ANTICOAGULANT ACTIVITY OF A SULFATED GALACTAN. SERPIN-INDEPENDENT EFFECT AND SPECIFIC INTERACTION WITH FACTOR Xa.

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Sulfated Galactan (SG) is a sulfated polysaccharide from the red algae *Botryocladia occidentalis* that showed a promising anticoagulant and antithrombotic activity. The anticoagulant action of SG in normal plasma is due to inhibition of FXa and/or thrombin by activation of serpins, antithrombin (AT) and/or heparin cofactor II (HCII). Surprisingly, SG retains its capacity to prolong the coagulation time using a serpin depleted plasma. SG is able to totally inhibit intrinsic tenase and prothrombinase complexes. These results show that SG has an alternative inhibitory activity than the ordinary AT/HCII dependent mechanism. Additionally, we investigated a possible role of Ca^{2+} on the SG catalysis of the FXa inhibition mediated by AT. Actually, it is proposed that Ca^{2+} ions induce the exposure of a specific heparin-binding exosite on FXa (FXa HBE). In the presence of Ca^{2+}, the inhibitory effect of SG:AT complex on native FXa increased approximately 200 fold as compared with the activity in the presence of EDTA. Inhibitory assays using FXa mutants at HBE showed that the effect SG:AT is mostly preserved in these mutants. However, a specific ligand of FXa heparin-binding exosite, Ixolaris, completely abolished the inhibitory effect of SG on the protease. Ixolaris prevents FXa binding to SG immobilized on a sepharose column. This result suggests that SG binds to FXa through FXa-HBE and, probably, inhibits prothrombinase complex through its interaction, impairing the complex assembly. Overall, these results indicate that SG acts as an anticoagulant through a different mechanism from that described for heparin.