

INVITED SYMPOSIUM IV

Molecular Initiation of Fibrinolysis.

HAGEMAN FACTOR (HF) DEPENDENT FIBRINOLYSIS. Allen P. Kaplan, Robert Mandle, Jr., Lewis D. Yecies, and Henry L. Meier. National Institutes of Health, Bethesda, Md.

The HF dependent fibrinolytic pathway is initiated by binding of HF (M.W. 80,000) and a complex of prekallikrein and high molecular weight (HMW) kininogen (M.W. 280,000) to negatively charged surfaces. A reciprocal reaction proceeds in which HF_A converts prekallikrein to kallikrein and kallikrein activates HF. The rate of each enzymatic reaction is augmented by HMW kininogen. The active site of HF, as assessed by incorporation of ^3H -DDFP, in the surface bound enzyme does not form upon binding in the presence or absence of HMW kininogen, but is generated upon activation by kallikrein. The product, HF_A (M.W. 80,000), is subsequently cleaved to liberate the active Hageman factor fragments (M.W. 28,000). Two forms of prekallikrein (M.W. 88,000 and 85,000) are cleaved by HF_A to yield kallikreins in which a heavy chain (M.W. 52,000) is disulfide linked to a light chain (36,000 or 33,000) and, for each molecular form, the active site is in the light chain. Kallikrein activates plasminogen (M.W. 94,000) to yield a plasmin consisting of a heavy chain (M.W. 58,000) disulfide linked to a light chain (M.W. 27,000) and again, the active site is in the light chain. Digestion of prekallikrein by kallikrein yields proenzymes of molecular weight 78,000 and 75,000 that appear to represent the previously described plasminogen proactivator. Factor XI circulates bound to HMW kininogen, is activated by HF_A in the presence of HMW kininogen, and factor XI_A -HMW kininogen activates HF. Thus factor XI may contribute to the gradual activation of HF and evolution of fibrinolytic activity in prekallikrein deficient plasma. A further role for factor XI_A as a plasminogen activator will be discussed.

THE MECHANISM OF ACTIVATION OF HUMAN PLASMINOGEN BY UROKINASE. Francis J. Castellino and Bernard N. Violand. The Department of Chemistry, The University of Notre Dame, Notre Dame, Indiana 46556.

When native human plasminogen (Glu-Pg) is activated to plasmin by urokinase (UK), at least two peptide bonds are cleaved in the process. Cleavage of one bond (I), located in the interior of the molecule, results in formation of the well known, two polypeptide chain plasmin; in which the chains are stabilized by a single disulfide bond. Cleavage of another bond (II), located at the original amino terminus of native Glu-Pg, results in covalent release of a peptide (P), of molecular weight ca. 8,000. The final plasmin obtained (Lys-Pm), is a molecule in which both bonds are cleaved. An intermediate plasminogen (Lys-Pg), in which only II is cleaved, and an intermediate plasmin (Glu-Pm), in which only I is cleaved, can be isolated under appropriate conditions. Our studies indicate that the activator, UK, is not capable of catalysis of bond II cleavage in either Glu-Pg, Glu-Pm, or the native plasmin heavy chain (Glu-H), isolated from Glu-Pm. On the other hand, plasmin readily accomplishes cleavage of II from all of the above molecules. Bond I cleavage is catalyzed only by UK, in this activation system. Physical and kinetic studies leads us to propose a mechanism for Glu-Pg activation by UK involving the following steps: (1), an initial UK catalyzed cleavage of I, forming Glu-Pm; (2), a Glu-Pm catalyzed cleavage of II, in an autocatalytic step, forming Lys-Pm; (3), a Glu-Pm or, more likely, Lys-Pm catalyzed cleavage of II from the remaining Glu-Pg, forming Lys-Pg; (4), a UK catalyzed cleavage of I in Lys-Pg, forming Lys-Pm. (Supported by grants from the N.I.H. and A.H.A.).