

EFFECT OF PHOTOOXIDATION ON HUMAN FIBRINOGEN. Y. Inada, B. Hessel and B. Blombäck. Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, S-10401 Sweden.

Photooxidation of human fibrinogen and of the amino terminal fragment N-DSK, obtained by cyanogenbromide treatment of fibrinogen, has been studied. Fibrinogen and N-DSK were illuminated in the presence of methylene blue. Polymerization of fibrinogen after addition of thrombin was abolished by illumination as measured spectrophotometrically. Fibrinopeptides were released at a normal rate from the photooxidized fibrinogen by the action of thrombin. During the course of the polymerization of fibrinmonomers, a short period of illumination gave rise to the inhibition of the subsequent polymerization reaction. Histidine residues in photooxidized fibrinogen and photooxidized N-DSK were shown to be modified by illumination. Illumination of thrombin activated fibrinogen-Sepharose (fibrinmonomer-Sepharose) and thrombin activated N-DSK-Sepharose in the presence of methylene blue resulted in loss of affinity to fibrinogen. On the other hand, photooxidized fibrinogen which had no polymerization activity showed the same affinity for fibrinmonomer-Sepharose as did nonoxidized fibrinogen. The latter result suggests that histidine residues in the N-DSK domain of fibrinogen play an important role in the polymerization process.

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FIBRINOPEPTIDE B AND AGGREGATION OF FIBRINOGEN. John R. Shainoff and Beatriz N. Dardik, Research Division, Cleveland Clinic, Cleveland, OH

Copperhead venom procoagulant enzyme, previously shown to remove fibrinopeptide B at faster rate than fibrinopeptide A, has been found to release little of the A peptide at temperatures below 14°. At low temperatures, tight aggregation of the derivative lacking B blocked release of A by the enzyme. Transient release of A occurred, but stopped as removal of B approached completion. Overall losses of A amounted to 8% and 2% from human and rabbit fibrinogen respectively. Resultant clots with A intact dissolved on warming to 37°, whereafter release of A resumed with secondary coagulation ensuing. When dissolved at 37° with PMSF-inactivated enzyme, the fibrin remained highly soluble (>12 mg/ml). Ultracentrifugation showed constant levels of 8S monomer together with 16S aggregates in amounts accounting for the total protein at concentrations down to 0.16 mg/ml, and monomer alone at lower concentrations. From changes in saturation level of monomer at lower temperatures, the enthalpy of aggregation appeared to be about half of the -50 kcal/mole associated with aggregation of regular fibrin. Other experiments indicate that removal of the A and B peptides unmasks separate aggregation sites, each of which place the monomers in alignment suitable for rapid crosslinking. The β -chain segment spanning residues 15-42 appears to be critically involved in the aggregation that follows release of B itself, because the aggregation does not occur when this segment is removed together with B. In early stages of reaction between fibrinogen and plasmin. The aggregation which follows release of A depends on a different site, because the early alterations inflicted by plasmin do not prevent coagulation of fibrinogen by thrombin. (Supported in part by USPHS Grant HL-16361).

FIBRINOGEN-COLLAGEN INTERACTIONS. A. Stemberger, F. Jilek*, H. Hörmann* and G. Blümel. Institut f. Experimentelle Chirurgie der Technischen Universität and Max-Planck-Institut f. Biochemie*, München, West Germany.

Recently fibrinogen-collagen sponge preparations have been used surgically for tissue adhesion (Austria, BRD, Swiss). Therefore fibrinogen-collagen interactions were studied by affinity-chromatography and by investigation of fibrin crosslink formation in presence of collagen.

Affinity chromatography of plasma on collagen-Sepharose yielded retention of fibrinogen on conjugates of native collagen type3 and denatured collagen type1. Vice versa these collagen types were adsorbed by fibrinogen-Sepharose.

Fibrinogen-collagen mixtures were clotted by adding thrombin, Ca^{2+} and factor 13. In contrast to dissolved collagen, collagen fibers interfered with fibrin crosslinking, i.e. - dimer formation was restricted as shown by acrylamid gel electrophoresis of the reduced coagulum. Covalent coupling of fibrin to collagen was not observed by DEAE and CM-cellulose chromatography.

The affinity of collagen to fibrin was demonstrated by adhesion of collagen sponges (containing thrombin) with 10% fibrinogen. Breaking strength (p/cm²) is dependent on sponge. Studies on collagen-fibrinogen tissue adhesions are in progress. Scanning electron microscopic pictures showed inclusion of fibrin into collagen sponges.

According to our results, collagen fibrinogen adhesion refer mainly to mechanic sticking. The affinity of plasma fibrinogen to collagen type3 will stimulate further investigations, since Gay et al. (Klin. Wschr. 53, 1975) have shown that collagen type3 is the main connective tissue component under endothelial cell-layers of arterial walls.