Purification and characterization of an anticoagulant peptide derived from fish protein hydrolysate

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Abstract

New anticoagulant compounds provides clinical perspectives for the prevention and treatment of blood related disorders[1]. An anticoagulant peptide which inhibit intrinsic pathway of human coagulation cascade was isolated from a protein hydrolysate of marine fish, yellowfin sole (*Limanda aspera*) frame. Screening studies on anticoagulant peptides employed protein hydrolysates with different proteases. Alpha-chymotrypsin mediated hydrolysate could prolong activated partial thromboplastin time (APTT) markedly but not the prothrombin time (PT) of *in vitro* coagulation tests, revealing the anticoagulant as an intrinsic pathway inhibitor. The coagulation activity was purified by a combination of chromatographic techniques. The purified inhibitor had a molecular weight of 3-5 KDa determined by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition. The specific factor inhibition activity in intrinsic pathway was determined by clotting assays which assess the ability of anticoagulant treated plasma to retard normalization of prolonged coagulation time of specific-factor deficient human plasma. The mechanism of coagulation of the new compound was elucidated by simple method of SDS-PAGE using the specific coagulation factors. It was observed that the anticoagulant could bind with intrinsic pathway coagulation factor with a higher molecular ratio. Further, dependency of Ca+ and other several cations on the binding of anticoagulant peptide with the coagulation factor was tested. These results speculate that the new peptide prolong the clotting time by binding to an intrinsic pathway coagulation factors.

References

1. Jeffrey, I. W., Jack, H. (2001), New anticoagulant drugs, *Chest* **119**, 95-107.