Structure, biology, evolution and medical importance of sulfated -fucans and -galactans

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Summary:

Sulfated fucans and galactans are strongly anionic polysaccharides found in marine organisms. Their structures vary among species, but their major features are conserved among phyla. Sulfated fucans are found in marine brown algae and echinoderms, whereas sulfated galactans occur in red and green algae, marine angiosperms, tunicates (ascidians) and sea urchins. Polysaccharides with 3-linked, $\beta$-galactose units are highly conserved in some taxonomic groups of marine organisms and show a strong tendency toward 4-sulfation in algae and marine angiosperms, and 2-sulfation in invertebrates. Marine algae mainly express sulfated polysaccharides with complex, heterogeneous structures, whereas marine invertebrates synthesize sulfated fucans and sulfated galactans with regular repetitive structures. These polysaccharides are structural components of the extracellular matrix. Sulfated fucans and galactans are involved in sea urchin fertilization acting as species-specific inducers of the sperm acrosome reaction. Because of this function the structural evolution of sulfated fucans could be a component in the speciation process. The algal and invertebrate polysaccharides are also potent anticoagulant agents of mammalian blood and represent a potential source of compounds for antithrombotic therapies.

Keywords: fucoidan / carrageenans / algae / marine invertebrate / anticoagulant / sea urchin fertilization / acrosome reaction / species-specificity / sulfated polysaccharides / sperm-egg recognition.

Running title: Sulfated -fucans and -galactans.
Introduction

With the exception of some mammalian polysaccharides, the sulfated fucans and galactans of algae and marine invertebrates are the most well-studied sulfated polysaccharides. In terms of total biomass, they are more abundant than glycosaminoglycans. As we will discuss below, these compounds exhibit a wide structural diversity and have intriguing biological functions.

In this review, we describe the main structural features of sulfated fucans and galactans isolated from marine organisms. The structures of the algal polysaccharides are complex and heterogeneous (Pereira et al. 1999; Berteau and Mulloy 2003). In contrast, polysaccharides from marine angiosperms and invertebrates have simpler, more homogeneous structures. They are composed of repeating units, which vary in a species-specific manner (Mourão 2004; Aquino et al. 2005; Mourão 2007). Here, we also discuss the evolutionary implications of these marine sulfated polysaccharides and their biological effects, focusing on the description of their natural role in echinoderm fertilization. Finally, we also describe the inhibition by these polysaccharides of mammalian blood coagulation, with emphasis on developing new therapeutic agents.

Heterogeneous sulfated fucans from algae

The first isolation of a sulfated fucan (originally denoted fucoidan) from marine brown algae (Phaeophyta) was reported by Killing in 1913. These sulfated polysaccharides can be extracted from algal cell walls with hot water (Percival and Ross 1950), acid solutions (Black 1954) or protease digestion (Martinez-Rumayor and Januzzi 2006; Leite et al. 1998). They can account for >40% of the dry weight of algal cell walls (Kloareg 1984). Sulfated fucans have been extensively studied in brown algae, and are present in all brown algae thus far investigated (Patankar et al. 1993; Nishino et al. 1995; Chevolot et al. 1999; Chizhov et al. 1999; Chevolot et al. 2001; Pereira et al. 1999; Bilan et al. 2002; Yoon et al. 2007). The composition of these molecules may vary according to the species (Percival and Ross 1950; Mian and Percival 1973), the extraction procedure (Mabeau et al. 1990), the season and climatic conditions (Black 1954; Von Holdt et al. 1955; Wort...
1955; Honya et al. 1999). These polysaccharides are absent, or occur only in minor amounts in green algae (Chlorophyta), red algae (Rhodophyta), and golden algae (Xanthophyta). Brown algal sulfated fucans are among the most abundant marine sulfated polysaccharides, since these algae dominate the near-shore environment in both number of species (1,500-2,000) and biomass.

The initial structural study of a sulfated fucan was that obtained from the common brown alga *Fucus vesiculosus*. Early on, Percival and Ross (1950) suggested a polysaccharide composed of $\alpha$-L-fucopyranose, mainly bound by 1→2 glycosidic linkages and sulfation at 4-position (Figure 1A). The simplicity of this structure was contested by Patankar and co-workers in 1993. Based on methylation analysis, these authors proposed that this sulfated fucan has a central core, composed of 4-sulfated or non-sulfated, 3-linked $\alpha$-L-fucopyranose units, with branches of non-sulfated fucose linked to the central core at position 2 or 4 (Figure 1B). Recently, studies based on high field NMR, revealed that the sulfated fucans from this and other brown algal species are composed equally of alternating units of 2,3-disulfated, 4-linked and 2-sulfated, 3-linked $\alpha$-L-fucopyranosyl units (Figure 1C). The heterogeneity of this polysaccharide results mainly from the occurrence of branches of non-sulfated fucose residues (Chevolot et al. 1999; Pereira et al. 1999). More recently, NMR analysis of sulfated fucans from other species of brown algae revealed unique structures (Figure 1D and E). In particular, occurrence of O-acetylation is also commonly present (Chizhov et al. 1999). Contradictions related to the structures of brown algal sulfated fucans arise from the difficulties in purifying these molecules as well as their highly complex structures due to presence of branching, random distribution of sulfation, different types of glycosidic linkages, and also the presence of other heterogeneities like acetylation, methylation and pyruvilation (Figure 1) (Chizhov et al. 1999; Bilan et al. 2007).

**Homogeneous, repetitive sulfated fucans of echinoderms**

In addition to brown algae, sulfated fucans are found in marine invertebrates. The first evidence showing sulfated fucans in sea urchins (Echinodermata, Echinoidea) was published ~60 years ago (Vasseur 1948), but no structural study was performed at this time. Since 1994, our laboratory has been performing a systematic structural analysis of the sulfated fucans from different species of sea urchins. Most of our work is on these molecules isolated from the jelly coats surrounding sea urchin eggs.
In contrast to the algal polysaccharides, sulfated fucans from sea urchins and sea cucumbers (Echinodermata, Holothuroidea) are easily purified and possess simple, unique structures of linear chains of α-L-fucose in well-defined repetitive patterns. Their specific sulfation patterns and the positioning of glycosidic bonds vary with the species (Figure 2).

The simplest sea urchin sulfated fucan, which is composed of \([3-\alpha-L-\text{Fuc-2(OSO}_3)-1]_n\), is found in the egg jelly of *Strongylocentrotus franciscanus* (Vilela-Silva et al. 1999). Other sea urchins so far studied possess sulfated fucans with different numbers of residues in the repeating units, which vary among the different species according to the position of the glycosidic linkages \([\alpha(1\rightarrow3)\text{ or } \alpha(1\rightarrow4)]\), and the sulfation sites (2-O- and/or 4-O-positions) (Figure 2).

**Sulfated galactans: the heterogeneity arises mostly due to complex sulfation patterns**

Marine sulfated galactans are widely abundant in red algae. Carrageenans and agarans are the most common sulfated galactans from macroalgae. The origin of the name carrageenan derived from a small village, Carragheen, on the Irish coast, where the carrageenan-bearing seaweed *Chondrus crispus* or “Irish moss” grows (Bixler 1994). The word agar (name proposed by Kuntsen et al. 1994, see also Lahaye 2001) originally derived from the word “agar”, which that means jelly in the Malay language (agar-agar). Both of these red algal polysaccharides usually have a linear backbone made of alternating 3-linked β-D-galactopyranose and 4-linked α-galactopyranose residues (Figure 3A), showing a ‘masked repeat’ unit of disaccharides similar to the animal glycosaminoglycans. The β-galactoses are always D-enantiomers, whereas the α-galactose residues may be present in the D- or L-configuration (Usov 1998). A substantial portion may also exist in the form of 3,6-anhydro derivatives (Figure 3A). Like sulfated fucans from brown algae, considerable structural variation in the red alga sulfated galactans occurs among different species and in samples collected at different environments, or in different seasons of the year (Pereira et al. 2005). Furthermore, various hydroxyl groups may be substituted by a sulfate ester, a methyl group or pyruvic acid (Usov 1998). The major structural variation in these polysaccharides is the sulfation pattern. The sulfate distribution along the galactose-backbone is quite heterogeneous as in animal glycosaminoglycans and the sulfate contents are markedly different between different species (Pereira et al. 2005).
Although the majority of red algal species express sulfated galactan with some heterogeneities, a minor number express homogeneous galactans, classically named as carrageenans and agarans. Carrageenans are traditionally classified by a Greek prefix according to the sulfation pattern and the presence of 3,6-anhydro bridge (carrageenose) on the 4-linked \(\alpha\)-D-galactose (van de Velde et al. 2004) (Figure 3A). We will not discuss variations in the structures of this class of polysaccharide since this topic has been extensively covered in several other reviews (Usov 1998, Lahaye 2001, van de Velde et al. 2004).

The carrageenans and agarans are extensively exploited due to their industrial applications. The wide uses of these sulfated polysaccharides are based on their unique properties to form strong aqueous gels. These molecules are the major hydrocolloids used as texturing agents for food. A small change from \(\alpha\)-D-galactopyranoses in carrageenans to \(\alpha\)-L-galactopyranoses in agaran is enough to promote great changes in the physical-properties of these molecules (Lahaye 2001). Other modifications in the backbone of the sulfated galactans can greatly change their physicochemical properties and, consequently, in industrial applications and biological activities. For example, high levels of 3,6-anhydro-\(\alpha\)-L-galactopyranosyl units in agar group polysaccharides (also known as agarose) and low sulfate contents are the major structural requirements for gelling (Lahaye 2001). Several types of these gels are widely exploited by industries in their attempt to obtain the best and specific gel formation under different conditions (regulated by temperature and the combination of ingredients) (Lahaye 2001).

Recently, sulfated galactans have also been characterized in green algal species, particularly those from the genus *Codium* (Matsubara et al. 2001; Bilan et al. 2007; Farias et al. 2008). In contrast to the repetitive disaccharides found in the sulfated galactans from red algae, the polysaccharides from green algae exhibit a backbone composed preponderantly of 4-sulfated, 3-linked \(\beta\)-D-galactopyranose units (Figure 3B). These green algal sulfated galactans can be highly pyruvilated at their non-reducing terminal residues, forming cyclic ketals such as 3,4-O-(1´carboxi)-ethylidene-\(\beta\)-D-Galp-1 (Bilan et al. 2007; Farias et al. 2008). Although they are more complex than those from red alga, evidence indicates the existence of a predominant structure and less structural variation as compared to those found in brown algal sulfated fucans (Figure 1).

Sulfated polysaccharides have not been described in vascular plants (angiosperm) until a recent report of a novel sulfated galactan isolated from the marine sea-grass, *Rupia maritima* (Angiospermae, Spermatophyta) (Aquino et al. 2005). The sea-grass is a group
of vascular flowering plants, which grow in highly saline marine environments. The structure of the sea-grass sulfated D-galactan is composed of regular tetrasaccharide repeating units (Figure 3C). Like red algae, the marine angiosperm polysaccharide contains both α- and β-D-galactose residues; however, these units are not distributed in an alternating order as found in red algal sulfated galactans.

In the marine environment, the sulfated galactans were also described in some species of invertebrates. In ascidians (also known as sea squirts or tunicates) (Urochordata, Asciidae), these polysaccharides contain a central core composed of 3-sulfated, 4-linked α-L-galactopyranosyl units (Figure 3D). Heterogeneity and variation among different ascidian sulfated galactans species arise from the occurrence of non-sulfated L-galactose or D-glucose branches (Mourão and Perlin 1987; Pavão et al. 1989; Santos et al. 1992). The discovery of sulfated galactans in tunicates was the first report of the existence of a polysaccharide composed exclusively of L-galactose residues (Mourão and Perlin 1987). Curiously, some biology textbooks refer to ascidians as the only animal possessing cellulose (Barnes 1980). The sulfated L-galactans, which are poorly soluble in water when highly branched, could possibly have been mistaken for cellulose in these studies performed several decades ago. Recent studies described genes responsible for cellulose synthesis in ascidians (Nakashima et al. 2004). A functional cellulose synthase was also reported in the invertebrate epidermis (Matthysse et al 2004). The enzyme catalyzes synthesis of 4-linked β-D-glucopyranose units. However, this enzyme may in fact be involved in the biosynthesis of sulfated α-L-galactans. The D-glucose is the best precursor of α-L-galactose units in ascidian polysaccharides (Mourão, 1991; Pavão et al 1994). Possibly, biosynthesis of the sulfated L-galactans involves incorporation of 4-linked β-D-glucopyranose followed by epimerization to α-L-galactopyranose units on the polysaccharide chain (Mourão and Assreuy 1995).

Sulfated galactans were also found in two species of sea urchins. One species (Echinometra lucunter) contains an egg jelly sulfated galactan composed of α-L-galactopyranosyl units, similar to those found in the ascidian polysaccharides. However, it is more homogeneous and clearly composed of linear chains of 2-sulfated, 3-linked repetitive units (Figure 3E) (Alves et al. 1997), instead of 3-sulfated and 4-linked residues. Another sea urchin species (Glyptocidaris crenularis) synthesizes a galactan composed of 3-linked galactopyranose units in the β-D-enantiomeric form, similar to those observed in
galactans from green algae. However, this sea urchin polysaccharide is very homogeneous, composed of alternating 2-sulfated and non-sulfated galactopyranose residues (Figure 3E) (our unpublished data).

How has the 3-linked, β-galactose unit occurred in marine organisms throughout the course of evolution?

A comparison among sulfated galactans from different organisms indicates that polysaccharides with the glycosidic linkage \( \beta(1\rightarrow3) \) are strongly conserved in some taxonomic groups of eukaryotes (rhodophytes, chlorophytes, angiosperms, echinoderms and mollusks). The sulfated galactans found among these phyla differ mainly in sulfation sites, with a strong tendency towards 4-sulfation in algae and marine angiosperms, and 2-sulfation in invertebrates. The 6-sulfation is dispersed in minor amounts throughout phylogeny. Similar distribution of sulfation pattern is not observed for the sulfated fucans. These observations provide grounds for speculation about the evolutionary history of sulfated polysaccharides.

The occurrence of the 3-β-D-GalP-1 unit in the sulfated galactan from the sea-urchin Glyptocidaris crenularis and its presence in sulfated galactans from green algae (Bilan et al. 2007; Farias et al. 2008) and from sea-grass (Aquino et al. 2005) (Figure 3) stimulated us to review the distribution of this structure in animal and plant kingdoms (Whittaker 1969) in order to propose a phylogenetic relationship of this unit (Figure 4). Although this comparison is based only on structural components of the sulfated galactans, which are products of action of several genes and biosynthetic enzymes, this taxonomic comparison might allow us to ask whether there is a relationship among the marine organisms that express sulfated 3-β-D-GalP-1.

Thus, the hypothetical cladogram (Figure 4) shows that the sulfated 3-β-D-GalP-1 units are preserved among species of specific phyla that inhabit the marine environment, including green algae, red seaweeds, marine sea-grass (Angiospermae, Spermatophyta), invertebrates (sea urchins, clams and tunicates) and vertebrates such as fishes that express keratan sulfate (Scudder et al. 1986).

Although the 3-β-D-GalP-1 unit has been preserved in the major phyla during evolution (with the only exception being brown algae), the preferential sulfation site on this
structure varies in a tendency toward 2-sulfation for animals, 4-sulfation for algae and marine angiosperms, and a dispersive distribution of 6-sulfation.

These observations raise the hypothesis that the galactosyltransferases responsible for the incorporation of 3-β-D-Galp-1 units in the biosynthesis of sulfated galactans have been maintained during evolution in specific phyla of marine organisms, but was allowed to vary in the distribution of sulfotransferases types. In favor of this hypothesis is the evidence that the basic backbones are the same, but with a variable position of sulfation that differs from species to species and from tissue to tissue. To some extent, these results are analogous to the biosynthesis of the glycosaminoglycans from vertebrates, where the glycosidic chains vary relatively little among polymers constructed in different tissues, organs and species. Modifications on the glycosidic core occur mostly after chain elongation, when the principal modification is the sulfation at different sites. Unfortunately, the biosynthesis of the sulfated galactans from marine organisms is virtually unknown and therefore it is not yet possible to compare the expression of these molecules. The alternative, and just as likely hypothesis, is that the presence of these sulfated galactans in such distantly related organisms is an example of independent, convergent evolution of biosynthetic pathways. This hypothesis is not based on gene sequence, not even on the sequence of proteins, and requires future work to propose a firm theory.

Biological relevance of marine invertebrate sulfated fucans and galactans

In the case of the invertebrates, these sulfated polysaccharides are components of the extracellular matrix. For example, the ascidians contain high concentrations of sulfated galactan as a component of the outer tunic, a protective layer enveloping the organism (Albano et al. 1990; Santos et al. 1992). Similarly, sulfated fucans from sea cucumbers also form part of the body wall (Mulloy et al. 1994; Ribeiro et al. 1994). In all of these cases, sulfated galactans or sulfated fucans occur in high concentrations in the extracellular matrix, which resemble the amount of glycosaminoglycans in proteoglycans found in the extracellular matrix of mammalian connective tissue (especially cartilage).

However, marine sulfated fucans and galactans have their own structural particularities. Firstly, they are more sulfated than vertebrate glycosaminoglycans such as chondroitin sulfate and dermatan sulfate, which contain one sulfate group per disaccharide unit. Perhaps, interactions between components of the extracellular matrix in marine organisms occur at higher salt concentrations than in vertebrates, and therefore require
polysaccharides with higher charge density. Secondly, glycosaminoglycans from mammalian extracellular matrices have molecular masses only between ~15 and ~60 kDa. The covalent complex of these mammalian chains with the core protein results in a high molecular mass complex (>100 kDa). In contrast, sulfated galactans and sulfated fucans from algae and invertebrates are themselves high molecular weight molecules. The attachment to a protein core still needs to be demonstrated and it is apparently irrelevant for the biological activities of this class of polysaccharide. In sea urchin egg jellies, the sulfated fucans have masses >1 million Da.

In addition to the sulfated polysaccharides found in the extracellular matrices of algae, marine angiosperms, ascidians and sea cucumbers, sulfated polysaccharides from sea urchins are also localized in the hydrated, usually transparent, jelly layer surrounding the eggs. The sea urchin egg jelly sulfated polysaccharides form a complex extracellular matrix containing the sulfated fucan complexed with many unknown proteins of both high and low molecular mass (Vacquier and Moy 1997). As described below, the egg sulfated fucan is intimately involved in gamete recognition (Mulloy et al. 1994; Ribeiro et al. 1994; Alves et al. 1997, 1998; Vilela-Silva et al. 1999, 2002; Hirohashi et al. 2002; Biermann et al. 2004; Mourão 2007).

A necessary event for the sea urchin fertilization is the sperm acrosome reaction (AR). The sea urchin AR involves the calcium-triggered exocytosis of the acrosome vesicle and the pH-induced polymerization of actin to form the ~1 μm long, finger-like, acrosomal process which protrudes from the anterior of the sperm head (Vacquier and Hirohashi 2004). When sperm approach the sea urchin egg, the sulfated fucan binds to sperm receptors which are homologs of human polycystin, the protein mutated in autosomal dominant polycystic kidney disease (Gunaratne et al. 2007). At least two pharmacologically distinct calcium channels open to allow calcium influx from the seawater (Darszon, Acevedo et al. 2006; Darszon, López-Martínez et al. 2006). The internal pH of the sperm also rises about 0.25 pH units due to sodium/proton exchange (de la Sancha et al. 2007). Both the calcium influx and pH rise are required for AR induction. The AR exposes the protein bindin which coats the acrosome process at the anterior tip of the sperm. The bindin attaches the sperm to the EBR1 receptor on the egg surface. Sperm bindin mediates both the species-specific attachment of sperm to egg and also the fusion of the plasma membranes of the two gametes (Vacquier et al. 1995; Cameron et al. 1996; Glaser et al. 1999; Kamei and Glabe 2003). The sequences of bindins are species-specific and have been shown to be subjected to positive selection (Zigler 2008).
The purified sulfated fucan of egg jelly, devoid of any detectable protein, will by itself induce the sperm AR (Vacquier and Moy 1997). Induction by the sulfated fucan is potentiated by a polysialic acid containing "sialoglycan" also isolated from egg jelly (Hirohashi and Vacquier 2002a). Large molecular mass sulfated fucan is needed to open both sperm calcium channels and degradation of its mass to ~60-kDa will open one channel, but not the other (Hirohashi and Vacquier 2002b).

A preliminary study indicated that AR was induced by sulfated polysaccharides from the sea urchin egg jelly (Segall and Lennarz 1979). The well-defined chemical structures of the sea urchin egg jelly sulfated fucans, and the observation that each species possesses a polymer with a different structure, suggests that these sulfated polysaccharides are the egg molecules involved in the species-specific induction of the sperm AR (Alves et al. 1997; Vilela-Silva et al. 2002, 2008). Indeed, when they were tested with homo-specific and hetero-specific sperm from species that co-inhabit the same area in Rio de Janeiro (sympatic species) we observed that sulfated polysaccharides are species-specific inducers of the AR (Alves et al. 1997) (Figure 5). This observation was confirmed as the study was extended to other species of the genus *Strongylocentrotus* (Hirohashi et al. 2002). The monosaccharide composition (galactose or fucose), the position of the glycosidic linkage (3- or 4-linked), the pattern of sulfation (at 2- or 4-positions), and the number of fucose moieties per repeating unit are all crucial for inducing the sea urchin sperm AR (Biermann et al. 2004; Mourão 2007). Independent work by Koyota et al. (1997) on starfish corroborates these findings on an additional class of echinoderms, the asteroids. They characterized an AR inducing substance (ARIS) isolated from a single species of starfish, *A. amurensis*. They showed that ARIS is a polysaccharide composed of 10-11 repetitive units of the pentasaccharide \((\rightarrow 4)\)−\(\beta\)-D-Xylp−(1→3)−\(\alpha\)-D-Galp−(1→3)−\(\alpha\)-L-Fucp−4(SO\(_4\))−(1→3)−\(\alpha\)-L-Fucp−4(SO\(_4\))−(1→4)−\(\alpha\)-L-Fucp−(1→)\(_n\).

(for more details about this biological event in starfish, see the review by Hoshi et al. 1994, and by Matsumoto et al. 2008).

One reason it is important to investigate the molecular details of AR induction by the egg jelly sulfated fucans is because it is very rare for a pure carbohydrate to induce a signal transduction event in animal cells. The demonstration that sulfated fucans induce the sperm AR in a species-specific manner led to additional experimentation with three related sympatic species of *Strongylocentrotus* (Figure 6). Egg jelly induces the AR species specifically in *S. droebachiensis* and *S. pallidus*. There are no other significant barriers to inter-specific fertilization between these two species. However, the AR in the
species *S. purpuratus* reacts non-specifically with the egg jellies of these two other species. However, hetero-specific fertilization is still blocked because the bindin protein of *S. purpuratus* does not attach the sperm to the eggs of the other two species (Biermann et al. 2004) (Figure 6).

As shown in the accompanying structural figures, the species-specific recognition of sulfated fucan with the sperm must be based on: the glycosidic linkage, pattern of sulfation, and size of the repeating unit. Small structural changes modulate an entire system of sperm-egg recognition and species-specific fertilization in sea urchins (Vilela-Silva et al. 2008). Thus, in addition to their known function in cell proliferation, coagulation, inflammation, angiogenesis, and viral infection, sulfated polysaccharides also mediate invertebrate fertilization (Figure 7).

**Could egg jelly sulfated fucans play a role in sea urchin speciation?**

Generally speaking, almost all genes involved in sexual reproduction evolve rapidly and many show that positive selection is involved in their divergence (Swanson and Vacquier 2002). Figure 6 depicts the phylogenetic relation and species divergence times of sea urchins as well as a summary of the structures of their egg jelly polysaccharides (Biermann et al. 2004; Vilela-Silva et al. 2008). Two of the Strongylocentrotid species, *S. purpuratus* and *S. droebachiensis* are very closely related. However, the former synthesizes the egg jelly fucan with a 1 to 3 glycosidic linkage, whereas the latter synthesizes a 1 to 4 linkage (Alves et al. 1997, 1998; Vilela-Silva et al. 2002). Females of both these species also make two distinct, female-specific, forms of these egg jelly polymers (Alves et al. 1998; Vilela-Silva et al. 2002). These observations suggest that the genes involved in the biosynthesis of these sulfated fucans may differentiate within a short evolutionary time. Thus, these genes have recently undergone a dramatic change during speciation in the *Strongylocentrotid* tree (Biermann et al. 2003; Lee 2003; Smith et al. 2006).

Cross-species fertilization can be prevented by one or more of the five steps those comprise the whole fertilization process: *i*) chemotaxis of the sperm to egg-released peptides (Kaupp et al. 2008), *ii*) induction of the sperm AR by the sulfated fucan, *iii*) binding of the acrosome process coated with bindin to the bindin receptor on the egg surface (Kamei and Glabe 2003), *iv*) penetration of the egg vitelline envelop, and *v*) fusion of the plasma membranes of the two gametes (Vacquier 1998). The synthesis of specific-specific structures of the sulfated fucans AR (Figure 7) could play a role in establishing the
pre-zygotic reproductive isolation that gave rise to these species. Phylogeny suggests that
S. droebachiensis and S. pallidus separated from S. purpuratus before their divergence
from each other. The bindin mechanism may have functioned as an isolation mechanism
in the earlier separation of these two sister species from S. purpuratus. A later speciation
event led to the formation of S. droebachiensis and S. pallidus, possibly due to
incompatibility of the sulfated fucans and AR induction (Figures 6 and 7).

Surprisingly, the egg jelly of S. droebachiensis contains a 1 to 4-linked sulfated
fucan, which is clearly distinct from those of the two closely related congenic species.
However, Arbacia lixula, which diverged from Strongylocentrotid species about 200 million
years ago, also has a 1 to 4-linked fucan (Figure 6). This similarity in glycosidic bond
among fucosyl residues is most probably due to convergent evolution. The occurrence
of the same sulfated fucan in these two very distantly related species is not relevant to their
cross-fertilization, because the populations of A. lixula and S. droebachiensis do not
overlap geographically. Nevertheless, this observation suggests that the gene for the
biosynthesis of 4-linked fucan may have been retained during evolution of S.
droebachiensis, but remained repressed or non-expressed until a period of strong natural
selection. This observation reminds us of a general concept in evolution, which states that
“the past of an organism not only determines its future, but also gives an enormous
reserve for a rapid modification, based on little genetic changes” (Gould 1984). Of course
we cannot exclude that similar carbohydrate structure (as 4-linked sulfated fucans) may be
synthesized by different genes.

The hypothesis that changes in the polysaccharide structure drive speciation of sea
urchins, as a result of fertilization incompatibility, requires future investigation, especially at
the genotype level, in order to propose a firm theory.

Anticoagulant and antithrombotic activities
Sulfated galactans and sulfated fucans exhibit potential pharmacological actions in
mammalian-systems. These include antiviral (Harrop et al. 1992), antimetastatic (Coombe
et al. 1987), antiangiogenic (Cumashi et al. 2007), antiinflammatory (Berteau and Mulloy
2003; Cumashi et al. 2007), antiadhesive (Berteau and Mulloy 2003; Cumashi et al. 2007),
anticoagulant (Pereira et al. 1999, Farias et al. 2000, Pereira, Melo, Mourão 2002, Pereira,
Vilela-Silva et al. 2002, Pereira et al. 2005, Mourão 2004; Cumashi et al. 2007) and
antithrombotic (Berteau and Mulloy 2003; Mourão and Pereira 1999; Mourão 2004,
Fonseca et al. 2008) activities. We will focus this review on the anticoagulant and
antithrombotic activities of these sulfated polysaccharides due to the pressing need for new antithrombotic drugs as a consequence of the increasing incidence of thromboembolic diseases - cardiovascular diseases are the leading cause of death (30% of total) in the world.

Heparin preparations are widely used for the treatment and prevention of arterial and venous thrombosis (Fareed et al. 2000). However, this polysaccharide has several limitations due to collateral effects and limited source of material (Mourão 2004). The situation is even more complex recently because of the alarming notification that heparin preparations have been contaminated with oversulfated chondroitin sulfate (Guerrini et al. 2008). This contaminant induces hypotension associated with kallikrein release when administered by intravenous injection (Kishimoto et al. 2008).

Sulfated fucans from brown algae (Berteau and Mulloy 2003) and also sulfated galactans from red algae (Pereira et al. 2005), and green algae (Matsubara et al. 2001) have been known for some time to act as modulators of coagulation. Most of their activities are mediated by both antithrombin and heparin cofactor II, although there is a particular case of a sulfated galactan from a specific green alga that exhibits a serpin-independent anticoagulant effect, possibly due to inhibition of fibrin polymerization (Matsubara et al. 2001). However, relatively few studies have interpreted the biological activity of sulfated fucans and sulfated galactans in terms of molecular structure.

The attempts to identify in the algal polysaccharide structural features necessary for their anticoagulant activity have been limited by the fact that algal sulfated fucans and sulfated galactans have complex, heterogeneous structures and their repeating sequences are not easily deduced. Only in the case of sulfated galactans isolated from two species of red algae has it been shown that the occurrence of 2,3-di-sulfated \( \alpha \)-galactose units is a critical structural motif in promoting the interaction of the polysaccharide with the plasma protease and the serpins (Pereira et al. 2005). Obviously, identification of specific structural requirements in the algal polysaccharides necessary for interaction with coagulation cofactors is an essential step for more rational development anticoagulant drugs from these compounds.

Several authors attempted to overcome this difficulty using either chemical oversulfation (Soeda et al. 1993) or desulfation (Haroun-Bouhedja et al. 2000) of native algal polysaccharides. The activity increases with increasing sulfate content (Boisson-Vidal
et al. 2000) and decreases when the native pattern of sulfation is disrupted by partial desulfation (Haroun-Bouhedja et al. 2000). In order to avoid the wide size dispersion observed for native, high-molecular-weight algal polysaccharides, several low-molecular-weight derivatives were prepared. The anticoagulant activity decreases with a decrease in the molecular size of the polysaccharide (Soeda et al. 1993). The main obstacle that continues to persist is that most of the low-molecular-weight derivatives still have complex and heterogeneous structures.

In contrast to most algal sulfated polysaccharides, the invertebrate carbohydrates constitute the most reasonable class of molecules to undergo structure-activity relationship studies. The regular and well-defined units in these compounds reveal clearly which type of sugar, glycosidic bonds and sulfate positions are responsible for the specific interaction with blood coagulation proteins that triggers the anticoagulant process (Mourão and Pereira 1999; Mourão 2004).

The results with the sulfated fucans and sulfated galactans from invertebrates reveal that the anticoagulant activity is not merely a consequence of their charge density and sulfate content. The structural requirement for interaction of these polysaccharides with coagulation cofactors and their target proteases are stereospecific (Pereira, Melo, Mourão 2002). First, the nature of the sugar residue (galactose or fucose) modifies markedly the anticoagulant activity as outlined by comparison between the active 2-sulfated, 3-linked α-L-galactan (Figure 3E) and the almost inactive 2-sulfated, 3-linked α-L-fucan (Figure 2H) (Pereira, Vilela-Silva et al. 2002). Second, the site of sulfation and/or position of the glycosidic linkage affect the activity, as indicated by comparison between the inactive 3-sulfated, 4-linked (Figure 3D) and the active 2-sulfated, 3-linked α-L-galactans (Figure 3E). Third, the occurrence of 2,4-di-sulfated units has an amplifying effect on the antithrombin-mediated anticoagulant activity of 3-linked α-L-fucans (Figure 1B). This is not merely a consequence of increased charge density. The anticoagulant activity increases ∼38-fold from a 2-sulfated, 3-linked α-L-fucan (Figure 2H) to a 2,4-disulfated α-L-fucan (Figure 2E), even though their sulfate content increases only ∼1.8-fold. Finally, specific sulfation sites are required for interaction with plasma serine-protease inhibitors. Notably, the occurrence of a single 4-sulfated unit in 3-linked α-L-fucan is the structural motif required to enhance inhibition of thrombin by heparin cofactor II, and the presence of exclusively 2-sulfated residues has a deleterious effect (Mourão 2004).
Prolongation of plasma coagulation time achieved by several sulfated polysaccharides is referred to as anticoagulant effect. However, the anticoagulant action of these compounds correlates only weakly with their antithrombotic properties (Björk and Lindahl 1982). Investigation of the antithrombotic activity requires the use of in vivo models of thrombosis in experimental animals, which is a laborious methodology. These models mimic different pathological conditions involved in thrombosis, such as decrease in blood flow, hypercoagulability state and lesion of the vascular endothelium.

Few studies report the antithrombotic activity of sulfated fucans and sulfated galactans. A low-molecular-weight fraction of algal sulfated fucan was shown to possess antithrombotic activity when tested on a venous thrombosis model in rabbits after intravenous (Mauray et al. 1995) or subcutaneous administration (Millet et al. 1999). Algal sulfated fucans were also tested on in vivo models of arterial surface damage. Intravenous administration of the polysaccharide prevents formation of microvascular thrombus induced by endothelial damage in arterioles and venules of mice (Thorlacius et al. 2000). The protective effect was attributable to the anticoagulant activity of the algal fucan. Sulfated fucan inhibits adhesion of radioactive labeled platelet and neutrophils to the surface of coronary arteries of pigs injured by angioplasty (Chavet et al. 1999). After perfusion of iliac arteries of rabbits with fluoro-labeled sulfated fucans, the labeling is mainly localized on the sites of injury (Deux et al. 2002).

Test of algal sulfated galactans on animal models of venous thrombosis revealed that these polysaccharides have a serpin-dependent anticoagulant activity due to inactivation of thrombin and factor Xa. But, these polysaccharides have also a pro-coagulant effect due to activation of factor XII. This last effect depends on the sulfation pattern of the polysaccharide. As a consequence of their anti- and pro-coagulant actions the algal galactans differ in their venous antithrombotic activities. Slight differences in the proportions and/or distribution of sulfated residues along the galactan chain is critical for the interaction between proteases, inhibitors and activators of the coagulation system, resulting in a distinct pattern in anti- and pro-coagulant activities as well as in the antithrombotic action. It is noteworthy that the algal sulfated galactans have no hemorrhagic effect even when tested at high doses (Fonseca et al. 2008).

The availability of sulfated galactans and sulfated fucans with well-defined structures and the possibility to compare their effects in a variety of in vivo models of
experimental thrombosis open new perspectives for the development of sulfated galactans and sulfated fucans as therapeutic agents.

Conclusions and perspectives

Sulfated fucans and sulfated galactans are widespread polysaccharides in marine organisms. These carbohydrates vary from species to species, but the major structural features tend to be conserved in each phylum. They have structural function as components of the extracellular matrix of algal and invertebrate tissues. They are also responsible for induction of the acrosome reaction in sea urchin sperm in a species-specific manner. Through this function, sulfated polysaccharides could play a role in the speciation process which establishes prezygotic reproductive isolation. The algal and invertebrate polysaccharides can also be assayed as alternative anticoagulant agents and represent a potential source of compounds for antithrombotic therapies. Indeed, all these data reveal that the biological actions of sulfated fucans and sulfated galactans do not simply depend on their negative charge density, but is also directly influenced by their structural features (sugar type, specific positions of sulfation and glycosidic linkage).

Further characterization of the action of these polysaccharides involves several challenges. A possible route to follow is the characterization of their binding to target proteins. With respect to their anticoagulant activities, the study of the interaction between polysaccharides with well-defined structures and purified proteins from the coagulation system is especially attractive. Computational modeling of the polysaccharide conformation using molecular dynamics may help to clarify these interactions. The action of sulfated polysaccharides as inducers of the sperm AR suggests the occurrence of plasma membrane receptor (Moy et al. 1996; Gunaratne et al. 2007). Experimental evidence suggests that suREJ1 is one receptor in the sea urchin sperm for the sulfated fucans from egg jelly coats (Vacquier and Moy 1997; Moy et al. 1996). Evidence for an egg receptor for sperm bindin has been shown for two species of sea urchin (Kamei and Glabe 2003). Certainly, studies of the specific interaction between the egg sulfated polysaccharide and the sperm receptor will define the regulation of sea urchin fertilization on a more refined molecular basis.
Preparation of oligosaccharides with well-defined chemical structures is always helpful for studies of carbohydrate-protein interaction. In this aspect we already described a procedure to obtain oligosaccharides from the sulfated fucans, which still retain the regular and repetitive structure (Pomin, Valente et al. 2005; Pomin, Pereira et al. 2005).

Possibly, the major challenge at this stage is the identification of the metabolic pathways involved in the biosynthesis of the invertebrate polysaccharides, especially those from sea urchins. This is not only a fascinating challenge in the carbohydrate field, but may also help to define the genetic basis for the sulfated polysaccharide mechanism of species recognition.

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Conflict of interest statement

None declared.
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Legends to figures

Fig. 1. Structures for the most dominant components of sulfated fucans from brown algae proposed during the years. These polysaccharides have very complex and heterogeneous structures and it is often not easy to determine any regular structure, even if they are present. (A) The 4-sulfated, 2-linked α-L-fucopyranoses was the first proposition by Percival and Ross (1950) as the major units of the fucoidan (name adopted at this time) from the common alga Fucus vesiculosus. In contradiction, after 43 years, Patankar et al (1993) proposed for this same species a more complex structure (B) composed of branched pentasaccharide repetitive units of 3-linked α-L-fucopyranose. The currently proposed structure for the sulfated fucan from this species (C), and also for Ascophyllum nodosum and Fucus evanescences (Chevolot et al. 1999, 2001; Bilan et al. 2002) is a polysaccharide preponderantly composed of 2- and/or 3-sulfated α-L-fucopyranosyl units with alternating 3- and 4-linkages with also occurrence of non-sulfated and branched units. (D) The sulfated fucan from Ecklonia kurome is a polymer of mostly 4-sulfated, 3-linked α-L-fucopyranosyl units (Nishino and Nagumo 1991). (E) The sulfated fucan from Chorda filum contains a quasi-regular structure of a repeating branched hexasaccharide unit but, with a variable 2- and 4-sulfation (Chizhov et al. 1999). The irregularities of the sulfated polysaccharides from E. kurome and C. filum (D and E) arise from the presence of non-sulfate and/or acetylated α-L-fucopyranosyl residues.

Fig. 2. Structures of the repeating units of the sulfated α-L-fucans from the cell wall of the sea cucumber (A) Ludwigothurea grisea (Mulloy et al. 1994) and from the egg jelly coat of sea urchins: (B) Lytechinus variegatus (Mulloy et al. 1994), (C) Strongylocentrotus pallidus (Vilela-Silva et al. 2002), (D) Arbacia lioxula (Alves et al. 1997), (E) Strongylocentrotus purpuratus isotype I and (F) isotype II (Alves et al. 1998), (G) Strongylocentrotus droebachiensis (Vilela-Silva et al. 2002) and (H) Strongylocentrotus franciscanus (Vilela-Silva et al. 1999). The species-specific structures vary in sulfation patterns (but, restricted at 2- and/or 4-positions), in glycosidic linkages: α(1→3) (A-C, E,F and H) and α(1→4) (D and G), and in number of residues of the repetitive units: tetrasaccharides (A-D), trisaccharide (F) and monosaccharides (E,G and F) however, they are all unbranched, linear polymers.
Fig. 3. Structures of the sulfated galactans from (A) red algae (Usov 1998, Lahaye 2001, Farias et al. 2000, Pereira et al. 2005), (B) green algae (Matsubara et al. 2001; Bilan et al. 2007; Farias et al. 2008), (C) sea grass (marine angiosperm) (Aquino et al. 2005), and marine invertebrates, such as (D) ascidians (also known as tunicates) (Mourão and Perlin 1987; Pavão et al. 1989, 1990; Santos et al. 1992; Albano et al. 1990) and (E) sea urchins (Alves et al. 1997).

Fig. 4. Schematic phylogenetic tree showing the proposed relationship among sulfated polysaccharides from marine organisms of different phyla. The structure 3-β-D-Galp-1 is identified by dark gray boxes. The 2-, 4- and 6-sulfations are indicated with light gray stripped, solid and dotted ellipses, respectively. Brown algae (Phaeophyta) exhibit polymers of α-L-fucose bound by (1→3) and (1→4) glycosidic linkages, and with different patterns of sulfation (Pereira et al. 1999; Berteau and Mulloy 2003). Red algae (Rhodophyta) exhibit sulfated galactans composed mainly of the sequence [3-β-D-Galp-1→4-α-D-Galp-1]n (Farias et al. 2000; Pereira et al. 2005). Most of them are composed of 4-α-D-3,6-AnGalp-1 (3,6-anidrogalactose residues) and 3-β-D-Galp-4(SO4)-1, as found in carrageenans, the most common sulfated polysaccharides from red algae (Murano et al. 1997). The preponderant residue of the sulfated galactans from green algae (Clorophyta) is 3-β-D-Galp-4(SO4)-1 (Bilan et al. 2007; Farias et al. 2008). The marine angiosperms (Angiospermae, Spermatophyta) exhibit the repeating sequence: [3-β-D-Galp-4(SO4)-1→3-β-D-Galp-4(SO4)-1→4-α-D-Galp-1→4-α-D-Galp-1]n, comprising structural features of algal and invertebrate sulfated polysaccharides (Aquino et al. 2005). In invertebrates, the sulfated polysaccharides from two species of sea urchins (Equinodermata, Echinoidea) E. lucunter (Alves et al. 1997) and Glyptocidaris crenularis (data not published) exhibit repeating sequences of [3-α-L-Galp-2(SO4)-1]n and [3-β-D-Galp-2(SO4)-1→3-β-D-Galp-1→]n, respectively. The clam Meretrix petechialis (Mollusca, Bivalvia) has a polysaccharide composed of the backbone 3-β-D-Galp-1, mainly 2-sulfated and to some extent 6-sulfated (Amornrut et al. 1999). Some species of ascidians (Urochordata, Asciidiacea) H. monus, S. plicata and Ascidia nigra, Clavelina oblonga (Santos et al. 1992; Albano et al. 1990; Pavão et al. 1989, 1990; respectively) exhibit the unit [4-α-L-Galp-3(OSO4)-1]n. The glycosaminoglycan keratan sulfate can be found in minor amounts as [3-β-D-Galp-6(OSO4)-1→4-β-D-GalNAc-6(OSO4)-1]n (Scudder et al. 1986) such as fishes (Teleostei, Chordata).
Fig. 5. Structures of sulfated polysaccharides from three sea urchin egg jelly that co-habit the harbor at Rio de Janeiro, Brazil, and their respective activities as inducers of the acrosome reaction in homologous or heterologous sperm. The species *A. lixula* and *L. variegatus* express sulfated fucans while *E. lucunter* expresses sulfated galactan. Data modified from Alves et al. (1997).

Fig. 6. Phylogenetic relationship and divergence times of sea urchin species and a summary of the structure of the polysaccharides from their egg jelly. Data modified from Bierrmann *et al.* 2004. The sulfated polysaccharide-mediated mechanism of egg-sperm recognition may have played an important role in the separation of *S. droebachiensis* from *S. pallidus*. The bindin mechanism may have functioned as an isolation mechanism on the earlier separation of the joint lineage from *S. purpuratus* (that exhibit two different isotypes I and II, see also Figure 2E and F). Myr = million years of evolutionary divergence (Bierrmann et al. 2004; Mourão 2007). Experiments show that in *Echinometra*, the sperm bindin bound to the egg is more important than AR induction (Metz et al. 1994), indicating a preponderance of the bindin mechanism of species recognition.

Fig. 7. Schematic representation of the two hierarchical steps in sea urchin gamete recognition. (A) Sulfated polysaccharide-based species recognition: the sperm acrosome reaction is induced when a sperm with the correct receptor type contacts specific sulfated polysaccharide in the egg jelly coat (red triangles). This reaction exposes the protein bindin (shown in blue). (B) The bindin paradigm: the protein bindin, coating the outside of the acrosomal process reacts with a matching egg membrane receptor. Data from Bierrmann *et al.* (2004).
A. *F. vesiculosus* \([2-\alpha-L-Fucp-1]_n\) proposed by Percival and Ross, 1950.

B. *F. vesiculosus* proposed by Patankar et al. 1993. \(\alpha-L-Fucp-1\) \(\downarrow\) 
\([3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\)
\(\alpha-L-Fucp-1\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\)
\(\text{SO}_3^-\) \(\text{SO}_3^-\) 

C. *A. nodosum* \([4-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1]_n\) 
*F. evanescens* \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\)
*F. vesiculosus* \(\text{diSO}_3^-\) \(\text{SO}_3^-\) 
Current proposition + branches of non sulfated \(-\alpha-L-Fucp\)

D. *E. kurome* \([3-\alpha-L-Fucp-1]_n\)
\(R = H \text{ or SO}_3^-\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\) \(\uparrow\)

E. *C. filum* \(R^1 = H \text{ or SO}_3^- \text{ or COCH}_3\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\)
\(R^2 = H \text{ or SO}_3^-\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\)
\([3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3-\alpha-L-Fucp-1\rightarrow 3]\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\)
\(R^1\) \(R^1\) \(R^1\) \(\alpha-L-Fucp-1\) \(R^1\) \(R^1\)
\(2\) \(2\) \(2\) \(2\) \(2\) 
\(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) \(\downarrow\) 
\(\text{diR}^2\) \(\text{R}^2\) 
\(R^2\) \(R^2\) 

Figure 1
Figure 2

A. *L. grisea*

\[
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
diSO_3^- \\
\end{array}
\quad
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\quad
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


B. *L. variegatus*

\[
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
diSO_3^- \\
\end{array}
\quad
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\quad
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


C. *S. pallidus*

\[
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
4 \\
\uparrow \\
SO_3^- \\
\end{array}
\quad
\begin{array}{c}
4 \\
\uparrow \\
SO_3^- \\
\end{array}
\quad
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


D. *A. lixula*

\[
[4-\alpha-L-Fucp-1 \rightarrow 4-\alpha-L-Fucp-1 \rightarrow 4-\alpha-L-Fucp-1 \rightarrow 4-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


E. *S. purpuratus I*

\[
[3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]

\[
\sim 80\% \ SO_3^-
\]


F. *S. purpuratus II*

\[
[3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1 \rightarrow 3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
diSO_3^- \\
\end{array}
\quad
\begin{array}{c}
4 \\
\uparrow \\
SO_3^- \\
\end{array}
\quad
\begin{array}{c}
4 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


G. *S. droebachiensis*

\[
[4-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


H. *S. franciscanus*

\[
[3-\alpha-L-Fucp-1]^n
\]

\[
\begin{array}{c}
2 \\
\uparrow \\
SO_3^- \\
\end{array}
\]


Figure 2
Figure 3

A) Red algae

\[
[3-\beta-D-Gal\beta-1\rightarrow 4-\alpha-Gal\beta-1]_n
\]

- 2-sulfation
- 4-sulfation

- enantiomer: D- (carrageenan) or L- (agaran)
- 3,6-anhydro ring (carrageenose and agarose)
- 2-sulfation
- 3-sulfation
- 6-sulfation

B) Green algae

\[
[3-\beta-D-Gal\beta-1]_n
\]

\[
\text{SO}_3^- \uparrow
\]

C) Sea grass

\[
[3-\beta-D-Gal\beta-1\rightarrow 3-\beta-D-Gal\beta-1\rightarrow 4-\alpha-D-Gal\beta-1\rightarrow 4-\alpha-D-Gal\beta-1]_n
\]

\[
\text{SO}_3^- \uparrow \text{SO}_3^- \uparrow
\]

D) Ascidians

\[
[4-\alpha-L-Gal\beta-1]_n
\]

\[
\text{SO}_3^- \uparrow
\]

- branches of \( \beta-D-Gal\beta \) and/or \( \alpha-L-Gal\beta \), depending on the species

E) Sea urchins

\[
[3-\alpha-L-Gal\beta-1]_n \quad [3-\beta-D-Gal\beta-1 \rightarrow 3-\beta-D-Gal\beta-1]_n
\]

\[
\text{SO}_3^- \uparrow \text{SO}_3^- \uparrow
\]
Figure 4

Eukaryotic ancestor

Brown algae

Red algae

Green algae

Angiosperms

Vertebrates (fishes)

Invertebrates

Tunicates

Keratan sulfate: $[3-\beta-D-Galp-6(SO_4)-1]_n \rightarrow 4-\beta-D-GalNAc-6(SO_4)-1]_n$

Ascidians: $[4-\alpha-L-Galp-3(SO_4)-1]_n$

Clam: $[3-\beta-D-Galp-2(SO_4)-1]_n + \text{minor 6-sulfate}$

Sea-urchins: $[3-\alpha-L-Galp-2(SO_4)-1]_n$

$[3-\beta-D-Galp-4(SO_4)-1]_n \rightarrow 3-\beta-D-Galp-2(SO_4)-1 \rightarrow 4-\alpha-D-Galp-1 \rightarrow 4-\alpha-D-Galp-1]_n$

$[3-\alpha-L-Fucp-2(SO_4)-1]_n$

$[3-\alpha-L-Fucp-2,3\text{di}(SO_4)-1]_n$

$[3-\beta-D-Galp-4(SO_4)-1]_n + \text{minor 6-sulfate}$
Figure 5

Sulfated polysaccharide from:

- A. lixula
- E. lucunter
- L. variegatus

Sperm from:

- A. lixula
- E. lucunter
- L. variegatus

Chemical structures:

- $[^{3-}\alpha-L-\text{Galp-1}]_n$
- $[^{3-}\alpha-L-\text{Fucp-1} \rightarrow ^{3-}\alpha-L-\text{Fucp-1} \rightarrow ^{3-}\alpha-L-\text{Fucp-1} \rightarrow ^{3-}\alpha-L-\text{Fucp-1}]_n$
- $[^{4-}\alpha-L-\text{Fucp-1} \rightarrow ^{4-}\alpha-L-\text{Fucp-1} \rightarrow ^{4-}\alpha-L-\text{Fucp-1} \rightarrow ^{4-}\alpha-L-\text{Fucp-1}]_n$

Acrosome Reaction (%): 50, 40, 30, 20, 10, 0
Sulfated polysaccharide mechanism

Species:
- *S. droebachiensis*
- *S. pallidus*
- *S. purpuratus*
- *S. franciscanus*
- *Lytechinus variegatus*
- *Echinometra lucunter*
- *Arbacia lixula*

**Structure of the sulfated polysaccharide**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Glycosidic linkage</th>
<th>Sulfation pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>L-Fuc 1→4</td>
<td>2S 2S</td>
</tr>
<tr>
<td>II</td>
<td>L-Fuc 1→4</td>
<td>2S 2S 2S 2S 2S</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→3</td>
<td>2S 2S 2S 2S 2S 2S</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→3</td>
<td>2S 2S 2S 2S 2S 2S</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→3</td>
<td>2S 4S (80%)</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→3</td>
<td>2S 4S 4S</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→3</td>
<td>2S 4S 4S</td>
</tr>
<tr>
<td></td>
<td>L-Gal 1→3</td>
<td>2S 2S</td>
</tr>
<tr>
<td></td>
<td>L-Fuc 1→4</td>
<td>2S 2S</td>
</tr>
</tbody>
</table>

Myr = Million years of evolutionary divergence

Figure 6
Figure 7