The factor IXa heparin-binding exosite is a cofactor interactive site: mechanism for antithrombin-independent inhibition of intrinsic tenase by heparin.

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Abstract

Therapeutic heparin concentrations selectively inhibit the intrinsic tenase complex in an antithrombin-independent manner. To define the molecular target and mechanism for this inhibition, recombinant human factor IXa with alanine substituted for solvent-exposed basic residues (H92, R170, R233, K241) in the protease domain was characterized with regard to enzymatic activity, heparin affinity, and inhibition by low molecular weight heparin (LMWH). These mutations only had modest effects on chromogenic substrate hydrolysis and the kinetics of factor X activation by factor IXa. Likewise, factor IXa H92A and K241A showed factor IXa-factor VIIIa affinity similar to factor IXa wild type (WT). In contrast, factor IXa R170A demonstrated a 4-fold increase in apparent factor IXa-factor VIIIa affinity and dramatically increased coagulant activity relative to factor IXa WT. Factor IXa R233A demonstrated a 2.5-fold decrease in cofactor affinity and reduced ability to stabilize cofactor half-life relative to wild type, suggesting that interaction with the factor VIIIa A2 domain was disrupted. Markedly (R233A) or moderately (H92A, R170A, K241A) reduced binding to immobilized LMWH was observed for the mutant proteases. Solution competition demonstrated that the EC(50) for LMWH was increased less than 2-fold for factor IXa H92A and K241A but over 3.5-fold for factor IXa R170A, indicating that relative heparin affinity was WT > H92A/K241A > R170A >> R233A. Kinetic analysis of intrinsic tenase inhibition demonstrated that relative affinity for LMWH was WT > K241A > H92A > R170A >> R233A, correlating with heparin affinity. Thus, LMWH inhibits intrinsic tenase by interacting with the heparin-binding exosite in the factor IXa protease domain, which disrupts interaction with the factor VIIIa A2 domain.

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