Plasma Clearance of Human Antiproteinase/Proteinase Complexes

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Evidence has been obtained, though frequently by an indirect manner, showing that \( \alpha_2 \)-M/proteinase complexes are quickly removed from circulation. Thus NIELSEN and GANROT [1] succeeded in achieving activation of the entire plasma plasminogen pool by massive streptokinase therapy. Plasmin was mainly recovered as an \( \alpha_2 \)-M/plasmin complex which disappeared from circulation within 24 h. During this period \( \alpha_2 \)-M dropped to half its initial concentration, no complex formation of plasmin with \( \alpha_1 \)-A was demonstrated. Fibrinogen degradation products were produced by the proteolytic activity of plasmin before the enzyme became fixed to \( \alpha_2 \)-M. These fragments may have influenced the clearance rate of \( \alpha_2 \)-M/plasmin complexes by a competitive overload of the reticuloendothelial system. Indeed, OHLSSON [2] observed a half-life of only 8 min when bovine trypsin complexed with canine \( \alpha_1 \)-M and \( \alpha_2 \)-M was injected to dogs. However, in this experiment a heterologous enzyme was used and the clearance speed might have been influenced by the foreign proteins which are always quickly removed from circulation. Thus it seemed necessary to check the half-life time in plasma of such complexes obtained by the interaction of human \( \alpha_2 \)-M with human enzymes. Indeed some information thus might be obtained in order to know whether preparations of injectable \( \alpha_2 \)-M which are now available might constitute a useful tool during an acute proteolytic unbalance such as massive fibrinolysis. No observations were made concerning the removal from circulation of \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-A) and inter-\( \alpha \)-trypsin inhibitor (ITI, protein \( \pi \)) proteinase complexes but the presence of “inactive” \( \alpha_1 \)-A in biological fluids has been reported. Now in contrast to \( \alpha_2 \)-M these inhibitors interact with the active sites of the proteolytic enzymes forming complexes devoid of any enzymatic (esterasic) activity. Thus some preliminary experiments concerning the plasmatic clearance of \( \alpha_1 \)-A/trypsin and ITI/trypsin were included in this study.

Materials and Methods

\( \alpha_2 \)-M was purified by precipitation with rivanol and exclusion chromatography on DEAE-cellulose as described earlier [3].

\( \alpha_1 \)-A was obtained by chromatography on DEAE-cellulose and preparative electrophoresis. ITI was prepared by chromatography on DEAE-cellulose, associated with precipitation steps.

All proteins were checked by analytical ultra-centrifugation, electrophoresis in acrylamide/agarose gels and immunoelectrophoresis. Plasminogen was obtained
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This highly purified material was converted to active plasmin by insoluble streptokinase [5]. An activated preparation of PPSB \(^1\) [6] was used as source of human thrombin. Human trypsin was a gift from Dr. TRAVIS. The complexes were obtained by saturating the antiproteinases with the proteolytic enzymes: plasmin and thrombin for \(\alpha_2\)M; trypsin for \(\alpha_1\)A and ITI until a slight proteolytic activity appeared indicating saturation of the inhibitors. This excess activity was blocked by addition of Kunitz' inhibitor (Iniprol Choay) to avoid any secondary degradation of the complexes.

Labelling of the antiproteinases by \(^{131}\)I and \(^{125}\)I (both these labels being used in the experiments with \(\alpha_1\)A and ITI) was achieved in the presence of chloramine T, about 10 \(\mu\)Ci being used for 15 mg of proteins [7].

The labelled material was injected into the right cubital vein whereas blood samples were taken from the left cubital vein after 5, 10, 15, 30, and 60 min, the intervals then being 2, 6 and 24 h; after that time samples were taken during 11 days at 24 h intervals for \(\alpha_2\)M, and 5 days for \(\alpha_1\)A and ITI. The radioactivity of the starting material was about 20–30 \(\mu\)Ci/ml and the injected volumes varied from 2–6 ml. All individuals received daily 20 drops of Lugol's solution 3 days preceding the injection of the labelled material and during the whole observation period.

Results

There was a striking difference between the results obtained for \(\alpha_2\)M and those concerning \(\alpha_1\)A and ITI: 75% of control \(\alpha_2\)M remained in the vascular compartment 1 h after injection, the half-life of this material being 135 h. The slopes obtained for total radioactivity were in close agreement with those corresponding to immunoprecipitable material. In opposition to these results \(\alpha_2\)M/proteinase complexes disappeared much more rapidly from the intravascular space [8]. After 1 h only 33% of the initial radioactivity was present in the circulation and the half-life of the remaining radioactivity dropped to 30 h. Furthermore when the radioactivity of the immunoprecipitable \(\alpha_2\)M alone was measured only 13% remained in the circulation after 1 h thus showing the rapid elimination of \(\alpha_2\)M/proteinase complexes. Less than 2% immunoprecipitable radioactive material remained in the circulation after 24 h. If total radioactivity is taken into account, 60% is found for control \(\alpha_2\)M after 24 h as compared with 16% for \(\alpha_2\)M/thrombin and 13% for \(\alpha_2\)M/plasmin. The slight dissociation between total radioactivity and that found after immunoprecipitation of the labelled complex indicates some fragmentation occurring only with the complexes.

The control \(\alpha_1\)A and ITI were labelled with \(^{125}\)I and injected to the same persons as the \(^{131}\)I labelled complexes. All these samples behaved in a similar way. No dissociation between “free” and complexed antiproteinase was seen either for \(\alpha_1\)A or for ITI. Furthermore, immunoprecipitation could only partially be achieved, 55% of ITI disappeared from the intravascular space after 3 h the same values being reached with \(\alpha_1\)A after 6 h. The remaining material had a plasma half-life of about 40 h for ITI and 47 h for \(\alpha_1\)A.

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\(^1\) Abbreviations: PPSB: Mixture of Prothrombin (factor II), Proconvertine (factor VII), Stuart factor (factor X), and Antihemophilic factor B (factor IX). \(\alpha_2\)M: \(\alpha_2\)-macroglobulin.