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ON THE IDENTITY OF PLATELET FIBRINOGEN WITH PLASMA FIBRINOGEN. H. Kaudewitz (1), A. Henschen (1) and R.E. Zimmermann (2). Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG (1) and Department of Physiology II, University of Münster, Münster, FRG (2).

It is a well established fact that fibrinogen occurs not only in blood plasma but also in the α-granules of the platelets. Recently, it has been shown that fibrinogen is synthesised in the megakaryocytes as well as in the liver. Plasma fibrinogen is derived from the liver, but platelet fibrinogen either exclusively or partially from the megakaryocytes. Conclusive, proteinchemical evidence for the identity of the fibrinogens from the two biosynthetic sources has previously not been produced. However, the two fibrinogen preparations have been shown to have the same overall peptide chain composition, to be thrombin-clottable and release fibrinopeptides of A- and B-type, and to react with antibodies against plasma fibrinogen. The two preparations differ in the way that platelet fibrinogen lacks the higher-molecular-mass γ-chain variant.

The aim of the present investigation was to conduct a detailed proteinchemical comparison between human plasma and platelet fibrinogen. For this purpose, fibrin(ogen)s from the two sources were mercaptolysed, alkylated and the three peptide chains isolated by reversed-phase high-performance liquid chromatography (HPLC). The peptide chains were then analysed directly for amino-terminal sequence and for carboxyterminal sequence by isolation of a terminal fragment. The HPLC-fingerprint patterns of the cyanogen bromide-cleaved chains were compared. The native fibrinogens were also treated with thrombin and the fibrinopeptide type distribution determined by reversed-phase HPLC. The carbohydrate side chain compositions were established by ion-exchange-chromatographic methods after acid hydrolysis. So far no previously unrecognized differences have been observed.

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CONFORMATIONAL EQUILIBRIA IN THE γ CHAIN COOH-TERMINUS OF HUMAN FIBRINOGEN. C.S. Cierniewski and A.Z. Budzynski. Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140 U.S.A.

Synthetic peptides and fragments cleaved from native fibrinogen are used in studies to localize binding sites for various ligands. We addressed the question how the native conformation of a selected γ chain segment is affected by scission of the original chain. The conformation of the γ chain COOH-terminus of intact fibrinogen and its various fragments containing this region has been compared by an immunochemical analysis. An antibody population specific for the native epitope within the γ391-405 segment was isolated by affinity chromatography on the corresponding synthetic peptide. Between 19.2 and 22.8% of antibodies were obtained from three different antisera indicating that this region represents one of the major epitopes of native fibrinogen. Anti-γ391-405 antibodies were used to determine the value of K_{conf}, the equilibrium constant for the interconversion of the non-native and native conformations of this epitope. The measurements were done using native fibrinogen, fragments D_1 and DD, γ chain and γ391-405 synthetic peptide. In addition, the effect of 5 M guanidine-HCl on the conformation of fragments D_1 and DD, which is known to abolish their antipolymerizing activity, was studied. Radioiodinated fibrinogen was used in the determination of K_{conf}, and quantitative analytical parameters, CI50% and CIs, calculated from competition between ^{125}I-fibrinogen and the fibrinogen derivatives under study for binding to the immunochemically purified antibody. The measurements indicated that the epitope is unperturbed by iodination of fibrinogen and that 38.5% of fragment D_1, 8.9% of fragment DD, 3.6% of the γ chain and less than 0.008% of the γ391-405 molecules adopt in aqueous solution the native conformation within the epitope. Denaturation of fragment D_1 with 5 M guanidine-HCl affected only slightly the conformation of this γ chain determinant. More significant changes in the conformation were observed when fragment DD was denatured. The results suggest that long-range interactions are necessary for the stabilization of the native structure in the region of fibrinogen that interacts with the antibody and which is in close vicinity to the polymerization site, crosslinking site, and platelet recognition site.

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INFLUENCE OF THE SUBENDOTHELIAL BASEMENT MEMBRANE (BM) COMPONENTS ON FIBRIN ASSEMBLY: EVIDENCE FOR A FIBRINOGEN BINDING SITE ON TYPE IV COLLAGEN. Jones, M.R. and Gabriel, D.A. The University of North Carolina at Chapel Hill, N.C., U.S.A.

Effective repair of vascular injury requires assembly of a secure fibrin patch at the injury site. A secure fibrin patch must be anchored to the injury site, else it becomes an embolus. Using enzyme linked immunoassay (ELISA), we have evaluated the binding of fibrinogen (F) to single as well as combinations of the subendothelial BM components including laminin (L), type IV collagen (C), and dermatan sulfate (D). Tests were performed using plates coated over night at either 4°C or 37°C for one hour with individual BM or combinations or BM components. Results indicate that F strongly binds to both D and type IV collagen. Binding of F to L was approximately 50% compared to C or D. Combinations of C, L and D were studied with mole ratios of L and D normalized to C. Thus, when the mole ratio of L was increased from 0.1 to 10 relative to C, the amount of F bound to the plate coated with C and L decreased by 30%. When both D and L were added to C and the binding of F to the coated plate evaluated, increasing the mole ratio of D from 1 to 10 relative to C and L (C:D:L=1:1:0-10), increased the binding of F two fold. If the mole ratio of L was increased by a factor of 10 compare to C, and D varied form 0 to a 10 mole excess compared to C, (C:L:D=1:10:0-10) the increase in F binding was only increased by 1/3. Therefore, L decreases the binding of F by C, while D strongly increases the binding of F. These data suggest that an important interaction between the endothelial basement membrane and fibrin exists and the mole ratio between CLD is critical in determining the extent of the interaction. The interaction between F and C,L,D may underlie the mechanism by which a fibrin clot is anchored and stabilized to the injury site.

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THROMBIN BINDING FRAGMENT E GENERATED DURING FIBRINOGENOLYSIS. C.A. Goodwin, M.F. Scully, V. Ellis and V.V. Kakkar. Thrombosis Research Unit, King's College School of Medicine & Dentistry, Denmark Hill, London SE5 8RX, UK.

Binding of fibrinogen by thrombin was measured by inhibition of amidolysis of S2238 and found to be 12 μM. Upon digestion of fibrinogen with plasmin (0.16ugs/mg fibrinogen) for 4 hours at 37°C, thrombin binding activity remained in the supernatants upon heat treatment. The thrombin binding activity in the dialyzed supernatant reached a maximum after two hours coinciding with maximal release of B 1-42 and 45-39 KDa chain fragments. Measured immunologically, levels of fragment E at this time were 45% of the maximum generated after 4 hours digestion. FPA levels in the dialyzed supernatant (measured by RIA and HPLC) after thrombin treatment, were zero and did not increase until 1½ hours after the beginning of digestion, reaching a maximum at 4 hours. The thrombin binding activity generated was stable to further plasmin action. Upon gel chromatography of 2 and 4 hour supernatants, thrombin binding activity coincided closely with fragment E, measured immunologically. Further purification showed the fragment to have Ki for thrombin amidolytic activity of 0.5μM. The fragment also inhibited the thrombin clotting time of plasma but did not affect fibrin monomer polymerization.The fragment was susceptible to very slow inactivation by thrombin but not arvin (though it did inhibit arvin amidolytic activity). A thrombin binding (thrombin inhibitory) fragment is therefore generated during the early stages of fibrinogenolysis and may be the result of protection by 45 and 39 KDa A α carboxy terminus fragments since E fragments generated in later stages (in the presence of 29 and 25 KDa fragments) do not have this property. These findings may give interesting new insight into thrombin/fibrinogen interaction.