Mutagenesis of thrombin selectively modulates inhibition by serpins heparin cofactor II and antithrombin III. Interaction with the anion-binding exosite determines heparin cofactor II specificity.

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Abstract

Thrombin is a multifunctional serine protease that plays a critical role in hemostasis. Thrombin is inhibited by the serpins antithrombin III and heparin cofactor II in a reaction that is dramatically accelerated by glycosaminoglycans. The structural basis of the interaction with these inhibitors was investigated by introducing single amino acid substitutions into the anion-binding exosite (R68E, R70E) and unique insertion loops (K52E, K154A) of thrombin. The rate of inhibition of these recombinant thrombins by antithrombin III and heparin cofactor II was determined in the absence and presence of glycosaminoglycan. The second order rate constant (k2) for inhibition by antithrombin III without heparin was 3.7 x 10(5) M-1 min-1 for wild-type thrombin; rates for the mutant thrombins varied less than 2-fold. For inhibition by antithrombin III with heparin, the rate constant was 4.5 x 10(8) M-1 min-1 for wild-type thrombin with no significant differences between any of the recombinant thrombins. In contrast, the rate constant for inhibition by heparin cofactor II without glycosaminoglycan was 4.3 x 10(4) M-1 min-1 for wild-type thrombin; rates were 10-fold slower for thrombin K52E and 2- to 3-fold slower for thrombins R68E and R70E. The rate constants for inhibition of wild-type thrombin by HCII in the presence of heparin or dermatan sulfate were 9.2 x 10(8) M-1 min-1 and 9.0 x 10(8) M-1 min-1, respectively. Compared to wild-type thrombin, the rate of inhibition by HCII with glycosaminoglycan was 5- to 15-fold slower for thrombins K52E and R70E and 50- to over 100-fold slower for thrombin R68E. Thrombin K154A was inhibited by heparin cofactor II with rates similar to wild-type thrombin in all assays. These results suggest that heparin cofactor II interacts with residue Lys-52 in the proposed S1' subsite and with residues Arg-68 and Arg-70 in the anion-binding exosite of thrombin, and that these interactions contribute to the molecular basis of heparin cofactor II specificity for thrombin.