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Recent advances in the study of the binding site of heparin to antithrombin

Pierre Sinaÿ

Laboratoire de Biochimie Structurale, UA 499, UFR — Faculté des Sciences, BP 6759, 45067 Orléans Cédex 2, France

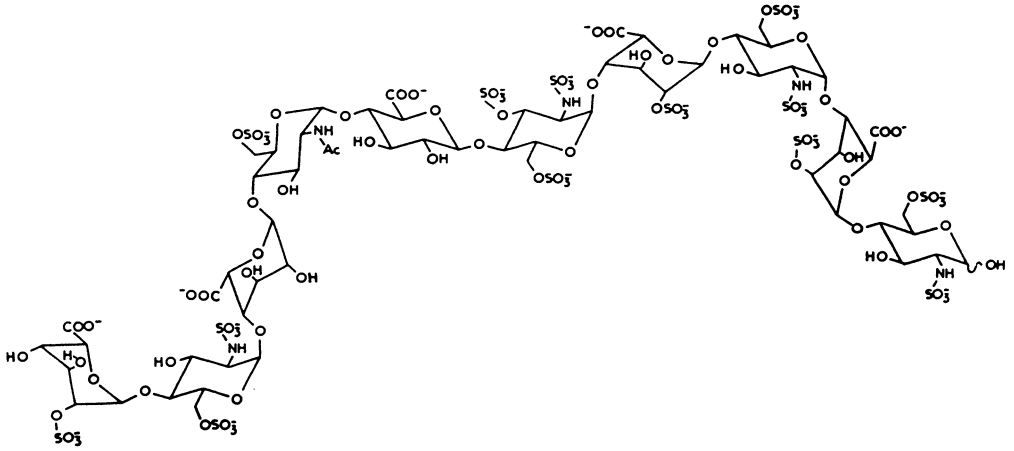
Abstract A unique pentasaccharide sequence of heparin has been proposed as the minimum structural requirement for binding to antithrombin and accelerating the inhibitory effect of this protein on a number of procoagulant proteases. This has been confirmed by the total chemical synthesis of the corresponding pentasaccharide, which indeed displayed a high affinity for antithrombin, resulting in a notably selective anti-Xa activity.

Another pentasaccharide has also been synthesized by a research group at the Choay Institute, whereby the unique 3-O-sulfate group was lacking. The product was completely inactive, demonstrating the key role of the 3-O-sulfate group in the binding site of heparin to antithrombin.

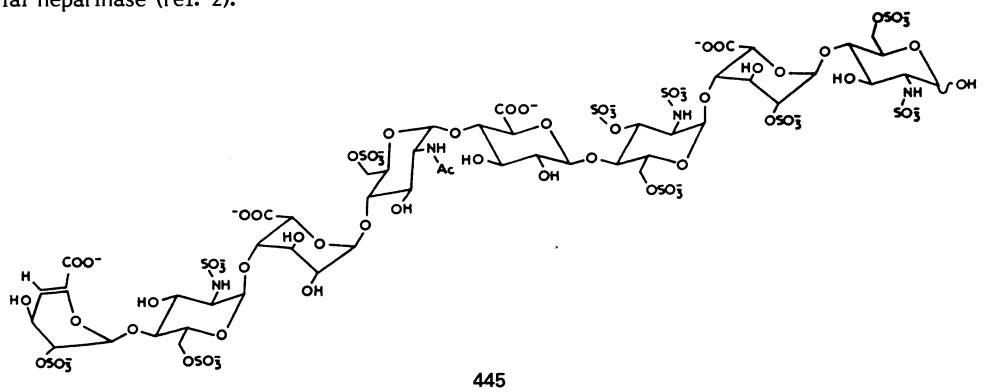
INTRODUCTION

Heparin, a sulfated polysaccharide belonging to the class of glycosaminoglycans, widely distributed in animal tissue, is currently used in therapy as an anticoagulant and antithrombotic agent. Heparin acts mainly by binding to antithrombin (AT) and accelerating the inhibitory effect of this protein on a number of procoagulant proteases. In order to determine the heparin sequence responsible for the specific binding to AT, the structure of various heparin fragments prepared by affinity chromatography and possessing high affinity for AT has been determined.

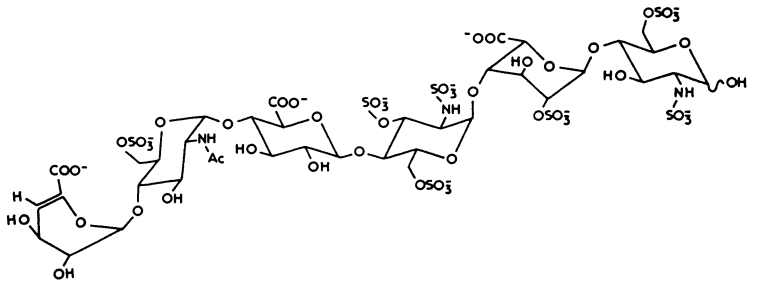
The following decasaccharide was first isolated (ref. 1) in very small amounts from a classical heparin preparation.



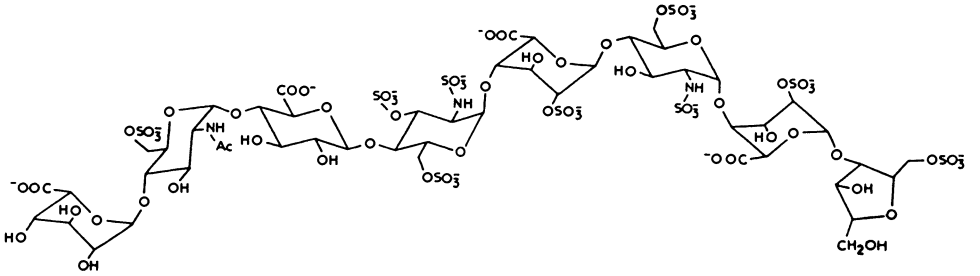
Another active oligosaccharide was subsequently obtained from partial depolymerization with bacterial heparinase (ref. 2).



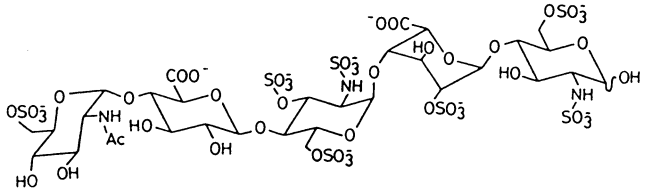
In order to get a smaller fragment, this octasaccharide was degraded in the presence of a high concentration of heparinase and the following hexasaccharide was obtained (ref. 3).



It is striking that this hexasaccharide has a high affinity for AT and displays a selective anti-factor Xa activity. Finally, the following active octasaccharide was obtained after partial nitrous acid deamination of heparin (ref. 4).



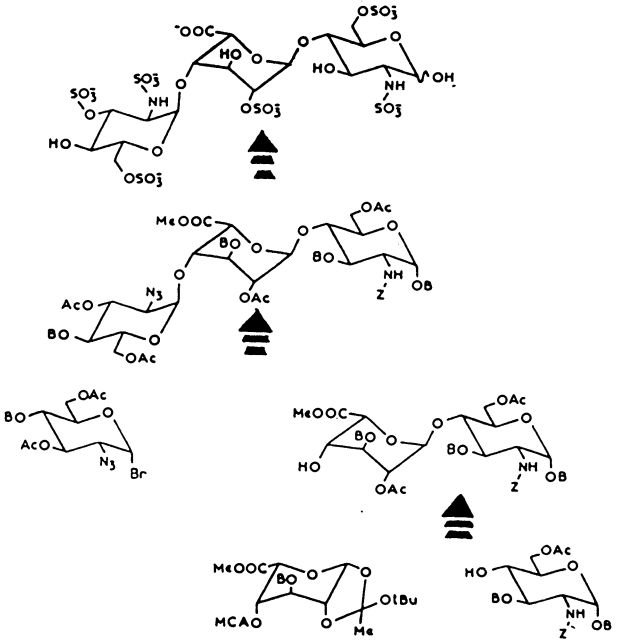
This structural work led to the hypothesis that the minimun sequence in heparin that binds to AT was contained in the following pentasaccharide DEFGH.



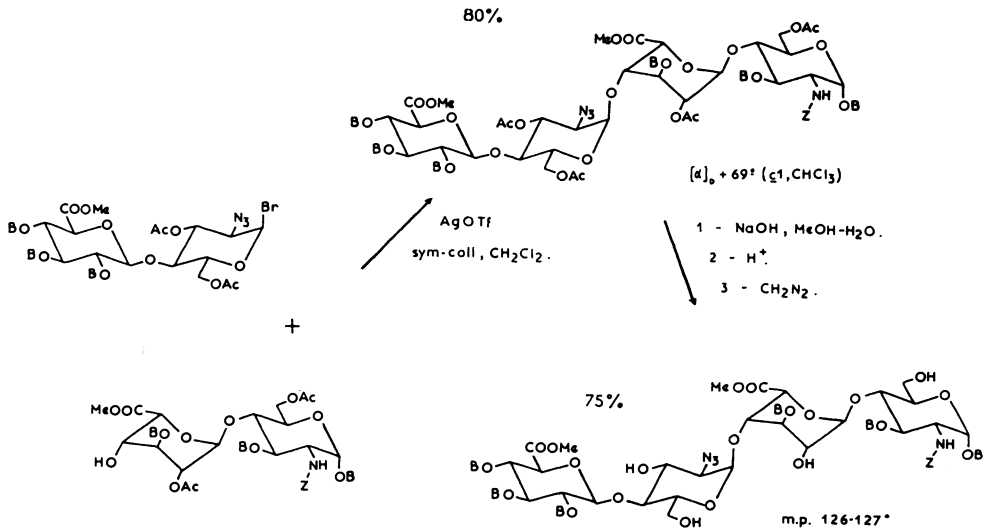
D E F G H

At this stage we launched a general program on the synthesis of various sulfated oligosaccharides.

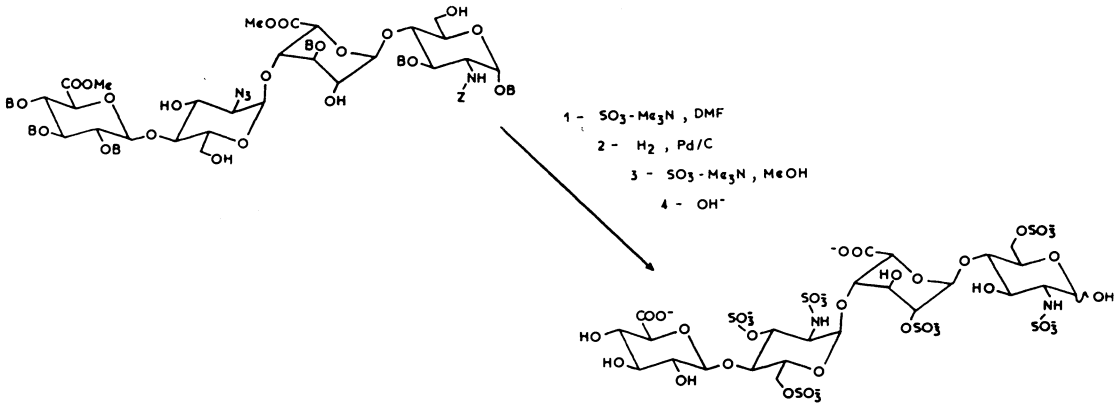
The next scheme delineates the strategy which was used by us in order to synthesize the trisaccharide DEF (ref. 5). This synthetic material has no affinity for AT.



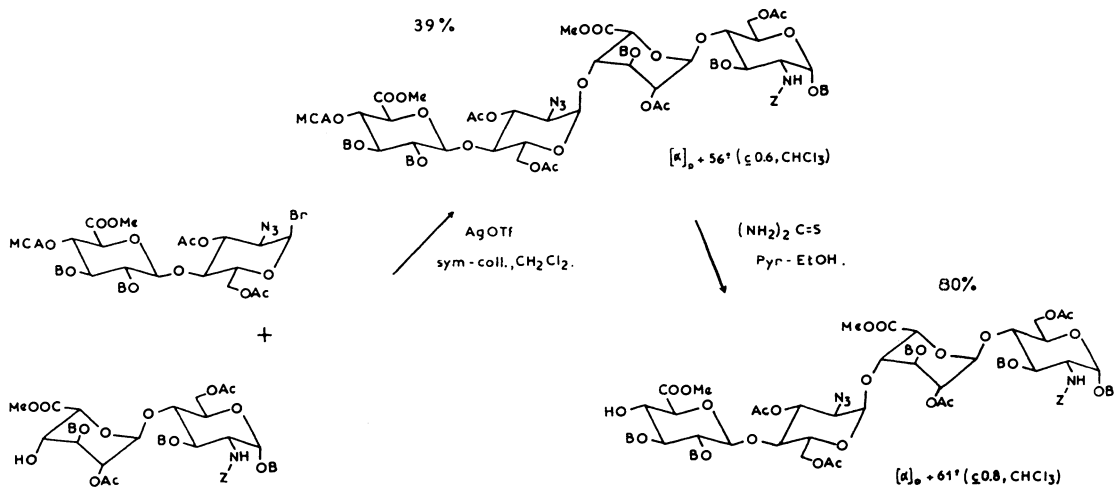
The tetrasaccharide EFGH was then selected as a synthetic target. A two-plus-two block methodology resulted in an efficient preparation of a suitably protected crystalline tetrasaccharide (m.p. 126-127°).



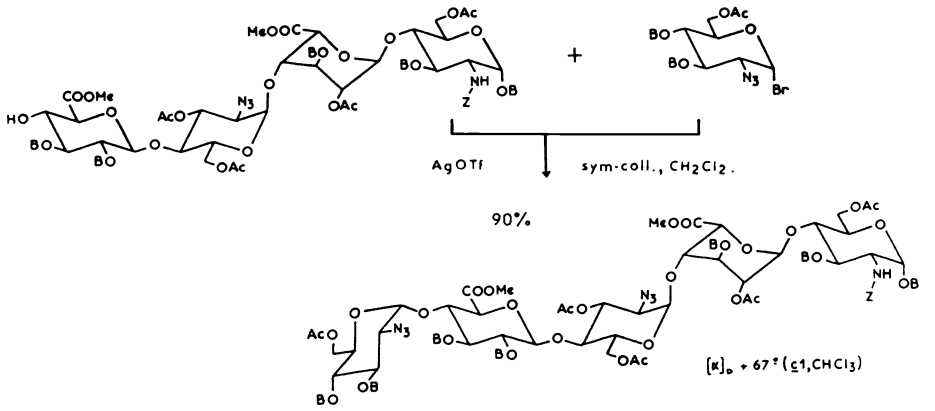
Sulfations and deprotection provided the fragment EFGH which again presented no affinity for AT. The tetrasaccharide DEF was also prepared and found to be inactive.



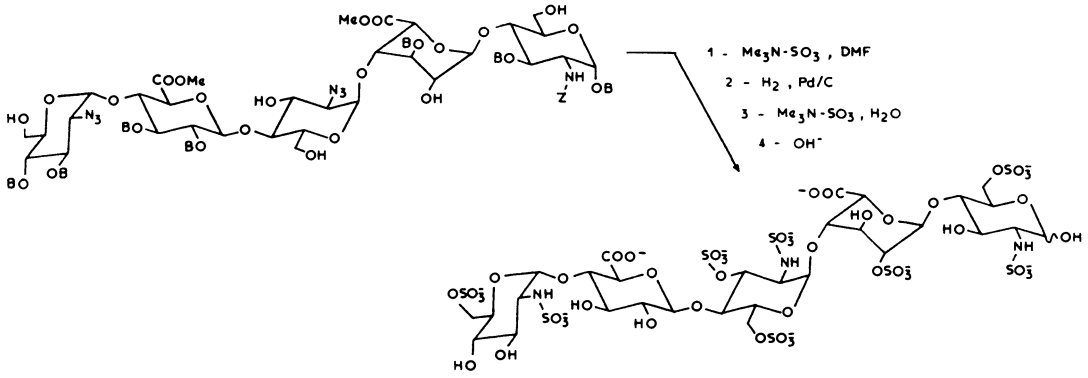
The total synthesis of the pentasaccharide DEFGH was then achieved. A tetrasaccharide was first prepared along the following lines.



Glycosylation of this derivative according to the azido procedure provided a protected pentasaccharide in excellent yield.



Alcaline deacetylation followed by esterification gave a polyol which was successfully sulfated and deprotected to give the traget molecule (ref. 6). Much to our pleasure, this compound displayed a high affinity for AT, resulting in a notably selective anti-Xa activity.



Through chemical synthesis, the availability of various oligosaccharides offered the unique opportunity of investigating the structure-activity relationship of heparin. The tetrasaccharide DEFG, which was synthesized along similar lines, displayed a rather weak anti-Xa activity in the presence of AT. A remarkable result was recently obtained by the research group at the Choay Institute when a pentasaccharide DEFGH was prepared whereby the 3-O position of unit F was not sulfated. This product was completely inactive, demonstrating the key role of the 3-O-sulfate group of F in the binding site of heparin to AT.

This piece of work illustrates the importance of oligosaccharide synthesis in the study of the relationship between the biosynthetic heterogeneities of polysaccharides and the corresponding biological functions. It is the result of a collaboration between the Choay Institute in Paris (J. Choay, M. Petitou), the Ronzoni Institute in Milano (B. Casu), the Macromolecular Chemical Institute of the CNR in Milano (D. Ferro) and our group (J.-C. Jacquinet, P. Sinaÿ).

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