Carbohydrate Research, 165 (1987) c1–c5
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Conformation of the pentasaccharide corresponding to the binding site of heparin to Antithrombin-III

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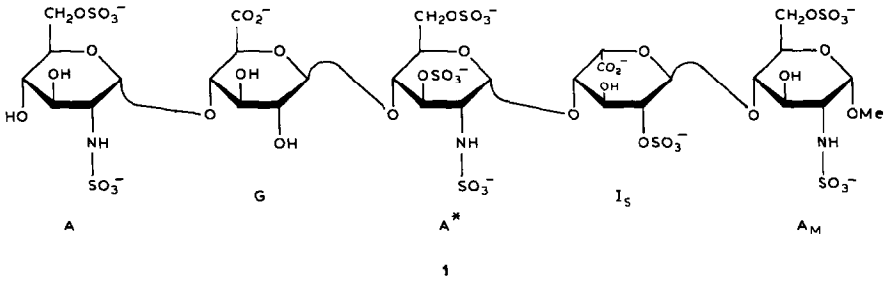
(Received January 9th, 1987; accepted for publication, April 16th, 1987)

The minimal binding site of heparin to Antithrombin-III (AT-III) is represented by a pentasaccharide[1–2] which was recently synthesised[3]. We now propose a model for the synthetic pentasaccharide[4] **1** based on force-field computations and n.m.r. (essentially n.O.e.) measurements.



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A major problem is the determination of the conformation of the iduronate residue I_S which has been a matter of controversy[5c,6]. Force-field computations indicated that, in addition to the $^1C_4$ and $^4C_1$ conformations, a third almost equienergetic conformation, the skew boat $^2S_0$, must be considered for the iduronate ring[5a].

The $^3J_{H,H}$ values for the iduronate residue in various mono- and oligosaccharides related to the heparin binding site for AT-III have been interpreted in

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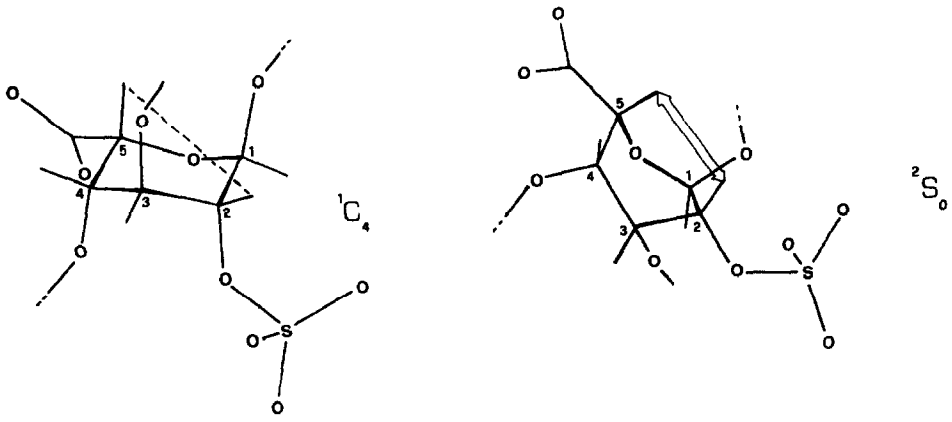

Fig. 1. The two conformations of I_S observed in heparin and inside oligosaccharide sequences. The short H-2/H-5 distance in the $^2S_0$ form is indicated.

terms of conformer populations[5d], the relative abundance of which is governed both by the sequence of monosaccharide units and by the pattern of substitution[5b,5d]. When present as an internal unit in an oligosaccharide, the iduronate residue is in equilibrium between the $^1C_4$ and $^2S_0$ conformations (Fig. 1).

Force-field computations carried out on **1** provided several models, and the procedure outlined below led to the selection of the most probable.

The φ,ψ energy maps were inspected first for the four pairs of interglycosidic torsional angles. Taking into account the three possible conformations of the iduronate ring, but neglecting differences in the side-chain conformations, sixteen conformers of the pentasaccharide were found with comparable energy (within 3.5 kcal.mol^{-1}).

Analysis of the $J_{H,H}$ values for **1** indicates no appreciable amount of the $^4C_1$ conformation of the iduronate ring[5d]; hence, the corresponding conformers were discarded. Furthermore, by restricting the energy range to 2 kcal.mol^{-1}, a series of conformers, characterised by the values $-7°$ and $39°$ for φ and ψ in the A–G interglycosidic linkage, can also be discarded.

Only the four remaining conformers were then considered; these corresponded to the $^1C_4$ and $^2S_0$ conformations for I_S, combined with the two possible φ,ψ angles for the I_S–A_M interglycosidic linkage. The values of φ and ψ for the four conformers are given in Table I, and the two most stable are shown in Fig. 2.

Using 500-MHz n.m.r. spectroscopy, both intra- and inter-residue negative n.O.e. effects were observed at 10° on a phase-sensitive NOESY map[7] (Fig. 3) and quantified by mono-dimensional n.O.e. difference spectroscopy. The n.O.e. observed between H-1 and H-2 of the three 2-amino-2-deoxyglucose residues was essentially the same (−16 to −17%), indicating isotropic tumbling. Particularly noticeable is the strong n.O.e. effect [−13%, *i.e.*, only slightly smaller than those (17–20%) observed for vicinal diequatorial protons] between H-5 and H-2 of residue I_S, as predicted for a prevalent $^2S_0$ conformation by the above calculations.

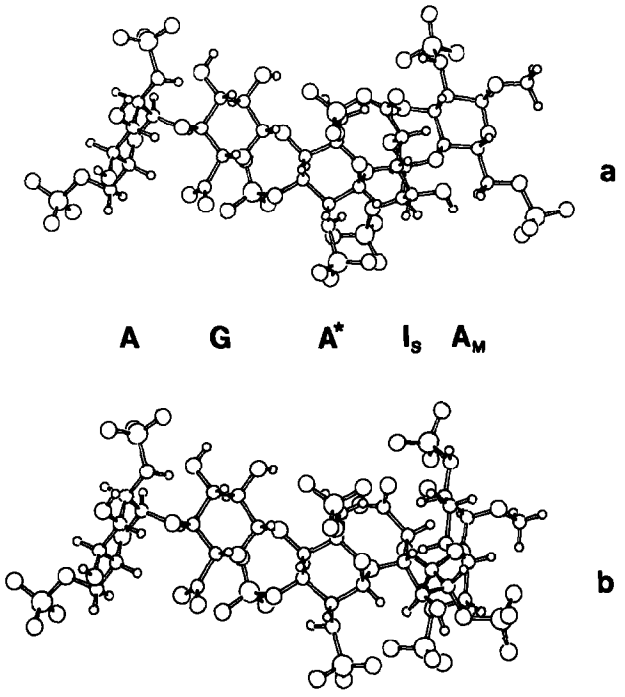

Fig. 2. The two most stable conformers computed for **1**: (a) minimum c I (according to Table I), corresponding to the $^1C_4$ conformation of I_S; (b) minimum s II (conformation ^2S_o).

TABLE I

INTERGLYCOSIDIC DIHEDRAL ANGLES φ(H-1–C-1)O-1–C-4) AND ψ(C-1–O-1)C-4–H-4) IN THE FOUR MORE STABLE CONFORMERS OF **1**, AND THEIR RELATIVE ENERGIES COMPUTED ACCORDING TO REFS. 5a AND 5d

Minimum[a]	$(\varphi_1; \psi_1)$	$(\varphi_2; \psi_2)$	$(\varphi_3; \psi_3)$	$(\varphi_4; \psi_4)$	*E (kcal.mol^{-1})*
c I	−36; −26	49; 4	−37; −33	24; −49	0.31
c II	−36; −26	49; 4	−35; −31	31; 19	1.34
s I	−36; −26	50; 4	−35; −41	34; −43	1.27
s II	−36; −27	48; 5	−38; −44	44; 9	0

[a]Minima c correspond to the $^1C_4$ conformation of I_S, whereas s refers to ^2S_o.

Indeed, in the skew-boat form, the H-2 to H-5 distance is the shortest interproton distance (2.3 Å, as compared with 2.8 Å for H-1 to H-3), whereas in the chair form it is 4.0 Å (see Fig. 1). This provides support for the occurrence of this conformer.

The present picture of the average conformation has the following features. (*a*) The A–G–A* sequence is quite rigid, owing to a single conformation of the glycosidic linkage. The present calculations indicate that the rigidity of the G–A* linkage arises from the β-D-glucuronate residue and from the interactions of the peculiar 3-*O*-sulfo group with O-5 and COO^- of G. (*b*) In the A*–I–A_M sequence

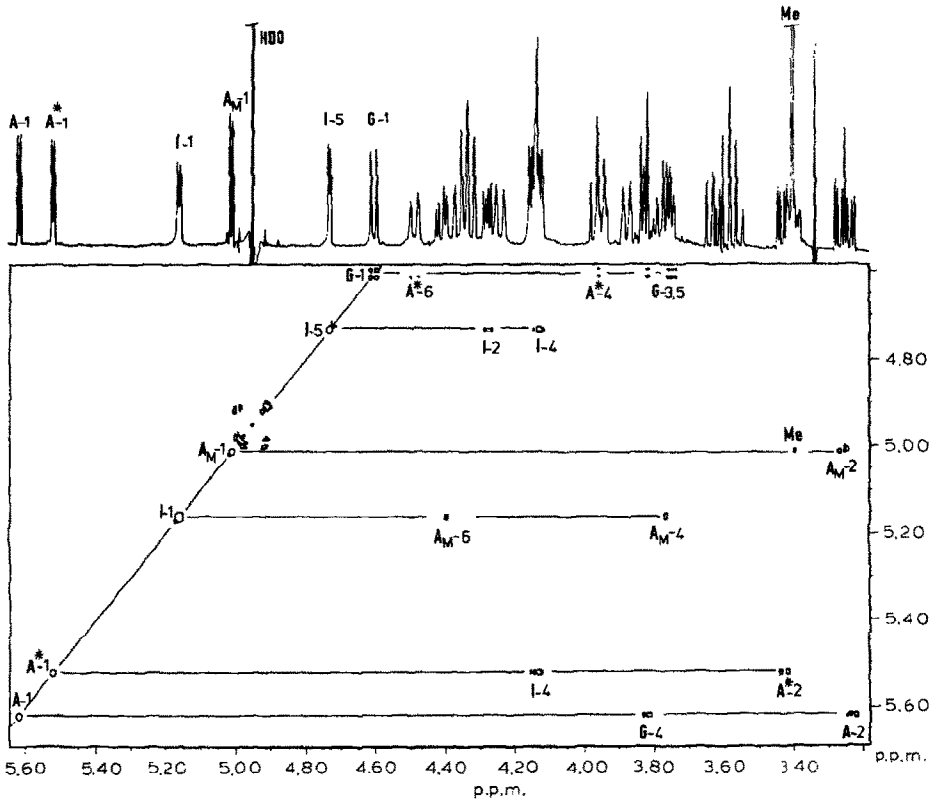

Fig. 3. 500-MHz phase-sensitive NOESY map (partial) of AGA*IA$_M$ in ^2H_2O at 20°. Only negative contours have been plotted.

(in the four conformers), the iduronate ring is in equilibrium between two conformations, the relative populations computed from the $^3J_{HH}$ values being $^2S_0$ ~60% and $^1C_4$ ~40%; the presence of the $^2S_0$ form is confirmed by the marked n.O.e. effect.

Thus, four main conformers are proposed as possible candidates for the pentasaccharide **1** in solution corresponding to the binding site to antithrombin. A more refined force-field and n.O.e. study is in progress to improve this model.

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