FREE COMMUNICATIONS XV

Thrombosis: Plasminogen Activator.

PRIMARY STRUCTURE OF PLASMINOGEN. SEPARATION OF TWO LYSINE-BINDING DOMAINS AND ONE NEOPLASMINOGEN (M.w. = 38,000). L. Sottrup-Jensen, T. E. Petersen and S. Magnusson, Department of Molecular Biology, University of Aarhus, Denmark.

The complete amino acid sequence of the heavy chain of human plasmin (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 K1-K5 (residues 83-161, 165-242, 255-333, 357-434 and 461-540, resp.) with the two kringle domains (residues 66-144 and 171-249) in bovine prothrombin. Of the two oligosaccharide groups one is glucosamine-based, attached to Asn-288, 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 electrophoretic variants of plasminogen. Degradation of native human plasminogen by porcine pancreatic elastase gave 14 small peptides (12 from the PAP region and two (residues 338-353 and 440-441, from between kringles 3 and 4 and 4 and 5, resp.), and three major fragments. Two of these, namely K1+2+3 (residues 79-337/353 (M.w. 29,843 plus)) and K4 (residues 354-439 (M.w. 9,687)) each contain a lysine-binding site. The third, neoplasminogen-Val-442 (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 (PAP+K1-K4) 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 Asp-546 - Gln-575, -S-S-bridged to Lys-660 - Tyr-671) is a substrate for urokinase, which cleaves the same Arg-Val-bond (560-561) as in native plasminogen.

AN INVENTORY OF PLASMINOGEN ACTIVATORS IN HUMAN PLASMA. C. Kluff, Gaubius Institute, Health Research Organization TNO, Herenstraat 5d, Leiden, The Netherlands.

Plasma plasminogen (pro)activators were activated and precipitated completely in a euglobulin fraction by means of dextran sulphate. Total activator activity was measured by adding flufenamate which rules out the influence of precipitated C1-inactivator on intrinsic activator system(s); extrinsic or vascular activator activity resistant to C1-inactivator was measured separately by adding extra C1-inactivator. In normal morning plasma, a total level of 100 ± 15 (n=50) arbitrary blood activator units (BAU)/ml was detected; after 15 min venous occlusion, plasma contained 249 ± 27 BAU/ml (n=7). The contribution of extrinsic activator amounted to a few percent in morning plasma and around 120 BAU/ml 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 Hageman trait (52 BAU/ml; n=3) and Fletcher trait plasma (44 BAU/ml; n=2). The factor XII-dependent component was revealed by the correction of the deficiencies with factor XII (1%) and purified plasma kallikrein (100,000 and 300,000 daltons, 50-75%), respectively. Fitzgerald trait plasma (84 BAU/ml; n=1) did not show an altered response. Activator activity (10-15 BAU/ml plasma) found in partially purified kallikrein preparations of 100,000 as well as 300,000 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).