A NOVEL OCTASACCHARIDE ISOLATED FROM THE MILD ACID HYDROLYSIS OF THE *ISOSTICHOPUS BADIONOTUS* SULFATED FUCAN

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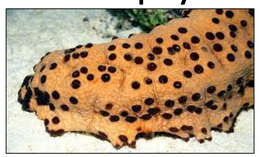
Abstract

Sea cucumber has traditionally been consumed as a tonic food in East Asia countries. Multiple biological activities, including anticoagulant, antithrombotic, angiogenic modulation and metastasis inhibition, have been reported for the sulfated glycans derived from the sea cucumber. Effects in coagulation and against viral infections have recently attracted considerable attention. The sulfated fucan from the sea cucumber specimen *Isostichopus badionotus* presents a linear tetrasaccharide repeating structure composed of the following sequence [→3Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→]$_n$. The crude polysaccharide from the dried *I. badionotus* was extracted by papain digestion and partially purified by ethanol precipitation. The crude polysaccharides were subjected to ion-exchange chromatography to isolate the sulfated fucan. The Sephadex G-15 was employed for desalting. Mild acid hydrolysis was performed by dissolving 30.0 mg of the purified sulfated fucan in 3.0 mL of 0.05 M H_2SO_4 for 10 hours. The hydrolyzed sulfated fucan was subjected to size-exclusion chromatography eluted with aqueous 10% EtOH in 1.0 M NaCl. The molecular weight of an octasaccharide was confirmed by mass spectrometry using a three-charged molecular ion. The obtained octasaccharide (830 µg) was dissolved in 150 µL of D_2O (99.8%) in a Shigemi tube and subjected to 1D ^1H and 2D COSY, TOCSY, HSQC, NOESY NMR experiments. In our research, we report for the first time, a stereospecific desulfation reaction during the mild acid hydrolysis of the *I. badionotus* sulfated fucan. In addition, a novel octasaccharide has been isolated from a controlled mild acid hydrolysis and structurally characterized by NMR, resulting the following sequence [→3Fuc(4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→3)-Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→]. The desulfation reaction that occurs at the fucose residue between 2- and 4-sulfated unit at the non-reducing residue during the mild acid hydrolysis was confirmed via cross-peak assignments in the ^1H-^1H COSY and ^1H-^{13}C HSQC NMR spectrum. This novel and chemically defined octasaccharide will be subjected to NMR experiments for assessment of its conformation in solution.

Extraction polysaccharide from the sea cucumber



Isostichopus badionotus

Papain digestion → Ethanol precipitation → Ion-exchange chromatography

Mild acid hydrolysis → Size exclusion chromatography → Obtained oligosaccharides

Mild acid hydrolysis

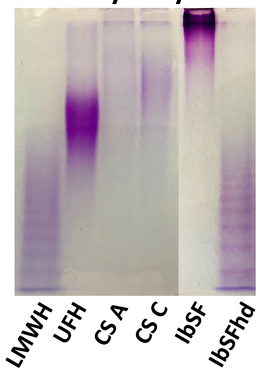


Figure 1. Mild acid hydrolysis of IbSF (30.0 mg) was performed by dissolving in 3.0 mL of 0.05 M H_2SO_4 for 10 hours. Depolymerized IbSF were subjected to electrophoresis on SDS-12% polyacrylamide gel. LMWH (low molecular weight of heparin), UFH (unfractionated heparin), CS A (chondroitin sulfate A), CS C (chondroitin sulfate C), IbSF (sulfated fucan from *I. badionotus*), IbSFhd (hydrolyzed sulfated fucan)

Size exclusion chromatography (Bio-Gel P-10)

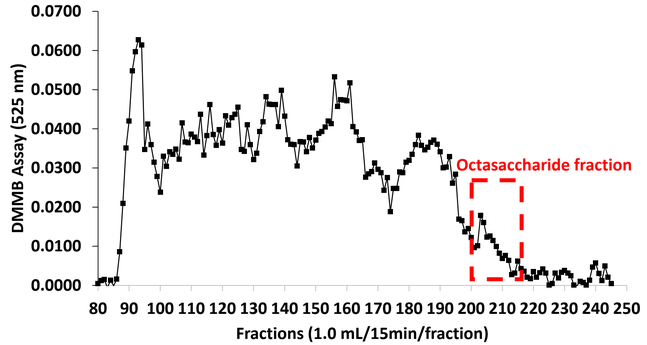


Figure 2. Mild acid hydrolyzed sulfated fucan was fractionated by size exclusion chromatography on Bio-Gel P-10 column (1.5 x 160 cm, 1 mL/15min/fraction) eluted with aqueous 10% EtOH in 1M NaCl. Octasaccharide fraction has desalted with Sephadex G-15 column.

Mass analysis

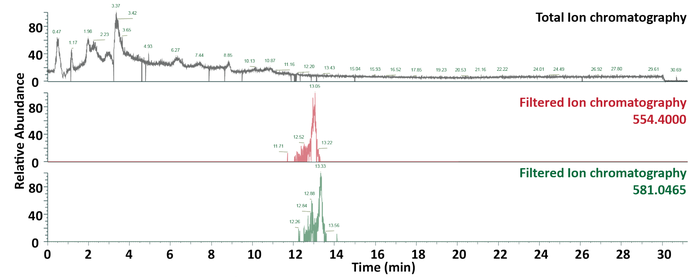


Figure 3. The molecular weight of an octasaccharide was confirmed by mass spectrometry using a three-charged molecular ion. The oligosaccharide was analyzed as a mixture composed of mono- and 2-desulfation oligosaccharides.

^1H NMR

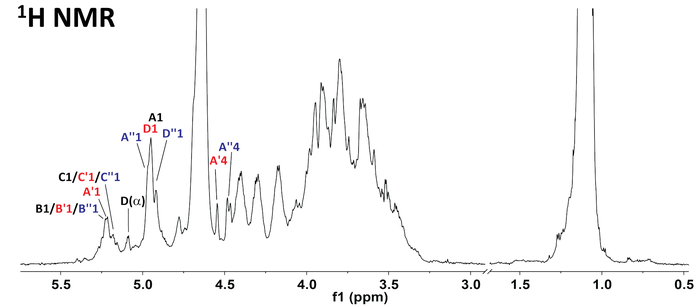
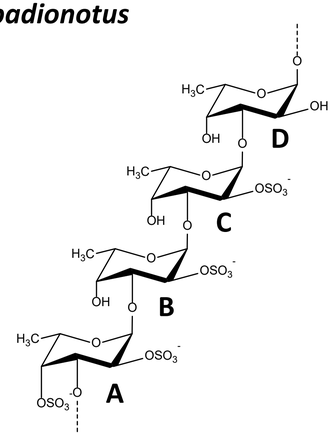


Figure 4. ^1H NMR spectra of octasaccharide structure of IbSF. The sample (815 ug) obtained by size exclusion chromatography fractionated on Bio-gel P-10 column. NMR sample of oligosaccharide prepared with 150 µL of 100% D_2O in SHIGEMI tube, analyzed at the 600 MHz Bruker instrument.

Structure of sulfated fucan from *I. badionotus*



[→3Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→]$_n$

Figure 5. The structure of the sulfated fucan from the *I. badionotus* body wall. Sulfated polysaccharides from sea cucumber (*I. badionotus*) have a tetrasaccharide repeating structure mono- di- sulfated repeating unit composed with α-L-fucan. The mono- and 2-desultion on the disulfate residue (A or/and A' unit) obtained during the mild acid hydrolysis was confirmed by mass analysis

Results

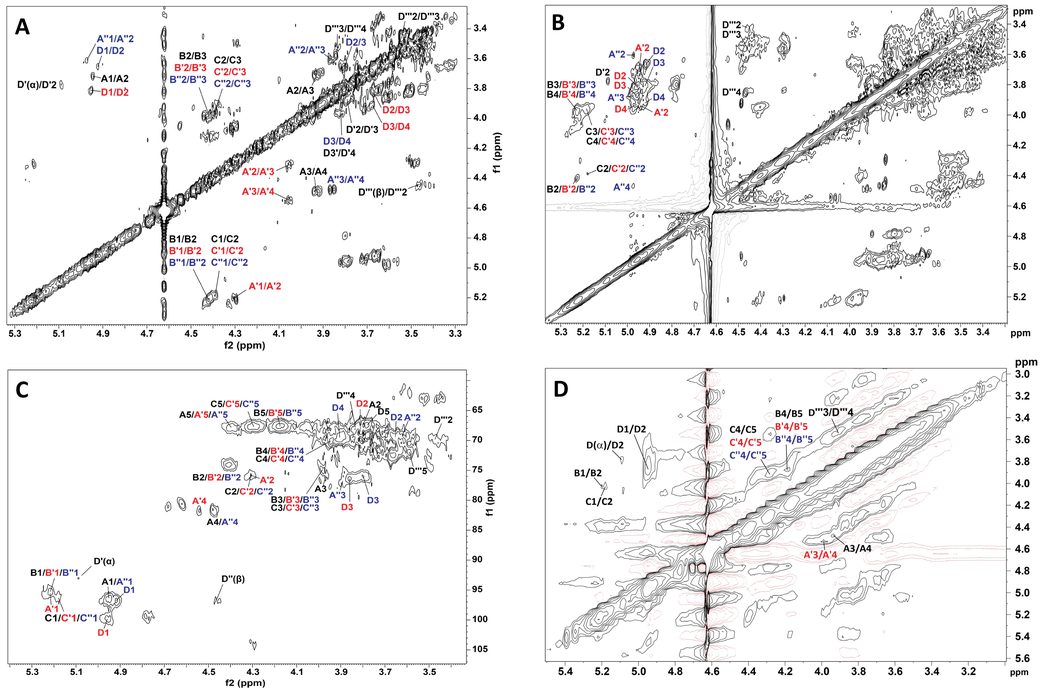


Figure6. From the 2D NMR experiment, **A**: ^1H-^1H COSY, **B**: ^1H-^1H TOCSY, **C**: ^1H-^{13}C HSQC, **D**: ^1H-^1H NOESY, confirmed that two different desulfated polysaccharide compounds generated during the mild acid hydrolysis. We confirmed for the first time a stereospecific desulfation reaction during the mild acid hydrolysis of the I. badionotus sulfated fucan. In addition, a novel octasaccharide has been isolated from controlled mild acid hydrolysis and structurally characterized by NMR. The structures are characterized by NMR, resulting in the following sequence [→3Fuc(4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→3)-Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→] as major, [→3Fuc(4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→3)-Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→] as minor components.

Table 1. ^1H and ^{13}C chemical shifts (δ, ppm) for the desulfated octasaccharides from the *I. badionotus* in 100% D_2O at 25°C. Resonances associated with the sulfation sites are highlighted in bold.

Residue	H1 (C1)	H2 (C2)	H3 (C3)	H4 (C4)	H5 (C5)	H6 (C6)
A	4.95	3.72	3.93	**4.49**	4.39	1.15
	(96.6)	(68.7)	(75.7)	**(81.6)**	(67.8)	(16.4)
A'	5.22	**4.31**	4.06	**4.55**	4.42	1.15
	(95.2)	**(75.8)**	(75.2)	**(81.8)**	(67.7)	(16.6)
B	5.25	**4.42**	3.99	3.86	4.18	1.09
	(95.7)	**(74.2)**	(75.0)	(70.2)	(67.5)	(16.2)
B'	5.25	**4.42**	3.99	3.86	4.18	1.09
	(95.3)	**(74.2)**	(75.0)	(70.2)	(67.5)	(16.2)
C	5.19	**4.31**	3.97	3.90	4.30	1.12
	(96.2)	**(75.8)**	(75.0)	(69.5)	(67.5)	(16.4)
C'	5.19	**4.31**	3.97	3.90	4.30	1.12
	(96.2)	**(75.8)**	(75.0)	(69.5)	(67.5)	(16.4)
D	4.96	3.81	3.87	3.97	3.76	1.13
	(100.0)	(68.5)	(76.5)	(70.0)	(67.5)	(16.4)
D'(α)	5.09	3.79	3.88	3.97	3.76	1.13
	(93.1)	(67.3)	(69.4)	(70.0)	(67.5)	(16.4)
D'''(β)	4.46	3.47	3.55	3.84	3.63	1.14
	(97.2)	(69.9)	(78.8)	(68.3)	(71.8)	(16.5)

Residue	H1 (C1)	H2 (C2)	H3 (C3)	H4 (C4)	H5 (C5)	H6 (C6)
A	4.95	3.72	3.93	**4.49**	4.39	1.15
	(96.6)	(68.7)	(75.7)	**(81.6)**	(67.8)	(16.4)
A''	4.97	3.62	3.86	**4.48**	4.42	1.15
	(96.6)	(69.4)	(76.3)	**(81.6)**	(67.7)	(16.6)
B	5.25	**4.42**	3.99	3.86	4.18	1.09
	(95.7)	**(74.2)**	(75.0)	(70.2)	(67.5)	(16.2)
B''	5.25	**4.42**	3.99	3.86	4.18	1.09
	(95.3)	**(74.2)**	(75.0)	(70.2)	(67.5)	(16.2)
C	5.19	**4.31**	3.97	3.90	4.30	1.12
	(96.2)	**(75.8)**	(75.0)	(70.2)	(67.5)	(16.4)
C''	5.19	**4.31**	3.97	3.90	4.30	1.12
	(96.2)	**(75.8)**	(75.0)	(70.2)	(67.5)	(16.4)
D	4.92	3.66	3.80	3.91	3.76	1.13
	(96.9)	(67.5)	(76.3)	(69.6)	(67.5)	(16.4)
D''(α)	5.09	3.79	3.88	3.97	3.76	1.13
	(93.1)	(67.3)	(69.4)	(70.0)	(67.5)	(16.4)
D'''(β)	4.46	3.47	3.59	3.84	3.63	1.14
	(97.2)	(69.9)	(78.8)	(68.3)	(71.8)	(16.5)

Discussion

The sulfated fucan compound was isolated from the I. badionotus by the DEAE cellulose column. The structure of sulfated fucan from I. badionotus presents a linear tetrasaccharide repeating structure composed as [→3Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→]$_n$. Mild acid hydrolysis was employed to get the low molecular sulfated fucan buiding block. The obtained oligosaccharide was analyzed by mass, and 1D and 2D NMR. The structure was characterized, resulting the following the sequence [→3Fuc(4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→3)-Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→] as a major components, and [→3Fuc(2S,4S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc(2S)-(α1→3)-Fuc-(α1→]$_2$ as a minor component. This novel and chemically defined octasaccharide will be subjected to NMR experiments for assessment of its conformation in solution.

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