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10:00 h

INTERACTIONS OF TRIPHENYL-METHANE COMPOUNDS WITH FIBRINOGEN AND FIBRIN MONOMERS. J-J. Ryckewaert and R. F. Doolittle. Department of Chemistry, University of California, San Diego La Jolla, CA U.S.A.

A synthetic compound, aurin tricarboxylic acid (ATA), in the concentration range of 10-100 nanomoles/ml exhibits the property of significantly shortening the thrombin clotting time of fibrinogen. Upon binding to fibrinogen or fibrin monomers, obtained either by the action of thrombin or reptilase on fibrinogen, ATA possesses the ability to accelerate the polymerization formation of fibrin. Polymerization studies of fibrin monomers at different ionic strengths show that ATA greatly enhances the lateral aggregation of fibrin polymers even at high ionic strength. Calcium ions do not influence the effects of ATA. Spectroscopic methods have been used to study and locate the binding sites in the different parts of the fibrinogen molecule. Compounds with structures that are closely related to that of ATA do not affect the polymerization process, with the exception of the parent compound aurin, which has a slight effect. ATA does not influence the binding ability of fibrinogen for the synthetic peptides: Gly-Pro-Arg and Gly-His-Arg, as was shown by equilibrium dialysis experiments with radioactive-labelled peptides. Nevertheless, in the presence of Gly-Pro-Arg or Gly-Pro-Arg-Pro, which are known to interfere with the polymerization of fibrin monomer, ATA becomes a potent inhibitor of the overall process of fibrin formation. This duality of behavior has permitted us to study the different steps of longitudinal and lateral association of fibrin monomers.

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10:15 h

THE MECHANISM OF INHIBITION OF FIBRIN ASSEMBLY BY FRAGMENT D. J. Williams, R. Hantgan, D. Knoll, J. McDonagh and J. Hermans. Departments of Biochemistry and Pathology, Univ. of North Carolina, Chapel Hill, NC 27514 U.S.A.

Measurements of clot rigidity and fiber thickness indicate that fragment D is a potent inhibitor of fibrin assembly. At physiological ionic strength, D concentrations in excess of 2 moles D/mole fibrinogen lead to a large decrease in clot rigidity and fiber thickness. Above 14 moles D/mole fibrinogen, gelation is inhibited. The molecular weight and radius of gyration of the fibers, determined by light scattering, confirm that short oligomers result, composed of 3 fibrin monomer molecules at 120 moles D/mole fibrinogen and 9 monomers at 14.4 moles D/mole fibrinogen. If D is added to a solution of long protofibrils, no inhibition is observed. Apparently the polymerization of monomers to protofibrils is blocked by D, but not lateral association.

Inhibition of protofibril growth was studied in 0.5 M NaCl, pH 7.4, where lateral association is limited by the ionic strength. Stopped-flow light scattering data show a small decrease in the initial rate of polymerization and a slightly prolonged $t_{\frac{1}{2}}$; the final intensity is less than that for a solution of long protofibrils. This result suggests that fragment D binds to the growing protofibrils with a small effect on the initial polymerization rate, but exerts its inhibitory effect by limiting the later stages of protofibril growth. Measurements of the length of the inhibited protofibrils, calculated from diffusion coefficients obtained by dynamic light scattering, confirm that polymers containing as few as 15 monomers have been obtained. Negative stain electron microscopy also shows a clear limitation of polymer growth under these conditions.

Fragment D interferes with fibrin formation by directly blocking the first assembly step: bimolecular polymerization of activated fibrin monomer molecules to form protofibrils. Fragment D apparently occupies a site normally occupied by a fibrin monomer molecule, thus forming a dead-end complex which cannot undergo further assembly.

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10:30 h

REGULATION OF FIBRIN FORMATION. B. Blombäck, B. Kudryk, M. Okada, S. Söderman and L. Therkildsen. Karolinska Institutet, Stockholm, Sweden and The New York Blood Center, N.Y.

Nossel et al. have shown that fibrin formation in vivo may be regulated by release of the Bβ 1-42 fragment from fibrinogen by plasmin. We have studied the effect of trypsin on the fibrin formation in vitro. Fibrinogen solutions (1 g/l) in Tris-imidazole buffer, pH 7.4, were incubated with trypsin (1:2,000, w/w) for 1-10 min. After incubation soybean trypsin inhibitor was added and clottable fibrinogen recovered by adding thrombin. Non-clottable fibrinogen in the supernatant was recovered by adding equal volume of ethanol. SDS-PAGE, before and after reduction, was performed on the above protein fractions as well as N-terminal analysis on N-DSK obtained from them by treatment with CNBr. Clot-supernatants were used in radioimmunoassay (RIA) for estimation of the following fragments: FPA, FPB, Bβ 15-42 and Hi2-DSK (peptide from COOH-terminal part of A α-chain). It was found that during incubation the fibrinogen progressively lost its coagulability. The clottable fraction appeared to have the same SDS-PAGE patterns as for intact fibrinogen. The non-clottable fraction showed progressive diminution of intact A α-chains but no degradation products could be observed. The B β-chain on SDS-PAGE appeared to be shifted towards the γ-chain. The RIA assays showed that concomitant with loss of coagulability there was release of peptides containing Bβ 15-42. Release of FPA and FPB occurred during tryptic digestion but no clotting occurred. Only small release of Hi2-DSK immunoreactivity occurred. NH_2-terminal analysis of N-DSK suggested that Bβ 42-43, Bβ 54-55 and a third, as yet unidentified bond, had been split by trypsin. These results suggest that tryptic cleavages in the Bβ and Aα chains destroy the polymerization sites which are unfolded in the molecule by release of FPA and FPB. Similar studies employing plasmin are now in progress.

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10:45 h

LOCALIZATION OF A FIBRIN POLYMERIZATION SITE. Stephanie A. Olexa and Andrei Z. Budzynski. Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia, Pennsylvania.

The formation of a fibrin clot is initiated after the proteolytic cleavage of fibrinogen by thrombin. The enzyme removes fibrinopeptides A and B, and generates fibrin monomer which spontaneously polymerizes. Polymerization appears to occur through the interaction of complementary binding sites on the NH_2- and COOH- terminal regions of the molecules since Fragment D_1, encompassing the COOH-terminals of the β and γ chains, binds to thrombin-treated NDSK which contains NH_2-terminals of the α, β and γ chains. A peptide of 4,200 molecular weight has been isolated from the γ chain remnant of fibrinogen Fragment D_1 which has the ability to bind to the NH_2-terminal region of fibrin monomer, thus inhibiting fibrin monomer polymerization. The peptide reduces the maximum rate and extent of the polymerization of thrombin or batroxobin fibrin monomer and increases the lag time of the reaction. The D_1 peptide does not interact with NDSK, fibrinogen or Fragment D_1 but it binds to thrombin-treated NDSK with a K_d of 1.45×10^{-6} M and approximately two binding sites per molecule of NDSK have been found. Fibrin monomer formed either by thrombin or batroxobin binds approximately two molecules of D_1 peptide per molecule of fibrin monomer, indicating that the complementary site is revealed by the loss of fibrinopeptide A. The NH_2-terminal amino acid sequence (Thr-Arg-Trp) and the COOH-terminal sequence (Ala-Gly-Asp-Val) of the D_1 peptide were determined. Therefore the γ 373-410 region of the fibrinogen molecule contains a polymerization site which is complementary to the thrombin-activated site on the NH_2-terminal region of fibrinogen. The binding site on the D_1 peptide has the characteristics of the polymerization site which is exposed and available on the COOH-terminal region of the fibrinogen molecule without any participation of thrombin.

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