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Amino Acid Sequence Studies on Plasmin-Derived Fragments of Human Fibrinogen: Amino-Terminal Sequences of Intermediate and Terminal Fragments†

Takashi Takagi and Russell F. Doolittle*

ABSTRACT: The progressive changes in amino-terminal sequence brought about by the digestion of human fibrinogen by plasmin have been studied. In addition, the limit products (fragments D and E) have been isolated and characterized in the same way. These studies have confirmed the generally accepted scheme of fibrinogen being changed into a large molecular weight fragment X, which in turn is converted into an intermediate fragment Y and a limit fragment D, followed by the breakdown of fragment Y into an additional fragment D and another core fragment E. Our data allow the precise identification of several of the junctions being attacked, including one in a region of the γ chain whose sequence has not previously been reported. The cleavages are not singular in any case, however, and, as suggested by others, intermediate species exist which correspond to "early D," "late D," etc. In addition to localizing the exact bonds split by plasmin, we have been able to sequentially position the core fragments relative to each other, since the γ-chain amino terminus of fragment D has been found to be contiguous to the known carboxy-terminal sequence of fragment E.

During the course of the last 15 years many different groups have studied the pattern of plasmin degradation of human fibrinogen (Nussenzweig *et al.*, 1961; Marder *et al.*, 1969; Gaffney and Dobos, 1971; Furlan and Beck, 1972; Pizzo *et al.*, 1972; Mills, 1972; Kowalska-Loth *et al.*, 1973; Mosesson *et al.*, 1973; *inter alia*). In recent years most of the data have been acquired by sodium dodecyl sulfate polyacrylamide gel electrophoresis. In general, the results have been consistent with a scheme whereby fibrinogen is first transformed into a somewhat smaller molecule, fragment X, which is then split asymmetrically into a fragment D and a larger fragment Y (Marder, 1970). Fragment Y is subsequently broken into a second fragment D and a limit fragment E. The pattern is consistent with a dimeric fibrinogen molecule in which the fragment E represents a central domain and the two fragments D are disposed symmetrically about a central axis. A few investigators have challenged this interpretation, especially with regard to the numbers of fragments D and E which result, Mosesson *et al.* (1973) contending that there is only one fragment D per molecule, and Plow and Edgington (1974) claiming that there are really two fragments E. The situation is confused in part by the fact that plasmin degradation is not an entirely specific process, and there are "early" and "late" species of each of the major fragments, the relative amounts of which depend on the exact conditions of digestion.

In this article we report a detailed study of the plasmin digestion course for human fibrinogen, combining the use of sodium dodecyl sulfate gel electrophoresis for monitoring the fragmentation progress with amino-terminal sequence studies for identifying cleavage points. Our data generally confirm previous notions of an asymmetric cleavage scheme which ultimately yields two fragments D and one fragment E. They add to previous reports in that we have identified precise cleavage points in several instances, including some fragmentation sites in regions whose amino acid sequences have not previously been reported.

Experimental Section

Materials

Human fibrinogen was prepared from blood bank plasma by a cold ethanol fractionation procedure (Doolittle *et al.*, 1967). U..S. Red Cross human plasmin in 50% glycerol (10 C.T.A. U/ml) was kindly provided by Dr. Alan Johnson, New York University Medical Center. Soybean trypsin inhibitor (SBTI)[1] was purchased from Worthington Bio-

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[1] Abbreviations used are: SBTI, soybean trypsin inhibitor; TATG, thioacetylthioglycolic acid; PAS, periodic acid–Schiff; Gdn· HCl, guanidine hydrochloride; SDS, sodium dodecyl sulfate.

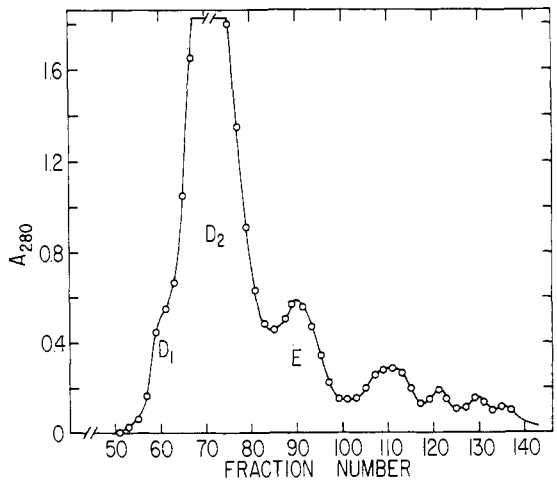

FIGURE 1: Separation of fragments D and E, produced by 15-hr digestion with plasmin, on Bio-Gel A 1.5m (2.5 × 90 cm) equilibrated and eluted with 4 M guanidine chloride. Flow rate, 21 ml/hr; fraction size, 3.5 ml. The peaks labeled D_2 and E were pooled separately and used for the amino-terminal sequence experiments (Table II).

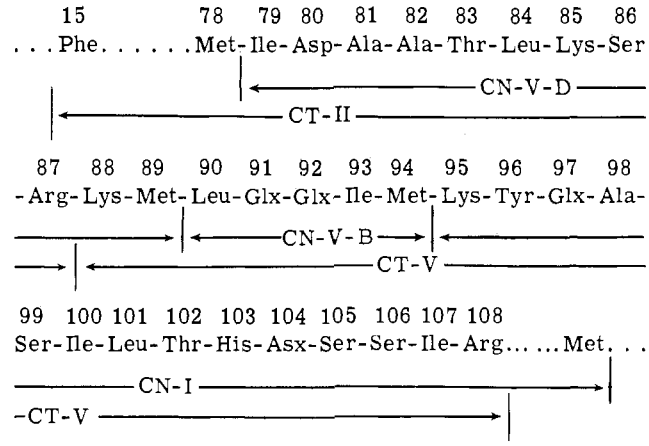

FIGURE 2: Previously unpublished amino acid sequence from human fibrinogen γ chains derived from cyanogen bromide fragments (CN) and overlapping citraconylated-tryptic peptides (CT). The sequence of residues 1–78 of the human γ chain has been published previously (Blombäck *et al.*, 1973).

chemicals. Thioacetylthioglycolic acid (TATG) was synthesized according to the procedure of Jensen and Pedersen (1961). Gdn · HCl was purchased from Heico, Incorporated. Trifluoroacetic acid was obtained from Matheson Coleman and Bell and was distilled before use.

Methods

Plasmin Digestion of Fibrinogen. The plasmin in 50% glycerol (10 C.T.A. U/ml) was mixed with human fibrinogen (10 mg/ml in 0.15 M NaCl–0.05 M Tris buffer (pH 7.5)) in a ratio of 1:15 by volume. Digestion was carried out at 22° (room temperature); at intervals, solid SBTI was added to a final concentration of 0.5 mg/ml in order to inhibit the plasmin. Samples were immediately placed at 60° for up to 30 min in order to heat-precipitate the major fragments (but not fragment E). The heat-treated specimens were centrifuged, the supernatants stored for subsequent study of the heat-soluble peptides released, and the precipitates examined by SDS gel electrophoresis and subjected to stepwise degradation for identification of amino-terminal sequences.

Sodium Dodecyl Sulfate Gel Electrophoresis. Aliquots of the heat-precipitated material and other samples were dissolved in a solution containing 8 M urea and 2% sodium dodecyl sulfate. If the preparations were to be examined in the reduced form, mercaptoethanol was added to a concentration of 10 μl/ml. All samples were incubated at 37° for 16–24 hr before being applied to 6 × 140 mm gels. Electrophoresis was conducted in a manner similar to that described by Weber and Osborn (1969); the running buffer was 0.1 M phosphate (pH 7.2) containing 0.1% sodium dodecyl sulfate. The running current was 8–9 mA/gel. The acrylamide concentration of the gels was varied between 4 and 5% depending on the requirements of the investigation. Gels were stained either by Coomassie Brilliant Blue for protein or the periodic acid–Schiff reagent for carbohydrate (Zacharius *et al.*, 1969).

Stepwise Degradation by the Thioacetylation Procedure. The amino-terminal sequences of the various preparations were determined with the thioacetylation procedure introduced by Mross and Doolittle (1971) and as modified by Takagi and Doolittle (1974). In outline form, the method involves coupling with TATG, suitable washing, cleavage with F_3CCOOH, and removal of the cyclic amino acid derivative with dichloroethane. The advantage of this particular stepwise degradation method is that the free amino acid is readily regenerated and can be quantified on a standard amino acid analyzer. As such it is particularly useful when more than one polypeptide chain is being examined simultaneously. The major disadvantage of the method is that serine and threonine undergo a β-elimination reaction as a function of the cyclization during cleavage and are not readily identified. In the present study this happened not to be a major drawback, only one instance of an undetected serine residue being involved in our primary conclusion.

Preparation of Terminal D and E Fragments. Terminal fragments D and E were prepared after plasmin digestion for 15 hr at room temperature (T = 22°). They were isolated by gel filtration on Bio-Gel A 1.5m (2.5 × 90 cm) equilibrated and eluted with 4 M guanidine (Figure 1). The designated peaks were pooled appropriately, exhaustively dialyzed against distilled water, and lyophilized. Each gave one major band on sodium dodecyl sulfate gel electrophoresis.

Previously Unpublished Sequences from Human Fibrinogen γ Chain. Although a full report on the amino acid sequence of major portions of the γ chain from human fibrinogen will appear elsewhere,[2] some of these data are important in the present study for pinpointing bonds cleaved by plasmin. Accordingly, an abbreviated description of these findings is included here. In this regard, γ chains were prepared from sulfitolyzed human fibrinogen and subjected to cyanogen bromide fragmentation (Sharp *et al.*, 1972). Alternatively, γ chains were citraconylated (Attasi and Habeeb, 1972) and then digested with trypsin. Peptides were isolated by gel filtration followed by further purification by paper electrophoresis; they were sequenced by standard techniques as described previously (Takagi and Doolittle, 1974). The peptides relevant to the present study are summarized in Figure 2. The sequences of α-chain residues 1–51 and γ-chain residues 1–78 have been reported previously (Blombäck *et al.*, 1972, 1973), as well as have preliminary announcements dealing with β-chain residues 1–115 (Blombäck and Blombäck, 1972). The residue numbering used

[2] T. Takagi and R. F. Doolittle, in preparation.

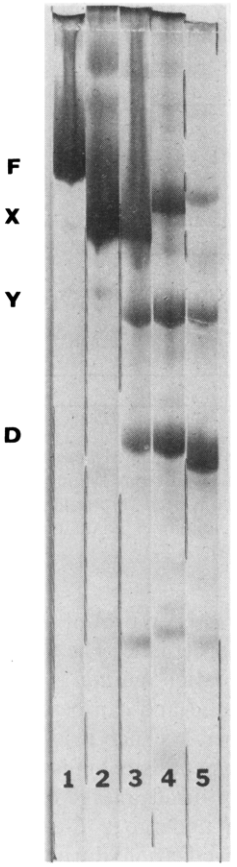

FIGURE 3: Sodium dodecyl sulfate gel electrophoresis of plasmin-digested human fibrinogen which had been heat-precipitated at intervals. The samples were taken from the same batches used to perform stepwise degradations (Table I). These are unreduced samples stained with Coomassie Blue. Gel 1, zero time; gel 2, 10-min digestion; gel 3, 30-min; gel 4, 60 min; gel 5, 120 min. F, fibrinogen; X, fragment X; Y, fragment Y; D, fragment D.

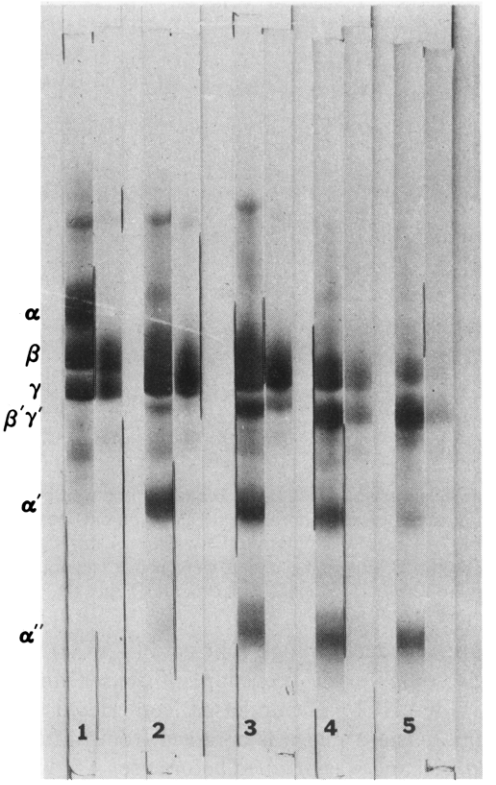

FIGURE 4: Sodium dodecyl sulfate gel electrophoresis of plasmin-digested human fibrinogen. The samples are the same ones as those shown in Figure 3 except that they have been reduced. In each pair, the left-hand gel was stained with Coomassie Blue and the right-hand one with the PAS stain. The chain assignments are consistent with those found by many other investigators (see text).

throughout the present article is consistent with and/or derived from those reports.

Results

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. The progress of the plasmin digestion of fibrinogen was readily followed by gel electrophoresis; in general our results were similar to those reported by many other laboratories. Hence, after 10-min digestion, virtually all of the starting fibrinogen was converted into another larger molecular weight species, fragment X (Figure 3). After 30 min, substantial amounts of fragments Y and D were present in the heat-precipitated material, and by 60 min these were the major components present. After 2-hr digestion fragment D was the predominant species. These designations were verified by a consideration of reduced samples which were stained both with Coomassie Brilliant Blue for protein and the PAS stain for carbohydrate (Figure 4); it has been established previously that only β and γ chains contain PAS-positive material (Gaffney, 1972; Pizzo *et al.*, 1972). Because fragment E remains in the supernatant under the conditions employed, the gels show only fragments X, Y, and D. The conversion of an "early D" to a "later D" was quite apparent between the 60- and 120-min time points.

Amino-Terminal Sequences. The amino-terminal sequences of the five preparations (0-, 10-, 30-, 60-, and 120-min time points) were determined by the thioacetylation procedure (Table I). The zero time sample (undigested fibrinogen) yielded a series of amino acids consistent with the known structures of the fibrinopeptide A—which is the amino-terminal segment of the fibrinogen α chain—and the amino-terminal sequence of the fibrinogen γ chain. Because the amino terminus of the β chain does not have a free α-amino group, degradation of that chain does not occur. Thus in the first cycle, only alanine and tyrosine were found, these being present at a 1:1 ratio (Table I). Similar results were obtained in steps 2–5 except for the serine which occurs at position α3 and the threonine at γ4, these two amino acids not being readily detected by the method.

10-Min Digestion. After 10-min exposure to plasmin under the described conditions, and at a time when gels indicated that the bulk of the fibrinogen had been converted into fragment X (Figure 3), alanine and tyrosine were still the only two amino acids detected in appreciable amounts, but the ratio of the two was now 2:1, the amount of alanine having doubled (Table I). Subsequent cycles exhibited a newly exposed sequence, Ala-Arg-Pro-Ala-Lys, which corresponds to positions 43–47 in the β chain. Thus, one of the first major attack points for plasmin is the arginyl–alanine bond at position β42–43 leading to the loss of a 42-residue segment from the amino terminus of the β chain. As shown by the gels, however, and as noted by many previous workers, the major loss in bulk during the conversion of fibrinogen to fragment X is due to cleavage in the carboxy-terminal half of the α chain. These pieces remained in the supernatant after heat treatment, however. They have been isolated and identified, as have peptides corresponding to the amino terminus of the β chain, and are the subject of another paper.[2]

Table I: Amino Acids Recovered from Five-Step Degradation of Various Stages of Plasmin Digestion of Human Fibrinogen.[a]

Plasmin Digestion Time (min)	TATG Degradation Cycle				
	First Residues	Second Residues	Third Residues	Fourth Residues	Fifth Residues
0	Ala (28) Tyr (24)	Asp (40) Val (28)	Ala (17)	Gly (21)	Glu (15) Gly (10) Arg (8)
10	Asp (10) Ala (48) Tyr (24)	Asp (16) Val (26) Arg (19)	Pro (10) Ala (19)	Gly (21) Ala (12)	Glu (12) Lys (7)9 Arg (9)3
30	Asp (29) Ala (61) Tyr (29) Lys (8)	Asp (53) Val (39) Arg (20)	Glu (18) Pro (16) Ala (27)	Asp (30) Glu (21) Gly (33) Ala (23) Leu (15) Tyr (13) Lys (14) Arg (10)	Asp (10) Glu (17) Gly (9) Val (13) Lys (16) Arg (13)
60	Asp (53) Ala (53) Tyr (24) Lys (13)	Asp (70) Val (43) Arg (19) Ile (13)	Glu (37) Ala (21) Pro (8)	Asp (18) Gly (15) Leu (11) Tyr (9) Arg (5)	Asp (13) Glu (12) Val (14) Lys (13) Arg (10)
120	Asp (93) Ala (41) Tyr (12) Lys (10)	Asp (86) Val (15) Ile (28) Arg (9)	Glu (49)	Asp (24) Leu (19) Tyr (16)	Asp (16) Val (15)

[a] The nanomoles of amino acid recovered at each step are given in parentheses; readings are direct from Autolab Integrator tape and are uncorrected for yield.

30-Min Digestion. After 30-min digestion, at which point the gels indicated the simultaneous existence of fragments X, Y, and D in the heat-precipitated fraction (Figures 3 and 4), at least six distinct chain types could be distinguished upon examination of the amino-terminal sequences. Two of these corresponded to the terminal sequences of the fibrinopeptide A (α chain) and the undigested γ chain, and a third was the sequence of β-chain residues 43–47, first detected after 10-min digestion. A fourth sequence deduced from the amino acids found was that of β-chain residues 54–58, indicating a subsequent plasmin cleavage at the lysyl–lysine constituting the β-chain 53–54 linkage. Two additional sequences were detected at this time which correspond to newly exposed end groups in the α and β chain of fragments X and Y which have been partially degraded but whose γ chains are still intact. Both the α and β chains of this intermediate begin with aspartic acid (Table III).

60-Min Digestion. By 60 min, the major components present in the heat-precipitated fraction are fragments Y and D (Figures 3 and 4). At this point, the terminal sequences of all three chains in fragment D could be identified, and the exact position of the cleavage in the γ chain pinpointed. Thus, the new sequence clear at this point is Ala-Ile-Glx-Leu, corresponding to γ-chain residues 63–66, and showing that plasmin has cleaved the lysyl–alanine linkage at positon γ62–63. The situation with regard to the terminal sequences of the α and β chains was less clear. The new sequence Asx-Asx-Glx appeared at this time, and the amounts indicated that both the α and β chains of one part of fragment Y begin with this same sequence. Steps 4 and 5 contained a variety of amino acids, and no firm conclusion for chain assignment can be made at this time (Table III).

120-Min Digestion. After 2-hr digestion, the end group pattern in the heat-precipitated fraction actually becomes simpler. The sequences corresponding to the amino-terminal segments of the α chain (*i.e.*, the fibrinopeptide A) and the virginal γ chain are almost completely absent, signaling the complete conversion of fragment Y to E and D (fragment E is in the supernatant fraction). The material remaining in the precipitate is virtually all intermediate fragment D (Figure 3). Its α- and β-chain termini both begin with the sequence Asx-Asx-Glx, and its γ-chain terminal sequence commences with the alanine at position 63, as evidenced by the continuing sequence Ile-Glx-Leu (Table III). As was the case in the 60-min sample, steps 4 and 5 contained a variety of amino acids and an unequivocal assignment of sequence for the α and β chains was not possible.

Terminal Fragment D. Purified fragment D produced after 15-hr digestion exhibited virtually the same amino-terminal sequence pattern for the α and β chains as did the material present in the heat-precipitated fraction at 120 min (Table II). In contrast, the γ chain of the "late" fragment D begins with the sequence (Ser)Arg-Lys-Met-Leu (Table III), which corresponds to residues γ86–90 (Figure 2). The 23-residue segment corresponding to amino acids γ63–85 has been completely lost sometime between the 120-min time point and the 15-hr termination, changing "intermediate D" into "late D." The bond cleaved in this instance is the lysyl–serine at position γ85–86.

Terminal Fragment E. Fragment E isolated from the 15-hr digestion ("late E") exists in two major forms (Table II). One of these has an α-chain piece which begins with the sequence Gly-Pro-Arg-Val-Val, which is the sequence exposed upon the release of the fibrinopeptide A (Table III).

Table II: Amino Acids Recovered from Five-Step Degradation of Fragments D and E of Human Fibrinogen.[a]

TATG Degradation Cycle				
First Residues	Second Residues	Third Residues	Fourth Residues	Fifth Residues
		Fragment D		
Asp (40.7)	Asp (27.6)	Glu (25.4)	Asp (22.9)	Asp (14.3)
	Arg (16.4)	Lys (12.2)	Met (11.9)	Glu (11.3)
		Tyr (12.9)	Tyr (18.8)	Val (17.8)
				Leu (18.2)
				Tyr (17.8)
		Fragment E		
Gly (27.1)	Pro (12.2)	Glu (99.6)	Val (12.3)	Val (17.5)
Val (25.0)	Val (132.6)	Ala (58.1)	Arg (62.6)	Lys / His (84.6)[b]
Tyr (49.5)		Arg (10.0)		
Lys (42.5)				Arg (47.6)

[a] The nanomoles of amino acid recovered at each step are given in parentheses; readings are taken directly from Autolab Integrator tape and are uncorrected for yield. [b] His and Lys were not well separated on analyzer in this run.

The other species has an α-chain moiety which begins with the sequence Val-Val-Glu-Arg-His, in correspondence with residues α20–24. This clearly demonstrates that a significant amount of the terminal tripeptide Gly-Pro-Arg (α17–19) is clipped off during the late stages of plasminolysis. Both of these late fragment E species have β chains which begin with lysine-54 and γ chains which still have the original amino-terminal tyrosine intact (Table III).

Discussion

Fibrinogen can be proteolytically digested into a characteristic set of fragments by plasmin—and, for that matter, by trypsin and chymotrypsin also. Moreover, the action of all three of these enzymes follows a virtually identical course during the early stages of digestion, indicating that the pattern of breakdown is very much influenced by the vulnerability of the substrate (Mihalyi, 1970). The nomenclature used to define the plasmin-derived fragments dates back to an early study by Nussenzweig *et al.* (1961) in which the five peaks emerging from a DEAE-cellulose chromatographic analysis of plasmin-digested fibrinogen were designated A–E. As it happened, peaks A–C represented a collection of many peptides chipped off during the course of the digestion, but fractions D and E represented substantial core fragments with molecular weights of approximately 80,000 and 50,000, respectively. The nomenclature for transient fragments produced during the course of the plasmin digestion of fibrinogen was introduced by Marder *et al.* (1969), who characterized the intermediate fragments X and Y. Marder (1970) also recognized the fundamentally asymmetric mode of cleavage and noted how compatible it was with the Hall and Slayter triglobular model of fibrinogen (Hall and Slayter, 1959). Thus, after the removal of peptide material from the parent fibrinogen model to yield fragment X, the next major conversion results in a fragment Y and a fragment D; the fragment Y is subsequently broken into a fragment E and a second fragment D. According to this scheme, then, fragment E would correspond to a central domain and the two fragments D to equitably disposed terminal units. Although there is not universal agreement on this scheme of events (Mosesson *et al.*, 1973; Plow and Edgington, 1974), most studies performed since that time, including this one, are consistent with those ideas. There is also substantial agreement that fragment E is composed of the amino-terminal regions of all six of the chains comprising fibrinogen. As such, it is a dimer and corresponds closely to the dimeric "disulfide knot" isolated after cyanogen bromide fragmentation of fibrinogen (Blombäck *et al.*, 1968; Marder, 1971). Many other studies have subsequently shown that fragment D is also comprised of portions of all three chain types, α, β, and γ. Depending on the stage of digestion and the particular investigating group the β-chain portion may range from 35,000 to 45,000 in molecular weight, the γ chain from

Table III: Amino-Terminal Pentapeptidyl Sequences Reconstructed from Stepwise Degradation Data.[a]

Digestion Time (min)	α-Chain Sequences	β-Chain Sequences	γ-Chain Sequences
0	Ala-Asp(Ser)Gly-Glu.. 1 2 3 4 5	PCA-..(blocked)... 1	Tyr-Val-Ala(Thr)Arg.. 1 2 3 4 5
10	Ala-Asp(Ser)Gly-Glu.. 1 2 3 4 5	Ala-Arg-Pro-Ala-Lys.. 43 44 45 46 47	Tyr-Val-Ala(Thr)Arg.. 1 2 3 4 5
30	Ala-Asp(Ser)Gly-Glu.. 1 2 3 4 5 Asx-Asx-Glx..	Ala-Arg-Pro-Ala-Lys.. 43 44 45 46 47 Asx-Asx-Glx.. Lys-Val-Glu-Arg-Lys.. 54 55 46 57 48	Tyr-Val-Ala(Thr)Arg.. 1 2 3 4 5
60	Ala-Asp(Ser)Gly-Glu.. 1 2 3 4 5 Asx-Asx-Glx..	Ala-Arg-Pro-Ala-Lys.. 43 44 45 46 47 Asx-Asx-Glx.. Lys-Val-Glu-Arg-Lys.. 54 55 56 57 58	Tyr-Val-Ala(Thr)Arg.. 1 2 3 4 5 Ala-Ile-Gln-Leu(Thr).. 63 64 65 66 67
120	Asx-Asx-Glx..	Asx-Asx-Glx..	Ala-Ile-Gln-Leu(Thr).. 63 64 65 66 67
Fragment D (15 hr)	Asx-Asx-Glx..	Asx-Asx-Glx..	(Ser)Arg-Lys-Met-Leu.. 86 87 88 89 90

[a] Prepared from the data presented in Tables I and II.

Table IV: Some Identified Cleavage Points in the Plasmin Digestion of Human Fibrinogen.

Digestion Stage	α-Chain Bonds	β-Chain Bonds	γ-Chain Bonds
Early		Arg–Ala 42 43	
Middle		Lys–Lys 53 54	Lys–Ala 62 63
Late	Arg–Gly 16 17 Arg–Val 19 20		Lys–Ser 85 86

25,000 to 40,000, and the α chain from 10,000 to 15,000. These values have to be viewed in the general context of a starting molecule in which the α chains have molecular weights in the range of 65,000–70,000, the β chains approximately 55,000, and the γ chains about 47,000 (McKee *et al.*, 1970).

There has been less agreement, however, on the exact residues included in the various fragments and which bonds are cleaved by plasmin as the reaction progresses, and these are the questions we set out to answer in the present study. The answers were made possible by the examination of amino-terminal sequences of the unfractionated digestion mixture at various stages of digestion. By using a quantitative method and proceeding through five cycles at each stage, unequivocal judgments about chain assignments could be made. Thus, six plasmin cleavage points have been precisely determined (Table IV) and the newly exposed sequences at two other junctions determined (Table III).

Two examples of how the approach has resolved previous ambiguities are in order. Budzynski *et al.* (1974), noting the report of a quantitative increase in the amount of amino-terminal alanine upon the conversion of fibrinogen to fragment X (Kierulf, 1972), rightly deduced on the basis of ancillary information that the split had to be in the amino-terminal region of the β chain. What they could not determine, however, was whether this was the result of cleaving the Arg–Ala at position 42–43, the Lys–Ala at 47–48 or the Lys–Ala at 58–59, since the size difference in fragments was below the resolution of their gels. Our study clearly shows that the cleavage point in question is the Arg–Ala at β42–43. Similarly, Kowalska-Loth *et al.* (1973) observed a small amount of amino-terminal valine in (late) fragment E. While considering the possibility that the valine was the result of the removal of the terminal Gly-Pro-Arg sequence at positions 17–19, they concluded that this was unlikely on other grounds, speculating instead that the valine was due to cleavage of the Lys–Val bond at β54–55. Our sequential degradation clearly shows that the amino-terminal valine represents the α-chain terminal sequence beginning at residue 20, the sequence Val-Val-Glu-Arg being established. Moreover, the amount of amino-terminal valine which is exhibited after 15-hr digestion is about as great as the amino-terminal glycine belonging to those fragment E molecules which have retained the terminal Gly-Pro-Arg (Table II).

Structural Significance of the Exact Cleavage Points. Since the fragments D and E both contain portions of all three chain types (α, β, and γ), the connections between them must be composed of at least three strands. *A priori* there was no reason to suppose that all three chains were cleaved synchronously. In fact, our data indicate that the α- and β-chain connections are severed between the fragments D and the central domain at a very early stage, but the units are held together by the γ chain alone until later in the digestion. The key bond which must be split in order to release a fragment D from the parent molecule or from an intermediate fragment Y is the Lys–Ala bond a γ62–63. These events are depicted diagramatically in Figure 5.

After their separation from parental fragment, both fragments D and E are further degraded. At the very least, fragment D loses a 23-residue segment from the amino-terminal portion of its γ-chain moiety. The gel electrophoresis patterns indicate that the β and γ chains may also be degraded at the carboxy terminus, as reported by others. Fragment E is degraded at the amino-terminal end of its α chain, both the fibrinopeptide A and the contiguous tripep-

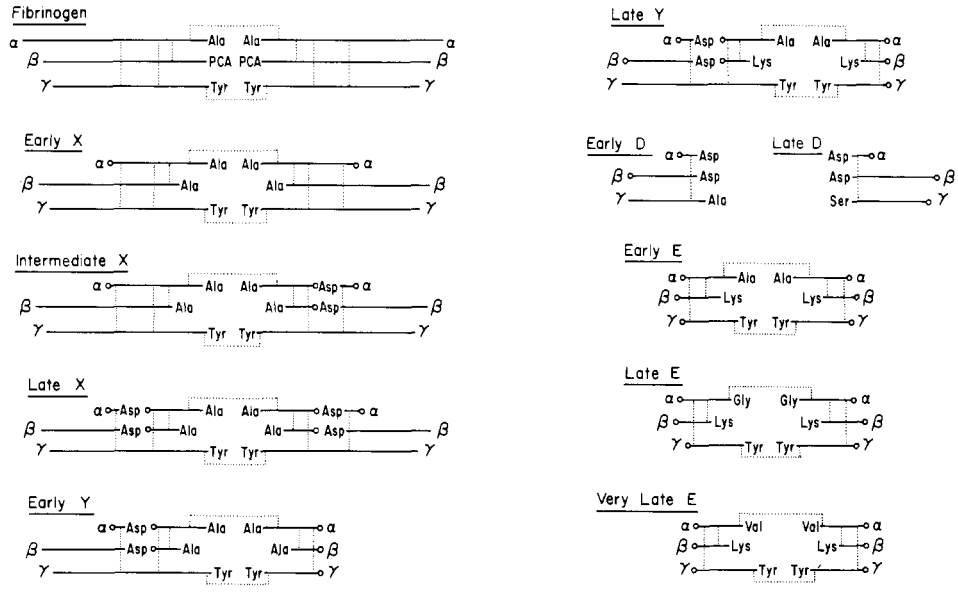

FIGURE 5: Schematic depiction of some of the events occurring during the plasmin digestion of human fibrinogen, showing amino termini of all chains in the parent molecule and major fragments. Broken lines denote interchain disulfide attachments.

tide Gly-Pro-Arg being extensively removed by exhaustive plasmin digestion.

We are still unsure of the exact position of the initial cleavages in α- and β-chain strands connecting the principal domains. On the basis of other data,[2] however, we know that the carboxy terminals of "late fragment E" are at positions α79, β118, and γ53. The molecular weights of chain fragments determined on gel electrophoresis suggest that the fragment D amino terminals must be very close to these residues, if not exactly contiguous. It is likely, also, that all of the initial cleavage points between these domains are close to each other in a spatial sense.

Acknowledgments

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