Glycoconj J (2014) 31:341–344
DOI 10.1007/s10719-014-9530-1

Anticoagulant motifs of marine sulfated glycans

Vitor H. Pomin

Received: 8 April 2014 / Revised: 3 May 2014 / Accepted: 5 May 2014 / Published online: 20 May 2014
© Springer Science+Business Media New York 2014

Abstract Sulfated polysaccharides, like the glycosaminoglycan (GAG) heparin, are known to exhibit anticoagulant properties when certain structural features are present. The structural requirement for this action is well-established for heparin, in which a pentasaccharide motif plays a key role for keeping the high-affinity interaction to antithrombin. Over the last years of this glycomic era, several novel anticoagulant sulfated glycans have been described. Those from marine sources have been awakening special attention mainly because of their impressive anticoagulant effects together with structural uniqueness. The commonest of these glycans are the sulfated fucans (SFs), the sulfated galactans (SGs), and the marine invertebrate GAGs like the fucosylated chondroitin sulfate and ascidian dermatan sulfate. Since these marine sulfated glycans do not bear within their polymeric chains the specific pentasaccharide motif of heparin, other structural features must be necessary to trigger the anticoagulant effect. The objective of this report is to present the anticoagulant motifs of the marine SFs, SGs and GAGs.

Keywords Carbohydrate-based drug development · Glycosaminoglycans · Sulfated fucans · Sulfated galactans · Sulfation pattern

Heparin: the antithombin high-affinity pentasaccharide is the structural requirement for the anticoagulant activity

Sulfated polysaccharides are endowed with multiple therapeutic functions such as anticoagulant [1, 2], antithrombotic [2], anti-inflammatory [3], antivirus [4], and anticancer [5] activities. These functions occur as a result from certain structural combinations of sulfation pattern and glycosylation type [6]. Anticoagulant activity is by far the mostly studied and desirable clinical activity of the sulfated polysaccharides. This is because of the high number of thromboembolic patients and deaths around the world each year. Heparin is the mostly used anticoagulant/antithrombotic agent in therapies involving the heart-and-blood diseases [7]. Heparin is a glycosaminoglycan composed mostly of alternating 4-linked α-L-iduronic acid (IdoA) and 4-linked α-D-glucosamine (GlcN). While IdoA units are mostly 2-sulfated, GlcN units are frequently *N*-sulfated (GlcNS) (Fig. 1). In addition, GlcN units can be also *N*-acetylated, *N*-unsubstituted (rarely), 3-sulfated (rarely), and 6-sulfated (commonly), as shown at Fig. 1. Sometimes, the unepimerized glucuronic acid (GlcA) rarely carrying 2-sulfation can also participate as a structural component. All these variations contribute significantly to enhance the structural heterogeneity of heparin. However, for the best anticoagulant activity of heparin, the presence of a pentasaccharide motif carrying the rare 3-*O*-sulfation is required (Fig. 2). This pentasaccharide exhibits high-affinity for antithrombin [8]. A synthetic version of this pentasaccharide, also known as fondaparinux, is a widely used drug available in the market. It was chemically synthesized and trademarked at the first time by GlaxoSmithKline under the commercial trade name Arixtra [9]. This pentasaccharide is the mostly known example of a biologically active structural motif of a sulfated glycan.

The novel marine sulfated glycans

Heparin is not the only sulfated polysaccharide possessed of anticoagulant activity. A large number of other sulfated polysaccharides exist, especially those of marine sources of very unique structures [10–12]. The commonest examples of marine sulfated glycans under research in this glycomic age are the sulfated fucans (SFs) [10], the sulfated galactans (SGs) [10], and the unique GAGs isolated from invertebrate

V. H. Pomin (✉)
Program of Glycobiology, Institute of Medical Biochemistry Leopoldo de Meis, University Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, R. Prof. Rodolpho Paulo Rocco, 255, HUCFF 4A01, Ilha do Fundão, Rio de Janeiro, RJ 21941-913, Brazil
e-mail: pominvh@bioqmed.ufrj.br

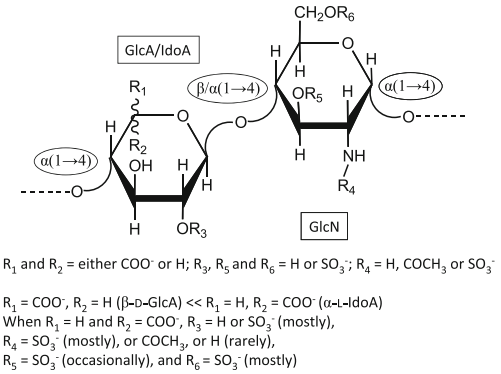

Fig. 1 Representative structure of the repeating disaccharide unit of heparin. It is composed of alternating 4-linked uronic acid (iduronic acid, IdoA, or glucuronic acid, GlcA) and 4-linked α-glucosamine (GlcN) units. Although heparin has some β-D-GlcA units, its major uronic acid type is α-L-IdoA with 2-*O*-sulfation. The amino group of the composing GlcN unit can be unsubstituted (NH_2), *N*-acetylated ($NHCOCH_3$), or *N*-sulfated ($NHSO_3^-$). While the former is the rarest substituent, the latter is the major substituent. The Glc*N*,6-disulfated residue is the commonest unit in heparin. Although the 3-*O*-sulfation at the GlcN unit occurs rarely, it is relevant for the anticoagulant activity of heparin due to its high-affinity for antithrombin. The glycosidic bonds are indicated in ellipses whereas monosaccharide types are indicated in *rectangles*

species like the unique dermatan sulfates [12] and the fucosylated chondroitin sulfate [11]. The SFs, mostly composed of α-L-fucopyranosyl units, can be isolated from brown algae, sea urchins and sea cucumbers. The SGs, mostly composed of α-L-, α-D-, or β-D-galactopyranosyl units, can be isolated from red and green algae, ascidian and sea urchins. The distinct GAGs like the dermatan sulfate, which exhibits a distinct pattern of sulfation if compared to the commonest mammalian dermatan sulfate; and the fucosylated chondroitin sulfate, which has a branched α-L-Fuc*p* unit linked to the 3-position of the backbone GlcA unit, are found respectively in ascidians and sea cucumbers. These marine biopolymers are heavily sulfated. The sulfation patterns vary from species-to-species.

The structural requirements involved in anticoagulation

Like heparin, the serine protease inhibitor (serpin)-dependent anticoagulant functions of the marine sulfated glycans are driven basically by enhancing inhibitory activities of the two major serpins of the blood, antithrombin (AT) and heparin cofactor II (HCII) over some blood cofactors like the proteases thrombin, factor IIa, and factor Xa. When certain structural requirements are present in the marine sulfated polysaccharides, they are able to trigger and/or catalyze the inhibitory activities of AT and HCII via two principal mechanisms. The template mechanism, in which the sulfated polysaccharide makes a molecular bridge between the serpin and the protease, enabling thus the formation of the ternary complex (sulfated polysaccharide-serpin-protease). And, the allosteric mechanism in which a sulfated glycan-induced conformational change of the serpin allows inhibition of the proteases. The occurrence and predominance of either one or both of these two mechanisms during the anticoagulant reactions are generally controlled by intrinsic structural features of the sulfated glycans [6].

Hence, not all marine SFs, SGs, and invertebrate GAGs present anticoagulant activities. Over the last 25 years, our laboratory has made great efforts to recognize and assign the molecules and species names capable to exhibit the anticoagulant effect [12, 13]. In our investigations, we were mostly interested in identifying also the respective structural features of these molecules involved in their anticoagulant potentials or different levels of response. Through a systematic analysis and comparative interpretation, we were able to point out these major structural requirements. They are the 2-sulfation

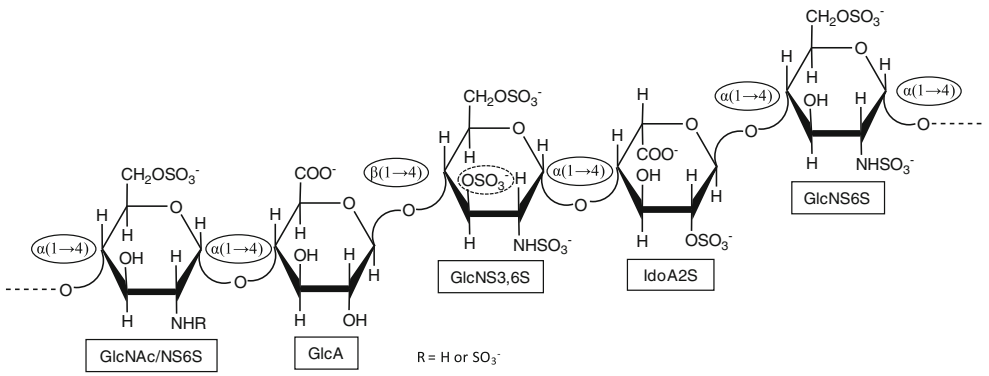

Fig. 2 Antithrombin (AT)-binding site composed of the following pentasaccharide structure [→4)-D-GlcNAc/NS6S-α-(1→4)-D-GlcA-β-(1→4)-D-GlcNS3,6S-α-(1→4)-L-IdoA2S-α-(1→4)-D-GlcNS6S-α-(1→]. The glycosidic bonds are indicated in ellipses, whereas monosaccharide types are indicated in rectangles. The abbreviations GlcNAc/NS6S; GlcA; GlcNS3,6S; and IdoA2S; stand for *N*-acetyl glucosamine/*N*-,6-disulfated glucosamine; glucuronic acid, *N*-, 3-,6-tri-sulfated glucosamine; and 2-sulfated iduronic acid; respectively. The 3-*O*-sulfation required for the antithrombin high-affinity is highlighted with the dash ellipse for fast notation. The glycosidic bonds are indicated in ellipses, whereas monosaccharide types are indicated in *rectangles*

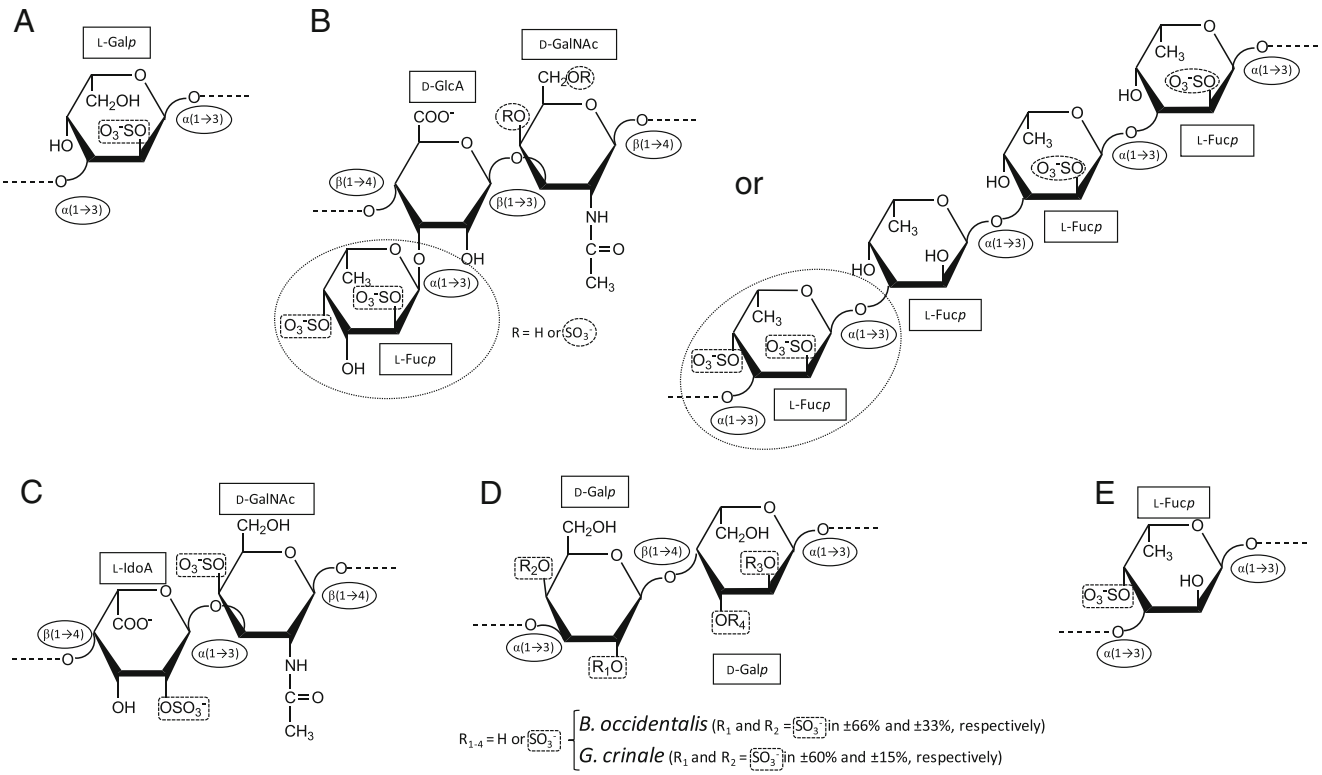

Fig. 3 General representation of the anticoagulant structures found in marine SFs, SGs and GAGs. **a** The 3-linked 2-sulfated α-L-Gal*p*-contained polysaccharide from the sea urchin *Echinometra lucunter* [14]. **b** The two anticoagulant molecules from the holothurian species *Ludwigothurea grisea*: the fucosylated chondroitin sulfate (*right panel*) and the SF (*left panel*), both containing the functional unit 2,4-disulfated α-L-Fuc*p* (indicated by dotted ellipses) [15]. **c** The ascidian dermatan sulfate composed of alternating 4-linked 2-sulfated α-L-IdoA residue and 3-linked 4-sulfated D-GalNAc unit isolated from the species *Styela plicata* [12]. **d** The red algal SGs composed of alternating β-3-linked and α-4-linked D-Gal*p* units within different sulfation content depending on the species (*Botriocladia occidentalis* vs *Gelidium crinale*) [16]. **e** The 3-linked 4-sulfated α-L-Fuc*p*-containing polymer seen in sea urchins and sea cucumber SFs [17]. The glycosidic linkages and monosaccharide types are represented in continuous ellipses and rectangles, respectively. Sulfation sites involved or not in the anticoagulant activity are shown in dashed rectangles and ellipses, respectively. The sugar units labeled as L-Gal*p*, D-GlcA, D-GalNAc, L-Fuc*p*, and L-IdoA stand for L-galactopyranose, D-glucuronic acid, *N*-acetyl D-galactosamine, L-fucopyranose, and L-iduronic acid, respectively. See Table 1 for biological outcome of these structures

in 3-linked α-L-SGs [14]. The 2,4-disulfation in α-L-fucopyranosyl units found either as composing units of certain sea urchin and sea cucumber linear SFs, or as branching units of the holothurian fucosylated chondroitin sulfate [15]. The levels of 4-sulfation at the galactosamine units combined with certain levels of 2-sulfation at the IdoA units in the ascidian dermatan sulfates [12]. The sulfation content in red algal SGs composed of equal and homogenous backbones [16]. And finally the 4-sulfation found in 3-linked α-L-SFs [17]. This latter enhances the inhibitory activity of HCII over IIa [17]. The four former structural requirements enhance both HCII and AT inhibitory activity over both coagulation proteases, IIa and Xa [12, 14–16]. These structures with anticoagulant activities are depicted in Fig. 3 for illustrative purposes. Table 1 also summarizes in a straightforward way these structural requirements of the marine sulfated glycans and the respective anticoagulant effects.

Table 1 Summary of the structural requirements, and effects in anticoagulation of the marine sulfated polysaccharides

Structural requirement	Consequence
2-sulfated 3-linked α-L-Gal*p* [14] 2,4-disulfation in Fuc*p* [15]	Enhance serpin (HCII and AT) inhibitory activity over the coagulation proteases (IIa and Xa)
4-sulfated GalNAc + 2-sulfated IdoA in ascidian dermatan sulfates [12]	
Sulfation content in red algal homogeneous SGs [16]	
4-sulfation in invertebrate 3-linked SFs [17]	Enhance HCII-dependent IIa inhibition

Conflict of interest The author states that he is not aware of any authorship, affiliations, memberships, funding, or financial holdings that might be perceived as damaged or as affecting the objectivity of the content of this material. The author declares no conflict of interest by any part.

Funding This study was supported by the grants Universal-14/2013-[470330/2013-9] and E-26/110.961/2013 from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), respectively. The content of this work is solely the responsibility of the author and does not necessarily represent the official views of the funding agencies.

References

1. Pereira, M.S., Mulloy, B., Mourão, P.A.S.: Structure and Anticoagulant Activity of Sulfated Fucans. Comparisom between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. J. Biol. Chem. **274**, 7656–7667 (1999)
2. Mourão, P.A.S.: Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. Curr. Pharm. Des. **10**, 967–981 (2004)
3. Cumashi, A., Ushakova, N.A., Preobrazhenskaya, M.E., D'Incecco, A., Piccoli, A., Totani, L., Tinari, N., Morozevich, G.E., Berman, A.E., Bilan, M.I., Usov, A.I., Ustyuzhanina, N.E., Grachev, A.A., Sanderson, C.J., Kelly, M., Rabinovich, G.A., Iacobelli, S., Nifantiev, N.E., Consorzio Interuniversitario Nazionale per la Bio-Oncologia, Italy: A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology **17**, 541–552 (2007)
4. Baba, M., Snoeck, R., Pauwles, R., de Clercq, E.: Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. Antimicrob. Agents Chemother. **32**, 1742–1745 (1988)
5. Vishchuk, O.S., Ermakova, S.P., Zvyagintseva, T.N.: Sulfated polysaccharides from brown seaweeds Saccharina japonica and Undaria pinnatifida: isolation, structural characteristics, and antitumor activity. Carbohydr. Res. **346**, 2769–2776 (2011)
6. Pomin, V.H.: Review: an overview about the structure-function relationship of marine sulfated homopolysaccharides with regular chemical structures. Biopolymers **91**, 601–608 (2009)
7. Hirsh, J., Anand, S.S., Halperin, J.L., Fuster, V., American Heart Association: Guide to anticoagulant therapy: Heparin: a statement for healthcare professionals from the American Heart Association. Circulation **103**, 2994–3018 (2001)
8. Petitou, M., Casu, B., Lindahl, U.: 1976–1983, a critical period in the history of heparin: the discovery of the antithrombin binding site. Biochimie **85**, 83–89 (2003)
9. Giangrande, P.L.: Fondaparinux (Arixtra): a new anticoagulant. Int. J. Clin. Pract. **56**, 615–617 (2002)
10. Pomin, V.H., Mourão, P.A.: Structure, biology, evolution, and medical importance of sulfated fucans and galactans. Glycobiology **18**, 1017–1027 (2008)
11. Pomin, V.H.: Holothurian chondroitin sulfate. Mar. Drugs **12**, 232–254 (2014)
12. Pavão, M.S., Aiello, K.R., Werneck, C.C., Silva, L.C., Valente, A.P., Mulloy, B., Colwell, N.S., Tollefsen, D.M., Mourão, P.A.: Highly sulfated dermatan sulfates from Ascidians. Structure versus anticoagulant activity of these glycosaminoglycans. J. Biol. Chem. **273**, 27848–27857 (1998)
13. Pomin, V.H.: Structure-function relationship of anticoagulant and antithrombotic well-defined sulfated polysaccharides from marine invertebrates. Adv. Food Nutr. Res. **65**, 195–209 (2012)
14. Pereira, M.S., Vilela-Silva, A.C., Valente, A.P., Mourão, P.A.: A 2-sulfated, 3-linked alpha-L-galactan is an anticoagulant polysaccharide. Carbohydr. Res. **337**, 2231–2238 (2002)
15. Fonseca, R.J., Santos, G.R., Mourão, P.A.: Effects of polysaccharides enriched in 2,4-disulfated fucose units on coagulation, thrombosis and bleeding. Practical and conceptual implications. Thromb. Haemost. **102**, 829–836 (2009)
16. Pereira, M.G., Benevides, N.M., Melo, M.R., Valente, A.P., Melo, F.R., Mourão, P.A.: Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? Carbohydr. Res. **340**, 2015–2023 (2005)
17. Pereira, M.S., Melo, F.R., Mourão, P.A.: Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? Glycobiology **12**, 573–580 (2002)