Discussion Letter

# A cross-linking mechanism for the heparin-enhanced antithrombin–thrombin reaction?

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Emerging evidence suggests that a simple cross-linking reaction through ionic interaction may be the primary cause of the heparin-accelerated antithrombin inhibition of α-thrombin.

*Heparin* *Antithrombin* *Thrombin*

## 1. INTRODUCTION

The molecular mechanism of heparin-enhanced antithrombin–thrombin reaction [1–4] is a debated subject. Three models are currently available to account for the action of heparin. (i) The heparin-antithrombin model [5–7] postulates that heparin binds to antithrombin and transforms antithrombin into a more effective inhibitor. (ii) The heparin-thrombin model [8–11] postulates that heparin binds to thrombin and makes thrombin more susceptible to antithrombin inhibition. (iii) The ternary (template) model [12–17] postulates that heparin must interact with both antithrombin and thrombin to enhance the inhibition. The fact that all 3 models are well sustained by experimental evidence suggests that a compromised working model must accommodate all experimental results supporting each of those models.

There is now growing evidence to indicate that the ternary model [12] may represent the compromise between the heparin-thrombin and heparin-antithrombin models as the practical working model. This evidence further implies that heparin accelerates the formation of the antithrombin–thrombin complex by acting primarily as a noncovalent cross-linking reagent, and that the acidic sulfate groups of heparin cross-link antithrombin and thrombin by interacting with the basic cross-linking sites of antithrombin and thrombin independently.

This evidence, derived from both chemical and kinetic studies, may be summarized as follows:

(i) It is now well demonstrated that heparin reacts with antithrombin [3,18,19] and thrombin [9,11,20–23] independently. The reactions require the acidic sulfate groups of heparin [16,24–26] and the basic amino acids (Lys and Arg) of both antithrombin [3,12,27,30] and thrombin [8,10,12,28]. In addition to heparin, sulfate polymers such as polyanetholesulfonate [27,29], dermatan sulfate [31,32], heparan sulfate [32] and pentosane polysulfate [33,34] also display heparin-like activity in enhancing antithrombin–thrombin reaction.

(ii) Chemical studies [8,10,12] have shown that the

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Thrombin refers to α-thrombin unless otherwise indicated

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heparin binding sites of antithrombin and thrombin are independent of their reactive site and active site, respectively. The sensitivity of heparin-enhanced antithrombin–thrombin reaction can be diminished or abolished by masking the heparin-binding site (through chemical modification of Lys and Arg) of antithrombin [3,12] and/or thrombin [8,10,12,28]. On the other hand, blocking of the active site of thrombin does not prevent heparin-mediated cross-linking of antithrombin and thrombin [12].

(iii) The most convincing evidence for the cross-linking mechanism comes from kinetic studies which have shown that interactions of both antithrombin and thrombin with heparin are involved in the expression of activity [14,17]. At fixed concentrations of antithrombin and thrombin, the reaction rates of antithrombin and thrombin always display a bell-shaped curve against the increase of heparin concentration [6,14,17,35–37]; i.e., there is an optimal heparin concentration for each reaction mixture with fixed concentrations of antithrombin and thrombin. The molar ratios of antithrombin/heparin/thrombin observed under those optimal conditions are strikingly close to 1 : 1 : 1 [14]. These results are characteristic of protein cross-linking phenomena: at low concentrations of heparin, both antithrombin and thrombin will compete for binding to heparin; at excess concentrations of heparin, both antithrombin and thrombin will be saturated with heparin; and the optimal cross-linking reaction occurs when the cross-linking reagent and proteins have approximately equal concentrations.

Finally, the binding mechanism of this proposed cross-linking model bears an interesting resemblance to the binding mechanism of the hirudin–thrombin complex [38]. Efficient inhibition of both hirudin and antithrombin requires interaction with $\alpha$-thrombin through the active site as well as an independent basic site [39,40]. This resemblance, together with the intriguing sequence homology of hirudin and fibrinopeptides [38], suggets that a common mechanism may account for the unique enzymatic specificity of $\alpha$-thrombin toward both protein inhibitors and protein substrates.

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