

PROTHROMBIN AND ABNORMAL (DES- $\gamma$ -CARBOXY) PROTHROMBIN: ASSESSMENT OF PLASMA LEVELS BY RIA IN DISORDERS OF HEPATIC VITAMIN K-DEPENDENT CARBOXYLATION. R.A. Blanchard, B.C. Furie, S.F. Kruger, and B. Furie. Tufts-NEMC and Tufts Univ. Sch. Med., Boston, MA.

The vitamin K-dependent blood coagulation zymogens contain  $\gamma$ -carboxyglutamic acid (Gla) in their amino terminal domains. The presence of these Gla residues is necessary for the physiologic activation of these zymogens to proteases. The Gla residues are synthesized from glutamic acids on precursor proteins in the liver by a vitamin K-dependent carboxylase. In treatment with vitamin K antagonists or in the presence of vitamin K-deficiency carboxylation is impaired and des- $\gamma$ -carboxy forms circulate in the blood. We have developed specific RIAs for human prothrombin (NPT) and for des- $\gamma$ -carboxy human prothrombin (APT) to evaluate the levels of these species in human plasma. These plasma assays measure only NPT despite the presence of APT or only APT despite the presence of NPT.

	Normal	Warfarin	Vit K Def	Hepatitis	Cirrhosis
APT ( $\mu$ g/ml)	0	29	62	1.4	0.5
Range	0	12-57	32-100	0-6	0.02-4
NPT ( $\mu$ g/ml)	100	18	28	78	58
Range	73-164	3-34	19-34	50-140	24-86
No. patients (N=19)	(N=23)	(N=4)	(N=16)	(N=22)	

These results suggest that total prothrombin synthesis is decreased in patients treated with warfarin and in patients with intrinsic liver disease compared to normals or to vitamin K-deficient patients. APT is not a component of normal plasma (>0.03 g/ml). However APT is present in 90% of the patients with intrinsic liver disease. In ten patients with liver disease in whom samples were obtained before and after vitamin K administration APT persisted in the plasma. This indicates impaired vitamin-K-dependent carboxylation in these disorders. We feel that the patterns of plasma NPT and APT in these disease states are characteristic and may prove useful in identifying and distinguishing liver disease from disorders of PT biosynthesis.

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COMPLEX FORMATION BETWEEN PROTHROMBIN AND STAPHYLOCOAGULASE. M.Lindhout, H.Hendrix and H.Hemker. Department of Biochemistry, Limburg University, Maastricht, the Netherlands.

Staphylocoagulase is a protein secreted by certain strains of *Staphylococcus aureus*. Addition of the extracellular protein to a human prothrombin preparation results in the generation of a thrombin-like activity.

Our investigations on the prothrombin-staphylocoagulase interaction was undertaken in order to establish the kinetics of the complex formation, the mechanism of activation of prothrombin by staphylocoagulase and the enzymatic properties of the complex.

Evidence for the formation of a equimolar complex between prothrombin and staphylocoagulase was obtained from gelelectrophoresis and titration experiments. The procedure, in order to study the kinetics of the complex formation, made use of a coupled assay in which D-Phe-Pip-L-Arg-p-nitroanilide hydrolysis by the complex was monitored. The second rate constant of the complex formation, as determined from pseudo-first order kinetics, is  $4 \times 10^6 \text{ sec}^{-1} \text{ M}^{-1}$ . The dissociation constant of the complex was determined to be orders of magnitude less than  $10^{-11} \text{ M}$ . Using  $^3\text{H}$ -DFFP, no exchange was observed between prothrombin in the complex with free prothrombin. Analysis of the complex by gelelectrophoresis in the presence of Na-dodecyl sulfate and mercaptoethanol shows that proteolysis cannot account for the generation of thrombin-like activity. Small amounts of degradation products (prethrombin 1) were found.

Thrombin-like activity was generated as a result of the interactions between staphylocoagulase and human prethrombin 1, human prethrombin 2 and bovine prethrombin 2. Indications for an interaction between staphylocoagulase and thrombin were obtained from experiments with anti-thrombin III and hirudin. Identical enzymatic properties of thrombin and the prothrombin-staphylocoagulase complex were observed towards small synthetic substrates and fibrinogen. No activity of the complex towards factor V, factor VIII and platelets was found.

PREFERENTIAL BINDING OF PROTHROMBIN FRAGMENTS ON MONOLAYERS CONTAINING PHOSPHATIDYLSELINE. M.F.LECOMPTÉ and I.R.MILLER Department of Membrane Research, the Weizmann Institute of Science, Rehovot, ISRAEL

Phospholipids from platelet membranes play a crucial role in the last step of the blood coagulation cascade, as a catalytic surface for the conversion of prothrombin into thrombin by the prothrombinase complex. It provides a favorable configuration of the required factors (factor Xa, prothrombin, factor Va), concentrated on its surface. The maximal biological activity is observed with mixtures of phospholipids containing phosphatidylserine, in presence of  $\text{Ca}^{++}$ .

In order to understand at the molecular level, which part of prothrombin interact with phospholipids, we studied the correlation between the number of prothrombin molecules or fragments of it adsorbed (by counting the surface radioactivity of  $^3\text{H}$ -protein) and their ability to penetrate into the phospholipid monolayer (measured by the increase of the lipid layer capacitance), by two recent complementary methods of direct surface measurement (radioactivity and electrochemistry), in function of  $\text{Ca}^{++}$  concentration and phospholipid composition.

We could detect parallelly the number of cystine residues (by electrochemical reduction) of the penetrating parts of the adsorbed protein. It occurs that adsorbed protein don't penetrate. The number of S-S groups reduced per adsorbed protein molecule can be deduced and its variation indicate different orientation of the molecules on the phospholipid surface. Indeed, it is the case at different  $\text{Ca}^{++}$  concentration, for prothrombin and fragment 1, which contain  $\gamma$ -carboxyglutamic groups.

We could also observe that penetration is specific of phospholipid composition for fragment 2 but not for thrombin and is not specific for both in function of the  $\text{Ca}^{++}$  concentration.

Results indicate that the ratio of penetration to the total number of adsorbed prothrombin molecules and fragment 1 is important for an optimal catalysis.

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IDENTIFICATION OF TWO DYSTHROMBINS DERIVED FROM PROTHROMBIN QUICK. R. A. Henriksen and W.G. Owen, Departments of Pathology and Biochemistry, University of Iowa, Iowa City, Iowa 52242, U.S.A.

A dysfunctional thrombin, Thrombin Quick, derived from the congenital dysprothrombin, Prothrombin Quick, had 30-40 percent of normal thrombin activity on the low molecular weight substrates, benzoylarginine ethylester, tosyl-gly-pro-arg-p-nitroanilide, and carbobenzoxylysine-p-nitrophenylester, but only 2-5 percent of normal activity in Factor V and Factor VIII activation and platelet aggregation. Titration with the high affinity competitive inhibitor of thrombin, dansyl-arginine-N-(3-ethyl-1,5-pentanediy)amide (DAPA) indicated an active site concentration of 0.4 per mole protein. Further studies indicated that the Thrombin Quick preparation could be eluted from sulfopropyl Sephadex as two components, one of which did not show fluorescence enhancement on treatment with DAPA nor did it possess tripeptidase activity. Further analytical chromatography on sulfopropyl Sephadex indicated that the two Thrombin Quick components are chromatographically distinct from thrombin and that their elution pattern is not altered by treatment with thrombin. Each of the Thrombin Quick components was rechromatographed in the presence of thrombin. There was no indication of interconversion of the two components. The results indicate that the presence of two components in Thrombin Quick is not related to the incomplete cleavage of a 13 residue peptide from the amino terminus of the thrombin A chain which occurs in normal thrombin. A synthetic substrate assay was used to determine relative activities of 0.38, 0.45 and 0.33 for Prothrombin Quick, Prethrombin 1 Quick and Thrombin Quick, respectively, compared to their normal counterparts. The simplest explanation for the occurrence of the two Thrombin Quick species is that they are derived from two genetic allomers of prothrombin.