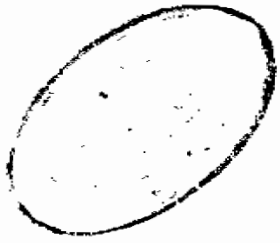
PROTEIN C AND ORAL ANTICOAGULANTS



THESIS

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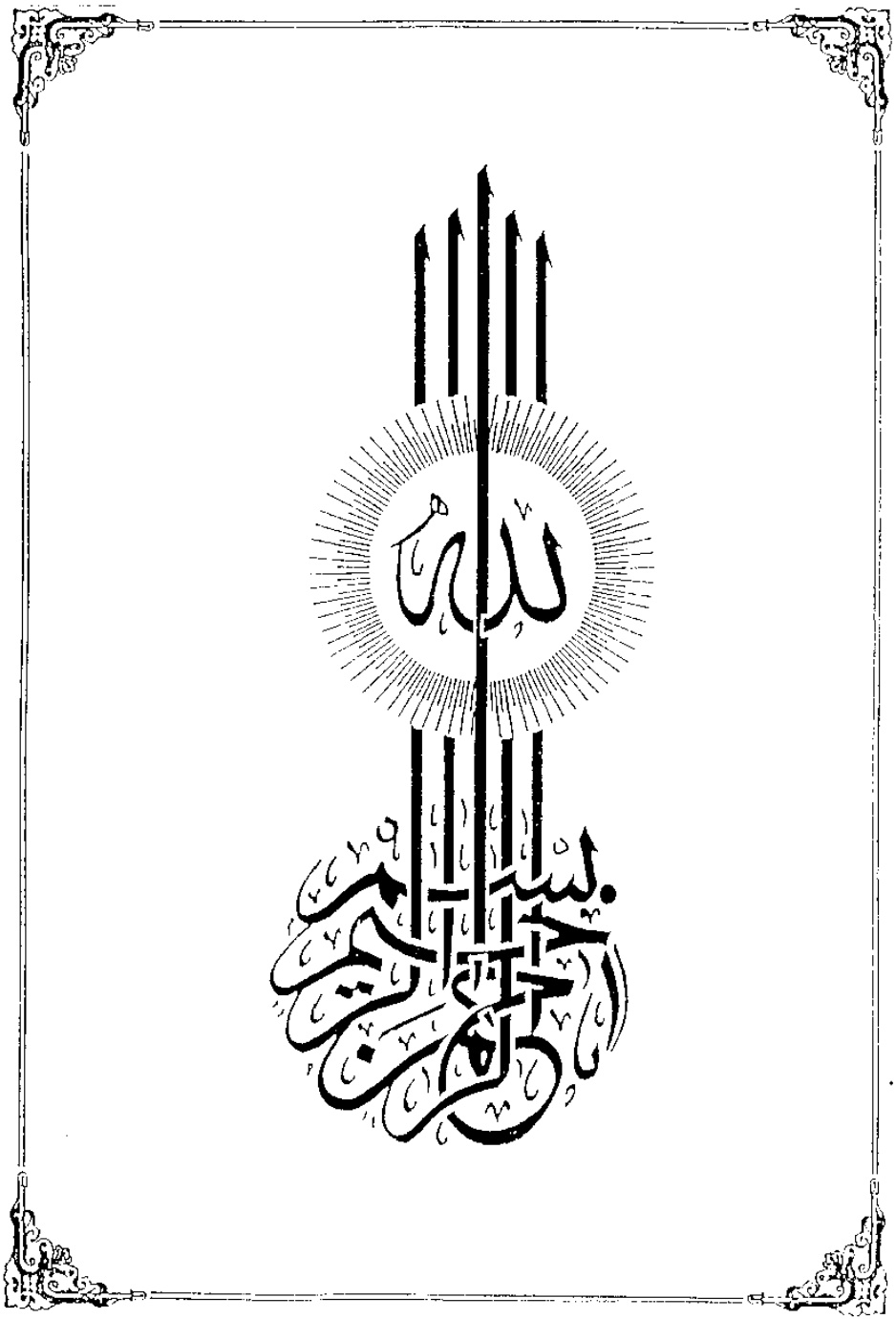


CONTENTS

INTRODUCTION AND HISTORICAL EVENTS

Protein C is the zymogen of a serine protease involved in blood coagulation that has been isolated from both human and bovine plasma.

In 1961 Seegers and coworkers showed that the digestion of crude bovine prothrombin with purified thrombin, leads to the development of potent anticoagulant activity. This anticoagulant protein was called autoprothrombin IIa as it was believed to be derived from prothrombin (Seegers et al., 1961).

It was in 1970 that Marciniak demonstrated that autoprothrombin IIa activity was not derived from prothrombin.

Later, Stenflo (1976) described a new protein on his studies on purified bovine vitamin K dependent coagulation proteins, he called it Protein C.

PC received its present name because it was purified from a protein fraction (pool C). This fraction was obtained after gradient elution of a prothrombin complex concentrate on a DEAE-Sephadex column (Kisiel, 1979).

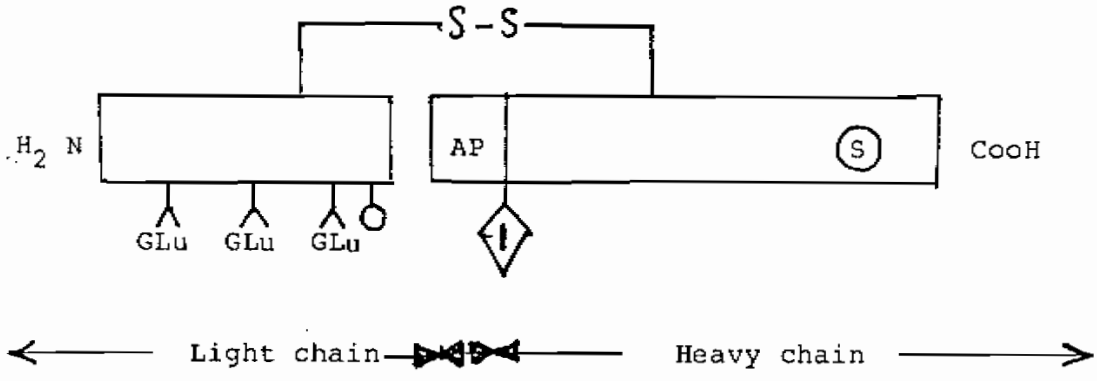
PC is a circulating plasma proanticoagulant (Stenflo, 1976; Kisiel, 1979). When physiologically activated, by thrombin-thrombomodulin complex (Esmon and Owen, 1981), it exerts an anticoagulant effect. This is accomplished through inactivation of active factor V and VIII (Kisiel et al., 1977) as well as enhancement of fibrinolysis (Zolton and Seegers, 1973; Comp & Esmon, 1981). Thus, it is involved in the fine balance of haemostatic mechanism.

PS is another vit K dependent protein, it is a cofactor of APC (Walker, 1980).

PC activation is enhanced by phospholipids and factor Va (Mann, 1984).

PC is inhibited by a glycoprotein present in plasma (Marlar & Griffin, 1980).

PC is a vitamin K dependent serine protease synthetised in the liver (Stenflo, 1976; Kisiel et al., 1977). It is depressed in hepatic dysfunction, and during therapeutic oral anticoagulants by vitamin K antagonists (Bertina et al., 1982; Vigano et al., 1983). This is best exemplified by patients bearing valvular prosthesis where long term oral anticoagulation is necessary to



◇ = Thrombin cleavage site

S = Active site serine

A.P. = Activation peptide

GLu = Gammacarboxyglutamic acid

= Carbohydrate side chains.

Figure (1) Protein C Biochemistry and activation (Triplett, 1985).

Bovine PC has a molecular weight of 54,300; it consists of 2 polypeptide chains of mol.wt. 43,800 and 23,500, linked by disufide bonds (Kisiel & Davie, 1981). It contains about 18% carbohydrates distributed on the heavy and light chains (Kisiel et al., 1976).

The complete amino acid sequence of bovine PC has been determined (Stenflo & Fernlund, 1982). It shows a high degree of homology with the other vitamin K dependent factors, especially in the amino terminal part of the light chain (Glu-containing region) and the carboxy terminal part of the heavy chain (active site containing region). The heavy chain is homologous to the other serine proteases. It contains the components of the charge relay system typical of the serine proteases, vis, histidine-56, asparatic acid-102, and serine-201 (Stenflo & Fernlund, 1982). The heavy chain has 3 carbohydrate side chains at aspargine residues 93, 154 & 170 (Stenflo & Fernlund, 1982).

Comparison of amino acid and carbohydrate composition of human and bovine Protein C:

Human and bovine PC are remarkably similar in composition with the notable exceptions of their histidine, valine and N-acetylglucosamine contents. In addition, Di Scipio & Davie (1979) recently have reported that

both human and bovine PC contain 10 residues of γ-carboxyglutamic acid per mole of protein.

Human PC appears to be more highly glycosylated than the bovine protein (23% carbohydrate human) including mannose, galactose, glucosamine, and neuraminic acid.

Amino-terminal sequence of human PC ; aspartic acid was identified as the amino-terminal residue in the heavy chain and no other amino acids were detected. This is the same residue found in the amino-terminus of the heavy chain of bovine PC . Despite this similarity, the next 17 amino terminal residues of the heavy chain were completely different from that observed in the heavy chain of bovine PC .

The amino terminal sequence of the 1st 18 residues of the heavy chain of PC and the equivalents per 44,000 g of protein were as follows: Asp (0.8), Pro (0.8), Glu (0.6), Asp (0.3), Glu (0.4), Glu (0.4), Asp (0.2), Glu (0.3), Val (0.4), Asp (0.3), Pro (0.2), Arg (0.1), Leu (0.3), Ile (0.2), Asp (0.1), Gly (0.1), Lys (0.1) and Val (0.2).

The amino acid sequence for the light chain was also determined and the equivalents per 21,000 g of protein were as follows: Ala (0.9), Asp (0.6), Ser (0.2),

Phe (0.9), Leu (0.9), Glu (not quantitated), Glu (not quantitated), Leu (0.4), Arg (0.2), Ile (0.3), Ser, Trp, Lys, and Glu.

Thus the first nine residues of the light chain of human PC are identical to that found in the light chain of bovine PC .

Perhaps the most significant difference resides in the apparant concentration of this protein in its respective plasma (Kisiel, 1979). While 4-8 mg of PC were consistently obtained from 15 litres of human plasma, 20-30 mgs of PC were isolated from an equivalent amount of bovine plasma.

Biosynthesis of PC and Role of Vitamin K:

It has been shown that the liver is the primary source of plasma proteins including those of coagulation and fibrinolytic system (William, 1977; Goldsmith et al., 1980).

The use of animal organ perfustion, cell culture and activity studies, supplied evidence that the liver parenchymal cells are the principle site of synthesis of most plasma proteins (Munns et al., 1976; Graves et al., 1982).

Based on clinical studies it has been assumed that most of the components of the PC system [PC & PS] are produced by the liver (Griffin et al., 1982; Fair & Revak, 1984).

Formation of vitamin K dependent factors appear to occur in two steps. First, a polypeptide chain is produced on the ribosomes in the hepatocytes, a step that does not require the presence of vit K. Then a second carboxyl group is inserted into the gamma carbon of glutamic acid residues in the polypeptide chain. Carboxylation is carried out by a carboxylase enzyme, and vit K is an essential cofactor of this reaction.

In this process, vit K is oxidized, but is conserved by a second enzyme, vit K reductase (Triplett, 1985).

Protein C contains γ-carboxyglutamic acid residues which are the sites of binding of calcium ions to this protein and are required for its function in blood coagulation.

Activation of Protein C:

Human PC exists in plasma as an inactive precursor that is readily converted to a serine enzyme by human ∝-thrombin. This activation is identical to that observed during the activation of bovine PC by bovine ∝-thrombin as monitored by SDS gel electrophoresis and amidase activity measurements.

In the activation of human PC by thrombin, the bond cleaved is the Arg-Leu bond between residues 12 & 13 in the amino terminal region of the heavy chain releasing a small peptide (Mr = 1000) (Kisiel et al., 1977).

Cleavage of the peptide by thrombin results in the formation of a new amino terminal sequence of Leu-Ile-Asp-Gly in the heavy chain of human activated PC . Thus, it seems highly probable that the basic mechanism for the proteolytic activation of human PC is similar to that observed in the activation of several other serine proteases including the Vit K-dependent coagulation factors.

Activation of these factors involves the cleavage of a peptide bond in the amino-terminal portion of the polypeptide chain containing the active site serine (Kraut, 1977).

In the case of human PC the new amino-terminal residue would be Leucine which would represent the first example

of an amino-terminal Leucine residue among the serine proteases. This activation of PC by thrombin is rather slow.

In 1981 Esmon & Owen discovered a cofactor, present on the surface of endothelial cells which increases the rate of thrombin catalyzed PC activation at least 20,000 folds.

This cofactor has many properties in common with the other known cofactor proteins involved in the activation of Vit K- dependent zymogens. These include a dependence on Ca^{++} for function, a high affinity for the enzyme component of the complex, and the ability to increase the maximum rate of the reaction.

The cofactor was called "Thrombomodulin" to emphasise its ability to change the specificity of thrombin (Esmon et al., 1982).

Thrombomodulin is a plasma membrane protein that does not require an intact phospholipid bilayer for biological activity.

Thrombomodulin remains bound to the cell surface during PC activition. Fig.2

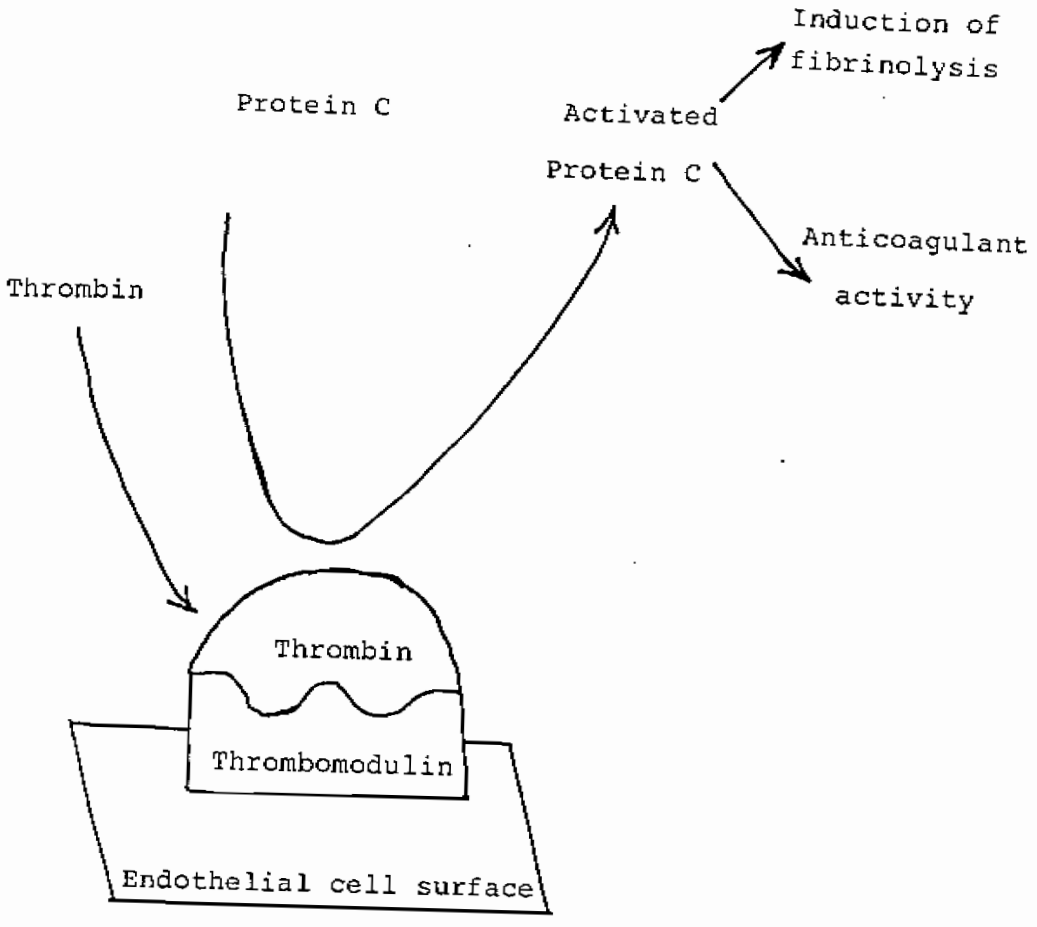


Fig (2) Proposed schematic model for in vivo protein C activation, (Comp. et al., 1982).