Heparin cofactor II is regulated allosterically and not primarily by template effects. Studies with mutant thrombins and glycosaminoglycans.

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Abstract

Besides its critical role in hemostasis, the serine protease thrombin also participates in wound healing, inflammation, and atherosclerosis. Thrombin is inhibited by the serpins antithrombin and heparin cofactor II (HCII) in reactions that are accelerated markedly by specific glycosaminoglycans. Following vascular injury, thrombin must be inhibited at both intravascular and extravascular sites that impose different constraints on the recognition of thrombin by these inhibitors. The present study examines the role of anion-binding exosite II of thrombin in the interaction with glycosaminoglycans and HCII. Acceleration of thrombin inhibition by serpins in the presence of glycosaminoglycans is proposed to occur by a template mechanism, in which inhibitor and protease bind simultaneously to the same glycosaminoglycan chain, facilitating their interaction. According to the template model, disruption of protease binding to glycosaminoglycan should significantly reduce acceleration of the inhibition. Specific mutations in exosite II (R89E, R245E, K248E, and K252E) disrupted thrombin binding to both dermatan sulfate and heparin, indicating that both glycosaminoglycans bind to a common site in exosite II. The same mutations markedly decreased the rate constant for thrombin inhibition by antithrombin-heparin (up to 100-fold) but had little effect on the rate constant for thrombin inhibition by HCII-heparin (7-fold maximal reduction) and no effect on the rate constant for thrombin inhibition by HCII-dermatan sulfate. These results are incompatible with a template model for thrombin inhibition by HCII and dermatan sulfate. In the presence of glycosaminoglycan, HCII and antithrombin interact with opposing thrombin exsites and use distinct mechanisms of glycosaminoglycan catalysis. Antithrombin employs a template mechanism that requires heparin to interact with thrombin exosite II, whereas HCII employs an allosteric mechanism that requires thrombin exosite I but is largely independent of exosite II. These findings have potential implications for glycosaminoglycan therapy and for the respective physiologic roles of HCII and antithrombin.