Harvey, C. L., Clericuzio, E., & Nussbaum, A. L. (1970) *Anal. Biochem. 36*, 413–421.
Kato, K.-I., Goncalves, J. M., Houts, G. E., & Bollum, F. J. (1967) *J. Biol. Chem. 242*, 2780–2789.
Kirtikar, D. M., & Goldthwait, D. A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2022–2026.
Kleihues, P., & Margison, G. P. (1974) *J. Natl. Cancer Inst. 53*, 1839–1841.
Kornberg, A. (1969) *Science 163*, 1410–1418.
Lawley, P. D., Orr, D. J., Shah, S. A., Farmer, P. B., & Jarman, M. (1973) *Biochem. J. 135*, 193–201.
Loveless, A. (1969) *Nature (London) 223*, 206–207.
Maitra, S. C., & Frei, J. V. (1975) *Chem.-Biol. Interact. 10*, 285–293.
Margison, G. P., & Kleihues, P. (1975) *Biochem. J. 148*, 521–525.
Mehta, J. R., & Ludlum, D. B. (1978) *Biochim. Biophys. Acta 521*, 770–778.
Nicoll, J. W., Swann, P. F., & Pegg, A. E. (1975) *Nature (London) 254*, 261–262.
O'Connor, P. J., Capps, M. J., & Craig, A. W. (1973) *Br. J. Cancer 27*, 153–166.
Pegg, A. E., & Hui, G. (1978) *Biochem. J. 173*, 739–748.
Strider, W., Harvey, C., & Nussbaum, A. L. (1968) *J. Med. Chem. 11*, 524–527.
Yoneda, M., & Bollum, F. J. (1965) *J. Biol. Chem. 240*, 3385–3391.

Effects of Fibrinopeptide Cleavage on the Plasmic Degradation Pathways of Human Cross-Linked Fibrin†

Stephanie A. Olexa* and Andrei Z. Budzynski

ABSTRACT: The presence of fibrinopeptide B in human fibrin has a significant effect on plasmic degradation pathways of cross-linked clots. Two types of fibrin were obtained from fibrinogen by incubation either with thrombin, to remove both fibrinopeptides A and B, or with batroxobin, to cleave fibrinopeptide A only. Fibrins obtained after various incubation times were characterized by the determination of the NH_2-terminal amino acids, the content of fibrinopeptides, and the extent of cross-linking. The fibrins were digested by plasmin and were analyzed by polyacrylamide gel electrophoresis. The presence and concentration of the (DD)E complex, as well as fragments E_1 and E_2, in the digests were dependent upon the loss of fibrinopeptide B from cross-linked fibrin. These degradation products, and also fragment DD, appear to be useful molecular markers of fibrinolysis.

Human fibrinogen is converted to fibrin through a limited proteolytic cleavage by thrombin, releasing two molecules of the fibrinopeptides A and B from a dimeric fibrinogen molecule (Bailey et al., 1951; Bettelheim & Bailey, 1952; Lorand, 1951, 1952; Blombäck & Yamashina, 1958; Gladner et al., 1959; Folk et al., 1959). The removal of fibrinopeptide A proceeds at a much faster rate than that of fibrinopeptide B (Bettelheim, 1956); however, the removal of fibrinopeptide A is sufficient for the fibrin polymerization to occur (Laurent & Blombäck, 1958). The investigation of the role of fibrinopeptide release was stimulated by the discovery of fibrinogen clotting enzymes in snake venoms. Reptilase and batroxobin from *Bothrops jararaca* and *Bothrops atrox*, respectively, cleave fibrinopeptide A (Laurent & Blombäck, 1958; Blombäck et al., 1957; Bilezikian et al., 1975) and form fine-structured clots which appear to contain fibrin monomers in an end-to-end arrangement (Laurent & Blombäck, 1958) which can be cross-linked by activated factor XIII (Furlan et al., 1976).

Blombäck et al. (1978) have reported that human fibrin isolated from blood clotted in glass tubes has a Gly/Tyr ratio of 1.3:1, indicating that very little fibrinopeptide B was cleaved by thrombin. The authors suggested that most fibrin formed in vivo would still contain a majority of the fibrinopeptide B. However, at present it is impossible to conclude if fibrinopeptide B is present in intravascular fibrin clots.

Digestion of human cross-linked fibrin by plasmin results in the formation of fragments DD and E and α polymer remnants as the major high molecular weight degradation products (Ferguson et al., 1975; Gaffney & Brasher, 1973; Gaffney et al., 1975; Hudry-Clergeon et al., 1975; Kopeć et al., 1973; Marder et al., 1976; Pizzo et al., 1973a,b). A complex between fragments DD and E, first observed by Gormsen & Feddersen (1973) and later by others (Gaffney & Brasher, 1973; Kopeć et al., 1973), contains one molecule of fragment DD and one molecule of fragment E (Olexa & Budzynski, 1979a). Close analysis of the fragment E from plasmic digests of cross-linked fibrin revealed that there are three species of fragment E, that is, E_1, E_2, and E_3 of molecular weight 60 000, 55 000, and 50 000, respectively (Olexa & Budzynski, 1979b). Fragments E_1 and E_2 can bind with fragment DD, forming the (DD)E complex; however, fragment E_3 cannot. Plasmic digestion of human cross-linked fibrin, which had been formed by extended incubation with thrombin and lacks all of the fibrinopeptides A and B, proceeds according to the following scheme (Olexa & Budzynski, 1979a):

$$(DD)E_1 \rightarrow (DD)E_2 \rightarrow DD + E_3$$

It has been suggested that the (DD)E complex is a nucleus of the fibrin polymerization sites (Hudry-Clergeon et al., 1975; Olexa & Budzynski, 1979b).

Since the differential loss of fibrinopeptide A or B affects the structure of the fibrin clot (Laurent & Blombäck, 1958),

† From the Specialized Center of Research in Thrombosis and Department of Biochemistry, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140. *Received July 9, 1979.* This work was supported by Grant No. HL14217 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, and by Grant No. 76826 from the American Heart Association.

0006-2960/80/0419-0647$01.00/0

it was of interest to see if the presence of fibrinopeptides would also affect the plasmic degradation pattern of the fibrin. The (DD)E complex is a unique degradation product of cross-linked fibrin; thus, it was of particular interest to see if this species could be used to monitor fibrinolysis regardless of the fibrinopeptide content of the fibrin. In the present investigation, fibrin was formed by thrombin or batroxobin and contained no fibrinopeptide A and varying amounts of fibrinopeptide B. The plasmic degradation patterns of these fibrins were examined.

Materials and Methods

Preparation of Fibrin. Human fibrinogen (Grade L, A. B. Kabi, Stockholm, Sweden) in 0.15 M ammonium acetate, pH 7.8, was supplemented with 0.05 M calcium chloride and 0.002 M mercaptoethanol. Factor XIII (Loewy et al., 1961) was activated with either thrombin (human α-thrombin kindly supplied by Dr. John W. Fenton, II, New York Department of Health, Albany, NY) or batroxobin (Pentapharm, Basle, Switzerland) and incubated at a final concentration of 2.5 units/mL for 1 h at room temperature. Then the mixture was added to the fibrinogen solution to a final concentration of 0.045 unit/mL of thrombin or batroxobin. Hirudin (Pentapharm, Basle, Switzerland; 10 ATU[1]/mL final concentration) was added to the fibrinogen and factor XIII preparations which were incubated with batroxobin to prevent any thrombin activity. The fibrinogen reaction mixtures were incubated at room temperature for various times. The reaction was stopped by the addition of phenylmethanesulfonyl fluoride (PMSF; Sigma, Saint Louis, MO; 0.001 M final concentration) and hirudin (10 ATU/mL final concentration). The fibrin was immediately wound onto a glass rod, immersed in 2 mL of 0.15 M ammonium acetate, pH 7.8, containing 0.001 M PMSF and 10 ATU/mL hirudin, and then freeze-dried. One milligram of fibrin was dissolved in 1 mL of 9 M urea, 3% $NaDodSO_4$, and 3% mercaptoethanol and then electrophoresed on 7% polyacrylamide gels containing 0.1% $NaDodSO_4$, under reducing conditions. The gels were scanned in a densitometer (Densicord 552, Photovolt, New York). Non-cross-linked fibrin was obtained as described before (Marder et al., 1976).

Determination of the NH_2-Terminal Amino Acids of Fibrin. The NH_2-terminal amino acids of fibrinogen and fibrin were determined by the dansylation method of Gray (1972) using $\sim$1 nmol of protein. Dansylated amino acids were analyzed by chromatography on polyamide sheets (Cheng Chin Trading Co., Taiwan) according to the method of Woods & Wang (1967) with the modification of Hartley (1970). Dansylated ϵ-lysine, *O*-tyrosine, and cysteine were used as markers for the completeness of the reaction.

Quantitation of Dansylated Amino Acids. For determination of the ratio of glycine to bis(dansyl)tyrosine as the NH_2-terminal amino acids in fibrin and fibrinogen, spots corresponding to glycine, alanine, bis(dansyl)tyrosine, and cysteine, as well as blank spots for a base line reading, were cut out and soaked in acetone–90% formic acid (9:1 v/v) overnight. The eluted amino acids were dried and then dissolved in 0.2 mL of ethyl acetate (Chen, 1967) and read in a spectrofluorometer (Farrand Optical Co., Valhalla, NY) with an excitation maximum of 332 nm and an emission maximum of 500 nm. The ratio of glycine to bis(dansyl)-tyrosine in fibrin incubated with thrombin for 24 h was equated to 2.0 and the ratio in fibrinogen was equated to 0. The ratios for the other fibrins were calculated from these figures. The fluorescence of cysteine was comparable in all preparations. Chromatography and quantitation of amino acids were done 4 times for each fibrin sample.

Measurement of Fibrinopeptides A and B in the Fibrin Supernates. Immediately after removal of the fibrin clot, absolute ethanol was added to the supernate and the fibrinopeptides in the solution were purified according to the method of Blombäck et al. (1966) with the modifications of Budzynski et al. (1975). The peptides were spotted on cellulose thin-layer chromatography sheets (Chromagram 6064, Eastman, Rochester, NY) and developed in pyridine–butanol–acetic acid–water (10:15:3:12) at room temperature for 4 h. Fibrinopeptides A and B were localized by Sakaguchi (Shainoff & Page, 1960) and ninhydrin (Moffat & Lytle, 1959) stains. The ratio of fibrinopeptide B to A in the fibrin supernates was determined by cutting unstained regions from a chromatographic plate which corresponded to the locations of the standard peptides, elution of the peptides with 1% potassium hydroxide, and quantitation of the arginine by the method of Szilágyi & Szabó (1958). Arginine (Sigma, St. Louis, MO) and purified fibrinopeptides A and B were used for the standard curves.

Plasmic Digestion. The cross-linked fibrins were washed several times to remove any excess PMSF and then digested by plasmin in the ratio of 10 CTA units/g of fibrin (10.2 CTA units/mL; kindly provided by Dr. David L. Aronson, Bureau of Biologics, Food and Drug Administration, Rockville, MD) as described (Olexa & Budzynski, 1979a). Fibrinogen and non-cross-linked fibrin were digested similarly except that plasmin was added in the ratio of 4 CTA units/g of protein.

Polyacrylamide Gel Electrophoresis. Proteins were analyzed in two electrophoretic systems: in 7% polyacrylamide gels containing 0.1% $NaDodSO_4$ according to the method of Weber & Osborn (1969) and in nondissociating medium in 9% polyacrylamide gels in Tris–glycine buffer by the method of Davis (1964). Approximately 10 μg of protein was applied per gel and staining was by the method of Fairbanks et al. (1971).

Results

Characterization of Fibrin. Human fibrinogen was incubated with thrombin or batroxobin in the presence of calcium and factor XIII for various times. The first strands of fibrin appeared at 1 min of incubation, $\sim$25% of the fibrinogen had clotted by 3 min, and by 9 min a solid clot had formed. At various times the action of thrombin or batroxobin was inhibited and the fibrin was removed. Unclotted fibrinogen was precipitated from the supernate with ethanol. The fibrin was characterized by three methods: determination of the NH_2-terminal amino acids of the fibrin, analysis of the released fibrinopeptides in the fibrin supernate, and measurement of the extent of factor XIIIa induced γ-chain cross-linked bonds (Table I).

The NH_2-terminal amino acids of fibrinogen are alanine, pyroglutamic acid, and tyrosine (Aα, Bβ, and γ chains, respectively). Cleavage of fibrinopeptides A and B by thrombin produces glycine as the new terminal amino acid on both the α and β chains (Blombäck & Yamashina, 1958). Therefore, fibrinogen should have a glycine/tyrosine ratio of 0 and fibrin which has lost 2 mol each of fibrinopeptides A and B per mol of fibrin monomer would have a glycine/tyrosine ratio of 2.0. In the present work the presence of glycine as an NH_2-terminal amino acid in the fibrinogen preparation was equal to or just above background levels. Therefore, very little, if any, of the fibrinogen had been converted to fibrin monomer prior to the

[1] Abbreviations used: ATU, antithrombin units; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; CTA, Committee on Thrombolytic Agents; KIU, kallikrein inhibitor units.

Table I: Characterization of Fibrin Formed by Thrombin and Batroxobin for Various Times

enzyme	time	Gly/bis (Dns)-Tyr[a]	FPB/FPA[b]	γ-γ/total γ[c]
thrombin	3 min	0.87[d]	0.01[d]	0.45[d]
thrombin	6 min	1.05	0.01	0.613
thrombin	10 min	1.075	0.015	0.916
thrombin	20 min	1.15	0.09	0.987
thrombin	1 h	1.41	0.21	0.991
thrombin	2 h	1.68	0.36	0.993
thrombin	6 h	1.89	0.76	0.990
thrombin	24 h	2.0	0.72	0.991
batroxobin	4 h[e]	1.03	0.015	0.988

[a] Ratio of glycine to tyrosine as NH_2-terminal amino acids; thrombin, 24 h, was equated to 2.0. Bis(Dns)-Tyr represents tyrosine carrying 2 mol of dansyl substitutes per mol of the amino acid. [b] Ratio of fibrinopeptide B to A in the fibrin supernate. [c] Ratio of γ chains in the dimerized form to total γ chains. [d] All the values presented are the average of four determinations. [e] Results on batroxobin fibrins formed after 1, 2, 4, 6, 10, and 24 h were the same, and therefore only the 4-h sample has been shown.

addition of enzymes. Similarly, fibrin which was incubated with thrombin for 24 h had no alanine as an NH_2-terminal amino acid. This indicates that thrombin cleaved all of the fibrinopeptide A during this incubation and suggests that the fibrinogen preparation had not been digested by plasmin in the NH_2-terminal region of the Bβ chain (Takagi & Doolittle, 1975).

After incubation with thrombin or batroxobin, insoluble fibrin was formed; therefore, at least fibrinopeptide A was probably removed from these fibrin monomers (Bailey et al., 1951; Bettelheim, 1956; Bettelheim & Bailey, 1952; Lorand, 1951, 1952; Ferry, 1952; Folk et al., 1959). Since the fibrin which had been incubated with thrombin for 3 min had a glycine/tyrosine ratio of 0.87 and no fibrinopeptide B was recovered from the supernate (Table I), then this fibrin must have lost ~2 mol of fibrinopeptide A and little, if any, fibrinopeptide B per mol of fibrin monomer. Trace amounts of alanine as an NH_2-terminal amino acid were present in this preparation, indicating that some fibrinogen may have been incorporated into the clot. As the time of incubation with thrombin increased, the amount of fibrinopeptide B in the supernate and the ratio of glycine to tyrosine as the NH_2-terminal amino acid increased proportionally (Table I). Fibrin which had reacted with thrombin for 6 h has lost all alanine as an NH_2-terminal amino acid and has a glycine/tyrosine ratio of 1.89, indicating that most, if not all, of the fibrinopeptides A and B have been cleaved. However, the ratio of fibrinopeptide A to B recovered from the supernate was 2:1.5, suggesting that the recovery of fibrinopeptide B is not as complete as that of fibrinopeptide A. This preferential loss of fibrinopeptide B is not unusual, although at the present time no explanation can be proposed (Blombäck et al., 1966). Fibrin formed by the action of batroxobin for 1, 2, 4, 6, 10, or 24 h was all similar, having lost very little, if any, fibrinopeptide B into the supernate and having a glycine/tyrosine ratio of ~1. In Table I only one representative batroxobin fibrin (4 h) is shown.

The amount of factor XIIIa induced cross-link bonds was analyzed by determining the ratio of γ chain in the dimerized form to total γ chains for each fibrin preparation by densitometric scanning of 7% polyacrylamide–$NaDodSO_4$ gels which were run under reducing conditions. By 10 min of thrombin incubation, almost all of the γ chains were cross-linked. It was found that the 10-min thrombin and all batroxobin fibrins have similar characteristics of NH_2-terminal amino acids, fibrinopeptides content, and extent of cross-linking of the γ chains (Table I); therefore, the properties of these preparations can be directly compared.

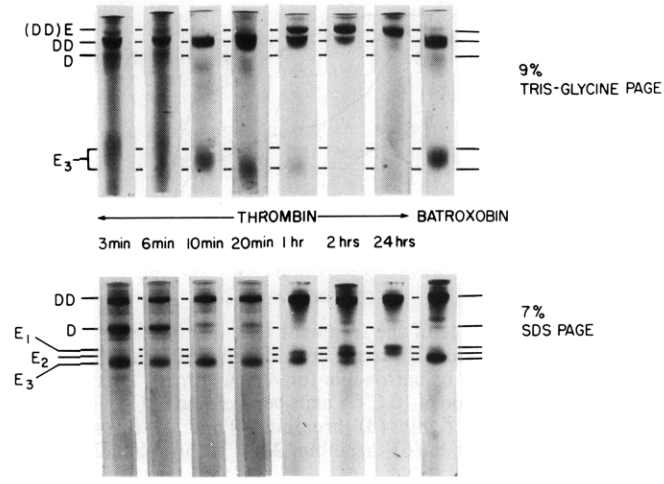

FIGURE 1: Demonstration that the (DD)E complex and fragments E_1 and E_2 are not formed in a digest of batroxobin fibrin. Tris–glycine–polyacrylamide gels (9%) and $NaDodSO_4$–polyacrylamide gels (7%) of 2-h plasmic digests of fibrin formed by the action of either thrombin or batroxobin for various times. Since all digests of batroxobin fibrin had the same electrophoretic pattern, only one of them (4 h) is shown (gel 8). The numbers between gels indicate the time of incubation with thrombin (gels 1–7). The heterogeneous appearance of fragment E_3 on Tris–glycine–polyacrylamide gel electrophoresis is due to the microheterogeneity of this species.

Plasmic Digestion of Fibrins. The cross-linked fibrins formed by thrombin or batroxobin were digested by plasmin in a ratio of 10 CTA units per g of fibrin. At various times aliquots of the supernate were removed from the digestion mixture and then analyzed on 9% polyacrylamide–$NaDodSO_4$ gels. The gels were scanned in a densitometer, and the relative proportions of various fractions were calculated.

The patterns on Tris–glycine and $NaDodSO_4$ gels of the 2-h plasmic digest of each of the fibrins were very different (Figure 1). Fibrin made by the action of thrombin for 3 or 6 min was not completely cross-linked; therefore, the digestion mixture contains a considerable amount of fragment D species in addition to fragments DD and E_3.[2] It appeared that as the time of fibrin incubation with thrombin increased the relative amount of the (DD)E complex produced after 2 h of plasmic digestion also increased (Figure 1). Concomitantly, the relative amount of fragments E_1 and E_2 in the digest increased in parallel with that of the (DD)E complex. Plasmic digestion of batroxobin fibrin produced only fragments DD and E_3 regardless of incubation time.

Figure 2 shows the percentage of the fragment DD moiety which is in the (DD)E complex at each digestion time for all of the cross-linked fibrins. Plasmic digestion of fibrin which has been made by the action of thrombin for 3, 6, or 10 min or by batroxobin produced very little, it any, (DD)E complex, even at very short incubation times (Figure 2). In contrast,

[2] Fragments E_1, E_2, and E_3 from cross-linked fibrin have molecular weights of 60 000, 55 000, and 50 000, respectively, and differ greatly in charge, reflected in their mobility on Tris–glycine–polyacrylamide gel electrophoresis. Fragments E_1 and E_2 maintain the ability to bind to fragment DD, forming a (DD)E complex, while fragment E_3 does not bind to fragment DD. In the present work, fragment E species were catagorized as E_1, E_2, or E_3 based on molecular weight determined by $NaDodSO_4$–polyacrylamide gel electrophoresis, mobility on Tris–glycine–polyacrylamide gel electrophoresis, and the ability to complex with fragment DD.

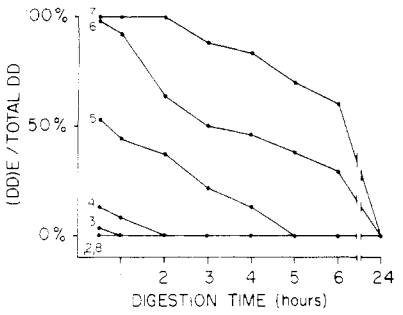

FIGURE 2: Percentage of the fragment DD moiety which is in the (DD)E complex in timed digests of cross-linked fibrin which had been formed by thrombin or batroxobin for various times. Digestion by plasmin was in the ratio of 10 CTA units per g of fibrin. (1) Thrombin, 3 min; (2) thrombin, 6 min; (3) thrombin, 10 min; (4) thrombin, 20 min; (5) thrombin, 1 h; (6) thrombin, 2 h; (7) thrombin, 24 h; (8) batroxobin, 1–24 h.

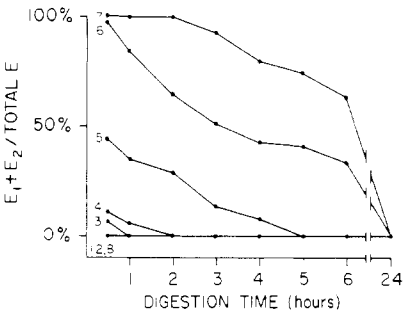

FIGURE 3: Percentage of fragment E species that are either E_1 or E_2 in timed digests of cross-linked fibrin. Digestion was by plasmin in the ratio of 10 CTA units per g of fibrin. (1) Thrombin, 3 min; (2) thrombin, 6 min; (3) thrombin, 10 min; (4) thrombin, 20 min; (5) thrombin, 1 h; (6) thrombin, 2 h; (7) thrombin, 24 h; (8) batroxobin, 1–24 h.

as the time of incubation with thrombin was increased, the amount of (DD)E complex formed during digestion increased in parallel. The digestion pattern of the fibrin formed by thrombin for 6 or 24 h is identical with that seen previously (Olexa & Budzynski, 1979a).

The percentage of the fragment E species that are present as fragments E_1 and E_2 in the timed digests of the fibrins corresponds to the relative amount of the (DD)E complex in the digest (Figure 3). As the time of incubation with thrombin increased, the amount of fragments E_1 and E_2 produced by plasmic degradation of the fibrin increased. Therefore, the loss of fibrinopeptide B from the fibrin enables the formation of fragments E_1 and E_2 which are recovered in a (DD)E complex.

The present evidence indicates that fibrin which has lost fibrinopeptide A only, but not fibrinopeptide B, regardless if formed by thrombin or batroxobin, has a unique plasmic degradation pattern. Upon digestion this fibrin forms fragments DD and E_3 as the primary soluble products rather than the (DD)E complex containing fragments E_1 and E_2. The formed fragment DD was capable of complexing when it was mixed with purified fragment E_1, but the fragment E_3 was not able to bind to purified fragment DD. Thus, it appears that the absence of the (DD)E complex was due to the lack of fragments E_1 and E_2 rather than to an effect on fragment DD. The appearance of the (DD)E complex as a plasmic product of cross-linked fibrin is inversely proportional to the loss of fibrinopeptide B in the fibrin. In cross-linked fibrin which has lost all of the fibrinopeptide B, the (DD)E complex is the primary soluble plasmic degradation product.

Plasmic Digestion of Fibrinogen and Non-Cross-Linked Fibrin. Fibrinogen which contained both fibrinopeptides A and B as well as non-cross-linked fibrin which was formed by thrombin action for 24 h and therefore lacked both fibrinopeptides A and B was digested by plasmin in the ratio of 4 CTA units per g of protein. The fragment E species from fibrinogen were treated with thrombin (1 unit/mg, 4 h at 37 °C) to remove all fibrinopeptides before comparison with fragments E_1, E_2, and E_3 from fibrin. Plasmic digestion of fibrinogen produced only fragment E_3; however, digestion of non-cross-linked fibrin produced small amounts of fragment E_2 in addition to fragment E_3 after very short digestion times. This fragment E_2 was quickly cleaved to fragment E_3. It is not known if fragment E_1 is formed during plasmic digestion of non-cross-linked fibrin and quickly degraded or if it is not formed at all. The patterns of fragment E species produced during plasmic digestion of fibrinogen and non-cross-linked fibrin are similar to those noted previously (Olexa & Budzynski, 1979b). The data indicate that non-cross-linked fibrin, even if all of the fibrinopeptides A and B have been removed, does not form a significant amount of fragments E_1 and E_2 upon plasmic digestion.

Discussion

Human cross-linked fibrin which lacks both fibrinopeptides A and B is digested by plasmin according to the scheme $(DD)E_1 \rightarrow (DD)E_2 \rightarrow DD + E_3$. Thus, the (DD)E complex is the primary soluble plasmic degradation product of cross-linked fibrin, while fragments DD and E_3 are the terminal products. This pattern is consistent, regardless of the plasmin/fibrin ratio; however, the rate of the terminal product formation depends upon the enzyme concentration (Olexa & Budzynski, 1979a).

The present work indicates that fibrinopeptides have a significant effect on the pattern of plasmic degradation of fibrin. The appearance of the (DD)E complex as an early digestion product is directly dependent upon the release of fibrinopeptide B from fibrin. Fibrin which has lost fibrinopeptide A only forms fragments DD and E_3 after plasmic action (Table I, Figures 2 and 3). Therefore, in vivo the plasmic degradation products of cross-linked fibrin would depend not only on the relative plasmin concentration but also on thrombin action as measured by the presence or absence of fibrinopeptide B.

During the plasmic digestion of fibrin the fragment E_1 moiety is extremely susceptible to further cleavage to fragment E_3. It appears that fragment E_1 is protected against proteolysis by being a part of the (DD)E complex since in non-cross-linked fibrin which has lost all fibrinopeptides A and B this species is quickly digested. In non-cross-linked fibrin the association of fragment E species with the aligned fragment D regions in the fibrin fiber or the formation of a weak (D)E complex during digestion may offer some protection to the fragment E species. Similarly, during the digestion of fibrinogen, species corresponding to fragments E_1 and E_2 may be formed but are probably rapidly degraded. Thus, fragments E_1 and E_2 can be associated with the degradation of cross-linked fibrin, specifically fibrin which has lost both fibrinopeptides A and B, and may be useful markers of fibrinolysis even in the presence of extensive fibrinogenolysis.

Ferry (1952) postulated that polymerization of fibrin monomer molecules takes place through two processes, initially by an end-to-end association of molecules forming a fibrin strand and secondarily by a lateral association of the strands. Steiner & Laki (1951) used light scattering to differentiate between the two steps in polymerization. Laurent & Blombäck (1958) suggested that fibrinopeptides A and B had different functions; that is, the release of fibrinopeptide A appeared to

promote end-to-end polymerization while removal of fibrinopeptide B led to lateral association of strands. Laudano & Doolittle (1978) showed that the tripeptide Gly-Pro-Arg, which is contiguous with fibrinopeptide A, can bind to fibrinogen and can inhibit fibrin monomer polymerization, indicating that this peptide may be an NH_2-terminal polymerization site. In a later work the same investigators demonstrated that Gly-His-Arg, which is the peptide following fibrinopeptide B, also binds to fibrinogen; however, it does not inhibit polymerization (Laudano & Doolittle, 1979). The present results indicate that the removal of fibrinopeptide B does reveal a site which affects the structure of the fibrin clot. The formation of the (DD)E complex as a plasmic degradation product appears to be regulated by the loss of fibrinopeptide B. Therefore, the binding sites on the NH_2-terminal region of fibrin monomer, i.e., on fragment E, which are complementary to those on fragment DD are likely to be revealed or augmented by the loss of fibrinopeptide B. Fragments E_1 and E_2 bind to the aligned fragment D regions in fragment DD forming the complex but do not bind to fibrinogen, fibrin monomer, or fragments X, Y, or D (Olexa & Budzynski, 1979b). These observations indicate that the binding sites on fragments DD and E_1 or E_2 which are holding together the (DD)E complex are not active during the end-to-end polymerization of fibrin but may be involved in side-to-side aggregation of fibrin strands.

References

Bailey, K., Bettelheim, F. R., Lorand, L., & Middlebrook, W. R. (1951) *Nature (London) 167*, 233–234.

Bettelheim, F. R. (1956) *Biochim. Biophys. Acta 19*, 121–130.

Bettelheim, F. R., & Bailey, K. (1952) *Biochim. Biophys. Acta 9*, 578–579.

Bilezikian, S. B., Nossel, H. L., Butler, V. P., Jr., & Canfield, R. E. (1975) *J. Clin. Invest. 56*, 438–445.

Blombäck, B., & Yamashina, I. (1958) *Ark. Kemi 12*, 299–319.

Blombäck, B., Blombäck, M., & Nilsson, I. M. (1957) *Thromb. Diath. Haemorrh. 1*, 76–86.

Blombäck, B., Blombäck, M., Edman, P., & Hessel, B. (1966) *Biochim. Biophys. Acta 115*, 371–396.

Blombäck, B., Hessel, B., Hogg, D., & Therkildsen, L. (1978) *Nature (London) 275*, 501–505.

Budzynski, A. Z., Marder, V. J., & Sherry, S. (1975) *Blood 45*, 757–768.

Chen, R. F. (1967) *Arch. Biochem. Biophys. 120*, 609–620.

Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.

Fairbanks, G., Steck, T. L., & Wallach, D. F. H. (1971) *Biochemistry 10*, 2606–2617.

Ferguson, E. W., Fretto, L. J., & McKee, P. A. (1975) *J. Biol. Chem. 250*, 7210–7218.

Ferry, J. D. (1952) *Proc. Natl. Acad. Sci. U.S.A. 38*, 566–569.

Folk, J. E., Gladner, J. A., & Laki, K. (1959) *J. Biol. Chem. 234*, 67–70.

Furlan, M., Seelich, T., & Beck, E. A. (1976) *Thromb. Haemostasis 36*, 582–592.

Gaffney, P. J., & Brasher, M. (1973) *Biochim. Biophys. Acta 295*, 308–313.

Gaffney, P. J., Lane, D. A., Kakkar, V. V., & Brasher, M. (1975) *Thromb. Res. 7*, 89–99.

Gladner, J. A., Folk, J. E., Laki, K., & Carroll, W. R. (1959) *J. Biol. Chem. 234*, 62–66.

Gormsen, J., & Feddersen, C. (1973) *Scand. J. Haematol. 10*, 337–348.

Gray, W. R. (1972) *Methods Enzymol. 25*, 121–138.

Hartley, B. S. (1970) *Biochem. J. 119*, 805–822.

Hudry-Clergeon, G., Marguerie, G., Pouit, L., & Suscillon, M. (1975) *Thromb. Res. 6*, 533–541.

Kopeč, M., Teisseyre, E., Dudek-Wojciechowska, G., Kloczewiak, M., Pankiewicz, A., & Latallo, Z. S. (1973) *Thromb. Res. 2*, 283–291.

Laudano, A. P., & Doolittle, R. F. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3085–3089.

Laudano, A. P., & Doolittle, R. F. (1979) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 38*, 792.

Laurent, T. C., & Blombäck, B. (1958) *Acta Chem. Scand. 12*, 1875–1877.

Loewy, A. G., Dunathan, K., Kriel, R., & Wolfinger, H. L., Jr. (1961) *J. Biol. Chem. 236*, 2625–2633.

Lorand, L. (1951) *Nature (London) 167*, 992–993.

Lorand, L. (1952) *Biochem. J. 52*, 200–203.

Marder, V. J., Budzynski, A. Z., & Barlow, G. H. (1976) *Biochim. Biophys. Acta 427*, 1–14.

Moffat, E. D., & Lytle, R. I. (1959) *Anal. Chem. 31*, 926–928.

Olexa, S. A., & Budzynski, A. Z. (1979a) *Biochemistry 18*, 991–995.

Olexa, S. A., & Budzynski, A. Z. (1979b) *J. Biol. Chem. 254*, 4925–4932.

Pizzo, S. V., Schwartz, M. L., Hill, R. L., & McKee, P. A. (1973a) *J. Biol. Chem. 248*, 4574–4583.

Pizzo, S. V., Taylor, L. M., Jr., Schwartz, M. L., Hill, R. L., & McKee, P. A. (1973b) *J. Biol. Chem. 248*, 4584–4590.

Shainoff, J. R., & Page, I. H. (1960) *Circ. Res. 8*, 1013–1022.

Steiner, R. F., & Laki, K. (1951) *Arch. Biochem. Biophys. 34*, 24–27.

Szilágyi, I., & Szabó, I. (1958) *Nature (London) 181*, 52–53.

Takagi, T., & Doolittle, R. F. (1975) *Biochemistry 14*, 940–946.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.

Woods, K. R., & Wang, K. T. (1967) *Biochim. Biophys. Acta 133*, 369–370.