FREE COMMUNICATIONS XV

Thrombosis: Plasminogen Activator.

Primary structure of plasminogen. Separation of two lysine-binding domains and one neo-lysine-binding unit (N.B. = 36,000). L. Potting, R. M. K. Engel, K. Petersen and P. den Supple, Department of Molecular Biology, University of Aarhus, Denmark.

The complete amino acid sequence of the heavy chain of human plasminogen (residues 1-560) has been established. The complete sequence of the light chain (residues 561-790) is accounted for but six overlaps remain to be established. We have located 17 of the 24 disulfide bridges. The remaining 7 can be assigned with high confidence on the basis of extensive homology of the light chain with serine proteases and of kringle domains KL-KS (residues 93-161, 165-242, 255-333, 357-434 and 461-540, resp.) with the two kringle domains (residues 66-144 and 172-249) in bovine gastrin. Of the two oligosaccharide groups one is glucosamine-based, attached to Asn-286, the other galactosamine-based, attached to Thr-345. The former is either present or absent, the latter is heterogeneous. This variability could account for six or more of the observed electro-phoretic variants of plasminogen. Degradation of native human plasminogen by porcine pancreatic elastase gave 14 small peptides (2 from the PAP region and two residues 330-353 and 440-444, from between kringle 3 and 4 and 4 and 5, resp.), and three major fragments. Two of these, namely KL2-3 (residues 79-137/353 (M.W. 25,643 plus) and KL3 (residues 354-439 (M.W. 9,687)) each contain a lysine-binding site. The third, neo-lysine-binding unit (residues 442-790) is activated faster than plasminogen (residues 1-790) with either urokinase or streptokinase indicating that the structure contained in residues 1-441 (PARM2-3) is not necessary for activation of plasminogen, and raising questions regarding a possible role for leukocyte elastase in changing the affinity of plasminogen/plasmin for other proteins by removing lysine-binding sites. A 42-residue fragment (residues Arg-546-Glu-575, -O-b-bridged to Lys-560-Tyr-571) is a substrate for urokinase, which cleaves the same Arg-Val bond (560-561) as in native plasminogen.


Plasma plasminogen (proactivator) were activated and precipitated completely in a 2-15% acrylamide fraction by means of dextran sulfate. Total activator activity was measured by adding flufenamate which rules out the influence of precipitated Cl-inactivator on intrinsic activator system(s); extrinsic or vascular activator activity resistant to Cl-inactivator was measured separately by adding extra Cl-inactivator. In normal morning plasma, a total level of 105 ± 15 (n=50) arbitrary blood activator units (BAU/m) was detected; after 15 min venous occlusion, plasma contained 245 ± 27 BAU/m (n=7). The contribution of extrinsic activator amounted to a few percent in normal plasma and around 120 BAU/m in occlusion plasma. The measured activator activity in morning plasma originates mainly from the intrinsic system(s) and was subdivided roughly into two equal components, one dependent on factor XII and one apparently independent of factor XII. The latter was found in Hagman trait (52 BAU/m; n=3) and Fletcher trait plasma (44 BAU/m; n=2). The factor XII-dependent component was revealed by the correction of the deficiencies with factor XII (1%) and purified plasma kallikrein (102,690 and 350,000 daltons, 50-75%), respectively. Fitzgerald's trait plasma (84 BAU/m; n=1) did not show an altered response. Activator activity (10-15 BAU/m plasma) was found in partially purified kallikrein preparations of 100,000 as well as 320,000 daltons seems to be a property of the kallikrein. Thus, the total amount of activator activity found in morning plasma is made up by a few percent extrinsic activator, 10-15% kallikrein, 30-40% factor XII-dependent activator and 40-50% unidentified component(s).