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DEMONSTRATION OF C$\bar{1}$-INHIBITOR COMPLEXES IN PLASMA BY HIGHLY SENSITIVE RADIOIMMUNOASSAYS USING A MONOCLONAL ANTIBODY AGAINST A NEODETERMINANT ON COMPLEXED C$\bar{1}$-INHIBITOR.

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Levels of factor XIIa- and kallikrein-C$\bar{1}$ inhibitor (C$\bar{1}$-Inh) complexes in plasma reflect activation of the contact system in vivo. Here, we report the development of radioimmunoassays (RIAs) for these complexes using a monoclonal antibody (mAb KOK12) that reacts with a neodeterminant exposed on C$\bar{1}$-Inh after interaction with proteases. mAb KOK12 was obtained by a fusion experiment with spleen cells of a mouse hyperimmunized with C$\bar{1}$-Inh complexes.
Experiments with purified C$\bar{1}$-Inh incubated with either C$\bar{1}$s or elastase revealed that the determinant for mAb KOK12 is exposed on complexed as well as proteolytically inactivated (modified) C$\bar{1}$-Inh.
Radioimmunoassays (RIAs) for the detection of factor XIIa-C$\bar{1}$-Inh and kallikrein-C$\bar{1}$-Inh complexes were performed as follows: mAb KOK12 was coupled to Sepharose and incubated with the sample to be tested. Binding of C$\bar{1}$-Inh complexes was detected by a subsequent incubation with ^{125}I-antibodies against factor XII or (pre)kallikrein.
With these RIAs, activation of 0.1% of factor XII or prekallikrein in plasma is easily detected.
Optimal conditions for blood sampling and processing were established, i.e. conditions that prevented any in vitro activation of factor XII and prekallikrein. Levels of factor XIIa-C$\bar{1}$-Inh and kallikrein-C$\bar{1}$-Inh complexes in plasma samples from normal donors were less than 0.1 U/ml (100 U/ml is the maximal amount of C$\bar{1}$-Inh complexes generated in pooled plasma by DXS). Considerably higher, and fluctuating levels were observed in patients with diseases such as septicaemia. These highly sensitive RIAs will facilitate studies concerning the role of the contact system in human pathophysiology.

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C1-INHIBITOR: STRUCTURE-ACTIVITY RELATIONSHIPS. A. de Agostini (1), F. Barja (1), S. Carrel (2), P. C. Harpel (3) and M. Schapira (1,4). Division de Rhumatologie, HCU, Geneva, Switzerland (1), Ludwig Institute, Epalinges, Switzerland (2), Hematology-Oncology Division, New York Hospital-Cornell Medical Center, New York, NY, USA (3) and Department of Pathology, Vanderbilt University, Nashville, TN, USA (4).

C1-inhibitor [C1-In] and other protease inhibitors of the serpin superfamily inactivate serine proteases by forming bimolecular enzyme-inhibitor complexes, a reaction that is associated with changes in the inhibitor conformation. To determine the significance of these changes, we have examined the influence of various treatments on the binding to C1-In of monoclonal antibody 4C3. This antibody was previously shown to bind to an epitope created during the reaction of C1-In with the Arg-specific protease plasma kallikrein [K]: the site for 4C3 was expressed on the K-C1-In complex, on C1-In cleaved at position P_1 and released from K-C1-In [C1-In*], but not on unreacted C1-In. The binding of 4C3 to the various forms of C1-In was now measured by radioimmunoassay and Western blot. Following inactivation by C1-In of the Arg-specific enzymes factor XII active fragment [XIIf] or C1s, the binding site for 4C3 was detectable on XIIf-C1-In, C1s-C1-In and C1-In*. However, when K or XIIf were incubated with heat-inactivated C1-In, both enzymes remained active, no complex was formed, and the site for 4C3 was not created. When C1-In was cleaved by neutrophil elastase [E] (a Met- or Val- specific protease that is not inhibited by C1-In), the 1st cleavage product C1-In' retained inhibitory activity (as shown by its ability to form a complex with XIIf) but did not bind 4C3. However, subsequent cleavage of C1-In' by E at position P_3 yielded C1-In'', a product which was inactive but bound 4C3. Thus, identical conformational changes of C1-In (as assessed by the emergence of the site for 4C3) are seen when C1-In inactivates its target enzymes while being cleaved at P_1 or when the inhibitor is catalytically inactivated by cleavage at P_3. Therefore, these changes are necessary but not sufficient for observing enzyme inactivation.

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FIBRIN-BINDING STUDIES OF PRO-UROKINASE (PRO-UK) USING SOLID PHASE FIBRIN PLATES. E. Anglés-Cano, R. Pannell*, and V. Gurewich*. INSERM U.143, Hôpital de Bicêtre, Paris, France and *Vascular Laboratory, Department of Biomedical Research, Tufts University School of Medicine, Boston, MA, USA.

Pro-UK is a single chain urokinase-type plasminogen activator (scu-PA) which has fibrin selective thrombolytic properties. However, quantitative data on pro-UK binding to fibrin and on the mechanism of its fibrin enhanced activation of plasminogen have been difficult to obtain. In the present study, a well defined fibrin network constructed on glutaraldehyde-activated PVC plates (Anal. Biochem. 153 : 201-210, 1986) and highly purified pro-UK (99 % scu-PA) were used. Binding was investigated as follows : varying dilutions of pro-UK in the presence of a trace amount of ^{125}I-labeled pro-UK in buffer without or with glu-plasminogen, plasmin or α-thrombin and in urine, plasma or serum, were incubated overnight at 4°C and then 2 h at 37°C in the fibrin plates. After washing, the wells were cut out and counted in a gamma-counter. The labeled pro-UK and the effect of enzymes on scu-PA were investigated by SDS-PAGE and autoradiography. In parallel experiments, the activity of the fibrin bound and unbound products was investigated spectrophotometrically by adding glu-plasminogen and a synthetic substrate selective for plasmin. The binding of pro-UK to fibrin was 1.7 $\pm$ 0.1 % in buffer and 0.2 $\pm$ 0.08 % in plasma, as determined from isotopic and spectrophotometric measurements. This binding is similar to that of (0.13 $\pm$ 0.05%) two-chain urokinase (plasmin-transformed scu-PA), but is extremely low compared to the specific binding of tPA (68 $\pm$ 4%). By contrast, in urine, 11.2 $\pm$4.47 % binding of pro-UK to fibrin was observed. Thrombin did not modify the binding but transformed scu-PA into a two-chain molecule which had lost activity. These data indicate that pro-UK has little affinity for fibrin under these conditions but that some binding may be induced by a co-factor which is present in urine. Confirmation that thrombin degrades scu-PA was obtained and it is suggested that this effect may help to regulate fibrinolysis.

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FIBRIN ACTIVATABLE UROKINASE (FA-UK): A LATENT FORM OF UK IN URINE RELATED TO A COMPLEX WITH AN INHIBITOR/FIBRIN-BINDING CO-FACTOR OF UK AND POSSIBLY OF PRO-UK. C. Dwivedi, R. Pannell and V. Gurewich. Vascular Laboratory, Department of Biomedical Research, St. Elizabeth's Hospital, Boston, MA 02135, USA.

The plasminogen activator activity in urine, expressed in IU, was found to be consistently 2-3 fold higher by fibrin plate assay than against amidolytic substrate (S-2444). Moreover, when the S-2444 assay was preceded by incubation of urine with soluble fibrin, a similar 2-3 fold increase in activity was found. The fibrin effect was dose-dependent and specific for various forms of soluble fibrin but not fibrinogen. The fibrin activatable activity was inhibited by antibodies to UK and was termed FA-UK. About 1/3 of the total UK activity in freshly voided urine was composed of FA-UK. The relative FA-UK content of urine was found to be enhanced by concentration. The FA-UK bound to fibrin/Celite. By gel filtration (S-200) of urine or by zymography, the MW of FA-UK was ~100K. Pretreatment of the samples with soluble fibrin prior to SDS-PAGE enhanced the amount but not the position of the FA-UK activity on the zymogram, indicating that the complex was not dissociated by fibrin. Pretreatment with hydroxylamine (1M) eradicated the FA-UK activity in urine. Addition of UK or pro-UK to urine followed by concentration (X10), increased the 100K band on the zymogram. Under conditions of this experiment, it was shown that little conversion of pro-UK to UK occurred suggesting that complexation occurs with pro-UK as well as with UK. Moreover, a ~100K FA-UK band on zymography was demonstrated afer addition of pro-UK to urine treated with DFP (5mM) or GGAck (20 μM) to inactivate UK.

It was concluded that a ~50K inhibitor in urine, with properties similar to an inhibitor described by Stump et al (JBC 261:12759, '86), acts as a co-factor for fibrin binding of UK and possibly also of pro-UK. It is speculated that this co-factor may contribute to the fibrin-specificity of pro-UK by localizing both it and its activated derivative, UK, to the fibrin surface.

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