NOVEL INSIGHTS INTO THE INTERACTIONS OF SULFATED ANTICOAGULANT POLYSACCHARIDES WITH THE THROMBIN/ANTITHROMBIN COMPLEX

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Introduction

Cardiovascular diseases are the leading cause of death and healthcare expenditure worldwide[1], highlighting the need for effective anticoagulant therapies like heparin[2]. Although clinically effective, heparin is associated with side effects and significant limitations, emphasizing the demand for alternatives with improved therapeutic profiles[3]. Among the most studied anticoagulant drugs in recent decades are thrombin-inhibiting molecules, which can act through direct or indirect mechanisms. The mechanism of action of these molecules can function through direct or indirect mechanisms[4]. Marine polysaccharides, such as sulfated galactan (sulfGal) from the sea urchin *Echinometra lucunter,* are promising alternatives due to their role as indirect thrombin inhibitors[5]. However, limited information is available regarding the atomistic and molecular mechanisms underlying the inhibitory activity of these sulfated polysaccharides. This study investigated the intermolecular interactions of sulfGal with the thrombin/antithrombin (TH/AN) complex, comparing its molecular affinity with that of heparin.

Material and Methods

The 16-residue sulfGal molecule was prepared using the Glycan Carbohydrate Builder and completed via the CHARMM-Gui interface. The 16-residue heparin structure was extracted from the Protein Data Bank (PDB ID: 1TB6)[6]. To generate the three-dimensional structure of the TH/AN complex, comparative modeling was performed using the Robetta server, with the similar structure 1TB6 as a template. The apo form of the complex underwent a 500 ns molecular dynamics (MD) simulation in an aqueous environment to refine its structural conformation. A clustering algorithm[7] was then applied to identify the most representative conformations from the MD trajectory. The selected conformation was subjected to molecular docking using GlycoTorch Vina[8], followed by an additional 500 ns of MD simulation using AMBER 2023[9] to refine the docking results and carbohydrate molecular poses.

Results and Discussion

The comparative modeling of the protein complex was based on the crystallographic model 1TB6 from the PDB, with ligands removed. Protein sequences of thrombin and antithrombin were obtained from UniProt, IDs P00734 and P01008, respectively. The model generated by the Robetta server was validated using a Ramachandran plot and QMEANDisco, obtained through the Swiss-Model server, with favorable values of >95% and 0.73 ± 0.05, respectively. The structure then underwent a final 5 ns MD simulation for refinement.

The 500 ns MD simulation of the TH/AN complex in its apo form allowed us to identify the presence of a transient cavity in the heparin binding region, suggesting a stable binding site. Molecular

docking results, performed on the main conformations of the TH/AN complex, indicated a bridge-like conformation for both the heparin and sulfGal molecules. Further 200 ns MD simulations of the TH/AN-heparin and TH/AN-sulfGal complexes indicated a higher affinity of heparin for the TH/AN complex relative to sulfGal throughout the trajectories. This higher affinity may correlate with the stronger anticoagulant activity of heparin, which is associated with an increased bleeding risk in patients.

Conclusion

The findings from this study provide valuable new insights into the affinity of sulfated polysaccharides, such as sulfGal and heparin, for the thrombin/antithrombin (TH/AN) complex, advancing our understanding of their intermolecular interactions. The results suggest that sulfated polysaccharides interact to TH/AN complex by the same bridge conformation but show different molecular affinities. Understanding the main interaction sites and the most stable molecular cavities in the TH/AN complex can be used in the design of future anticoagulants.

Acknowledgments

The authors would like to thank the financial support of CAPES, CNPq, FAPERJ, UFRJ and UFF. The fellowships granted by CNPq, CAPES (Financial code: 001), and FAPERJ are gratefully acknowledged.

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