THE N-TERMINAL SEQUENCE OF THE LIGHT CHAIN DERIVATIVE OF BOVINE PLASMIN

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SUMMARY: Activation of bovine plasminogen by urokinase occurred by limited proteolysis of peptide bonds resulting in the formation of active plasmin. Electrophoresis of reduced plasmin on sodium dodecylsulfate (SDS)-polyacrylamide gel revealed the two-chain structure of bovine plasmin. The heavy and light chains were separated by dialysis of reduced and carboxymethylated (RCM)-plasmin against deionized water. By the Edman procedure, the N-terminal sequence of the light chain was concluded to be Ile-Val-Gly-Gly-, which was homologous to that of bovine trypsin.

A homology in N-terminal sequences liberated during the activation of zymogens was observed between bovine trypsin, Ile-Val-Gly-Gly- (1); bovine chymotrypsins A and B, Ile-Val-Asn-Gly- (2,3); porcine elastase, Val-Val-Gly-Gly- (4) and bovine thrombin, Ile-Val-Gln-Gly- (5). This homology seemed to be related to the functional homology of these proteolytic enzymes (6). Plasmin, a major proteolytic enzyme in blood plasma, has a specificity very similar to that of trypsin, and the activation of plasminogen was shown to be caused by the limited proteolysis of a specific peptide bond (7). Robbins *et al.* (7) reported that valine, which was not found as the N-terminus of human plasminogen, was present as the N-terminus of activated plasmin, but the successive amino acid sequence has not yet been reported. Therefore, it was of interest to see whether this sequential homology is also found in the newly liberated N-terminal sequence of plasmin. Groskopf *et al.*

found that the two chains of human plasmin were easily separated by dialysis of RCM-human plasmin against 2 mM NH_4HCO_3 (8). In this paper, the two chains of bovine plasmin, the heavy and light chains, were separated by Groskopf's method with a slight modification, and it was found that the N-terminal sequence of the light chain of bovine plasmin was homologous to that of bovine trypsin.

MATERIALS AND METHODS

Bovine plasminogen was purified from the euglobulin fraction of bovine plasma as described previously (9). SDS-polyacrylamide gel electrophoresis was performed by the method of Shapiro et al. (10). Gels were stained with 0.2% Coomassie brilliant blue in methanol-acetic acid-water (5:1:5, v/v/v) and stain was removed by dialysis against 7.5% acetic acid-5% methanol solution (11). The N-terminal amino acid sequence was determined by the modification of the Edman procedure described by Blombäck et al. (12). After thin layer chromatography on silica gel plates, phenylthiohydantoin (PTH)-amino acids were identified under ultraviolet light with a fluorescent indicator (Silica gel F_{254}, Merck Co.).

RESULTS

Isolation of bovine plasmin and separation of its two polypeptide chains. Bovine plasminogen was activated by urokinase by incubation of purified plasminogen (100 mg) with 500 units of urokinase (Green Cross Co., Japan) in 3 ml of 0.04M Tris-0.02M lysine buffer, pH 8.5, for 15 min at 37°C. The incubation mixture was passed through a column (2 x 160 cm) of Sephadex G-100 equilibrated with 0.1M acetic acid, and two main protein peaks, active plasmin and a residual inactive protein fragment, were obtained. The two-chain structure of

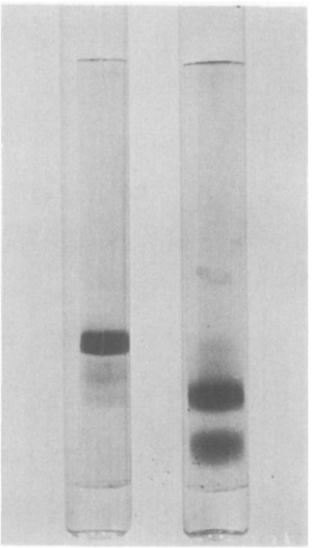

Fig. 1. SDS-Disc electrophoresis of plasmin. Plasmin (50 μg) dissolved in 0.1 ml of 8 M urea in 1% SDS (a), or in 0.1 ml of 8 M urea-0.1 M mercaptoethanol-1% SDS (b) was subjected to SDS-disc electrophoresis in 0.2 M phosphate buffer, pH 7.2, containing 0.1% SDS at 7 mA per tube for 3 hr at room temperature following the procedure of Shapiro et al.(9). In order of their increasing mobilities and decreasing molecular size, the materials in the two bands were named the light and heavy chains.

bovine plasmin was evident on analysis of reduced plasmin by SDS-disc electrophoresis, and materials in the two bands were named the heavy and light chains (Fig. 1). To prepare RCM-bovine plasmin, lyophilized bovine plasmin (15 mg) was completely reduced in 1 ml of 0.1M mercaptoethanol-6M guanidine hydrochloride-1M triethanolamine buffer, pH 9.0 at 37°C for 6 hr and submitted to carboxymethylation with 500 mg of monoiodoacetic acid in 0.5 ml of 3N NaOH. Dialysis of the RCM-plasmin against deionized water for 6 hr resulted in the formation of a precipitate which was shown to be the light chain derivative by SDS-electrophoresis (Fig. 2). The heavy chain was recovered

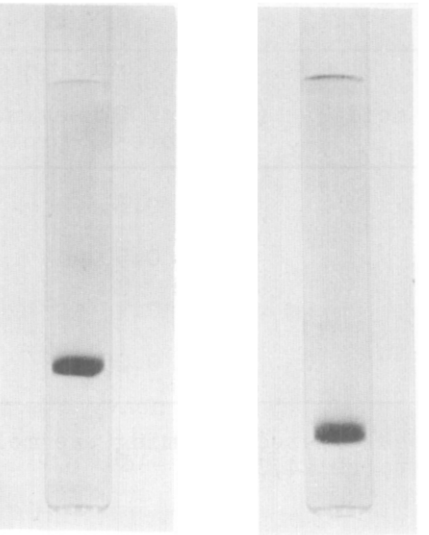

Fig. 2. SDS-Disc electrophoresis of the polypeptide chains separated. Aliquots containing 20 μg of each polypeptide chain obtained by dialysis of RCM-plasmin were dissolved in 0.1 ml of 8 M urea in 0.1% SDS and subjected to electrophoresis on SDS-polyacrylamide gel in 0.2 M phosphate buffer, pH 7.2 containing 0.1% SDS at 6 mA per tube at room temperature for 2.5 hr. For determination of the molecular weight, RCM-derivatives of standard proteins (bovine serum albumin, egg albumin, pepsin, chymotrypsinogen A and trypsin) were simultaneously subjected to electrophoresis. The molecular weight of the two polypeptide chains were calculated by the procedure of Shapiro et al.(9) from the relative electrophoretic mobilities.

in the supernatant. The molecular weight of the heavy and light chain derivatives were calculated to be approximately 35,000 and 24,000, respectively, from the results of SDS-electrophoresis.

N-Terminal sequence of the light chain derivative. The light chain derivative of bovine plasmin (4 mg) was subjected to Edman degradation. The N-terminal amino acid was identified as isoleucine by co-chromatography of the PTH-derivative with an authentic sample of PTH-Ile. Table I summarizes the amino

Table I. Edman degradation of the light chain

Step	PTH-amino acid	Recovery[1] (mole of PTH-amino acid per mole of protein)
1	Ile	0.65
2	Val	0.50
3	Gly	0.34
4	Gly	0.25

1) The values were calculated assuming the molecular weight of the light chain to be 24,000.

acids identified as PTH-derivatives during sequential Edman degradation. Thus, the N-terminal sequence of the light chain of bovine plasmin was found to be Ile-Val-Gly-Gly.

DISCUSSION

The present paper shows that the N-terminal sequence of the light chain of bovine plasmin is homologous to that of bovine trypsin. Bergström reported that the N-terminal sequence of bovine plasminogen was Asp-Ile(Leu)-Ile(Leu)-Asp (13), so it seems likely that the Ile-Val-Gly-Gly sequence becomes the N-terminus as the result of activation of bovine plasminogen by urokinase. N-Terminal isoleucine is essential for the proteolytic activity of bovine trypsin (14), bovine chymotrypsin (15) and bovine thrombin (6), so cleavage of the peptide bond involving an isoleucine, which becomes the N-terminus of the light chain, is probably an essential step for formation of the functionally active bovine plasmin.

As in the case of N-terminal sequences of four amino acids in five proteolytic enzymes, bovine trypsin, bovine chymotrypsins A and B, bovine thrombin and porcine elastase,

Val^2 and Gly^4 were also present in the N-terminal sequence of bovine plasmin.

The difference that isoleucine is present in the N-terminal amino acid sequence of the light chain of bovine plasmin and valine in that of human plasmin seems to be due to species-specificity. The N-terminal amino acid of plasminogen was also different, being lysine in human (7) and aspartic acid in bovine material (13).

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