THE AMINO ACID SEQUENCE OF A 27-RESIDUE PEPTIDE RELEASED FROM THE α-CHAIN CARBOXY-TERMINUS DURING THE PLASMIC DIGESTION OF HUMAN FIBRINOGEN

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SUMMARY: The amino acid sequence of a 27-residue peptide released during the early stages of the plasmin digestion of human fibrinogen has been determined. The corresponding cyanogen bromide fragment has also been isolated from the purified α-chains of fibrinogen, although a separable fraction of those chains lack the fragment, evidently because of in vivo degradation. The peptide is the carboxy-terminal segment of native α-chains.

During the course of characterizing the cyanogen bromide fragments of the human fibrinogen α-chain, we isolated a peptide which did not contain homoserine and which therefore corresponded to the carboxy-terminal peptide. We were especially interested in this peptide since we had already determined the sequences of the carboxy-terminal cyanogen bromide fragments of the β- and γ-chains of human fibrinogen and found them to have structurally homologous segments (1-3). Beyond that, the carboxy-terminus of the α-chain is of considerable interest because of its recognized vulnerability to proteolysis by plasmin and other proteases (4). Accordingly, we subjected fibrinogen to a brief exposure to plasmin and searched specifically for a peptide with properties similar to those of the α-chain carboxy-terminal cyanogen bromide fragment. Because of the unusually high histidine content of the peptide, we were able to identify the appropriate plasmic peptide on our first attempt. Fortuitously, the plasmin-generated peptide is only one amino acid longer than the cyanogen bromide fragment, which is to say it has an amino-terminal methionine residue.

EXPERIMENTAL

Preparation of the Cyanogen Bromide Fragment. Human fibrinogen prepared according to a previously described procedure (5) was reduced and alkylated and the preparation chromatographed on a carboxymethyl cellulose column (Fig. 1). Two distinguishable α-chain peaks were pooled separately, dialyzed exhaustively against distilled water, and lyophilized, following which they were subjected to fragmentation with cyanogen bromide (6). The cyanogen bromide digest was lyophilized and then dissolved in 10% acetic acid and fractionated on a Sephadex G-50 column (2.5 x 90 cms) equilibrated and developed with the same solvent.[1] The small and medium molecular weight peptides were purified further by paper electrophoresis at pH 2 and 5. It was observed that one of the α_2 pools exhibited a band which was absent in the case of α_1 (Fig. 2). The peptide did not contain homoserine and thus represented the carboxy-terminus of those chains (Table I).

Isolation of the Corresponding Plasmic Fragment. To approximately 220 mg of human fibrinogen dissolved in 20 mls of 0.15 M NaCl, 0.05 M Tris, pH 7.5, was added 0.4 mls of human plasmin (Kabi) in 50% glycerol. After 10 min. at room temperature, the digestion was halted by the addition of 4 mg of solid soybean trypsin inhibitor (Worthington), as well as by placing the reaction mixture at 65° for 10 min. The extent of digestion was observed by electrophoresing the digests on SDS gels (Fig. 3).

The released peptides were separated from the precipitated parent protein by centrifugation (20,000 x g,10 min); the supernatant was passed over a Sephadex G-50 column (2.5 x 100 cms.) equilibrated and developed with 0.1 M ammonium bicarbonate. The small and medium molecular weight pools were subjected to paper electrophoresis and the strips scanned for histidine-containing peptides with the Pauly reagent (8). A peptide with the same mobility as the cyanogen bromide fragment described above was isolated

[1] A complete report dealing with the characterization of each of these pools will appear elsewhere (7).

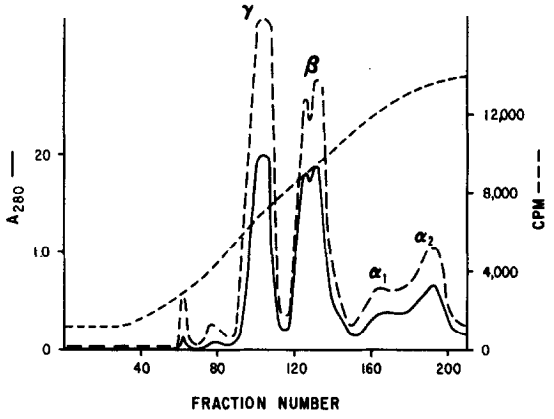

Fig. 1 Carboxymethyl cellulose chromatography (2.5 x 20 cms) of human fibrinogen which has been reduced (dithiothreitol) and alkylated (14-C iodoacetic acid). The starting buffer is 0.01 M sodium acetate, pH 5.2, in 8 M urea. The elution gradient was achieved by mixing equal volumes (400 mls each) of starting buffer and a limit buffer composed of 0.15 M sodium acetate, pH 5.2, in 8 M urea.

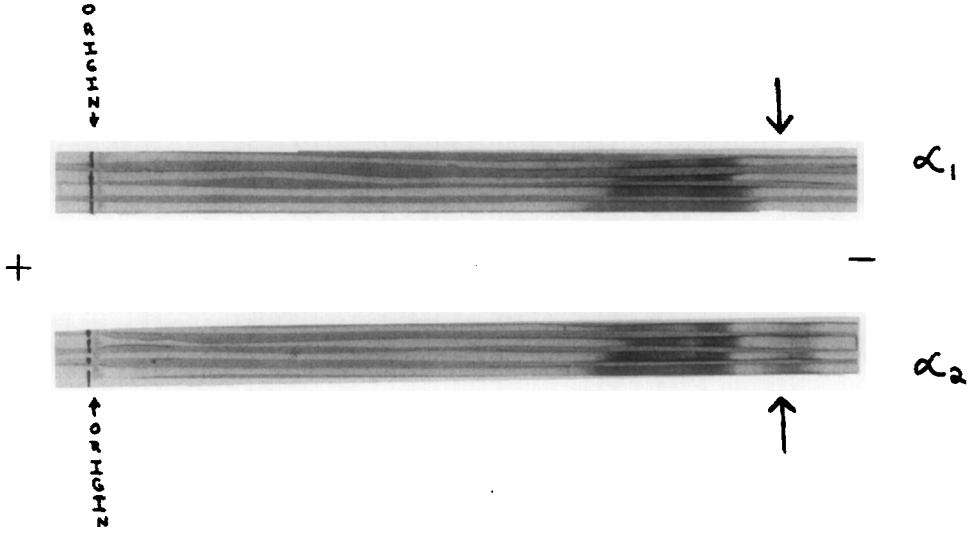

Fig. 2 Paper electrophoretic guidestrips stained with ninhydrin showing a peptide in one of the cyanogen bromide pools of α_2 chains which is absent from the corresponding pool of α_1 chains.[2] Electrophoresis conditions: pH 2.0, 300 V, 3 hrs.[1]

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and found to be the corresponding plasmic fragment. Its amino acid composition is identical with the cyanogen bromide peptide except for the presence of one additional residue, a methionine which was subsequently shown to be at the amino-terminus (Table I).

TABLE I. Amino Acid Compositions of Carboxy-Terminal Cyanogen Bromide and Plasmin Fragments from Human Fibrinogen α-Chains[a]

	CNBr Peptide	Plasmin Peptide
	HαCN5c	HFPL3A2
Aspartic acid	2.1 (2)	2.2 (2)
Threonine	1.7 (2)	1.6 (2)
Serine	3.1 (3)	3.1 (3)
Glutamic acid	3.2 (3)	3.2 (3)
Proline	1.0 (1)	0.8 (1)
Glycine	3.3 (3)	3.2 (3)
Alanine	4.1 (4)	4.0 (4)
Valine	0.9 (1)	0.9 (1)
Methionine		0.8 (1)
Histidine	2.8 (3)	3.0 (3)
Lysine	2.1 (2)	2.8 (2)
Arginine	2.0 (2)	1.8 (2)
Total Residues	26	27

[a]Expressed as residues per mole. Neither of the peptides contains isoleucine, leucine, tyrosine, phenylalanine, tryptophan or cysteine. All results obtained on a Spinco Model 119 Amino Acid Analyzer employing a three-buffer, single column system.

Amino Acid Sequence Determination. The amino acid sequences of the two peptides (i.e., the cyanogen bromide and plasmic fragments) were, in the main, determined with an automatic solid phase sequencer of our own design (9). Preparations were attached to aminoalkylated glass beads (Corning) using phenylene diisothiocyanate (10) or a water soluble carbodiimide (11), and then degraded stepwise by a thioacetylation procedure (11). In addition, the peptides and certain subpeptides were subjected to the DNS-PITC procedure (12). In this regard, the cyanogen bromide fragment was digested with trypsin and three main subpeptides characterized (Table I). A thermolysin digest of the largest tryptic peptide also yielded a key confirmatory peptide (Thr-Lys).

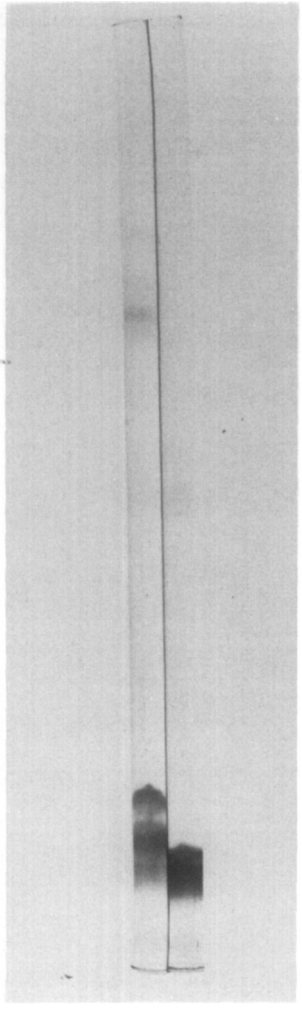

Fig. 3 Dodecyl sulfate (SDS) gel electrophoresis of fibrinogen before and after minimum digestion with plasmin. Electrophoresis conditions: 4% acrylamide gels, 9 mAmps/gel, pH 7.2 (14).

DISCUSSION

The complete amino acid sequence of the plasmin-derived peptide is shown in Fig. 4, along with the carboxy-terminal sequences of the β- and γ-chains of human fibrinogen. The α-chain cyanogen bromide fragment is almost the same size as the γ-chain cyanogen bromide fragment, the peptides being composed of 26 and 27 residues respectively. Also, both peptides have carboxy-terminal valine, an observation consistent with a report that the carboxy-

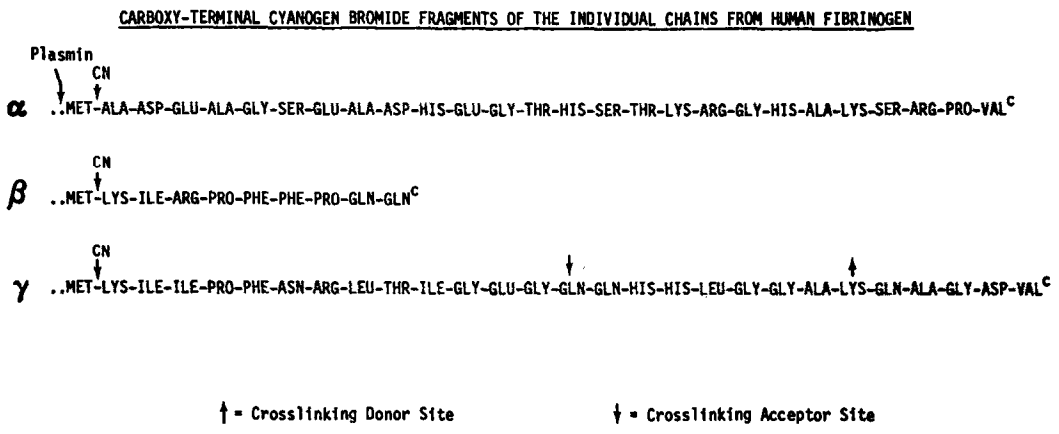

Fig. 4 Amino acid sequence of the carboxy-terminal segment of human fibrinogen α-chains compared with the corresponding peptides from β-chains (3) and γ-chains (1,2).

terminal residues of the α- and γ-chains are both valine (13). Except for these features, however, any further resemblance must be regarded as marginal at best, in contrast to the strong homology existing between β- and γ-chains (Fig. 4).

It is generally accepted that the carboxy-terminal halves of fibrinogen α-chains are very exposed and vulnerable to proteolytic attack. As long ago as 1970, Mills and Karpatkin (4) reported that the α-chains of human fibrinogen were heterogeneous when examined by SDS gel electrophoresis, there being at least two predominant species differing by approximately 3000 in molecular weight. Moreover, the larger of these could be converted into a form indistinguishable from the smaller by short-term treatment of fibrinogen with plasmin (4). It is noteworthy that the molecular weight of our plasmic peptide calculated from its constituent amino acids is 2,859. So far as we know, the isolation and characterization of two distinct α-chain types corresponding to the bands reported by Mills and Karpatkin (4) has not been reported previously. Although the SDS gel system we use (14) is not so highly resolving that we would feel comfortable attaching a value to the

small size difference between our α_1 and α_2-chains, there is little doubt that the difference between them is the absence of the plasmin-released 27-residue carboxy-terminal peptide.

In recent reports a major emphasis has been placed on a much larger molecular weight moiety released during the plasminolysis of fibrinogen, that being a part of the α-chain referred to as fragment H (15) or fragment A (16). This material has a remarkable amino acid composition, being predominantly composed of polar amino acids. A cyanogen bromide fragment corresponding to this large molecular weight material released by plasmin has also been reported (17), and it will be of interest to find if the equivalent fragment from α_1-chains is missing homoserine.

It should be noted that fibrinogen prepared from freshly collected plasma contains both α_1 and α_2 chains, suggesting that removal of this 27-residue peptide occurs under ordinary circumstances in vivo. As such, a radioimmuno-assay for this peptide employing appropriately directed antibodies might be a very simple way to monitor day-by-day plasmin activity at a stage before more extensive degradation of fibrinogen and/or fibrin has occurred. Similarly, antibodies directed to the carboxy-terminus of this peptide may be useful as probes for characterizing the domains of influence of these unusual polar extremeties.

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REFERENCES

1. Chen, R. and Doolittle, R.F. Biochemistry, 10:4486-4491 (1971).
2. Sharp, J.J., Cassman, K.G. and Doolittle, R.F. FEBS Letters, 25:334-336 (1972).
3. Takagi, T. and Doolittle, R.F. Biochim. Biophys. Acta, 386:617-622 (1975).
4. Mills, D. and Karpatkin, S. Biochem. Biophys. Res. Comm., 40:206-211 (1970).
5. Doolittle, R.F., Schubert, D. and Schwartz, S.A. Arch. Biochem. Biophys., 118:456-467 (1967).

6. Gross, E. Methods Enzymol., 11: 238-255 (1967).
7. Doolittle, R.F., Cassman, K.G., Cottrell, B.A., Friesner, S., Hucko, J., and Takagi, T. In preparation.
8. Smith, I. in Chromatographic and Electrophoretic Techniques, Vol. 1, p. 296. Interscience: New York, 1960.
9. Doolittle, L.R., Mross, G.A., Fothergill, L. and Doolittle, R.F. In preparation.
10. Laursen, R.A., Horn, M.J. and Bonner, A.G. FEBS Letters, 21:67-70 (1972).
11. Mross, G.A. and Doolittle, R.F. Fed. Proc., 30:1241 abs. (1971).
12. Gray, W.R. Methods Enzymol., 25:333-344 (1972).
13. Okude, M. and Iwanaga, S. Biochim. Biophys. Acta, 251:185-196 (1971).
14. Weber, K. and Osborn, M. J. Biol. Chem., 244:4406-4412 (1969).
15. Harfenist, E.J. and Canfield, R.E. Biochemistry, 14:4110-4117 (1975).
16. Takagi, T. and Doolittle, R.F. Biochemistry, 14:5149-5156 (1975).
17. Gollwitzer, R., Becker, U., Hahn, E. and Timpl, R. FEBS Letters, 36:281-284 (1973).