

Elucidating the specificity of non-heparin-based conformational activators of antithrombin for factor Xa inhibition

Qudsia Rashid,
Mohammad Abid¹,
Mohamad Aman
Jairajpuri

Departments of Bio-Sciences, Protein Conformation and Enzymology Lab, ¹Organic Synthesis Lab, Jamia Millia Islamia, New Delhi, India

Address for correspondence:

Dr. Mohamad Aman Jairajpuri, Department of Bio-Sciences, Protein Conformation and Enzymology Lab, Jamia Millia Islamia (A Central University), Jamia Nagar, New Delhi - 110 025, India.
E-mail: mjairajpuri@jmi.ac.in

Abstract

Introduction: Antithrombin, the principal inhibitor of coagulation proteases, requires allosteric activation by its physiological cofactor, heparin or heparin sulfate to achieve physiologically permissible rates. This forms the basis of heparin's use as a clinical anticoagulant. However, heparin therapy is beset with severe complications, giving rise to the need to search new non-heparin activators of antithrombin, devoid of these complications and with favorable safety profiles. **Materials and Methods:** We chose some representative organic compounds that have been shown to be involved in coagulation modulation by affecting antithrombin and applied a blind docking protocol to find the binding energy and interactions of the modified (sulfated) versus unmodified organic scaffolds. **Results and Conclusion:** Increased sulfation plays a key role in shifting the specificity of organic compounds like quercetin, diosmin, rutin, mangiferin, isomangostin, Trapezifolixanthone and benzofuran towards the heparin binding site (HBS). However, in hesperetin and tetrahydroisoquinoline, sulfation shifts the specificity away from HBS. We have further tried to elucidate changes in the binding affinity of quercetin on account of gradual increase in the number of hydroxyl groups being substituted by sulfate groups. The results show gradual increase in binding energy with increase in sulfation. A theoretical screening approach is an ideal mechanism to predict lead molecules as activators of antithrombin and in determining the specificity for antithrombin.

Key words: Antithrombin, autodock, flavonoids, heparin, PyMOL

INTRODUCTION

Antithrombin (ATIII), a member of serine protease inhibitor (serpin) super family of proteins, is the principal inhibitor of many coagulation proteases, especially factor Xa and thrombin.^[1,2] It plays a critical role in the prevention of thrombosis by regulating these key enzymes of coagulation cascade.^[3] However, its reaction with both the proteases is very slow under physiological

conditions unless activated by its physiological activator heparin or heparin sulfate. Heparin accelerates the antithrombin inhibition of these proteases by several 100-folds which forms the basis of heparin based anticoagulant therapy.^[4,5]

In the absence of its cofactor heparin, two residues, P14 and P15 (Gly379-Ser380) at the N-terminal of the reactive center loop (RCL) of antithrombin are embedded within the body of the antithrombin owing to its minimal rate of factor Xa inhibition.^[2] The accelerating effect of heparin on the inhibition of the substrate proteases is achieved by an allosteric activation of antithrombin for inhibition of factor Xa and via a bridging mechanism by the formation of a ternary complex between antithrombin, thrombin and heparin for the efficient inhibition of thrombin. The conformational activation of antithrombin induces approximately 300-folds faster inhibition of factor Xa,

Access this article online

Quick Response Code:



Website:
www.jnsbm.org

DOI:
10.4103/0976-9668.127282

while the bridging mechanism contributes a massive, approximately 2500-folds acceleration of thrombin inhibition.^[6,7] Although the pentasaccharide suffices for the accelerated inhibition of factor Xa, for the bridging mechanism, a minimum of 18 saccharides including the pentasaccharide sequence is critical for achieving heparin mediated thrombin inhibition [Figure 1].^[8-10]

The heparin binding site (HBS) of ATIII is comprised of positively charged residues of helices A and D and the N-terminal region.^[11] A specific sequence and sulfation pattern present in a fraction of heparin and heparin sulfate (DEFGH) chains promote a high affinity interaction by inducing a large-scale conformational change in antithrombin. DEFGH binds to pentasaccharide-binding site (PBS) of ATIII in the domain comprising positively charged residues: Arg47, Lys114, Lys125 and Arg129. On the other hand, the full-length heparin in addition to PBS binds to the extended region formed by Arg132, Lys133 and Arg136 at the C-terminal end of helix D, known as extended

heparin binding site (EHBS).^[11-18] The conformational changes on account of heparin binding include extension of helix D by forming a 2-turn helix (helix P) at the N-terminal end, straightening of a small kink which is present in helix D prior to heparin binding and a 1.5-turn extension of helix D toward the C-terminal end.^[11,19] These changes in the HBS are also conferred to the RCL^[20] [Figure 2].

Although heparin is being used as the main stay of anticoagulant universally for the past 7 decades, it is beset with many complications including hemorrhage, thrombocytopenia, osteoporosis and inconsistent patient response, owing to its polyanionic, polymeric and polydisperse nature.^[21-24] In order to reduce the side effects of heparin, several non-heparin based small molecules have been investigated for their antithrombin activation potential,^[25-30] where the elementary precept of these studies is the heparin/pentasaccharide–antithrombin interaction mechanism. It has also been proved that ATIII binds to a specific site on the heparin molecule and that

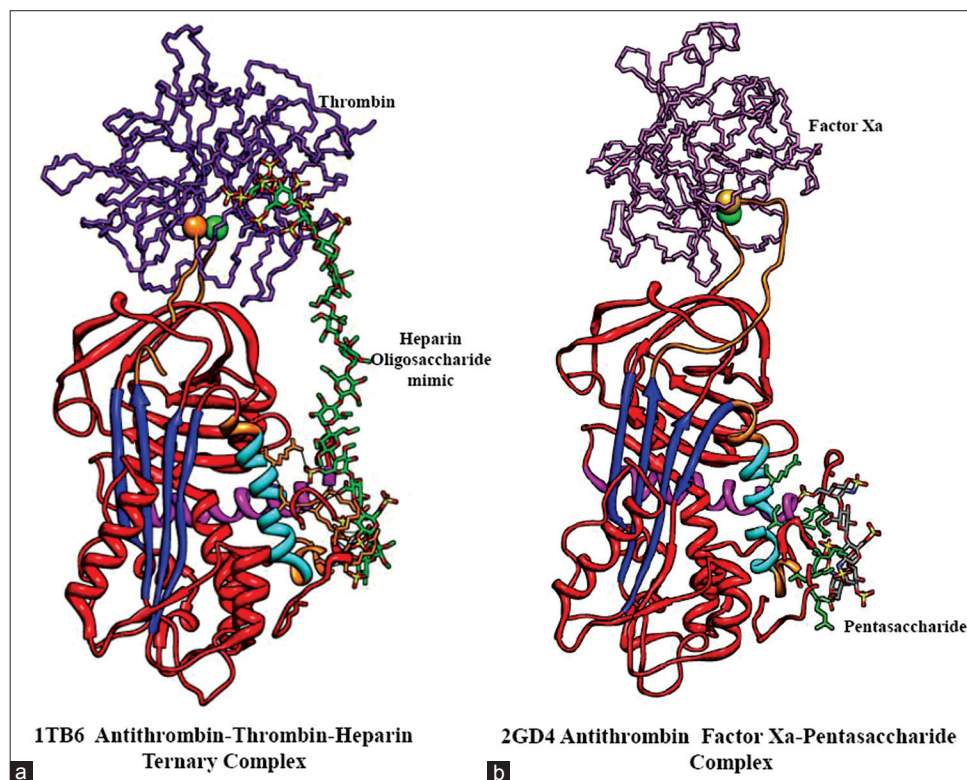


Figure 1: The structure of an antithrombin–thrombin–heparin ternary complex taken from PDB 1TB6 (a) and antithrombin factor Xa–pentasaccharide complex taken from PDB 2GD4 (b) Shows the crystal structures of the Michaelis complex between (a) antithrombin–thrombin–heparin ternary complex taken from PDB 1TB6 and (b) antithrombin factor Xa–pentasaccharide complex taken from PDB 2GD4. Thrombin inhibition involves non-allosteric activation, figure shows that thrombin inhibition occurs due to the interaction of thrombin and AT with full-length heparin through a bridging mechanism of activation. Some negative charges available at the full-length heparin chain binds non-specifically to the exosite (positively charged region) of thrombin. Inhibition of factor Xa involves allosteric activation by a heparin pentasaccharide. Circulating ATIII interacts with the high affinity pentasaccharide sequence in full-length heparin via the heparin-binding site forming a complex with endothelial heparin, this leads to the exposure of the RCL, which recognizes factor Xa and is known to provide a conformational activation mechanism. Molecular graphic images were produced using the UCSF Chimera package from the Resource for biocomputing, visualization and informatics at the University of California, San Francisco

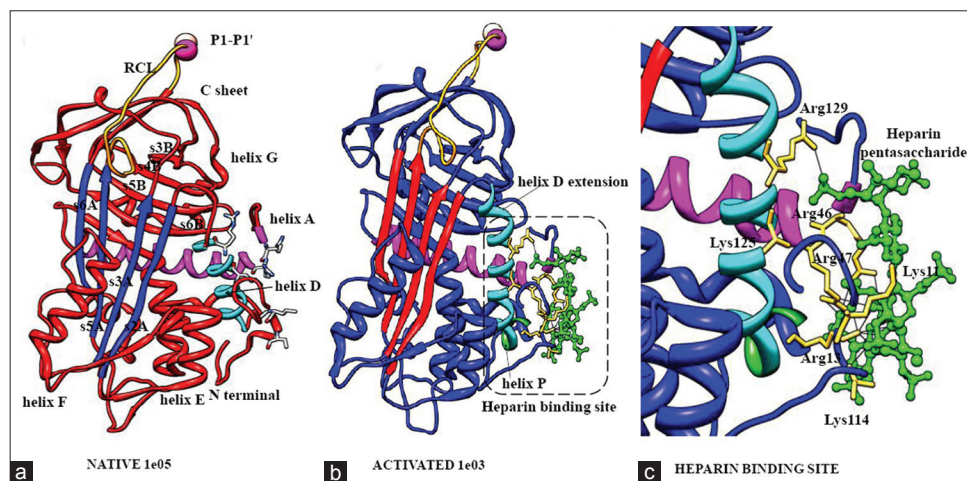


Figure 2: Conformational changes in cofactor (heparin) bound antithrombin and residues involved in cofactor interaction. Structures of native and pentasaccharide bound forms of antithrombin. Antithrombin is depicted in cartoon diagram in its (a) native (1E05) (b) and pentasaccharide bound activated (1E03). The native (1E05) circulating antithrombin in blood shows several regions that are important in controlling and modulating conformational changes. The reactive center loop (RCL) is involved in protease recognition and conformational transformation as strand 4A after inhibition. (a, b) Shows the key structural differences between native and pentasaccharide bound states. It illustrates the heparin-dependent conformational changes in antithrombin, like extension of helix D by forming a 2-turn helix (P helix) at the N-terminal end, a 1.5-turn extension of helix D toward the C-terminal end. Movement of strand 3A and strand 5A and expulsion of RCL. The P1-P1' (Arg-Ser) residues and the heparin pentasaccharide are shown as balls and sticks, respectively. (b, c) Shows the basic residues in the heparin-binding site (HBS) that interacts with the pentasaccharide (the HBS is indicated by a box in (b)): Lys-11 and Arg-13 in the N-terminal end; Arg-46 and Arg-47 in the helix A; and Lys-114, Phe-121, Phe-122, Lys-125 and Arg-129 in the region of the helix D. The figures were produced using Chimera

the anticoagulant activity of heparin should be related to the probability of finding this site in the molecules of the preparation.^[31] Frequent attempts have been made so far towards the design of non-heparin activators of ATIII.^[25-30] A computational approach in the initial screening strategy is an ideal method for discovering lead scaffolds.

Binding specificity of various polyphenolic scaffolds to antithrombin in the HBS is the best indicator of its conformational activation potential. In this study, we propose that a theoretical screening approach for identifying alternative non-heparin activators of antithrombin for factor Xa inhibition is an ideal strategy to identify lead compounds and modify them for an appreciable activation of antithrombin. A screening strategy has been applied using a blind docking protocol to find the binding energy and interactions of the modified versus unmodified organic scaffolds. We chose some representative organic compounds that have been shown to be involved in coagulation modulation by affecting antithrombin.

MATERIALS AND METHODS

Ligand and protein preparation

The X-ray crystal structure of antithrombin 1e05^[32] was acquired from research collaboratory for structural bioinformatics (RCSB) (<http://www.rcsb.org/pdb/>). I chain which is the inhibitory monomer chain was extracted from the dimeric structure and used as a representation

for the native conformation. The structures of the non-sulfated parent ligands were acquired from National Center for Biotechnology Information (NCBI) Pubchem compound (<http://www.ncbi.nlm.nih/pccompound>). The hydroxyl groups in each parent molecule were substituted by a sulfate group (OSO_3^-) to generate the corresponding polysulfated ligands [Figure 3]. The corresponding modified structures (sulfates) were drawn in ChemDraw 3D ultra 8.0 software (Molecular Modeling and analysis; Cambridge soft Corporation, USA [2003]).^[33] The 3-dimensional coordinate files in protein data bank (PDB) format of these ligands were generated using the NCI's Online SMILES Translator and Structure File Generator.^[34] Autodock 4.0 was used for molecular docking of the ligands/organic scaffolds to ATIII.^[35] Antithrombin (1e05) pdb file was imported into Autodock tools (ADT), all water molecules were removed, polar hydrogens were added, kollman charges were assigned to all atoms and Gasteiger charges were calculated. The ligand pdb files were also imported into ADT, polar hydrogens were added and Gasteiger charges were calculated; the rigid root and the rotatable bonds were defined by the Autotors tool of ADT. Affinity grids with grid maps of $58 \times 60 \times 58$ points and $1.00\text{\AA}$ grid point spacing were centered on whole protein encompassing the active site using the autogrid tool of ADT.

Docking

Autodock was used to evaluate ligand binding energies over the conformational search space by Lamarckian genetic algorithm with long run using maximum evaluations over a

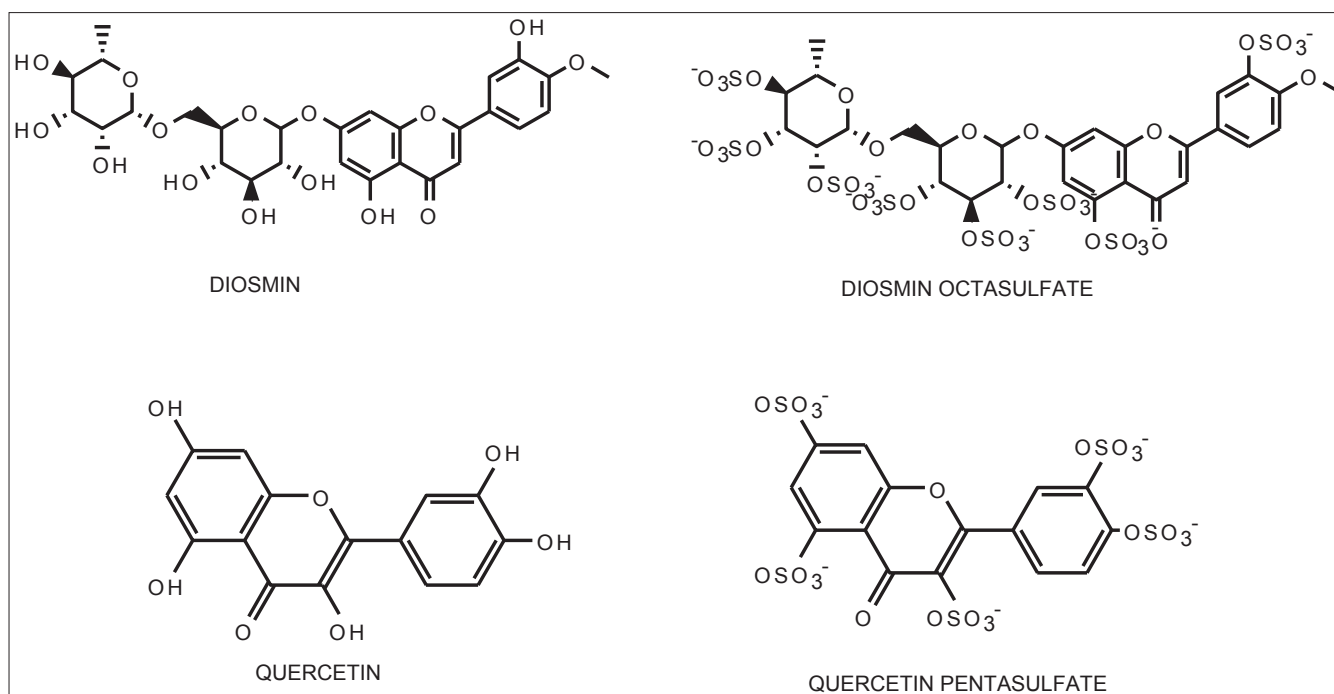


Figure 3: Molecular structures of some native and sulfated ligands. The structures were drawn in ChemDraw Ultra 8.0, all the hydroxyl (-OH) groups in the parent compounds were substituted by sulfate (-OSO_3^-) groups to generate the corresponding polysulfated molecules

population of 150 individuals. Default docking parameters were used (population size of 150 individuals, maximum number of energy evaluations 25,000,000, maximum number of generations 27,000, elitism (the number of top individuals that are guaranteed to survive into the next generation) of 1, mutation rate of 0.02 and a crossover rate of 0.8). In the output log file, we have considered the minimum energy conformation state of each ligand showing binding affinity in kcal/mol. Root mean square deviation (RMSD) values were calculated relative to the best mode and used only movable heavy atoms. Images of ligand and antithrombin (1E05 I chain) bound complexes were prepared using PyMOL program^[36] and polar contacts between them were noted down.

RESULTS

Flavonoids, xanthenes, dihydroxybenzofurans (DHP) and tetrahydroisoquinoline were sulfated at specific location to target antithrombin to compare their binding energies and specific interactions with their corresponding non-sulfated molecules. The interactions computed using Autodock have shown that blind docking can easily distinguish between the changes in specificity due to sulfation.

The results shown in Table 1 and Figure 4 indicate that the specificity of flavonoids can shift either away or inside the HBS on account of sulfation. Quercetin on sulfation showed affinity towards heparin-binding residues in the

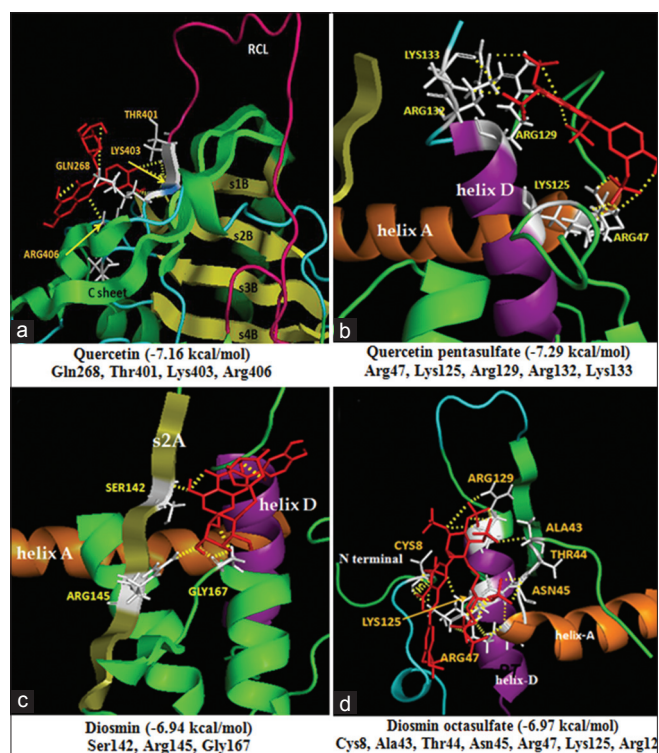


Figure 4: Binding affinity and polar contacts of native and sulfated quercetin and diosmin with antithrombin. Binding affinity and polar contacts of native and sulfated quercetin (a, b) and diosmin (c, d) with antithrombin. Images of ligand and antithrombin (1E05 I chain) bound complexes were prepared in PyMOL program and polar contacts between them were noted down. The structures were drawn in ChemDraw Ultra8.0. All the hydroxyl groups in each parent are substituted by a sulfate group (OSO_3^-) to generate the corresponding polysulfated ligands

helix A, helix D and N-terminal, whereas the unsulfated quercetin binds away from the HBS. Sulfation in diosmin increased the overall binding in the HBS with involvement of helix A, helix D and N-terminal residues. In contrast to these, hesperetin shifted its binding away from the HBS on sulfation [Figure 4 and Table 1]. Non-sulfated hesperetin binds ATIII in strand 2A in the EHBS region which is involved in the propagation of conformational change on account of heparin binding. Sulfation of hesperetin switches the affinity away from the HBS where it now binds the C-sheet with a binding energy of -6.8 kcal/mol.

Non sulfated ligands; quercetin, rutin, mangiferin, isomangostin, trapezifolixanthone and benzofuran bind away from the HBS, however, a sulfation-induced switch in the binding specificity takes place where their corresponding sulfated ligands bind specifically in the HBS. Significant increase in the binding energy is observed in rutin for the HBS on account of sulfation. These results

indicate that sulfated quercetin, diosmin & rutin and unsulfated hesperetin are effective leads for enhancing ATIII-dependent factor Xa inhibition rates.

We also determined whether the specificity switch takes place on complete sulfation or gradually with the increasing extent of sulfation. Quercetin with 1, 2, 3, 4 and sulfate groups were docked with antithrombin [Table 2]. The results showed that a gradual increase in the extent of sulfation switches the specificity to the HBS with progressive increase in the binding energy. Binding energy of quercetin with maximum sulfation is highest with involvement of N-terminal, helix A and helix D in binding. These results clearly indicate that a sulfation based specificity switch either inside or away from the HBS can be used as an initial screening to test a range of organic scaffolds. Specific increase in the affinity on account of sulfation or other modifications can also be detected.

Table 1: Comparison of minimum binding energies and interacting residues of ligands and their corresponding sulfates with antithrombin

Ligand	Unmodified/non-sulfated		Sulfated	
	Binding energy (kcal/mol)	Interacting residues	Binding energy (kcal/mol)	Interacting residues
Quercetin	-7.16	Gln268, Thr401, Lys403, Arg406. (s2B, others)	-7.29	Cys8, Arg47, Lys125, Arg129, Lys133 (N-terminal, helix A, helix D, PBS)
Hesperetin	-6.59	Arg132, Lys133, Ala134, Lys136, Ser138, Leu140, Ser142.(EHBS, s2A)	-6.81	Lys228, Lys257 (C sheet, others)
Diosmin	-6.94	Ser142, Arg 145, Gly167 (s2A, others)	-6.97	Cys8, Ala43, Thr44, Asn45, Arg47, Lys125, Arg129 (N-terminal, helix A, helix D, PBS)
Rutin	-1.25	Arg259, Asp277, Glu271, Thr280, Asp309 (s1B, s2B, s3B, helix H)	-4.90	Gln38, Arg46, Arg49 (helix A)
Mangiferin	-6.75	Val355, Ala356, Glu357, Arg359 (others)	-5.97	Cys8, Asn45, Arg47, Lys125, Arg129 (N-terminal, helix A, helix D, PBS)
3-Isomangostin	-6.10	Tyr131, Glu163 (helix A, others)	-6.65	Arg132, Lys125, Arg47, Asn45 (helix A, helix D others)
Gartanin	-5.96	Arg406, Pro288 (others)	-7.45	Arg393, Lys275, Lys228, Lys257 (RCL, C sheet, others)
Trapezifolixanthone	-6.71	Asn428, Arg 324 (s6A, others)	-6.59	Cys8, Arg47, Lys125, Arg129 (N-terminal, helix A, helix D, PBS)
Benzofuran	-5.62	Asn418, Arg413 (s6B, s6A)	-6.94	Cys8, Arg47, Lys125, Arg129 (N-terminal, helix A, helix D, PBS)
Tetrahydro isoquinoline	-5.48	Cys8, Arg47, Lys125 (N-terminal, helix A, helix D, PBS)	-8.01	Lys226, Ser227, Lys228, Lys275 (s3A, s2B, others)

PBS: Pentasaccharide-binding site; EHBS: Extended heparin-binding site; RCL: Reactive center loop. Table shows comparison of minimum binding energies (lowest docked energy from each cluster) of ligands and their polysulfates with antithrombin (1e05). It also shows the residues (and domain) involved in interaction. The binding energies were noted from the final output file of each docking run and the interacting residues were determined from images of ligand and antithrombin (1E05 I chain) bound complexes prepared using PyMOL program and polar contacts between them were noted down

Table 2: Change in binding specificity with respect to varying degree of sulfation in quercetin

Ligand	Quercetin	Quercetin monosulfate	Quercetin disulfate	Quercetin trisulfate	Quercetin tetrasulfate	Quercetin pentasulfate
Binding energy (kcal/mol)	-7.16	-4.92	-5.83	-5.62	-6.05	-7.29
Interacting residues	Gln268, Thr401, Lys403, Arg406	Arg132, Ala134, Lys136, Lys 193	Cys8, Arg47, Lys125, Arg129, Lys133	Lys125, Arg129, Lys133	Lys198, Trp189, Lys188, Arg145, Gln171	Arg47, Lys125, Arg129, Arg 132, Lys133

Table shows the change in specificity on gradual increase in extent of sulfation in quercetin

DISCUSSION

The functional mimics of heparin without its adverse effects are highly desirable as alternative therapy for antithrombin activity modulation. Heparin polysaccharide is decorated by numerous ionic groups, viz sulfate and carboxylate groups. The average disaccharide in heparin contains 2.5 sulfate groups and a carboxylate group with an average charge density of approximately 0.4-0.5 charges per Å.^[37] It has been observed that replacing a specific sulfate group of the pentasaccharide with a phosphate group dramatically reduces its antithrombin binding. Therefore, it is strongly believed that replacing the sulfate groups in the binding domain of heparin with other anions nullifies its anticoagulant activity.^[27] The free energy of binding of antithrombin–heparin interaction is a cumulative of 40% ionic and 60% non-ionic interactions.^[37] The contribution of non-polar residues is maximal, the non-ionic interaction involving non-polar groups continue to remain largely unknown. Although extensive literature is available about the contribution of positively charged residues, arginine and lysine in the heparin-binding domain of antithrombin are involved in initial heparin binding and conformational activation.^[38] The molecular basis of the predominant non-ionic contribution in heparin interaction needs to be determined for more appropriate design considerations.

Sulfated organic molecules are gaining importance as modulators of many physiological processes, including inhibition of coagulation. Based on various organic scaffolds, many molecules have been designed which demonstrate antithrombin activation, the key tenet of these newly designed synthetic antithrombin activators is the requirement of high sulfate content and appropriate charge density. Non-saccharide organic scaffolds may lead to new anticoagulants with effective safety profiles. These scaffolds with lesser charge density compared to heparins are anticipated to recognize antithrombin with higher non-ionic binding energy and may be expected to cause minimal cross-reactivity with other proteins. Flavonoids, xanthenes and tetrahydroisoquinoline scaffolds show promise when sulfated at specific locations.

CONCLUSION

Non-heparin based conformational activators of antithrombin that can enhance factor Xa inhibition activity without the underlying side effects are envisaged to be of great importance in anticoagulation therapy. However, lack of understanding of its specificity and structure function modulation of antithrombin hampers the screening of a large range of compounds.

A screening strategy to test and increase binding specificity is a better option to test a large range of compounds before undertaking elaborative experimental studies.

ACKNOWLEDGMENTS

The research in the lab is supported by grants from Department of Biotechnology, University Grant Commission and Indian Council of Medical Research, Government of India. We acknowledge DST-FIST for departmental computer support. QR is supported by CSIR Senior Research Fellowship.

REFERENCES

1. Olson ST, Björk I. Regulation of thrombin activity by antithrombin and heparin. *Semin Thromb Hemost* 1994;20:373-409.
2. Olson ST, Björk I, Sheffer R, Craig PA, Shore JD, Choay J. Role of the antithrombin-binding pentasaccharide in heparin acceleration of antithrombin-proteinase reactions. Resolution of the antithrombin conformational change contribution to heparin rate enhancement. *J Biol Chem* 1992;267:12528-38.
3. Huntington JA, McCoy A, Belzar KJ, Pei XY, Gettins PG, Carrell RW. The conformational activation of antithrombin. A 2.85-Å structure of a fluorescein derivative reveals an electrostatic link between the hinge and heparin binding regions. *J Biol Chem* 2000;275:15377-83.
4. Björk I, Olson ST. Antithrombin. A bloody important serpin. *Adv Exp Med Biol* 1997;425:17-33.
5. Rosenberg RD, Damus PS. The purification and mechanism of action of human antithrombin-heparin cofactor. *J Biol Chem* 1973;248:6490-505.
6. Olson ST, Swanson R, Raub-Segall E, Bedsted T, Sadri M, Petitou M, *et al.* Accelerating ability of synthetic oligosaccharides on antithrombin inhibition of proteinases of the clotting and fibrinolytic systems. Comparison with heparin and low-molecular-weight heparin. *Thromb Haemost* 2004;92:929-39.
7. Olson ST, Björk I. Predominant contribution of surface approximation to the mechanism of heparin acceleration of the antithrombin-thrombin reaction. Elucidation from salt concentration effects. *J Biol Chem* 1991;266:6353-64.
8. Carrell RW, Stein PE, Fermi G, Wardell MR. Biological implications of a 3 Å structure of dimeric antithrombin. *Structure* 1994;2:257-70.
9. Li W, Johnson DJ, Esmon CT, Huntington JA. Structure of the antithrombin-thrombin-heparin ternary complex reveals the antithrombotic mechanism of heparin. *Nat Struct Mol Biol* 2004;11:857-62.
10. Johnson DJ, Li W, Adams TE, Huntington JA. Antithrombin-S195A factor Xa-heparin structure reveals the allosteric mechanism of antithrombin activation. *EMBO J* 2006;25:2029-37.
11. Jin L, Abrahams JP, Skinner R, Petitou M, Pike RN, Carrell RW. The anticoagulant activation of antithrombin by heparin. *Proc Natl Acad Sci U S A* 1997;94:14683-8.
12. Ersdal-Badju E, Lu A, Zuo Y, Picard V, Bock SC. Identification of the antithrombin III heparin binding site. *J Biol Chem* 1997;272:19393-400.
13. Desai U, Swanson R, Bock SC, Björk I, Olson ST. Role of arginine 129 in heparin binding and activation of antithrombin. *J Biol Chem* 2000;275:18976-84.
14. Schedin-Weiss S, Desai UR, Bock SC, Gettins PG, Olson ST, Björk I. Importance of lysine 125 for heparin binding and activation of antithrombin. *Biochemistry* 2002;41:4779-88.
15. Arocas V, Bock SC, Raja S, Olson ST, Björk I. Lysine 114 of antithrombin is of crucial importance for the affinity and kinetics of heparin pentasaccharide binding. *J Biol Chem* 2001;276:43809-17.

16. Arocas V, Turk B, Bock SC, Olson ST, Björk I. The region of antithrombin interacting with full-length heparin chains outside the high-affinity pentasaccharide sequence extends to Lys136 but not to Lys139. *Biochemistry* 2000;39:8512-8.
17. Desai UR, Petitou M, Björk I, Olson ST. Mechanism of heparin activation of antithrombin. Role of individual residues of the pentasaccharide activating sequence in the recognition of native and activated states of antithrombin. *J Biol Chem* 1998;273:7478-87.
18. Huntington JA, Olson ST, Fan B, Gettins PG. Mechanism of heparin activation of antithrombin. Evidence for reactive center loop preinsertion with expulsion upon heparin binding. *Biochemistry* 1996;35:8495-503.
19. van Boeckel CA, Grootenhuys PD, Visser A. A mechanism for heparin-induced potentiation of antithrombin III. *Nat Struct Biol* 1994;1:423-5.
20. Desai UR. New antithrombin-based anticoagulants. *Med Res Rev* 2004;24:151-81.
21. Gray E, Mulloy B, Barrowcliffe TW. Heparin and low-molecular-weight heparin. *Thromb Haemost* 2008;99:807-18.
22. Rabenstein DL. Heparin and heparan sulfate: Structure and function. *Nat Prod Rep* 2002;19:312-31.
23. Lefkou E, Khamashta M, Hampson G, Hunt BJ. Review: Low-molecular-weight heparin-induced osteoporosis and osteoporotic fractures: A myth or an existing entity? *Lupus* 2010;19:3-12.
24. Januzzi Jr JL, Jang IK. Fundamental concepts in the pathobiology of heparin-induced thrombocytopenia. *J Thromb Thrombolysis* 2000;10:7-11.
25. Gunnarsson GT, Desai UR. Designing small, nonsugar activators of antithrombin using hydrophobic interaction analyses. *J Med Chem* 2002;45:1233-43.
26. Gunnarsson GT, Desai UR. Exploring new non-sugar sulfated molecules as activators of antithrombin. *Bioorg Med Chem Lett* 2003;13:679-83.
27. Monien BH, Desai UR. Antithrombin activation by nonsulfated, non-polysaccharide organic polymer. *J Med Chem* 2005;48:1269-73.
28. Monien BH, Cheang KI, Desai UR. Mechanism of poly (acrylic acid) acceleration of antithrombin inhibition of thrombin: Implications for the design of novel heparin mimics. *J Med Chem* 2005;48:5360-8.
29. Correia-da-Silva M, Sousa E, Duarte B, Marques F, Carvalho F, Cunha-Ribeiro LM, *et al.* Polysulfated xanthenes: Multipathway development of a new generation of dual anticoagulant/antiplatelet agents. *J Med Chem* 2011;54:5373-84.
30. Raghuraman A, Liang A, Krishnasamy C, Lauck T, Gunnarsson GT, Desai UR. On designing non-saccharide, allosteric activators of antithrombin. *Eur J Med Chem* 2009;44:2626-31.
31. Hopwood J, Höök M, Linker A, Lindahl U. Anticoagulant activity of heparin: Isolation of antithrombin-binding sites. *FEBS Lett* 1976;69:51-4.
32. McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. *J Mol Biol* 2003;326:823-33.
33. ChemDraw Ultra, Version 8.0, Cambridge Software, 2003. Available from: <http://www.cambridgesoft.com> [Accessed October 30, 2012].
34. Online SMILES Translator and Structure File Generator, ????. Available from: <http://www.cactus.nci.nih.gov/translate> [Accessed October 30, 2012].
35. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al.* AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 2009;30:2785-91.
36. DeLano WL. 2002. The PyMOL Molecular Graphics System. San Carlos, CA, USA: DeLano Scientific; [Accessed October 30, 2012].
37. Jairajpuri MA, Lu A, Desai U, Olson ST, Björk I, Bock SC. Antithrombin III phenylalanines 122 and 121 contribute to its high affinity for heparin and its conformational activation. *J Biol Chem* 2003;278:15941-50.
38. Olson ST, Björk I, Bock SC. Identification of critical molecular interactions mediating heparin activation of antithrombin: Implications for the design of improved heparin anticoagulants. *Trends Cardiovasc Med* 2002;12:198-205.

How to cite this article: Rashid Q, Abid M, Jairajpuri MA. Elucidating the specificity of non-heparin-based conformational activators of antithrombin for factor Xa inhibition. *J Nat Sc Biol Med* 2014;5:36-42.

Source of Support: Nil. **Conflict of Interest:** None declared.