Preliminary structural characterization, anti-inflammatory and anticoagulant activities of chondroitin sulfates from marine fish cartilage

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Chondroitin sulfates isolated from cartilage of five marine fish species: Atlantic salmon (*Salmo salar*), Greenland shark (*Somniosus microcephalus*), blackmouth catshark (*Galeus melastomus*), birdbeak dogfish (*Deania calcea*), and Arctic skate (*Amblyraja hyperborea*), were characterized in detail by ^{1}H and ^{13}C NMR spectroscopy. The complete signal assignments for carbohydrates were made and the relative contents of the key structural units were estimated by 2D homonuclear and heteronuclear NMR spectroscopy (COSY, TOCSY, NOESY, HSQC, and HMBC). The average length of the polysaccharide chain was evaluated from the integrated intensity ratio of the terminal and internal monosaccharide residues. The anti-inflammatory and anticoagulant activities of the specimens were studied. Chondroitin sulfates from salmon and Arctic skate exhibit considerable anti-inflammatory activity. All specimens manifest weak anticoagulant activity. The results of the present study indicate that chondroitin sulfates deserve more detailed investigation as potential anti-inflammatory agents.

Key words: chondroitin sulfate, structure, anticoagulant activity, anti-inflammatory activity, NMR spectra.

Sulfated polysaccharides, for example, hexosaminoglycans, fucoidans, *etc.*, have a broad spectrum of biological activities due to strong ionic interactions with various protein receptors (see, for example, Refs 1—6) and, consequently, can be considered as promising pharmaceutical substances capable of regulating important physiological processes, such as blood coagulation, the development of the inflammation, angiogenesis, and adhesion of cells and viruses.[3—8] However, the therapeutic use of these polysaccharides is substantially limited because of the low selectivity of their biological action and the structure heterogeneity, which makes the standardization of these polysaccharides difficult. Therefore, the search for new polysaccharides of this type free of the above-mentioned drawbacks is an important problem.

Chondroitin sulfates comprise a group of linear sulfated natural polysaccharides belonging to hexosaminoglycans. The carbohydrate chain of chondroitin sulfates consists of repeating disaccharide units including alternating 3-substituted *N*-acetyl-β-D-galactosamine (GalNAc) and 4-substituted β-D-glucuronic acid (GlcA) residues.[5,6] Depending on the character of sulfation of the repeating dis-

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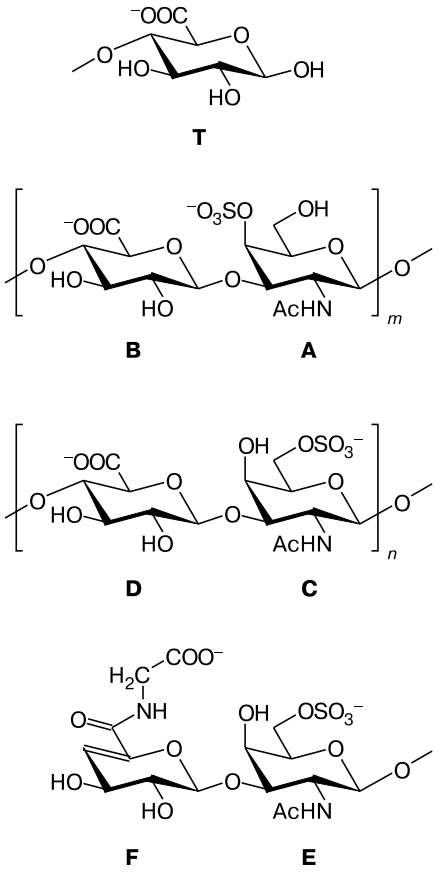

Fig. 1. Fragments identified in the polysaccharides based on one- and two-dimensional ^{1}H and ^{13}C NMR spectra.

accharide units, different types of chondroitin sulfates are distinguished.[5,6] Thus chondroitin sulfates A and C contain disaccharide units with 4- and 6-sulfated galactosamine residues, respectively (see the repeating units B—A and D—C in Fig. 1).

The earlier studies of chondroitin sulfates from various sources have shown that these polysaccharides are not only structural components of connective tissues but also play an important role in the regulation of intercellular interactions.[5,6,9] This class of polysaccharides, which are abundant in cell surface proteoglycans and intercellular matrix, perform signaling functions with different growth factors,[10] P- and L-selectins, and chemokines.[11] In addition, these polysaccharides play a critical role in the development and regeneration of central nervous system cells[12,13] and are receptors for various pathogens, including viruses, parasites, and bacteria.[14,15] It was shown that the functions of chondroitin sulfates are closely related to the positions of sulfate groups in the polysaccharide chain.[5,6,11,16] Therefore, investigations of the structural features and biological activities of a series of practically available polysaccharides is a topical problem.

Experimental

Isolation and pre-purification of chondroitin sulfates. The isolation from cartilage tissues and pre-purification were performed according to a known method.[17] Sharks and skates were collected as a whole and delivered to the laboratory in the frozen state. Salmon heads were delivered after processing at Murmansk plants in the non-frozen state, immediately processed, or frozen. Shark, skate, and salmon cartilage was extracted, purified from residual surrounding tissues (muscle and connective proteins and bones), and ground using a PU-0.6 universal driving gear connected to an MS 2-70 meat grinder (Soyuztorgmash, USSR) or a 1094 homogenizer (Tecator, Sweden).

Polysaccharides were isolated as follows. The raw material was subject to alkaline hydrolysis with 0.2 *M* NaOH at 37 °C for 3 h. Then the solution of chondroitin sulfate and proteins was separated from the precipitate by centrifugation using an Avanti J-25 centrifuge (Beckman, USA) at 5000 rpm for 30 min.

In the next step, the enzymatic hydrolysis was carried out with the use of a complex proteolytic enzyme preparation (EP) prepared at the Laboratory of Biochemistry and Technology of the N. M. Knipovich Polar Research Institute of Marine Fisheries and Oceanography from the hepatopancreas of red king crab (*Paralithodes camtschaticus*) acclimatized in the Barents Sea. The proteolytic activity of the EP was determined by measuring hydrolysis of a 1% sodium caseinate solution as the model substrate and expressed in μmoles of tyrosine liberated after the treatment with the EP (1 g) at 37 °C and pH 7.0 for 10 min. The amount of tyrosine was determined by spectrophotometry (λ = 280 nm) from the corresponding calibration curve. The proteolytic activity of the EP with respect to sodium caseinate was approximately 400 μmol of tyrosine per gram. The solution (pH 7.0—7.5) obtained after centrifugation was diluted with water (1 : 1) and heated to 50 °C. The enzyme preparation was added at the beginning of hydrolysis and 3 h after. The weight ratio of the EP and the raw material was 0.006 : 1 in both steps; the total hydrolysis time was 5 h. The hydrolysate was centrifuged using an Avanti J-25 centrifuge at 5000 rpm for 30 min and then filtered through a membrane with a pore size of 0.45 μm.

Chondroitin sulfate was precipitated from the hydrolysate by the addition of a double volume of ethanol. After 20 h, the precipitate was separated by centrifugation at 5000 rpm for 30 min. Then the precipitate was washed with ethanol and again centrifuged. Then chondroitin sulfate thus obtained was dried in air and then in a drying oven at a temperature not exceeding 60 °C. The yields of the chondroitin sulfates from different sources based on the weight of the raw material are given in Table 1.

Additional purification of chondroitin sulfates. An ion exchange resin (Amberlite IR-120 (Na^+), 200 mg) was added to an aque-

Table 1. Yields of pre-purified chondroitin sulfates

Raw material	Yield (%)
Salmon (*Salmo salar*)	10.31
Greenland shark (*Somniosus microcephalus*)	13.96
Blackmouth catshark (*Galeus melastomus*)	7.53
Birdbeak dogfish (*Deania calcea*)	6.66
Arctic skate (*Amblyraja hyperborea*)	15.51

ous solution of pre-purified chondroitin sulfate (20 mg in 2 mL of water), and the mixture was stirred at room temperature for 2 h. The resin was filtered off and washed with water. Chondroitin sulfate was isolated from the filtrate by chromatography on Sephadex G-15 gel (60×3 cm). The aliquots of the fractions were deposited on Kieselgel 60 F254 silica gel plates (Merck). The plates were sprayed with a 10% solution (v/v) of 85% phosphoric acid in ethanol and heated at 150 °C. The presence of carbohydrates in the fractions was evidenced by characteristic carbonization. After lyophilization of carbohydrate-containing fractions, purified chondroitin sulfate was obtained as a white amorphous powder (11—14 mg).

NMR spectroscopy. The NMR spectra were recorded on Bruker Avance 600 and Bruker DRX 500 spectrometers at 303—306 K after the single lyophilization of the samples from D_2O followed by their dissolution in 99.96% D_2O (2—3% solutions). The spectra were calibrated against sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (internal standard, δ_H 0). The spectra were processed with the use of the Bruker TopSpin 2.1 software package. The parameters used for measuring 2D NMR spectra of polysaccharides have been described earlier.[18] The TOCSY experiment was recorded with a mixing time (MLEV17 spin-lock) of 200 ms; the ROESY experiment, with a mixing time (ROE spin-lock) of 200 ms. The HMBC experiment was measured with a delay or 60 ms for long-range coupling constants evolution.

Influence of samples on blood coagulation. The anticoagulant action of chondroitin sulfates was measured as the doubling of the activated partial thromboplastin time (2APTT) induced by the sample according to a conventional procedure.[19] The normal-hemostasis reference human plasma (80 μL) was mixed with a solution (20 μL) of chondroitin sulfate (0—200 μg per sample) in 0.9% NaCl and the mixture was incubated at 37 °C for 1 min. The reagent (100 μL) containing a mixture of soybean phospholipids and an activator (ellagic acid) was incubated at 37 °C for 2 min, and then a 0.025 *M* $CaCl_2$ solution (100 μL) preheated at 37 °C was added to the mixture. The clotting time was detected visually. The 2APTT value corresponding to the polysaccharide concentration, at which the time of fibrin clot formation (activity) is doubled, is expressed in *n* μg of chondroitin sulfate per milliliter of the sample.

Model of acute peritoneal inflammation in rats. The peritoneal inflammation in rats was induced according to a method described earlier.[20,21] A 9.0% solution of peptone (Moscow Chemical Company Laverna) in 0.9% NaCl (8 mL) was administered intraperitoneally to female Wistar rats (*ca.* 250 g) under ether narcosis. Solutions of chondroitin sulfate (0.3 mL in sterile 0.9% NaCl) was administered to a rat femoral vein under ether narcosis 15 min after the injection of peptone. Sterile 0.9% NaCl (0.3 mL) was administered intravenously to control animals. After 3 h, the animals were decapitated under ether narcosis. The abdominal cavity was washed with a medium (30 mL) containing PBS, heparin 60 unit mL^{-1}, 0.02% EDTA, and 0.03% bovine serum with intense peritoneal massage. The total cell number in the washing liquid was counted in Goryaev's chamber. To calculate the number of neutrophils, the cell suspension was centrifuged at 400 g for 10 min. The concentrated suspension was diluted with a whole bovine serum (1 : 1). Smears were made and stained by the Pappenheim method. The percentage of neutrophils in the smears was determined by counting 6—8 hundred cells. The total number of neutrophils in the exudate was calculated from the percentage of neutrophils and the total cell number.

Results and Discussion

We studied five specimens of chondroitin sulfates isolated from cartilage of salmon (*Salmo salar*), Greenland shark (*Somniosus microcephalus*), blackmouth catshark (*Galeus melastomus*), birdbeak dogfish (*Deania calcea*), and Arctic skate (*Amblyraja hyperborea*).

The ^1H NMR spectra of the polysaccharides contained broadened signals at δ 3.3—0.7, which were impossible to unambiguously identify and which could be assigned to aliphatic impurities present in the samples. Because of this, the samples of chondroitin sulfates were additionally purified by treatment with the Amberlite IR-120 (Na^+) ion-exchange resin and chromatography on Sephadex G-15 gel. After this purification, the spectra of the samples contained, if at all, a substantially smaller number of unidentified signals.

The ^1H (Fig. 2) and ^{13}C NMR spectra were recorded for all samples. The positions of the signals in the spectra of different samples are identical, but the spectra differ in the integrated intensity. Therefore, to determine the structures of polysaccharides, we chose one sample isolated from salmon cartilage. The one-dimensional (1D) ^1H and ^{13}C NMR spectra of this sample were analyzed with the use of two-dimensional (2D) NMR experiments, such as homonuclear $^1H/^1H$ COSY, TOCSY, and ROESY and heteronuclear $^1H/^{13}C$ HSQC and HMBC.

The anomeric region of the 2D $^1H/^{13}C$ HSQC spectrum (Fig. 3, *a*) shows two groups of intense signals. The chemical shifts for one group of signals are at about δ 4.57/102.4; for another group, at about δ 4.48/105.4. In each group, several signals with similar chemical shifts were distinguished. An analysis of the homonuclear 2D COSY and TOCSY spectra revealed the positions of the signals for other protons of these monosaccharide residues (Table 2) corresponding to the signals for the anomeric protons and carbon atoms in the HSQC spectrum (see Fig. 3, *a*). In addition, an analysis of the homonuclear 2D spectra allowed us to determine the order of the magnitude (large/small) of the intraresidue coupling constants $^3J_{H,H}$. The assignment of the signals in the ^{13}C NMR spectrum (see Table 2) was made based on the additionally recorded HSQC spectrum with the use of the chemical shifts in the ^1H NMR spectra.

Based on the ^1H and ^{13}C chemical shifts of monosaccharide residues and the intraresidue coupling constants between protons, the first group of signals (δ ≈ 4.57/102.4) in the anomeric region of the HSQC spectrum was assigned to galactosamine residues (β-D-GalN, the residues A and C, see Fig. 1, Table 2); the second group (δ ≈ 4.48/105.4), to glucuronic acid residues (β-D-GlcA, the residues B and D,

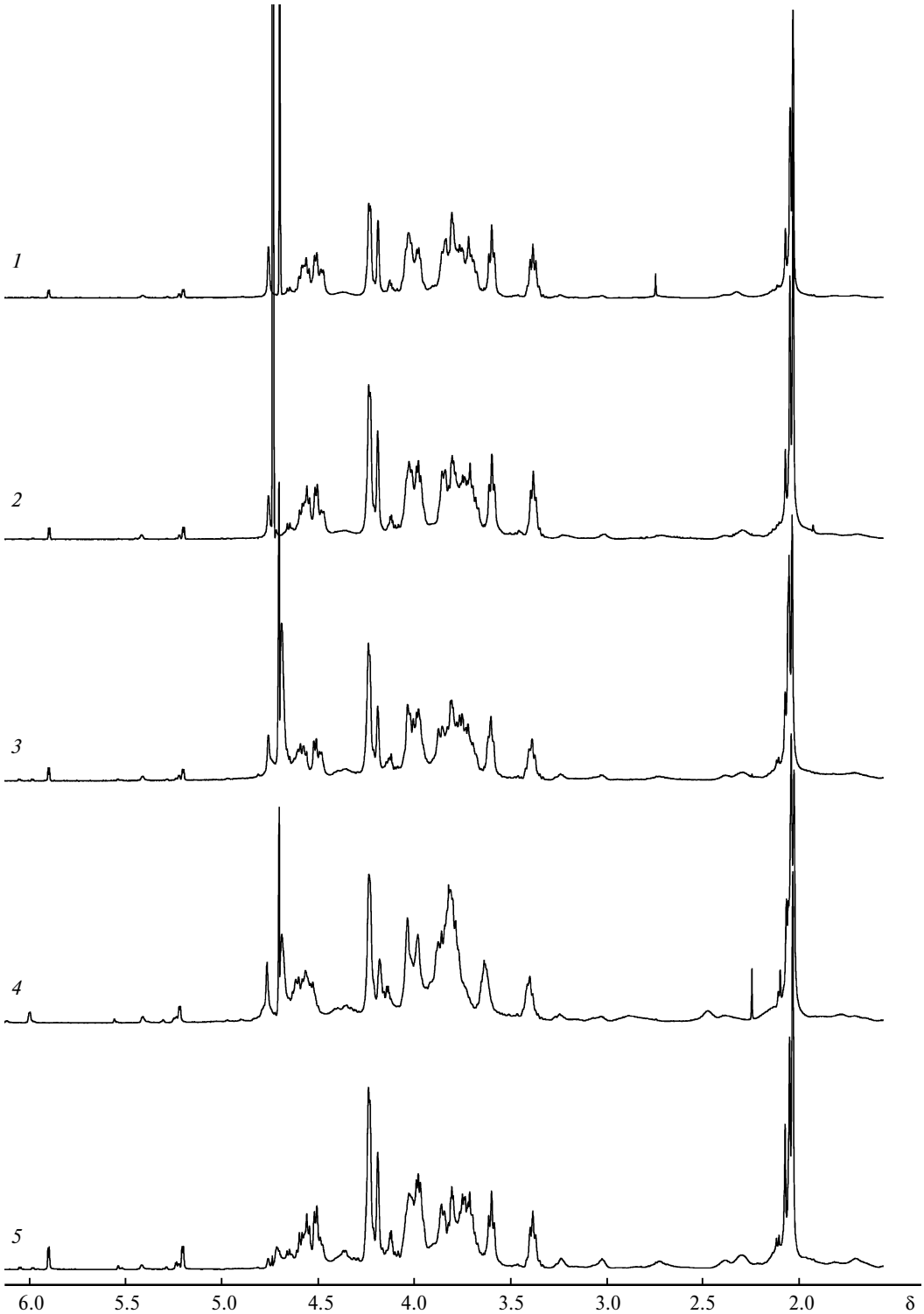

Fig. 2. ^{1}H NMR spectra (303—306 K) of chondroitin sulfates from salmon (*Salmo salar*) (*1*), Greenland shark (*Somniosus microcephalus*) (*2*), blackmouth catshark (*Galeus melastomus*) (*3*), birdbeak dogfish (*Deania calcea*) (*4*), and Arctic skate (*Amblyraja hyperborea*) (*5*).

see Fig. 1, Table 2). The ^{1}H and ^{13}C chemical shifts for the β-D-GalN residues indicate that the latter are completely N-acetylated, mainly 6-O-sulfated, and, to a lesser extent, 4-O-sulfated. The positions of the acetate groups were confirmed by the nuclear Overhauser effect (NOE) experiments. Thus there are correlations between the pro-

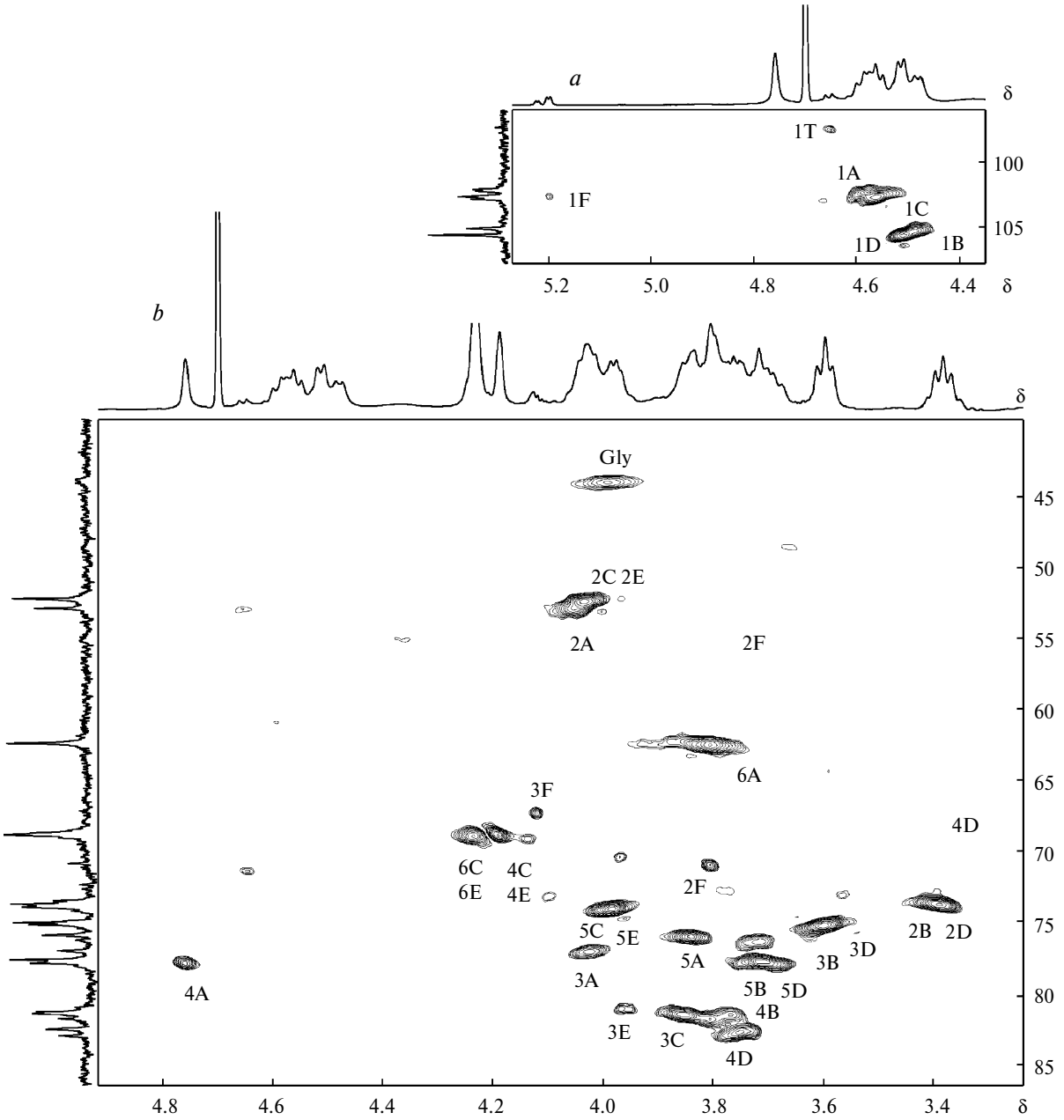

Fig. 3. 2D HSQC spectra of chondroitin sulfate from cartilage of salmon (*Salmo salar*): *a*, the region of signals for anomeric protons and carbon atoms, *b*, the region of signals for ring protons and carbon atoms (except for the anomeric atoms).

tons H(2) of the β-D-GalN residues and the methyl protons of *N*-acetyl groups in the ROESY spectrum.

The sequence of monosaccharide residues in the polysaccharide chain of chondroitin sulfates was determined based on analysis of the $^{1}H/^{13}C$ HMBC spectrum (see Fig. 1). The HMBC spectrum has the correlations $H(1_B)/C(3_A)$, $H(1_A)/C(4_B)$, $H(1_D)/C(3_C)$, and $H(1_C)/C(4_D)$ between atoms separated by glycosidic bonds. Based on these correlations, we divided the main residues into two blocks $(A—B)_m$ and $(C—D)_n$ (see Fig. 1). The block structure of the chain was confirmed by the absence of the correlations $H(1_A)/C(4_D)$ and $H(1_C)/C(4_B)$. In the case of the block structure of polysaccharides, the number of glycosidic bonds between different blocks may be relatively small and, consequently, the correlations corresponding to the interactions between atoms across these glycosidic bonds may not be observed in the HMBC spectra.

The anomeric region of the 2D $^{1}H/^{13}C$ HSQC spectrum (see Fig. 3, *a*) shows, in addition to the main signals,

Table 2. Chemical shifts in the ^1H and ^{13}C NMR spectra of polysaccharides (D_2O, 306 K)

	Residue	δ											
		^1H						^{13}C					
		H(1)	H(2)	H(3)	H(4)	H(5)	H(6,6′)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
T	→4)-β-D-GlcA-OH	4.65	3.36	3.63	3.74	3.74	—	97.6	—	—	—	—	175.7[a]
A	→3)-β-D-GalNAc[b]-(1→	4.59	4.03	4.03	4.75	3.83	3.80	102.3	52.8	76.8	77.6	75.8	62.3
B	→4)-β-D-GlcA-(1→	4.47	3.38	3.59	3.78	3.70	—	105.2	73.9	75.2	81.8	77.9	175.7[a]
C	→3)-β-D-GalNAc[b]-(1→	4.56	4.03	3.86	4.18	3.98	4.24	102.7	52.3	81.5	68.9	74.0	68.9
D	→4)-β-D-GlcA-(1→	4.50	3.38	3.59	3.74	3.70	—	105.7	73.9	75.2	82.9	77.9	175.5[a]
E	→3)-β-D-GalNAc[b]-(1→	4.59	4.03	3.95	4.18	3.98	4.24	102.3	52.3	81.1	68.9	74.0	68.9
F	4-Deoxy-4,5-dehydro--α-L-*mpeo-threo*-hexurono-pyranosyl[c]-(1→	5.20	3.80	4.13	4.90	—	—	102.6	70.8	67.1	108.5	145.9	172.7

[a] The signal assignment can be changed to opposite.
[b] The chemical shifts of the NAc groups: δ_H, 2.00—2.05; δ_C, 23.9 and 176.3.
[c] The chemical shifts of the glycine residue: δ_H, 3.98; δ_C, 43.8 and 175.7.

two weak signals 1 T (δ 4.65/97.6) and 1 F (δ 5.20/102.6). Based on the data from the $^1H/^1H$ COSY and TOCSY spectra, the signal 1 T was assigned to the β-D-GlcA residue located at the reducing end of the polysaccharide chain. It should be noted that the signals corresponding to other monosaccharide residues at the reducing end of the chain were absent in the spectra. However, this fact can be attributed not to the complete absence of these residues in the sample but to the low intensity of the corresponding signals.

An analysis of the COSY and TOCSY spectra showed that the proton corresponding to the signal 1 F in the HSQC spectrum is involved in the closed spin system with three other protons (see Table 2), which are coupled by spin-spin interactions. In the HSQC spectrum, the first three protons of this system, $H(1_F)$, $H(2_F)$, and $H(3_F)$, correlate with the carbon atoms $C(1_F)$, $C(2_F)$, and $C(3_F)$ whose chemical shifts are characteristic of carbohydrates. The chemical shift of the $C(1_F)$ signal is virtually identical to that of the C(1) signal of the glucuronic acid residues B and D. The chemical shifts of the $C(2_F)$ and $C(3_F)$ signals correspond to unsubstituted carbon atoms.

The carbon atom $C(4_F)$ whose chemical shift is not typical of pyranoside derivatives (δ 108.5, see Table 2) corresponds to the proton $H(4_F)$. Let us also note that the correlations at δ 5.20/145.9 and δ 4.90/145.9 with the same carbon atom in the $^1H/^{13}C$ HMBC spectrum correspond to the protons $H(1_F)$ and $H(4_F)$, respectively. Based on these data, it was concluded that the residue F is 4-deoxy-4,5-dehydro-α-L-*threo*-hexuronopyranose ($\delta_{C(5F)}$ = 145.9) and is located at the nonreducing end of the polysaccharide chain (see Fig. 1).

In the $^1H/^{13}C$ HMBC spectrum, there is a correlation at δ 5.20/81.1 between the proton $H(1_F)$ and the carbon atom $C(3_E)$, which is indicative of the presence of the (1→3)-glycosidic bond between the residues F and E (see Fig. 1). Let us also note that the galactosamine residue E is 6-O-sulfated. Based on the available spectroscopic data, we did not find evidence for the presence of the bond between the terminal residue F and the 4-sulfated galactosamine residue, which is apparently attributed to a small amount of these fragments in the polysaccharide chain.

After the assignment of the signals of monosaccharide residues, only one signal with a significant intensity at δ 3.98/43.8 remained unidentified in the HSQC spectrum. The correlations between the proton corresponding to this signal and other protons of the system are absent in the COSY, TOCSY, and ROESY spectra. An analysis of the edited HSQC spectrum showed that this signal can be assigned to the CH_2 group. It was also noted that the ^{13}C chemical shift corresponding to the signal under consideration in the HSQC spectrum is characteristic of the carbon atom bound to the nitrogen atom. Based on the available data, it was suggested that this signal corresponds to the CH_2 group of glycine.

Our hypothesis was later confirmed by the results of amino acid analysis. According to these data, the sample under consideration contains a substantial amount of glycine and a small amount of other amino acids. In the $^1H/^{13}C$ HMBC spectrum, there are two correlations between the protons of the CH_2 group of glycine and the carbonyl carbon atoms at δ 3.98/175.7 and 3.98/172.7. The former correlation corresponds to the interaction with the carboxy carbon atom of glycine; the second correlation is, apparently, assigned to the interaction with the carbon atom C(6) of the unsaturated uronic acid residue. Based on these observations, we suggested that all glycine residues are amide substituents at the C(6) atoms of the terminal residues F (see Fig. 1). This hypothesis is confirmed by the fact that the intensity of the signal of the

CH_2 group of glycine is proportional to the intensity of the signals for the terminal residues F in the spectra of the samples under study. To the best of our knowledge, similar structural moieties have not been found earlier in chondroitin sulfates. It should be noted that the NMR spectroscopic data for the monosaccharide units A—D and E (see Fig. 1) are in satisfactory agreement with the spectroscopic characteristics of the corresponding types of chondroitin sulfates.[22,23] This is why we did not specially determine the absolute configurations of the monosaccharide residues in the polysaccharides under study. We assigned the D configuration to these residues by analogy with all known chondroitin sulfates. This characteristic feature was beyond doubt taking into account the sources of the polysaccharides under study and the fact that their NMR spectra are identical to those published earlier for chondroitin sulfates.[22,23]

An analysis of the integrated intensities of the signals in the ^{1}H NMR spectra, to be more precise, the intensity ratios of the proton $H(4_F)$ and the protons $H(2_B) + H(2_D) + H(2_T)$, provides an estimate of the chain length of the polysaccharide products (Table 3). It can be seen that chondroitin sulfates isolated from different sources differ in the chain length. It should be noted that the above-described analysis is qualitative because the products can contain not only polysaccharide chains terminated by the F-type moiety but also a certain amount of polysaccharides terminated by units of other types. It should also be noted that we failed to determine the ratio of the signals belonging to the main units A—B and C—D in the polysaccharides due to the strong overlap of these signals in the ^{1}H NMR spectra (see Fig. 1). We did not ascertain whether individual polysaccharide chains consist of units of the same type (*i.e.*, certain chains are composed only of the units A—B or C—D) or they include both types of units within the polysaccharide chains.

Table 3. Anti-inflammatory and anticoagulant activities of chondroitin sulfates from different fishes

Source of chondroitin activity	Chain length[a]	Anti-inflammatory activity			2APTT[b]
		n[c]	N ($\cdot 10^6$)[d]	I (%)[e]	
Control		9	74.0±12.5		
Salmon (*Salmo salar*)	53	4	19.6±1.6	73.5	>700
Greenland shark (*Somniosus microcephalus*)	49	3	47.4±0.7	35.9	190
Blackmouth catshark (*Galeus melastomus*)	27	4	33.2±7.4	55.1	128
Birdbeak dogfish (*Deania calcea*)	32	3	40.6±14.8	45.1	249
Arctic skate (*Amblyraja hyperborea*)	20	4	15.6±3.8	78.9	385

[a] The polysaccharide chain length (the number of disaccharide units) was qualitatively evaluated by ^{1}H NMR spectroscopy (see the text).
[b] 2APTT is the doubling of the activated partial thromboplastin time (μg mL^{-1}).
[c] n is the number of laboratory animals (rats) in the group.
[d] N is the number of neutrophils per rat.
[e] I is the inhibition.

We investigated the anticoagulant and anti-inflammatory activities of the chondroitin sulfates isolated in the present study. The anti-inflammatory activity was determined in *in vivo* experiments as the inhibition of peritoneal inflammation in rats. In the case of peptone-induced acute inflammation in rats, the number of neutrophils in peritoneal cavities increased to $(74.0 \pm 12.5) \cdot 10^6$ (the reference group, nine animals). The intravenous administration of chondroitin sulfates led to a substantial decrease in the number of neutrophils (see Table 3). When administered in an amount of 4 mg per kilogram of the rat weight, chondroitin sulfates from different sources showed a substantial difference in the degree of inhibition varying from 35.9% to 78.9%. The polysaccharides isolated from Arctic skate and salmon better inhibited the development of the inflammation (78.9% and 73.5%, respectively). Chondroitin sulfates isolated from different sharks are characterized by a relatively low anti-inflammatory activity (35.9%—55.1%).

The anticoagulant activity of the chondroitin sulfates isolated in the present study was investigated with the use of the test on the increase in the activated partial thromboplastin time (APTT) characterizing the combined influence of the inhibitor on the factors of the intrinsic coagulation cascade (see Table 3). All chondroitin sulfates showed insignificant activity. The polysaccharides from shark cartilage exhibited the highest activity (128—249 μg mL^{-1}), whereas the anticoagulant properties of the polysaccharides from salmon and Arctic skate were much less pronounced (385 and 700 μg m^{-1}, respectively).

A comparison of the anticoagulant activity of chondroitin sulfates under study with the anti-inflammatory action of these substances did nor reveal correlations between two biological activities. Apparently, these two types of activity are determined by different structural features of polysaccharides. A substantial difference in the biological activity of the series of the compounds under investigation does not directly correlate with the structural parameters determined by NMR spectroscopy. However, further investigation of the most active chondroitin sulfates from salmon and Arctic skate cartilage could be important for the elucidation of the possibility of their use for the design of agents with anti-inflammatory activity.

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