IMMUNOLOGICAL CHARACTERISATION OF A α CHAIN FRAGMENTS OF HUMAN FIBRINOGEN.

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In an investigation of the early cleavage fragments resulting from fibrin(ogen)olysis, we have examined the nature of small peptides arising from the C-terminal part of the A α chain of human fibrinogen.

Antiserum to the carboxymethylated A α chain of human fibrinogen has been prepared, and used to study the A α -related antigens from (i) a plasmin digest of human fibrinogen, (ii) human serum, obtained from urokinase-treated normal plasma, and (iii) normal human serum. Using immunodiffusion and immunoelectrophoresis with anti-A α antiserum, along with polyacrylamide gel electrophoresis, at least two non-identical A α -related antigens have been detected in a final plasmin digest of human fibrinogen. The largest of these antigens (MW 26,000) has been isolated and named A α -RA (26,000). This may be similar to the previously-described fragments, Hi2-Ala, fragment A or fragment H. A α -related antigen has been produced by *in vitro* fibrinogenolysis in plasma and has been detected in the serum. In addition, small quantities of A α -related antigen have been found in untreated normal human serum.

These results suggest that anti-A α antiserum is likely to be a useful reagent for the study of the early plasmin-derived fragments of fibrin(ogen)olysis *in vitro* and *in vivo*.

LOCALIZATION AND CHARACTERIZATION OF THE CLEAVAGE-ASSOCIATED NEOANTIGEN IN THE E DOMAIN OF FIBRINOGEN. E.F. Plow and T.S. Edgington. Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

Plasmin cleavage of fibrinogen to generate fragment X partially exposes a specific cryptic molecular site, fg-E_{neo}. This site in the E domain of the molecule is further exposed during subsequent cleavage. We now report on localization of this site which provides an incisive marker for the structural and conformational changes associated with plasmin cleavage of fibrinogen. Fg-E_{neo} was stable to reduction and alkylation and the chains of the E fragment were separated by ion exchange chromatography on DEAE-cellulose. An active component was obtained and subjected to molecular exclusion chromatography on Sephadex G-50 to insure removal of intact fg-E. A fg-E_{neo} positive chain was recovered and identified as E γ with respect to amino-terminal tyrosine, amino acid composition, and immunochemical analysis. The fg-E_{neo} site was stable to tryptic degradation, and tryptic peptides were prepared and separated by multiple molecular exclusion chromatographic steps. Final separation of two peptides of similar size was achieved on the basis of carbohydrate content by affinity chromatography on Concanavalin A. Only the active peptide was bound by the lectin. Purity and identification of the active tryptic peptide as γ 36-53 was established by amino acid composition and sequence. These results establish that this region of the γ chain of fibrinogen is not present at the hydrated surface of the native molecule but that, in association with plasmin cleavage and conformational changes, this site is progressively exposed and provides a dynamic marker of the cleavage sequence.