CHAPTER 12

Structure–Function Relationship of Anticoagulant and Antithrombotic Well-Defined Sulfated Polysaccharides from Marine Invertebrates

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Contents

Abstract

Marine sulfated polysaccharides (MSPs), such as sulfated fucans (SFs), sulfated galactans (SGs), and glycosaminoglycans (GAGs) isolated from invertebrate animals, are highly anionic polysaccharides capable of interacting with certain cationic proteins, such as

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(co)-factors of the coagulation cascade during clotting-inhibition process. Primarily, these molecular complexes between MSPs and coagulation-related proteins seem to be driven mostly by electrostatic interactions. However, through a systematic comparison using several novel well-defined sulfated polysaccharides composed of repetitive oligosaccharides with clear sulfation patterns, it was proved that those molecular interactions are essentially regulated by the stereochemistry of the glycans (which depends on a conjunction of anomeric configurations, sugar types, conformational preferences, glycosylation, and sulfation sites), rather than just a mere consequence of the electronegative density charges (mainly from number of sulfate groups). Here, we present an overview about the structure–function relationship of the invertebrate MSPs with regular structures as potential anticoagulant and antithrombotic agents, as pathologies related to the cardiovascular system are one of the major causes of mortality in the world.

I. INTRODUCTION TO MARINE SULFATED POLYSACCHARIDES: A HIGH TENDENCY FOR REGULAR CHEMICAL STRUCTURES IN INVERTEBRATES

The efforts in both structural and functional studies of marine sulfated polysaccharides (MSPs) have been increasing significantly over the past 10 years (Pomin, 2008; Pomin and Mourão, 2008). This attention is a consequence of the large interest in novel sulfated polysaccharides (SPs) such as glycosaminoglycans (GAGs). GAGs are of particular interest to researchers looking for alternative and/or safer sources of heparin (Mourão and Pereira, 1999). Heparin is the most clinically exploited anticoagulant over the past 50 years (Fareed *et al.*, 2000). Until the early 1990s, sulfated fucans (SFs), homopolymers of fucopyranosyl units exclusively in α-L-form (Pomin, 2008; Pomin and Mourão, 2008), and sulfated galactans (SGs), homopolymers of α-L- or α– and/or β-D-galactopyranosyl units (Pomin, 2009) were usually extracted and characterized from the cell walls of the three major groups of macroalgae. Phaeophyta (brown algae) expresses SFs, while Rhodophyta (red algae) and Chlorophyta (green algae) both express SGs. Historically, algal SFs and SGs are used mainly in clinical tests and large-scale extractions for industrial purposes. Recently however, new and interesting sources of these compounds have been found in the extracellular matrices of certain marine invertebrates (Mourão, 2004, 2007; Mourão and Pereira, 1999; Pomin, 2008, 2009; Pomin and Mourão, 2008). In contrast with most algal sulfated polysaccharides, the invertebrate polymers exhibit highly regular chemical structures (Figs. 12.1 and 12.2A–D), which make it easier to correlate their biological functions to their respective structural features (Pomin, 2008;

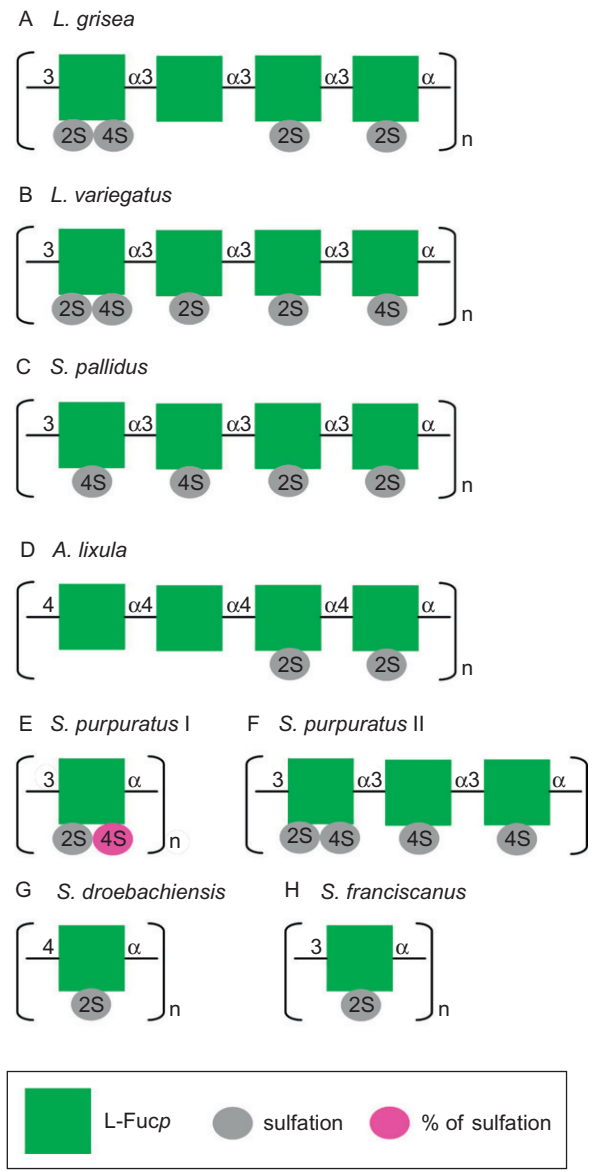

FIGURE 12.1 Chemical structures of repeating units of the SFs from the cell wall of the sea cucumber (A) and from the egg jelly coat of sea urchins (B–H). These polysaccharides are composed of α-L-fucopyranosyl units (green squares). The species-specific structures vary in sulfation patterns (exclusively 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), trisaccharides (F), and monosaccharides (E, G, and H), but they are all linear. The numbers over the black thin lines represent the glycosidic linkage. The gray and violet ellipses represent the right and % of sulfation, respectively. The numbers

Pomin and Mourão, 2008; Vilela-Silva *et al*., 2008). This is of enormous benefit to the entire field of glycobiology. At one time, it was relatively hard to accurately correlate the structure–activity relationship for the majority of SPs. This same advantage is also observed for some marine invertebrate GAGs (Fig. 12.2F). In contrast, many mammalian GAGs exhibit a large variety of sulfation patterns (Gandhi and Mancera, 2008) and consequentially demand much more effort to assay their biological activities, determine their specific structural features, and subsequently propose a dependable structure–function relationship.

The MSPs with well-defined chemical structures (SFs, SGs, and GAGs in Figs. 12.1 and 12.2) are extracted from echinoderms (Echinodermata) like sea cucumbers (Holothuroidea) (Mulloy *et al*., 2004) and sea urchins (Echinoidea) (Pomin and Mourão, 2008; Vilela-Silva *et al*., 2008) or ascidians (Urochordata, Ascidiacea) (Mourão and Perlin, 1987; Pavão, 2002; Pavão *et al*., 1995, 1998; Santos *et al*., 1992; Vicente *et al*., 2001). The SFs (Fig. 12.1B–H) and SGs (Fig. 12.2A and B) are widely spread throughout the jelly coat that surrounds sea urchin eggs. As discussed below, these particular MSPs are physiologically responsible for controlling the external fertilization in sea urchins through a very restrict species-specific manner (Mourão, 2007; Vilela-Silva *et al*., 2008). Among all the MSPs with well-defined chemical structures described so far (Figs. 12.1 and 12.2), there is just a single description from the body wall of the sea cucumber (Fig. 12.1A) (Mulloy *et al*., 2004), a few examples of SGs isolated from the tunic of ascidians (Fig. 12.2C and D) (Mourão and Perlin, 1987; Pavão *et al*., 1998; Santos *et al*., 1992), and some reports from specific red algal cell walls (Fig. 12.2E) (Fonseca *et al*., 2008; Pereira *et al*., 2005). All of the remaining structures (Figs. 12.1B–H and 12.2A and B) belong to the egg jelly coat of sea urchins (Alves *et al*., 1997, 1998; Mulloy *et al*., 2004;

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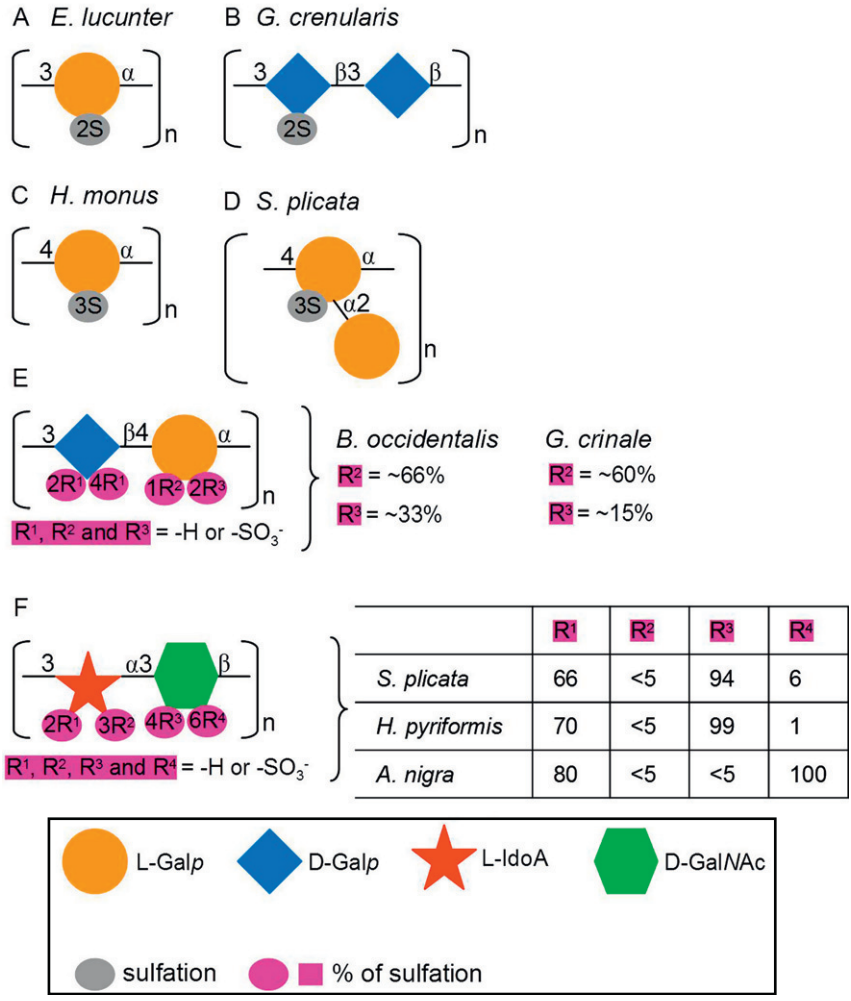
before *S* inside the ellipses represent the sulfation position. The structures are the following: (A) *Ludwgothurea grisea* [→3)-α-L-Fuc*p*-2,4(OSO_3^-)-(1→3)-α-L-Fuc*p*-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→]$_n$ (Mulloy *et al*., 2004); (B) *Lytechinus variegatus* [→3)-α-L-Fuc*p*-2,4(OSO_3^-)-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→3)-α-L-Fuc*p*-4(OSO_3^-)-(1→]$_n$ (Pomin and Mourão, 2008); (C) *Strongylocentrotus pallidus* [→3)-α-L-Fuc*p*-4(OSO_3^-)-(1→3)-α-L-Fuc*p*-4(OSO_3^-)-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→]$_n$ (Vilela-Silva *et al*., 2002); (D) *Arbacia lixula* [→4)-α-L-Fuc*p*-2(OSO_3^-)-(1→4)-α-L-Fuc*p*-2(OSO_3^-)-(1→4)-α-L-Fuc*p*-(1→4)-α-L-Fuc*p*-(1→]$_n$ (Alves *et al*., 1997); (E) *Strongylocentrotus purpuratus*-I ∼80% [→3)-α-L-Fuc*p*-2,4(OSO_3^-)-(1→]$_n$ and ∼20% [→3)-α-L-Fuc*p*-2(OSO_3^-)-(1→]$_n$ and (F) *S. purpuratus*-II [→3)-α-L-Fuc*p*-2,4(OSO_3^-)-(1→3)-α-L-Fuc*p*-4(OSO_3^-)-(1→3)-α-L-Fuc*p*-4(OSO_3^-)-(1→]$_n$ (Alves *et al*., 1998); (G) *Strongylocentrotus droebachiensis* [→4)-α-L-Fuc*p*-2(OSO_3^-)-(1→]$_n$ (Vilela-Silva *et al*., 2002); and (H) *Strongylocentrotus franciscanus* [3)-α-L-Fuc*p*-2(OSO_3^-)-(1→]$_n$ (Vilela-Silva *et al*., 1999).



	R1	R2	R3	R4
S. plicata	66	<5	94	6
H. pyriformis	70	<5	99	1
A. nigra	80	<5	<5	100

FIGURE 12.2 Chemical structures of the repeating units of SGs from the egg jelly coat of sea urchins (A and B), from the tunic of ascidians (C and D), and from the red algae (E); and of the repeating units of DSs from ascidians (F). These polysaccharides are composed of L-galactopyranoses (orange circles), D-galactopyranoses (blue diamond), L-iduronic acids (red star), and D-*N*-acetylgalactosamines (green hexagons). The numbers over the black thin lines represent the glycosidic linkage. The gray and violet boxes and ellipses represent the right and % of sulfation, respectively. The numbers before *S* and *R* inside the ellipses represent the positions of sulfation or radicals. The superscripted numbers after *R* represent the respective radical. The structures are the following: (A) *Echinometra lucunter* (Alves *et al.*, 1997); (B) *Glyptocidaris crenularis* [→3-α-L-Gal*p*-2(OSO_3^-)-1→3-α-L-Gal*p*-1→]$_n$ (Castro *et al.*, 2009); (C) *Herdmania monus* [→4)-α-L-Gal*p*-3(SO_3^-)-(1→]$_n$ (Santos *et al.*, 1992); (D) *Styela plicata* {→4)-α-L-Gal*p*-2[→1)-α-L-Gal*p*-3(OSO_3^-)]-3(OSO_3^-)-(1→}$_n$ (Mourão and Perlin, 1987); (E) both *Botriocladia occidentalis* and *Gelidium crinali* express [3-β-D-Gal*p*-1→4-α-Gal-1→]$_n$ with different sulfation contents (Fonseca *et al.*,

Vilela-Silva *et al.*, 1999, 2002). The body of some species of ascidians also contains peculiar GAGs that show distinct sulfation patterns from mammalian GAGs (Pavão *et al.*, 1995, 1998) as exemplified with the tunicate dermatan sulfates (DSs) in Fig. 12.2F.

II. THE INTERACTION OF MSPS WITH COAGULATION (CO)-FACTORS: PREVENTION OF BLOOD COAGULATION

Unrelated to the natural biological role as inducers of fertilization, the SFs and SGs also exhibit potential pharmacological actions in mammalian systems. Among several clinical activities: antiviral, antimetastatic, antiangiogenic, anti-inflammatory, antiadhesive (Coombe *et al.*, 1987; Cumashi *et al.*, 2007; Harrop *et al.*, 1992), the anticoagulant and antithrombotic actions are the most explored so far (Farias *et al.*, 2000; Pomin, 2008). This is due to a pressing need for new antithrombotic drugs as a consequence of the increasing incidence of thromboembolic diseases. In fact, cardiovascular diseases are the leading cause of death worldwide (30% of total causes) (Becker *et al.*, 2007; Berteau and Mulloy, 2003; Cumashi *et al.*, 2007; Farias *et al.*, 2000; Mourão, 2004; Mourão and Pereira, 1999, Pereira *et al.*, 1999, 2002a,b, 2005; Pomin, 2008, 2009; Pomin and Mourão, 2008).

The SFs and SGs show great advantages as alternative sources for anticoagulant therapies, especially because of the massive use of heparin (Fareed *et al.*, 2000). This mammalian GAG has the highest negative charge density ever seen for a natural molecule. It is also well known to present several limitations due to collateral effects and limited sources of material (Mourão, 2004). The situation has been complicated due to the alarming discovery of heparin preparations that 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).

The new MSPs (Figs. 12.1 and 12.2) offer some advantages over heparin. They show considerably low contamination levels of virus and/or prions because they are exclusively extracted from marine sources. Contamination in clinical solutions of heparin can occur more easily, as this compound is extracted from mammalian sources, like porcine and bovine intestinal mucosa and bovine lung (Mourão, 2004). Moreover, the invertebrate MSPs are promising to be more useful clinical reagents than algal

2008; Pereira *et al.*, 2005); and (F) the DS from *Styela plicata*, *Halocynthia pyriformis*, and *Ascidian nigra* are composed of [4-α-L-IdoA-1→3-β-D-GalNAc-1]$_n$ with also different sulfation patterns (Pavão *et al.*, 1995, 1998).

SPs due to their regular and well-defined structures (Mourão, 2004; Pomin, 2008; Pomin and Mourão, 2008), even though algal molecules have been utilized more until now.

In addition to all the benefits described above, the very clear and regular structures of invertebrate SFs (Fig. 12.1) and SGs (Fig. 12.2A–D), the algal SGs (Fig. 12.2E), and the ascidians GAGs (Fig. 12.2F) enable determination of well-defined structure–function relationships (Pomin, 2008; Pomin and Mourão, 2008). This knowledge allows prediction of the major molecular features involved in molecular interactions such as those between the MSPs and blood (co)-factors, similarly used for the fertilization assays mentioned in the previous section. The anticoagulant action of the SPs resides mainly in the potentiation of natural inhibitors of plasma proteases. The plasma proteases include activated factor II (IIa, commonly known as thrombin) and activated factor X (Xa). Inhibitors include the serpins (antithrombin, AT; and heparin cofactor II, HCII). The catalysis of SPs can be classified into two distinct mechanisms: (i) the allosteric change of the serpins induced by the SPs and (ii) the template mechanisms, where the glycosidic chain of the SPs can act as a ''bridge'' that brings together both protease and serpin. The mechanisms for AT and HCII are illustrated in Fig. 12.4A and B, respectively.

Next, we will describe particular cases of how each specific structural feature of the MSPs (Figs. 12.1 and 12.2) can individually account for different levels of activities and interactions with serpins and/or blood proteases (Fig. 12.3). This results in differing kinetics to prevent clot formation. The particular structural contributions of the MSPs can be properly recognized when the anticoagulant properties of these biopolymers are systematically compared. In addition, the results might offer qualitatively accurate data. This systematic comparison was carried out by two *in vitro* anticoagulant assays: (i) the APTT (activated partial thromboplastin time) which measures the general anticoagulant effect where all the plasma proteins are included and (ii) the direct measurement of the inhibition level of factors IIa or Xa by AT or HCII in the presence of the MSPs. Results from these assays are compiled in Table 12.1.

III. DECODING THE REGULATING STRUCTURAL FEATURE OF MSPS IN INTERACTIONS WITH COAGULATION (CO)-FACTORS

A. An example of influence by sugar type

When we compare all the structures in Figs. 12.1 and 12.2, we can trace structural similarities and differences for the SFs and SGs. For example, both SF from *Strongylocentrotus franciscanus* (Fig. 12.1H) and SG from

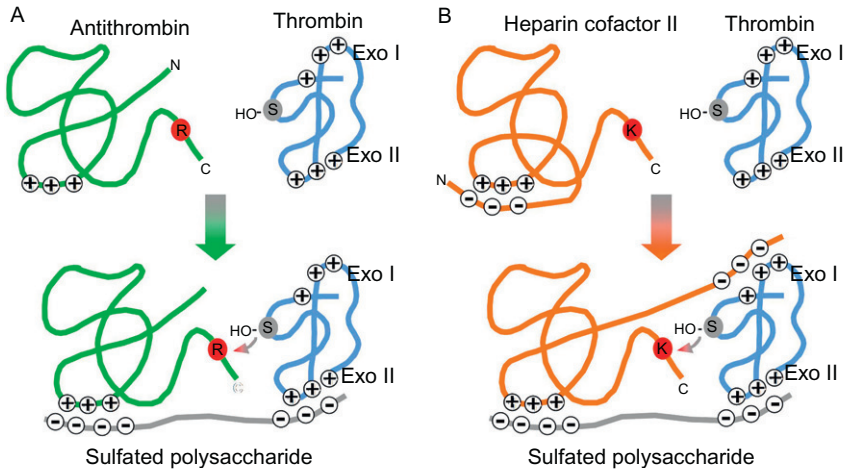


FIGURE 12.3 Molecular schematic representation of the anticoagulant mechanisms of SPs. The thrombin (IIa, in blue) can be inhibited by serpins such as antithrombin (AT, in green) or heparin cofactor-II (HCII, in orange). In both cases, the SP (gray line) brings together the serpins and the protease-IIa mainly through electrostatic interactions of their opposite charges. In the thrombin, this charged cluster is the EXO II. Next, the hydroxyl groups of a serine (S) residue from thrombin will bind to the C-terminus of the serpins, actually to an arginine (R) residue of the AT (A), or to a lysine (K) residue in the case of the HCII (B). In the bind states, a conformational change will occur in both serpins, although this change is more predominant and necessary at the HCII case. (B) Note that the N-terminus of the HCII will interact also with the EXO I of IIa through also electrostatic contacts. With the examples described throughout the text, it is clear that the template mechanism between SPs (SFs, SGs, GAGs), serpins (AT, HCII), and protease (IIa) has differential stabilities or formation kinetics directly related to the structural features of the SPs.

Echinometra lucunter (Fig. 12.2A) present the same sulfation pattern (exclusive and entirely 2-sulfated), the same anomeric configuration (α-form), the same glycosidic linkage (1→3), and the same molecular mass (∼100 kDa). Their single difference is their sugar type (fucopyranose or galactopyranose, respectively) (Pomin, 2008). Interestingly, this single structural difference is itself enough to promote great changes in the anticoagulant properties of these homopolysaccharides. The 2-sulfated α-galactan from *E. lucunter* exhibits a significant anticoagulant activity (APTT of 20 IU mg, although almost 10-fold less than UFH, Table 12.1). The specific anticoagulant assay with the purified proteases revealed that this SG enhances both IIa and factor Xa inhibition by either AT or HCII (Table 12.1). However, the anticoagulant effect of 2-sulfated α-fucan from *S. franciscanus* is exclusively based on catalysis of AT inhibition over factor Xa, although it

TABLE 12.1 Anticoagulant activities of MSPs measured by APTT[a] and by IC_{50} for thrombin (IIa) and factor Xa inhibition in the presence of antithrombin (AT) or heparin cofactor II (HCII) (Fonseca *et al.*, 2008; Mourão, 2004; Mourão and Pereira, 1999; Pavão *et al.*, 1998; Pereira *et al.*, 2005)

				IC_{50} (μg/mL)		
Polysaccharide	Source	Structure (figure)	APTT (IU/mg)	IIa/AT	IIa/HCII	Xa/AT
3-linked sulfated α-L-fucans	*S. purpuratus* (I)	1E	76	0.3	0.3	2
	S. purpuratus (II)	1F	10	0.9	2	ND
	S. pallidus	1C	18	>500	>500	>500
	L. variegatus	1B	3	>500	>500	>500
	S. franciscanus	1H	~2	>500	>500	250
	L. grisea	1A	<1	>500	>500	>500
4-linked sulfated α-L-fucans	*S. droebachiensis*	1G	<1	ND	ND	ND
	A. lixula	1D	~2	150	150	>500
Sulfated α-L-galactans	*E. lucunter*	2A	20	3	6	20
	H. monus	2C	~2	>500	>500	>500
	S. plicata	2D	<1	>500	>500	>500
Algal SGs (Fonseca *et al.*, 2008; Pereira *et al.*, 2005)	*B. occidentalis*	2E	93	0.02	1.1	2.5
	G. crinali		65	0.02	25	1.5
Ascidian DSs (Pavão *et al.*, 1998)	*S. plicata*	2F	8	Inactive	0.31	Inactive
	H. pyriformis		11	Inactive	0.35	Inactive
	A. nigra		<5	Inactive	320	Inactive
Mammalian standard DSs (Pavão *et al.*, 1998)	Native		2	nr[b]	3	nr
	Oversulfated		13	nr	2	nr

[a] The activity is expressed as international units/mg using a parallel standard curve based on the International Heparin Standard (193 units/mg).
[b] Nonreported.

is 12.5-fold less active than the α-SG. This single effect over the Xa/AT system explains the much lower activity of the compound from *S. franciscanus* (APTT of ∼2IUmg^{-1}, 100-fold less active than UFH), as the anti-Xa activity has a relatively minor influence on the APTT. This is an illustrative and typical example of a sugar-type-dependent biological effect of polysaccharides.

B. An example of preferential conformation binding

Interaction energies obtained from molecular dynamic simulations are statistically equivalent for both 2-sulfated α-L-galactan from *E. lucunter* (Fig. 12.2A) and 2-sulfated α-L-fucan from *S. franciscanus* (Fig. 12.1H). This would be expected based on the structural similarities of these two compounds, especially conformations in solution. However, they revealed markedly different interactions for binding with the complex AT/IIa. The explanation for this result is the extreme difference in orientations on the AT-binding site (∼90°, Fig. 12.4C and D). Such difference in orientation upon binding to AT explains the lack of activity of SF under the same experimental conditions and fully supports the expected bridging mechanism in the activity of SG, as previously described (Melo *et al.*, 2004). Thus, both compounds are capable of interacting with AT as indicated by theoretical results and experimental data showing that

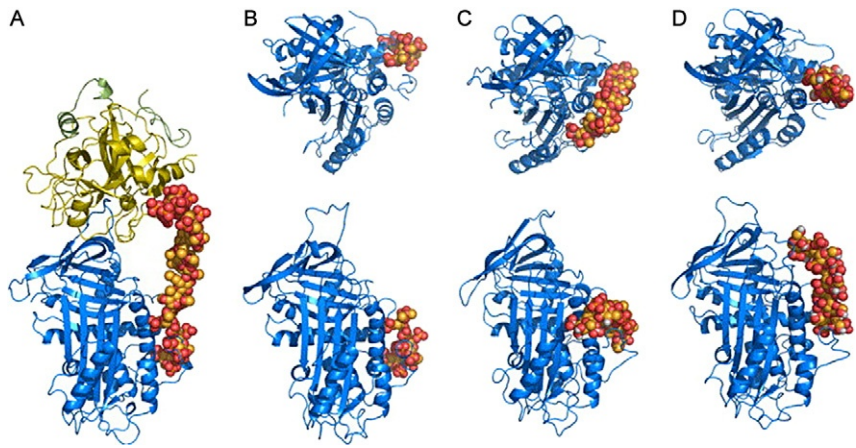


FIGURE 12.4 Structures of the complexes between different SPs and AT. (A) Ternary complex between AT, thrombin, and an heparin derivative (PDB ID 1TB6); (B) AT bounded to the synthetic pentasaccharide (PDB ID 1E03); (C) final structure from a 5-ns MD of AT complexed to a SF decasaccharide with pyranose rings; (D) final structure from a 5-ns MD of AT complexed to a SG decasaccharide with pyranose rings. For (B)–(D), two orientations of the complexes are presented. Data from Becker *et al.* (2007).

both polysaccharides are retained on an AT-affinity column. However, only the complex formed by the SG and the AT (Fig. 12.4D) allows further interaction with IIa. An analogous complex is the ternary complex between heparin-AT-IIa (Fig. 12.4A) which supports the potentiation of this protease inactivation by AT in the presence of the SG. This is an example of how the orientational constraints in solution can influence spatial preferences of the SPs for their different bindings with target proteins (Melo *et al.*, 2004). It is obvious that the swap of the sugar type will drastically change the conformation of the polymer as well, implying a different conformational binding as explained in this section.

C. An example of influence by sulfation pattern

Based on this same systematic comparison, the SGs from the red algal species, *Botryocladia occidentalis* and *Gelidium crinale*, exhibited identical backbones and the same chain size. However, there are slight differences in their sulfation patterns (Fig. 12.2E). As a consequence of this difference, the two algal SGs differ in their anticoagulant and venous antithrombotic activities as previously described (Fonseca *et al.*, 2008). SGs from *G. crinale* exhibit procoagulant and prothrombotic effects in low doses (up to 1.0 mg/kg body weight). In high doses (>1.0mg/kg), this polysaccharide inhibits both venous and arterial thrombosis in rats. In contrast, SG from *B. occidentalis* is a very potent anticoagulant and antithrombotic compound in low doses (up to 0.5mg/kg body weight), inhibiting venous experimental thrombosis, but these effects are reverted in high doses. Conversely, arterial thrombosis is only inhibited at high doses (>1.0 mg/kg) of the polysaccharide from *B. occidentalis*. These results indicate that slight differences in the proportions and/or distribution of sulfated residues along the galactosyl chain may be critical for the interaction between proteases, inhibitors, and activators of the coagulation system, resulting in a distinct pattern in anti- and procoagulant activities and in the antithrombotic action. As summarized in Table 12.1, these structural differences account for the 30% difference in anticoagulant activity (APTT) of these algal macromolecules and even greater difference in catalytic effect of the sulfated polysaccharide on HCII-mediated anti-IIA activity.

Indeed, the structural requirement for the interaction of these SPs with the coagulation cofactors and their target proteases and inhibitors are stereospecific (Mourão, 2004; Mourão and Pereira, 1999; Pomin, 2008; Pomin and Mourão, 2008). The site of sulfation has a major impact on activity. This can be illustrated by the fact that 2,4-di-sulfated units have an amplifying effect on the AT-mediated anticoagulant activity in the series of 3-linked α-L-fucans (Fig. 12.1; Table 12.1). Specific sulfation sites are required for the interaction with plasma serine-protease inhibitors. Note the occurrence of the 4-sulfated unit content in the

3-linked α-L-fucans: *Lytechinus variegatus* (a single 4-sulfated unit/tetrasaccharide—Fig. 12.1B), *Strongylocentrotus pallidus* (a double 4-sulfated unit/tetrasaccharide—Fig. 12.1C), and *Strongylocentrotus purpuratus*, isotype II (a double 4-sulfated unit/trisaccharide—Fig. 12.1F). This 4-sulfation is the structural motif required to enhance the inhibition of IIa by HCII. In contrast, the presence of 2-sulfated residues seems to have a deleterious effect on HCII-mediated anti-IIA activity of the polysaccharides (Mourão, 2004).

Ascidian DSs with the backbone structure [4-α-L-IdoA-1→3-β-D-GalNAc-1]$_n$ but with different patterns of sulfation (Fig. 12.2F) also reveal different anticoagulant activities (Table 12.1) (Pavão *et al.*, 1995,1998; Vicente *et al.*, 2001). They are also useful and reliable cases to describe the influence of sulfation sites in biological actions. All three ascidian DSs have a high content of 2-*O*-sulfated α-L-iduronic acid residues but differ in the pattern of sulfation of the *N*-acetyl-β-D-galactosaminyl units. *Styela plicata* and *Halocynthia pyriformis* have 4-*O*-sulfated units, but in *Ascidian nigra*, they are 6-*O*-sulfated. This collection of ascidian DSs, where the extent and position of sulfate substitution have been widely characterized, was examined in anticoagulant assays. DS from *A. nigra* has no discernible anticoagulant activity, which indicates that 4-*O*-sulfation of the *N*-acetyl-β-D-galactosamine is essential for that activity (Table 12.1). In contrast, DSs from *S. plicata* and *H. pyriformis* are potent anticoagulants due to potentiation of thrombin inhibition by HCII. These ascidian DSs have ∼10- and ∼6-fold higher activity with HCII than native and oversulfated mammalian DSs, respectively (Table 12.1). They have no effect on factors IIa- or Xa inhibition by AT. These highly sulfated ascidian DSs show that they require very selective sulfation sites for promoting the HCII interactions, and consequently, their highly specific anticoagulant properties are intimately related to their sulfation levels (Table 12.1 vs. Fig. 12.2F).

IV. REMARK CONCLUSIONS

The example cases in this chapter have clearly shown how the structural features of marine homopolysaccharides (SFs and galactans) can be evaluated for interactions with coagulation proteins (acting as promising anticoagulant or antithrombotic reagents). It was observed that the carbohydrate–protein complexes are indeed stereospecific and not a mere consequence of charge density. It is important to note that the glycosidic structural characteristics examined individually in this chapter do not really account independently and/or exclusively for the biological actions or molecular interactions. Obviously, the active or inactive conformations of these homopolysaccharides arise from the presence of all structural features together, which comprise the ''final code'' required for

high-affinity interactions with the target proteins. However, specific or major structural features of the carbohydrates certainly act as preponderant regulators in the biological functions as succinctly described in this work.

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