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ON DIFFERENT MOLECULAR FORMS OF PLASMINOGEN ACTIVATOR INHIBITOR
I. Carlsson, J. Chmielewska and B. Wiman. Department of Clinical Chemistry, Karolinska Hospital, S-104 01 Stockholm, Sweden.

The production of plasminogen activator inhibitor (PAI) by the human cell-lines Hep G2 and HT 1080 have been studied by immunochemical and functional methods. In conditioned medium collected after 2h, the PAI seemed to be almost fully active, but with increasing incubation time the activity was gradually lost, in spite of that the PAI-antigen content increased continously. The active PAI form can be separated from the inactive form by gel-filtration. The inactive form behaves as a low M_r (about 50,000) component in the absence and in the presence of sodium dodecylsulphate. In contrast, the active form of PAI behaves as a high M_r (>300,000) compound in the absence of sodium dodecylsulphate but as a low M_r compound in its presence. The low M_r inactive PAI has been purified to homogeneity from HT 1080 conditioned medium, collected in the absence of fetal calf serum. This was achieved by chromatography on Concanavalin A-Sepharose, gel-filtration on Sephacryl S-300 and affinity chromatography on insolubilized monoclonal antibodies against PA-inhibitor. On treatment of this form of the inhibitor with 4 mol/L Guanidinium chloride, the activity was regained, but its gel-filtration behaviour was unchanged in the absence of serum/plasma (M_r about 50,000). Addition of plasma or serum prior to the gel-filtration, changed the elution pattern of PAI towards a high M_r form. The reason for this behaviour is not yet fully understood, but the most plausible explanation is the presence of a high M_r PAI-binding protein in plasma/serum. This hypothesis is presently being explored.

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ASSOCIATION OF PLASMINOGEN ACTIVATOR INHIBITOR(PAI-1) WITH THE MEMBRANE AND EXTRACELLULAR MATRIX OF HUMAN ENDOTHELIAL CELLS.
E.G. Levin and L. Santell, Scripps Clinic and Research Foundation, La Jolla, CA, USA.

The cytosol, membrane-containing, and extracellular matrix(ECM) fractions of cultured human endothelial cells were isolated and analyzed for the presence and levels of PAI-1. Cells($4x10^6$) released intact from the substratum had 70% (2.85±0.16 units)of their total inhibitor activity in the soluble portion of cell homogenate with the remaining activity(1.26±0.30 units) in the 100,000xg pellet.The ECM contained over twice as much activity as the total cellular inhibitor(9.82±0.35 units).Similar results were obtained with ECM after cell removal by 0.5% Na deoxycholate and hypotonic buffer treatment. Analysis of all three samples by SDS-PAGE and reverse fibrin autography showed inhibitor activity at an identical position on the gel corresponding to Mr=46000.Immunoblot analysis demonstrated that this inhibitor activity represented PAI-1. Inhibitor was not removed from the membrane by treatment with high salt,EDTA,divalent cations, low ionic strength buffer or sonication but was dissociated in the presence of detergents, guanidine HCl, or high pH. In contrast,ECM-associated PAI-1 was not affected by any of these treatments. Pulse-chase experiments indicated that the inhibitor was associated only transiently with the ECM in the presence of cells. None of the PAI-1 deposited during the labeling period was observed after 24 hrs. Incubation of the isolated ECM with protolytically active tPA resulted in a decline in the intensity of the Mr=46000 inhibitor band and the appearance of a Mr=44000 band and a Mr=110000 tPA-inhibitor complex band. Both of these bands were also found in the supernatent. The Mr=44000 band represented PAI-1 as shown by immunoprecipitation studies, although it did not contain anti-tPA activity. Thus, ECM-bound PAI-1 is susceptible to cleavage and release by tPA but does not in all cases form a stable complex with the protease. Elastase had a similar effect: following incubation, all of the PAI-1 was released from the ECM into the supernatent and was converted to the Mr=44000 inhibitor form. Collagenase, heparinase, and chondroitinase ABC had no effect. Therefore, PAI-1 is susceptible to inactivation and release from ECM by several proteases.

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PURIFICATION AND CHARACTERIZATION OF PAI-1 FROM HUMAN ENDOTHELIAL CELLS.
N.A. Booth (1), I. R. MacGregor (2), N. R. Hunter (2) and B. Bennett (1), Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD, Scotland (1) & SNBTS HQ Laboratories, 2 Forrest Road, Edinburgh EH1 2QN, Scotland (2)

Platelet α-granules and endothelial cells contain an inhibitor of plasminogen activator, which inhibits both t-PA and u-PA. The inhibitor (PAI-1) is detectable after SDS-PAGE and zymography on fibrin/plasminogen/u-PA detector gels. We have purified endothelial PAI-1 by a simple two-step procedure. Serum-free conditioned medium from human umbilical vein endothelial cells, grown in microcarrier culture, was fractionated on Sephadex CM-50, a cation exchanger, followed by gel filtration on Sephacryl S-200. Aprotinin was included throughout the procedure to maintain the activity of the inhibitor. The PAI-1 was purified 2000-fold with a recovery of about 7%. The purified protein had a specific activity of 8500 U/mg protein and the activity could be stimulated 14-fold by 4M guanidine. The purified PAI-1, of M_r 48000, was a single-chain glycoprotein. The product was apparently homogeneous on a silver-stained SDS-polyacrylamide gel, the protein band co-migrating with PAI activity. Further, a rabbit antiserum raised against the purified PAI-1 revealed only a single band on immunoblots of material from each stage of the purification. The immunoglobulin fraction of the antiserum, incorporated into the detector gel for zymographic analysis, neutralised the inhibitor from plasma, platelets and endothelial cells, confirming their identity. Preincubation of PAI-1 from these sources with the immunoglobulin prevented formation of a complex with t-PA or u-PA. This purification procedure, in which no denaturants are employed, provides a homogeneous preparation of PAI-1 that is useful for studies on the stimulatory effects of denaturants. The antiserum raised has allowed the development of a sensitive ELISA, specific for PAI-1.

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EVIDENCE FOR THE OCCURRENCE IN HUMAN PLASMA OF A COMPLEX OF UROKINASE (UK) AND PLASMINOGEN ACTIVATOR INHIBITOR TYPE I (PAI-1).
G. Dooijewaard, C.P.M. Gillis, R.J. van der Hoeven, D.C. Rijken and V.W.M. van Hinsbergh. Gaubius Institute TNO, Leiden, the Netherlands.

In plasma three forms of UK may occur: proUK, active UK and an inactive complex of UK with inhibitor(s). Recently we developed 1. an ELISA which measures the antigen of the three forms with equal efficiency and gives the total UK, irrespective of its molecular form, and 2. a BIA, which measures the activity of the immuno-immobilized material before (UK) and after activation with plasmin (proUK + UK). With these assays the concentrations of proUK, UK and UK.Inhibitor complex in plasma were estimated to be 2.1, < 0.1 and 1.1 ng/ml, respectively. The identity of the inhibitor involved remained unexplained. In this study we investigated the possibility that it is PAI-1. First we titrated PAI-1-containing serum-free culture medium of endothelial cells with increasing amounts of UK, measured the concentration of the remaining free UK by BIA and calculated the concentration of UK.PAI-1 formed. A linear and proportional relationship between UK.PAI-1 and the amount of medium titrated was established. Then we analysed the obtained titration curves in the ELISA and found closely overlapping straight lines with no evidence for any antigen to have been titrated away. This confirms that the UK.PAI-1 formed, indeed, is measured in the ELISA with equal efficiency as the free UK and affirms the possible presence of UK.PAI-1 in plasma. Hereafter we set out for the development of a UK.PAI-1 ELISA, which first singles out the UK antigen from the plasma, then senses the PAI-1-containing part with anti-PAI-1 antibodies conjugated with biotin and thereafter detects the concentration with streptavidin-HRP. Calibration curves were made with in vitro prepared UK.PAI-1 added to UK immuno-depleted plasma. Preliminary data give 0.8 ng/ml for the UK.PAI-1 in human plasma, which explains the bulk of the 1.1 ng/ml UK.Inhibitor complex.