

. Biochemistry. 2007 Jul 3;46(26):7886-95. Epub 2007 Jun 12.

# The heparin-binding exosite is critical to allosteric activation of factor IXa in the intrinsic tenase complex: the role of arginine 165 and factor X.

[Misenheimer TM](#), [Buyue Y](#), [Sheehan JP](#).

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

## Abstract

Heparin inhibits the intrinsic tenase complex (factor IXa-factor VIIIa) via interaction with a factor IXa exosite. To define the role of this exosite, human factor IXa with alanine substituted for conserved surface residues (R126, N129, K132, R165, N178) was characterized. Chromogenic substrate hydrolysis by the mutant proteases was reduced 20-30% relative to factor IXa wild type. Coagulant activity was moderately (N129A, K132A, K126A) or dramatically (R165A) reduced relative to factor IXa wild type. Kinetic analysis demonstrated a marked reduction in apparent cofactor affinity (23-fold) for factor IXa R165, and an inability to stabilize cofactor activity. Factor IXa K126A, N129A, and K132A demonstrated modest reductions (approximately 2-fold) in apparent cofactor affinity, and accelerated decay of intrinsic tenase activity. In the absence of factor VIIIa, factor IXa N178A and R165A demonstrated a defective  $V_{max(app)}$  for factor X activation. In the presence of factor VIIIa,  $V_{max(app)}$  varied in proportion to the predicted factor IXa-factor VIIIa concentration. However, factor IXa R165A had a 65% reduction in the  $k_{cat}$  for factor X, suggesting an additional effect on catalysis. The ability of factor IXa to compete for physical assembly into the intrinsic tenase complex was enhanced by EGR-chloromethylketone bound to the factor IXa active site or addition of factor X, and reduced by selected mutations in the heparin-binding exosite (N178A, K126A, R165A). These results suggest that the factor IXa heparin-binding exosite participates in both cofactor binding and protease activation, and cofactor affinity is linked to active site conformation and factor X interaction during enzyme assembly.

PMID: 17563121 [PubMed - indexed for MEDLINE]