Heparin Action

The objective of this editorial is to describe recent progress in our understanding of the biochemical mechanism of heparin's anticoagulant action and to consider ways in which this new knowledge may alter our current methods of monitoring heparin therapy.

During the coagulation of blood, thrombin is produced from its precursor, prothrombin, by the concerted action of Factor V and activated Factor X (Factor Xa) in the presence of a lipid surface and calcium ions. Thrombin consists of a heavy and a light polypeptide chain connected by disulfide crosslinks. Once this proteolytic enzyme is generated, it specifically cleaves two pairs of unique arginine-glycine peptide bonds in fibrinogen, and initiates the development of a fibrin clot. The clotting activity of thrombin is critically dependent upon a group of amino acid residues which constitute its active center region and predominant among these is a highly reactive serine on the heavy polypeptide chain.

Thrombin gradually loses its enzymatic activity when added to defibrinated plasma or serum. At the beginning of this century, Contjean, Morowitz, Howell, and others recognized this phenomenon and postulated that antithrombin, a specific inhibitor of thrombin, must be present in plasma under normal physiological conditions. By 1916, McLean had isolated heparin from the liver as well as the heart and demonstrated its potent anticoagulant action. Twenty three years later, Brinkhous et al. showed that the anticoagulant effects of heparin occurred only in the presence of a plasma component which they termed heparin cofactor.

In the 1950's, the work of Lyttleton, Waugh and Fitzgerald, and Monkhouse, France, and Seegers, indicated that plasma antithrombin activity and plasma heparin cofactor activity are intimately related. These investigations suggested that heparin acts to accelerate, by 50 to 100 fold, the rate at which antithrombin neutralizes thrombin. In 1968, this hypothesis was substantiated by Abildgaard who obtained small amounts of a purified human plasma protein which functions in both capacities. More recently, our work has provided convincing physiochemical evidence that antithrombin activity and heparin cofactor activity are properties of a single molecular species. Furthermore, we have demonstrated by immunoprecipitation of this protein from plasmas that this inhibitor is responsible for most, if not all, of the thrombin neutralizing activity and heparin cofactor activity of normal blood.

We have studied the neutralization of thrombin by antithrombin, utilizing purified human protein preparations, and have demonstrated that the equivalence point of the reaction occurs at a 1:1 stoichiometric ratio of enzyme to inhibitor. The addition of heparin to these components does not appreciably alter the equivalence point, but does dramatically accelerate the rate of the reaction.

We have also directly monitored the physical formation of a thrombin-antithrombin complex. As we expected from our estimates of reaction stoichiometry, the complex has a molecular weight approximately equal to the sum of the molecular weights of thrombin and antithrombin. In the absence of heparin, complex formation occurs at a relatively slow rate consonant with the gradual inhibition of enzymatic activity. In the presence of heparin, complex formation is virtually instantaneous.

The formation of this enzyme-inhibitor complex, both in the presence and absence of heparin, is critically dependent upon the active serine center of thrombin. If this residue is blocked by the addition of diisopropylfluorophosphate, interaction between thrombin and antithrombin is totally inhibited. As we would expect from the known specificity of thrombin, the reactive site of the inhibitor which binds the active serine center of the enzyme contains an arginine residue. Modification of this group on antithrombin with 2,3 butanedione or 1,2 cyclohexanedione virtually eliminates the ability of this protein to inhibit thrombin in the presence or absence of heparin.

Furthermore, given the highly acidic nature of heparin, one would expect that positive groups on antithrombin such as ε-amino lysyl residues form the binding site for this negatively charged anticoagulant. The chemical alteration of these residues with o-methylisourea prevents the binding of heparin to this protein and suppresses the acceler-
tion of inhibitor action by this anticoagulant. However, the slow progressive neutralization of thrombin by antithrombin is not appreciably affected.\textsuperscript{13}

Thus antithrombin neutralizes the activity of thrombin by complex formation via a reactive site (arginine)--active center (serine) interaction. Heparin binds to the lysyl residue of antithrombin and accelerates this interaction. The dramatic increase in the rate of complex formation is most probably dependent upon a heparin-induced conformational alteration of the inhibitor which renders the reactive site arginine more accessible to the active center serine of thrombin. Indirect evidence for this heparin-dependent conformational event has been obtained.

The coagulation cascade is composed of a series of linked proteolytic reactions. At each stage of this mechanism, a parent zymogen is converted to a corresponding serine protease which catalyzes a subsequent zymogen-serine protease transition. Thus of the seven proteins directly or indirectly involved in the conversion of prothrombin to thrombin, four proteins are ultimately activated to serine proteases (Factor XIa, Factor Xa, Factor IXa, and Factor X) and two proteins are cofactors for these proteolytic events (Factor V and Factor VIII).\textsuperscript{14, 15} The characterization of human Factor VIIa remains to be determined.

Previous investigators have shown that Factor Xa is neutralized by antithrombin and heparin.\textsuperscript{16, 17, 18} On the basis of this evidence and our knowledge of the biochemical mechanism of antithrombin action, we predicted that all serine proteases produced within the coagulation cascade would be neutralized by this inhibitor and that heparin would accelerate each of these interactions.

To test this concept, we have examined the interaction of antithrombin and heparin with partially purified Factor XIa, a serine protease which is generated at an early stage of the coagulation cascade. The physical properties and substrate specificity of Factor XIa differ markedly from those of Factor Xa and thrombin. We have been able to show that Factor XIa is slowly neutralized by antithrombin in the absence of heparin and rapidly inhibited by antithrombin in the presence of this anticoagulant.\textsuperscript{19} Furthermore, Aronson, utilizing antithrombin prepared by us, has shown that partially purified Factor IXa is neutralized by antithrombin and heparin in a similar fashion (personal communication).

Thus current evidence strongly favors our concept of heparin's multiple actions within the coagulation system and suggests that the remaining serine proteases of the clotting mechanism might be inactivated by this acidic mucopolysaccharide and its protein cofactor. As pure human coagulation factor proteins become available, we hope to rigorously test this hypothesis for each step of the coagulation cascade.

This generalized view of the action of heparin may require a substantial revision of our current usage of this acidic mucopolysaccharide. Although the mechanism of heparin's action suggests that \textit{in vivo} suppression of serine proteases should determine the adequacy of anticoagulant therapy, current techniques for monitoring heparin dosage measure only the extent to which it has "activated" plasma antithrombin. Present assays quantitate this phenomenon by determining the degree to which plasma from a heparinized patient inhibits the \textit{in vitro} addition of an arbitrary amount of either thrombin\textsuperscript{20} or Factor Xa\textsuperscript{21} or else the extent to which this plasma prevents the \textit{in vitro} generation of an arbitrary amount of serine proteases during kaolin activation (activated partial thromboplastin time).\textsuperscript{22} In either case no attempt is made to judge the effect of the heparin activated plasma antithrombin in inhibiting the serine proteases of the coagulation cascade generated within the patient's circulatory system. This critique of present methodology is pertinent to situations in which either standard dosages of heparin are employed after thrombus formation or low dosages are administered prior to clot development.

Several groups of investigators are establishing methods for the direct or indirect measurement of \textit{in vivo} levels of the serine proteases active in coagulation. Nossel and his co-workers have developed an assay for the detection of fibrinopeptide A which is released from fibrinogen by thrombin.\textsuperscript{23} Our laboratory is currently engaged in establishing techniques for the direct as well as indirect measurement of \textit{in vivo} levels of thrombin and other serine proteases of the coagulation system. As these and other sophisticated assays become available, we shall shift from our present approach in which arbitrary amounts of heparin are employed to grossly inhibit the clotting mechanism to one in which a minimum dosage is administered to adjust each stage of the coagulation cascade to a specific level of activity. This rigorous control of anticoagulant therapy should permit optimally effective usage of heparin medication with a minimal incidence of bleeding complications.
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