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Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex by an allosteric mechanism.

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Abstract

Phosphorothioate oligonucleotides (PS ODNs) prolong the activated partial thromboplastin time in human plasma by inhibition of intrinsic tenase (factor IXa-factor VIIIa) activity. This inhibition was characterized using ISIS 2302, a 20-mer antisense PS ODN. ISIS 2302 demonstrated hyperbolic, mixed-type inhibition of factor X activation by the intrinsic tenase complex. The decrease in $V(\max(\text{app}))$ was analyzed by examining complex assembly, cofactor stability, and protease catalysis. ISIS 2302 did not inhibit factor X activation by the factor IXa-phospholipid complex, or significantly affect factor VIII-phospholipid affinity. Inhibitory concentrations of ISIS 2302 modestly decreased the affinity of factor IXa-factor VIIIa binding in the presence of phospholipid ($K(D) = 11.5$ vs 4.8 nM). This effect was insufficient to explain the reduction in $V(\max(\text{app}))$. ISIS 2302 did not affect the in vitro half-life of factor VIIIa, suggesting it did not destabilize cofactor activity. In the presence of 30% ethylene glycol, the level of factor X activation by the factor IXa-phospholipid complex increased 3-fold, and the level of chromogenic substrate cleavage by factor IXa increased more than 50-fold. ISIS 2302 demonstrated partial inhibition of factor X activation by the factor IXa-phospholipid complex, and chromogenic substrate cleavage by factor IXa, only in the presence of ethylene glycol. Like the intact enzyme complex, ISIS 2302 demonstrated hyperbolic, mixed-type inhibition of chromogenic substrate cleavage by factor IXa ($K(I) = 88$ nM). Equilibrium binding studies with fluorescein-labeled ISIS 2302 demonstrated a similar affinity ($K(D) = 92$ nM) for the PS ODN-factor IX interaction. These results suggest that PS ODNs bind to an exosite on factor IXa, modulating catalytic activity of the intrinsic tenase complex.

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