

HOMOLOGUES OF FIBRIN X OLIGOMERS. R. Hafter, H. Graeff, R.v. Hugo. I. Frauenklinik der Universität München, FRG.

Crosslinked fibrin derivatives signalize intravascular coagulation. D-dimer, Y-D and X oligomers are observed in plasma from obstetric patients with severe coagulation disorder. They are also found in ascitic fluid from patients with advanced ovarian cancer and can be produced *in vitro* by simultaneous action of thrombin, plasmin and factor XIII with fibrinogen. The study was aimed to evaluate the subunit structure of separated molecular entities. The derivatives were separated by 4% SDS-PAGE preceded in case of the *in vivo* products by gel filtration and/or by immunoabsorption technique. The gels were sliced at the respective migration positions and derivatives therein reelectrophoresed on 7.5% gels after reduction. Subunit characterisation revealed that D-dimer is composed of the chain remnants  $\gamma^1$ - $\gamma^1$ ,  $\beta^2$ ,  $\alpha^2$ , while Y-D is composed of  $\gamma$ - $\gamma^1$ ,  $\beta^2$ ,  $\alpha^1$ ,  $\alpha^2$ , besides  $\alpha^E$ ,  $\beta^E$  and  $\gamma^E$ . Crosslinked X oligomers are composed of  $\gamma$ - $\gamma$ ,  $\gamma$ - $\gamma^1$ ,  $\beta$ ,  $\beta^2$ ,  $\beta^2$ ,  $\alpha^1$  and  $\alpha^2$  besides  $\alpha^E$ ,  $\beta^E$  and  $\gamma^E$ . Three possible combinations of plasmin degraded and undegraded dimeric  $\gamma$ -chains were observed *in vivo* and *in vitro*:  $\gamma$ - $\gamma$ ,  $\gamma$ - $\gamma^1$  and  $\gamma^1$ - $\gamma^1$ . The ratio of degraded ( $\gamma^1$ ) to undegraded  $\gamma$ -chains in dimeric  $\gamma$ -chain patterns indicates the mol. structure of the respective derivative. Two X oligomers could be demonstrated in which the ratio of  $\gamma$ - $\gamma$  to  $\gamma$ - $\gamma^1$  in terms of stain intensity was either 1:1 or 2:1. Their subunit compositions are in accordance with structures describable as D-X-Y and D-X-X-Y. Their molecular weights, calculated from the subunit compositions are 476,000 and 716,000 respectively. - It is proposed that crosslinked X oligomers exist as a homologous family with increasing X fragment content.

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POLYMERIZATION SITES OF DESIALATED FIBRINOGEN. P.M. Allison and N.U. Bang. Lilly Laboratories for Clinical Research, Depts. of Medicine and Pathology, Indiana University School of Medicine, Indianapolis, IN, USA

It has long been known (Laki and Chandrasekhar, Nature, 197:1267, 1963) that the enzymatic removal of sialic acid (SA) from fibrinogen (F) shortens the thrombin clotting time. Martinez et al. (J. Lab. Clin. Med., 89:367, 1977) demonstrated that this phenomenon is due to enhanced fibrin monomer polymerization. To more accurately identify the mechanisms involved we examined the binding of normal F, V. cholerae neuraminidase - treated F (NF) and their plasmin digests. Mean SA content of F of 4.5 residues / molecule decreased to unmeasurable levels in NF with a concomitant 10s shortening of the thrombin clotting time. F, NF, and plasmin digests of F (FP) and NF (NFP) were subjected to fibrin-monomer-Sepharose chromatography (FMSC) essentially according to Kudryk and Blombäck (J. Biol. Chem., 249:3322, 1974) and effluent fractions analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions. Mean values for F retained on FMSC and eluted with acid urea buffer was 61%, for NF 86%, for FP 65%, for NFP 78%. In NFP and FP 3 fragments D ( $D_1$ ,  $D_2$ ,  $D_3$ ) of  $M_r$  103K, 89K, and 83K were discerned by SDS-PAGE.  $D_1$  in FP and NFP was almost quantitatively retained on FMSC, whereas little, if any,  $D_2$  was retained from either digest. FMSC resulted in poor retention of  $D_2$  for FP and virtually complete retention of this fragment from NFP. Fragments E were not retained on FMSC of either FP or NFP. The data suggests that desialation of F exposes additional polymerization sites present in  $D_2$  but not  $D_3$  of F.

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SIGNIFICANCE OF TRYPTOPHAN RESIDUES IN FIBRINOGEN IN FIBRIN POLYMER FORMATION. A. Matsushima, Y. Saito, K. Okano and Y. Inada. Laboratory of Biological Chemistry, Department of Chemistry, Tokyo Institute of Technology, Tokyo 152, Japan.

Human fibrinogen and its fragment were modified with 2-hydroxy-5-nitrobenzyl bromide (HNBB) or with  $H_2O_2$ -dioxane to see the functional significance of tryptophan residues in the fibrinogen molecule. Tryptophan residues were modified with HNBB in a stepwise manner. Modification of two out of the total 64 tryptophan residues in the molecule did not reduce the polymerization activity with thrombin but further modification of the next two residues led to a complete loss of the polymerization activity. The latter two tryptophan residues modified were in Fragment E and the position may be either Trp 33 or Trp 41 in the  $\alpha$ -chain in the fibrinogen molecule.

Fragment D inhibited the association activity of fibrin monomer. This inhibitory effect was lost completely by the modification of approximately six out of the total 21 tryptophan residues in the fragment with  $H_2O_2$ -dioxane. These results indicate the significance of tryptophan residues in Fragments D and E of fibrinogen molecule in fibrin polymer formation.

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SELF-ASSEMBLY OF FIBRIN MONOMERS MEASURED BY QUASIELASTIC LIGHT-SCATTERING. D.E. Guinnup and J.S. Schultz. Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, U.S.A.

Purified fibrin monomer solutions were prepared in 1 M sodium bromide by alternation of precipitation and solubilization with pH adjustments between 6.0 and 5.3. The final preparation, at pH 5.3, was checked for monodispersity and purity by quasielastic laser light-scattering and was found to have a translational diffusion coefficient of  $2.14 \times 10^{-7}$  cm<sup>2</sup>/sec and a radius of gyration of 286 Å, in excellent agreement with published data.

The kinetics of fibrin self-assembly was monitored *in-situ* by measuring the autocorrelation function and mean intensity of scattered light with time after abruptly changing the pH from 5.0 to 6.3 in 1 M NaBr. The data were most consistent with an assembly model wherein fibrin associated with itself in an overlapping staggered end to end configuration. The evolving particle size distribution of fibrin fibrils was obtained by deconvoluting the autocorrelation functions obtained over a 30 minute period and unexpectedly revealed that larger aggregates, containing greater than about 6 fibrin units, were greatly favored over smaller aggregates, dimers, trimers, etc. Also, while the rate of fibrin assembly appeared to be proportional to the square of the monomer concentration, consistent with simple aggregation theory, the probability of a collision between particles resulting in the growth of a fibril was very small.

Additional *in-situ* experiments under laminar shear conditions in the physiological range showed the same pattern of fibril size distribution, except that the rate of self-assembly decreased about 30% as shear increased to 1000 sec<sup>-1</sup>.