FIBRINOLYTIC EFFECT OF ONE-CHAIN TISSUE-TYPE PLASMINOGEN ACTIVATOR. H. Johannessen, P.E. Nielsen, K. Piipponen and L.C. Petersen, Novo Research Institute, Bagsvaerd, Denmark.

The fibrinolytic properties of authentic one- and two-chain recombinant t-PA were compared to those of a plasmin resistant one-chain tPA analogue, t-PA-GLy735, which is point mutated in Arg959 of the activation site. The proteins were characterised by reversed phase HPLC, reduced SDS-PAGE, and their concentrations determined by the BCA (modified Lowry) method. When equivalent conc. of these enzymes were tested for fibrinolytic activity by means of clot lysis and fibrin plate lysis methods, the results found for two-chain tPA were consistently 50% higher than one-chain tPA forms. The time course for plasmin catabolised one-chain tPA cleavage during fibrin clot lysis was determined by means of 125I-tPA. The cleavage was not instantaneous, and one-chain tPA may account for a considerable fraction of the total amount of plasmin formed. This is confirmed by similar experiments with 125I-

**PLASMINOGEN ACTIVATORS**

**1607**

**1608**


Protein determination by composition conscious amino acid analysis (CCAAA) is generally applicable to polypeptides with known amino acid composition. Traditionally, in protein determination by AAA the mass of protein substance in a preparation is quantitated by simple summation of the mass of all amino acid residues found, although some amino acids e.g. Trp, Ser, Thr, Cys are unsatisfactorily determined. In this study we have explored the possibility of improving protein determination by AAA in cases where the amino acid composition of the preparation is precisely known from sequence data. In CCAAA protein determination the molar amounts of amino acids residues known to be present in the polypeptide. The mean of these values is multiplied with the theoretical molecular weight as calculated from the sequence and their concentrations determined. Amino acids selected for calculation and amino acid analysed. Amino acids selected for calculation and amino acid analysis are those with a high biological function and have been recommended as reference method for protein determination of well defined protein preparations. The result from CCAAA was within 10% of that found gravimetrically. The absorbity of t-PA was estimated to 1.75 (g/ml)^-1.

In summary, composition conscious amino acid analysis (CCAAA) is recommended as reference method for protein determination of well defined protein preparations where the amino acid composition is known.

Up to now the three-dimensional structure of t-PA or parts of this enzyme is unknown. Computer modeling methods can predict the spatial structure of the enzymatic part of t-PA is predicted on the hypothesis, the three-dimensional backbone structure of the being similar to the backbone structure of other serine proteinases. The t-PA model was built up in three steps:

1) Alignment of the t-PA sequence with other serine proteinases. Comparison of enzyme structures available from Brookhaven Protein Data Bank proved elastase as a basis for modeling.

2) Exchange of amino acids of elastase differing from the t-PA sequence. The replacement of amino acids was performed such that backbone atoms overlapped completely and side chains superpose as far as possible.

3) Modeling of insertions and deletions. To determine the spatial arrangement of insertions and deletions parts of related enzymes such as chymotrypsin or trypsin were used whenever possible. Otherwise additional amino acid sequences were folded to a B-turn at the surface of the proteins, where all insertions or deletions are located. Finally the side chain torsion angles of amino acids were optimised to prevent close contacts of neighbouring atoms and to improve hydrogen bonds and salt bridges.

The resulting model was used to explain binding of arginine 560 of plasminogen to the active site of t-PA. Arginine 560 interacts with Asp 189, Gly 193, Ser 195 and Ser 214 of t-PA (chymotrypsin numbering). Furthermore interaction of chromogenic substrate S 2238 with the active site of t-PA was studied. The need for D-configuration of the hydrophobic amino acid at the N-terminus of this tripeptide derivative could be easily explained.