Carbohydrate Research 456 (2018) 5–9

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

A highly regular fucan sulfate from the sea cucumber *Stichopus horrens*

Nadezhda E. Ustyuzhanina*, Maria I. Bilan, Andrey S. Dmitrenok, Elizaveta Yu. Borodina, Nikolay E. Nifantiev, Anatolii I. Usov**

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, Moscow 119991, Russia

ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form
27 November 2017
Accepted 6 December 2017
Available online 7 December 2017

Keywords:
Fucan sulfate
Sea cucumber
Stichopus horrens
Structure
Anticoagulant activity

ABSTRACT

A highly regular fucan sulfate **SHFS** was isolated from the sea cucumber *Stichopus horrens* by extraction of the body walls in the presence of papain followed by ion-exchange and gel permeation chromatography. **SHFS** had MW of about 140 kDa and contained fucose and sulfate in the molar ratio of about 1:1. Chemical and NMR spectroscopic methods were applied for the structural characterization of the polysaccharide. **SHFS** was shown to have linear molecules built up of 3-linked α-L-fucopyranose 2-sulfate residues. Anticoagulant properties of **SHFS** were assessed *in vitro* in comparison with the LMW heparin (enoxaparin) and totally sulfated 3-linked α-L-fucan. **SHFS** was found to have the lowest activity, and hence, both sulfate groups at *O*-2 and *O*-4 of fucosyl units seem to be important for anticoagulant effect of sulfated homo-(1 → 3)-α-L-fucans.

1. Introduction

Sulfated polysaccharides are the subject of chemical and biological research due to their various important physiological activities. Marine organisms are known as the rich source of such polysaccharides, brown algae and echinoderms being the most readily available raw material for isolation of polysaccharides containing sulfated fucose residues as the main structural components. Brown algal polysaccharides, named fucoidans, usually are mixtures of several sulfated polymers having complex monosaccharide composition and non-regular structures [1–3], and this feature mostly prevents elucidation of structure-activity relationships [4–7]. Echinoderms contain two classes of sulfated polysaccharides. Fucosylated chondroitin sulfates were found in the body walls of holothuria (sea cucumbers) [8], whereas representatives of another class, sulfated homofucans (fucan sulfates), are present in both see cucumbers and see urchins. Fucan sulfates isolated from different sea urchins were shown to possess regular molecules built up of oligosaccharide repeating units, mostly of fucose tetrasaccharides differing in amount and position of sulfate groups. Being components of egg jelly, the fucan sulfates with defined species-specific structures play a very important role in cell-cell recognition accompanying fertilization process in sea urchins [9]. Similar apparently regular fucan sulfates were obtained from the body walls of several sea cucumbers, such as *Telenota ananas* [10], *Acaudina molpadioides* [11], *Pearsonothuria graeffei* [12], *Isostichopus badionotus* [13] and *Holothuria tubulosa* [14]. More complex structures with branched pentasaccharide repeating units were suggested for fucan sulfates from *Apostichopus japonicus* [15], *Holothuria edulis* and *Ludwigothuria grisea* [16]. Structures of these holothurian fucan sulfates were also found to be species-specific. It is evident from comparative biological activity studies, that all the structural features of sulfated fucans, such as linear or branched carbohydrate moiety, sulfation pattern and several macromolecular characteristics, are important for the biological activity of these biopolymers [17–19].

The sea cucumber *Stichopus horrens* is a species widely distributed in tropical waters. Similarly to other holothuria, it contains two types of sulfated polysaccharides, fucosylated chondroitin sulfate and fucan sulfate. Structure of a fucosylated chondroitin sulfate isolated from this species was studied recently using infrared spectroscopy [20]. The present paper describes isolation of fucan sulfate **SHFS** from the body walls of sea cucumber *Stichopus horrens*, elucidation of its structure and assessment of anticoagulant activity.

* Corresponding author.
** Corresponding author.
E-mail addresses: ustnad@gmail.com (N.E. Ustyuzhanina), usov@ioc.ac.ru (A.I. Usov).

https://doi.org/10.1016/j.carres.2017.12.001
0008-6215/

2. Results and discussion

A mixture of water-soluble sulfated polysaccharides was isolated from the body walls of *Stichopus horrens* by conventional solubilization in the presence of papain [21], followed by addition of cetyltrimethylammonium bromide to precipitate the sulfated components, which were then transformed into water-soluble sodium salts. Further the crude polysaccharide sample was fractionated by anion-exchange chromatography on DEAE-Sephacel using stepwise elution with increasing sodium chloride solutions. According to monosaccharide composition of fractions, fucosylated chondroitin sulfate and fucan sulfate were eluted with 1.0 and 1.5 M NaCl, respectively. To accomplish further purification of fucan sulfate, eluate at 1.5 M NaCl was desalted and then subjected to gel permeation chromatography. This procedure made it possible to eliminate some fucosylated chondroitin sulfate due to its lower molecular weight. The major polysaccharide fraction **SHFS** contained Fuc and sulfate in a molar ratio of 1.0:0.95 together with traces of other monosaccharides. The L-configuration of fucose was confirmed by coincidence of results of quantitative determination of the monosaccharide with L-fucose dehydrogenase and by GLC.

Structure of **SHFS** was elucidated using NMR spectroscopic methods. Spectra of **SHFS** looked like spectra of a monosaccharide (Figs. 1 and 2), for example, there were only six carbon atom signals in the ^{13}C NMR spectrum. The presence of fucose in **SHFS** was confirmed by the characteristic values of chemical shifts of C-6 (δ 16.2) and H-6 (δ 1.29) in the ^{13}C and ^{1}H NMR spectra, respectively. The α-configuration of the fucosyl units was proved by the characteristic values of chemical shifts of C-1 (δ 96.0) and H-1 (δ 5.40) in the ^{13}C and ^{1}H NMR spectra, respectively.

The presence of only six cross-peaks in the HSQC spectrum confirmed the regularity of the polysaccharide (Fig. 3-I). The correlation H-1—H-2 in the COSY spectrum (Fig. 3-II) indicated the position of H-2 signal in a low-field region (δ 4.60). Such value of the chemical shift evidenced the presence of sulfate at C-2 in a fucosyl residue. The position of H-3 (δ 4.14) was determined by the correlation H-2—H-3 in the COSY spectrum. Using the HSQC spectrum, the signal of the respective C-3 atom was found out in a low field region (δ 74.9), indicating the position of glycosylation at *O*-3. The presence of (1 → 3)-linked α-fucosyl residues was also confirmed by the correlation H-1—H-3 in the ROESY spectrum (Fig. 3-III). Full assignment of the signals is shown in Table 1. Hence, the structure of **SHFS** is represented as →3)-α-L-Fuc2S-(1→ (Fig. 4). The NMR data of **SHFS** are correlated well with those obtained previously for sulfated fucan **SFFS** of similar structure isolated from the sea urchin *Strongylocentrotus franciscanus* [22] and with related synthetic tetrasaccharide **1** (Fig. 4) built up of the repeating 2-O-sulfated (1 → 3)-linked α-L-fucosyl residues [23] (Table 1). Small systematic deviations in the values of the chemical shifts are connected with different registration conditions of the spectra and with application of different internal standards.

Evaluation of molecular weight of **SHFS** was performed by TSK gel chromatography using an appropriate analytical column calibrated with pullulans. Previously it was demonstrated that pullulans could be applied as standards for MW estimation of sulfated polysaccharides, such as heparin, using eluant with high ionic strength [24]. The molecular weight of **SHFS** was assessed as ~140 kDa.

Anticoagulant activity of **SHFS** was assessed *in vitro* in comparison with the LMW heparin (enoxaparin) and fucan sulfate **PPFS** built up of 3-linked α-L-fucopyranose 2,4-disulfate residues. The latter polysaccharide was obtained by sulfation of a fucoidan fragment isolated from the brown seaweed *Punctaria plantaginea* [25] and demostrated significant effect on blood coagulation. The influence of all three samples on the intrinsic pathway of coagulation was evaluated in the activated partial thromboplastin time (APTT) assay. It was shown (Fig. 5) that polysaccharide **SHFS** possessed moderate anticoagulant activity, slightly lower than that of enoxaparin (2APTT were 3.92 ± 0.15 and 3.21 ± 0.14 μg/ml, respectively), while totally sulfated fucan **PPFS** with the same carbohydrate skeleton was significantly more active (2APTT was 2.71 ± 0.12 μg/ml). These results indicate that the presence of sulfate groups both at *O*-2 and *O*-4 of fucosyl units seems to be important for anticoagulant effect of sulfated homo-(1 → 3)-α-L-fucans. At the same time it should be noted that **SHFS**, having MW of about 140 kDa, differed considerably in this parameter, which may be important for anticoagulant activity, from preparations taken for comparison (both **PPFS** and enoxaparin contain about 20 monosaccharide residues per molecule on average [25]).

3. Conclusion

It is known that the body walls of *Stichopus horrens* contain sulfated fucan and fucosylated chondroitin sulfate [20], but, in contrast to the data of Myron et al. [20], the fucan sulfate content in our sample prevailed over the content of fucosylated chondroitin sulfate. The polysaccharides were resolved by ion-exchange chromatography, and fucan sulfate **SHFS** was purified further by gel-permeation chromatography. **SHFS** was shown to have rather simple composition, containing practically equimolecular amounts of fucose and sulfate, and simple NMR spectra. Therefore, structural analysis of **SHFS** was based mainly on the spectral evidence. A highly regular structure was proved for the polysaccharide molecules, which are built up of 3-linked α-L-fucopyranose 2-sulfate residues. A polysaccharide of similar structure was found earlier as a component of egg jelly of the sea urchin *Strongylocentrotus franciscanus* [22]. Anticoagulant activity of fucan 2-sulfate was shown to be markedly lower than that of corresponding 2,4-disulfate, and hence, both sulfate groups in totally sulfated linear 3-linked α-L-fucans seem to be important for effective anticoagulant action. More detailed investigation of biological activity of sulfated polysaccharides and related oligosaccharides [23,30,31] in dependence not only on sulfation pattern, but also on molecular masses and other structural features, will be the subject of our future investigation.

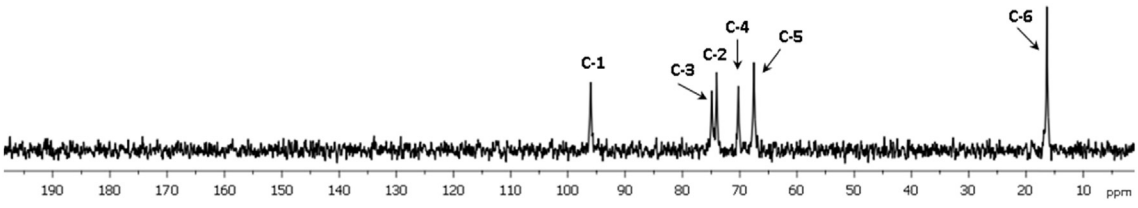

Fig. 1. ^{13}C NMR spectrum of fucan sulfate **SHFS**.