Differences in the Clotting of Lamprey Fibrinogen by Lamprey and Bovine Thrombins

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(Received 4 March 1964)

1. Improved methods for the purification of lamprey thrombin and fibrinogen are presented. 2. Lamprey thrombin releases two fibrinopeptides from lamprey fibrinogen during the transformation into fibrin. Bovine thrombin releases only one of these, a peptide referred to as fibrinopeptide B. The differences in the by-products of fibrin formation are reflected in the different *N*-terminal amino acid compositions of the two types of fibrin. 3. The fibrinopeptide that is not removed from the lamprey fibrinogen by bovine thrombin can subsequently be released by treatment of that fibrin with lamprey thrombin. 4. Under the conditions used, lamprey thrombin releases both fibrinopeptides at about the same rate. 5. The differences in interaction among these pairs of related proteins are extreme manifestations of the phenomenon loosely referred to as 'species specificity'.

Previous reports on the properties of lamprey (*Petromyzon marinus*) thrombin and fibrinogen have indicated that the fundamental chemistry of their interaction is similar to that of mammalian thrombin-fibrinogen systems (Doolittle, Oncley & Surgenor, 1962; Doolittle, Lorand & Jacobsen, 1963). As in mammalian systems, the clotting of lamprey fibrinogen appears to depend on the release of fibrinopeptide material effected by the proteolytic cleavage of arginyl-glycine peptide bonds. Apart from this central action, however, lamprey thrombin and fibrinogen have been found to differ from their mammalian homologues in several ways, and the interaction of either of these with their mammalian counterparts results in delayed clotting times. Further, the clotting of bovine fibrinogen by lamprey thrombin, a reaction that proceeds very slowly, has been reported to occur with release of only one of the two known bovine fibrinopeptides (Doolittle *et al.* 1962).

The accumulation of larger amounts of lamprey blood plasma than were previously available has made it possible to extend this study in greater detail. The present paper deals with improved methods for the purification of lamprey thrombin and fibrinogen and describes differences in the fibrinopeptide material resulting from the clotting of lamprey fibrinogen by lamprey thrombin on the one hand and bovine thrombin on the other.

MATERIALS AND METHODS

Lamprey blood plasma. Citrate-treated lamprey blood plasma (approx. 9 vol. of blood/vol. of 4% trisodium citrate) was frozen and shipped by air from the U.S.A. Blood had been drawn from animals entering various New England (U.S.A.) streams at the beginning of the spawning season. One batch of about 3 l. of plasma was collected specifically for this study, the blood being drawn and processed according to the methods described by Doolittle *et al.* (1962). This plasma is referred to below as batch I. Another batch of approx. 8 l. of lamprey plasma was generously provided by workers studying lamprey haemoglobin. This plasma, referred to below as batch II, was of necessity heavily haemolysed, and the yield and quality of fibrinogen was always inferior to that obtained from batch I.

Fibrinogen. The lamprey fibrinogen was prepared by modifications of the Cohn ethanol fractionation scheme (Cohn *et al.* 1946) and the employment of an $(\text{NH}_4)_2\text{SO}_4$ precipitation step (Laki, 1951). A fraction I precipitate was prepared as described by Doolittle *et al.* (1962) and dissolved in one-half of the original plasma volume of 0.2 M-NaCl containing 0.04% of trisodium citrate. Then 1 vol. of saturated $(\text{NH}_4)_2\text{SO}_4$ (adjusted to neutral pH with conc. NH_3) was added to 3 vol. of the fraction I solution at room temperature. The resulting precipitate was collected by centrifugation and redissolved in one-third of the original plasma volume of 0.1 M-ammonium acetate. Reprecipitation of the fibrinogen was effected under conditions of 8% (v/v) ethanol at -3° . The precipitate was dissolved in 0.15 M-ammonium acetate, pH 7.0, and the preparation was ready for clotting. On some occasions the final ethanol precipitation step was repeated one or more times. The fibrinogen preparation from batch I always contained more than 95% of clottable protein, as determined by measuring

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the extinction (at 280 $m\mu$) of the clot liquor after clotting. About 1 g. of purified fibrinogen was obtained from each litre of the citrate-treated plasma. The clottability of the fibrinogen obtained from the batch II plasma was usually between 85 and 92%. Only about 0.5 g. of fibrinogen was obtained from each litre of the citrate-treated plasma in this case.

Lamprey thromboplastin. Thromboplastin for use in the conversion of lamprey prothrombin into thrombin was prepared from lamprey skin that had been stored frozen for up to 2 years (Doolittle & Surgenor, 1962). A 20 g. (wet wt.) portion of the frozen skin was minced well into 100 ml. of cold 0.2 M-sucrose and homogenized in a Waring Blendor (Turmix) for 5 min. The Blendor was rinsed with 100 ml. of distilled water and the washings were added to the homogenate. The preparation was centrifuged at 800 g for 15 min. and the precipitated material discarded. The active supernatant fluid could be stored frozen at -20° for extended periods.

Thrombin. The unavailability of fresh lamprey plasma necessitated the preparation of prothrombin from the supernatant fluid of fraction I, rather than by immediate adsorption from fresh oxalate-treated plasma on to $BaSO_4$ (Doolittle *et al.* 1962). A fraction corresponding to Cohn fractions II + III (Cohn *et al.* 1946) was obtained by the addition of cold 50% (v/v) ethanol to give a final concentration of 25%. The preparation was stirred gently at -5° , centrifuged, and the precipitate dissolved in one-third of the original plasma volume of 0.08 M-NaCl. The prothrombin could not be adsorbed on to a $Mg(OH)_2$ suspension under conditions where mammalian thrombins are known to be adsorbed (Seegers, Loomis & Vandenbelt, 1945). Because of this, the prothrombin was converted into thrombin at this stage by the addition of lamprey thromboplastin, imidazole buffer and Ca^{2+} ions. In a typical preparation, 200 ml. of the dissolved fractions II + III, representing 600 ml. of citrate-treated plasma, were mixed with 20 ml. of thromboplastin suspension, 20 ml. of 0.1 M-imidazole buffer, pH 7.4, and 50 ml. of 0.1 M- $CaCl_2$. Maximum thrombin activity was usually generated in 30–60 min. at room temperature. Samples were removed periodically and the time necessary to clot fibrinogen solutions was measured.

A preliminary concentration of the thrombin activity was obtained by precipitating an inactive fraction under conditions of 10% (v/v) ethanol at -2° . The thrombin was subsequently precipitated by the addition of an equal volume of 50% ethanol to the supernatant fluid at -5° . After centrifugation, the precipitate was dissolved in a small volume of distilled water and stored at -20° for further use. The preparation also contained powerful fibrinolytic activity, and both lamprey and bovine fibrin clots formed by the action of this crude material tended to lyse after prolonged incubations.

Considerable purification was obtained by gel-filtration of the crude thrombin preparation on Sephadex G-100 (Pharmacia, Uppsala, Sweden). The thrombin activity lags behind the main protein peak (Fig. 1), and the subsequent purification is about 20-fold when calculated on the basis of thrombin activity/unit of extinction at 280 $m\mu$. Fibrinolytic activity emerges from the column with the main protein peak. The thrombin peaks were pooled and freeze-dried. The material was dissolved in an original volume of distilled water and freeze-dried a second time to

Fig. 1. Gel-filtration of a crude lamprey thrombin preparation on a Sephadex G-100 column (2.5 cm. $\times$ 80 cm.). A 10 ml. sample of a solution containing about 400 mg. of protein was applied. The equilibrating and eluting solution was 0.1 M-ammonium acetate containing 0.02% of BRIJ (detergent). The temperature was 5° . The flow rate was 34 ml./hr. The fractions were assayed for clotting activity on bovine fibrinogen. ○, Thrombin activity expressed as reciprocal of clotting time (min.) $\times 10^3$; ●, extinction at 280 $m\mu$.

remove the ammonium acetate. The doubly-dried white powder contained roughly the equivalent of 50 N.I.H. thrombin units/mg. when assayed on lamprey fibrinogen.

The action of lamprey thrombin on bovine fibrinogen, as reported by Doolittle *et al.* (1962), results in clotting times that are 30–50 times greater than when lamprey fibrinogen is used as a substrate. To conserve lamprey fibrinogen, however, columns were frequently monitored with bovine fibrinogen as a substrate. In those cases the clotting times were measured in minutes instead of seconds.

Further purification of the gel-filtered thrombin was accomplished with Amberlite IRC-50 resins by the method of Rasmussen (1955). Both XE-64 (200–400 mesh) and XE-97 (200–400 mesh) types of resin were found to be suitable. The degree of purification was generally about tenfold (Fig. 2), but the yields were not good and never greater than 50%. Some denaturation may occur in the process, which must be conducted at room temperature. Phosphate salts were removed by passing the freeze-dried thrombin peak, dissolved in a small volume of water, over a Sephadex G-25 column (2.5 cm. $\times$ 50 cm.) equilibrated and developed with 0.1 M-ammonium acetate.

Clotting. For conversion of the fibrinogen into fibrin, the fibrinogen concentration of solutions was adjusted to about 0.4% with 0.15 M-ammonium acetate, and thrombin equivalent to 1–2 N.I.H. units/ml. added. In the rate studies, only one-tenth as much thrombin was added. Usually the fibrin gels were kept at room temperature for about 1 hr., after which the fibrin was removed on a glass rod and the clot liquor filtered. The fibrin was washed with water, the washings being added to the clot liquor and the pooled solution freeze-dried twice. The washed fibrin was frozen until used for *N*-terminal amino acid analyses. The residue obtained after the freeze-drying of the clot liquors was dissolved in a small volume of water and transferred to Pyrex centrifuge tubes. The tubes were heated in a boiling-water bath in an effort to remove traces of unclotted proteins. Any coagulum that formed was removed by centrifugation, and, when necessary, the precipitate was washed and the

Fig. 2. Chromatography of semi-purified lamprey thrombin on an Amberlite IRC-50 (XE-97; 200–400 mesh) column (0.9 cm. $\times$ 32 cm.). A 2 ml. sample was adsorbed with 0.05 M-sodium phosphate buffer, pH 6.9, and eluted with 0.3 M-sodium phosphate buffer, pH 8.0. The temperature was approx. 22°. The fractions were assayed on lamprey fibrinogen. ○, Thrombin activity expressed as reciprocal of clotting time (sec.) $\times 10^4$; ●, extinction at 280 μ .

procedure repeated on the washings. This treatment was found to be necessary only for preparations derived from the plasma designated batch II. After a final freeze-drying, the residues were dissolved in small amounts of water for electrophoretic analysis.

Paper electrophoresis of freeze-dried clot liquors. Paper electrophoresis was carried out with an LKB model 3276 horizontal-type apparatus. An appropriate volume of the dissolved residues was streaked across the centre line of 4 cm.-wide paper strips (Schleicher and Schull no. 2043). A 0.1 M-pyridine-acetic acid buffer, pH 4.1, was used in all experiments. The usual conditions were 300 v (8.5 v/cm.) at room temperature for 4 hr. The papers were dried in an oven at 80° and then stained with a ninhydrin reagent (McMenamy, Lund & Oncley, 1957). Tests for ninhydrin-negative peptides were performed by the chlorination method (Rydon & Smith, 1952). Strips were also developed with a Sakaguchi reagent (Acher & Crocker, 1952). Where the peptides were to be eluted, guide strips about 2 mm. wide were cut from the edge of papers and stained appropriately. The conditions employed for characterization of the eluted material, as well as for its purification on a larger scale, are described by Doolittle (1965).

N-Terminal analysis of fibrinogens and fibrins. The phenyl isothiocyanate method of Edman (1950) was used, as applied to mammalian fibrinogens by Blombäck & Yamashina (1958). The PTH-amino acids* were quantitatively determined by using the paper-chromatographic system of Sjöquist (1960), the spectra of the eluted PTH-amino acids being determined on a Beckman DK-2a ratio recording spectrophotometer. The difficulty of separating PTH-aspartic acid from PTH-serine was overcome by taking advantage of the relatively harsh conditions

during cleavage and conversion (1 N-HCl at 100° for 1 hr.). Recovery experiments revealed that the labile serine derivatives were effectively destroyed in the process. With lamprey fibrin A (lamprey fibrin formed through the action of lamprey thrombin), which was first shown to be free of N-terminal aspartic acid, N-terminal serine was successfully measured by changing the cleavage and conversion conditions to those suggested by Fraenkel-Conrat & Harris (1954) in which the conditions are 3 N-HCl at room temperature for 3.5 hr. In no cases were the aqueous phases examined for PTH-arginine and PTH-histidine.

Positive identification of PTH-aspartic acid and PTH-glutamic acid was obtained by alkaline hydrolysis of the derivatives in 50 μ l. of 0.2 M-Ba(OH)₂ at 110° for 20 hr. in sealed glass tubes. The hydrolysates were spotted directly on paper strips and electrophoresis was conducted as described above. All reagents were washed and redistilled as recommended by Edman (1950) and Sjöquist (1960). Protein determinations on samples from the various preparations were determined either by dry-weight measurements on extensively dialysed samples or spectrophotometrically at 280 μ assuming $E_{1\%}^{1\text{cm}}$ to be 15 for the fibrinogen and urea-dispersed fibrins (cf. Blombäck & Blombäck, 1956).

Rate of disappearance of fibrinogen end groups. In those experiments designed to follow the rate of change of the various end groups during the transformation of lamprey fibrinogen into fibrin, the conditions were essentially the same as those employed by Blombäck & Laurent (1958) in their studies on mammalian fibrinogens. The fibrinogen concentration in these experiments was about 0.1%, and the pH was maintained at 7.4 with 0.1 M-imidazole buffer. Clotting was performed with enough lamprey thrombin to ensure that the first fibrin threads appeared after about 3 min. The phenyl isothiocyanate coupling time was extended to 3.5 hr. at room temperature to ensure complete coupling to the solid-phase fibrin, and two 50% acetone washes were introduced to ensure removal of the larger lamprey fibrinopeptide.

Other materials. The bovine thrombin was prepared by Dr S. Magnusson and contained about 300 N.I.H. units/mg. Bovine fibrinogen (fraction 1–4) was a generous gift from Dr B. Blombäck.

RESULTS

N-Terminal amino acids. Lamprey fibrinogen was found to contain N-terminal aspartic acid and glutamic acid by the phenyl isothiocyanate method (Table 1). The identification of these two amino acids was verified by alkaline hydrolysis of the PTH-amino acids to yield free aspartic acid and glutamic acid. N-Terminal serine would not be recovered by the procedure used to analyse the fibrinogen.

Lamprey fibrin produced by the action of lamprey thrombin, designated fibrin A, contained only N-terminal glycine when studied by the Blombäck & Yamashina (1958) modification of the Edman method (Table 1). The number of glycine end groups roughly corresponded to the sum of N-terminal aspartic acid and glutamic acid residues in the fibrinogen. In addition, N-terminal serine

* Abbreviation: PTH-amino acids, phenylthiohydantoin derivatives of amino acids.

Table 1. *N*-Terminal amino acids found in lamprey fibrinogen and fibrins by the phenyl isothiocyanate method

Fibrin A was produced with lamprey thrombin, fibrin B with bovine thrombin, and fibrin C by treating fibrin B with lamprey thrombin. Fibrin A-X is identical with fibrin A, but the conditions of analysis were changed to a method more suitable for the recovery of PTH-serine. Trace indicates less than 0.2 mole/330 000 g.; — indicates no spot detectable on paper chromatograms for those PTH-amino acids. Values were calculated on basis of 75% recovery for PTH-glycine and 60% for other PTH-amino acids (cf. Blombäck & Yamashina, 1958). Because of the different conditions employed in analysing fibrin A-X, the yields in that experiment are not strictly comparable with the other values listed.

Preparation	Plasma batch	<i>N</i> -Terminal amino acid (moles/330 000 g.)				
		Glutamic acid	Aspartic acid	Glycine	Serine	Alanine
Fibrinogen	Ic	1.9	1.7	—	—	—
	IIb	2.2	2.2	Trace	—	—
Fibrin A	IIa	0.3	Trace	3.9	—	Trace
	IIb	Trace	Trace	5.2	—	0.5
Fibrin B	Ib	Trace	1.7	1.6	—	—
	IIb	Trace	1.4	2.5	—	Trace
Fibrin C	Ia	—	Trace	4.4	—	—
Fibrin A-X	IIId	—	—	2.6	1.7	—

was detected in fibrin A (fibrin A-X in Table 1) when milder conditions were employed.

Identical results were obtained with the semi-purified (Sephadex G-100 only) and the further purified (Amberlite, IRC-50) lamprey thrombin preparations. In a few preparations, clotted with crude lamprey thrombin, small amounts of PTH-alanine were also detected after sustained digestions. It is likely that the *N*-terminal alanine represented an end group exposed by a fibrinolytic enzyme.

The clots formed by the action of purified bovine thrombin on lamprey fibrinogen were termed fibrin B. These preparations did not gel as firmly as duplicate preparations clotted with lamprey thrombin, even though enough bovine thrombin was often added to induce shorter clotting times than occurred in the lamprey-lamprey preparations. Only the glutamic acid end groups disappeared from the lamprey fibrinogen during the clotting effected by bovine thrombin (Table 1), an equivalent amount of *N*-terminal glycine appearing in the fibrin. The aspartic acid end groups were not significantly changed during the clotting process.

Identification of fibrinopeptides released from lamprey fibrinogen. Since the *N*-terminal amino acids of fibrins A and B were different, it was reasonable to expect that something was released during the formation of fibrin A that was not released during the formation of fibrin B. Only one major lamprey fibrinopeptide had previously been reported (Doolittle *et al.* 1962). In the present experiments, a second fibrinopeptide was found to be released by lamprey thrombin. This peptide is only slightly electronegative at pH 4.1 and did not move far

Fig. 3. Positions of ninhydrin-positive (N) and Sakaguchi-positive (S) materials from clot liquors on paper-electrophoresis strips. (a) Clot liquor after clotting with lamprey thrombin; (b) clot liquor after clotting with bovine thrombin; (c) reference amino acids. Cross-hatched areas were strongly evident; stippled areas varied with the quality of the fibrinogen preparation. Electrophoresis was carried out in 0.1 M-pyridine-acetic acid buffer, pH 4.1, at 300 v for 4 hr.

from the origin during paper electrophoresis (Fig. 3). For reasons that became apparent later, the peptide is now designated fibrinopeptide A. Under the same conditions of electrophoresis, the previously reported 'major' lamprey fibrinopeptide (Doolittle *et al.* 1962), referred to below as fibrinopeptide B, moved towards the anode with a mobility similar to that of glutamic acid (Fig. 3). The Sakaguchi-positive fibrinopeptide B was identified in the clot liquors of both fibrin A and fibrin B. Small amounts of other ninhydrin-positive material were usually found at and around the origin of the paper strips, the amount of contamination varying

with the quality of the fibrinogen preparation. Some of these contaminants were found to be free amino acids. When a given fibrinogen preparation was divided in half, however, and half clotted by lamprey thrombin and half by bovine thrombin, the only detectable difference between the two resulting paper strips was the presence of the ninhydrin-positive strongly Sakaguchi-positive fibrinopeptide A in the lamprey-lamprey preparation.

Conversion of fibrin B into fibrin A. It was decided to find out if lamprey fibrin B, produced by the action of bovine thrombin, could be converted into fibrin A by the action of lamprey thrombin, the more so since a large amount of lamprey fibrinogen had been clotted with bovine thrombin under the assumption that the reaction products of the lamprey and mammalian thrombins were the same (Doolittle *et al.* 1962). Characterization of the newly discovered fibrinopeptide A would be considerably facilitated if the peptide could be released from fibrin B. Accordingly, fibrin B, which had been stored frozen, was minced into small pieces with scissors and suspended in 0.2 M-ammonium acetate. Lamprey thrombin equivalent to 1 N.I.H. thrombin unit/ml. was added, and the fibrin pieces were stirred at room temperature for 3 hr. The fibrin particles were filtered off and the filtrate was freeze-dried. The material contained traces of free amino acids and other contaminating substances but was rich in fibrinopeptide A. Control experiments were run simultaneously in which were stirred (a) the minced fibrin B alone, (b) fibrin B with additional bovine thrombin present, and (c) the lamprey thrombin alone. Very small amounts of fibrinopeptide A were identified in the freeze-dried filtrates of (a) and (b) and none in (c). The same contaminating substances were found in the (a) and (b) controls as were found in the experimental mixtures. It was assumed that the small amounts of fibrinopeptide A detected in the two fibrin B controls arose from the action of contaminating lamprey thrombin in the original fibrinogen preparation. The only *N*-terminal amino acid found in this doubly-degraded lamprey fibrin, termed fibrin C in Table 1, was glycine. The amounts found were approximately the same as were found for fibrin A, and twice that of fibrin B. Conditions appropriate for the detection of *N*-terminal serine were not employed.

Rate of change of *N*-terminal residues during the formation of fibrin A. It was decided to find out if there was any significant difference in the rate of disappearance of the different end groups during the production of fibrin A, since in the two mammalian systems that have been studied (Blombäck & Laurent, 1958; Shainoff & Page, 1960) fibrinopeptide A is released much more rapidly than is fibrinopeptide B. At pH 7.4, the aspartic acid and

Fig. 4. Changes in the *N*-terminal amino acid pattern during clotting of lamprey fibrinogen by lamprey thrombin. A single preparation of fibrinogen was divided into four equal portions and the same amount of thrombin added to each. The clotting reactions were stopped at intervals by the addition of pyridine containing phenyl isothiocyanate. The first strands of fibrin appeared in the 4 min. and 6 min. preparations at about 3 min. *N*-Terminal residues are expressed as percentages of total end groups recovered at each time-interval. Incubation was at 23°, pH 7.4 and 1.0-2.0, the fibrinogen concentration being 1.2%. *N*-Terminal serine would not be detected by this method, nor was the aqueous phase examined for PTH-arginine and PTH-histidine. Full details are given in the text. O, PTH-glutamic acid; Δ, PTH-aspartic acid; ●, PTH-glycine.

glutamic acid end groups disappear from lamprey fibrinogen at about the same rate (Fig. 4), indicating that the lamprey thrombin releases both fibrinopeptides concurrently.

DISCUSSION

The results reported above extend preliminary observations on the mechanism of fibrinogen conversion in one of the most primitive vertebrates extant, the lamprey. On the basis of these results some previous conclusions have had to be revised. Thus the by-products of fibrinogen conversion are different, depending on whether lamprey or bovine thrombin is used as the clotting agent. In both cases one very electronegative peptide is released. This peptide, now designated fibrinopeptide B, is the previously reported 'major' fibrinopeptide. The second peptide, liberated only by lamprey thrombin, was not detected in previous experiments with smaller amounts of materials. It is likely that the peptide was not distinguished from the contaminatory material that frequently occurs

at the origin of the paper-electrophoresis strips in systems employing less than 97% of clottable fibrinogen. Fibrinopeptide A is not identical with the previously reported 'minor' peptide (Doolittle *et al.* 1962), a contaminant that was found to disappear when the fibrinogen preparations were purified to greater than 95% of clottable protein.

The distribution of *N*-terminal amino acids in lamprey fibrinogen has also had to be revised. In previous work (Doolittle *et al.* 1963) *N*-terminal amino acid analysis with the Sanger fluorodinitrobenzene reagent revealed that lamprey fibrinogen contained aspartic acid end groups that were replaced by glycine during the transformation into fibrin. In addition, both the fibrinogen and fibrin contained *N*-terminal serine. The discovery of *N*-terminal glutamic acid in lamprey fibrinopeptide B led to a re-examination of the end groups in the fibrinogen and fibrin and the subsequent discovery of the fibrinopeptide A. It is possible that the failure to detect the glutamic acid end group when the Sanger method was used can be attributed to difficulties in distinguishing DNP-glutamic acid from DNP-aspartic acid by paper chromatography. In that report, however, the quantitative values indicated a clear difference in the *N*-terminal amino acids of the fibrins, depending on whether

lamprey or human thrombin had been used to effect the conversion. The fibrin preparation clotted with human thrombin still contained *N*-terminal aspartic acid and had only about half as much glycine as had the fibrins clotted with lamprey thrombin.

Confirmation of the previously reported serine end groups in the lamprey fibrinogen and fibrins was hindered somewhat by the difficulties inherent in the measurement of PTH-serine in the presence of PTH-aspartic acid. With lamprey fibrin A, however, all of the aspartic acid end groups were shown to be removed. It was then possible to demonstrate the presence of the serine end group by changing to milder cleavage and conversion conditions. The serine end group was positively identified by its characteristic spectrum after heating the paper chromatogram and eluting the derivative (Sjöquist, 1960). The existence of *N*-terminal serine in fibrin A corroborates the detection of *N*-terminal serine in both lamprey fibrin and fibrinogen when the Sanger method was used. It seems likely that this peptide chain in lamprey fibrinogen and fibrin corresponds to the one in mammalian fibrinogens and fibrins that ends in tyrosine (cf. Blombäck & Yamashina, 1958).

Scheme 1. Summary of changes occurring in lamprey fibrinogen when exposed to lamprey or bovine thrombins. Full details are given in the text.

The distribution of end groups in lamprey fibrinogen and fibrins on a mole-per-weight basis is similar to that reported for several mammalian fibrinogens (Blombäck & Yamashina, 1958). These facts seem consistent with the report that the sedimentation coefficient ($S_{20,w}$ 8s) of lamprey fibrinogen is similar to that of mammalian fibrinogens (Doolittle *et al.* 1962).

A summary of the changes occurring during the transformation of lamprey fibrinogen into different forms of fibrin is depicted in Scheme 1. On the basis of the *N*-terminal amino acid studies, it was expected that fibrinopeptide A should have aspartic acid as its *N*-terminal residue whereas fibrinopeptide B should have *N*-terminal glutamic acid. This conclusion was subsequently verified (Doolittle, 1965).

The interaction of thrombins and fibrinogens from lower vertebrates with fibrinogens and thrombins from mammals proceeds to completion in terms of the amount of fibrinogen that is converted into fibrin. The rates at which the interaction and subsequent conversion take place, however, are subject to hindrances usually attributed to 'species specificity', by which it is inferred that the thrombins and fibrinogens from one group of animals are mutually co-adapted. In spite of this, there is growing evidence that all vertebrate fibrinogen molecules have sprung from a common ancestral type. In all mammals that have been examined, only arginyl-glycine peptide bonds are split by thrombin (Blombäck & Yamashina, 1958; Blombäck & Doolittle, 1963). With the lamprey fibrinogen conversion, only glycine end groups appear on the fibrin after the removal of the fibrinopeptides, and evidence is presented by Doolittle (1965) to show that the *C*-terminal amino acid of both peptides is arginine. In spite of this common linkage being split in mammalian and lamprey fibrinogens, the interaction of the distantly related thrombins in both cases removes only one of the fibrinopeptides, whereas the animal's own thrombin, or one from a closely related animal, removes at least two peptides. One must conclude from this that the specificity of various thrombins, though probably specific for arginyl-glycine links, can also be

narrowed by other aspects of the fibrinogen structure. Some indications of what factors might be involved in such a structural specificity are provided by the amino acid-sequence studies reported by Doolittle (1965).

I express my gratitude to Professor E. Jorpes and Dr B. Blombäck, in whose Laboratories these experiments were conducted. It is a pleasure to thank the many students at Amherst College (U.S.A.) who collected lamprey blood for this study, and Dr W. Love of the Marine Biological Laboratory, Woods Hole, U.S.A., for his gift of lamprey plasma. The technical assistance of Mr P. Nilsson and Miss Gunilla Croneborg was invaluable. This investigation was supported by U.S. Public Health Service Fellowship no. HE-14057 and by Grant no. HE7379-01, U.S. National Institutes of Health, to Dr B. Blombäck.

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