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0426 IMMUNOCHEMICAL STUDIES ON ANTITHROMBIN III

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Depressed Antithrombin III (AT) levels increase thrombic tendency in man, therefore value in assaying this protein has been established. Immunochemical analysis of AT in clinical disease has however proved controversial, consequently systematic studies were undertaken to rationalize the requirements necessary to optimise these methods in particular electro-immunoassay. The known binding affinity of AT for heparin has been exploited to differentiate high affinity AT from its inhibitor - protease complexes and has resulted in reports stating that heparin added to the agar gel prior to electrophoresis significantly reduces the time required for completion of antigen/antibody complexes. Our studies however have demonstrated that the antibody required for quantitative analysis must be capable of not only reacting with "native" antigenic determinants of AT but also with "neo" antigens that are exposed when inhibitor-protease complexes are formed. Heparin should not be used in the test protocol, for it has a paradoxical effect on immunoprecipitation in gels, masking some antigenic determinants of unbound - high affinity AT on one hand, and appear to disrupt the immunoprecipitin "rocket" formed with the inhibitor-protease complexes during electrophoresis on the other.

0427 STRUCTURE OF THE ANTITHROMBIN III-BINDING SITE IN HEPARIN

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Fragments with high affinity for antithrombin III (AT), composed of 12 to 16 monosaccharide units, were isolated from heparin after partial chemical or enzymatic depolymerization of the polysaccharide. Analysis of such fragments based on identification of deamination products suggested that nonsulfated L-iduronic acid (a minor constituent) is essential for the anticoagulant activity of heparin. The location of this unit in the AT-binding sequence was determined by periodate oxidation. Furthermore, an N-sulfate group essential for activity was located by structural analysis of partially N-desulfated fragments retaining high affinity for AT. It is proposed that the AT-binding sequence in heparin has a variable structure containing certain nonvariable regions. A tentative structure for this sequence is presented, with indication of identified constant and variable regions.

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0428 STRUCTURE OF HEPARIN AND RELATED GLYCOSAMINOGLYCANS

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The structure of heparin is largely accounted for by disaccharide sequences of α1,4-linked 2-O-sulphated L-iduronic acid and N,6-O-disulphated D-glucosamine. However, the insertion of other residues(especially D- glucuronic acid, non-sulphated L-iduronic acid and N-acetylated D-glucosamine) leads to hybrid structures. Also the other iduronic acid-containing glycosaminoglycans are structurally heterogeneous. Heparan sulphates contain variable amounts of heparin-like blocks, and dermatan sulphate contains also chondroitin -like segments.

Available evidence on the structure and conformation of the above glycosaminoglycans will be discussed in terms of the availability of individual functional groups for interaction with plasma proteins.