A novel antithrombin domain dictates the journey’s end of a proteinase

DOI 10.1074/jbc.H117.787325

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Edited by Norma Allewell

Antithrombin (AT) is an anticoagulant serpin that irreversibly inactivates the clotting proteinases factor Xa and thrombin by forming covalent complexes with them. Mutations in its critical domains, such as those that impair the conformational rearrangement required for proteinase inactivation, increase the risk of venous thrombosis. Águila et al. characterize for the first time the destabilizing effects of mutations in the region of AT that makes contact with the proteinase in the final acyl-enzyme complex. Their work adds new insight into the unique structural intricacies of the inhibitory mechanism.

Inhibitory serpins (serine proteinase inhibitors) belong to a superfamily of proteins with a characteristic fold of three β sheets, eight or nine α helices, and a reactive center loop (RCL)² that contains a specific proteinase cleavage site (1). Of the ~1,500 identified serpin gene sequences, 36 are present in humans where they perform diverse functions. Antithrombin (AT), one of the 29 known human inhibitory serpins, is a major regulator of hemostasis, as inhibition of its targets thrombin and factor Xa (FXa) leads to a reduction in clotting. Inhibitory serpins are metastable: They undergo one of the largest known conformational changes upon forming covalent complexes with their proteinase targets (2). Whereas the importance of the RCL structure in this conformational change is well documented, much less is known about the structural requirements of residues in the base of the serpin, in particular the region that becomes the “landing place” for the inactivated proteinase in the covalent complex. Águila et al. (3) now present clinical and biochemical proof that this region in the serpin is critical for inhibitory function and may be considered a new regulatory domain.

Serpin action has been likened to that of a mousetrap. In the Michaelis complex, the proteinase is docked to the RCL of the native serpin; upon cleavage of the reactive site in the RCL, an acyl-enzyme intermediate is formed, and the N-terminal RCL fragment inserts as an extra strand into the principal β sheet of AT, rapidly translocating the attached proteinase 180° to the distal end of the serpin (Fig. 1, A and B). In this energetically favorable and stable complex, the proteinase active site is distorted, and the serpin acts as a suicide substrate (1, 2, 4, 5). Proteinase translocation, final docking, and distortion are steps in the inhibitory pathway of the branched serpin mechanism (Fig. 1C). Disturbing the serpin structure favors the alternative side of the branched mechanism, the substrate pathway, in which the acyl-enzyme intermediate completes hydrolysis to form a cleaved serpin and regenerated proteinase.

The elaborate structure of inhibitory serpins makes them particularly prone to dysfunction caused by mutations. Moderate and even mild AT deficiency, as observed in patients with heterozygous mutations, significantly increases the risk of deep vein thrombosis and pulmonary embolism. In type II AT deficiency, normal antigen levels are present but the inhibitory activity is reduced due to mutations in critical regions of the serpin. RCL mutations increase the contribution of the substrate pathway by affecting RCL insertion and proteinase translocation. Relative rates of kinetic trapping of the stable complex (k₅) and the substrate reaction (k₄) are altered, and the stoichiometry of inhibition (equal to (k₅ + k₄)/k₄) becomes larger than 1 (6). Dissociation of the final serpin-protease complex (k₅) also increases this stoichiometry, and artificial perturbation of the serpin-proteinase interface has been shown to destabilize the final complex (7). Águila et al. (3) were the first to identify natural, destabilizing AT mutations at this interface in patients with AT type II deficiency (3) and to propose two potential mechanisms for impaired FXa and thrombin inactivation by these mutants.

In their new study, Águila et al. (3) consider five previously undescribed mutations outside key functional domains associated with type II AT deficiencies, I207T, L340F, S349P, S365L, and H369Y (Fig. 1D), and investigate two of these sites in depth. A patient with the S365P mutation was previously identified as type I-deficient, with poor AT expression. Moreover, Ser365 and Ile397 are highly conserved and are located in the area of contact with the protease in the covalent complex and near the RCL. Despite these features, it was not intuitively obvious how these residues would impact the inhibition mechanism. Studying these residues therefore provides an opportunity to assign functional roles to new regions of AT as well as to differentiate between mechanisms of increased substrate partitioning dur-
The importance of understanding the structure and function of serpins lies in their role in proteinase inhibition and the implications of mutations in disease. The figures illustrate the complex mechanism of proteinase inactivation by serpins, with a focus on the role of specific residues such as Ser365 and Ile207. The diagrams show the interaction between serpins and proteinases, highlighting the substrate pathway and the implications of mutations for the inhibition stoichiometry.

**References**


**Editors’ Pick Highlight: A novel antithrombin domain**

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