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The regulation of factor IXa by supersulfated low molecular weight heparin.

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Abstract

Supersulfated low molecular weight heparin (ssLMWH) inhibits the intrinsic tenase (factor IXa-factor VIIIa) complex in an antithrombin-independent manner. Recombinant factor IXa with alanine substitutions in the protease domain (K126A, N129A, K132A, R165A, R170A, N178A, R233A) was assessed with regard to heparin affinity in solution and ability to regulate protease activity within the factor IXa-phospholipid (PL) and intrinsic tenase complexes. In a soluble binding assay, factor IXa K126A, K132A, and R233A dramatically (10-20-fold) reduced ssLMWH affinity, while factor IXa N129A and R165A moderately (5-fold) reduced affinity relative to wild type. In the factor IXa-PL complex, binding affinity for ssLMWH was increased 4-fold, and factor X activation was inhibited with a potency 7-fold higher than predicted for wild-type protease-ssLMWH affinity in solution. In the intrinsic tenase complex, ssLMWH inhibited factor X activation with a 4-fold decrease in potency relative to wild-type factor IXa-PL. The mutations increased resistance to inhibition by ssLMWH in a similar fashion for both enzyme complexes (R233A > K126A > K132A/R165A > N129A/N178A/wild type) except for factor IXa R170A. This protease had ssLMWH affinity and potency for the factor IXa-PL complex similar to wild-type protease but was moderately resistant (6-fold) to inhibition in the intrinsic tenase complex based on increased cofactor affinity. These results are consistent with conformational regulation of the heparin-binding exosite and macromolecular substrate catalysis by factor IXa. An extensive overlap exists between the heparin and factor VIIIa binding sites on the protease domain, with residues K126 and R233 dominating the heparin interaction and R165 dominating the cofactor interaction.

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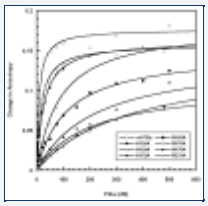

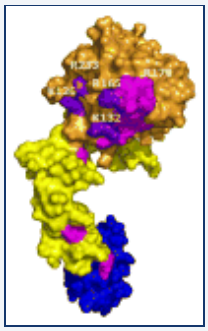

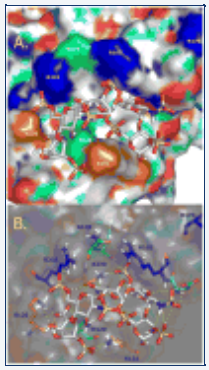

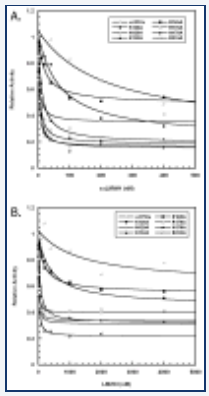

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