

**0015****08:45 h**

RAPID ISOLATION OF NATIVE UROKINASE FROM NORMAL HUMAN URINE  
K. Huber, J. Kirchheimer and B. R. Binder, Dept. of Medical Physiology, University of Vienna, Vienna, Austria.

Urokinase (UK) has been isolated to complete homogeneity starting mainly from commercially available prepurified preparations. When native human urine was the starting material, high amounts of urine were necessary because of low yields due to time consuming and complicated procedures. Furthermore, without addition of inhibitors a shift to lower molecular weight ( $M_r$ ) forms could not be avoided. In order to develop a rapid isolation procedure yielding final UK preparations of complete homogeneity and as unaltered as possible, we avoided concentration procedures at the beginning of the preparation as well as antibody columns because of high activity losses and binding of non active UK antigen, respectively, and employed two affinity chromatography steps.

Two liters of native human urine were dialysed and adsorbed to gelatine-Sepharose; UK was completely bound and could be eluted with 0.1M Tris-HCl buffer, 0.7M  $\text{CaCl}_2$ , pH 7.4. Active material was pooled, dialysed, and adsorbed to agmatine-Sepharose. Again, activities applied were totally bound and could be eluted with 0.1M phosphate buffer, 0.4M KCl, pH=7.4. Active material was freeze dried and gel filtrated on Sephadex G-150 in 0.1M Tris-HCl buffer, 1M NaCl, pH=8.0. During the whole isolation procedure Tween-80 (0.1%) was used in the buffer systems. Gelatine (0.02%) was present during dialysis. The procedure resulted in a UK preparation with a  $M_r$ =56,000 (SDS-PAGE) of complete homogeneity, and with a yield of 10-20% calculated from the starting material. Specific activity was about 16 pmoles of active enzyme per  $\mu\text{g}$  of protein ( $^3\text{H}$ -DFF incorporation). The final product cleaved Glu-plasminogen to plasmin with an apparent  $K_M$ =1 $\mu\text{M}$  and a  $k_{\text{cat}}$ =0.5  $\text{s}^{-1}$ .

The method described is a simple and efficient procedure resulting in a product with a specific activity comparable to those described for completely purified preparations and in a  $M_r$  form identical to that predominantly present in native urine.

**0017****09:15 h**

ON THE CONVERSION OF HIGH MOLECULAR WEIGHT UROKINASE TO THE LOW MOLECULAR WEIGHT FORM. Grant H. Barlow, Charles W. Francis and Victor J. Marder. Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA.

The conversion of high molecular weight urokinase (HMW) to low molecular weight urokinase (LMW) by plasmin *in vitro* has been studied. The two molecular weight forms of urokinase were separated by SDS polyacrylamide gradient gel electrophoresis and active enzyme extracted from gel segments into isotonic saline after slicing the gel at 5 mm intervals. Extracts from gel segments were analyzed by the fibrin plate method, and electrophoretic separation of the two forms were shown to be complete by comparison with the migration of purified standards and by the absence of lytic zones between the peaks of activity. HMW was incubated with plasminogen and fibrinogen for various time intervals from 2.5 to 10 minutes, enzymatic activity inhibited with aprotinin, and the samples subjected to electrophoresis. Conversion from HMW to LMW was apparent in as little as 2.5 minutes and continued for the 10 minute duration of the experiments. Similar experiments starting with LMW showed no change in molecular weight. Incubation of HMW without plasminogen resulted in no conversion to LMW implying that this reaction was not autocatalytic. The same conversion may occur *in vivo* during therapeutic administration of urokinase when a "lytic state" is produced and plasmin activity is present. Possible conversion of HMW to LMW *in vivo* will need to be considered in evaluating the relative therapeutic efficacy of different urokinase preparations and in interpreting the results of clinical trials.

**0016****09:00 h**

PURIFICATION OF A NEW HIGH MW SINGLE CHAIN FORM OF UROKINASE (UK) FROM URINE. S.S. Husain, V. Gurewich, and B. Lipinski. Vascular Laboratory, Department of Research, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, MA. 02135.

Purified UK exists in 2 forms, a high MW (55,000 daltons) and a low MW (33,000 daltons) enzyme. The former is composed of 2 chains held together by disulfide bonds and is believed to be a precursor of the latter. Little affinity for fibrin has been ascribed to either form. We have purified a third form of UK using affinity chromatography on fibrin-celite, a method which we developed to purify the major plasminogen activator from blood. When freshly voided urine was exposed to fibrin-celite, approximately 20% of the UK present was tightly bound to the fibrin. This high affinity UK (HAUK) was eluted in a sharp peak with arginine (0.2 M). Purification was achieved by gel filtration (Sephadex G-200) of the activator peak. SDS gel electrophoresis showed a single band (56,000 daltons) which remained intact after exposure to reducing agents, indicating that HAUK has a single chain structure and may be the native form of UK. The specific activity of HAUK is relatively low (40,000-50,000 CTA u/mg) suggesting that it may be a proactivator. Freshly voided urine and a rapid isolation procedure are necessary if degradation of HAUK is to be avoided. The unique high fibrin affinity of HAUK, which is not shared by the other 2 forms of UK, may make it a more specific and efficient fibrinolytic agent by confining and concentrating the enzymatic activity to the fibrin surface. The attachment of a suitable radiolabel to HAUK may also provide a useful tool for the detection of intravascular fibrin thrombi.

**0018****09:45 h**

ISOLATION AND CHARACTERIZATION OF A PLASMINOGEN ACTIVATOR (PA) FROM HUMAN MYOCARDIAL TISSUE. B.R. Binder, G. Reissert and R. Beckmann, Dept. Med. Physiol. Univ. Vienna, Austria.

Pig heart is one of the major sources for isolation of PAs. However, no attempt has been made to isolate PA from human heart. It was the aim of the study to isolate a PA from human myocardial tissue and to compare it with the vascular plasminogen activator (VPA) derived from cadaver vessel eluates.

Human heart was obtained from cadavers and myocardial tissue was prepared, homogenized, and after extraction with a neutral buffer, a pH=4.2 extract was obtained containing about 60-70% of PA activity originally present in the homogenate. The latter extract was made 2 M with  $(\text{NH}_4)_2\text{SO}_4$  at pH=7.0 precipitating all of the activator activity. PA activity could be purified to apparent homogeneity by reverse ammonium sulfate gradient solubilization followed by hydrophobic interaction chromatography on octyl-Sepharose, affinity chromatography on arginin-Sepharose and gel filtration on Sephadex G-150. The material obtained showed a single band in SDS polyacrylamide gel electrophoresis corresponding to  $M_r$ =70,000 and the region of the gel where PA activities could be eluted.

Kinetic analysis of the purified activator with synthetic paranitroanilide substrates revealed a  $K_M$  of 0.8 mM, 4 mM, and 0.4 mM for H-D-Ile-Pro-Arg-paranitroanilide, H-D-Val-Gly-Arg-paranitroanilide, and H-D-Phe-Aze-Arg-paranitroanilide, respectively, which were almost identical to those obtained with the VPA using the same substrates. Plasminogen activation with the myocardial PA showed in a purified system a strong dependence on the presence of fibrin as it could be observed with VPA.

From the similar isolation characteristics, the molecular weight, the amidolytic activities, and the fibrin effect on plasmin formation it can be concluded that the PA isolated from human myocardial tissue is similar or identical to the VPA.