Biochimica et Biophysica Acta 1830 (2013) 4719–4733

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Biosynthesis and function of chondroitin sulfate

Tadahisa Mikami, Hiroshi Kitagawa *

Department of Biochemistry, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan

ARTICLE INFO

Article history:
Received 2 April 2013
Received in revised form 3 June 2013
Accepted 6 June 2013
Available online 14 June 2013

Keywords:
Chondroitin sulfate
Glycosaminoglycan
Glycosyltransferase
Proteoglycan
Sulfotransferase
Biosynthesis/catabolism

ABSTRACT

Background: Chondroitin sulfate proteoglycans (CSPGs) are principal pericellular and extracellular components that form regulatory milieu involving numerous biological and pathophysiological phenomena. Diverse functions of CSPGs can be mainly attributed to structural variability of their polysaccharide moieties, chondroitin sulfate glycosaminoglycans (CS-GAG). Comprehensive understanding of the regulatory mechanisms for CS biosynthesis and its catabolic processes is required in order to understand those functions.
Scope of review: Here, we focus on recent advances in the study of enzymatic regulatory pathways for CS biosynthesis including successive modification/degradation, distinct CS functions, and disease phenotypes that have been revealed by perturbation of the respective enzymes *in vitro* and *in vivo*.
Major conclusions: Fine-tuned machineries for CS production/degradation are crucial for the functional expression of CS chains in developmental and pathophysiological processes.
General significance: Control of enzymes responsible for CS biosynthesis/catabolism is a potential target for therapeutic intervention for the CS-associated disorders.

1. Introduction

Chondroitin sulfate (CS) is a representative sulfated glycosaminoglycan (GAG) that is widespread on cell surfaces and within extra/pericellular matrices in the form of proteoglycans (CSPGs), where at least one CS side chain is covalently attached to a panel of core proteins. CSPGs have been implicated not only in diverse physiological events such as cytokinesis, morphogenesis, and neuronal plasticity, but also in pathological processes including skeletal disorders, glial scar formation after brain injury, and infections with viruses and bacteria [1–3]. Mounting evidence indicates that most of functions of CSPGs are largely exerted through the CS moieties, with core proteins seeming to act merely as a scaffold [1–3]. Notably, CS moieties *in vivo* vary considerably in the size and the number of CS chains per core protein and in the position and degree of sulfation. Such structural complexity is able to support multifaceted functions of CSPGs. Here, we summarize the functional importance of CS chains, focusing primarily on how they are constructed by distinct biosynthetic machineries.

2. Conventional biosynthetic scheme of CS chains

CS is a class of sulfated GAGs that are linear polysaccharides consisting of repeating disaccharide units composed of uronic acid and *N*-acetylhexosamine. Because of their characteristic disaccharide units [(–4GlcAβ1–3GalNAcβ1–)$_n$], where GlcA and GalNAc represent glucuronic acid and *N*-acetylgalactosamine, respectively, CS can be classified as galactosaminoglycan (Fig. 1). The assembly of CS chains occurs in endoplasmic reticulum/Golgi compartments, and is initiated by the synthesis of the so-called GAG-protein linkage region, GlcAβ1–3Galβ1–3Galβ1–4Xylβ1–*O*-Ser, which is covalently linked to specific serine residues embedded in different core proteins [1,3,4]. The tetrasaccharide structure of the linkage region is assembled through the sequential stepwise addition of individual monosaccharide units, single Xyl (xylose), two successive Gal (galactose), and single GlcA residues, by the corresponding specific glycosyltransferases, XylT (xylosyltransferase) [5,6], GalT-I (β1,4-galactosyltransferase I) [7,8], GalT-II (β1,3-galactosyltransferase II) [9], and GlcAT-I (β1,3-glucuronyltransferase I) [10–12], respectively (Table 1 and Fig. 2). The first GalNAc transfer to the non-reducing terminal GlcA residue in the tetrasaccharide linkage region by GalNAcT-I (GalNAc transferase I) triggers the synthesis of chondroitin (Chn) backbone [1,3,4]. The repetitive disaccharide region characteristic of CS is synthesized by the alternate additions of GlcA and GalNAc residues through the actions of GlcAT-II (GlcA transferase II) and GalNAcT-II (GalNAc transferase II), respectively (Fig. 2) [1,3,4]. It should

Abbreviations: C4ST, chondroitin 4-*O*-sulfotransferase; C6ST, chondroitin 6-*O*-sulfotransferase; ChABC, chondroitinase ABC; ChGn, chondroitin GalNAc transferase; Chn, chondroitin; ChPF, chondroitin polymerizing factor; ChSy, chondroitin synthase; CS, chondroitin sulfate; D4ST, dermatan 4-*O*-sulfotransferase; DS, dermatan sulfate; DS-epi, GlcA C-5 epimerase (DS epimerase); EXT, exostosin; EXTL, EXT-like; FAM, family with sequence similarity; GAG, glycosaminoglycan; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GalNAc4S-6ST, GalNAc 4-sulfate 6-*O*-sulfotransferase; GalNAcT, GalNAc transferase; GalT-I, β1,4-galactosyltransferase-I; GalT-II, β1,3-galactosyltransferase-II; GlcA, glucuronic acid; GlcAT-I, β1,3-glucuronyltransferase-I; GlcAT-II, GlcA transferase-II; GlcNAc, *N*-acetylglucosamine; GlcNAcT, GlcNAc transferase; HA, hyaluronan; HNK-1, human natural killer-1; HS, heparan sulfate; HSV, herpes simplex virus; HYAL, mammalian hyaluronidase; PG, proteoglycan; Ser, serine; TM, thrombomodulin; UST, uronyl 2-*O*-sulfotransferase; Xyl, xylose; XylT, xylosyltransferase

* Corresponding author. Tel.: +81 78 441 7569; fax: +81 78 441 7571.
E-mail address: kitagawa@kobepharma-u.ac.jp (H. Kitagawa).

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.06.006

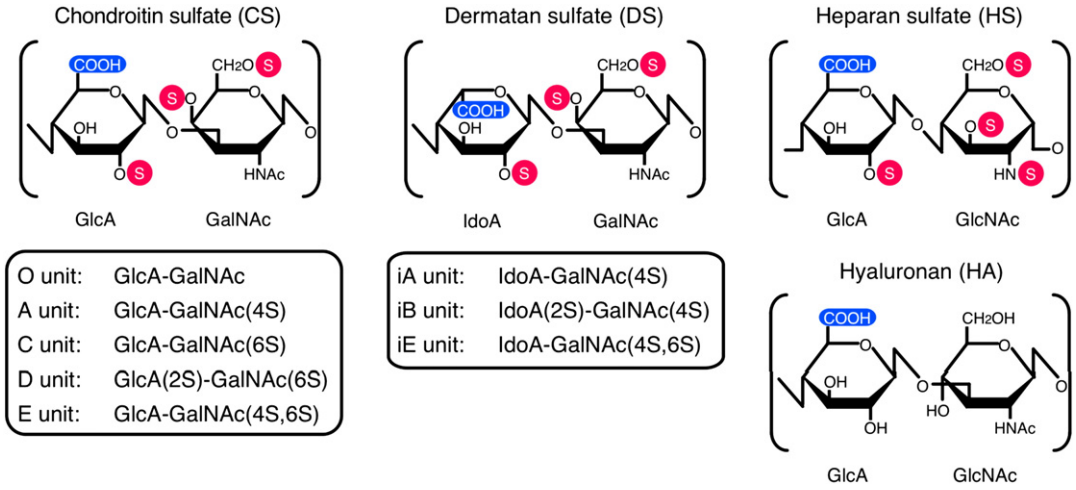

Fig. 1. Typical disaccharide units found in CS, DS, HS, and HA GAG chains. CS chains are constituted of GlcA and GalNAc residues. DS is a stereoisomer of CS including IdoA instead of or in addition to GlcA. HS chains comprise GlcA and GlcNAc residues. These sugar residues can be esterified by sulfate at various positions as indicated by "S" enclosed by a circle. In contrast, HA is also a linear polymer composed of the repeating disaccharide unit [(−4GlcAβ1–3GlcNAcβ1−)$_n$], and is similar in structure to non-sulfated CS, Chn. The disaccharide units of CS chains are classified into O, A, C, D, and E units on the basis of their sulfation patterns, and iO, iA, iE, and iB units are the isomeric counterparts found in DS chains. 2S, 4S, and 6S represent the 2-*O*-, 4-*O*-, and 6-*O*-sulfate group, respectively. The abbreviation "i" in DS disaccharide units stands for IdoA.

be noted that the tetrasaccharide linkage region is shared with another sulfated GAG, heparan sulfate (HS) that consists of repetitive disaccharide unit [(−4GlcAβ1–4GlcNAcα1−)$_n$] including *N*-acetylglucosamine (GlcNAc) as an *N*-acetylhexosamine, thereby being classified as glucosaminoglycan (Fig. 1). Therefore, the addition of GlcNAc instead of GalNAc residue triggers the synthesis of HS on the linkage region, indicating that

Table 1
CS biosynthetic/catabolic enzymes in human.

Enzymes (activity)	Abbreviation	Gene symbols (synonym)		Chromosomal location	mRNA accession number
Glycosyltransferases involved in synthesis of the tetrasaccharide linkage region					
Xylosyltransferase	XylT	*XYLT1*		16p12.3	NM_022166
		XYLT2		17q21.33	NM_022167
β1,4-Galactosyltransferase-I	GalT-I	*B4GALT7*		5q35.2–q35.3	NM_007255
β1,3-Galactosyltransferase-II	GalT-II	*B3GALT6*		1p36.33	NM_080605
β1,3-Glucuronyltransferase-I	GlcAT-I	*B3GAT3*		11q12.3	NM_012200
Glycosyltransferases involved in synthesis of the repeating disaccharide region of CS chains					
Chondroitin synthase (GalNAcT-II, GlcAT-II)	ChSy-1	*CHSY1*		15q26.3	NM_014918
	ChSy-2	*CHSY2*	(*CSS3*)	5q23.3	NM_175856
	ChSy-3	*CHSY3*	(*CHPF2*) (*CSGLCA-T*)	7q36.1	NM_019015
Chondroitin polymerizing factor (GalNAcT-II, GlcAT-II)	ChPF	*CHPF*	(*CSS2*)	2q35	NM_024536
Chondroitin GalNAc transferase (GalNAcT-I, GalNAcT-II)	ChGn-1	*CSGALNACT1*		8p21.3	NM_018371
	ChGn-2	*CSGALNACT2*		10q11.21	NM_018590
Sulfotransferases and epimerases					
Chondroitin 4-*O*-sulfotransferase	C4ST-1	*C4ST1*	(*CHST11*)	12q	NM_018413
	C4ST-2	*C4ST2*	(*CHST12*)	7p22	NM_018641
	C4ST-3	*C4ST3*	(*CHST13*)	3q21.3	NM_152889
Dermatan 4-*O*-sulfotransferase	D4ST-1	*D4ST1*	(*CHST14*)	15q15.1	NM_130468
Chondroitin 6-*O*-sulfotransferase	C6ST-1	*C6ST1*	(*CHST3*)	10q22.1	NM_004273
Uronyl 2-*O*-sulfotransferase	UST	*UST*		6q25.1	NM_005715
GalNAc 4-sulfate 6-*O*-sulfotransferase	GalNAc4S-6ST	*GALNAC4S-6ST*	(*CHST15*) (*BRAG*)	10q26	NM_015892
Glucuronyl C-5 epimerase	DS-epi1	*DSE*	(*SART2*)	6q22	NM_013352
	DS-epi2	*DSEL*		18q22.1	NM_032160
Enzymes modifying the tetrasaccharide linkage region					
Xylose 2-*O*-kinase	XylK	*FAM20B*	(*gxk1*)	1p25	NM_014864
Galactose 6-*O*-sulfotransferase		*C6ST1*	(*CHST3*)	10q22.1	NM_004273
Exostosin-like glycosyltransferase 2 (GlcNAcT-I)	EXTL2	*EXTL2*		1p21	NM_001439
Uronyl 3-*O*-sulfotransferase		*HNK1ST*	(*CHST10*)	2q11.2	NM_004854
Chondroitin sulfate hydrolases					
endo-β-*N*-acetylgalactosaminidase	HYAL-1	*HYAL1*		3p21.3	NM_033159
	HYAL-4	*HYAL4*	(*CSHY*)	7q31.3	NM_012269
	SPAM1	*SPAM1*		7q31.3	NM_003117