Differences between uterine and melanoma forms of tissue plasminogen activator

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Tissue plasminogen activator purified from human uterine tissue exhibits differences in N-terminal starting positions in relation to the melanoma cell plasminogen activator usually studied. A new starting position is compatible with an additional N-terminal processing apart from those already known. Like the melanoma activator, the uterine activator was found to yield protein chains starting at either of two positions. One of these was identical between uterine and melanoma activators, whereas the other was unique in each case. The most abundant starting position for the uterine preparation was at a valine residue, apparently from cleavage of a Gln–Val bond, and corresponding to Val-7 of the longest form of the melanoma activator chain.

Amino acid sequence analysis *N-terminal proteolysis* *Protein processing*

1. INTRODUCTION

The structure of tissue plasminogen activator (t-PA) has recently been extensively studied. Bacterial clones containing activator cDNA sequences have been isolated [1,2] and an amino acid sequence of 527 residues was deduced, with serine as the suggested N-terminal amino acid [1]. Structural studies of the purified protein from the melanoma cell line confirmed the structure deduced [3] but also showed the presence of 4 different heterogeneities [4–6].

If purified under the protection of a protease inhibitor (aprotinin), the product obtained was a single-chain polypeptide [7,8] which was found to be heterogeneous in the N-terminus. About 50% of the molecules were 3 residues longer (L-chains) than the rest (S-chains) [6]. This heterogeneity was apparently associated with a Ser/Gly microheterogeneity in position L-4 (equal to S-1). Treatment of the single-chain activator with plasmin causes cleavage of the polypeptide approximately in the middle to give two disulfide connected chains (A and B) from the N- and C-terminal parts, respectively [4]. In addition, plasmin also cleaves off the differential tripeptide of the original N-terminal part, thus removing the S/L difference. Finally, two variants of tissue plasminogen activator can be separated on arginine–Sepharose (variant I and II in order of elution), having different mobilities on SDS–polyacrylamide gel electrophoresis. Their size difference resides in the A-chain [5]. All these data from t-PA have been derived from products of a melanoma cell line (Bowes).

We have studied here the N-terminal sequences of single-chain and two-chain preparations of human uterine tissue plasminogen activator. The uterine activator was recovered in still another different form from those known before for t-PA, suggesting one further differential proteolysis in the N-terminal region.

Abbreviation: t-PA, tissue-type plasminogen activator

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2. MATERIALS AND METHODS

Human uterine t-PA was isolated from uteri obtained at autopsy. Purification was performed essentially as in [8] with the only difference being the use of Tween 80 (0.1 g/l) instead of Triton X-100. The preparations usually gave a mixture of single-chain and two-chain molecules even when aprotinin was present during purification. One preparation, however, was essentially single-chain t-PA and was selected for N-terminal sequence analysis together with a preparation containing a mixture of both forms. The latter preparation is denoted two-chain form here.

For reduction and *S*-carboxymethylation, the purified enzyme (about 200 μg of single-chain activator and 1300 μg of the two chain form) was dissolved in 400 μl of 1.4 M Tris–HCl (pH 8.3) and 200 μl of 0.8% SDS. Dithiothreitol (10 μl, 500 mM) was added and, after incubation under nitrogen at 37°C for 2.5 h, also 150 μl of 100 mM iodo[2-^{14}C]acetate (24 μCi). Alkylation was allowed to proceed for 1.5 h at 37°C and was stopped by the addition of 20 μl 2-mercaptoethanol. The product was desalted on a Sephadex G-25 column (0.5 × 66 cm) equilibrated in 0.1% SDS. The material corresponding to the radioactive protein peak was pooled (7 ml), concentrated under a stream of nitrogen to 600 μl and applied for degradation in a liquid phase Beckman 890D sequencer. Phenylthiohydantoins were identified and quantitated using reverse-phase high-performance liquid chromatography (Hewlett Packard 1084B) with an acetonitrile gradient system [9].

3. RESULTS

Results from sequence analyses of single- and two-chain activator preparations are shown in table 1. The single-chain material gave two amino acid sequences in different yield, with a main structure having valine as the N-terminal amino acid. The amino acid sequence showed that this residue corresponds to valine in position 7 of the 'long' (L-chain [6]) single-chain melanoma t-PA. The other structure, constituting about 30% of the molecules, had serine as the N-terminal residue, corresponding to position 4 in the t-PA L-chain structure (equivalent to position 1 of the S-chain [6]). Relationships between the uterine t-PA structure (U) now determined and the melanoma ac-

Table 1

Results of sequence analyses of single- and two-chain preparations of uterine tissue plasminogen activator

Cycle	Single-chain activator Residue identified	Two-chain activator Residue identified		
		A-chain	B-chain	
1	Val(1.7)/Ser	Val(15.6)/Ser	Ile(15.4)	
2	Ile(2.0)/Tyr(0.6)	Ile(16.1)/Tyr(3.2)	Lys(7.3)	Leu(7.7)
3	Cys(Cm)/Glx	Cys(Cm)/Glx	Gly	
4	–/Val(0.5)	Arg/Val(4.8)	Gly	Val(8.9)
5	Asp/Ile(0.7)	Asp/Ile(3.6)	Leu(17.0)	Ala(6.6)
6	Glu	Glu	Phe(13.4)	
7	Lys(0.7)	Lys(6.9)	Ala(16.7)	Leu(5.0)
8	Thr	Thr	Asp	
9	Glx	Gln	Ile(13.0)	
10	Met(0.8)	Met(3.8)	Ala(12.6)	

Values show nmol recovered of stable PTH-derivatives from applications of 3 nmol (single-chain preparation) and 20 nmol (two-chain preparation) of polypeptide. As shown, the single-chain preparation gave two sequences in different yield (relative yield 3:1), whereas 3 structures were identified in the two-chain preparation (relative yield 5:1:5). In some positions, residues corresponding to a fourth structure were also identified (last column). Valine in cycle 4 (with a sum of 13 7 nmol) is present in two structures and listed with separate recoveries in the same relative ratio as Ile/Ala in cycle 5

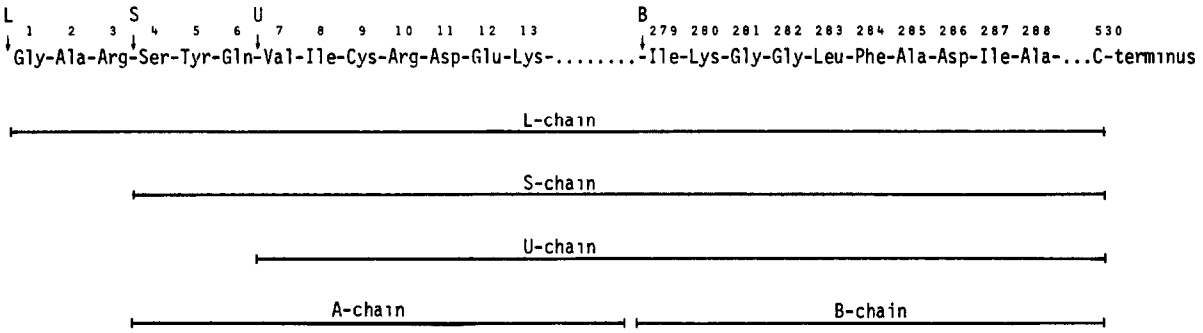

Fig.1. Summary of all characterized cleavage sites in tissue plasminogen activator. L and S are cleavage sites found in single-chain melanoma activator preparations [4,6] and U denotes the new cleavage site found in both single- and two-chain preparations of the uterine activator (here). B-cleavage converts the single-chain molecule into a two-chain form. Numbers are from [1] after adjustment according to the results of [6].

tivator structures (L and S) previously reported are shown in fig.1. The two-chain uterine activator revealed the same two sequences as the single-chain form (about 80% of the non-B-chains had Val as N-terminal residue) together with a third amino acid sequence, which was identified as the N-terminal region of the B-chain.

These data show the occurrence of a new and previously unknown cleavage in the N-terminal part of human t-PA. In addition, the cleavage in the middle of the molecule producing the A- and B-chains (B-cleavage) is shown to be identical for two-chain uterine activator and plasmin-treated melanoma activator. A summary of cleavage sites discovered in different t-PA preparations is shown in table 2 and further outlined in fig.1. It may be added that for the two-chain preparation low but significant amounts of residues not matching any of the sequences derived from cleavages at L, S, U or B sites (fig.1) were also recovered (table 1): cycle 2 (Leu), 5 (Ala), 7 (Leu) and possibly 4 (Val, which was recovered in higher yield than expected from the A-chain alone, cf. table 1). Since this structure does not correspond to other regions of t-PA either, it probably reflects a contaminating peptide present in the two-chain preparation.

Table 2

Summary of all reported cleavage sites and proteolytic processings of tissue plasminogen activator (t-PA)

	L	S	U	B
Single-chain melanoma t-PA	+	+	−	−
Two-chain melanoma t-PA	−	+	−	+
Single-chain uterine t-PA	−	+	+	−
Two-chain uterine t-PA	−	(+)	+	+

Parentheses indicate cleavage in low yield (i.e., less than 25% of major cleavage site(s)). Sites L, S, U and B are shown in fig.1

4. DISCUSSION

Analyses of t-PA preparations from human uterine tissue and melanoma cells have previously demonstrated a high degree of general similarity in amino acid composition, fibrin binding and immunoreactivity [7]. Our results now demonstrate a new cleavage in single-chain uterine t-PA, suggesting an additional processing, different from those known before. Noticeably, the cleavage occurs even in the absence of the B-cleavage that produces the two-chain form, because Val-7 is also the major N-terminus of single-chain preparations. The new cleavage is therefore probably not caused by plasmin but still appears to be surprisingly specific. The protease(s) causing this 'U'-type cleavage is apparently not present or active in preparations from the melanoma cell cultures since no such cleavage has been demonstrated for the melanoma activator.

Finally, in relation to different N-terminal processings, it may be noticed that a pattern with L-chains and 3 residues shorter S-chains similar to the t-PA pattern was found for the N-terminal part

of high-M_r urokinase [10], and also that residues 6 and 7 (Gln-Val) are identical for human t-PA and urokinase. It will be interesting to find out if these similarities are pure coincidence or if they reflect functional properties of plasminogen activators, and if the presently defined new cleavage may also occur in urokinase preparations. Regardless of functional possibilities, the N-terminal multiplicity of human t-PA is considerable (fig.1) and greater than previously anticipated.

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