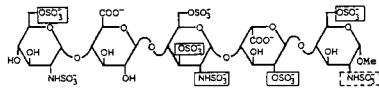


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MOLECULAR CONFORMATION OF THE PENTASACCHARIDE CORRESPONDING TO THE BINDING SITE OF HEPARIN TO ANTITHROMBIN III. J. Choay (1), M. Petitou (1), B. Perly (2), B. Casu (3), M. Ragazzi (4) and D. Ferro (4). Institut Choay, Paris, France (1), C.E.A., Gif-sur-Yvette, France (2), Istituto "G. Ronzoni", Milano, Italy (3), Istituto di Chimica delle Macromolecole, Milano, Italy (4).

The knowledge of the tridimensional structure of the sequence required in heparin for binding to antithrombin III (AT) is essential for the understanding of the molecular basis of the anticoagulant and antithrombotic activity of this glycosaminoglycan. This problem was approached by a combined experimental (chemical synthesis and Nuclear Magnetic Resonance, "NMR") and theoretical (force field calculations) study of the pentaaccharide I reproducing the minimal sequence for high affinity binding to AT.



NMR data (interproton coupling constants and nuclear-overhauser-enhancement) were used to test a number of possible conformations of I proposed on the basis of molecular energy calculation. In particular, these studies, confirming the results obtained by force field calculations, showed that the sulfated iduronic residue (I_5) conformation is an equilibrium between the chair 1C_4 and the skew boat 2S_0 , and permitted to discard models with the I_5 residue in the alternate chair 4C_4 . It is of interest that the relative population of the 1C_4 and 2S_0 conformers of I_5 in sequence I (40:60) is reversed with respect to that found for the same residue in the regular sequences of heparin (60:40), such a reversal being largely associated with electrostatic interactions caused by the unique 3-O-sulfo group of the preceding amino sugar residue. The most favoured models of I are characterized by a cluster of anionic groups including all the sulfate groups proposed as essential for full expression of the activity (full line square on the formula) but one (dotted line square on the formula), plus the carboxylate group of the glucuronic acid residue.

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ROLE OF THE HIGH-AFFINITY PENTASACCHARIDE IN HEPARIN ACCELERATION OF ANTITHROMBIN III INHIBITION OF THROMBIN AND FACTOR Xa. Steven T. Olson (1), Ingemar Bjork (1), Paul A. Craig (1), Joseph D. Shore (1) and Jean Choay (2), Henry Ford Hospital, Detroit, Michigan 48202 USA (1) and Institut Choay, Paris, France (2).

The high-affinity heparin pentaaccharide (H_5) and an 8000 Mr high-affinity heparin (H_{26}) have been compared with respect to their interaction with antithrombin III (AT) and their accelerating effect on AT inhibition of thrombin (T) and factor Xa by rapid kinetic and equilibrium binding studies at pH 7.4, 25°C. K_s of .068 μM at I 0.15 and 0.57 μM at I 0.3 were determined for the AT- H_5 interaction, which were 5 and 2.5-fold weaker, respectively, than affinities determined for H_{26} . Comparison of the kinetics of binding of H_5 and H_{26} to AT at I 0.15 under pseudo-first order conditions ($[H]_0 \gg [AT]_0$) demonstrated a saturable dependence of the observed rate constant for both reactions with indistinguishable limiting rate constants of 700 +/-120 s^{-1} and 520 +/-90 s^{-1} , but somewhat different K_s for the initial binding interaction of 20 and 29 μM for H_5 and H_{26} , respectively. These results indicate that H_5 induces the same conformational change in AT as the larger heparin, but that the rate of reversal of this conformational change is greater for H_5 which is the basis for its weaker AT affinity. Bimolecular rate constants for neutralization of factor Xa and thrombin by AT- H_5 and AT- H_{26} complexes were determined by p-aminobenzamide displacement under pseudo-first order conditions ($[AT-H] \gg [T]$ or $[Xa]$). I-independent values of .62 $\mu\text{M}^{-1}s^{-1}$ were obtained for Xa inhibition by AT- H_5 at I 0.15 and 0.3, compared to I-dependent values of 1.4 and 0.91 $\mu\text{M}^{-1}s^{-1}$ for AT- H_{26} . For thrombin inhibition by AT- H_5 , and I-independent enhancement of 1.6-fold in the bimolecular rate constant from .0098 to .016 $\mu\text{M}^{-1}s^{-1}$ was observed, in sharp contrast to the marked I-independent enhancement by AT- H_{26} of the bimolecular rate constant ranging from 4000 to 200-fold at I 0.15 and 0.3, respectively. These results are consistent with a primary ionic strength-independent contribution of the AT conformational change to heparin enhancement of factor Xa but not thrombin neutralization by AT, with an ionic strength-dependent component for both reactions, compatible with a differential role for a protease-heparin interaction. Supported by grant HL-30237

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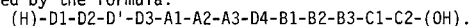
MOLECULAR CLONING OF THE HUMAN GENE FOR VON WILLEBRAND FACTOR AND IDENTIFICATION OF THE TRANSCRIPTION INITIATION SITE. Carolyn J. Collins (1), Richard B. Levene (1), Christina P. Ravera (2), Markar J. Dombalagian (2), David M. Livingston (1), Dennis C. Lynch (1), Dana-Farber Cancer Institute, Boston, MA, USA (1) and Meloy Laboratories, Springfield, VA, USA (2).

Most patients with von Willebrand's disease appear to have a defect affecting the level of expression of the von Willebrand factor (vWf) gene. Thus, an understanding of the pathogenesis of von Willebrand's disease will require an analysis of the structure and function of the vWf gene in normals and in patients. To begin such analyses, we have screened a human genomic cosmid library with probes obtained from vWf cDNA and isolated a colinear segment spanning =175 kb in five overlapping clones. This segment extends =25 kb upstream and =5 kb downstream of the transcription start and stop sites for vWf mRNA, implying the vWf gene has a length of =150 kb. Within one of these clones, the vWf transcription initiation sites have been mapped. A portion of the promoter region has been sequenced, revealing a typical TATA box, a downstream CCAAT box, and a perfect downstream repeat of the 8 base pairs containing the major transcription start site. Primer extension analysis suggests that sequences contained within the downstream repeat of the transcription start site may be used as minor initiation sites in endothelial cells. Transfection studies are underway to evaluate the role of sequences within this promoter region in gene regulatory activity. Comparative restriction analyses of cloned and chromosomal DNA segments strongly suggests that no major alterations occurred during cloning and that there is only one complete copy of the vWf gene in the human haploid genome. Similar analyses of DNA from vWf-expressing endothelial cells and non-expressing white blood cells suggests that no major rearrangements are associated with vWf gene expression. Finally, cross hybridization patterns among seven mammalian species suggests a strong conservation of genomic sequences encoding the plasma portion of vWf, but a lower degree of conservation of sequences encoding the N terminal region of provWf.

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VON WILLEBRAND FACTOR (vWF) PRO-POLYPEPTIDE IS REQUIRED FOR vWF MULTIMER FORMATION. C.L. Verweij, M. Hart and H. Pannekoek, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Dept. of Molecular Biology, Amsterdam, The Netherlands.

The von Willebrand factor (vWF) is a multimeric plasma glycoprotein synthesized in vascular endothelial cells as a pre-pro-polypeptide with a highly repetitive domain structure, symbolized by the formula:



A heterologous expression system, consisting of a monkey kidney cell line (COS-1), transfected with full-length vWF cDNA, is shown to mimic the constitutively, secretory pathway of vWF in endothelial cells. The assembly of pro-vWF into multimers and the proteolytic processing of these structures is found to proceed along the following, consecutive steps. Pro-vWF subunits associate to form dimers, a process that does not involve the pro-polypeptide of pro-vWF. This observation is derived from transfection of COS-1 cells with vWF cDNA, lacking the genetic information encoding the pro-polypeptide, composed of the domains D1 and D2. Pro-vWF dimers are linked intracellularly to form a regular series of multimeric structures that are secreted and cannot be distinguished from those released constitutively by endothelial cells. The presence of the pro-polypeptide, embedded in pro-vWF, is obligatory for multimerization since the deletion mutant lacking the D1 and D2 domains fails to assemble beyond the dimer stage. It is argued that the D domains are involved in interchain interactions.