MIXED POSTERS IX
Thrombosis

CHARACTERIZATION OF PURIFIED URINARY TRYPsin INHIBITORS. A. Takada, Y. Takada, K. Minakata, and H. Sumi. Department of Physiology, Hamamatsu University, School of Medicine, Hamamatsu, Japan.

Human urine was passed through Celite 545 column, and the non adsorbed eluate was adsorbed to bentonite, then eluted with 2% pyridine. The eluate was precipitated with ammonium sulfate, and the precipitate was dissolved, then passed through DEAE-Sephadex A-50, trypsin-Sepharose and Sephadex G-200. There were three types of urinary trypsin inhibitors (UTI) of Mr. of 65,000, 45,000, and 25,000 respectively. No difference in inhibitory spectra was observed among these three UTI's. Purified UTI inhibited trypsin, chymotrypsin, and kallikrein. The inhibition of chymotrypsin did not exceed 50%, even at the high concentration of UTI. UTI inhibited plasmin to a lesser extent but no inhibition of thrombin, C13, C14 or urokinase was observed. UTI had 7% carbohydrate and no H1S, PRG, TRP was found in aminoacid analysis. UTI was not identical to Α1,A but related to inter-κ-trypsin inhibitor immunologically. Some persons excreted both UTI and Α1,A in the urine although most people did only UTI. Since Α1,A is not found in the urine of most people, UTI may not be the same as inter-κ-trypsin inhibitor which is filtered into the urine.


Among several thrombin inhibitors in normal plasma, antithrombin III (AT-III) and Α1-macroglobulin (α1-M) are especially important. However, there has been no appropriate method to determine AT-III and α1-M separately. This study was carried out to differentiate the actions of these two inhibitors of thrombin in plasma. By using single radial immunodiffusion method, we observed that AT-III was adsorbed to bentonite, although α1-M was not. It was necessary to incubate 1 ml of oxalated plasma with 300 mg of bentonite at 37°C for 15 minutes to remove AT-III completely. Supernatant plasma contains neither AT-III nor fibrinogen which affects antithrombin activity of plasma. From these results, it is evident that antithrombin activity of plasma adsorbed with bentonite was attributed to that of α1-M. Antithrombin activities of plasma adsorbed with bentonite in 20 healthy subjects were significantly correlated with concentrations of α1-M in plasma, measured immunologically (r=0.87, p<0.001). It is reasonable to presume that difference between antithrombin activity of derethanized plasma by heating at 56°C for 5 minutes and that of bentonite treated plasma mainly indicates activity of AT-III. These differences measured in 20 healthy individuals were significantly correlated with plasma AT-III levels, assayed immunologically (r=0.59, p<0.01).