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Heparin inhibits the intrinsic tenase complex by interacting with an exosite on factor IXa.

Sheehan JP, Kobbervig CE, Kirkpatrick HM.

Department of Medicine/Hematology, University of Wisconsin-Madison, 53706, USA.

jps@medicine.wisc.edu

Abstract

The specific molecular target for direct heparin inhibition of factor X activation by intrinsic tenase (factor IXa-factor VIIIa) was investigated. Comparison of size-fractionated oligosaccharides demonstrated that an octasaccharide was sufficient to inhibit intrinsic tenase. Substitution of soluble dihexanoic phosphatidylserine (C6PS) for phospholipid (PL) vesicles demonstrated that inhibition by low-molecular weight heparin (LMWH) was independent of factor IXa-factor VIIIa membrane assembly. LMWH also inhibited factor X activation by the factor IXa-PL complex via a distinct mechanism that required longer oligosaccharides and was independent of substrate concentrations. The apparent affinity of LMWH for the factor IXa-PL complex was higher in the absence of factor VIIIa, suggesting that the cofactor adversely affected the interaction of heparin with factor IXa-phospholipid. LMWH did not interact directly with the active site, as it failed to inhibit chromogenic substrate cleavage by the factor IXa-PL complex. LMWH induced a modest decrease in factor IXa-factor VIIIa affinity [K(D(app))] on PL vesicles that did not account for the inhibition. In contrast, LMWH caused a substantial reduction in factor IXa-factor VIIIa affinity in the presence of C6PS that fully accounted for the inhibition. Factor IXa bound LMWH with significantly higher affinity than factor X by competition solution affinity analysis, and the K(D(app)) for the factor IXa-LMWH complex agreed with the K(I) for inhibition of the factor IXa-PL complex by LMWH. Thus, LMWH binds to an exosite on factor IXa that antagonizes cofactor activity without disrupting factor IXa-factor VIIIa assembly on the PL surface. This exosite may contribute to the clinical efficacy of heparin and represents a novel target for antithrombotic therapy.

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