1576

CHARACTERIZATION OF LIMITED PROTEOLYTIC DIGESTS OF TISSUE PLASMINOGEN ACTIVATOR . G. Teshima, R. Harris, R. Keck, A. Meunier, J. Burnier and B. Keyt. Genentech, Inc. 460 Pt. San Bruno Blvd., South San Francisco, California 94080 U.S.A.

Tissue plasminogen activator (tPA) is a single chain glycoprotein of 527 amino acids consisting of structural domains homologous to other plasma proteins ("finger","epidermal growth factor", "kringles" and "protease"). Unlike zymogens of other serine proteases, tPA in the single chain form (1-527), has amidolytic and fibrinolytic activity. However, the amidolytic activity is enhanced when tPA is cleaved by plasmin at the Arg275-Ile276 bond to yield the disulfide bonded two chain form. We used trypsin to study the structure and function of tPA by limited digestion. Aliquots of tPA (1 mg/ml) were digested at pH 7 with varying amounts of trypsin (1:10,000, 1:1000, 1:100 and 1:10; enzyme to substrate ratio). The dilute solutions of trypsin (1:10,000) were effective at completely converting one chain tPA to the two chain form, but little additional proteolysis was observed on SDS-PAGE. The proteolytic fragments of tPA were isolated by reduction and carboxymethylation (RCM), SDS gel electrophoresis and reversed phase HPLC. The RCM polypeptides were identified by amino acid composition and sequence. Specific antisera were prepared against peptide antigens of tPA including (1-27), (1-275), (276-527) and (502-525). Immunoblotting experiments with the tryptic digests of tPA indicated that the region (1-275) is more susceptible to proteolytic attack than the protease (275-527). Specific cleavage sites were identified at positions 7, 10, 27 and 40. Partially digested tPA preparations were tested for enzymatic activity as determined by hydrolysis of the peptide substrate S-2288 or by clot lysis. Limited proteolysis at the amino terminus was correlated with significant loss of fibrinolytic activity but minimal effect on the amidolytic activity. Increased tryptic digestion resulted in complete loss of amidolytic activity and significant reduction in antigenic activity as determined by polyclonal anti-tPA ELISA. These results are consistent with the amino terminal "finger" domain being in part responsible for the fibrin-binding specificity of tPA. Limited tryptic digest of tPA, cleaves first at Arg-275, then subsequently cleaves the "finger" with associated loss of fibrinolytic activity.

1577

STUDIES ON THE DIRECT PROTEOLYTIC ACTION OF HUMAN TISSUE PLASMINOGEN ACTIVATOR ON HUMAN FIBRONECTIN AND VITRONECTIN. S. Wasi (1), S. Juodvalkis (1), P. Alles (1), and J.E. Aubin (2). National Reference Laboratory, Canadian Red Cross B.S. (1) and MRC Group in Periodontal Physiology, University of Toronto (2), Toronto.

The ability of cells to make or break specific attachments to extracellular matrix (ECM) and other cells is important in cell migration, proliferation and wound repair. Specific attachment proteins believed to be involved in mediating these interactions comprise functional domains joined by protease sensitive segments. Proteases can conceivably modulate cellular interactions by releasing functional domains from parent molecules. Tissue plasminogen activator (t-pA) is known to participate in various pathophysiological processes. That t-pA may also act directly on structural proteins has not been investigated. We have studied the direct proteolytic action of melanoma t-pA on fibronectin (FN), vitronectin (VN) and laminin (LN). These were incubated with t-pA for 0 to 48 h in 50 mM Tris HCl, pH 7.4. The cleavage products were separated on polyacrylamide slab gels and blotted onto nitrocellulose strips. FN and VN fragments with cell attachment properties were identified by incubating the strips with human gingiva fibroblasts and staining with Amido black. Monoclonal antibodies to FN were used to identify heparin, cell and gelatin binding fragments. VN was converted to a major 55 Kd product as a function of time. Lower molecular weight species migrating at 45 Kd, 30 Kd and 15 Kd positions were also identified. Most of these fragments possessed cell attachment properties. LN became susceptible to t-pA digestion after denaturation with H_2O_2. The catalytic activity of t-pA could be inhibited in the presence of nitrophenyl-p-guinidino benzoate (a synthetic inhibitor of plasminogen activator), whereas O-phenanthroline (a metalloprotease inhibitor), α 2-antiplasmin and trasylol had no effect. A monoclonal IgG preparation (HI 72 A_1, kindly provided by Dr. David J. Loskutoff) that specifically inhibits t-pA also inhibited the protelyotic action of t-pA on FN. These data suggest that direct proteolytic action of t-pA on adhesive proteins may modulate cellular behaviour in various normal and pathological conditions which involve dynamic interactions between cells and ECM and where plasminogen activator levels are elevated either transiently or permanently, for example during tissue remodelling, wound-related repair and thrombolytic therapy.

1578

EFFECT OF THE LIPOPROTEIN FRACTION FROM PLASMA-EUGLOBULIN ON THE PLASMINOGEN ACTIVATION. K.Deguchi(1), H.Suzuki(1), H.Wada(1), S.Shirakawa(1), Y.Iga(2) and S.Kameyama(2), The 2nd Department of Internal Medicine, School of Medicine, Mie University, Tsu-city, Mie(1) and Central Research Labs Green Cross Corp., Osaka (2), Japan.

The effect of the lipoprotein fraction on fibrinolysis was assessed in an in vitro system containing plasminogen and tissue plasminogen activator(t-PA). The fraction were separated from plasma-euglobulin by the sucrose density gradient ultracentrifugation at 90,000 g, 5°C for 48 hours. The fraction corresponding to high density lipoprotein(HDL), especially its subfraction HDL_3 (1.125 d 1.121), did not promote any amidolytic activity on ^{125}S-2251.However,it induced amidolytic activity in the presence of t-PA and even stronger activity in the presence of plasminogen and t-PA. the potentiating effect of this subfraction on the plasminogen activation by t-PA or urokinase was also evidenced on the plasminogen free fibrin plate. The HDL_3 fraction may contain plasminogen and a potentiating substance involved in the conversion of plasminogen to plasmin by t-PA or urokinase.

The maximum level of plasminogen in the HDL_3 fraction was 30 % in both its activity evaluated by chromogenic[3] assay and its antigen evaluated by the immunodiffusion method. And the pattern of the plasminogen in this subfraction on the crossed immunoelectrophresis agaist anti-human plasminogen rabbit serum was similar to that in normal plasma. Furthermore, the latex aggregation test revealed the presence of fibrinogen and/or its degradation products in this subfraction, their levels has no effect on plasminogen activation by t-PA. The HDL_3 fraction treated with heat at 56°C for 15 min. retained the activity.

We conclude from the above findings that the fraction corresponding to HDL,especially HDL_3,from plasma-euglobulin contains a substance that potentiates the plasminogen activation by t-PA or urokinase, in addition to plasminogen.

1579

New high capacity lysine-agarose based on divinylsulfone activated agarose. A. Lihme (1), C. S. Nielsen (1) and U. Christensen (2). The Protein Laboratory (1) and Chemical Laboratory IV (2), University of Copenhagen, Sigurdsgade 34, DK-2200 Copenhagen, Denmark.

It is generally acknowledged that plasminogen is best purified on some kind of immobilized lysine. We here present results on plasminogen purification using a new type of lysine-agarose affinity chromatography. Divinylsulfone activated agarose[1] were used for alpha-N-immobilization of L-lysine ("Minileak", KemEuTec,P.O. Box 613, DK-2200). The intrinsic features of this matrix are[2,3]:

a) High density of active groups, high coupling capacities(40-50mM) of $-NH_2$,-SH,-OH-groups.
b) Very high mechanical stability
c) Practically no leaking of immobilized ligands.

The matrix is useful for fast and effeicient protein-purification in automated systems. Using a FPLC-system and a variety of exp. conditions lysine-minileak, compared with lysine-Sepharose (Pharmacia) consistently yielded more than tvice the amount of plasminogen from plasma and serum.

1) L. Sundberg and J. Porath, (1975) Portides Biol. Fluids, Proc. Colloq., 23, 49-62.
2) A. Lihme et al.,(1986) J. Chromatogr.,376, 299-305.
3) H.J. Allen and E.A.Z. Johnson,(1975)Carbohydr. Res. 58, 253-265.