

HUMAN PROTHROMBIN/PHOSPHOLIPIDS INTERACTION, COMPETITIVE EFFECT OF AN ANTICOAGULANT VENOM PHOSPHOLIPASE A<sub>2</sub>. G. A. Boffa, R. Benarous, M. C. Boffa.  
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Published online: 2019-04-16 The anticoagulant activity of the phospholipase A<sub>2</sub> isolated from a Viperidae venom was explained by the formation of a complex with phospholipid at its protein binding site (1). The anticoagulant effect was reversible with specific antiphospholipase A<sub>2</sub> immunoglobulins.

The interaction between <sup>125</sup>I prothrombin and phospholipid in the presence of calcium was impaired by the phospholipase. A competitive effect between prothrombin and the phospholipase was demonstrated. It did not appear sufficient to explain the anticoagulant property of the enzyme. This effect was abolished after photo-oxidation of the phospholipase. In the presence of another Viperidae phospholipase A<sub>2</sub>, devoid of anticoagulant activity, no competitive effect was observed.

The presence of several sites responsible for anticoagulant, catalytic and recognition properties have been looked for by various partial chemical denaturation processes.

(1) *Biochim. Biophys. Acta* 1976, 429, 828-852.

PREPARATION OF A NONTHROMBOGENIC PROTHROMBIN COMPLEX CONCENTRATE. Sudhish Chandra and Milan Wickerhauser, Blood Research Laboratory, American National Red Cross, Bethesda, Maryland, USA

The major problem faced in the preparation of prothrombin complex concentrates (PTC) for clinical use is the activation of clotting factors. This activation may cause thrombo-embolic complications when such a preparation is administered to the patients with liver impairment or factor IX deficiency. We have developed a large scale procedure which minimizes this problem and leads to a substantially safer preparation. Plasma supernatant, after recovery of cryoprecipitate, is adsorbed with kaolin to remove contact factors. Kaolin supernatant is fractionated by precipitation with 10% polyethylene glycol 4000 (PEG) to remove fibrinogen, plasminogen and factors that might contribute to the undesirable activation of PTC. PTC is then extracted from the 10% PEG supernatant by batchwise adsorption and elution with DEAE cellulose. The eluting buffer is enriched with a small amount of antithrombin III to maintain the stability of the product against possible activation. The eluted PTC is concentrated by lyophilization, desalted on a Sephadex G-50 column, sterilized and lyophilized under aseptic conditions. The final concentrate was 70-fold purified and the recovery was 30% over starting plasma in terms of factor IX activity. The preparation was non-thrombogenic *in vitro* by the nonactivated partial thromboplastin time test and *in vivo* by the stasis model (Wessler et al *J. Appl. Physiol.* 14, 943, 1959) using 90 F IX units/Kg. body weight of the rabbit.

STRUCTURAL CHARACTERISTICS OF PROTHROMBIN. Daniel A. Walz and Walter H. Seegers. Department of Physiology, Wayne State University, Detroit, Michigan, U.S.A. 48201.

The amino acid sequences of human and bovine prothrombin fragments 1 and 2 have been completed. In addition, partial sequence information has been obtained on the respective fragments from chicken prothrombin. Fragment 1 from human (155 amino acids), bovine (156 amino acids) and chicken (approximately 161 amino acids) contains the vitamin K-dependent calcium binding region (residues 1-32) and is a highly conserved region of the fragment. Fragment 2 from human (118 amino acids), bovine (118 amino acids) and chicken (113 amino acids) is apparently undergoing a more rapid rate of nucleotide substitution than is fragment 1. The A-chain of the enzyme from human (36+13 amino acids), bovine (49 amino acids) and chicken (49 amino acids) have a highly variable amino-terminal portion (residues 1-13) and a conserved portion (residues 14-49). Calculations for fragment 1 indicate the presence of 3  $\alpha$ -helical regions (23.2%), 9  $\beta$ -sheet regions (34.2%) and 10  $\beta$ -turns (25.8%). The calcium binding region is an  $\alpha$ -helical region of the molecule. Fragment 2 has 5  $\alpha$ -helical regions (38.1%), 4  $\beta$ -sheet regions (19.5%) and 9  $\beta$ -turns (30.5%). The differing rates of substitution found for fragments 1 and 2 are having no apparent effect on the calculated  $\alpha$ -helical or  $\beta$ -sheet regions within prothrombin and may account for the limited, conserved activation bonds available within the prothrombin molecule. (Supported in part by a grant from the National Institutes of Health, HL-03424-19 and the McGregor Foundation of Detroit).