FREE COMMUNICATIONS XIII

Coagulation: Prothrombin Structure, Synthesis and Activation.

MODULES OF STRUCTURE AND FUNCTION IN THE EVOLUTION OF PROTHROMBIN, PLASMINOGEN AND FACTORS X and IX. S. Magnusson, L. Sottrup-Jensen and T. E. Petersen, Department of Molecular Biology, University of Aarhus, Denmark.

Comparison of the amino acid sequences of prothrombin (Magnusson et al.), factor X_1 (Enfield et al.: light chain; Titani et al.: heavy chain), plasminogen (Wiman: PAP; Sottrup-Jensen et al.: heavy chain region; Wallén & Wiman, Sottrup-Jensen et al.: light chain region) and what is known about factor IX (Fujikawa et al.) indicates that these zymogens contain three major structure modules of separate evolutionary origin: 1) A C-terminal serine protease module is common to all four zymogens and to other serine proteases. 2) The "middle" module is either a kringle structure (duplicated in prothrombin, quintiplicated in plasminogen; all seven kringle structures mutually homologous), or a "pseudo-kringle" structure (one in factor X, residues appr. 45-142 of the light chain; probably one in factor IX, both mutually homologous). 3) The "N-terminal" module is either the vitamin-K dependent region (residues 1-41/42) of prothrombin, factor X and factor IX (all mutually homologous), or the PAP-region of plasminogen. Apparently then different variants of type 2) and 3) modules and also different numbers of type 2) modules can be "fused" to a C-terminal serine protease module during evolution to produce large serine protease zymogens with strictly defined binding affinities and activation characteristics. The "three-module" evolutionary origin of these zymogens leads to the assumption that each module in the zymogen has its own tertiary structure, largely independent of the rest of the molecule. This view is strengthened by the fact that large (1 mm x 0.5 mm x 0.5 mm) high-resolution (2.8 Å) crystals of native A-fragment (residues 1-156) from bovine prothrombin have been obtained (Olsson, Lindkvist, Sottrup-Jensen, Petersen & Magnusson).

STUDIES INVOLVED IN THE DEVELOPMENT OF A SPECIFIC RADIOIMMUNOASSAY FOR PLASMA PROTHROMBIN. H. Hassouna and W.H. Seegers. Wayne State University School of Medicine, Detroit, Michigan, USA.

Antibodies directed against purified prothrombin, its activation products profragment 1 and profragment 2, its intermediate derivative prethrombin 1, and its enzyme portion thrombin were raised in rabbits. Antigenically reactive regions of each purified product were screened for crossreactivity and each antiserum tested for enzyme inhibition activity. Monovalent antisera to the native molecule have populations of antibodies that react with the purified and the iodinated I^{125} profragment 1, profragment 2, prethrombin 1, but not with thrombin. Profragment 1, profragment 2, and prethrombin 1 have one similar antigenic determinant site in common. Antisera to prethrombin 1 also crossreact with thrombin, implying the unmasking of a determinant site. Fresh oxalate and citrate plasma was tested on immunodiffusion against all specific antisera. The intact prothrombin molecule and each one of its reaction products, including thrombin, was found in plasma; they can be identified separately by their differing mobilities on immunoelectrophoresis. Activation of prothrombin in plasma is inhibited by incubation of plasma with antibodies to the native molecule or to profragment 1, but not to prethrombin 1 or profragment 2. Purified thrombin added to its antiserum loses its activity on fibrinogen. Effect of addition of crossreacting fragments on the binding of I^{125} prothrombin to its antiserum was studied. The response curve of profragment 1 is identical to that of prothrombin; profragment 2 and prethrombin 1 inhibit only 10% of binding, thrombin has no effect. To avoid nonspecific effects, standard curves and incubates are run in 1/10 dilutions of prothrombin-free plasma. Prethrombin 1 and thrombin are assayed in a carefully dialyzed barium carbonate adsorbed plasma.