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Structure of a fucoidan from the brown seaweed *Fucus serratus* L.☆

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Abstract—A fucoidan consisting of L-fucose, sulfate and acetate in a molar proportion of 1:1:0.1 and small amounts of xylose and galactose were isolated from the brown seaweed *Fucus serratus* L. The fucoidan structure was investigated by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy of its desulfated and de-O-acetylated derivatives as well as by methylation analysis of the native and desulfated polysaccharides. A branched structure was suggested for the fucoidan with a backbone of alternating 3- and 4-linked α-L-fucopyranose residues, →3)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→, about half of the 3-linked residues being substituted at C-4 by trifucoside units α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-(1→. Minor chains built up of 4-linked α-fucopyranose and β-xylose residues were also detected, but their location, as well as the position of galactose residues, remained unknown. Sulfate groups were shown to occupy mainly C-2 and sometimes C-4, although 3,4-diglycosylated and some terminal fucose residues may be nonsulfated. Acetate was found to occupy C-4 of 3-linked Fuc and C-3 of 4-linked Fuc in a ratio of about 7:3.

Keywords: Fucoidan; NMR; Seaweed; Brown algae; *Fucus serratus*

1. Introduction

Sulfated fucans present in brown algae and some marine invertebrates have a wide variety of biological activities.[2] Invertebrate polysaccharides have usually simple ordered structures differing in the specific sulfation patterns and/or position of glycosidic linkages within their oligosaccharide repeating units.[3,4] As a result, the structures of these repeating units can be determined unambiguously, especially by using high-field NMR spectroscopy,[5] and hence, important correlations between structures and biological activities of polysaccharides may be outlined.[6,7] In contrast, algal sulfated fucans, usually named fucoidans, have much more complex and heterogeneous structures devoid of regularity.[8] There is only one example of an algal polysaccharide, a highly sulfated fraction of fucoidan from *Fucus distichus*, which has a ^{13}C NMR spectrum corresponding to a structure with repeating disaccharide units: →3)-α-L-Fuc*p*(2,4-di-SO_3^-)-(1→4)-α-L-Fuc*p*(2SO_3^-)-(1→, but even in this case some minor deviations from the regular structure were observed using chemical methods of structural analysis.[9] Several fucoidans isolated from closely related species of brown algae belonging to the same order Fucales seem to have similar backbones of alternating 3- and 4-linked α-L-fucopyranose residues, although the regularity of their molecules is masked by random sulfation and acetylation.[10,11] Representatives of Chordariales[12] and Laminariales[13,14] may have another backbone built up of 3-linked α-L-fucopyranose residues. Branched structures were postulated for several fucoidans,[12,14,15] but the presence of sulfate groups often prevents the unambiguous identification of branching points and determination of

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their position. Several minor monosaccharide constituents (galactose, xylose, etc.) were found in many fucoidan preparations, but their structural significance, as a rule, also remains unknown.[16]

In order to elucidate structural features of fucoidans responsible for their biological activities, we continue the investigation of sulfated polysaccharides from different species of brown algae. The present work is devoted to the structural analysis of a fucoidan isolated from *Fucus serratus* L.

2. Results and discussion

Water-soluble polysaccharides were extracted from the defatted[17] biomass of *F. serratus* and fractionated by anion-exchange chromatography (see Section 4). The yields and composition of the four fractions obtained are given in Table 1. Fraction F_4, which was essentially a homofucan sulfate containing fucose and sulfate in a molar ratio of about 1:1 and only traces of other monosaccharide constituents, was subjected to structural analysis.

The IR-spectrum of F_4 contained an intense absorption band at 1240 cm^{-1} (S=O) common to all the sulfate esters. An additional sulfate absorption band at 824 cm^{-1} (C–O–S, secondary equatorial sulfate) and a shoulder at 844 cm^{-1} (C–O–S, secondary axial sulfate) indicated that the majority of sulfate groups occupy positions 2 and/or 3, and only a minor part of sulfate is located at position 4 of fucopyranose residues.

Like many other native algal fucoidans, fraction F_4 had a very complex ^{13}C NMR spectrum, which was difficult to interpret completely (Fig. 1). It contained several intense signals in the anomeric (97–102 ppm) and high-field (16.5–16.7 ppm) regions, which are typical of α-fucopyranosides. The signals at 21–22 ppm confirmed the presence of *O*-acetyl groups. Unfortunately, the ^1H NMR spectrum of F_4 was poorly resolved, so we could not apply 2D procedures to assign other resonances in the ^{13}C NMR spectrum of the native polysaccharide.

Several chemical modifications were carried out to simplify the structure of F_4. Three modified polysaccharide preparations were obtained as the result of desulfation (deS), deacetylation (deAc) and both desulfation and deacetylation (deSdeAc). Molar proportions of constituents of F_4 and modified preparations are given in Table 2. Deacetylation was carried out by treatment of polysaccharides with aqueous ammonia.[14] A solvolytic desulfation procedure[18] was used to remove sulfate groups. The yield of desulfated polysaccharide (deS) was 34.8% of theoretical value. The preparation still contained about 4% of residual sulfate. High negative values of optical rotation of deAc and deSdeAc were consistent with α-configuration of L-fucopyranose residues in these polysaccharides.

Both ^1H (Fig. 2) and ^{13}C (Fig. 3) NMR spectra of desulfated and de-O-acetylated polysaccharide (deSdeAc) were resolved enough to apply 2D spectroscopy for the assignment of resonances in the 1D spectra. Analysis of COSY, TOCSY and HSQC (Fig. 4) spectra revealed the presence of α-fucose, β-xylose and β-galactose residues

Table 1. Yields and composition of fucoidan fractions obtained by ion-exchange chromatography of crude polysaccharide preparation (F)

Fraction	Yield, % of F	Neutral monosaccharides (%)					Sulfate (SO_3Na) (%)	Acetate (CH_3CO) (%)
		Fuc	Xyl	Gal	Man	Glc		
F_1	23.2	—	—	—	2.8	92.8	—	—
F_2	2.6	35.3	10.2	2.1	4.0	3.3	14.8	0.7
F_3	18.1	54.8	4.0	2.6	1.4	0.6	21.9	0.7
F_4	43.9	46.6	1.5	1.6	—	—	31.8	1.1

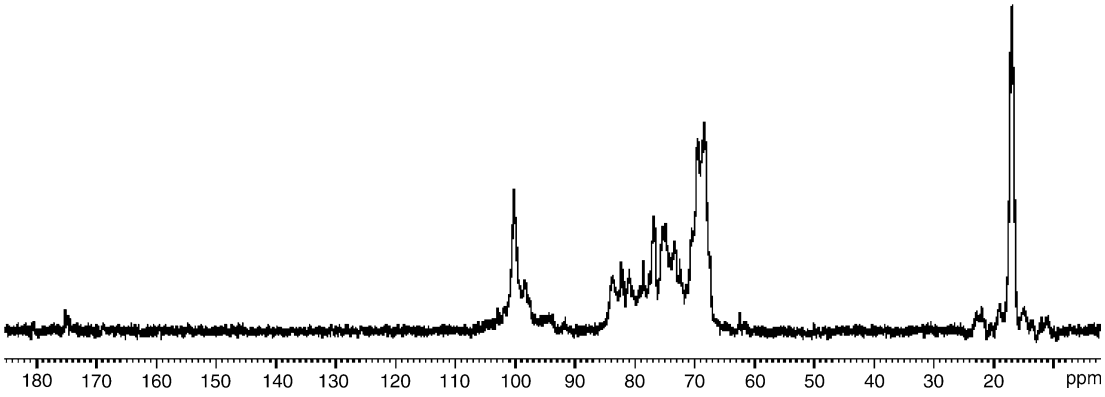

Figure 1. The ^{13}C NMR spectrum of native fucoidan F_4 (recorded at 333 K).

Table 2. Composition (molar proportions) and optical rotation of polysaccharide preparations

Sample	Fuc	Xyl	Gal	SO_3Na	$[\alpha]_D^{20}$ (° in H_2O)
F_4	49	2	1	48	ND
deAc	47	2	3	48	−135.0 (*c* 1.0)
deS	61	15	17	7	ND
deSdeAc	55	20	20	5	−105.9 (*c* 0.6)

in the sample. Two main types of α-fucose derivatives were detected that correspond to 3-O-glycosylating and 4-O-glycosylating residues (Fig. 4). Several anomeric signals of different intensities were found for both types of α-fucose residues (*P*, *Q* for 3-O-glycosylating and *X*, *Y*, *Z* for 4-O-glycosylating ones) in the ^{1}H NMR spectrum [see the ^{1}H projection of the HSQC spectrum (Fig. 4)]. These data were interpreted as an indication of the presence of several different types of fucose residues **A**–**H** (see structures **1**–**3** in Scheme 1 and Table 3) according to the following considerations. The intense signals *Q* and *Y* should correspond to the anomeric protons of the main fucoidan chain built up of α-L-fucopyranose residues with alternating (1→3) and (1→4)-linkages (cf.[11]). The modes of substitution in this chain were confirmed by NOE experiments, such as 2D NOESY and ROESY. These spectra contained correlation peaks 5.08/3.94 and 5.08/4.00 corresponding to H-1′/H-3 and H-1′/H-4 interactions usual for (1→3)-linked fucobioside fragments as well as 4.98/3.87 and 4.98/1.30 corresponding to H-1′/H-4 and H-1′/H-6 interactions for (1→4)-linked fucobioside fragments. It was suggested that smaller signals *P* and *X* correspond to the anomeric protons of branches of the main fucoidan chain. The presence of branches was confirmed by signals of terminal α-L-fucopyranose residues in the spectra (see the ^{1}H and ^{13}C NMR chemical shifts for residue **G** in Table 3) and by the presence of a correlation peak 3.90/75.0 in the HSQC spectrum of deSdeAc corresponding to the correlation H-3/C-3 of 3,4-di-O-glycosylated α-L-fucose (residue **C** in Table 3). The latter assignment was confirmed by analysis of the spectra of a model synthetic branched trifucoside (see below). Thus, we may suppose that deSdeAc molecules are built up mainly of structures **1** and **2** (see Scheme 1). Structure **1**

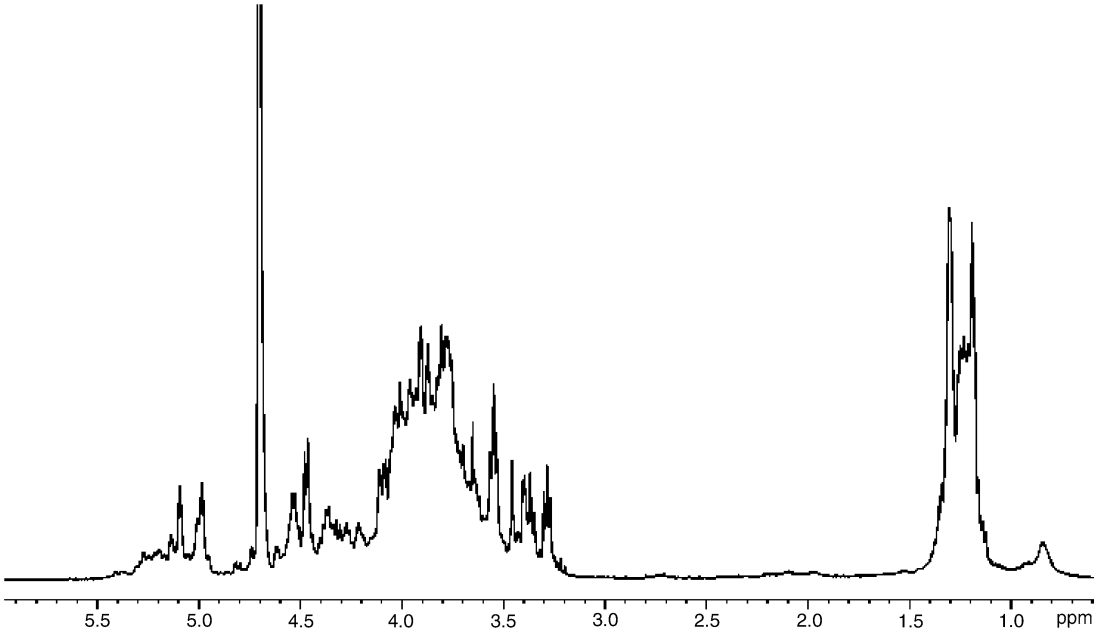

Figure 2. The ^{1}H NMR spectrum of desulfated and deacetylated polysaccharide (deSdeAc).

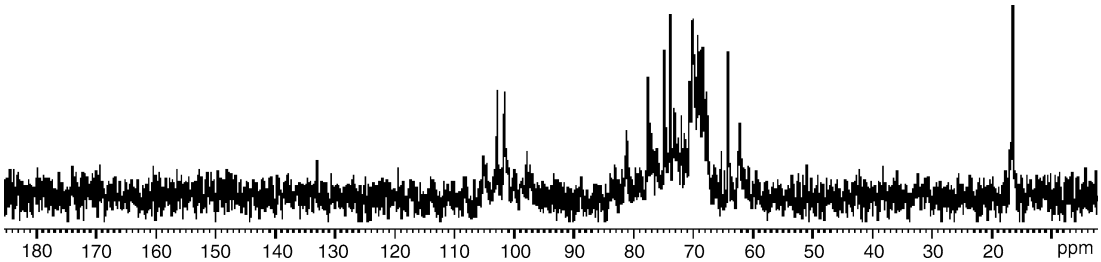

Figure 3. The ^{13}C NMR spectrum of desulfated and deacetylated polysaccharide (deSdeAc).

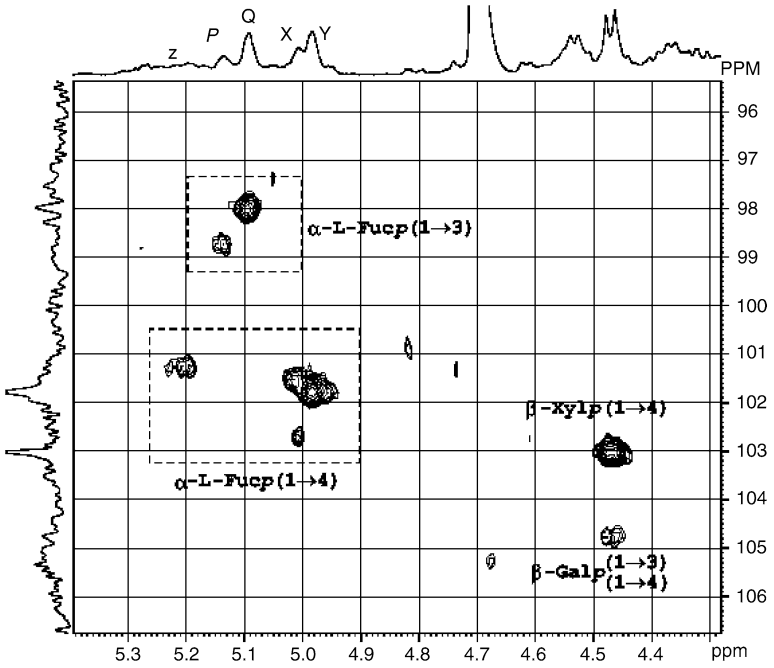

Figure 4. A part of 2D HSQC spectrum containing anomeric signals of desulfated and deacetylated polysaccharide (deSdeAc).

A **B**

→3)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→

1

C **D**

→3)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→
4
↑
α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*

G **F** **E**

2

H

→4)-α-L-Fuc*p*-(1→4)-

3

R_1
↓
2
→3)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*(2SO_3)-(1→
4
↑
R_2

(a) (about 50%): R_1= SO_3^-, R_2= H
(b) (about 50%): R_1= H,
R_2= α-L-Fuc*p*-(1→4)- α-L-Fuc*p*(2SO_3)-(1→3)-α-L-Fuc*p*(2SO_3)-(1→,
the terminal residue may also be 4-monosulfated or 2,4-disulfated.

4

Scheme 1.

is a chain containing α-L-fucopyranoside residues with alternating (1→3) and (1→4) glycosidic linkages. Structure **2** has the same backbone, but 3-O-glycosylated residues of this chain are substituted at C-4 by side chains. Table 3 gives all the ^{1}H and ^{13}C NMR chemical shifts for these structures. Analysis of the HSQC spectrum confirmed substitution of residues **B** and **D** at C-4 (downfield location of C-4 resonances at 81.5 ppm), residue **A** at C-3 (signal C-3 at 77.5 ppm) as well as residue **C** at both C-3 and C-4 (downfield location of C-3 and C-4 resonances at 75.0 and 81.0 ppm, respectively). The structure and the length of the side chain in fragment **2** were established by 2D NOE experiments and positions of correlation peaks in HSQC spectra. Thus the NOE experiments showed that the terminal residue **G** was linked to C-4 of residue **F** (a correlation peak H-1′/H-4 at 5.01/3.90 ppm), whereas residue **F** was linked to C-3 of residue **E** (correlation peak H-1′/H-3 at 5.14/3.97 ppm). No correlation peaks were observed between anomeric proton of residue **E** and ring protons of residue **C** due to their overlap with the peaks belonging to other residues in NOESY and ROESY spectra. However, residue **E** could be assigned unambiguously as linked to C-4, because the chemical shift of C-1 for residue **E** was 101.9 ppm, and it could be suggested that residue **E** was linked to C-4 of residue **C**. Finally, taking into account the ratio between intensities of signals of anomeric protons belonging to backbone and to side chain residues (Fig. 4, signals *P*:*Q*:*X*:*Y* in approximate ratio of 1:2:1:3), it may be concluded that the polysaccharide molecules contain about equal amounts of structures **1** and **2**, and hence, the majority of side chains contain three fucose residues.

In addition, there was a small broad signal *Z* in ^{1}H NMR spectrum (Fig. 4) corresponding to the anomeric

Table 3. NMR data for desulfated and deacetylated fucoidan (deSdeAc)

Structure	Residue	^{1}H Chemical shifts (ppm)					
		H-1	H-2	H-3	H-4	H-5	H-6
1 ~50%	**A** →3)-α-L-Fuc*p*-(1→4)-	4.98	3.90	3.94	4.00	4.53	1.18
	B →4)-α-L-Fuc*p*-(1→3)-	5.08	3.87	4.04	3.87	4.36	1.30
2 ~50%	**C** →3,→4)-α-L-Fuc*p*-(1→4)-	4.96	3.83	3.90	3.90	4.56	1.31
	D →4)-α-L-Fuc*p*-(1→3)-	5.08	3.87	4.04	3.87	4.36	1.30
	E →3)-α-L-Fuc*p*-(1→4)-	4.98	3.90	3.97	3.97	4.53	1.18
	F →4)-α-L-Fuc*p*-(1→3)-	5.14	3.83	4.03	3.90	4.36	1.30
	G α-L-Fuc*p*-(1→4)-	5.01	3.98	3.96	3.82	4.27	1.20
3	**H** →4)-α-L-Fuc*p*-(1→4)-	5.20	3.78		3.99		1.23
		^{13}C Chemical shifts (ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
1 ~50%	**A** →3)-α-L-Fuc*p*-(1→4)-	101.9	68.5	77.5	70.4	67.9	16.5
	B →4)-α-L-Fuc*p*-(1→3)-	98.0	70.2	70.2	81.5	69.0	16.5
2 ~50%	**C** →3,→4)-α-L-Fuc*p*-(1→4)-	101.9	70.2	75.0	81.0	69.6	16.5
	D →4)-α-L-Fuc*p*-(1→3)-	98.0	70.2	70.2	81.5	69.0	16.5
	E →3)-α-L-Fuc*p*-(1→4)-	101.9	68.5	77.5	70.4	67.9	16.5
	F →4)-α-L-Fuc*p*-(1→3)-	98.7	70.0	70.4	81.0	69.0	16.5
	G α-L-Fuc*p*-(1→4)-	101.5	69.1	70.1	73.2	68.7	16.5
3	**H** →4)-α-L-Fuc*p*-(1→4)-	101.2	70.0		82.0		16.5

protons of (1→4)-linked chain of α-L-fucopyranose residues (structure **3**). However, the content of (1→4)-linked chains in deSdeAc was small, so the assignment of its subspectra was only tentative. Table 3 (residue **H**) represents the ^{1}H and ^{13}C NMR chemical shifts for some of the signals of this chain. The mode of substitution in this chain was confirmed by NOE experiments (correlation peaks 5.20/3.99 and 5.20/1.23 corresponding to H-1′/H-4 and H-1′/H-6 interactions) and by positions of glycosylating (C-1) and glycosylated (C-4) carbon atoms in the HSQC spectrum (101.2 and 82.0 ppm, respectively).

Analysis of β-xylose signals present in the 2D spectra revealed (1→4)-linked β-xylopyranose residues; the subspectrum of terminal xylose residues was also observed (for signal assignments see[19]). The ratio of terminal and internal xylose residues was in the range of 1:4–1:8, based on the intensity of signals in the 2D HSQC spectrum. The NOE spectra contained correlation peaks at 4.40/3.99 and 4.40/1.31 ppm corresponding presumably to H-1′/H-4 and H-1′/H-6 xylose–fucose interactions, respectively. Thus, we suppose that short chains (of about six residues) of (1→4)-linked β-xylan may be present as branches or sometimes may terminate the (1→3, 1→4)-linked fucoidan chains. Analysis of β-galactose signals revealed (1→3) and (1→4)-linked β-galactose residues. No correlations were obtained between galactose and fucose and/or xylose residues in the NOE spectra. It was concluded that fucoidan preparation analyzed in the present study contained a small amount of (1→3, 1→4)-linked galactan, which was accidentally not separated during the purification steps. The distinct signals corresponding to xylans and galactans were only observed in the spectra of desulfated polysaccharides (deSdeAc and deS), where the relative contents of these monosaccharides were increased due to degradation of some fucoidan molecules under solvolytic desulfation conditions (Table 2). In contrast, spectra of sulfated preparations F_4 and deAc contained very weak signals of xylose residues and practically no signals belonging to galactose residues.

Thus, according to spectral evidence, the main part of desulfated and de-O-acetylated fucoidan (deSdeAc) contains a linear backbone of alternating (1→3)- and (1→4)-linked α-L-fucopyranose residues, in which a part of 3-O-glycosylated fucose residues (about 50%) is substituted at C-4 by trifucoside side chains. The polysaccharide backbone is similar to the backbone of the fucoidan from brown seaweed *Fucus evanescens* described previously[11] where no branches were detected. However, a revision of the NMR spectra of the *F. evanescens* fucoidan with account of the spectral data of deSdeAc of *F. serratus* made it possible to identify some 3,4-disubstituted α-L-fucopyranose residues in the former polysaccharide. The presence of 3,4-branching points in the fucoidan structures was also confirmed by comparison of ^{13}C NMR chemical shifts for the carbon atoms at (1→3) and (1→4) glycosidic linkages in the polysaccharide with the chemical shifts of the corresponding carbon atoms in the synthetic propyl 3,4-di-*O*-α-L-fucopyranosyl-α-L-fucopyranoside[20] (Table 4). It should be noted that some deviations in signal positions in the spectra of polysaccharide and of model trifucoside were observed, but the differences in chemical shift values for both linkages did not exceed 1.1 ppm. Most probably, these spectral differences are connected with

Table 4. ^{13}C NMR data for 3,4-branched fragment of deSdeAc fucoidan and synthetic propyl 3,4-di-*O*-α-L-fucopyranosyl-α-L-fucopyranoside[20]

Sample	Linkage	C-1′	C-4
deSdeAc Fucoidan		101.9	81.0
Trifucoside	(1→4)	102.1	80.2
Differences		−0.2	0.8
		C-1′	C-3
deSdeAc Fucoidan		98.0	75.0
Trifucoside	(1→3)	97.1	76.1
Differences		0.9	−1.1

the different conformations of model 3,4-branched trifucoside and respective fragments within the polysaccharide chain. Similarly, spectral differences in formally identical residues **B** and **D**, on the one hand and residue **F**, on the another hand, may be explained by different mobilities of the residues belonging to backbone and to side chains of the polysaccharide.

NMR spectra of de-O-acetylated polysaccharide (deAc) were analyzed to localize the positions of sulfate groups. The full resonance assignments for ^{1}H and ^{13}C NMR spectra of deAc were not made. However, sulfate was shown to occupy all positions 2 of both 3- and 4-linked α-L-fucopyranose residues, as evidenced from the low-field shifts of H-2 and C-2 resonances in the 2D HSQC spectrum (correlation peaks at 4.58/74.8 and 4.50/76.7 ppm, respectively). At the same time, only a part (~50%) of terminal fucose residues has sulfate at C-2 (correlation peaks at 4.41/77.0 ppm in the 2D HSQC spectrum). Low-field shifts of H-4 and C-4 of some 3-linked and some terminal residues showed that additional sulfate occupies C-4 (correlation peaks at 4.96/81.0 and 4.64/82.3 ppm, respectively, in the 2D HSQC spectrum). However, all the 3,4-disubstituted and some terminal fucose residues were found to be free of sulfation.

NMR spectra of desulfated fucoidan (deS) were analyzed to estimate the molar proportion of fucose and acetate (1:0.1) and to determine the positions of *O*-acetyl groups. The full resonance assignments for ^{1}H and ^{13}C NMR spectra of deS were not made. Acetate was found at C-4 of 3-linked residues (70%) as well as at C-3 of 4-linked residues (30%), as followed from the position and intensities of characteristic low-field shifts of the corresponding proton and carbon resonances (correlation peaks at 5.45/71.5 and 5.31/73.2 ppm, respectively, in the 2D HSQC spectrum).

Methylation of polysaccharides was used to confirm the spectral data on their structure. Native fucoidan (F_4) and desulfated fucoidan (deS) were methylated with methyl iodide in the presence of sodium hydroxide in methyl sulfoxide.[21] Methylated polysaccharides were hydrolyzed, and the resulting mixtures of partially methylated monosaccharides were analyzed as alditol acetates by GLC–MS.[22]

The molar ratios of partially methylated fucitol acetates obtained from desulfated fucoidan (deS) were as follows: acetates of 2,3,4-tri-*O*-methyl-:2,3-di-*O*-methyl-:2,4-di-*O*-methyl-:2-*O*-methyl-:3-*O*-methyl- or (and) 4-*O*-methyl-fucitol, 17:37:33:8:4. This set of fucose derivatives was consistent with the (1→3, 1→4)-backbone of deSdeAc. Evidently, a rather high content of nonreducing terminal fucose residues may be explained by marked degradation of the starting polysaccharide F_4 under solvolytic desulfation conditions, but the proportion between monomethyl and dimethyl derivatives corresponded approximately to one branching point per about every seven chain residues, thus confirming the presence of structures **1** and **2** in roughly equal amounts. Detection of 3- or 4-*O*-methylfucitol suggested that side chains may occupy not only C-4, but also C-2 of the backbone, although this latter position of branches was not observed in the NMR spectra. In addition, some 3- or 4-*O*-methylfucitol may be formed due to the presence of some residual sulfate at positions 2 in deS, although the low-level of this sulfate prevented detection of its position by NMR spectroscopy as well.

The molar ratios of partially methylated fucitol acetates obtained from the native fucoidan (F_4) were as follows: acetates of 2,3,4-tri-*O*-methyl-:2,3-di-*O*-methyl-:3,4-di-*O*-methyl-:2-*O*-methyl-:3-*O*-methyl-fucitol and fucitol, 3:6:7:3:24:57. These results corresponded to highly substituted and/or branched fucoidan molecules, so the exact interpretation of them in terms of polysaccharide structure was rather difficult.

3. Conclusion

According to several recent publications,[9–11] the common structural feature of fucoidans isolated from the algae belonging to the order Fucales is the backbone built up of alternating 3- and 4-linked α-L-fucopyranose residues. Linear structures were suggested for some polysaccharides, and a highly branched fucoidan fraction was also found in *Ascophyllum nodosum*.[15] The latter polysaccharide contained more 1→3 than 1→4 linkages, side chains consisted of single and multi-unit fucosyl residues and were attached at C-2 of the backbone. The polysaccharide described in the present paper is different from these known fucoidans in several respects. As many other polysaccharides obtained from Fucales, it contains a backbone of alternating 3- and 4-linked α-L-fucopyranose residues, but (1→4) linkages slightly prevail over (1→3) ones, and hence, some chains built up of contiguous (1→4)-linked α-L-fucopyranose residues could be found by analysis of NMR spectra. The molecules are branched, one branching point being present on average in every heptasaccharide fragment. Side chains are represented mostly by trifucoside units and are linked to O-4 of 3-linked fucose residues of

the main chain. Short (1→4)-β-xylan fragments may also be present as side chains. According to methylation results, some side chains may be attached to C-2 of the backbone. On average every fucose residue in the native polysaccharide is monosulfated. Sulfate groups are distributed nonuniformly, being mostly attached to O-2, but sometimes also to O-4; disulfated and unsulfated residues are also present (structure **4**, see Scheme 1). In addition, the native polysaccharide is acetylated containing one acetate per every 10 fucose residues and *O*-acetyl groups occupy C-4 and C-3 in a ratio of about 7:3.

The carbohydrate moiety of the fucoidan may be represented essentially as combination of structures **1** and **2** in equal amounts. Based on the complex NMR spectra of deSdeAc, these two structures seem to be randomly distributed along the molecule. Structural heterogeneity resulting from nonstoichiometric substitution by sulfate and acetate, as well as from random distribution of branches prevents description of the fucoidan as a regular polymer containing repeating units of definite chemical structure. Nevertheless, we hope that unique structural features of the fucoidan from *F. serratus* found in the present work will help to explain some of its properties including biological activity.

4. Experimental

4.1. General methods

Quantitative determination of monosaccharides, acetate and sulfate, gas–liquid chromatography, recording of IR spectra and optical rotation measurements were carried out as described previously.[9,11] NMR spectra were recorded using a Bruker DRX-500 spectrometer at 303 K (at 333 K for native fucoidan F_4). Samples were deuterium-exchanged by lyophilization two times with D_2O and then examined as 2–3% solutions in 99.97% D_2O, TSP (δ_H 0 ppm) and methanol (δ_C 50.15 ppm) were taken as the internal standards. The parameters used for 2D experiments were described previously.[11] The TOCSY spectra were acquired with 200 ms duration of MLEV17 spin-lock; the ROESY spectra were acquired with 200 ms duration of spin-lock, the NOESY spectra were acquired with 300 ms duration of mixing time, the HMBC spectra were recorded with 60 ms delay for evolution of long-range couplings.

4.2. Isolation of fucoidan

The alga *F. serratus* was collected from the littoral of the Barents Sea (Dalnie Zelentsy, the Murmansk region) in August of 2001, dried in air and then in vacuum for 2 weeks. The milled algal biomass (61.2 g, particles about 0.25 mm) was treated at room temperature with a 4:2:1 MeOH–$CHCl_3$–H_2O mixture to remove coloured matter, washed with acetone, filtered and vacuum dried to yield 40.9 g (66.8%) of defatted biomass. This material and 2% aqueous $CaCl_2$ solution (5 × 250–300 mL) were mechanically stirred at 85 °C for 5 h. The extracts were collected by centrifugation, combined, dialyzed and lyophilized to give crude polysaccharide fraction (F), yield 10.0 g (24.4% of dry defatted biomass), composition: fucose, 32.8%; glucose, 24.4%; SO_3Na, 18.9%; xylose, 2.9%; galactose, 2.6%; mannose, 1.8%. An aqueous solution of F (1.55 g in 50 mL) was placed on a column (24 × 4 cm), containing DEAE-Sephacel (Pharmacia) in Cl^--form, and eluted with water followed by NaCl solutions of increasing concentration (0.5, 1.0 and 1.5 M), each time up to the absence of a positive reaction of eluate for carbohydrates[23] with phenol and concd H_2SO_4. All the solutions obtained were dialyzed and lyophilized, yields of fractions F_1–F_4 being 0.36, 0.04, 0.28 and 0.68 g, respectively. Composition of these fractions is given in Table 1.

4.3. Chemical modifications of polysaccharides

Solvolytic desulfation of F_4 (as pyridinium salt) was carried out as described earlier.[13] Yield of desulfated fucoidan (deS) containing 3.9% of residual SO_3Na was 30 mg from 130 mg of the starting material. Samples of F_4 and deS were treated with aqueous ammonia at 37 °C to remove acetyl groups.[14] Methylation of fucoidans followed by hydrolysis and GLC–MS of partially methylated fucitol acetates was performed as previously described.[13,14]

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