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Facile analysis of contents and compositions of the chondroitin sulfate/dermatan sulfate hybrid chain in shark and ray tissues

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ABSTRACT

The chondroitin sulfate (CS)/dermatan sulfate (DS) hybrid chain was extracted from specific tissues of several kinds of sharks and rays. The contents and sulfation patterns of the CS/DS hybrid chain were precisely analyzed by digestion with chondroitinases ABC and AC. All samples predominantly contained the A- and C-units. Furthermore, all samples characteristically contained the D-unit. Species-specific differences were observed in the contents of the CS/DS hybrid chain, which were the highest in Mako and Blue sharks and Sharpspine skates, but were lower in Hammerhead sharks. Marked differences were observed in the ratio of the C-unit/A-unit between sharks and rays. The contents of the CS/DS hybrid chain and the ratio of the C-unit/A-unit may be related to an oxidative stress-decreasing ability.

1. Introduction

The demand for chondroitin sulfate (CS) in health food supplements and for medical use is increasing. CS is obtained from the tissues of sharks and porcine animals. However, since the shark population is decreasing due to overfishing and porcine animals are susceptible to infectious diseases, we have been seeking alternative resources to these animals. Marine products such as fishes and squids are promising because they are abundant and not markedly affected by infectious diseases. The contents and sulfation patterns of the CS/dermatan sulfate (DS) hybrid chain as well as hyaluronic acid derived from fishes and squids have already been clarified.[1,2] In order to find an alternative animal to sharks as a resource of CS, it is important to know the contents and sulfation patterns of CS from sharks.

CS belongs to the proteoglycan family, each member of which has a linear glycan chain composed of the repeating disaccharide unit, -4)β-D-GlcA(1–3)β-D-GalNAc(1-. Some of the C-5 of GlcA in the CS chain is epimerized to IdoA in order to form a DS unit, and, thus, the repeating disaccharide unit is expressed as -4)α-L-IdoA(1–3)β-D-GalNAc(1-. Naturally occurring CS often forms a CS/DS hybrid chain. CS/DS hybrid chains in fish are mostly composed of A- and C-units at concentrations of several hundred milligrams per 100 g of dry-defatted tissue. CS from squids characteristically contains ~ 30% of the E-unit. The amount of CS in squids is similar to that in fish.

CS has been investigated in shark and ray species by other groups. Fürth and Bruno initially isolated CS from the cartilage of sharks and rays in 1937.[3] In 1963, Seno and Meyer isolated CS-B and hyaluronic acid from the skin of sharks (Blue sharks and Sandbar sharks).[4] However, a precise compositional analysis was not performed until much later. Sugahara's group reported the isolation of specific oligosaccharides from shark cartilage.[5a–i] Oligosaccharides containing the D structure have been shown to exhibit neurite outgrowth-promoting activities[5e] and binding activities to CS antibodies.[5h,i] Sugahara's group subsequently determined the molar ratio of CS disaccharides in the cartilage and liver of Blue sharks (*Prionace glauca*). The chondroitinase ABC digestion of CS afforded ΔO, ΔC, ΔA, ΔD, ΔB, and ΔT.[5f,g] The cartilage and liver of Blue sharks were found to contain 30~50% of the A-unit, but smaller amounts of the D-unit. These parts also contained 6~18% of the B-unit and

Abbreviations: CS, chondroitin sulfate; DS, dermatan sulfate; GAG, glycosaminoglycan; GlcA, glucuronic acid; GalNAc, *N*-acetyl-galactosamine; ΔO, ΔHexA(1–3)β-D-GalNAc; ΔA, ΔHexA(1–3)β-D-GalNAc(4S); ΔC, ΔHexA(1–3)β-D-GalNAc(6S); ΔD, ΔHexA(2S)(1–3)β-D-GalNAc(6S); ΔE, ΔHexA(1–3)β-D-GalNAc(4,6-diS); ΔK, ΔHexA(3S)(1–3)β-D-GalNAc(4S); ΔT, ΔHexA(2S)(1–3)β-D-GalNAc(4,6-diS); O, -4)β-D-GlcA(1–3)β-D-GalNAc(1-; iO, -4)α-L-IdoA(1–3)β-D-GalNAc(1-; A, -4)β-D-GlcA(1–3)β-D-GalNAc(4S)(1-; iA, -4)α-L-IdoA(1–3)β-D-GalNAc(4S)(1-; B, -4)β-D-GlcA(2S)(1–3)β-D-GalNAc(4S)(1-; iB, -4)α-L-IdoA(2S)(1–3)β-D-GalNAc(4S)(1-; C, -4)β-D-GlcA(1–3)β-D-GalNAc(6S)(1-; iC, -4)α-L-IdoA(1–3)β-D-GalNAc(6S)(1-; D, -4)β-D-GlcA(2S)(1–3)β-D-GalNAc(6S)(1-; iD, -4)α-L-IdoA(2S)(1–3)β-D-GalNAc(6S)(1-; E, -4)β-D-GlcA(1–3)β-D-GalNAc(4,6-diS)(1-; iE, -4)α-L-IdoA(1–3)β-D-GalNAc(4,6-diS)(1-; T, -4)β-D-GlcA(2S)(1–3)β-D-GalNAc(4,6-diS)(1-; iT, -4)α-L-IdoA(2S)(1–3)β-D-GalNAc(4,6-diS)(1-.

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10~23% of the E-unit. In contrast, fin cartilage has been reported to contain small amounts of the B- and E-units, but 15% of the D-unit (species not shown).[5i] Toida's group recently isolated the CS/DS hybrid chain from the fins of deep-sea sharks, and demonstrated that the fins contained hyaluronic acid and the O-, C-, A-, B-, and D-units.[6] It has not yet been determined whether these differences are due to the species of shark or tissue examined.

Tsegenidis and co-workers investigated CS in ray species. The chondroitinase digestion of the skin from *Raja clavata* afforded ΔO, ΔC, ΔA, ΔD, and ΔK at 3, 4, 61, 13, and 20%, respectively.[7] Dellias et al examined the ratio of CS disaccharides in the skin of 4 ray species. Enzymatic digestion of the skins from *D. americana, D. guttata*, and *A. narinari* mainly afforded ΔA (50, 51, and 47%, respectively) and ΔB (30, 33, and 24%, respectively), and, to a lesser extent, ΔD (3, 2, and 8%, respectively), while the skin from *P. motoro* afforded ΔA (75%), ΔB (6%), but not ΔD.[8]

To the best of our knowledge, the CS/DS hybrid chain has not yet been examined in other tissues of sharks and rays. Although analytical studies have been performed using a limited number of shark species, the total contents of the CS/DS hybrid chain in these tissues currently remain unknown. Therefore, we herein isolated the CS/DS hybrid chain from a number of tissues in five shark species and three ray species, some of which have been utilized for the manufacture of CS, and then analyzed the contents and compositions of disaccharide units.

2. Materials and methods

Blue sharks (*Prionace glauca*) were caught off the coast of Fukuejima, Nagasaki, Mako sharks (*Isurus oxyinchus*) off the coast of Ishinomaki, Miyagi, Smooth hammerhead (*Sphyrna zygaena*) and Scalloped hammerhead (*Sphyrna lewini*) sharks off the coast of Yushima, Kumamoto, Dusky sharks (*Carcharhinus obscurus*) off the coast of Narushima, Nagasaki, Stingrays (*Dasyatis akajei*) at Nakaumi, Tottori, and Sharpspine skates (*Okamejei acutispina*) and Kwangtung skates (*Dipturus kwangtungensis*) in the Sea of Japan, off the coast of Tottori, Japan. The tissues of sharks and rays examined in this study were summarized in Table 1. Each material was minced before use.

DEAE-cellulose was purchased from Wako Pure Chemical (Osaka, Japan). Dialysis cellulose tubing (MWCO 12,000–16,000) was from Viskase Companies, Inc. (Darien, IL). Chondroitinase ABC (EC 4.2.2.4) and chondroitinase AC (EC 4.2.2.5) were from Sigma Aldrich (Tokyo, Japan) and IBEX Pharmaceuticals Inc. (Montréal, Canada), respectively. The LH-20 gel and amino-bound PA-03 (PA-G) column were from GE Healthcare, Bio-Science AB (Uppsala, Sweden), and YMC

Table 1
Contents and sulfation patterns of the CS/DS hybrid chain in shark and ray tissues

Name	Tissue	Content of CS/DS hybrid (mg) /100 g defatted dry tissue	Sufation patterns of the disaccharide unit																					
			GlcA- + IdoA-type (%)								GlcA-type (%)				IdoA-type (%)				GlcA-type (%)		IdoA-type (%)			
			A	C	D	E	A	C	D	E	A	C	D*	E	iA	iC	iD*	iE	A	C	iA	iC	iD	iE
Blue shark	Tail fin	955	27	57	13	3					11	49	0	0	16	8	13	3						
	Dorsal fin	1061	27	56	14	3					8	45	0	0	19	11	14	3						
	Pectoral fin	1863	32	48	20	0					7	33	0	0	25	15	20	0						
	Spine	1823	20	67	13	0					5	52	0	0	15	15	13	0						
Mako Shark	Tail fin	1582	37	44	17	2					13	42	0	0	24	2	17	2						
	Dorsal fin	1670	37	40	20	3					11	35	0	0	26	5	20	3						
	Pectoral fin	1376	38	38	21	3					13	30	0	0	25	8	21	3						
	Spine	1330	33	48	18	1					11	46	0	0	22	2	18	1						
Dusky shark	Tail fin	767	39	36	20	5					11	25	0	0	28	11	20	5						
	Dorsal fin	439	41	33	21	5					6	16	0	0	35	17	21	5						
	Spine	458	38	41	19	2					16	36	0	0	22	5	19	2						
Smooth Hammerhead	Tail fin	502	42	32	22	4					10	22	0	0	32	10	22	4						
	Dorsal fin	189	42	31	23	4					11	23	0	0	31	8	23	4						
	Spine	1064	43	36	19	2					14	28	0	0	29	8	19	2						
Scalloped Hammerhead	Dorsal fin	395	53	23	19	5					20	21	0	0	33	2	19	5						
	Pectoral fin	997	57	17	21	5					15	17	0	0	42	0	21	5						
	Spine	368	52	24	21	3					10	12	0	0	42	12	21	3						
Stingray	Head	1608	24	66	10	0					9	55	0	0	15	11	10	0						
	Spine	573	26	62	11	1					9	49	0	0	17	13	11	1						
	Fin	628	27	59	12	2					7	41	0	0	20	18	12	2						
Sharpspine skate	Head-Tail	1296	28	61	10	1					10	47	0	0	18	14	10	1						
	Fin	1172	31	55	12	2					12	48	0	0	19	7	12	2						
Kwangtung skate	Head-Tail	882	32	57	9	2					11	44	0	0	21	13	9	2						
	Fin	597	35	52	12	1					12	41	0	0	23	11	12	1						

[a] The value of the iD-unit needs to be considered as a mixture of the D- and iD-units (see details in the text).