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REVIEW

Biological function of unique sulfated glycosaminoglycans in primitive chordates

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Abstract Glycosaminoglycans with unique sulfation patterns have been identified in different species of ascidians (sea squirts), a group of marine invertebrates of the Phylum Chordata, sub-phylum Tunicata (or Urochordata). Oversulfated dermatan sulfate composed of [4-α-L-IdoA-(2-O-SO_3)$^{-1}$ → 3-β-D-GalNAc(4-OSO_3)$^{-1}$]$_n$ repeating disaccharide units is found in the extracellular matrix of several organs, where it seems to interact with collagen fibers. This dermatan sulfate co-localizes with a decorin-like protein, as indicated by immunohistochemical analysis. Low sulfated heparin/heparan sulfate-like glycans composed mainly of [4-α-L-IdoA-(2-OSO_3)$^{-1}$ → 4-α-D-GlcN(SO_3)$^{-1}$ (6-O-SO_3)$^{-1}$]$_n$ and [4-α-L-IdoA-(2-O-SO_3)$^{-1}$ → 4-α-D-GlcN(SO_3)$^{-1}$]$_n$ have also been described in ascidians. These heparin-like glycans occur in intracellular granules of oocyte assessory cells, named test cells, in circulating basophil-like cells in the hemolymph, and at the basement membrane of different ascidian organs. In this review, we present an overview of the structure, distribution, extracellular and intracellular localization of the sulfated glycosaminoglycans in different species and tissues of ascidians. Considering the phylogenetic position of the subphylum Tunicata in the phylum Chordata, a careful analysis of these data can reveal important information about how these glycans evolved from invertebrate to vertebrate animals.

Keywords Glycosaminoglycans · Dermatan sulfate · Heparin/heparan sulfate · Invertebrates · Primitive chordates · Ascidians

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Abbreviations

ECM	Extracellular matrix
GAGs	Glycosaminoglycans
CS	Chondroitin sulfate
DS	Dermatan sulfate
Hep	Heparin
HS	Heparan sulfate
KS	Keratan sulfate
GlcN	D-glucosamine
Gal	Galactose
GalN	D-galactosamine
GlcA	D-glucuronic acid
IdoA	L-iduronic acid
hplc	High performance liquid chromatography

Introduction

Most of the glycosaminoglycans (GAGs) are glycosylation products of specific proteins known as proteoglycans [1].

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They consist of a linear sequence of repeating disaccharide units formed by an amino sugar (*N*-acetylglucosamine, *N*-acetylgalactosamine) and an uronic acid (glucuronic acid or iduronic acid) or galactose. The disaccharide units are usually modified by the addition of sulfate groups to the *N*-acetylhexosamines, hexuronic acids and galactose [2]. For example, vertebrate GAGs such as chondroitin sulfate (CS) (GalNAc β → 4 GlcA β1 → 3) and its glucuronic acid C5-epimer, dermatan sulfate (DS) (GalNAc β → 4 GlcA β 1 → 3 / GalNAc β1 → 4 IdoA α1 → 3), can be O-sulfated at carbon 4 and/or 6 of the *N*-acetylgalactosamine and at carbon 2 of the main hexuronic acid [2]. In vertebrate heparin (Hep)/heparan sulfate (HS) (GlcNAc α1 → 4 GlcA α1 → 4 / GlcNAc α1 → 4 IdoA α1 → 4), *O*-sulfation occurs mostly at the carbon 6 of the *N*-acetylglucosamine, where 3-*O*-sulfation can also be present forming the antithrombin-binding site, and at carbon 2 of the iduronic acid. Additionally, the glucosamine can also be N-sulfated [3].

Vertebrate keratan sulfate (KS) is a linear polymer of [GlcNAc β1 → 3 Gal β1 → 4]-repeating disaccharide units. It can contain a C6-O-sulfation in the *N*-acetylglucosamine and/or the galactose units [4]. Hyaluronic acid (HA) is the only non-sulfated vertebrate GAG that does not result from a glycosylation process. It is a linear polymer formed by [GlcNAc β1 → 4 GlcA β1 → 3]-repeating disaccharide units [5].

Specific repeating disaccharide units and their various oligosaccharide domains regulate the binding of several proteins/glycoproteins, growth factors/growth factor receptors, and therefore are considered key players in the biological activity of GAGs [6–9]. Their implication in the activity of various matrix metalloproteinases has also been recently described [10].

Sulfated glycans similar to those found in vertebrate GAGs have been reported in major Phyla of the animal kingdom, occurring in Arthropoda, Mollusca, Annelida, Urochordata, Echinodermata, Coelenterata, and Porifera [11, 12]. Urochordate animals of the Class Ascidiacea, or ascidians are marine invertebrates and the closest relatives of vertebrates [13]. As expected from the presence of GAG-like polymers in these invertebrates, several genes similar to the chondroitin/dermatan sulfate-specific sulfotransferases have been identified in the genome of the ascidian *Ciona intestinalis*. They were found to be differently expressed resulting in a tissue-specific expression pattern [14]. These genes are expressed in the developing notochord and in the brain of *Ciona intestinalis* embryos where they are mainly involved in the morphogenetic movement of notochord cells.

In this review, we present an overview of the structure, distribution, extracellular and intracellular localization of the sulfated GAGs in different species and tissues of ascidians. Considering the phylogenetic position of the subphylum Tunicata in the phylum Chordata, a careful analysis of these data can reveal important information about how these glycans evolved from invertebrate to vertebrate animals.

Ascidian dermatan sulfate-like glycans

Mammalian-derived DSs are mainly composed of repeating disaccharide units (α-L-IdoA4 → β-D-GalNAc3-)$_n$ that carry esterified by sulfation groups in positions that vary among DSs from different cell types and tissues, as well as their physiological roles [2]. However, the common sulfation patterns involve mainly sulfation at C4 of GalNAc, which in several cases may be as high as 75–95 % of the total disaccharides, a small to considerable portion of C6 sulfated GalNAc (15–20 %) and a low percentage of C2 mono-*O*-sulfated IdoA structures (3–6 %) [15].

Different from mammalian DSs, in ascidians, different sulfation patterns have been identified. (Fig. 1 and Table 1). These sulfation patterns may even vary between the ascidian species. It is noticeable that all ascidians derived DSs contain much higher esterified sulfates than the mammalian ones. From a historic perspective, the occurrence of CS/DS, the occurrence of chondroitin/dermatan sulfate in ascidians was first reported in 1977 by Cassaro and Dietrich [11]. However, the disaccharide analysis of the GAGs of this urochordate was only described 17 years later, in 1994. A major di-sulfated disaccharide unit, representing about 60 % of the total, was identified by paper chromatography of the products formed after complete chondroitinase ABC degradation of the DS isolated from the stolidobranchia ascidian *Styela plicata* [16]. Further high performance liquid chromatograpy (HPLC) analysis of the disaccharides and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) of native purified dermatan sulfates from *S. plicata* and *Halocynthia pyriformis* (another stolidobranchia ascidian) identified the di-sulfated disaccharide units as [4-α-L-IdoA-(2-O-SO_3)$^{-1}$ → 3-β-D-GalNAc(4-OSO_3)$^{-1}$]$_n$ [17]. These disaccharide units are considered a distinguishing feature of DSs obtained from ascidians of the Order Stolidobranchia. In contrast, the ascidians *Phallusia nigra* [18] and *Ciona intestinalis* of the Order Phlebobranchia possess DSs predominantly consisted of [4-α-L-IdoA-(2-O-SO_3)$^{-1}$ → 3-β-D-GalNAc(6-O-SO_3)$^{-1}$]$_n$ di-*O*-sulfated disaccharides, which is rarely found in more primitive ascidians of the Order Stolidobranchia. Additionally, fast-atom bombardment mass spectrometry analysis confirmed sulfation at carbon 2 of the iduronic acid and at carbon 6 of the *N*-acetylgalactosamine [19] in the DS isolated from *P. nigra*. Although the occurrence of 6-*O*-sulfation at the *N*-acetylgalactosamine in DSs of Phlebobranchia ascidians is unusual, DS specific 6-*O*-sulfotransferase gene was identified in *C. intestinalis* [14]. The main types of DSs found in ascidians are shown in Table 1.

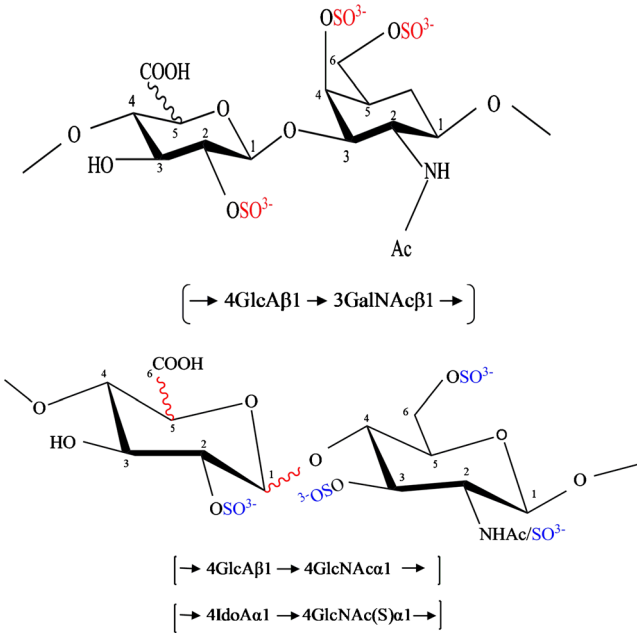

Fig. 1 Disaccharide repeating units of chondroitin/dermatan sulfates (upper panel) and heparin/heparan sulfates (bottom panel). All possible sulfation position identified in various species are shown. Their combination may form non,- mono-, di- and tri-sulfated disaccharide units. *N*-sulfonylation of GlcN is also present in heparin structures. DSs derived from ascidian composed of the disaccharide unit β(1 → 4)L-IdoAα(1 → 3)D-GalNAcβ(1 → 4). In DS derived from ascidian *S. plicata and H. pyriformis*, sulfate groups are mainly identified in positions at C2 of IdoA and C4 of GalNAc, whereas in *P. nigra* in positions C2 and C6, respectively. The percentage of sulfates in DSs of ascidians at C3 of IdoA accounts for a portion below 5 %. Although the sulfation at C4 of GalNAc in mammalian DS comprise the major sulfation pattern as with the two of the three ascidian-derived DSs, the major differences in sulfation between mammalian and ascidian-derived DSs is in the high number of esterified sulfates at C2 in ascidian. Mammalian-derived Hep/HS is composed of alternating repeating disaccharide units of a uronic acid (either β-D-GlcA or α-L-IdoA) linked to α-D-GlcNAc/GlcNHS. Monosaccharides within the unit as well as disaccharide units bind each other with a 1 → 4 glycosidic linkage. Units are mainly sulfated at C6 of GlcNAc, whereas various portions at C3 of GlcNAc and *N*-sulfonylation are also present depending on the cell types, tissues and pathophysiological roles of Hep/HS. Units may also sulfated mainly at C2 of IdoA and rarely at C3

Ascidian heparin-like glycans

Mammalian-derived HeP/HS is composed of alternating repeating disaccharide building blocks of a hexuronic acid (either β-D-GlcA or α-L-IdoA) linked to *N*-acetyl- or *N*-sulfonylated-α-D-glucosamine (GlcNAc/GlcNHS). Monosaccharides within the unit as well as disaccharide units bind each other with a 1 → 4 glycosidic linkage. Units are mainly sulfated at C6 of GlcNAc, whereas various portions at C3 of GlcNAc and *N*-sulfonylation, as well as at C2 and C3 of IdoA, are also present depending on the cell types, tissues and pathophysiological roles of Hep/HS [6, 7, 15].

At the biosynthetic levels of HS it is noticing that the *O*-sulfation occur mainly at the units containing the epimeric form (IdoA), since sulfotransferases prefer those NS backbones, which are rich in IdoA sequences in the HS [2, 15, 20].

From the historical point of view, the occurrence of a heparin-like glycan in ascidians was first reported in 1999 [21]. Heparin was isolated from the body of the stolidobranchia ascidian *S. plicata* and identified by agarose gel electrophoresis before and after degradation with nitrous acid and heparin lyase [21]. Heparin has also been detected in the body of the phlebobranchia ascidian *Phallusia nigra* by agarose gel electrophoresis before and after specific enzymatic degradation with heparinase I (*unpublished data*). Disaccharide analysis has yet to be performed on the *P. nigra* heparin.

Fine HPLC analysis of the disaccharides derived by enzymatic degradation with heparin and heparan sulfate lyases of the *S. plicata* glycan indicated that the invertebrate Hep is a heterogeneous polymer composed mainly of the disaccharide $[4\text{-}\alpha\text{-L-IdoA-}(2\text{-OSO}_3)^{-1} \rightarrow 4\text{-}\alpha\text{-D-GlcN}(\text{SO}_3)^{-1}(6\text{-O-SO}_3)^{-1}]_n$ (~53 %), followed by $[4\text{-}\alpha\text{-L-IdoA-}(2\text{-O-SO}_3)^{-1} \rightarrow 4\text{-}\alpha\text{-D-GlcN}(\text{SO}_3)^{-1}]_n$ (~22 %), $[4\text{-}\alpha\text{-L-IdoA-1} \rightarrow 4\text{-}\alpha\text{-D-GlcN}(6\text{-O-SO}_3)^{-1}]_n$ (~14 %) and $[4\text{-}\alpha\text{-L-IdoA1} \rightarrow 4\text{-}\alpha\text{-D-GlcN}(\text{SO}_3)^{-1}]_n$ (~11 %) [21]. Further detailed analysis of the *S. plicata* heparin by one-dimensional ^{1}H NMR and interpretations of two-dimensional ^{1}H/^{1}H COSY (correlation spectroscopy) and TOCSY (total correlation spectroscopy), and ^{1}H/^{13}C HMQC (heteronuclear multiple quantum coherence) correlation spectra confirmed the previous disaccharide analysis [22]. The main types of Hep/HS found in ascidians are summarized in Table 1.

It is also worth noticing that recent NMR structural analyses revealed an uncommon structure of HS with that of mammals and even of the mollusk heparonoids. Specifically the marine mollusk *N. nodosus* derived HS exhibits a unique structure in terms of sulfation pattern. Th is HS is characterized by the absence of L-IdoA, contain high percentage of D-GlcNAc, whereas the portion of the sulfated D-GlcA residues at C2 and C3 is very low [15, 23].

Dermatan sulfate and heparin distribution in *S. plicata* tissues

In order to understand the biological roles of GAGs in ascidians, we expanded the structural study of these molecules to the tissue level. We determine the distribution of the 2,4-*O*-di-sulfated dermatan and heparin in tissues from different organs (intestine, heart, pharynx and mantle) [24]. DS and Hep were detected in all organs, but their percentages were different. Whereas DS prevails in the pharynx, followed by mantle, heart and intestine, Hep is the main GAG in the intestine, followed by heart, mantle and pharynx.

Table 1 Distribution of sulfates in dermatan sulfates and heparin/heparan sulfate in different species of ascidians

Order	Species	Sulfates	References
Stolidobranchia	*Styela pilicata*	DS (2-OSO_3 → 4-OSO_3)	[16, 17]
		HS mainly (2-OSO_3) → (N-SO_3) (6-OSO_3) and (2-OSO_3) → (N-SO_3)	[21, 22]
	Hyalocynthia pyriformis	DS (2-OSO_3 → 4-OSO_3)	[17]
Phlebobranchia	*Phallusia nigra*	DS (2-OSO_3 → 6-O-SO_3) (predominant type)	[18] [19]
	Ciona intestinalis	DS (2-OSO_3 → 6-O-SO_3) (predominant type)	[18]

DS (IdoA → GalNAc)

HS (IdoA → GlcN)

DS, dermatan sulfate; HS, heparan sulfate; IdoA, L-iduronic acid; GlcNAc, N-acetyl-D- galactosamine; GlcN, D-glucosamine

Interestingly, the degree of sulfation of the DS differs slightly among the organs. In comparison to pharynx and mantle, intestine and heart have DSs with higher percentage of 2,4-di-sulfated units On the other hand pharynx and mantle have Heps with higher percentage of tri-*O*-sulfated units, followed by heart and intestine.

To determine the tissue localization of the oversulfated dermatan and the heparins, sections of the intestine and the pharynx of the ascidian *S. plicata* were stained with the cationic dye 1,9-dimethylmethylene blue, before or after incubation of tissue sections with chondroitinase ABC. The characteristic metachromatic staining of the dye revealed that sulfated glycans are diffusely distributed throughout the extracellular matrix and inside epithelial cells of the intestine and pharynx. However, after chondroitinase ABC treatment the extracellular matrix metachromatic staining was completely abolished while that of the epithelial cells remained intact [24]. These results indicated that the oversulfated dermatan occurs in the extracellular matrix while the Hep occurs inside the epithelial cells.

The presence of GAGs in intestine and pharynx epithelial cells was confirmed utilizing staining with the anti-Hep mouse monoclonal antibody ST-1. Notably this antibody, which has been raised against Hep complexed to *Salmonella Minnesota* recognizes an epitope in the intact unmodified molecule of Hep [25]. In addition, by using the same approach, we also detected Hep in accessory cells, named test cells, that reside in the periviteline space of ascidian oocytes [22] and in a granulocyte cell, which circulates in the ascidian hemolymph [26]. Biochemical and fine structure $^{1}H/^{13}C$ NMR analysis of the GAGs isolated from the test cells and the hemocytes confirmed that they are in fact Hep [22, 26].

Searching for the function of GAGs in ascidians

Extracellular oversulfated dermatan

DS is synthesized as a glycosylation product of a specific protein named decorin, which is a genetically related member of the Small Leucine-Rich Proteoglycan (SLRP) family [27]. Decorin and other SLRP members have been shown to interact with extracellular matrix molecules resulting in the maintenance of the matrix integrity. For example, Decorin binding to collagen type I and II controls fibrillogenesis [28]. In ascidians, DS is present in the extracellular matrix, where collagen is also present [16].

Phylogenetically, ascidians and mammals are related. Since both of them are chordate animals, there is a high probability that they share similar molecules with common functions. Therefore, we wondered whether the oversulfated dermatan would be linked to a decorin-like protein core and if it would interact with type I collagen in the extracellular matrix of ascidian tissues. Using a biochemical approach, we showed that collagen is present in intestine, heart, pharynx and mantle of *S. plicata*. Confocal microscopy images of intestine and pharynx sections stained with a collagen-specific method revealed that collagen occurs at a similar location as the oversulfated dermatan. Moreover, immunohistochemical analysis with anti-decorin specific antibody indicated the co-localization of collagen, DS and a decorin-like protein in the extracellular matrix of the ascidian tissues [29].

Finally, immunogold electron microscopy with anti-decorin and anti-type I collagen antibodies showed the co-localization of type I collagen and decorin in the extracellular matrix of ascidian tissues. Biochemical analysis also indicated that the oversulfated 2,4-*O*-di-sulfated dermatan is linked to a

protein core in the ascidian tissues [29]. Overall, these results strongly suggest that similar to mammalians, ascidian DS is synthesized on a decorin-like core protein, which interacts with type-I collagen, and probably controls the extracellular matrix organization.

Intracellular Hep

In high Chordates, highly sulfated GAGs, such as Hep and oversulfated chondroitin are linked to a serglycin core protein, which is mainly found as an intracellular product of immunologic cells that either reside in the tissue or circulate in the blood [30, 31]. The two prototypes of these immunologic cells are mast cells, which are tissue-resident, and basophils, which circulate in blood. These cells share some characteristics such as intracellular granules containing serglycin, histamine and several serine proteases [32]. In mast cell, serglycin is glycosylated with Hep and in basophil with oversulfated chondroitin [30]. This finding raises the hypothesis that the ascidian heparin-containing cells could be the primitive counterparts of mammalian mast cells and basophils. Several additional biochemical and functional evidences obtained from these cells supported this hypothesis.

Ascidian test cell- is it a primitive mast cell ancestor?

During the study of GAGs distribution in ascidian tissues we found a Hep-containing cell that resides in the tissue - the oocyte test cell [22], which is believed to originate from blood cells [33]. The function of the test cells remains an open question, although some authors believe these cells secrete factors important for the oocyte development, while others associate test cells with the release of substances that provide hydrophilic properties to the tunic [34]. Lastly, there is also the hypothesis that links them to processes of formation of the larval tunic [35, 36].

A rather peculiar functional aspect of the ascidian test cells that may indicate a new hypothesis for their biological function, is the fact that the compound 48/80, a potent stimulator of mammalian mast cell degranulation, also promotes degranulation of test cells [22]. Furthermore, tryptase activity was detected in the supernatants of pure preparations of test cells after degranulation with compound 48/80, indicating that degranulation is associated with protease release, which is also observed in mammalian mast cells. Finally, an ultrastructural study revealed that intracellular granules of *S.plicata* test cells are formed by elongated filaments of serial globules with an electron-lucent circle and a central electron-dense region, differing structurally from those found in mammalian mast cells. However, similar to the mammalian counterpart cell, immunocytochemistry with anti-Hep and anti-histamine antibodies showed that Hep and histamine co-localize at the border of the filaments in the ascidian test cell granules [37]. Overall, these data suggest that the ascidian oocyte test cells are ancient ancestors of mammalian mast cells, involved in defense mechanisms. Therefore, in primitive chordates Hep would be involved in the storage of mediators (histamine) and enzymes (tryptases) in cytoplasmic granules of specialized cells, a similar role observed in recent chordates such as mammals [38].

Ascidian hemolymph granulocyte- is it a primitive basophil ancestor involved in innate immune response?

As mentioned before, in recent chordates intracellular Hep and oversulfated chondroitin occur in some types

Table 2 Distribution of glycosaminoglycans in the ascidian *S.plicata*: Organ and tissue localization and possible function

Glycosaminoglycan	Organ	Tissue localization	Possible function
Dermatan sulfate-like[a] [4-α-L-IdoA-(**2-O-SO$_3$**)$^{-1}$– > 3-β-D-GalNAc(**4-OSO$_3$**)$^{-1}$]n (main disaccharide Unit)	Pharynx (63 %) Mantle (19.4 %) Heart (10.3 %) Intestine (7.3 %)	- Extracellular matrix (co-localized with decorin-like protein and collagen	Maintenance of extracellular matrix integrity?
Heparin/heparan sulfate-like[b] [4-α-L-IdoA-(**2-OSO$_3$**)$^{-1}$– > 4-α-D-GlcN(**SO$_3$**)$^{-1}$(**6-O-SO$_3$**)$^{-1}$]n (main disaccharide Unit) and [4-α-L-IdoA-(**2-O-SO$_3$**)$^{-1}$– > 4-α-D-GlcN(**SO$_3$**)$^{-1}$]n	Pharynx (63 %) Mantle (20.2 %) Heart (23.3 %) Intestine (46 %)	- Test cells - Epithelial cells at the lumen of intestine and pharynx	- Test cells: Storage of histamine and tryptases in intracellular granules? - Epithelial cells: yet to be studied
Heparin/heparan sulfate-like[c] [4-α-L-IdoA-(**2-OSO$_3$**)$^{-1}$– > 4-α-D-GlcN(**SO$_3$**)$^{-1}$(**6-O-SO$_3$**)$^{-1}$]n and [4-α-L-IdoA-(**2-O-SO$_3$**)$^{-1}$– > 4-α-D-GlcN(**SO$_3$**)$^{-1}$]n (equal amounts)	Hemolymph	Granulocytes (basophil-like cells)	Storage of histamine and tryptases in intracellular granules?

[a] Reference [24]

[b] Reference [22, 24]

[c] Reference [26]

of immunologic cells that either reside in the tissues (mast cells) or circulate in the blood (basophils) [30, 31]. Considering the ascidian phylogeny and taking into account that a Hep-containing cell similar to mammalian mast cells was detected in *S. plicata* tissues, the hypothesis that a basophil-like cell containing intracellular GAGs would be circulating in the hemolymph of this invertebrate chordate was raised.

Five cell types are present in the hemolymph of *S.plicata* and classified as univacuolated and multivacuolated cells, amebocyte, hemoblast and granulocyte [26]. Immunoelectron microscopy showed that Hep and histamine colocalize in intracellular granules of only one type of hemocyte, the granulocyte [26]. Further structural analysis of the granulocyte heparin revealed that the GAG is composed mainly of approximately equal amounts of [4-α-L-IdoA-(2-OSO_3)$^{-1}$ → 4-α-D-GlcN(SO_3)$^{-1}$(6-O-SO_3)$^{-1}$]$_n$, and [4-α-L-IdoA-(2-OSO_3)$^{-1}$ → 4-α-D-GlcN(SO_3)$^{-1}$]n disaccharide units [26].

Interestingly, the granulocyte in the hemolymph of *S. plicata* is morphologically related to vertebrate basophils. A central electron-dense region was observed in the ascidian granulocyte granules, which is a characteristic mainly found in granulocytes of higher vertebrates, such as reptiles and mammals [39, 40]. On the other hand, in granulocytes from more primitive vertebrates, such as fish and bufonid, this electron-dense core is not observed in the granules.

In mammal basophils, serglycin is glycosylated with oversulfated chondroitin [31]. Since we found Hep in the granules of the *S.plicata* granulocyte, instead of chondroitin, we asked whether this Hep would be linked to a serglycin core protein. Using immune-electron microscopy with an antibody against the serglycin carboxy-end protein and a gold-labeled secondary antibody we showed that out of the 5 hemocytes types found in the ascidian hemolymph, only the Hep-containing granulocyte reacted positively with the anti-serglycin antibody. Gold particles associated with anti-serglycin were restricted to intracellular granules at a similar region where Hep and histamine are present. This strongly suggests the presence of a basophil-like cell in the hemolymph of *S.plicata* that contains histamine and serglycin proteoglycan linked to Hep chains. This cell may be a primitive form of basophil, which during evolution maintained some biochemical characteristics but lost the capacity to synthesize Hep and acquire that of synthesize oversulfated chondroitin sulfate.

Mammalian mast cells and basophils express complex and partially overlapping roles in acquired and innate immunity. These include both effector cell and, potentially, immunoregulatory activities [41]. In ascidians, the hemocytes circulate in the hemolymph and perform a wide variety of functions, such as phagocytosis, recognition of self and non-self molecules, repair of damaged tissues, and induction of blood-cell aggregation to prevent blood-fluid loss [42].

Concluding remarks

In this review, the structure and biological functions of the sulfated GAGs in different species and tissues of ascidians are presented and discussed. Taking into account the phylogenetic position of the subphylum Tunicata in the phylum Chordata, such structural analysis may shed light on the evolution of ascidian GAGs from invertebrate to vertebrate animals. A careful analysis of the structural and functional data of the Urochordata GAGs is also given and summarised in the Table 2, suggesting that although the structure of DS and Hep are different, their physiological functions have been conserved during the evolution of the Chordates.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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