PRIMARY STRUCTURE OF HUMAN PLASMINOGEN. EVIDENCE FOR GENE DUPLICATION IN THE HEAVY CHAIN AND POSSIBLE HOMOLOGY WITH FIBRINOGEN

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1. Introduction

Activation of human plasminogen is a two-step process involving loss of a 77-residue fragment from the N-terminus and cleavage of an Arg–Val bond about two-thirds of the way along the peptide chain [1–3]. The resulting plasmin consists of a heavy chain (mol. wt approx. 60 000) and a light chain (mol. wt 25 000) linked by a disulfide bond. The light chain contains the catalytic site of plasmin and is homologous with trypsin and other serine proteases [4]. Little is known of the structure and function of the heavy chain. For this reason we have begun sequence studies on the heavy chain. Having sequenced about 30% of the molecule, we are able to report extensive internal homology within the heavy chain, which suggests that the process of gene duplication has occurred during the evolution of plasminogen.

2. Materials and methods

Human plasminogen (lysine form [5]) was prepared from Cohn fraction $III_{2,3}$ by the method of McClintock et al. [2].

A 1.5 g sample of plasminogen dissolved in 50 ml of 70% formic acid was cleaved with 3.5 g of cyanogen bromine for 16 hr in the dark under nitrogen. The solution was lyophilized and the resulting peptide mixture reduced and carboxymethylated with iodoacetate. Separation of peptides was accomplished by gel filtration on Sephadex G-50 in 2.5% acetic acid and chromatography on DEAE cellulose and DEAE Biogel.

Overlapping methionine peptides were obtained by digestion of reduced, carboxymethylated plasminogen with trypsin, followed by separation of the peptides on Sephadex G-25, DEAE Biogel, and Dowex-50 columns.

Sequencing was accomplished by solid-phase Edman degradation [6,8]. Peptides were attached to resins by the homoserine lactone method [9] for cyanogen bromide peptides and the diisothiocyanate method [10] for lysine peptides. Large peptides (> 80 residues) were attached to aminopropyl glass [11]. Phenylthiohydantoins were analyzed by thin-layer chromatography and HI hydrolysis to amino acids.

3. Results and discussion

Cyanogen bromide cleavage of plasminogen gave 7 peptides originating from the heavy chain, containing approximately 15, 15, 19, 86, 90, 115 and 165 residues, respectively. These were aligned by comparison with overlapping tryptic peptides (to be published elsewhere). N-Terminal sequences were obtained by solid-phase Edman degradation [6,9,11]. C-Terminal sequences of cyanogen bromide peptides were determined by first cleaving at lysine and arginine with trypsin and coupling the mixture to the sequencing resin by the homoserine lactone procedure [9]. Since only the homoserine peptides are coupled by this procedure, the C-terminal is thus selectively attached and prior separation from the other peptides is unnecessary.

Using the strategy outlined above, we were able to determine the C-terminal sequence of the 86-residue cyanogen bromide peptide CB-2 as well as peptide CB-3 (fig. 1). The overlapping tryptic peptide T-1 permitted alignment of these two peptides.

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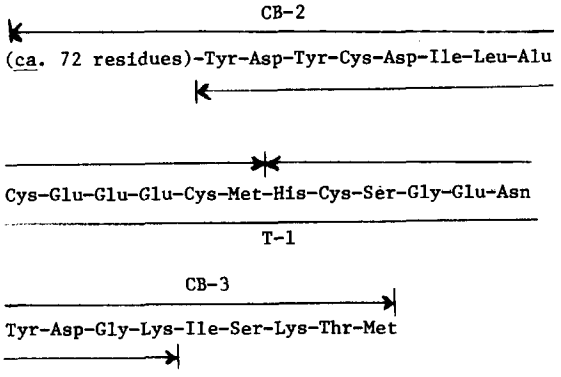

Fig.1. Alignment of cyanogen bromide peptides.

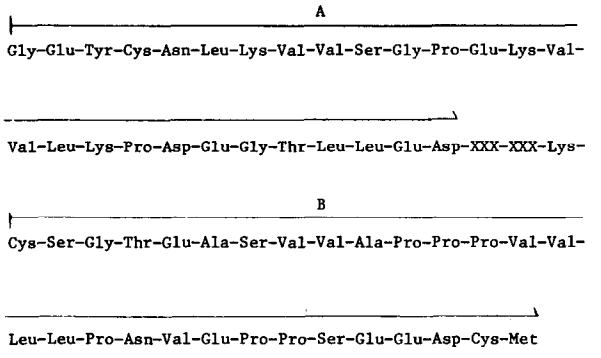

Fig.2. Partial sequence of the C-terminal portion of CB-6, isolated after tryptic cleavage of the maleylated peptide. Part A was sequenced after removal of maleyl groups from lysine. Part B was sequenced after selective attachment of the C-terminal homoserine peptide following cleavage of CB-6 with trypsin.

Peptide CB-6, containing 90 amino acids, was blocked at lysine with maleic anhydride and cleaved with trypsin. Chromatography gave a 60-residue segment containing homoserine, whose partial sequence is shown in fig.2.

Comparison of our results with those of Wiman and Wallén [1] on the pre-activation peptide showed a striking similarity in a region which is characterized by several half-cystine and glutamic acid residues (fig.3). The homology amounts to about 25%, or 40% including conservative mutations, and is much more than is likely to have occurred by chance alone.

Another example of internal homology is seen in peptide CB-6 as aligned in fig.4.

These findings provide strong evidence that a process of gene duplication, such as recently proposed for prothrombin [12], occurred during the evolution of plasminogen. However, more sequence data must be obtained before a mechanism can be postulated.

Even more interesting is an apparent similarity between the plasmin peptides and the 'disulfide knot'

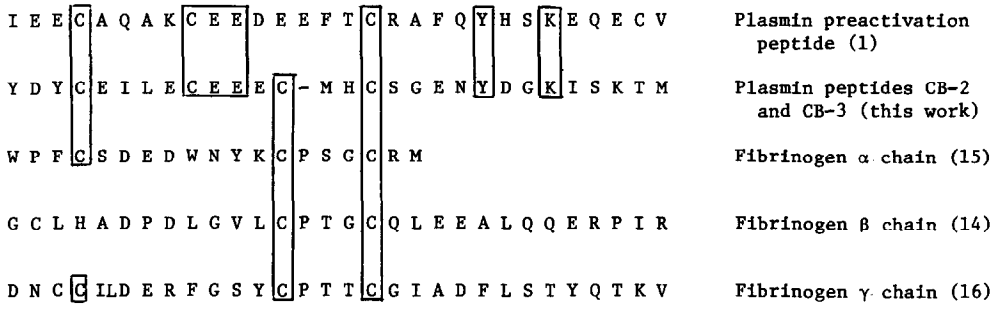

Fig.3. Alignment of plasmin peptides showing internal homology with fibrinogen peptides.

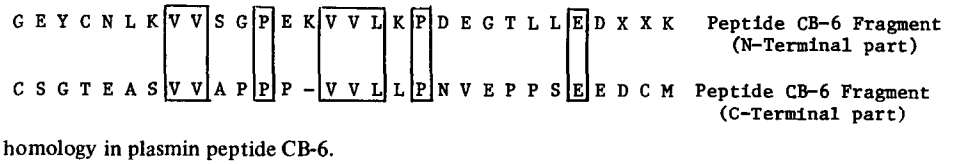

Fig.4. Internal homology in plasmin peptide CB-6.

region of fibrin chains (fig.3)' The homology is much less evident here, but the differences between the plasmin and fibrin chains are not much greater than between the fibrin chains themselves. In view of close functional relationship between fibrinogen and plasminogen and their strong affinity for one another during clot formation [13], it is possible that these molecules have a common ancestor. More sequence data is required to test this hypothesis.

Acknowledgments

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References

[1] Wiman, B. and Wallén, P. (1975) Eur. J. Biochem. 50, 489–494.
[2] McClintock, D. K., Englert, M. E., Dziobkowski, C., Snedeker, E. H. and Bell, P. H. (1974) Biochemistry 13, 5334–5344.
[3] Walther, P. J., Steiman, H. M., Hill, R. L. and McKee, P. A. (1974) J. Biol. Chem. 249, 1173–1181.
[4] Groskopf, W. R., Hsieh, B., Summeria, L. and Robbins, K. C. (1969) J. Biol. Chem. 244, 359–365.
[5] Summeria, L., Arzadon, L., Bernabe, P. and Robbins, K. C. (1973) J. Biol. Chem. 248, 2984–2991.
[6] Laursen, R. A. (1971) Eur. J. Biochem. 20, 89–102.
[7] Laursen, R. A. (1975) in: Immobilized Enzymes, Antigens, Antibodies and Peptides, (H. H. Weetall, ed.) Marcel Dekker, New York, in press.
[8] Laursen, R. A., Bonner, A. G. and Horn, M. J. (1975) in: Instrumentation in Amino Acid Sequence Analysis, (R. N. Perham, ed.) Academic Press, New York, in press.
[9] Laursen, R. A., Horn, M. J. and Bonner, A. (1972) FEBS Lett. 21, 67–70.
[10] Horn, M. J. and Laursen, R. A. (1973) FEBS Lett. 36, 285–288.
[11] Wachter, E., Machleidt, W., Hofner, H. and Otto, J. (1973) FEBS Lett. 35, 97–102.
[12] Hewett-Emmett, D., McCoy, L. E., Hassouna, H. I., Reuterby, J., Walz, D. A. and Seegers, W. H. (1974) Thromb. Res. 5, 421–430.
[13] Bang, N. U. (1971) in: Thrombosis and Bleeding Disorders, (Bang, N. U., Beller, F. K., Deutsch, E. and Mammen, E. F., eds.) p 292 Academic Press, New York.
[14] Söderquist, T. and Blombäck, B. (1971) Naturwissenschaften 58, 16–33.
[15] Blombäck, B., Hessel, B., Iwanaga, S., Reuterby, J. and Blombäck, M. (1972) J. Biol. Chem. 247, 1496–1512.
[16] Blombäck, B., Grondahl, N. J., Hessel, B., Iwanaga, S. and Wallén, P. (1973) J. Biol. Chem. 248, 5806–5820.