ON THE IDENTIFICATION OF POLYMERIC SITES IN HUMAN FIBRINOGEN PEPTIDE CHAINS. B. Lipinski, J. Ewaskiewicz, s. Wilhelm, J. Ewaskiewicz.
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Human fibrinogen has repeatedly been shown to occur in a great number of different molecular forms. Some types of heterogeneity are evident already from variations in solubility properties and in electrophoretic behaviour of the total molecule and of its peptide chain components. The reason for these variations are partly known. Thus, degradation of the C-terminal parts and phosphorylation of two serine residues gives rise to heterogeneity in the Aa-chain. Differences in the synthesis of the carbohydrate side chain causes heterogeneity both in the N- and the Y-chain. Differences in chain length at the C-terminus of the Y-chain are responsible for additional variation. All these variants are expected to exist in each humanbeing. An other category of human fibrinogen variants may be due to genetic polymorphism within the population, i.e. the presence of inherited, common, normal variants. Seven sites of microheterogeneity have so far been tentatively identified, mainly by disagreements between protein and DNA sequence analyses. Three of the sites are located in the Aa-chain (positions 47, 296 and 310), three in the B-chain (positions 162, 296 and 148) and one in the Y-chain (position 88). The aim of the present study was to identify these sites on the proteinchemical level in pooled plasma as well as in plasma from single individuals and especially various members of the same family. For this purpose suitable fibrinogen fragments containing the tentatively microheterogenous sites were isolated after cleavage of fibrinogen with cyanogen bromide, trypsin and/or chymotrypsin by repeated fractionations by means of conventional and reversed-phase high-performance liquid chromatography and counter-current distribution. The components were characterised by N-terminal sequence and amino acid composition. Polymorphism in human fibrinogen has previously only once been identified by restriction fragment length analysis in a non-transcribed region of the Aa-chain locus but never in the transcribed regions, i.e. the peptide chains. The present investigation will allow the estimation of the number of peptide chain haplotypes and the possible correlation to other genetic variants of fibrinogen. Complex formation between thrombin and fibrinogen requires interaction between thrombin and the sum of the M of thrombin and fragment E. This shows that human thrombin forms a complex with fibrinogen fragment E. Hence, we can conclude that only the C-terminal part of the fibrinogen molecule is necessary for interaction with thrombin. Under reducing conditions, the complex of thrombin with fragment E produced four bands on gel electrophoresis. One was thrombin; the remaining three were complexes of thrombin with fragment E chain remnants. To investigate this further, carboxymethylated human fibrinogen chains Aα, Bβ and γ were purified and coupled to Sepharose 4B. 125I-Thrombin was applied on the three columns. Nearly all radioactivity was bound to the three affinity columns and was eluted with higher NaCl concentration. We can infer that complex formation between thrombin and fibrinogen requires interaction between thrombin and all three fibrinogen chains. To find which amino acid residues are responsible for interaction with fibrinogen, human thrombin was coupled to Affi-Gel 102 and Affi-Gel 202 through thrombin's carboxyl and amino groups, respectively. We observed binding of this fibrinogen and fibrinogen fragments to Affi-Gel 102 column, indicating that lysine residues and perhaps the N-terminal of the thrombin molecule interact with fibrinogen. When thrombin gel chromatography with amino groups, there was no interaction between thrombin and fibrinogen or fragment E.

HUMAN PLASMA FIBRINOGEN MOLECULAR WEIGHT VARIANTS: CHARACTERIZATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND IDENTIFICATION BY SEQUENCE ANALYSIS. E. Miller and A. Henschen. Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG.

Human plasma has been shown to contain several fibrinogen variants which differ from each other in molecular weight. The three most common forms, with molecular weights of 340 kD, 305 kD and 270 kD, have been reported to change considerably in their relative amounts during certain pathophysiological processes such as acute phase reactions, disseminated intravascular coagulation, fibrinolytic disorders, liver disease and cancer. Though it has been suggested that the differences in molecular weight are due to degradation of one or both, respectively, of the carboxyterminal parts of the Aa-chains, the removed or altered segments have so far never been precisely determined. Consequently, it has called for possible candidates about the origin of the variants. The aim of this study was to isolate the various molecular weight variants from plasma and to characterize their peptide chain components proteinchemically. Fibrinogen was first isolated from plasma by glycine precipitation and the variants were then separated by stepwise ammonium sulfate precipitation, taking advantage of their different solubility.

The peptide chain component of the various fractions were isolated by reversed-phase high-performance liquid chromatography (HPLC). The N-termini were identified by direct sequence analysis. Chemical and enzymatic cleavages of the peptide chains resulted in fragment mixtures which were compared with the corresponding mixtures obtained from human fibrinogen by HPLC Fingerprinting. Finally, the fragments were subjected to N-terminal sequence and amino acid analysis so that the exact N-termini of the peptide chains, especially in the lower molecular weight variants with the use of the ambiguous amino acid sequence data, might also be drawn about the origin of the lower molecular weight variants and about the mechanism by which they may be formed.

COMPLEX FORMATION BETWEEN THROMBIN AND FIBRINOGEN OR FIBRINOGEN DEGRADATION PRODUCTS (FDP). E. Kacsmarek, and J. McDonagh. The Department of Pathology, Beth Israel Hospital, Harvard Medical School, Boston, MA, U.S.A.

To identify the part of the fibrinogen molecule which interacts with thrombin, binding of human thrombin to plasma FDP was analyzed. 1-thrombin was incubated with FDP, purified fibrinogen fragment D or fragment E in the presence of 0.2 glutaraldehyde. Incubation showed only one dark band, the molecular weight (M) of which was identical to that of thrombin, indicating no complex formation between thrombin and fragment D. Incubation with 1-thrombin and fibrinogen fragment E, two dark bands were observed; the electrophoretic mobility of the first was the same as that of thrombin and of the second band was equal to the sum of the M of thrombin and fragment E. This shows that human thrombin forms a complex with fibrinogen fragment E. Hence, we can conclude that only the C-terminal part of the fibrinogen molecule is necessary for interaction with thrombin. Under reducing conditions, the complex of thrombin with fragment E produced four bands on gel electrophoresis. One was thrombin; the remaining three were complexes of thrombin with fragment E chain remnants. To investigate this further, carboxymethylated human fibrinogen chains Aα, Bβ and γ were purified and coupled to Sepharose 4B. 125I-Thrombin was applied on the three columns. Nearly all radioactivity was bound to the three affinity columns and was eluted with higher NaCl concentration. We can infer that complex formation between thrombin and fibrinogen requires interaction between thrombin and all three fibrinogen chains. To find which amino acid residues are responsible for interaction with fibrinogen, human thrombin was coupled to Affi-Gel 102 and Affi-Gel 202 through thrombin's carboxyl and amino groups, respectively. We observed binding of this fibrinogen and fibrinogen fragments to Affi-Gel 102 column, indicating that lysine residues and perhaps the N-terminal of the thrombin molecule interact with fibrinogen. When thrombin gel chromatography with amino groups, there was no interaction between thrombin and fibrinogen or fragment E.