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ON THE KINETICS OF THE INHIBITION OF PLASMINOGEN ACTIVATORS BY THE PLASMINOGEN ACTIVATOR INHIBITOR PAI-1 J.Chmielewska and B. Wiman, Department of Clinical Chemistry and Blood Coagulation, Karolinska Hospital, S-104 01 Stockholm, Sweden.

The kinetics of the inhibition of the following plasminogen activators: one- and two-chain tissue plasminogen activator (t-PA) and low and high molecular weight urokinase (UK) by PAI-1 was studied. For this purpose direct systems were employed and the reactions were studied in the presence of different concentrations of plasminogen activator chromogenic substrates. The second-order rate constant of the association reaction was estimated from the initial decline in plasminogen activator activity. Determination of the rate constants in the absence of substrates was performed by plotting the rate constants versus the substrate concentrations and extrapolation to zero concentration. The rate constants with all plasminogen activators were very similar and estimated as $2 - 4 \times 10^7\ M^{-1} \times s^{-1}$. The reactions were also studied in the presence of 6-aminohexanoic acid, lysine, arginine, guanidinium chloride (final concentrations for all substances about 1 mmol/L) and heparin (10 mg/L), without any significant effect on the rate constants. The effect of soluble fibrin (bathroxobin-digested fibrinogen in urea) at 10 - 300 nmol/L was also studied. With one-chain t-PA the rate constant was decreased about 10-fold with the highest fibrin concentration and about 2-fold at 30 nmol/L. In contrast, the reactions with urokinase or two-chain t-PA were not influenced by fibrin at these concentrations. These findings may have a physiological significance: the one-chain t-PA adsorbed to the fibrin surface and actively involved in fibrinolysis would be protected against inactivation by PAI. This phenomenon adds further to the physiological fibrin specificity of one-chain t-PA.

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DETERMINATION OF INTERMEDIATES, PRODUCTS AND CLEAVAGE SITE IN THE REACTION BETWEEN PLASMINOGEN ACTIVATOR INHIBITOR 2 (PAI 2) AND UROKINASES. U. Kiso (1), H. Kaudewitz (1), A. Henschen (1), B. Åstedt (2), E.K.O. Kruithof (3) and F. Bachmann (3).Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG (1), Dpt. of Obstetrics & Gynaecology, University Hospital, Lund, Sweden (2) and Hematology Div., Dpt. of Medicine, University Hospital Center, CHUV, Lausanne, Switzerland (3).

Several specific inhibitors for both urokinase-type and tissue-type plasminogen activators have in recent years been isolated from various organs and cell lines. The inhibitors isolated from human placenta and from the human histiocytic lymphoma cell line U-937 have been shown to be immunologically related.They are denoted as plasminogen activator inhibitor type 2 (PAI 2). It has earlier been demonstrated by gel electrophoresis that during the inhibition reaction at first complexes are formed between activator and inhibitor and then the inhibitor is cleaved and the complex can be dissociated.

In the present study the reactions between urokinase and PAI 2 were analysed by means of reversed-phase high-performance liquid chromatography (HPLC) and SDS-polyacrylamide gelelectrophoresis (PAGE). Both high-molecular-weight (54 kDa) and low-molecular weight (33 kDa) urokinase (UK) were used. The PAI 2 was derived from placenta and from U-937 cells. It has a molecular size of 45 kDa. Equimolar amounts of UK and PAI 2 were incubated for 15 min at room temperature. On HPLC and SDS-PAGE analysis new components, corresponding to the UK-PAI 2-complexes, appeared and both UK and PAI 2 disappeared. The apparent sizes of the complexes were approx. 82 kDa and 62 kDa, depending on the type of UK used. The HPLC retention times of the complexes were higher than those of UK or PAI 2. The complexes were stable under the conditions employed for the analyses, but could be completely dissociated by incubation for 1 h at pH 10 and 37°. The released UK behaved identical with the UK starting material. However, the released PAI 2 had decreased in molecular size according to the PAGE analysis, and on HPLC an additional component appeared. As PAI 2 has a blocked N-terminus and the new component shows an N-terminal sequence it must correspond to the C-terminal part of PAI 2. The sequence defines thus the reactive site for the cleavage by the plasminogen activator.

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PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1: STUDIES ON STRUCTURE AND REGULATION

P.A. Andreasen (1), A. Riccio (2), L.R. Lund (1), K.G. Welinder (3), F. Blasi (2), and K. Danø (1).

(1) Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark; (2) International Institute of Genetics and Biophysics, CNR, Naples, Italy; (3) Institute of Biochemical Genetics, University of Copenhagen, Denmark.

Human plasminogen activator inhibitor type-1 is an Mr~54,000 protein which specifically inhibits urokinase-type (u-PA) and tissue-type (t-PA) plasminogen activators. During inhibition, u-PA and t-PA convert PAI-1 to an inactive form with Mr ~50,000. We have determined the amino-terminal amino acid sequence of native and converted PAI-1, and isolated and partly sequenced PAI-1 cDNA. The data show that the conversion of PAI-1 consists of cleavage of an Arg-Met bond 33 residues from the carboxy-terminus, thus localizing the reactive center of the inhibitor to that position, and identifying PAI-1 as an "arg-serpin". PAI-1 activity is known to be influenced by a number of agents; we have studied the mechanisms of the stimulation of PAI-1 activity by transforming growth factor-β (TGF-β) and the synthetic glucocorticoid dexamethasone in human WI-38 lung fibroblasts and HT-1080 fibrosarcoma cells. By the use of PAI-1 cDNA, TGF-β was found to course a rapid increase in PAI-1 mRNA level in WI-38 cells, reaching a maximal 50-fold enhancement after 8 hours. Dexamethasone caused a 10-fold increase in PAI-1 mRNA in HT-1080 cells, which was detectable after 4 hours and became maximal after 16 hours. In both cases, the 3.4 as well as the 2.4 Kb-PAI-1-mRNA species were increased. Quantitative studies on the effect of these agents on PAI-1 protein levels in cell extracts and culture media by ELISA gave results consistent with the effects on PAI-1 mRNA. These studies suggest that TGF-β and glucocorticoids may exert important controls over plasminogen activation-mediated extracellular proteolysis through an enhancement of PAI-1 gene transcription.

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PLASMINOGEN ACTIVATOR INHIBITOR (PAI) ACTIVITY AS WELL AS PAI-1 AND PAI-2 ANTIGEN IN HEALTHY INDIVIDUALS AND HOSPITALIZED PATIENTS.

E.K.O. Kruithof, G. Nicoloso, A. Gudinchet and F. Bachmann. Laboratoire central d'hématologie, CHUV, Lausanne, Switzerland.

The activity of plasminogen activators (PA) is regulated in part by PA-inhibitors. Two distinct PAIs have been identified: PAI-1 and PAI-2. To be able to measure these proteins specifically we have purified PAI-1 and PAI-2, raised antisera and developed specific radioimmunoassays (RIAs), with detection limits in plasma of 10 and 15 ng/ml, respectively.
In a population of over 350 individuals, PAI activity correlated well with PAI-1:Ag ($r=0.699$), but not with PAI-2:Ag ($r=0.070$). In healthy individuals PAI-1 concentrations varied considerably (between 10 and 85 ng/ml, median 29 ng/ml). During venous occlusion (VO) applied in 40 healthy individuals, average PAI-1 in plasma increased from 27 before, to 43 and 59 ng/ml after 10 and 20 min VO ($p < 0.001$, Wilcoxon test). Overall fibrinolytic activity increased 15 fold, and t-PA:Ag levels eightfold. Increased PAI activity and PAI-1 antigen levels with respect to controls were found in patients with cardiovascular or thromboembolic disease, malignancies, hepatic insufficiency, after major trauma and in the postoperative period. This wide spectrum of pathologies with increased PAI-1 levels supports previous suggestions that PAI-1 behaves as an acute phase reactant. In patients undergoing extracorporeal circulation PAI activity and PAI-1 antigen were measured before and 1 h, 1 d and 7 d after the operation. Highest values of activity and antigen were observed 1 h postoperatively. These values remained elevated the day after the operation, but returned to preoperative levels within 7 days.
PAI-2 antigen concentrations were at or below the detection limit in all controls and the majority of patients. Very high levels of PAI-2 (above 30 ng/ml) were only observed in pregnant women and in patients with hepatocellular carcinoma.
The specific measurement of PAIs thus show increased levels in a variety of clinical conditions. The availability of specific RIAs permits to conduct prospective studies and to evaluate to what degree elevated PAI-1 and PAI-2 concentrations are correlated with the stage of the disease, its prognosis and the risk to develop thromboembolic complications.