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

The fibrinolytic properties of authentic one- and two-chain recombinant tPA were compared to those of a plasmin resistant one-chain tPA analogue, tPA-Gly_{275}, which is point mutated in Arg_{275} 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 values found for two-chain tPA were consistently 50% higher than one-chain tPA forms. The time course for plasmin catalysed one-chain tPA cleavage during fibrin clot lysis was determined by means of ^{125}I-tPA. The cleavage is 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 ^{125}I-tPA-Gly_{275}, which is essentially intact one-chain tPA at the time of fibrin clot lysis. The effect of tPA activation site cleavage was also studied using plasmin coupled to sepharose beads. Plasmin sepharose was removed at different time intervals, and tPA was tested for amidolytic activity with > Glu-Gly-Arg-pNA and fibrinolytic activity as measured by fibrin clot lysis time as well as by fibrin plate methods. The results indicate that in the presence of fibrin, plasminogen can be activated by one-chain tPA at a considerable rate. On the other hand, the fact that the fibrinolytic activity measured by conventional assays is lower with one-chain tPA than with two-chain tPA should be considered when of these methods are used for standardization.

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PREDICTION OF THE THREE-DIMENSIONAL STRUCTURE OF THE ENZYMATIC PART OF T-PA. A. Heckel and K.M. Hasselbach. Dr. Karl Thomae GmbH, Birkendorfer Str. 65, D-7950 Biberach, West Germany

Up to now the three-dimensional structure of t-PA or parts of this enzyme is unknown. Using computer graphical methods the spatial structure of the enzymatic part of t-PA is predicted on the hypothesis, the three-dimensional backbone structure of t-PA being similar to that of other serine proteases. The t-PA model was built up in three steps:

1) Alignment of the t-PA sequence with other serine proteases. 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 overlapp 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 ß-turn at the surface of the proteine, 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 2288 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.

1609

t-PA PROTEIN DETERMINATION: IMPROVED ACCURACY THROUGH COMPOSITION CONSIOUS AMINO ACID ANALYSIS (CCAAA). A. Brändström and M. Rånby. Biopool AB, Box 4025, S-900 04 Umeå, Sweden

Protein determination by composition consious 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 that are known to be quantitatively recovered are divided by the number of residues known to be present in the polypeptide. The mean of these values is identified as the molar quantity of polypeptide in the sample. In addition, the standard deviation of the mean serves as identification and homogeneity control. In this study the accuracy of the method was checked gravimetrically.

CCAAA was applied on t-PA prepared from cultured Bowes melanoma cells by immunopurification on PAM1-Sepharose 6BFF and subsequent gelfiltration in 1 mol/L NH_4HCO_3 on Sephacryl SA-200. About 2 µg of lyophilized protein was hydrolysed in 6 mol/L HCl under vacuum for 24 hours at 110°C and amino acid analysed. Amino acids selected for calculation of the molar quantity were (number of residues within parentheses) Asp+Asn(50), Glu+Gln(52), Pro(29), Ala(32.5), Val(25), Leu(39), His(16), Lys(21) and Arg(35.5). To obtain the gravimetric quantity the molar quantity was multiplied with the theoretical molecular weight as calculated from the sequence and with the carbohydrate content taken into account.
For t-PA the standard deviation of the mean molar quantity typically was less than 5%. The result from CCAAA was about 10% higher than that obtained from conventional AAA. The result from CCAAA was within 10% of that found gravimetrically. The absorbtivity of t-PA was estimated to 1.75 $(g/L)^{-1}cm^{-1}$.
In summary, composition consious amino acid analysis (CCAAA) is recommended as reference method for protein determination of well defined protein preparations where the amino acid composition is known.

1610

COMPARISON OF SINGLE-CHAIN AND TWO-CHAIN RECOMBINANT TISSUE-PLASMINOGEN ACTIVATOR (t-PA) IN VITRO. TURNOVER OF SINGLE-CHAIN t-PA IN ANIMALS AND MAN. G. Nguyen, L. Bara, M. Samama, J. Conard, P. Van Dreden, P. Desnoyers, A.V. Bendetowicz. Laboratoire Central d'Hématologie, Hôtel-Dieu, Paris, France.

Two preparations of recombinant t-PA obtained from mammalian cells (Boehringer Ingelheim) containing mainly single-chain forms t-PA (sct-PA > 95%) or two-chain forms t-PA (tct-PA > 70%) were compared in vitro for their thrombolytic and fibrinogenolytic properties.

Thrombolytic activities studied on human hanging clots were similar for both preparations, at plasma concentrations ranging from 62.5 to 1,000 IU/ml. Fibrinogenolysis appeared for both preparations at concentrations higher than 500 IU/ml, after a 90 min incubation at 37°C. For higher concentrations, fibrinogenolysis was dose-dependent, and there was a trend with sct-PA to provoke less fibrinogenolysis.

The turnover of sct-PA was studied in rabbits and cats. After an intravenous bolus injection of 0.2 mg/kg, t-PA activity and antigen showed a double decay disappearance curve. Initial and secondary phases had similar t.1/2 for the activity and the antigen, as well as in both species, which were respectively estimated about 2 min and 20 min.

Although the biological and immunological clearance rates were not significantly different, sct-PA antigen values were much higher than the activity.

Sct-PA clearance rate was also measured in patients with acute myocardial infarction, treated with intravenous infusion of sct-PA, 100 mg over 3 hours, of which 60 mg during the first hour (n=4). t-PA antigen was regularly measured within 20 min after the end of the first hour infusion.

A biphasic disappearance curve was observed, the first phase was extremely rapid with a t.1/2 of 3.2 ± 1.2 min, and the secondary phase had a t.1/2 of 10.4 ± 2.6 min.