagulant activity.

1. INTRODUCTION

Fucoidans are sulfate polysaccharides derived from marine brown seaweed containing mainly fucose and sulfate groups with other residues such as galactose, xylose, glucose, manose and uronic acids. Fucoidans were reported to possess various biological effects *in vitro* and *in vivo* such as anti-inflammatory, anticoagulant, antithrombotic, antiviral including anti-HIV, immunomodulatory, antioxidant and antitumor [1]. Vietnam has a coastline of about 3600 km with the climate varying from subtropical in the northern part to tropical in the southern part of the country, very suitable for different seaweed species to grow. The genus *Sargassum* is the largest natural seaweed resource of Vietnam including about 50 species [2]. In this report we study structural characteristics and anticoagulant activity of sulfate polysaccharide from the brown

alga *Sargassum aliqualium* and relationships between the sulfate polysaccharide structure and biological activity.

2. EXPERIMENTS

2.1. Materials

The alga was collected in June 2015 from Nha Trang bay. The sample was washed in seawater to remove mud, sand and other substances and then air-dried at room temperature and milled to fine powder.

2.2. Extraction and Purification of Fucoidan: The method of Bilan *et al*. [3]

2.3. Chemical Analysis: monosaccharides [3], sulfate content [4] and uronic acid [5]

2.4. Anion-Exchange Chromatography [3]

2.5. NMR spectroscopy: NMR experiments were performed on Bruker AVANCE 600.

2.6. Chemical methosd: Desulfation [6], methylation analysis [7]

2.7. Anticoagulant activity assay [8]

3. RESULTS AND DISCUSSION

The results of the monosaccharide compositions of fucoidan extracted from *Sargassum aliqualium* were summarized in Table 1. The main components were: fucose, galactose, sulfate, uronic acid along with a minor amount of xylose, glucose and mannose.

Table1. Chemical composition (%w) of fucoidan extractd from *Sargassum aliqualium.*

Sample	Fuc (%)	Xyl,%	Man,%	Glc,%	Gal,%	UA,%	SO_3Na,%
FSA	9.2	2.2	2.0	2.2	8.5	12.6	22.9

Table 2. Yields and composition of fucoidan fractions.

NaCl	H%	Fuc,%	Xyl,%	Man,%	Glc,%	Gal,%	UA,%	SO_3Na,%
0.5M	4.7	4.5	4.9	4.3	6.7	12.0	27.9	5.9
1.0M	**21.9**	**15.9**	**5.7**	**3.5**	**2.2**	**11.3**	**13.6**	**21.8**
1.5M	9.8	19.2	4.6	2.2	1.7	25.5	5.3	29.2
2.0M	2.3	31.2	2.9	1.0	0.8	17.3	1.8	31.5

The crude fucoidan was fractionated into four fractions by using anion – exchange chromatography with NaCl as eluent. All four fractions were sent to chemical analysis; the results were summarized in Table 2. The fraction 0.5 M containing high concentration of uronic acid and glucose can be explained by the presence of alginate and laminaran in the

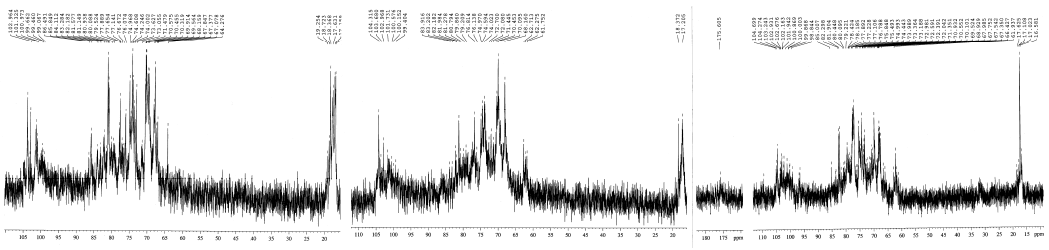
sample. The fraction 1 M had the highest yield and contained complicated chemical compositions including galactose, fucose, xylose and a small amount of other sugars. There were also a large portion of sulfate groups and uronic acid. Turning to the fraction 1.5 M, the main components were fucose, galactose sulfate and other sugars in small quantity. Finally, the lowest yielded fraction 2.0 M contained only fucose, galactose and sulfate.

The monosaccharide composition of the samples after desulfation was given in Table 3. The desulfated yield of fraction 1.0 M and 1.5 M were 56.6 % and 44 % correspondently. However, the content of fucose in fractions after desulfation was reduced.

Table 3. Yields and composition of desulfated fucoidan fractions 1.0 M and 1.5 M.

	Fuc,%	Xyl,%	Man,%	Glc,%	Gal,%	UA,%	SO_3Na,%
1.0 M	15.9	5.7	3.5	2.2	11.3	13.6	21.8
1.0 MdeS	8.1	4.0	7.8	4.3	14.0	30.6	2.8
1.5 M	19.2	4.6	2.2	1.7	25.5	5.3	29.2
1.5 MdeS	12.8	5.3	2.7	1.8	31.6	10.4	4.1

The ^{13}C NMR spectra of sulfated polysaccharides were complicated (Figure 1). To clarify, the fraction 2 M contained at least 6 signals in the anomeric region (105 - 95 ppm) and specific signals for the methyl group of fucose (20 - 17 ppm), while the CH_2OH group's signals of galactose were completely missing in the area around 62 ppm (Figure 1a). This means that fraction 2 M contained many branches. The distribution of monomers on polysaccharide backbone as well as the position of the sulfate groups and glycoside bonds on the monomers were unrepeated. According to the analyzing results of chemical constituents and NMR spectrum (Figure 1b), the structure of sulfated polysaccharide in fraction 1.5 M was more complicated than that of fraction 2 M. In the anomeric region, there existed some additional signals and there were signals in the region 63 - 61 ppm belonging to non-substituent CH_2OH group. It should be noticed that most of the spectra of desulfated polysaccharides were more complicated than the spectra of original polysaccharides. On the ^{13}C NMR of fraction 1M (Figure 1c), the intense signals in 17 ppm area belonged to methyl group of the fucose. In the anomeric region (110 - 95 ppm), the appearance of some signals with similar intensity demonstrated the presence of various types of sugars and glycoside bonds in the study sample. In addition, there were also the signals of non-substituent CH_2OH group in the region 63-61 ppm and carboxyl group of uronic acids at 175.6 ppm.



(a) (b) (c)

Figure1. ^{13}C NMR spectra of fucoidan fractions 2 M (a), 1.5 M (b) and 1 M (c).

The results of methylation analysis were given in Table 4. The products of methylation included three xylose derivatives, 9 fucose derivatives and 20 hexoses derivatives such as mannose and galactose. From Table 4, it was noticed that there existed methylated derivatives of fucositol with high content of fucofuranosyl. Until now, fucofuranosyl was only found in a type of fucoidan extracted from *Flagelliformis chordaria* seaweed [9]. In most of the methylated samples, the fucose residues were substituted in 2 or 3 positions by the sulfate groups. Otherwise, they might create glycoside links in the backbone or branches of the polysaccharides. In case of 1.5 MdeS, mannose content was small, which can be claimed that almost all methylated hexoses were galactose. The monomers were linked together through 1-6 and 1-4 linkages. In fraction 1.0 MdeS, the mannose content is roughly equivalent to galactose content. But the difference between these two sugars could not be detected by MS spectrum and led to more difficulties in interpreting the results of the methylation samples of fraction 1 M and 1.0 MdeS.

Table 4. Methylation analysis of fucoidan fractions 1.0 M, 1.0 MdeS, 1.5 M and 1.5 MdeS.

Position of O-methyl groups in	Deduced positions of substitution	**1.0 M**, mol%	**1.0 MdeS**, mol%	**1.5 M**, mol%	**1.5 MdeS**, mol%
Xyl:					
2,3,4	Xyl*p*→	3	10	2	4
2,4	→3Xyl*p*→	2	-	tr.	
2,3(3,4)		tr.	3*	4	7
Fuc:					
2,3,5	Fuc*f*→	1	1	2	1
2,3,4	Fuc*p*→	3	14	4	7
2,3	→4(5)Fuc*p*(*f*)→	6	6	3	4
3,5	→2Fuc*f*→	-	-	3	-
2,4	→3Fuc*p*→	6	6	3**	6
2	→3,4(5)Fuc*p*(*f*)→	6	1	11	2
3	→2,4(5)Fuc*p*(*f*)→	9	1	7***	-
4	→2,3Fuc*p*→	-	3	+	-
Fuc	→2,3,4Fuc→	4	-	9	-
Hex:					
2,3,4,6-Man	Man*p*→	tr.	2	tr.	1
2,3,4,6-Gal	Gal*p*→	1	5	2	12
3,4,6	→2Hex*p*→	-	5	-	-
2,3,6	→4Hex*p*→	4	**10**	2	15
2,3,6	→4Hex*p*→	3	4	1	2
2,4,6	→3Hex*p*→	4	6	1	-
2,3,4	→6Hex*p*→	2	4	8	21
2,6	→3,4Hex*p*→	**7**	-	7	2
4,6	→2,3Hex*p*→	8	9	1	3
3,6+4,6		3	-	tr.	
3,6	→2,4Hex*p*→	3	3	tr.	2
2,3	→4,6Hex*p*→	2	2	1	2
2,4	→3,6Hex*p*→	5	3	10	
3,4+2,4		-	-		3
2	→3,4,6Hex*p*→	5	tr.	10	
4	→2,3,6Hex*p*→	-	1		
3	→2,4,6Hex*p*→	-	1		
3(4)		9		2	-
3(4)		9		2	2
Gal		1	-	5	4

*Mixture (2,3+3,4) ratio ~ 3:1. **Mixture (2,4+3,4). ***Contained some 4-O-Me-Fuc

Anticoagulant activity similar to heparin of the sulfated polysaccharide fractions extracted from SA seaweed was compared with Clexane (low molecular weight heparin was used as control sample). Testing techniques were described in previous study [8]. The results showed that fraction 0.5 M was inactive while the anticoagulant activity increased from fraction 1.0 M to 2.0 M, which was suitable to the increase of sulfate content (Figure 2). 2APTT values (the concentration of the substance at which the blood clot formation was slowed down by half), of the fractions of sulfate polysaccharide were higher than Clexane.

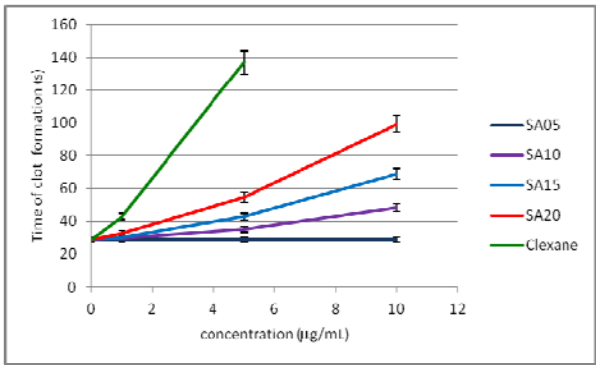


Figure 2. Anticoagulant activity of fucoidan from *Sargassum aliqualium*.

4. CONCLUSIONS

Four fractions of sulfate polysaccharide extracted from the brown alga *Sargassum aliqualium* (Fucales, sargassaceae) were fractionated by using anion – exchange chromatography. Their structural characteristics were determined by desulfation, methylation and NMR spectroscopic methods. The results showed that four sulfate polysaccharides have branched structure, their chemical compositions are galactose, fucopyranose, fucofuranose, mannose, some other monosaccharides and sulfate groups in different positions. The sulfate polysaccharides from *Sargassum aliqualium* showed anticoagulant activity and higher sulfate content fraction has higher activity.

Acknowledgments. This work was financially supported by the Vietnam Academy of Science and Technology (VAST), project number VAST.HTQT.Nga.02.15-16. The authors are grateful to Dr. Le Nhu Hau who identified brown seaweed species.

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TÓM TẮT

ĐẶC ĐIỂM CẤU TRÚC VÀ HOẠT TÍNH CHỐNG ĐÔNG TỤ MÁU CỦA SULFATE POLYSACCHARIDE TÁCH CHIẾT TỪ LÒAI RONG NÂU *SARGASSUM ALIQUALIUM*

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Bằng phương pháp sắc kí trao đổi ion đã thu được 4 phân đoạn từ sulphate polysaccharide tách chiết từ loài rong Sargassum aliqualium. Đặc điểm cấu trúc của 4 phân đoạn này đã được nghiên cứu bằng các phương pháp desulfate hóa, methyl hóa và phổ NMR. Kết quả cho thấy sulfate polysaccharide có chứa nhiều mạch nhánh với các thành phần đường khác nhau bao gồm các gốc galactose, fucopyranose, fucofuranose, mannose và lượng nhỏ các đường khác với nhóm sulfate tại các vị trí khác nhau. Kết quả thử hoạt tính chống đông tụ máu cho thấy các sulfate polysacchride thể hiện hoạt tính chống đông tụ máu và hoạt tính này tăng cùng với sự tăng hàm lượng sulfate.

Từ khóa: sulfate polysaccharide, *Sargassum aliqualium* , cấu trúc, hoạt tính chống đông tụ máu.