Vol. 102, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1981 Pages 46-52

ISOLATION OF HUMAN VAL_{354}-PLASMINOGEN AS AN ELASTOLYTIC FRAGMENT OF HUMAN GLU_1-PLASMINOGEN

James R. Powell and Francis J. Castellino

Department of Chemistry, University of Notre Dame,
Notre Dame, Indiana 46556

Received July 16, 1981

Summary: Limited elastolysis of native human plasminogen affinity chromatography variants 1 and 2 results in the formation of a number of polypeptide fragments that can be purified by a combination of Sepharose-lysine affinity chromatography and gel filtration. Two newly identified fragments, both of which retain their lysine binding properties, are the kringles 1-4 region, from variants 1 and 2, and an activatible fragment with an NH_2-terminal sequence of VAL-GLN-ASP, termed VAL_{354}-plasminogen.

INTRODUCTION

Human GLU_1-Pg[1] is synthesized de novo as a single polypeptide chain of 790 amino acids (1-3). The primary sequence has been shown to have considerable homology to that of prothrombin and both molecules have been shown to contain triple disulfide loop regions of approximately 80 residues each; two in the case of prothrombin and five in human plasminogen, which have been termed "kringle" structures (4). Upon elastolytic digestion of plasminogen, it was originally shown that three discrete fragments could be purified, consisting of the K1-3 region, K4 and a fragment termed VAL_{442}-Pg (3), containing the kringle 5 region on its amino terminus.

[1] Abbreviations: GLU_1-Pg, LYS_{77}-Pg, VAL_{354}-Pg and VAL_{442}-Pg-human plasminogens possessing GLU_1, LYS_{77}, VAL_{354} and VAL_{442} as the amino terminal amino acid residues, respectively; K1-3 and K1-4-plasminogen fragments containing the amino acid sequence of TYR_{79}-VAL_{337}, VAL_{353} and TYR_{79}-ALA_{439}, respectively; K4-plasminogen fragment containing the sequence of VAL_{354}-ALA_{439}; NP, NH_2-terminal plasminogen fragment comprising residues GLU_1-VAL_{78}; ϵ-ACA-ϵ-amino caproic acid; PTI-pancreatic trypsin inhibitor; PTH-phenylthiohydantoin.

0006-291X/81/170046-07$01.00/0

Detailed studies have outlined the kinetic and biophysical parameters of VAL_{442}-Pg and its activation product, VAL_{442}-plasmin (5, 6); the ϵ-ACA binding properties of the kringle loop regions (7, 8); and the effect of ϵ-ACA on the thermal unfolding of these fragments (9).

As part of our continuing studies regarding the structure-function relationships of plasminogen and plasmin, we have investigated the possibility of obtaining elastolytic fragments which contain additional combinations of kringle structures and have expanded these studies to plasminogen variants 1 and 2, individually. This communication represents a report of the major findings.

MATERIALS AND METHODS

Proteins. GLU_1-Pg and LYS_{77}-Pg have been purified as variants 1 and 2 by affinity chromatography (10), as modified by Brockway and Castellino (11). Native streptokinase was purified from Kabikinase (AB Kabi), as outlined previously (12) and urokinase (Abbokinase) was generously provided by Dr. W. H. Holleman of Abbott Laboratories.

Elastase digestion. GLU_1-Pg or LYS_{77}-Pg variants 1 and 2 were subjected to limited elastolysis, in separate digestions, as described previously (6, 9), except that the 15 minute digest was performed in 50 mM Tris·HCl/50 mM lysine, pH 8.0 at room temperature.

Amino acid sequence analysis. The semi-micro procedure for manual Edman degradations generally followed that of Peterson et al. (13), with the substitution of two benzene/ethyl acetate (1:1, v:v) extracts after evaporation of the coupling buffer in a N_2 stream. Approximately 50-100 nmol of each intact protein was subjected to three sequence cycles in this manner. The PTH-derivatives were identified by two methods. The first was HPLC identification on a Beckman Liquid Chromatography System described previously (14), using a 4.6 mm x 250 mm Altec Ultrasphere-ODS reverse phase column. A linear gradient program, consisting of 80% (v/v) of 10 mM sodium acetate, pH 5.0/20% methanol, as the starting buffer, and 40% v/v of 10 mM sodium acetate, pH 5.0/60% methanol, as the limit buffer, was applied over a 20 minute interval. The final conditions were maintained for 10 minutes. The flow rate, throughout, was 1.0 ml/minute, at room temperature. The NH_2-terminal residues were quantitated by comparison with a standard mixture of PTH-amino acids. The identifications were checked qualitatively by fluorescent polyamide TLC (15).

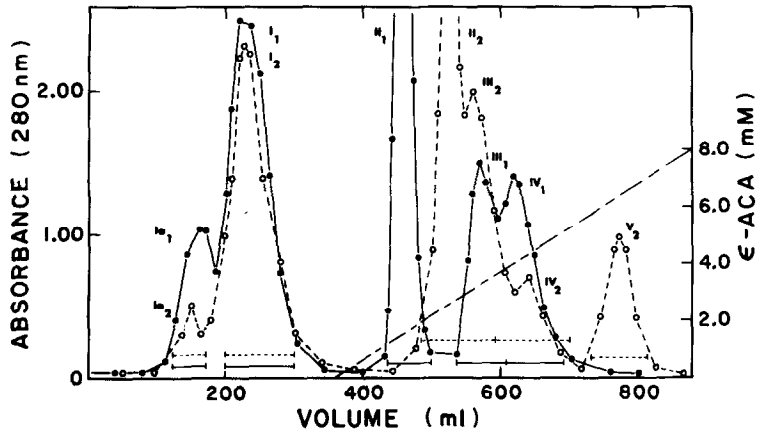

Fig. 1. Elution of the elastolysis fragments of GLU_1 or LYS_{77}-plasminogen variants 1 (●) and 2 (o) from a 2.5 x 25 cm column of Sepharose-4B - L-lysine. The pooled protein fractions are shown in the horizontal bars and the concentration of ε-ACA by the slanted, dashed line. The subscripts 1 and 2 refer to pools derived from plasminogen variants 1 and 2.

RESULTS AND DISCUSSION

The affinity chromatography profiles of 15 minute elastase digests of human plasminogen variants 1 and 2 are shown in Figure 1. The pool subscripts 1 and 2 refer to fragments prepared from variants 1 and 2, respectively. Pools Ia_1, Ia_2, I_1 and I_2 interact very weakly with the affinity ligand. Based upon amino terminal sequence analysis and SDS gel electrophoresis, pools Ia_1 and Ia_2 constitute NP; whereas, pools I_1 and I_2 correspond to VAL_{442}-Pg (3). The ε-ACA gradient partially resolves a number of components from each variant; the earliest eluting peak (II_1) being the only homogeneous fragment and identified as the $K1\text{-}3_1$ region, which consists of residues TYR_{79}-VAL_{337} and TYR_{79}-VAL_{353}.

Figure 2 displays the gel filtration behavior of pools III_1 and IV_1. Pool III_1 nearly exclusively contains fragment $K4_1$ (VAL_{354}-ALA_{439}). Pool IV_1 contains three components; the largest of which is undigested

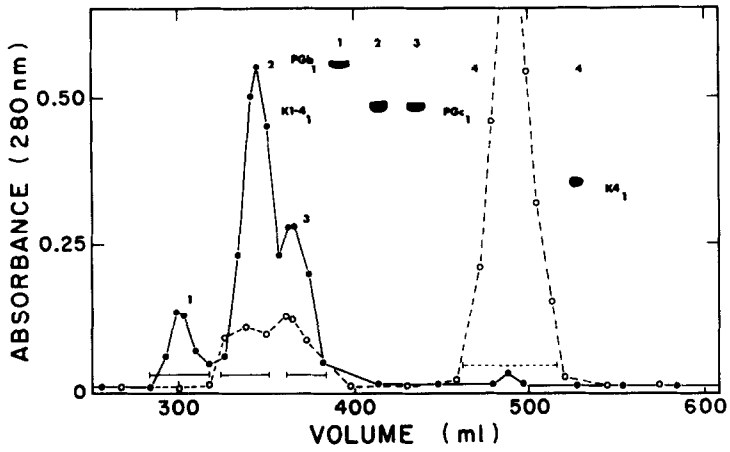

Fig. 2. Gel filtration profiles of protein pools III_1 (o) and IV_1 (•) from Figure 1 on a 2.5 x 90 cm column of Sephacryl S-200 equilibrated in 50 mM Tris·HCl/100 mM lysine, pH 7.4 at 4°. The reduced SDS gel insets refer to protein pools corresponding to the numbered peaks. Again, the subscripts 1 and 2 refer to the plasminogen carbohydrate variant from which the pool was isolated. Pgb refers to LYS_{77} or TYR_{79} plasminogen and Pgc to VAL_{354}-plasminogen.

plasminogen (peak 1). Following this, peak 2 is identified as the $K1\text{-}4_1$ fragment (TYR_{79}-ALA_{439}) and when further digested with elastase yields fragments $K1\text{-}3_1$ and $K4_1$ (data not shown). The remaining peak 3 is a fully activatible plasminogen, termed VAL_{354}-Pg_1, containing kringles 4,5 plus the latent plasmin light chain. SDS gel electrophoretograms of each purified component are shown in the inset.

Figure 3 shows the gel filtration profiles of the ϵ-ACA gradient pools II_2-III_2, IV_2 and V_2, from Figure 1. Pool II_2-III_2 is well-resolved into $K1\text{-}3_2$ (peak 5) and $K4_2$ (peak 6). Pool IV_2 is composed of VAL_{354}-Pg_2 (peak 3) and $K4_2$ (peak 4). Pool IV_2 contains undigested plasminogen (peak 1) and the $K1\text{-}4_2$ fragment (peak 2). Again, SDS gel electrophoretograms of each purified fragment are shown in the inset.

SDS gels depicting the activation of VAL_{354}-Pg_2 to VAL_{354}-Pm are shown in Figure 4A (gels 3 and 4). Here, when compared to the activation

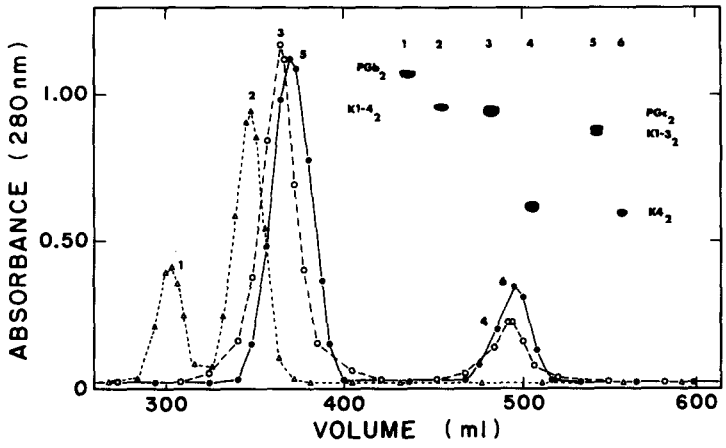

Fig. 3. Gel filtration of protein pools II_2-III_2 (●), IV_2 (o), and V_2 (Δ) as described in Figure 2. The reduced SDS gel insets again refer to the numbered peaks.

products of GLU_1-Pg (gel 6), it is seen that the light chain of VAL_{354}-Pm, which contains the plasmin active site, is intact, and that the heavy chain is considerably reduced in size. The VAL_{354}-Pg species can be readily converted to VAL_{442}-Pg by treatment with elastase (Figure 4B). The products (gel 3) are VAL_{442}-Pg and K4, and after treatment with urokinase or streptokinase, the familiar heavy chain fragment (K5) and light chain of VAL_{442}-Pm appear (gel 4). A similar pattern is observed for VAL_{354}-Pg_1 (data not shown).

Amino terminal amino acid sequence analysis of VAL_{354}-Pg clearly shows that the first three residues are VAL-GLN-ASP. Given the molecular weight of this molecule, of approximately 48,000, and the fact that carboxy-terminal terminus of VAL_{354}-Pg is identical to GLU_1-Pg, the above three residues could only occur at sequence positions 354, 355 and 356 in intact plasminogen, clearly establishing the identity of the degraded form of native plasminogen.

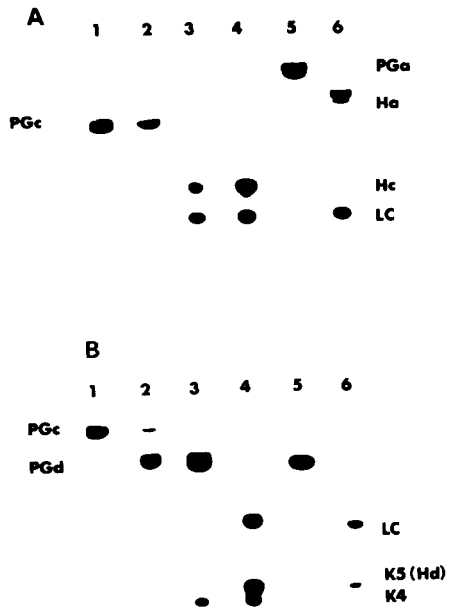

Fig. 4. (A) Reduced SDS gels displaying the activation of VAL_{354}-Pg (Pgc) variants 1 and 2. The VAL_{354}-Pg (1.5 mg/ml) was activated for 30 minutes at 37° in 50 mM Tris·HCl/100 mM lysine, pH 7.4, containing 100 KIU/ml Trasylol and 300 CTA units/ml urokinase. The gels depict: 1) VAL_{354}-Pg variant 1; 2) VAL_{354}-Pg variant 2; 3)-4) the heavy (HC) and light chains (LC) of VAL_{354}-Pg variant 1 and 2, respectively; and 5)-6) GLU_1-Pg (Pga) and the urokinase activation chains, respectively, for comparison. (B) Elastase conversion of VAL_{354}-Pg variant 2 to VAL_{442}-Pg. The gels represent: 1) VAL_{354}-Pg; 2) 2 hour digest; 3) 3 hour digest; 4) 30 minutes after urokinase addition; 5)-6) VAL_{442}-Pg and the urokinase activation chains, respectively, for comparison. Conversion parameters are given in Materials and Methods.

At least three important contributions have been made by this report: 1) The original study of the elastolysis of human plasminogen by Sottrup-Jensen et al. (3) was performed on plasminogen which was not previously separated into its two affinity chromatography variants, leading to confusion as to the origin of the derived fragments - a condition now solved by this communication; 2) the identification of a new heavy chain fragment, K1-4; and 3) the identification of a new degraded plasminogen, VAL_{354}-Pg containing kringles 4, 5 and the latent plasmin light chain. Since the kringle

regions of plasminogen are important to several of its properties, e.g., ϵ-ACA binding sites (7,8) fibrin binding sites (16), effect of ϵ-ACA on the conformation (17) and activation (18) of GLU_1-Pg, and on its interaction with α_2-antiplasmin (19), the existence of these new fragments should aid in our understanding of these important interactions.

ACKNOWLEDGEMENTS

This study was supported by Grant HL-13423 from the National Institutes of Health.

REFERENCES

1. Wiman, B. (1973) Eur. J. Biochem. 39, 1 9.
2. Wiman, B. (1977) Eur. J. Biochem. 76, 129.
3. Sottrup-Jensen, L., Claeys, H., Zajdel, M., Petersen, T. E. and Magnusson, S. (1978) Progress in Chemical Fibrinolysis, Vol. 3, pp. 191-209, Raven Press, New York.
4. Magnusson, S., Sottrup-Jensen, L., Petersen, T. E., Dudek-Wojciechowska, G. and Claeys, H. (1976) Proteolysis and Physiological Regulation, pp. 203-208, Academic Press, New York.
5. Christensen, U., Sottrup-Jensen, L., Magnusson, S., Petersen, T. E. and Clemmensen, I. (1979) Biochim. Biophys. Acta 655, 422-481.
6. Powell, J. R. and Castellino, F. J. (1980) J. Biol. Chem. 255, 5329-5335.
7. Lerch, P. G., Rickli, E. E., Lergier, W. and Gillessen, D. (1980) Eur. J. Biochem. 107, 7-13.
8. Lerch, P. G. and Rickli, E. E. (1980) Biochim. Biophys. Acta 625, 374-478.
9. Castellino, F. J., Ploplis, V. A., Powell, J. R. and Strickland, D. K. (1981) J. Biol. Chem. 256, 4778-4782.
10. Deutsch, D. and Mertz, E. T. (1970) Science 170, 1095-1096.
11. Brockway, W. J. and Castellino, F. J. (1972) Arch. Biochem. Biophys. 151, 194-199.
12. Castellino, F. J., Sodetz, J. M., Brockway, W. J. and Siefring, G. E. (1976) Methods in Enzymol. 45, 244-257.
13. Peterson, J. D., Nehrlich, S., Oyer, P. E. and Steiner, S. F. (1972) J. Biol. Chem. 247, 4866-4871.
14. Morris, J. M., Blatt, S., Powell, J. R., Strickland, D. K. and Castellino, F. J. (1981) Biochemistry, in press.
15. Summers, M. R., Smythers, G. W. and Oroszlan, S. (1973) Anal. Biochem. 53, 624-628.
16. Rakozci, J., Wiman, B. and Collen, D. (1978) Biochim. Biophys. Acta 540, 295-300.
17. Violand, B. N., Sodetz, J. M. and Castellino, F. J. (1975) Arch. Biochem. Biophys. 170, 300-305.
18. Thorsen, S. (1975) Biochim. Biophys. Acta 393, 55-65.
19. Wiman, B., Boman, L. and Collen, D. (1978) Eur. J. Biochem. 87, 143-146.