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Purification and Characterization of Anticoagulant Protein from Ark Shell, *Scapharca broughtonii*

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Introduction

The physiological systems that control blood fluidity are both complex and elegant. Blood must remain fluid within the vasculature and yet clot quickly when exposed to nonendothelial surfaces at sites of vascular injury. There are two principle mechanisms to control a delicate balance in higher organisms (Davie & Ratnoff, 1964). Present evidence suggests that the intrinsic pathway play an important role in the growth and maintenance of fibrin formation in the coagulation cascade while a second overlapping mechanism, called the extrinsic pathway, is critical in the initiation of fibrin formation. Coagulation factors is in two mechanisms, and in order to clot blood, they are activated by a cooperation with Ca^{2+}, phospholipid and vitamin K etc. . For example, the human placental anticoagulant protein (PAP of PAP- I), which is a Ca^{2+}-dependent phospholipid binding protein (Funakoshi et al., 1987) inhibited the activity of factor Xa, so that it prolonged fibrin formation. We wondered whether any other protein was involved in regulation of the coagulant system as an anticoagulant protein from natural organisms. Natural agents would have not harmful side-effects in comparision with chemically synthesized materials such as warfarin, aspirin, phenindione, etc.. But anticoagulant agents from natural, especially marine organisms have hardly been researched except for polysaccharides from marine algae.

In this study, a novel and natural anticoagulant protein (APS) with predominant activity was purified from ark shell, *Scapharca broughtonii*, and identified as the inhibitor of factor IX (Christmas factor) in the human blood coagulant cascade.

Materials and Methods

The APS (Anticoagulant Protein from ark shell, *Scapharca broughtonii*) was purified by ammonium sulfate precipitation, DEAE-Sephadex A-50 column, Sephadex G-75, DEAE-Sephacel column and Biogel P-100 from the flesh of shell. To measure inhibitory activity on the intrinsic and extrinsic coagulation pathway, the aPTT (activated Patial Thromboplastin Time) and PT (Prothrombin Time) assay was used, respectively. And assay of Factor IX inhibitory activity was used for the measurement of specific-inhibited factor.

Results and Summaries

The APS was purified from the soluble fraction in the flesh of ark shell by 20~80% ammonium sulfate precipitation, anion exchange (DEAE-Sephadex A-50, DEAE-Sephacel) and gel fitration (Sephadex G-75, Biogel P-100) column chromatography. It had approximately the molecular weight of 26kDa by 7.5% SDS-PAGE. Coagulant inhibitory effects of the APS on both intrinsic and extrinsic pathways were examined. The APS prolonged coagulation time over ten times than control (normal plasma) on the aPTT assay, and it showed that the APS inhibited the coagulant factors in a intrinsic system. So we examined which factor was inhibited on intrinsic pathway, in the factor IX was negatively controlled to about 27% by the APS while control (normal plasma) expressed 100%. The APS inhibited factor IX as strong as it could induce hemophilia B (below 25% is referred to hemophilia B) in a human plasma, pathologically (Davie et al., 1987).

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