Available online at www.sciencedirect.com
ScienceDirect
*Mendeleev Commun.*, 2015, **25**, 420–421
Mendeleev Communications

# Computational study of the possible formation of the ternary complex between thrombin, antithrombin III and fucosylated chondroitin sulfates

**Alexey G. Gerbst, Nadezhda E. Ustyuzhanina and Nikolay E. Nifantiev***

*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 8784; e-mail: nen@ioc.ac.ru*

DOI: 10.1016/j.mencom.2015.11.006

**Computer docking studies suggest an explanation for better anticoagulant activity of fucosylated chondroitin sulfates as compared to non-fucosylated ones due to the formation of the ternary complex with thrombin and antithrombin III.**

Sulfated glycosaminoglycans from mammalians, first of all heparin and its derivatives, are extensively applied in medical practice as anticoagulant drugs. However, their side effects such as hemorrhage and heparin-induced thrombocytopenia stimulate further search for anticoagulants of another nature.[1,2] Fucosylated chondroitin sulfates (FCS) from echinoderms form a separate class of glycosaminoglycans which possess potent anticoagulant and antithrombotic effects.[3] The backbone of these biopolymers is built up of alternating (1→4)-linked β-D-glucuronic acid and (1→3)-linked and selectively 4- and/or 6-O-sulfated *N*-acetyl β-D-galactosamine residues (Figure 1). Significant difference of FCS as branched biomolecular systems[4] from linear chondroitin sulfates is the presence of fucose branches at O-3 of glucuronic acid. It is noteworthy, that structures of FCS vary accordingly to the type of echinoderms species.[3,5] Structural variations were observed in degree and pattern of sulfation, number of branches and molecular weight.

Several studies demonstrated that the level of anticoagulant activity of FCS exceeded that of heparinoids.[6] Recently it was shown that distinct structures of the α-fucose units in FCS do not affect their anticoagulant activity.[7] It is important that non-fucosylated chondroitin sulfate did not exhibit effect on blood coagulation.[8] In case of heparinoids, it is known that their anticoagulant properties are determined by their ability to form ternary complexes with thrombin and antithrombin III (ATIII) as it was demonstrated experimentally for an 18-saccharide mimetic SR123781[7] (Figure 2). It was possible to suggest that FCS could

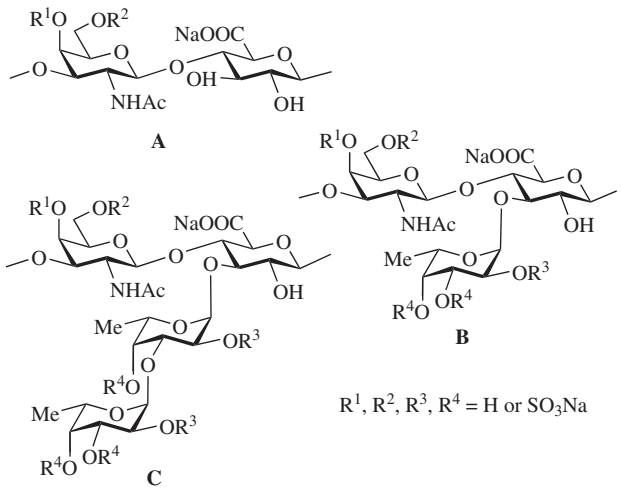



**Figure 1** Fragments of linear chondroitin sulfate (**A**) and of two fucosylated chondroitin sulfates (**B**) and (**C**).

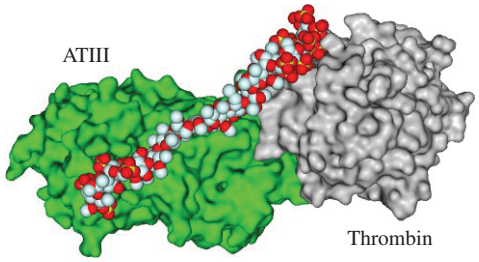



**Figure 2** Ternary complex formed by thrombin (gray), ATIII (green) and heparin mimetic SR123781 (crystallographic structure from PDB, code 1TB69).

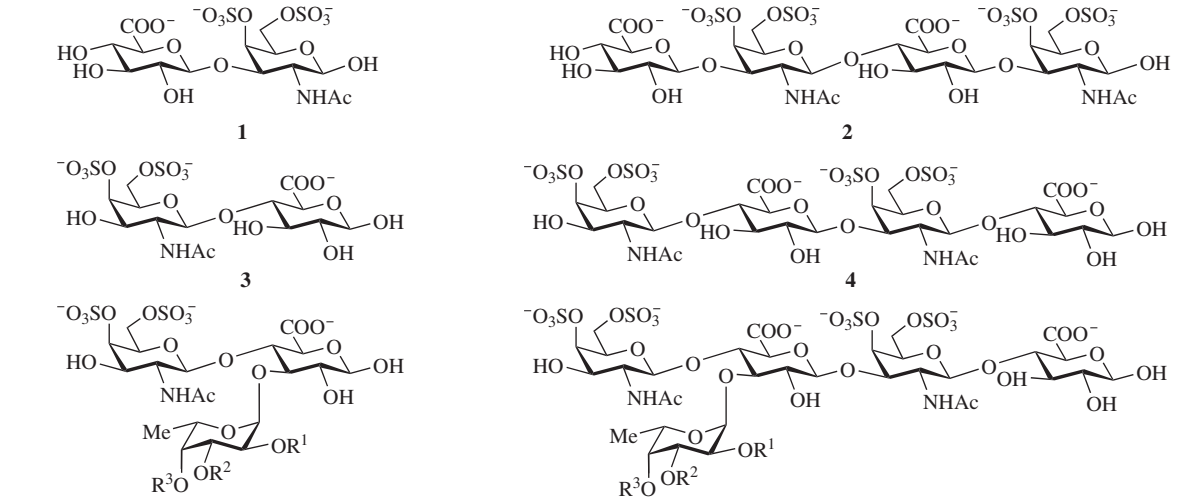
exhibit anticoagulant activity through similar mechanism. To prove this assumption, we have performed computer docking study with the use of a series of model selectively O-sulfated oligosaccharides (**1**–**16**) (Figure 3) representing fragments both with and without fucose residue which are repeatedly encountered in chondroitin sulfate and FCS chains. The aim of this study was to evaluate possible affinity of these oligosaccharides towards thrombin and ATIII binding sites.

The stability of the studied oligosaccharide–protein complex is determined by the strength of interaction between the inhibitor and the active sites of thrombin and ATIII and ability of the inhibitor to interact simultaneously with each of proteins. It can thus be supposed that linear chondroitin sulfate **A** is unable to form such ternary complexes, while upon introduction of a fucose residue **B** or **C** it becomes an effective coagulation inhibitor.

The docking experiments were performed with the use of Autodock 4.0 software[10] modified for parallel execution.[11] Genetic algorithm with standard parameters was employed. Number of runs was set to 20. From the crystallographic structure it was deduced that active sites of thrombin and ATIII accommodated four monosaccharide residues of the SR123781 chain. Thus, docking of tetrasaccharide active fragments of SR123781 to the mentioned active sites was performed first to verify the docking methodology. The crystallographic binding poses were reproduced with reasonable accuracy (RMSD ~ 1.5 Å). Then model oligosaccharides **1**–**16** were studied. The calculated energies are given in Table 1.

Since the binding sites both in thrombin and ATIII are located on the protein surface and not buried inside, docked molecules had sufficient freedom during calculations to generate large number of docked poses. The pose with the lowest energy was chosen in each case.

It can be seen from Table 1 that while free energies of binding towards ATIII are generally of the same order without any clear



**1** **2** **3** **4**

**5** $R^1 = R^2 = R^3 = H$
**6** $R^1 = R^2 = R^3 = SO_3^-$
**7** $R^1 = SO_3^-$, $R^2 = R^3 = H$
**8** $R^1 = R^3 = H$, $R^2 = SO_3^-$
**9** $R^1 = R^2 = H$, $R^3 = SO_3^-$
**10** $R^1 = R^3 = SO_3^-$, $R^2 = H$
**11** $R^1 = R^2 = R^3 = H$
**12** $R^1 = R^2 = R^3 = SO_3^-$
**13** $R^1 = SO_3^-$, $R^2 = R^3 = H$
**14** $R^1 = R^3 = H$, $R^2 = SO_3^-$
**15** $R^1 = R^2 = H$, $R^3 = SO_3^-$
**16** $R^1 = R^3 = SO_3^-$, $R^2 = H$

**Figure 3** Model compounds used for docking experiments.

**Table 1** Calculated free energies of binding for the model compounds **1**–**16** to ATIII ($E_{AT}$) and thrombin ($E_T$) active sites (kcal mol$^{-1}$).

| Compound | $E_{AT}$ | $E_T$ | Compound | $E_{AT}$ | $E_T$ |
|---|---|---|---|---|---|
| **1** | –8.6 | –5.0 | **10** | –7.0 | –8.7 |
| **2** | –7.2 | –5.5 | **11** | –8.9 | –6.3 |
| **3** | –9.3 | –5.6 | **12** | –7.7 | –6.2 |
| **4** | –7.7 | –4.4 | **13** | –9.7 | –7.0 |
| **5** | –7.8 | –7.6 | **14** | –8.8 | –7.3 |
| **6** | –7.5 | –8.3 | **15** | –9.6 | –8.0 |
| **7** | –8.9 | –8.0 | **16** | –8.5 | –5.6 |
| **8** | –7.2 | –7.2 | SR123781 | –9.2 | –6.0 |
| **9** | –9.4 | –8.5 | | | |

dependence on whether a fucose is present in the molecule or not, for binding with thrombin there is a significant difference. Compounds that do not contain a fucose residue (**1**–**4**) exhibit lower values of binding energies. Upon the introduction of fucose, free energies of binding increase especially in cases of smaller ligands with a sulfate group at position O-4 of the fucose ring (compounds **9**–**10**). The average error of the Autodock scoring function is about 2.1 kcal mol$^{-1}$ according to the program manual. Thus it can be seen that the increase should be considered significant in case of docking to the thrombin binding site, while affinities to ATIII are more or less equal for all the studied fragments. This means that when these fragments are present in the chondroitin chain, binding to ATIII occurs regardless of the presence of fucose, while binding to thrombin in the absence of fucose is weak or impossible and such ternary complex holding together thrombin and ATIII cannot be formed.

Considering the abovesaid on the topology of active sites and large degree of freedom for the ligands, no distinct tendencies about additional interactions formed by the fucose residue were observed. It could be thought that its role was just in making a branch with a new negatively charged monosaccharide residue. In that way the length of the main chain remained the same while more positively charged groups surrounding binding site could be involved.

In our opinion, these results might present a possible explanation why linear chondroitin sulfates which have no side fucose units have not been found to show anticoagulant activity so far. While binding to ATIII seems possible in all cases, binding of the same non-fucosylated chain to the thrombin is much weaker, which leads to the instability (or maybe impossibility at all) of the corresponding ternary complex. To verify this result, we have started the synthesis[12] and conformational analysis[13] of oligosaccharides related to different linear and branched fragments of chondroitin sulfates and FCS, as well as molecular modelling of their binding to protein receptors other than thrombin and ATIII. The results of these studies will be reported elsewhere.

This work was supported by the Russian Science Foundation (grant no. 14-13-01325).

## References

1 A. Greinacher, *N. Engl. J. Med.*, 2015, **373**, 252.
2 P. A. S. Mourão, *Mar. Drugs*, 2015, **13**, 2770.
3 V. H. Pomin, *Mar. Drugs*, 2014, **12**, 232.
4 V. P. Ananikov, E. A. Khokhlova, M. P. Egorov, A. M. Sakharov, S. G. Zlotin, A. V. Kucherov, L. M. Kustov, M. L. Gening and N. E. Nifantiev, *Mendeleev Commun.*, 2015, **25**, 75.
5 P. Myron, S. Siddiquee and S. Al Azad, *Carbohydr. Polym*., 2014, **112**, 173.
6 L. Luo, M. Wu, L. Xu, W. Lian, J. Xiang, F. Lu, N. Gao, C. Xiao, S. Wang and J. Zhao, *Mar. Drugs*, 2013, **11**, 399.
7 G. R. Santos, B. F. Glauser, L. A. Parreiras, E. Vilanova and P. A. Mourão, *Glycobiology*, 2015, **25**, 1043.
8 V. B. Krylov, A. A. Grachev, N. E. Ustyuzhanina, N. A. Ushakova, M. E. Preobrazhenskaya, N. I. Kozlova, M. N. Portsel, I. N. Konovalova, V. Yu. Novikov, H.-Ch. Siebert, A. S. Shashkov and N. E. Nifantiev, *Russ. Chem. Bull., Int. Ed*., 2011, **60**, 746 (*Izv. Akad. Nauk, Ser. Khim.*, 2011, 731).
9 W. Li, D. J. Johnson, C. T. Esmon and J. A. Huntington, *Nat. Struct. Mol. Biol.*, 2004, **11**, 857.
10 R. Huey, G. M. Morris, A. J. Olson and D. S. Goodsell, *J. Comput. Chem.*, 2007, **28**, 1145.
11 P. Khodade, R. Prabhu, N. Chandra, S. Raha and R. Govindarajan, *J. Appl. Crystallogr*., 2007, **40**, 598.
12 N. E. Ustyuzhanina, P. A. Fomitskaya, A. G. Gerbst, A. S. Dmitrenok and N. E. Nifantiev, *Mar. Drugs*, 2015, **13**, 770.
13 A. G. Gerbst, A. S. Dmitrenok, N. E. Ustyuzhanina and N. E. Nifantiev, *Mar. Drugs*, 2015, **13**, 936.

*Received: 31st July 2015; Com. 15/4697*