

Thursday, July 16, 1981

## Symposium XII

# Fibrinogen, Structure and Function

15:30-17:30 h

Dominion Ballroom

### 0787

FIBRINOGEN BIOSYNTHESIS: IN VITRO TRANSLATION, GLYCOSYLATION AND TRANSLOCATION OF FIBRINOGEN PEPTIDE CHAINS. G.M. Fuller and J.M. Nickerson. Department of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, T.X.

Fibrinogen is a hepatically derived plasma glycoprotein that is composed of three pairs of nonidentical chains linked together by complex sets of disulfide bridges. In an effort to understand the molecular and cellular processes of translating and assembling this important multichained protein we have utilized an in vitro translating system using mRNA's for rat fibrinogen. Highly specific antibodies to fibrinogen and to each chain have been developed and used to immunoprecipitate the nascent  $\alpha$ ,  $\beta$ , and  $\gamma$  polypeptides. We have also used a rat hepatoma cell line which synthesizes and secretes fibrinogen to prepare nonglycosylated but processed fibrinogen subunits. SDS/PAGE analysis of the translation products clearly show that each polypeptide has a "signal" peptide located at its amino terminal end. The size of the signal peptide is different for each chain. These results demonstrate that separate mRNA's exist for each of the fibrinogen subunits. Temporal analysis of the glycosylation of the  $\beta$  and  $\gamma$  chain reveal that the  $\gamma$  chain receives its Asn-linked carbohydrate as an early cotranslational event. The  $\beta$  chain's core carbohydrate moiety is near the end of the polypeptide and our evidence shows that the glycosylation event likely occurs posttranslationally. When microsomal membranes are added to an on-going translation system, all three of fibrinogen's polypeptides translocate into the cisternal space, with an apparent equal stoichiometry. Additional experiments suggest that fibrinogen assembly occurs as a cotranslational process.

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INTERACTION OF FIBRIN AND TISSUE PLASMINOGEN ACTIVATOR. P. Wallén and M. Rånby. Department of Physiological Chemistry, Umeå University, Umeå, Sweden.

Fibrin itself has a marked influence on fibrinolysis induced by tissue plasminogen activator (TA) indicating a specific interaction. The interaction between fibrin and TA is manifested in two ways, 1) a marked stimulating effect of fibrin on the activation of plasminogen; 2) physical adsorption of TA on fibrin. By measurement in a sensitive analytical system in which the generation of plasmin is followed by a chromogenic substrate it has been shown that TA is a rather poor activator of plasminogen. In the presence of fibrin the kinetics of the activation is dramatically changed. A stimulation up to 1000-fold is obtained at low plasminogen concentrations. As for activation of native plasminogen (Glu-plasminogen) there is a decrease of  $K_m$  (about 15-fold) as well as an increase of  $k_c$  (about 80-fold). Fibrinogen has comparatively little effect on the activation rate (at the most 10-fold). The amidolytic activity of TA, using an activator sensitive substrate, is not influenced by fibrin indicating that the effect is not due to conformational changes in the active site region of TA.

By varying the concentration of fibrin in the test system it has been demonstrated that the stimulation effect increases suddenly at a fibrin concentration of about 0.01  $\mu$ M. It was suggested that this value represents the dissociation constant of the TA-fibrin complex. However, the amount of fibrin necessary for the adsorption of TA in purification experiments indicates a significantly higher dissociation constant (about 0.4  $\mu$ M). An important difference between the purification experiments and the studies on fibrin stimulation is that plasminogen (plasmin) is absent in the former studies. The formation of a triple complex between fibrin, plasminogen and TA may be the explanation for a more efficient binding of TA in the kinetic studies.