FREE COMMUNICATIONS XIII

Coagulation: Prothrombin Structure, Synthesis and Activation.

MOLECULES 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 (Entfeld et al.), light chain (Tittani et al.), heavy chain, plasminogen (Wiman et al.) and what is known about factor IX (Putikka et al.) indicates that these systems contain three major structural modules of separate evolutionary origin: 1) A C-terminal serine protease module is common to all four systems and to other serine proteases. 2) The "middle" module is either a kringle structure (duplicated in prothrombin, quintuplicated in plasminogen; all seven kringle structures mutually homologous), or a "pseudo-kringle" structure (in factor X, residues 1-41/42) of prothrombin, factor X and factor IX (all mutually homologous), or the FAP-region of plasminogen. Apparently, the different variants of type 3) modules can be "fused" to a C-terminal serine protease module during evolution to produce large serine protease systems with strictly defined binding affinities and activation characteristics. The "three-module" evolutionary origin of these systems leads to the assumption that each module in the system 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, Lindqvist, Sottrup-Jensen, Petersen & Magnusson).

STUDIES INVOLVED IN THE DEVELOPMENT OF A SPECIFIC RADIOIMMUNOASSAY FOR PLASMA PROTHROMBIN. K. Massony and M.H. Speepers. 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 prothrombin 1, and its enzyme portion thrombin were raised in rabbits. Antibodies to the native molecule have populations of antibodies that react with the purified and the iodinated 125I profragment 1, profragment 2, prothrombin 1, but not with thrombin. Profragment 1, profragment 2, and prothrombin 1 have one similar antigenic determinant site in common. Antibodies to prothrombin 1 also crossreact with thrombin, implying the masking of a determinant site. Prothrombin and citrated plasma was tested on immunodiffusion against all specific antibodies. The intact prothrombin molecule and each one of its reaction products, including thrombin, was found in plasma, and these can be identified separately by their differing mobilities on immuno-electrophoresis. Activation of prothrombin in plasma is inhibited by incubation of plasma with antibodies to the native molecule or to profragment 1, but not to prothrombin 1 or profragment 2. Purified thrombin added to its antisera loses its activity on fibrinogen. Effect of addition of crossreacting fragments on the binding of 125I prothrombin to its antibodies was studied. The response curve of profragment 1 is identical to that of prothrombin; profragment 2 and prothrombin 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. Prothrombin 1 and thrombin are assayed in a carefully dialyzed barium carbonate adsorbed plasma.