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Review

Dermatan sulfate: Recent structural and activity data

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ABSTRACT

DS is a natural, complex polysaccharide which plays an important role in cell growth, differentiation, morphogenesis, cell migration, and bacterial/viral infections. Although its clinical use is limited, DS performs interesting biological activities, which should help in the development of DS-based therapeutics, such as drugs for parasitic and viral infections, regenerative medicine, anti-tumor drugs, or simply as a marker of significant disease progression. Biological activities of DS chains are likely to involve various growth factors, and specific DS chains having distinctive structures and properties are able to recruit factors and/or potentiate their activities, suggesting that minute amounts of functional DS chains can be utilized as active biomolecules. To this aim, new bioactive sources of DS may represent potential drugs for future research and development, as well as for a better understanding of the structure–function relationship, and would enable the production of this polysaccharide with its distinctive composition, structure and biological activities.

Contents

1. Introduction

Dermatan sulfate (DS) is a macromolecule belonging to a class of natural, structurally complex, sulfated, linear polymers named glycosaminoglycans (GAGs) (Jackson, Busch, & Cardin, 1991; Laremore, Zhang, Dordick, Liu, & Linhardt, 2009; Sasisekharan, Raman, & Prabhakar, 2006; Sugahara et al., 2003). There are many types of GAGs generally grouped into four groups: (1) hyaluronic acid or hyaluronan; (2) keratan sulfate; (3) chondroitin sulfate (CS)/DS, and (4) heparan sulfate/heparin. They are biosynthesized as polysaccharides of repeating disaccharides with an *N*-acetylhexosamine, *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine as one of the sugars. The alternating sugar is glucuronic acid (GlcA) with the exception of keratan sulfate which

Abbreviations: APC, activated protein C; BDNF, brain-derived growth factor; CS, chondroitin sulfate; DS, dermatan sulfate; FGF, fibroblast growth factor; GAGs, glycosaminoglycans; GDNF, glial-derived growth factor; GlcA, glucuronic acid; GalNAc, *N*-acetylgalactosamine; HARP, heparin affin regulatory peptide; HB-EGF, heparin binding EGF-like growth factor; HCII, heparin cofactor II; HGF-FS, hepatocyte growth factor/scatter factor; HPLC, high-performance liquid chromatography; KGF, keratinocyte growth factor; IdoA, iduronic acid; IFN-γ, interferon-γ; IL-8, interleukin-8; IP-10, γ-interferon inducible protein-10; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory peptides 1α; MK, midkine; PDGF, platelet-derived growth factor; PF4, platelet factor 4; PGs, proteoglycans; PTN/HB-GAM, pleiotrophin; SAX, strong anion exchange; SDS-1β, stromal cell-derived factor-1β; SLC, secondary lymphoid tissue chemokine; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

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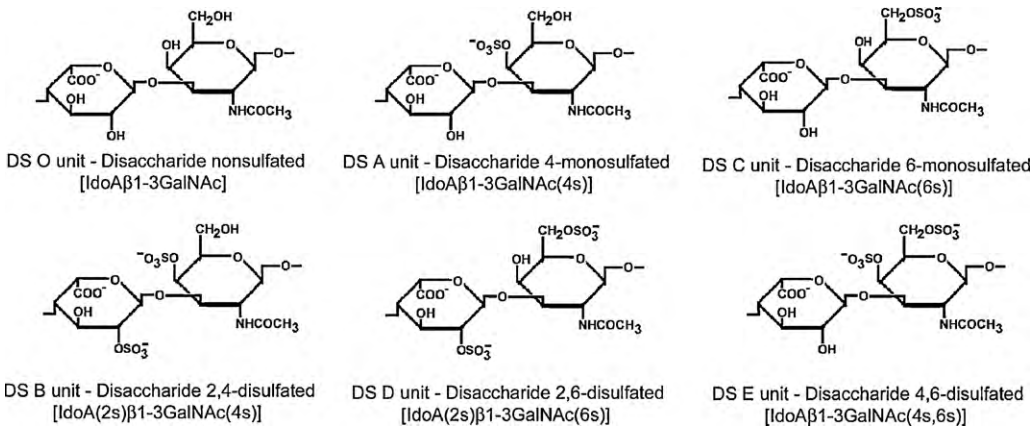

Fig. 1. Structures of typical disaccharides forming dermatan sulfate chains. GalNAc: N-acetyl-galactosamine. IdoA: L-iduronic acid.

contains galactose instead. Hyaluronic acid is not further modified, whereas the other groups are modified by: (1) the addition of *O*-sulfate groups on various hydroxyls (the three classes); (2) 5-epimerization of some GlcA residues to form iduronic acid (IdoA) residues (DS, heparan sulfate, heparin), and (3) removal of acetyl residues from some hexosamines replaced with *N*-sulfates (heparan sulfate and heparin) (Jackson et al., 1991). With the exception of hyaluronic acid, GAGs are often attached to a protein core resulting in macromolecules named proteoglycans (PGs) found inside cells, on their surfaces or in the extracellular matrices, playing important roles in a variety of diseases (Hardingham & Fosang, 1992). Furthermore, they are involved in the important role of cell–cell interaction, binding a variety of biologically important proteins and localizing these at the cell surface.

DS, produced by extraction and purification from different animal tissues, has several fundamental biological activities, as well as pharmacological properties, making it an experimental therapeutic agent to modulate a variety of these biological processes (Linhardt & Hileman, 1995; Mammen, Walenga, & Fareed, 1991; Trowbridge & Gallo, 2002; Volpi, 2006). Furthermore, newly identified sources of DS may represent potential drugs for future research and development and would enable the production of DS with a distinctive disaccharide composition, structure and biological activity.

2. DS structure

DS, also known as chondroitin sulfate B (CSB), is found in a wide variety of tissues in virtually all animals. It is a linear polysaccharide assembled as disaccharide units containing GalNAc or IdoA joined by β-1,4 or 1,3 linkages, respectively (Fig. 1). The presence of IdoA in DS distinguishes it from CS composed of GlcA. The *O*-sulfo groups are most commonly found on the 4-position of GalNAc residues and occasionally on the 6-position of *N*-acetylhexosamine and the 2-position of IdoA units. Monitoring of both the epimerization process and the sulfation reactions is not a random process but rather reflects a controlled enzymatic system for encrypting functional information into the polymer. For DS, the informational sequence is thus composed of three variables at the uronic acid position (IdoA or 2-*O*-sulfated IdoA) and four variables at the hexosamine (GalNAc or 4-*O*-sulfated GalNAc or 6-*O*-sulfated GalNAc or 4-*O*- and 6-*O*-disulfated GalNAc) (Fig. 1). DS sequence information is further influenced by variatale expression through the PG core protein in specific developmental and physiological conditions (Trowbridge & Gallo, 2002; Yamada & Sugahara, 2008). As a consequence, the variable total length of the polysaccharide chain, variable placement of IdoA, variable sulfation, and multiple alternatives for core proteins dictate the level of complexity of DS and DS-containing PGs. This variable DS chain length, disaccharide composition, and sulfation determine binding affinity and control functional interactions with potential protein partners.

3. DS biology and specific interactions

DS is covalently attached via an *O*-xylose linkage to serine residues of core protein to form DSPGs. The two best studied DSPGs are the small leucine-rich PGs called decorin and biglycan (Hardingham & Fosang, 1992; Trowbridge & Gallo, 2002). Both contain small protein cores and both are secreted matrix proteins, and they are composed of a few GAGs, 1 and 1–2 DS chains, respectively. However, depending on the cellular context, other PGs traditionally thought to contain exclusively other GAGs may occasionally contain DS (Trowbridge & Gallo, 2002). As a consequence, although several PGs have been shown to contain DS, it is not correct to conclude that these PGs are always DSPGs or that other PGs cannot exist with DS.

Accumulated evidence implies important biological functions of DS (and CS) chains in coagulation, cell proliferation, differentiation, migration, tissue morphogenesis, organogenesis, infection, and wound repair (Sugahara et al., 2003; Trowbridge & Gallo, 2002; Yamada & Sugahara, 2008). DS (and DSPG) interacts with a wide variety of molecules including (but not limited to) coagulation factors, matrix molecules, growth factors, protease inhibitors, cytokines, chemokines, adhesion molecules, and pathogen virulence factors via specific saccharide domains within the chains (Table 1). For many of these proteins, the precise binding site to DS or core protein has not been identified (Trowbridge & Gallo, 2002). However, because of their sulfation structure, most heparin-binding growth factors are presumed to interact with DS (and CS). Furthermore, DSPG–protein interactions are complicated by the fact that many interactions occur between the PG protein core and the binding protein rather than the polysaccharide. However, the particular functional domain structures, which are formed by combinations of the various disaccharide units (see Fig. 1), may participate in specific binding to bioactive molecules.

A phylogenetic tree of the distribution of sulfated GAGs in the animal kingdom is available in Medeiros et al. (2000). Along with heparan sulfate that is a ubiquitous component of all tissue-organized metazoan, CS/DS also have a widespread distribution. In invertebrates, CS/DS is found in sevaral taxa, namely porifera, cnidaria, rotifera, molluscs, crustacean, annelida, echinoderma, and insecta. However, as observed for vertebrate DS samples, the structure, molecular mass and anticoagulant activity vary according to the species analyzed. Invertebrate species are a rich source of CS/DS (and other sulfated GAGs) with novel structures (Cassaro

Table 1
Binding interactions of DS and DSPGs.

DS (CS)-binding proteins	GAG(s) bound	Biological effects related to the binding protein
ECM components		
Type II collagen	CS/DS type E	Primary osteoarthritis
Type V collagen	CS/DS type E	Tuberous sclerosis
Tenascin-X	DS	Collagen matrix stability
Opticin	DS	Binding to growth hormone
Coagulation factors		
Heparin cofactor II	DS	Anticoagulation
Thrombin	DS	Anticoagulation
APC	DS	Anticoagulation
Growth factors		
FGF-2, -10, -16, -18	DS/CS type E	Wound healing, angiogenesis-related diseases
KGF/FGF-7	DS/CS	Tissue repair, cancer
HB-EGF	DS/CS	Arteriosclerosis, liver cancer, wound healing
HGF/SF	DS	Cellular proliferation, organogenesis, liver diseases, breast cancer, myocardial infarction
MK	DS/CS/CS type E	Cancer
PTN/HB-GAM	DS/CS/CS type E	Cancer
PDGF	DS/CS	Arteriosclerosis, malignant tumor
VEGF	DS/oversulfated CS	Diabetic retinopathy, solid tumor, rheumatoid arthritis
TGF-β	DS	Growth regulation, cancer, liver fibrosis, scleroderma, etc.
GDNF	DS/CS type E	Extensive aganglionosis
BDNF	DS/CS type E	Autism
HARP	DS	Maturation of nerve cells, tumor growth
Chemokines		
IFN-γ	DS/CS	Antiviral and anti-tumor actions, receptor for INF-γ
IL-8	DS/CS	Arthritis, sepsis, acute, nephritis, etc.
MIP-1α	DS/CS	Myeloma bone disease
MCP-1	DS/CS	Atherosclerosis, multiple sclerosis
SLC	DS/oversulfated CS	Inflammation
IP-10	DS/oversulfated CS	Inflammation, cancer
SDF-1β	DS/oversulfated CS	Hematopoiesis
PF4	DS/oversulfated CS	Hematopoiesis, angiogenesis
Cell adhesion molecules		
CD-44	DS/oversulfated CS	Inflammation, malignant tumor
L-selectin	DS/oversulfated CS	Leukocyte adhesion deficiency
P-selectin	DS/oversulfated CS	Inflammation, thrombosis
RANTES	DS	Modulation of inflammatory response, allergy
Von Willebrand factor		Von Willebrand disease
Virus protein		
Glycoprotein C	DS/CS type E	*Herpes simplex* virus infection
Miscellaneous		
β-Amyloid peptide (Aβ)	DS	Alzheimer disease
α-Defensin	DS	Increased infectivity
Low-density lipoprotein	DS	Atherosclerotic plaque stabilization
LPL	DS	Hyperlipoproteinemia type I
EC-SOD	DS	Oxidant-mediated diseases
Borrelia burgdorferi adhesions	DS	Increased infectivity

et al., 1977; Hovingh & Linker, 1982; Nader et al., 1984; Volpi & Maccari, 2009). For example, a unique DS, different from that one extracted from *Scapharca inaequivalvis* (Volpi & Maccari, 2009) (see below), has been isolated from the body of the ascidian *Ascidia nigra* (Cassaro & Dietrich, 1977). The predominant structure was found composed of the disaccharide disulfated in position 2 of IdoA and 6 of GalNAc residues having no discernible anticoagulant activity and low ability to potentiate HCII. This result along with other studies implies that DS has a large structural variation depending on the origin.

4. Potential therapeutic application of DS

4.1. Anticoagulant and antithrombotic activities

A well-studied DS binding interaction occurs with heparin cofactor II (HCII). This serpin acts by inhibiting the procoagulative activity of thrombin and this effect is enhanced 1000-fold in the presence of DS having the power to form a stable ternary complex between the serpin and the protease (Liaw, Becker, Stafford, Fredenburgh, & Weitz, 2001). When intact DS is partially depolymerized, minimum fragments of octasaccharides and hexasaccharides bearing sulfate groups on C2 of IdoA and on the C4 of GalNAc are found to be required for the maintenance of HCII-mediated antithrombin activity (Maimone & Tollefsen, 1990). With a different DS source, two disaccharide species, IdoA-2S-GalNAc-4S and GlcA-GalNAc-4,6diS, were primarily identified as DS having the capacity to activate HCII (Linhardt, Al-Hakim, Liu, Kim, & Fareed, 1991). As a consequence, due to its specific interactions with this serpin, DS has anticoagulant powers. Furthermore, the discovery of these properties has focused attention on endogenous GAGs localized in the vasculature in addition to heparin found in mast cells.

While HCII-mediated antithrombin activity may play a role in the anticoagulant properties of DS, other less understood biochemical mechanisms are also involved in its antithrombotic capacity. In fact, the antithrombotic activity of DS can be correlated with an increased level of sulfation, an increased content of IdoA residues

(Linhardt et al., 1994) and a required minimum molecular weight. A high content of IdoA also results in an increased flexibility of DS, enhancing its ability to self-associate and possibly to interact with plasma proteins. However, although the anticoagulant activity of DS is significantly less than that of heparin, its venous antithrombotic activity appears to be significantly higher. Thus, the hemorrhagic properties of DS are greatly reduced when compared to heparin. This has made DS an interesting target in the development of new therapeutic agents for the prevention of thrombosis.

DS may also influence coagulation by enhancing the effects of activated protein C (APC) (Table 1) (Trowbridge & Gallo, 2002), an endogenous inhibitor of the clotting cascade. At a physiologically relevant concentration, DS is able to enhance APC activity more than other GAGs tested. Moreover, DS fractions with the highest charge density enhance APC activity more than unfractionated DS. These findings further suggest that DS oligosaccharide size and sulfation determine binding affinity and lead to different physiological consequences.

4.2. DS for infections

DS (and related PGs) have been associated with the capacity to modify resistance to infectious disease. In fact, decorin has been identified as a binding target for *Borrelia burgdorferi* (Table 1), the etiologic agent in Lyme disease, and decorin-deficient mice show an increased resistance to this infection (Brown et al., 2001).

DS also appears to be involved in infection through mechanisms independent of microbial adherence. Pathogenesis of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus pyogenes* involves release of proteinases able to degrade DS-containing PGs (Schmidtchen, Frick, & Björck, 2001). The free DS binds to and inactivates the neutrophil-derived cationic antimicrobial peptide α-defensin, a small cationic peptide essential for resistance to infection. As a consequence, inactivation of this peptide through binding to DS would be an effective strategy to increase pathogenesis.

4.3. DS in wound repair

As is well known, DS is a major constituent of the skin and, considering its location and activity in coagulation and cell growth, and its capacity to interact with several proteins, the function of DS in wound repair is an active area of investigation.

Following skin injury, the synthesis of several PGs is rapidly induced in a cell-specific pattern, such as syndecan-1 on endothelial cells in the wound and syndecan-4 and decorin throughout the dermis (Trowbridge & Gallo, 2002). Furthermore, evaluation of the soluble PG from wounds has shown that the major GAG present in this environment is DS produced in large amounts (Penc et al., 1998). Additionally, several functional studies of the wound repair response have also directly implicated DS in a few essential critical steps for the repair of injury. For example, DS derived from wounds activates endothelial leukocyte adhesion through stimulation of ICAM-1, and wound DSPGs are a potent promoter of the activity of FGF-2 (see Table 1), a growth factor essential to several aspects of the repair response (Trowbridge & Gallo, 2002). Finally, a close relation between innate immune defense of wounds and PG synthesis has been demonstrated (Trowbridge & Gallo, 2002).

4.4. DS in the central nervous system (CNS)

The CNS is a rich and complex source of PGs composed of DS/CS chains. Particularly high levels of CS/DSPGs have been observed during brain development having the capacity to finely tune expression patterns suggesting important functions at all stages of development (Rauch & Kappler, 2006; Sugahara & Mikami, 2007; Yamada & Sugahara, 2008). CS/DSPG levels are also elevated under various pathological conditions both in the CNS and in the periphery mainly associated with wound repair processes. In Alzheimer's disease, DS/CS localize to the characteristic lesions (Rauch & Kappler, 2006) and have been shown to be potent enhancers of amyloid fibrillogenesis (Sugahara & Mikami, 2007).

CS/DS promote the outgrowth of neurites in embryonic rat brain neurons, and CS E and oversulfated DS also promote the outgrowth of neurites in hippocampal neurons. Furthermore, oversulfated CS disaccharides as well as human brain-derived appican PG have been implicated in the support of neurite outgrowth. On the basis of these findings, CS/DSPGs have a very complicated role in promoting CNS repair or inhibiting neurite outgrowth depending on neuronal cell types and the fine structure of the polysaccharide chains by mainly interacting with growth factors and neurotropic factors as well as structural components of cells or ECM (Table 1). In fact, oversulfated DS (and CS) have been isolated from developing brains which are able to interact with a variety of different cytokines with high affinity and may modulate the activity of these molecules (Trowbridge & Gallo, 2002).

The sulfation pattern of DS/CS chains directly influences the binding to bioactive proteins and regulates CNS repair. In fact, analysis of primary neurons grown on DS/CS substrata revealed a good correlation between their characteristic sulfation patterns and neuritogenic properties (Castillo, Lukito, Wight, & Snow, 1999). These findings suggest that neuritogenic activities of the oversulfated DS/CS chains are dependent on the sulfation patterns rather than their simple charge densities. Thus, the sulfation profile of these chains is a crucial factor regulating the proliferation of neural stem cells in the CNS through interaction with growth factors. As a consequence, DS/CS poly- and/or oligosaccharides containing such active sequences may have great therapeutic potential. To support this view, recent studies have demonstrated that the degradation products of DS/CSPGs were highly effective in promoting axonal growth, enhancing microglial activation, and controlling T-cell functions. Furthermore, sulfated monosaccharides and disaccharides from DS/CS have been used to compete with the intact CS and other GAGs for binding β-amyloid, indicating that they may be effective inhibitors of the GAG-induced amyloid formation (Salinero, Moreno-Flores, & Wandosell, 2000). Additionally, the interesting effects observed after the alteration of the CS/DSPG function with a specific polysaccharide-degrading enzyme in models of CNS damage (Rolls et al., 2004) have also stimulated pharmacological interest in these molecules. On the basis of these findings, DS/CS polysaccharides from various sources and their degradation products and related oligosaccharides (and specific degrading enzymes) are expected to have pharmacological applications in the future.

4.5. DS as a potential anti-tumor drug

A clear correlation between the accumulation of CS/DSPGs and cancer progression has been demonstrated, although the biological role of these macromolecules in the progression has not been clearly determined. Tumor stroma and tumor fibrotic tissues contain abnormally high concentrations of CSPGs, especially in the form of PGs versican and decorin (Wegrowski & Maquart, 2004). The DS chains on these core proteins in colon adenocarcinoma are replaced by CS chains, the molecular size of GAG chains is decreased, and the sulfation pattern is altered (Theocharis, 2002). Disaccharides containing 4-*O*-sulfated GalNAc (mainly associated with DS), which predominate in normal tissues, become minor constituents in malignant tumors, but amounts of nonsulfated disaccharide units and disaccharides containing 6-*O*-sulfated GalNAc (mainly present in CS chains) are elevated. Furthermore, in invading melanoma, the accumulation of CS is associated with the appearance of a larger quantity of disaccharide E (see Table 1). These

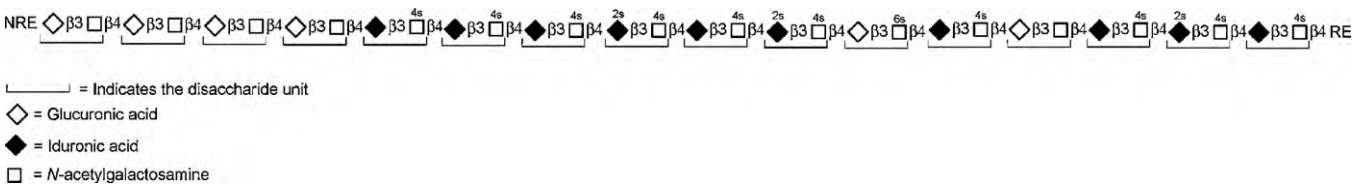

Fig. 2. Structure of *S. inaequivalvis* DS. A single typical chain is shown with some sequence heterogeneity. The non-reducing end (NRE) and reducing end (RE) of the polysaccharide chain are indicated.

results strongly suggest that DS (and CS) chains play some role in tumor progression.

As previously reported, DS(CS)PGs have been shown to interact with many different soluble and membrane-associated molecules as well as with a variety of growth factors and cytokines (Table 1). Most of the DS(CS)-binding growth factors are involved in cancer or tumor formation (Trowbridge & Gallo, 2002). As a consequence, fine structural alterations of DS chains and properties in a tumor would modify the activities of effective protein factors and influence the biology of cancer cells, contributing to cell growth and migration.

Although the specific sequences responsible for the binding of DS to several molecules have not been determined, such sequences are molecular targets for cancer diagnosis and therapeutics. In fact, detection of DS oligomer sequences will be instrumental for the identification of cancer malignancy and for any possible related therapy. To support this approach, an antibody, i.e. WF6, raised against shark aggrecan, specifically recognizes an epitope in CS chains. This epitope was investigated using an oligosaccharide microarray, and two binding CS octasaccharide sequences were identified (Pothacharoen et al., 2007; Smetsers et al., 2004). Furthermore, the serum concentration of the WF6 epitope was found to be highly increased in ovarian cancer (Pothacharoen et al., 2006), thus providing a useful biomarker for cancers and other disorders of the ovary. Similarly, CS/DS oligosaccharides having specific properties to bind effective molecules on tumor progression may inhibit the metastasis and proliferation of the tumor cells by neutralizing growth factors and cell adhesion molecules. The detection and structural identification of such CS/DS saccharides will make an important contribution to cancer therapy. Finally, a fucosylated CS isolated from sea cucumber was proved to inhibit the attachment of carcinoma cells to immobilized P- and L-selectins as well as the lung colonization by adenocarcinoma cells in an experimental metastasis mouse model (Borsig et al., 2007), suggesting that invertebrate polysaccharides may be a potential alternative to mammalian GAGs for blocking metastasis and inflammatory reactions without undesirable (in particular for heparin) side effects.

5. New recent DS structural characterization

According to previously reported evidence, an emerging paradigm in the modulation of biological functions is the specific interaction between DS (and GAGs) and numerous proteins at the cellular and extracellular level. These interactions modulate protein functions and thus regulate fundamental biological processes. However, given the complexity and the challenges involved in decoding the structure–function relationship of GAGs in their physiological context, it is important to adopt an integrated glycomic approach. Despite these challenges, there has been remarkable progress in the development of tools for the isolation and structural characterization of GAGs (and DS). One of the major areas of progress on this front is the development of sensitive analytical techniques to accurately define structure and properties, such as mass and composition, of DS oligosaccharides.

By applying multianalytical approaches, a unique DS was purified from marine clam *S. inaequivalvis* (Volpi & Maccari, 2009) and characterized for structure and properties. From a quantitative point of view, this DS was found to be composed of approx. 51% of disaccharide monosulfated in position 4 of the GalNAc, 21% of the disulfated disaccharide B, typical of DS, disulfated in position 2 of the HexA and 4 of the hexosamine, and trace amounts of the other two disulfated disaccharides, of the trisulfated disaccharide, and of the monosulfated disaccharide in position 6 of the GalNAc. Furthermore, significant amounts, 2.3%, of the monosulfated disaccharide in position 2 of the HexA were found, and approx. 17% of the nonsulfated disaccharide was observed. The charge density, evaluated as sulfate groups per disaccharide unit, was calculated to be approx. 1.1 due to the presence in particular of the disulfated disaccharide B. Furthermore, *S. inaequivalvis* GAG was mainly composed of IdoA with 45% of the total disaccharides containing this uronic acid found to be monosulfated in position 4 of the GalNAc and ~50% belonging to the disulfated disaccharide B typical of DS. On the contrary, GlcA was found to be mainly associated with the nonsulfated disaccharide and the disaccharide monosulfated in position 6 of the GalNAc. Finally, ~20% of the total disaccharides, mainly associated with the nonsulfated disaccharide, were found to be located at the non-reducing end of the DS chains. The molecular mass parameters of *S. inaequivalvis* DS were also determined by HPSEC, showing a mean value of 27,140, with a polysaccharide chain composed of about 55–58 disaccharide units, considering a mean mass value of 477 for disaccharide monosulfated (and the presence of rather similar percentages on nonsulfated and disulfated disaccharides).

On consideration of the previously reported structural data, IdoA was mainly found in disaccharides monosulfated in position 4 of GalNAc and disulfated in position 2 of the IdoA and 4 of GalNAc

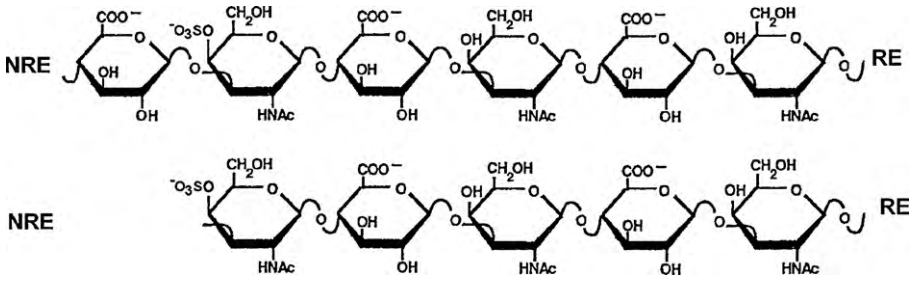

Fig. 3. Structure of the non-reducing end (NRE) of *S. inaequivalvis* DS and the saturated monosaccharide produced by the action of chondroitin ABC lyase and detected by HPLC–ESI-MS. RE: reducing end. Modified and reprinted with permission from Volpi and Maccari (2009).

(disaccharide B typical of DS, Di2,4dis) (see Fig. 2). On the contrary, GlcA was found to be mainly associated with the nonsulfated disaccharide (Di0s) with the remainder forming low percentages of monosulfated disaccharides in position 4 or 6 of GalNAc (Di6s or Di4s). Treatment with the exolytic lyase specific for CS produced a large percentage of the nonsulfated disaccharide and it is possible to suppose that these domains, rich in nonsulfated disaccharide and GlcA, are located close to the non-reducing end of the *S. inaequivalvis* DS (Fig. 3). Furthermore, the presence of saturated hexasaccharide derived from the non-reducing terminus of the intact DS ending with a HexA residue was also observed. Finally, according to Fig. 3, domains rich in nonsulfated disaccharides formed by GlcA units having a monosulfated disaccharide (possibly sulfated in position 4 of GalNac) or a monosulfated monosaccharide (possibly GalNac4SO_4) at the non-reducing end were assigned (Volpi & Maccari, 2009).

HCII-mediated thrombin inhibition *in vitro* has been demonstrated using mammalian DS isolated from several animal sources (Halldórsdóttir, Zhang, & Tollefsen, 2006; Maimone & Tollefsen, 1990; Osborne, Daniel, Desilva, & Seymour, 2008). The structural characteristics of DS required for high affinity binding to HCII are repeating disulfated disaccharide units of either IdoA2SO_4–GalNAc4SO_4 (Maimone & Tollefsen, 1990) or GlcA/IdoA–GalNAc4,6SO_4 (Halldórsdóttir et al., 2006). *S. inaequivalvis* DS possesses a high HCII activity quite similar to that of several DS samples purified from mammalian tissues (Mascellani et al., 1993) probably due to the presence of a high content of the disulfated disaccharide IdoA2SO_4–GalNAc4SO_4. We should also consider that the variations in the relative percentage of the IdoA2SO_4–GalNAc4SO_4 disaccharide within the different tissue-derived DS do not appear to correlate directly with the HCII-mediated thrombin inhibition (Osborne et al., 2008). Finally, other studies have also indicated that the IdoA/GlcA ratio (Casu, Guerrini, & Torri, 2004) may also contribute significantly to the capacity of DS to inhibit HCII-mediated thrombin activity, as a high content of IdoA also results in an increased flexibility of DS, enhancing its ability to self-associate and possibly to interact with plasma proteins.

6. Conclusions

GAGs constitute a considerable fraction of the glycoconjugates located on cellular membranes and in the extracellular matrix of virtually all mammalian tissues but also other Vertebrates and many Invertebrates. Although heparin/heparan sulfate tend to be emphasized as the most biologically active GAGs, DS is particularly interesting for further studies and applications because it is expressed in many tissues and it is the predominant glycan present in skin. In fact, DS and DSPGs have been implicated in cardiovascular disease, tumorigenesis, infection, wound repair, and fibrosis. Furthermore, growing evidence suggests that this GAG is an important cofactor in a variety of cell behaviours, in the development of the CNS, and it also acts as a receptor for various pathogens. Biological activities of DS chains possibly involve various growth factors and chemokines, and these functions are closely associated with the sulfation patterns and characteristics of the polysaccharide chains.

Due to its above mentioned intriguing biological activities, DS may help in the development of targeted therapeutics, in particular for parasitic and viral infections, regenerative medicine based on the idea that minute amounts of functional poly(oligo)saccharides can be utilized for tissue regeneration, and anti-tumor drugs, in particular in tumor cell proliferation and metastasis.

As reported above, DS (and other GAGs) functions and possible therapeutic applications are closely related to its specific structure and properties as well as distinctive oligosaccharide sequences inside the polymer chains. The detection and identification of such DS sequences would be an important contribution to therapy. GAGs (and DS) research is currently at a stage where there is a need for an integrated system or glycomics approach to define their structure–function relationship. With this aim, new bioactive sources of DS, such as Invertebrates, may represent potential drugs for future research and development and would enable the production of this polysaccharide having a distinctive composition, structure and biological activities. In fact, *S. inaequivalvis* DS displays a peculiar structure, in particular due to the presence of significant amounts of nonsulfated disaccharide, to the elevated percentage of the disaccharide B and to the presence of not previously reported low amounts of the disaccharide monosulfated in position 2 of the HexA (Di2s). Furthermore, this GAG was found to have a high charge density value of 1.10 increasing to 1.54 in the case of the carbohydrate backbone solely composed of IdoA residues due to the very high content of the disaccharide B. Additionally, *S. inaequivalvis* DS was also found to contain approx. 25% of disaccharides having GlcA residues mainly associated with the nonsulfated disaccharide (Di0s) and located close to the non-reducing end. Finally, a high HCII activity rather similar to that of several DS samples purified from mammalian tissues was demonstrated for DS from *S. inaequivalvis*. All these particular structural characteristics make this polysaccharide particularly interesting for further studies and applications and for a better understanding of their structure–function relationship.

References

Borsig, L., Wang, L., Cavalcante, M. C., Cardilo-Reis, L., Ferreira, P. L., Mourão, P. A., et al. (2007). Selectin blocking activity of a fucosylated chondroitin sulfate glycosaminoglycan from sea cucumber. Effect on tumor metastasis and neutrophil recruitment. *Journal of Biological Chemistry*, *282*, 14984–14991.

Brown, E. L., Wooten, R. M., Johnson, B. J., Iozzo, R. V., Smith, A., Dolan, M. C., et al. (2001). Resistance to Lyme disease in decorin-deficient mice. *Journal of Clinical Investigation*, *107*, 845–852.

Cassaro, C. M., & Dietrich, C. P. (1977). Distribution of sulfated mucopolysaccharides in invertebrates. *Journal of Biological Chemistry*, *252*, 2254–2261.

Castillo, G. M., Lukito, W., Wight, T. N., & Snow, A. D. (1999). The sulfate moieties of glycosaminoglycans are critical for the enhancement of beta-amyloid protein fibril formation. *Journal of Neurochemistry*, *72*, 1681–1687.

Casu, B., Guerrini, M., & Torri, G. (2004). Structural and conformational aspects of the anticoagulant and anti-thrombotic activity of heparin and dermatan sulfate. *Current Pharmaceutical Design*, *10*, 939–949.

Halldórsdóttir, A. M., Zhang, L., & Tollefsen, D. M. (2006). N-acetylgalactosamine 4,6-O-sulfate residues mediate binding and activation of heparin cofactor II by porcine mucosal dermatan sulfate. *Glycobiology*, *16*, 693–701.

Hardingham, T. E., & Fosang, A. J. (1992). Proteoglycans: Many forms and many functions. *FASEB Journal*, *6*, 861–870.

Hovingh, P., & Linker, A. (1982). An unusual heparan sulfate isolated from lobsters (*Homarus americanus*). *Journal of Biological Chemistry*, *257*, 9840–9844.

Jackson, R. J., Busch, S. J., & Cardin, A. D. (1991). Glycosaminoglycans: Molecular properties, protein interactions, and role in physiological processes. *Physiological Reviews*, *71*, 481–539.

Laremore, T. N., Zhang, F., Dordick, J. S., Liu, J., & Linhardt, R. J. (2009). Recent progress and applications in glycosaminoglycan and heparin research. *Current Opinion in Chemical Biology*, *13*, 633–640.

Liaw, P. C., Becker, D. L., Stafford, A. R., Fredenburgh, J. C., & Weitz, J. I. (2001). Molecular basis for the susceptibility of fibrin-bound thrombin to inactivation by heparin cofactor II in the presence of dermatan sulfate but not heparin. *Journal of Biological Chemistry*, *276*, 20959–20965.

Linhardt, R. J., Al-Hakim, A., Liu, S. Y., Kim, Y. S., & Fareed, J. (1991). Molecular profile and mapping of dermatan sulfates from different origins. *Seminars in Thrombosis and Hemostasis*, *17*(Suppl 1), 15–22.

Linhardt, R. J., Desai, U. R., Liu, J., Pervin, A., Hoppensteadt, D., & Fareed, J. (1994). Low molecular weight dermatan sulfate as an antithrombotic agent. Structure–activity relationship studies. *Biochemical Pharmacology*, *47*, 1241–1252.

Linhardt, R. J., & Hileman, R. E. (1995). Dermatan sulfate as a potential therapeutic agent. *General Pharmacology*, *26*, 443–451.

Maimone, M. M., & Tollefsen, D. M. (1990). Structure of a dermatan sulfate hexasaccharide that binds to heparin cofactor II with high affinity. *Journal of Biological Chemistry*, *265*, 18263–18271.

Mammen, E. F., Walenga, J. M., & Fareed, J. (Eds.). (1991). *Seminars in thrombosis and hemostasis*. New York/Stuttgart: Theme Medical Publishers.

Mascellani, G., Liverani, L., Bianchini, P., Parma, B., Torri, G., Bisio, A., et al. (1993). Structure and contribution to the heparin cofactor II-mediated inhibition of thrombin of naturally oversulphated sequences of dermatan sulphate. *Biochemical Journal*, *296*, 639–648.

Medeiros, G. F., Mendes, A., Castro, R. A., Bau, E. C., Nader, H. B., & Dietrich, C. P. (2000). Distribution of sulfated glycosaminoglycans in the animal kingdom: Widespread occurrence of heparin-like compounds in invertebrates. *Biochimica et Biophysica Acta*, *1475*, 287–294.

Nader, H. B., Ferreira, T. M. P. C., Paiva, J. F., Medeiros, M. G. L., Jeronimo, S. M. B., Paiva, V. M. P., et al. (1984). Isolation and structural studies of heparan sulfates and chondroitin sulfates from three species of molluscs. *Journal of Biological Chemistry*, *259*, 1431–1435.

Osborne, S. A., Daniel, R. A., Desilva, K., & Seymour, R. B. (2008). Antithrombin activity and disaccharide composition of dermatan sulfate from different bovine tissues. *Glycobiology*, *18*, 225–234.

Penc, S. F., Pomahac, B., Winkler, T., Dorschner, R. A., Eriksson, E., Herndon, M., et al. (1998). Dermatan sulfate released after injury is a potent promoter of fibroblast growth factor-2 function. *Journal of Biological Chemistry*, *273*, 28116–28121.

Pothacharoen, P., Siriaunkgul, S., Ong-Chai, S., Supabandhu, J., Kumja, P., Wanaphirak, C., et al. (2006). Raised serum chondroitin sulfate epitope level in ovarian epithelial cancer. *Journal of Biochemistry*, *140*, 517–524.

Pothacharoen, P., Kalayanamitra, K., Deepa, S. S., Fukui, S., Hattori, T., Fukushima, N., et al. (2007). Two related but distinct chondroitin sulfate mimetope octasaccharide sequences recognized by monoclonal antibody WF6. *Journal of Biological Chemistry*, *282*, 35232–35246.

Rauch, U., & Kappler, J. (2006). Chondroitin/dermatan sulfates in the central nervous system: Their structures and functions in health and disease. *Advances in Pharmacology*, *53*, 337–356.

Rolls, A., Avidan, H., Cahalon, L., Schori, H., Bakalash, S., Litvak, V., et al. (2004). A disaccharide derived from chondroitin sulphate proteoglycan promotes central nervous system repair in rats and mice. *European Journal of Neuroscience*, *20*, 1973–1983.

Salinero, O., Moreno-Flores, M. T., & Wandosell, F. (2000). Increasing neurite outgrowth capacity of beta-amyloid precursor protein proteoglycan in Alzheimer's disease. *Journal of Neuroscience Research*, *60*, 87–97.

Sasisekharan, R., Raman, R., & Prabhakar, V. (2006). Glycomics approach to structure–function relationships of glycosaminoglycans. *Annual Review of Biomedical Engineering*, *8*, 181–231.

Schmidtchen, A., Frick, I. M., & Björck, L. (2001). Dermatan sulphate is released by proteinases of common pathogenic bacteria and inactivates antibacterial α-defensin. *Molecular Microbiology*, *39*, 708–713.

Smetsers, T. F., van de Westerlo, E. M., ten Dam, G. B., Overes, I. M., Schalkwijk, J., van Muijen, G. N., et al. (2004). Human single-chain antibodies reactive with native chondroitin sulfate detect chondroitin sulfate alterations in melanoma and psoriasis. *Journal of Investigative Dermatology*, *122*, 707–716.

Sugahara, K., Mikami, T., Uyama, T., Mizuguchi, S., Nomura, K., & Kitagawa, H. (2003). Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. *Current Opinion in Structural Biology*, *13*, 612–620.

Sugahara, K., & Mikami, T. (2007). Chondroitin/dermatan sulfate in the central nervous system. *Current Opinion in Structural Biology*, *17*, 536–545.

Theocharis, A. D. (2002). Human colon adenocarcinoma is associated with specific post-translational modifications of versican and decorin. *Biochimica et Biophysica Acta*, *1588*, 165–167.

Trowbridge, J. M., & Gallo, R. L. (2002). Dermatan sulfate: New functions from an old glycosaminoglycan. *Glycobiology*, *12*, 117R–125R.

Volpi, N. (Ed.), (2006). *Advances in pharmacology*. North America/Europe/Middle East/Asia/Australia/Africa/Latin America: Elsevier Science.

Volpi, N., & Maccari, F. (2009). Structural characterization and antithrombin activity of dermatan sulfate purified from marine clam *Scapharca inaequivalvis*. *Glycobiology*, *19*, 356–367.

Wegrowski, Y., & Maquart, F. X. (2004). Involvement of stromal proteoglycans in tumour progression. *Critical Reviews in Oncology Hematology*, *49*, 259–268.

Yamada, S., & Sugahara, K. (2008). Potential therapeutic application of chondroitin sulfate/dermatan sulfate. *Current Drug Discovery Technologies*, *5*, 289–301.