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HEREDITARY HYPOFIBRINOGENEMIA WITH HEPATIC STORAGE OF FIBRINOGEN. F. Callea (1), R. de Vos (2), R. del Bono (1), O. Tortora (3), A. Albertini (1), A. Henschen (3) and V. J. Desmet (2). Histochemistry Unit, Department of Clinical Chemistry and RIA Spedali Civili Brescia, Italy (1), Laboratory of Histochemistry and Cytochemistry Catholic University of Leuven, Belgium (2), Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG (3).

An Italian family suffering from a novel hereditary disease, i.e. hypofibrinogenemia connected with hepatic storage of fibrinogen, has been identified. A similar disorder has previously been described in two German families. The discovery originated from the finding of a massive hepatic storage of fibrinogen in a liver biopsy specimen from a 65 years old female who during a laparotomy unexpectedly turned out to display cirrhosis. Identification of the stored material as fibrinogen was carried out by specific immunostaining applied on both light and electron-microscopic level. The stored fibrinogen appeared in the form of eosinophilic, pale, irregularly shaped inclusions within the cytoplasm of the hepatocytes. E.M. showed that the inclusions correspond to dilated cisternae of the RER filled by densely packed, curved, tubular structures arranged in a fingerprint-like fashion. Similar features of fibrinogen storage were not detected in any of 500 consecutive liver biopsy specimens examined for this purpose by appropriate techniques. Plasma fibrinogen (thrombin clottable fibrinogen) was found to be 35 mg/dl (n.v. 100-300 mg/dl), and remained persistently very low (20-40 mg/dl) over a follow-up period of two years. The immunoreactive fibrinogen was found to be two-three times as high as the clottable. The patient showed no clinical defect of hemostasis or impaired wound healing. Eight out of 19 family members also had persistently low plasma fibrinogen levels (45-80 mg/dl) over the same follow-up period. Hypofibrinogenemia with hepatic storage of fibrinogen seems to represent a further endoplasmic reticulum storage disease in addition to α-1-antitrypsin deficiency, the plasma deficiency being due to defective secretion of the protein from the hepatocytes. A genetically determined molecular abnormality may be responsible for the defective intracellular transport and subsequent storage at the primary site of protein synthesis, the RER. Hepatic storage of fibrinogen is likely to predispose to development of liver cirrhosis, but neither to defective hemostasis nor impaired wound healing.

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SEPARATION OF FIBRINOGEN FRAGMENTS ON GLYPROARGPROLYS-FRACTOGEL. C. Kuyas, A. Haeberli, P.W. Straub. Dept. of Medicine, Inselspital, University of Bern, Switzerland.

The tetrapeptide GlyProArgPro, which corresponds to the newly exposed N-terminal sequence of fibrin α-polypeptide chain after the action of thrombin, has a binding site in the C-terminal part of the γ-chain as suggested by several authors. Using GlyProArgProLys-Fractogel (GPRPK) chromatography we tried to isolate a fibrinogen fragment, obtained with different enzymes and conditions, which includes the binding site for GPRP. Human fibrinogen was digested by plasmin in presence and absence of Ca-ions, and the resulting lysates were applied in 0.05 M triethanolamine (TEA), 0.1 M NaCl pH 7.4 to the GPRPK-Fractogel. The gel was washed extensively with TEA-buffer and the adsorbed protein was eluted with 6M urea in TEA-buffer. The protein containing fractions were analyzed immunologically with anti-fragment E- and with anti-fragment D antibodies. Human fibrinogen was also digested with endopeptidase Arg-C in 0.1M $NaHCO_3$ pH 8.0 at 37°C over night. The enzyme cleaves fibrinogen to fragments, one of which comprising the γ-chain sequence 275-375 which is said to contain the fibrin polymerization site. The Arg C-lysate was chromatographed on GPRPK-Fractogel. All fragments were analyzed by SDS-electrophoresis and by reversed-phase HPLC.
Fragment D_1 was the only fibrinogen fragment which was adsorbed on GPRPK-Fractogel. All other assayed fibrinogen fragments obtained by enzymatic cleavage, showed no affinity to GPRPK-Fractogel. These results demonstrate that for the binding of GPRP to fibrinogen a conformationally intact γ-chain remnant of the fragment D is required.
One step chromatography using GPRPK-Fractogel can thus also be used to isolate fragment D_1 in high purity from plasmin lysates of fibrinogen.

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THE DYNAMIC STRUCTURE OF FIBRINOGEN PROBED BY SURFACE LABELLING AND CHEMICAL CROSS-LINKING. Angela Apap-Bologna, Ailsa Webster, Fiona Raitt and Graham Kemp, Department of Biochemistry and Microbiology, University of St. Andrews, St. Andrews, Scotland.

There is much controversy regarding the conformation of fibrinogen. Several models have been proposed ranging from an almost linear trinodular arrangement to a globular conformation. Consequently, it has been suggested that fibrinogen has a flexible structure where the actual conformation is influenced by its environment - one major factor being calcium concentration. Although the importance of tightly bound calcium ions (Kd ~ 1uM) to fibrinogen structure is well established, the role of the larger number of low affinity sites (Kd ~ 1mM) is still a matter of debate. We have utilised the techniques of radio-active photoaffinity surface labelling and chemical cross-linking to probe the molecule's conformation under different conditions. Studies were carried out in an attempt to provide information on: (1) The relative locations of the major domains within the fibrinogen molecule (2) The regions of the chains which are exposed on the surface (3) The dependence of the conformation on the solvent composition with particular reference to the effect of calcium concentration. Our results indicate that the central, or E domain of the molecule is partially buried and that the conformation of fibrinogen is certainly influenced by changes in solvent composition. Increasing calcium concentration in the millimolar range results in an increase in the proportion of intermolecular cross-linking, mainly through the [A]α chains. There have been several reports that the C-terminal regions of the [A]α chains are in close association, forming a fourth domain within the molecule. Our results suggest that calcium ions promote the dissociation of this domain.

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DIRECTED MUTAGENESIS OF HUMAN FIBRINOGEN: Aα CHAIN SUBSTITUTIONS THAT ALTER THROMBIN CLEAVAGE AND ANTIBODY RECOGNITION. S.T. Lord. Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 U.S.A.

The initial event in fibrin clot formation is the thrombin catalized cleavage of the Aα chain of fibrinogen between Arg16 and Gly17, releasing fibrinopeptide A. Previous data indicate that most of the information required for thrombin recognition and cleavage of the Aα chain lies within the amino terminal 51 residue CNBr fragment. In order to use protein engineering techniques to study the interaction of thrombin with the Aα chain, we have constructed a plasmid expression vector which encodes a tripartite protein consisting of amino acids 1-50 of the Aα chain of human fibrinogen followed by 60 amino acids of chicken collagen, and the beta-galactosidase protein from Escherichia coli. The codons for an initiator methionine and amino acids 1-50 were assembled from 7 oligonucleotides. Protein blot analysis of bacterial lysates of cells induced to synthesize this tribrid protein show a single band (MW_r = 125,000) crossreactive with a monoclonal antibody, Y-18, which recognizes the Aα chain of fibrinogen but not the products of thrombin cleavage. When these lysates are incubated with thrombin, fibrinopeptide A is released as demonstrated both by protein blot analysis and radioimmunoassay. By including one heterogeneous oligonucleotide in the assembly process, we have constructed plasmids which encode specific amino acid substitutions within residues 1-23. One of these substitutions, Gly14 to val, significantly alters both cleavage by thrombin and recognition by Y-18. Substitution of ilu for Arg23 alters neither thrombin cleavage nor monoclonal recognition while substitution of leu for Arg16 alters thrombin cleavage, but not recognition by Y-18.