Amino Acid Sequence Studies on the α Chain of Human Fibrinogen. Covalent Structure of the α-Chain Portion of Fragment D†

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ABSTRACT: The α-chain portion of fragment D has been purified from an exhaustive plasmic digest of human fibrinogen. The major polypeptide species has 91 amino acid residues, although a small amount of a 97-residue chain representing an earlier digestion stage remains. The amino acid sequence of the first 44 residues was determined by stepwise degradation with an automatic solid-phase sequencer. Another large stretch of sequence was revealed by the finding that the α chain of fragment D overlaps the cyanogen bromide fragments αCNIVA and αCNIII (Doolittle, R. F., Cassman, K. G., Cottrell, B. A., Friezner, S. J., Hucko, J. T., and Takagi, T. (1977), *Biochemistry 16* (preceding paper in this issue)). The automatic sequencer results were confirmed and extended by the isolation and characterization of 18 of 19 expected tryptic peptides from the fragment D α chain. As a result, almost the entire sequence has been obtained. The overlap with key cyanogen bromide fragments has also allowed us to propose an order for the first 198 residues of the fibrinogen α chain. A striking homology with the γ chain and β chain is apparent which has interesting structural implications.

Long-term digestion of mammalian fibrinogens with the enzyme plasmin results in a set of isolable core fragments, the chief of which have been designated fragments D and E in line with their relative elution positions from DEAE[1]-cellulose (Nussenzweig et al., 1961). These digestion products have been studied by very many groups, and the following statements represent a generally held view of the situation. Fragment E is a dimeric moiety, molecular weight about 50 000, and is comprised of disulfide-linked segments from the amino-terminal regions of all three pairs of the constituent chains of fibrinogen ($\alpha_2\beta_2\gamma_2$). Fragment D has a molecular weight in the range of 80 000 and is made up of disulfide-linked portions of the three chain types ($\alpha'\beta'\gamma'$). In the cases of both fragments D and E, the extent of digestion varies with the precise conditions, and at any given time a progressively digested series of products may coexist (e.g., Takagi and Doolittle, 1975a).

In this article, we report the purification and characterization of a species of fragment D from an exhaustively digested preparation of human fibrinogen. The molecular weight of the fragment is approximately 80 000, and its constituent chains have molecular weights of 11 000 (α chain), 28 000 (γ chain), and 37 000 (β chain). We have determined the amino terminals and amino acid compositions of each of the three chain types. In addition, we have completed almost all of the amino acid sequence of the α-chain peptide (n = 91–97 residues), in part by an extended run on a solid-phase amino acid sequencer, and the bulk of the remainder by overlapping cyanogen bromide fragments from purified fibrinogen α chains (Doolittle et al., (1977)). These studies have revealed a striking homology with corresponding segments of the γ and β chains and suggest how the various chains in fibrinogen are arranged in this region of the molecule.

Experimental Section

Materials and Methods. The chemicals used in this study and the analytical procedures employed have been described in the preceding article of this issue (Doolittle et al., 1977).

Preparation and Characterization of Fragment D. Human fibrinogen was dissolved in 0.15 M NaCl, 0.05 M Tris, pH 7.2, at a concentration of 10 mg/mL. Plasmin (Kabi, 10 casein units/mL in 50% glycerol) was added in a ratio of 1:14 (v/v) and digestion was allowed to take place for 6 h at room temperature (22 °C). At the end of that period, the digest was transferred to dialysis tubing and dialyzed overnight at 4 °C against 0.005 M sodium bicarbonate–carbonate buffer, pH 8.5, during which time the final stages of plasmic digestion occurred. The digest was chromatographed on DEAE-cellulose (Figure 1) under conditions similar to those employed originally by Nussenzweig et al. (1961). Peaks were pooled and aliquots removed for examination by sodium dodecyl sulfate gel electrophoresis. The remainders of the pools were dialyzed extensively against distilled water at 4 °C before freeze-drying. At least three different pools contained material corresponding to the moiety generally considered to be fragment D; we have designated these early-eluting D (EED) or D_1, regularly-eluting D (RED) or D_2, and late-eluting D (LED) or D_3. The data in this report pertain only to the regularly-eluting D—which in fact was the major species—unless stated specifically otherwise. The apparent molecular weight of this unreduced material, as determined by sodium dodecyl sulfate gel electrophoresis, was 80 000, being only slightly larger than an unreduced preparation of ovotransferrin (molecular weight 78 000).

Reduction and Carboxymethylation of Fragment D. Generally, 20–50 mg of fragment D was reduced by dissolving the protein in 6 M guanidinium chloride, 0.2 M Tris, pH 8.2, containing 0.005 M DTE, following which carboxymethylation with radioactive iodoacetic acid was conducted in exact accordance with the regimen described in the preceding paper of this issue for the reduction and carboxymethylation of fi-

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[1] Abbreviations used are: PAS, periodic acid–Schiff; TATG, thioacetylthioglycolic acid; DTE, dithioerythritol; Dns, 8-dimethylamino-1-naphthalenesulfonyl; CNBr, cyanogen bromide; DEAE, diethylaminoethyl; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

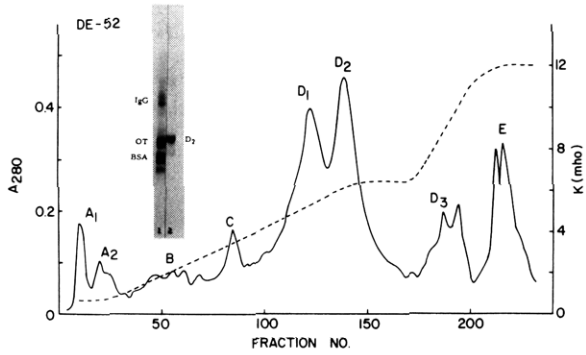

FIGURE 1: DEAE-chromatography of plasmic digest of human fibrinogen. The DEAE-cellulose column (2.5 × 30 cm) was equilibrated with 0.01 M $NaHCO_3/Na_2CO_3$ buffer, pH 8.9. After the sample (200 mg) was applied to the column and washed in well with starting buffer, a two-part gradient was begun. The first portion of the gradient was achieved with 400 mL each of starting buffer and a first limit buffer of the same pH and HCO_2^-/CO_3^{2-} concentration, but containing 0.09 M NaCl. At the completion of that elution, the second part of the gradient was achieved with 150 mL each of the first limit buffer and a final limit buffer containing 0.17 M NaCl. Flow rate = 60 mL/h; fraction size = 6.0 mL. The inset shows a sodium dodecyl sulfate gel (5% acrylamide) of the fragment D preparation used for isolation of α-, β-, and γ-chain fragments. Gel 1 reference materials (IgG = rabbit γ-globulin; OT = ovotransferrin; BSA = bovine serum albumin); gel 2, fragment D_2. Both gels stained with Coomassie blue.

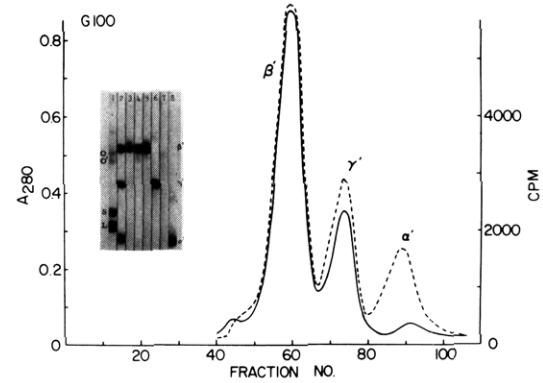

FIGURE 2: Isolation of reduced and carboxymethylated chains from fragment D by gel filtration on Sephadex G-100 (2.5 × 70 cm) equilibrated and developed with 10% acetic acid. In this particular experiment, 26 mg of treated fragment D was applied to the column. Flow rate = 20 mL/h; fraction size = 2.6 mL. Radioactivity from [^{14}C]carboxymethylcysteines was determined by counting 0.1-mL aliquots of alternate fractions. The inset shows the corresponding sodium dodecyl sulfate gels (8% acrylamide) of the starting material and various pools. Gel 1, reference substances (O and O′ = ovalbumin; L = lysozyme, and S = soybean trypsin inhibitor); gels 2 and 3, reduced fragment D; gels 4 and 5, fragment D β chain; gels 6 and 7, fragment D γ chain; gel 8, fragment D α chain. Gels 1, 2, 4, 6, and 8 were stained with Coomassie blue; gels 3, 5, and 7 were stained by the PAS procedure.

brinogen (Doolittle et al., 1977). After dialysis against water, the entire contents of the dialysis bag—including any precipitated material—was lyophilized.

Separation of Fragment D Chains. The freeze-dried carboxymethylated material was dissolved in 10% acetic acid and the constituent β-, γ-, and α-chain portions were separated by gel-filtration chromatography on Sephadex G-100 (Figure 2). Fractions from each of the three peaks were pooled appropriately and aliquots were subjected to sodium dodecyl sulfate gel electrophoresis and amino-terminal identification by both the Dns-Cl and TATG procedures, and their total amino acid compositions were determined. Although this report is primarily concerned with the α-chain portion of fragment D, the relative stage of plasmic digestion may be better appreciated if the molecular weights and end groups of the β and γ chains are also known. In this regard, sodium dodecyl sulfate gel electrophoresis gave molecular weights of 37 000, 28 000, and 11 000 for the β, γ, and α chains, respectively. The amino-terminal of the β chain was aspartic acid, the γ chain methionine (Met-89), and the α chain valine. In the case of the α chain, a small amount (ca. 10%) of aspartic acid was also found.

Stepwise Degradation of the Fragment D α Chain with an Automatic Solid-Phase Sequencer. Two milligrams of isolated α chain from fragment D (RED) was attached to amino-derivatized glass beads by the lysine-attachment method (Laursen et al., 1972). The preparation was subjected to 40 rounds of degradation with the automatic solid-phase sequencer (L. Doolittle et al., 1977). During the first several cycles of the extended run a small but detectable shadow sequence was observable which corresponded to a chain with six additional residues at its amino terminus. In a second run, 2 mg of α chain from early-eluting D (D_1) was treated the same way; 40 cycles with the sequencer gave the same results. The amino acid compositions of the α chains from these two kinds of fragment D were also indistinguishable.

The sequence obtained in these runs overlapped sequences determined for two cyanogen bromide fragments derived from fibrinogen α chains (Doolittle et al., 1977). Thus, the D α chains began with the valine which is residue 20 of αCNIVA, and, beginning with lysine-38, the sequence was the same as the amino-terminal segment of αCNIII. Moreover, the extra six residues observed as a minor contaminant in the fragment D α chains corresponded to residues 14–19 of αCNIVA. Because many other workers have reported data dealing with less extensively digested fragments D, we have begun our numbering of the D α chain with the amino terminus of the minor contaminant. It should be clear, however, that the major species we have studied here begins at residue 7 (Figure 3).

Enzymatic Digestion of the α Chain from Fragment D. Approximately 2 mg of fragment D α chain was digested with trypsin (4 h, 37 °C), and the peptides were purified by paper electrophoresis and, in some cases, by butanol–acetic acid–water chromatography. The amino acid compositions of the peptides recovered are tabulated in Table II. Approximately half of the peptides were identical with peptides recovered from α-chain cyanogen bromide fragment CNIVA, and the other half from CNIII. Only one unique peptide was found which was not found in one or the other of the two CNBr digests, and that was the methionine-containing junction peptide T3A1.

Finally, the carboxy terminus of the fragment D α chain was characterized by a series of digestions with carboxypeptidase B (0, 15, and 60 min at 37 °C). The results indicated a certain amount of heterogeneity at the carboxyl end, somewhat more than half of the chains apparently ending in arginine, and the remainder with lysine.

Discussion

In this and the preceding article of this issue, we have reported the amino acid sequence of a large portion of the fibrinogen α chain, which, when added to previously reported results, brings to almost half the amount of known sequence for that chain. The sequence includes virtually all of the α-chain portion included in fragment D, although—as is true for all the major fragments generated by plasmin digestion—there is a degree of heterogeneity produced during the cleavage, and the boundaries of that fragment are not altogether firm. Thus,

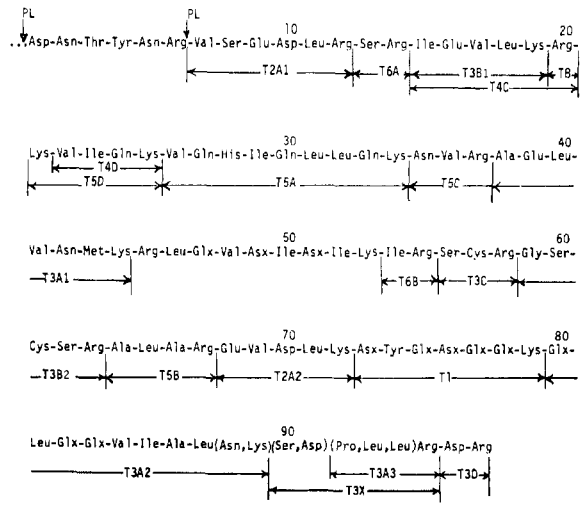

FIGURE 3: Proposed amino acid sequence of α-chain portion of fragment D. The bulk of the α chain characterized in this report actually begins with the valine residue at position 7, but a small portion (ca. 10%) of the material also had the additional six-residue sequence shown at the amino terminus. Similarly, digestion with carboxypeptidase B indicated that a substantial percentage of the chains is missing the carboxy-terminal eight residues shown. Residues 7–50 were mainly established with an automatic sequencer, the small contamination with the incompletely digested longer chain (i.e., residues 1–6) being revealed as a "shadow sequence". Residues 44–87 were determined on another sequencer run on an overlapping CNBr fragment (CN-III, preceding paper in this issue), as were residues 1–31 confirmed on another CNBr fragment (CIVA, preceding paper in this issue). The amino acid compositions of the tryptic peptides set off with arrows are tabulated in Table II.

TABLE I: Amino Acid Composition of α Chain Portion of Fragment D.

Amino Acid	αD[a]	Sequence Residues[b]	Hexapeptide Tail[c]	Collen et al.[d]
Asp[e]	11.3	11	3	14 (12.9)
Thr	0.6		1	1 (0.9)
Ser	5.7	6		5 (5.2)
Glu[e]	16.0	15		16 (16.0)
Pro	1.4	1		1 (0.7)
Gly	1.8	1		1 (1.2)
Ala	4.2	4		4 (3.7)
Cys	2.1	2		2 (1.5)
Val	8.2	9		9 (8.4)
Met	1.0	1		1 (0.9)
Ile	6.7	7		7 (7.0)
Leu	11.2	12		12 (12.4)
Tyr	1.5	1	1	2 (1.6)
Phe	0.3			
His	1.1	1		1 (1.2)
Lys	8.4	9		9 (8.6)
Arg	9.8	11	1	12 (11.8)
Trp				
Total	(91.4)	91	6	97 (94.0)

[a] Average of three analyses; values are relative to glutamic acid = 16.0. [b] Residues from reconstructed sequence (Figure 3). [c] The hexapeptide tail is an amino-terminal segment present on a small percentage of α chains in this digest. [d] From Collen et al. (1975). The integer values are our interpretation of their published data, which are given in parentheses. [e] Aspartic acid and glutamic acid values include asparagine and glutamine, respectively.

the exact nature of the carboxy terminus remains uncertain, and the amino terminus varies with the stage of digestion, beginning with aspartic acid-104 in its earlier stages and with valine-110 in late stages. Indeed, other investigators have reported the existence of both these chains, Collen et al. (1975) reporting a fragment D with an α chain beginning with an aspartic acid, and Furlan and Beck (1975) finding an amino-terminal valine in a more extensive digestion. It should be noted in passing that Collen et al. (1975) reported the first 12 residues of their fragment D α chain and that their sequence differs slightly from the corresponding segment in our sequence. Hence, their eighth residue was reported as a valine, whereas we find serine at that position (α-111). Because of the apparent contradiction, we have repeated that portion of the sequence several times, and we have also isolated the tryptic fragment (n = 6 residues) on numerous occasions. There is no doubt in our minds that the residue is serine in our α chains.

The structure of vertebrate fibrinogen has remained enigmatic over the years despite intense investigation on many fronts. At the very least, the unravelling of its amino acid sequence ought to support or refute one or more of the many models conceived for that structure. We believe the data in this report provide support for some earlier suggested structures, especially that conceived by Hall and Slayter (1959) on the basis of shadowcast electron micrographs.

In this regard, the most interesting feature of the amino-terminal third of the fibrinogen α chain (Figure 4) is a 110-residue stretch between two braces of cysteine residues. First, the sequence is in almost exact register with the corresponding segment of the γ chain (Figure 5). It is also homologous with those sections of the β chain which have been determined so far.[2] These observations lend further credence to our earlier expressed notions about the three nonidentical chains of fibrinogen being descended from a common ancestor (Doolittle, 1970, 1973; Takagi and Doolittle, 1975).

Secondly, its sequence is highly "helix-permissive" over long stretches; there are no proline residues at all between cysteine-49 and cysteine-158. In fact, all the data point to this region being a three-stranded coiled coil in which nonpolar side chains are turned into the center of the rod and which connects the central and distal domains of the fibrinogen molecule. Indeed, Astbury and co-workers (Bailey et al., 1943) found that fibrinogen displayed an x-ray pattern similar to the keratin–myosin group in that it had a 5.1-Å repeat unit. Such data were subsequently interpreted as indicative of coiled α helices (Pauling and Corey, 1953; Crick, 1952). Later, Cohen (1961) suggested that in all these proteins (the k-m-e-f group) the coiled coils might be serving as interdomainal connections. It is our contention that the coiled coils in fibrinogen begin immediately after the unique interchain connections involving cysteine-45 and -49 in the α chain, and terminate at the interchain connections involving cysteine-158 and -162.

Quite apart from the structure of fibrinogen itself, some comment is in order about how such a structure lends itself to proteolysis by plasmin. So far, we have definitely identified ten plasmin attack points in the α chain (Table III). The question arises as to whether the specificity of the enzyme is paramount or whether its attack is primarily governed by structural restraints in the fibrin(ogen) substrate.

The fact that a similar series of more or less refractory core fragments could be obtained by digesting fibrinogen with a variety of proteases led Mihalyi to propose that these digestions were attributable more to the vulnerability of the substrate than to the specificity of the enzymes (Mihalyi and Godfrey, 1963; Mihalyi, 1970). Indeed, he was among the first to recognize that it was the facile snipping of interdomainal con-

[2] R. F. Doolittle, K. G. Cassman, B. A. Cottrell, D. Goldbaum, D. Trovato, and K. Watt (in preparation).

TABLE II: Amino Acid Compositions of Tryptic Peptides Isolated from α-Chain Portion of Fragment D.[a]

Amino Acid	T2A1	T6	T3B1	T4C	T5D	T4D	T5A	T5C	T3A1	T3C	T3B2	T5B	T2A2	T1	T3A2	T3A3	T3D
Asp[b]	1.1							0.9	1.1				1.0	2.2	0.8		1.0
Thr																	
Ser	0.7	1.1								0.9	1.8						
Glu[b]	1.1		1.3	1.1	1.1	1.1	3.0		1.0				1.4	3.0	2.5		
Pro																0.8	
Gly											1.1						
Ala									0.7			2.0			1.3		
Cys[c]										0.9	0.8						
Val	1.0		1.1	1.1	0.9	1.2	1.3	1.0	1.1				0.7		1.3		
Met									+								
Ile		0.8	0.8	0.9	1.0	0.9	1.0								0.7		
Leu	1.3		0.6	0.6			2.0		1.3			1.0	0.6		2.2	2.2	
Tyr														0.7			
Phe																	
His							0.7										
Lys			1.3	1.3	2.1	0.9	1.0		0.8				1.1	1.1	1.2		
Arg	0.9	2.1		1.1				1.0		1.2	1.2	1.0				1.0	1.0
Total residues	6	4	5	6	5	4	9	3	7	3	5	4	5	7	9	4	2
Position in sequence	7–12	13–14 54–55	15–19	15–20	21–25	22–25	26–34	35–37	38–44	56–58	59–63	64–67	68–72	73–79	80–89	92–95	96–97
Approximate yield (%) (uncorrected)[d]	7	10	6	8	3	5	6	13	2	11	8	18	7	22	2	5	18

[a] Presented as molar ratios. [b] Aspartic acid and glutamic acid values include asparagine and glutamine, respectively. [c] Measured as carboxymethylcysteine. [d] Peptides with two characters (e.g., T1) were analyzed after one electrophoresis; those with three characters (e.g., T3C) after elution and a second electrophoresis; and those with four characters (e.g., T3A3) after two electrophoreses and butanol–acetic acid–water chromatography.

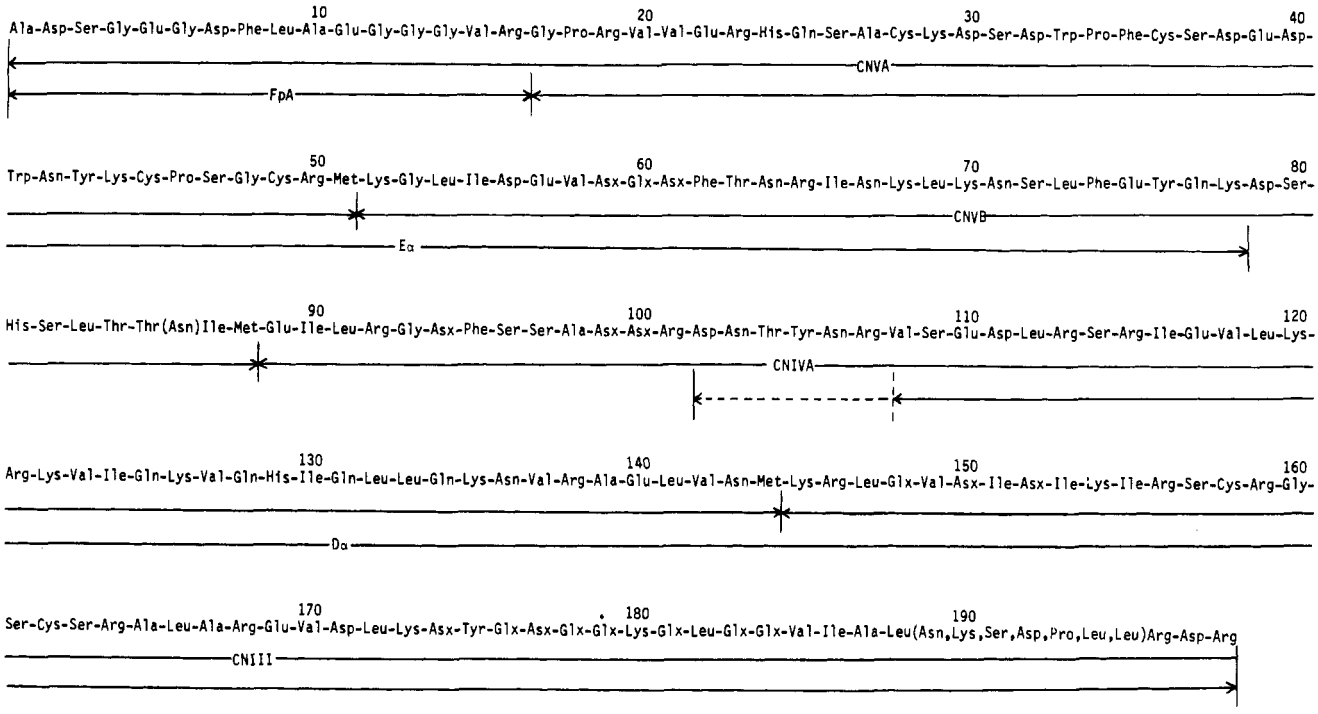

FIGURE 4: Proposed amino acid sequence of the first 198 residues of the fibrinogen α chain showing cyanogen bromide fragments (CN series), major plasmin peptides (Eα and Dα), and fibrinopeptide A (FpA).

nections which led to a set of core fragments consistent with the Hall and Slayter model (1959), a concept clearly and explicitly laid out by the subsequent experiments of Marder and co-workers (Marder, 1970). The problem is that perfectly coiled coils ought to have an inherent stability and be very resistant to proteolytic attack. It is in this regard that we point

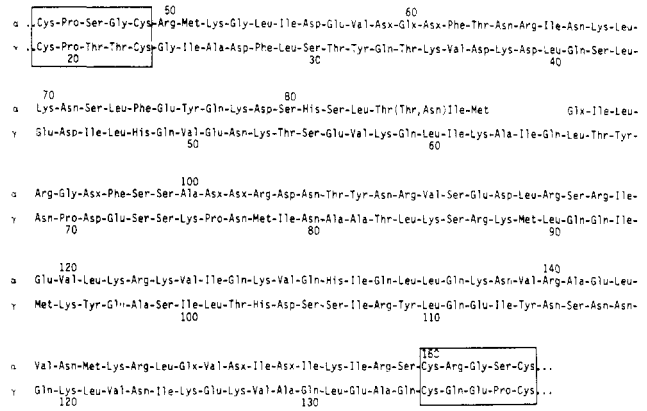

FIGURE 5: Amino acid sequences of α and γ chains between two braces of cysteines holding the (three) chains together. Note that the proline content of these regions is very low and that the sequences are highly "helix-permissive". Also, note that many nonpolar residues occur in register and at a frequency concordant with their being oriented to the middle of a three-stranded coiled coil. In addition to data provided in the present and preceding paper of this issue (Doolittle, et al., 1977), the following other data are used: residues α 45–51 (Iwanaga et al., 1969); α 52–78 (Takagi and Doolittle, 1975c); γ 19–78 (Iwanaga et al., 1968); γ 79–108 (Takagi and Doolittle, 1975a). In addition, Henschen and colleagues (Henschen and Lottspeich, 1975; Henschen and Warbinek, 1975) have reported the sequences of residues γ 108–138, although their sequence differs from the one shown here at positions 121 and 124 and does not include γ 139 (R. F. Doolittle, T. Takagi, B. Bouma, B. A. Cottrell, and S. J. Friezner, unpublished data).

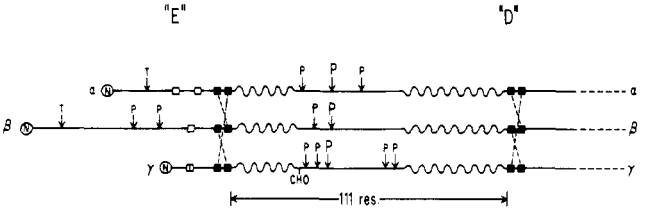

FIGURE 6: Schematic depiction of the connections between fragments E and D and the proposed disulfide registration (- - -) for the α, β, and γ chains. The thrombin attack points for releasing the fibrinopeptides A and B are represented by T. The primary plasmin attack points are represented by P. The second brace of cysteines on the β chain has not yet been firmly established.

up the potentially nonhelical nature of certain central portions of the three chain segments (Figure 6). Once the initial clips have been made, then the three chains would tend to unravel and be more susceptible to progressive digestion from their exposed ends.

In summary, we have determined the amino acid sequence of a large portion of the fibrinogen α chain, including those segments which occur in fragments D and E. The data are not only consistent with the notion that all three fibrinogen chains have descended from a common ancestor, but also with the existence of a three-stranded coiled coil stretching across an approximately 110-residue interdomainal connection. The connection may have a nonhelical central portion, however, which serves as the central focus for plasmin attack during fibrinogenolysis or fibrinolysis.

Acknowledgments

We thank Marcia Riley for preparing all the fibrinogen used in these studies and Danielle Goldbaum and Dennis Trovato for assistance in the operation of the automatic sequencer. We are also very grateful to Larry Doolittle for constructing the automatic sequencer.

TABLE III: Ten Identified Plasmic Cleavages Which Occur in α Chains during Fibrinogenolysis[a]

Val-Arg-Gly-Pro. .	15 16 17 18	(late)	
Pro-Arg-Val-Val. .	18 19 20 21	(very late)	
Gln-Lys-Asp-Ser. .	77 78 79 80		
Asx-Arg-Asp-Asn. .	100 101 102 103		
Asn-Arg-Val-Ser. .	106 107 108 109	(late)	
Arg/Lys-Met-Lys. .	208 209 210		
Phe-Lys-Ser-Gln. .	220 221 222 223		
Trp-Lys-Ala-Leu. .	231 232 233 234		
Met-Arg-Met-Glu	240 241 242 243		
Arg/Lys-Met-Ala. .	583 584 585	(earliest)	

[a] In addition to data presented in this report, the table includes plasmic cleavages identified in Takagi and Doolittle (1975a,b) and Cottrell and Doolittle (1976).

Supplementary Material Available

Supplementary data are available for all three papers of this series in this issue (63 pages). Ordering information is given on any current masthead page.

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Amino Acid Sequence Studies on the α Chain of Human Fibrinogen. Isolation and Characterization of Two Linked α-Chain Cyanogen Bromide Fragments from Fully Cross-Linked Fibrin†

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ABSTRACT: Fully cross-linked human fibrin was digested with cyanogen bromide and the resulting fragments were characterized and compared with the fragments produced upon cyanogen bromide treatment of fibrinogen α chains. The largest molecular-weight fraction isolated by gel filtration on Sephadex G-150 was reduced and alkylated, and upon rechromatography on Sephadex G-150 it eluted at the same place as the original material. This large molecular weight fraction was subjected to amino acid analysis and the amino-terminal sequences of its constituent chains were determined by both dimethylaminonaphthyl sulfonation (Dns) and thioacetylation procedures. The identified sequences corresponded to two cyanogen bromide fragments previously found in α chains isolated from fibrinogen, one of which has a molecular weight of about 30 000 and the other 6000. The latter is thought to be the carboxy-terminal penultimate cyanogen bromide fragment of the α chain.

Under physiological conditions, the thrombin-catalyzed transformation of fibrinogen to fibrin is accompanied by a fibrin stabilization phenomenon whereby factor XIII—itself activated by thrombin—introduces a series of ε (γ-glutamyl) lysine covalent cross-links. One set of these leads to the existence of γ-γ dimers in fibrin after it is reduced to its constituent chains (Chen and Doolittle, 1969; Takagi and Iwanaga, 1970), the attachment being reciprocal in nature and the connections being interposed between the antiparallel carboxy-terminal segments of neighboring molecules (Chen and Doolittle, 1970, 1971). Another set of cross-links involves α chains, and—in contrast to γ-γ dimer formation—the ensuing product is multimeric in nature (McKee et al., 1970). β chains are not involved in fibrin cross-linking (Chen and Doolittle, 1969).

The existence of α-chain multimers in cross-linked fibrin implies that more than one connection site exists in α chains, and Pisano et al. (1971) have shown definitively that as many as 6 mol of cross-link can exist per mol of starting fibrinogen in fully cross-linked fibrin, 2 of which are presumably involved in γ-chain cross-linking and 4 in α-chain connections. Since the fibrinogen molecule is a dimer, this reduces to one acceptor site on γ chains and two on α chains. Indeed, evidence for two different α-chain *acceptor* sites has been brought forth by several laboratories (Chen, 1970; Doolittle et al., 1972; Takagi and Doolittle, 1975; Ferguson et al., 1975). One of these has been found to be located in a midsection region between residues 200 and 250 (Takagi and Doolittle, 1975); the other is located more distally toward the carboxy terminus (Takagi and Doolittle, 1975; Ferguson et al., 1975).

Knowing the amino acid sequence around the midsection acceptor site, and knowing that this acceptor site was situated in a cyanogen bromide fragment which consists of only three residues (Pro-Gln-Met), we undertook an investigation to find that tripeptide in a cyanogen bromide digest of fully cross-linked fibrin to see if we might find a donor fragment attached to it. We also knew from other studies ongoing in our laboratory that none of the cyanogen bromide peptides of the γ chain and probably none of the β chain have amino-terminal proline. Accordingly, we thought that we could locate the cross-linked material on the basis of amino-terminal analysis alone. Unfortunately, the only amino-terminal proline we found was in the smallest molecular weight fractions, where it would be expected if it were not cross-linked. Instead, we found large molecular weight material involving two other α-chain cyanogen bromide fragments, both of which are described individually in a preceding paper in this issue (Doolittle et al., 1977). One of these, if not disulfide bound or involved in cross-linking, ought to emerge in a small-medium molecular-weight fraction. The other is the largest molecular-weight fraction remaining after cyanogen bromide fragmentation of α chains.

Experimental Procedures

Materials and Methods. With the following exceptions, the materials used and the analytical procedures employed have

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