Allosteric Effects Potentiating the Release of the Second Fibrinopeptide A from Fibrinogen by Thrombin*

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Fibrin formation depends on the release of the two N-terminal fibrinopeptides A (FPA) from fibrinogen, and its formation is accompanied by an intermediate, α-profibrin, which lacks only one of the FPA. In this study, we confirm that the maximal levels of α-profibrin found over the course of thrombin reactions with human fibrinogen are only half of what would be expected if the first and second FPA were being released independently with equal rate constants. The rapidity of release of the fibrinopeptides by thrombin had been shown to depend on an allosteric transformation that is induced when Na⁺ binds to a site defined by the 215–227 residues of thrombin, a transformation that results in the exposure of its fibrinogen-binding exosites transforming the thrombin from a slow to a fast acting form toward fibrinogen. When choline was substituted for sodium to transform thrombin to its slow form, the maximal levels of α-profibrin rose to those expected for independent release of the two FPA. Thus, it is only the fast thrombin that releases the second FPA fast, and that fast release only occurs when both FPA are present because of a partial coupling of its release with that of the first FPA. The release of the FPA from purified α-profibrin with the first FPA already missing is no faster than the release of any FPA. Surprisingly, we also found that slow thrombin became increasingly transformed to a fast form in the absence of sodium when the fibrinogen was elevated to high concentrations. This potentiation by concentrated fibrinogen also occurs with the recombinant mutant thrombin (Y225P), which is otherwise slow in both the presence and absence of Na⁺. The potentiation of thrombin by fibrinogen must be short-lived so that the thrombin reverts to its slow acting form in the interin among encounters with other fibrinogen molecules in dilute fibrinogen solutions lacking Na⁺, whereas at high fibrinogen concentrations the thrombin encounters other molecules before it reverts back to the slow form.

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MATERIALS AND METHODS
Fibrinogen grade 3 from Enzyme Research Laboratories (www.enzymeresearch.com) was purified further by gel chromatography on 8% non-cross-linked agarose (www.xcbeads.com) to remove aggregates. The anti-fibrinogen (a17–23) monoclonal antibody described previously (18) was a generous gift from Dr. Alphonse G. Merigan (The Rockefeller University, New York, NY). Solutions of thrombin were Ortho Diagnostics (Raritan, NJ) and Enzyme Research Laboratories. Thrombin was also generously provided by John Fenton (New York State Department of Health, Albany, NY). Recombinant wild-type (rWT) and mutant Y225F thrombins were as described previously (19).

The reagents of thrombin with fibrinogen were carried out at ambient temperature and pH 7.4 (0.02 M Tris) in parallel with the fibrinogen in either choline (Ch+) chloride to transform thrombin to its slow form or in sodium chloride (0.2 M) to predisperse the fast form. All solutions contained 0.1% polyethylene glycol 8000 to minimize adsorptive losses of thrombin and GP-RNH2 (2 or 6 mM) to suppress the coagulation of the fibrin. The thrombin preparations were used as supplied without removing packaged Na+ by dialysis, which could alter the thrombin concentrations. Based on prior studies (19), the sodium added with the thrombin (maximally 3 mM with Fibrinex® at 1.2 units/ml) would blunt the fast → slow transition of thrombin in the choline solutions by ~10%. The reactants and diluents were dispensed separately for each time point, and reactions were terminated with 20 μl phenylprolylarginine chloromethylketone. The analyses of levels of α-fibrinopeptide and fibrin produced in the course of thrombin reactions were carried out by GPPhoresis and immunoprobing as described previously (5). To promote full application of protein onto electrophoretic gels for quantitation of all derivatives, the samples were chilled and admixed with 0.5 volume of 9 M urea (3 M final) just prior to sample application.

GPPhoresis is an electrophoretic method that uses GP-RNH2 as an aggregation inhibitor to stage the separation of fibrin monomer in a distinct band that is clear of fibrinogen and α-fibrinopeptide. The α-fibrinopeptide co-migrates with the fibrinogen and is measured distinctly from fibrinogen by immunopanning with anti-fibrinogen (a17–23) antibody, which cross-reacts equivalently with α-fibrinopeptide and fibrin but not fibrinogen. The fractional content of fibrin/fibrinogen (p/T) was assessed from scans of Coomassie Blue-stained gels (Gradipure stain, www.bioexpress.com). Relative amounts of α-fibrinopeptide/fibrin (p/T) were assessed by immunopanning gels with anti-fibrinogen (a17–23) monoclonal antibody labeled with either 125I or horseradish peroxidase for imaging. The immunoprobing was carried out directly in the agarose gels without blotting (20). The imaging of radioactivity was performed with a Molecular Dynamics PhosphorImager (www.mdyx.com) and analyzed with their ImageQuant software. Densitometry data from phosphorimaging scans were scanned (Scanmaker 4, www.microtek.com) and analyzed using SigmaScan and PeakFit software (www.spss.com). Because of a sigmoidal relationship between antigen concentration and antibody retention, scans were non-linear at very low and high levels of antigen, and estimates of relative rates of α-fibrinopeptide and fibrin production accordingly were based on differences in reaction time or thrombin concentrations required to produce nearly equal levels of immunostaining. Values for relative rates of α-fibrinopeptide production were specified as the ratio of the peaks (ACh/Afibrinogen) of scans from two or more lanes of the electrophorograms for reactions in Ch+ versus one or more closest matching lane(s) for the reactions in Na+. Scans of inverted images of Coomassie Blue-stained 125I-labeled fibrinogen standards yielded peaks with areas proportional to phosphorimaging scans. The fractional content of α-fibrinopeptide/fibrinogen (p/T) was calculated from (p/f) × (f/T), where the values of p/f were determined from immunostaining and those of f/T were determined from Coomassie Blue staining.

As is well known, no gel-staining method can be considered reproducible. Thus, all of our comparisons, all of which were relative, were made between lanes within a gel but never between gels. Furthermore, the immunostaining was always direct within the gels and never by Western blotting, because fibrinogen and especially fibrin do not blot-transfer well. Numerous experiments were carried out for all of the conditions described here, and the ones selected for illustration were chosen because of their lowest background after immunoprobing. Background immunostaining varied widely, because we were probing thick gels (1.5 mm) and washout of unbound antibody was variable. For example, the experiment in Fig. 1 was repeated three times with three different thrombin sources and yielded essentially the same results in all experiments.

The maximal value of p/T observed over the course of reactions was used to calculate the relative rate (k1/k2) of release of the first and second FPA according to Equation 1 as described previously (5),

\[
\ln(p/T)_{\text{max}} = \frac{h_2}{h_1 - h_2} \ln(h_2) \quad (\text{Eq. 1})
\]

except when k1 = k2, where (p/T)max = 1/λ = 0.37. Equation 1 (adapted from Ref. 21) assumes that the overall reaction is is sequential with fibrinogen (φ) being converted to the intermediate α-fibrinopeptide (p) with a first-order rate constant k1 accompanied by conversion of the α-fibrinopeptide to fibrin (f) with a first-order rate constant k2. If the reactions are not purely sequential, the apparent value for the rate constant k2 will differ from the rate constant for the conversion of purified α-fibrinopeptide to fibrin. We emphasize that the conversion of fibrinogen to fibrin is not purely sequential and that Equation 1 is used only as a test of the sequentiality. A preceding study by others (22) raises the possibility that the overall reaction is purely sequential. However, our own preceding study (5) indicates that k2 = 3k1, raising the possibility that it might not be purely sequential. The existence of α-fibrinopeptide indicates that there is some sequentiality. The comparison of α-fibrinopeptide formation by slow versus fast thrombin was simply conceived as a possible quantitative approach to demonstrating the difference between pure versus partial sequentiality.

Fibrinopeptide A release was calculated from the production of the α-fibrinopeptide (p) and fibrin (f) in the course of the reactions based on Equation 2. However, the fibrinopeptides were measured directly by high pressure liquid chromatography (16) in a comparison of thrombin reactions with fibrinogen and the purified α-fibrinopeptide, FPA = p + 2f (Eq. 2) with there being one FPA released for each molecule of p and two FPA released for f. The α-fibrinopeptide was purified essentially as described previously (23) using gel chromatography (8% agarose at pH 8.6) of partial thrombin/fibrinogen mixture to separate it from fibrinogen (verified with radioiodinated fibrinogen tracer) and then suctioning (200 mm Hg) α-fibrinopeptide along with a wash through a slab of 3.5% agarose gel (36 cm2 × 2.5-mm deep) to filter out contaminating fibrin.

We greatly improved the recovery of the α-fibrinopeptide to ~80% from the suctioning step by 1) adding 1.2 molar GP-RNH2 to the α-fibrinopeptide concentrate (~8 mg/ml) before suctioning it (one-eighth slab volume) through the agarose filter slab and 2) collecting the wash from the filtering gel directly through the vent of the suctioning platens (an anodized specially milled 6 × 10 inches2 gel dryer) instead of collecting it into an underlying recipient gel described previously (23).

RESULTS
All of the measurements in this study were relative comparing fast and slow allosteric forms of thrombin by varying either thrombin concentrations or reaction times so that we were looking at nearly equal levels of the reaction products, α-fibrinopeptide and fibrin. The measurements are based on Coomassie Blue staining and on immunoprobing with anti-fibrinogen (a17–23) antibody, both of which follow sigmoidal relationships that can vary from one experiment to the next. Thus, comparisons based on nearly equal levels of products helped remove uncertainties over differences in rates.

Large Differences in k1 and k2, k1/k2 for Fast and Slow Thrombin in Dilute Fibrinogen—Reactions with fibrinogen at 0.3 mg/ml showed that only fast thrombin releases the second FPA at an apparent rate faster than the release of the first. As shown (Fig. 1), the plateau level of α-fibrinopeptide (pmax) in Ch+ solutions was two times that in Na+ solutions. The level of

2 For example, if 4 × thrombin in Ch+ was required to yield a nearly equal amount of α-fibrinopeptide as 1 × thrombin in Na+ at a given reaction time and if the peak area for the product in Ch+ was 10% greater than in Na+, the relative rate in Na+/Ch+ was calculated as h2/h1 = (4X + 0.1X)/IX = 4.1.
Coombie

Anti-α(17-23)

Scans gels with maximal profibrin

**FIG. 1.** Electropherograms showing high levels of α-profibrin in reaction mixtures of thrombin (1.2 units/ml) and dilute fibrinogen (0.3 mg/ml) in either 0.2 M Ch⁺ or Na⁺ solutions. Upper, Coomassie Blue-stained gel shows levels of fibrin (f) and mixed fibrinogen/profibrin (φ + p) bands used for calculating p/T = φ/φ + p) over the course of the reactions. The reaction (J) above the fibrin bands is a sample sharpening boundary between a 1% application gel and 4.25% resolving gel. The immunostained anti-fibrin(α17-23) gel shows levels of α-profibrin (p) and fibrin used for determining p/T and calculated p/T = φ/φ + p/T. The maximal plateau level of α-profibrin appearing in the reaction provides a measure of the relative rate constants (kφ/kp) for the release of the first and second FPA. The lower tracing compares scans of reaction mixtures at time points 90 s in Na⁺ (——) and 480 s in Ch⁺ (——), where the α-profibrin reached its maximum in the two sets of reactions. The maximal level of α-profibrin in Ch⁺ was twice as high as in Na⁺ and was in accord with its faster conversion to fibrin in Na⁺. The maxima occur at plateaus that are quite broad, and scans on the next, earlier, and later reaction mixtures were quite similar to that shown.

α-profibrin rose to four-tenths of the initial fibrinogen (p/T) with Ch⁺ substituted for Na⁺, a value essentially equal to the value (1/e) that would be expected from Equation 1 if the first and second FPA are being released independently with equal rate constants (kφ/kp = 1). The value of p/T determined for the reactions in Na⁺ was 0.18 – 0.2 (a range comparing 90- and 120-s Na⁺ scans with the 480-s Ch⁺ scan) (5). These values for p/T are essentially the same as the value 0.2 determined earlier by different methods, a value indicating that the second FPA is released with an apparent rate constant three times that of the first FPA as calculated from Equation 1. Similar results were obtained in four other series of dilute fibrinogen reactions, which included the use of three different sources of thrombin, Fibrindex, Fenton, and rWT.

The calculated rates of FPA release, based on one FPA per α-profibrin and two FPA per α-fibrin equivalents, indicated that the initial rate (kφ) was on the order of 4–5 times slower in Ch⁺ than the kφ in Na⁺. This 4–5-fold slower rate was less than the factor of 7 slower rate established (16) for reactions in the absence of sodium. The lesser difference observed here arose from a small amount of Na⁺ (3 m) added to the Ch⁺ solutions with the Fibrindex thrombin and was also attributed in large part to a higher concentration of fibrinogen (4 ×) used in the reactions with the dilute fibrinogen here as indicated from studies with more concentrated fibrinogen.

Concentrated Fibrinogen Promotes Transition of Slow Thrombin to the Fast Acting Form—Reactions of thrombin with fibrinogen at physiologic concentration (3 mg/ml) in Ch⁺ versus Na⁺ were initially carried out with the thrombin at five times the greater concentration in Ch⁺ than in Na⁺ because of the anticipated slower reactivity in Ch⁺. However, as shown (Fig. 2), instead of producing nearly equal quantities of α-profibrin at equal time points with 5X thrombin in the Ch⁺, both the α-profibrin and fibrin production were much faster than anticipated. Taking the differing thrombin concentrations into account and comparing reaction products at 30 s in Ch⁺ versus Na⁺, we calculated that the initial rate (kφ) of FPA release in Ch⁺ had jumped to 54–57% of that in Na⁺. The reactivity of thrombin had narrowed from 4–5 times faster in Na⁺ with dilute fibrinogen to only 2 times faster with the fibrinogen at physiologic concentration. A repeat analysis (data not shown) using thrombin at equal concentrations in Na⁺ and Ch⁺ gave the same result. What was also intriguing was that the plateau level of α-profibrin (Fig. 2, 120 s) in Ch⁺ became essentially equal to that in Na⁺ (p/T = 0.2), whereas it had been twice as high (p/T = 0.4) with dilute fibrinogen in Ch⁺. The lower p/T provided an indication that the release of the second FPA had accelerated relative to the first with the more concentrated fibrinogen in Ch⁺. To determine whether the higher fibrinogen concentration was responsible for the lower p/T and the accelerated production of p in Ch⁺, we repeated the experiment (Fig. 3) with a very high concentration of fibrinogen (16 mg/ml).

The test (Fig. 3), along with four others with fibrinogen at 16
mg/ml with both plasma-derived and recombinant wild-type thrombin, showed that the initial rate of production of p in Ch + was to 74–76%, based on scans for two reaction times, of the rate in Na . A logistic plot (Fig. 4) of changes in FPA release in choline versus sodium (f Ch , k Ch ) at varying fibrinogen concentrations suggests that the relative rates asymptotically approach values near 0.05 with infinitely dilute fibrinogen and 0.87 with infinitely concentrated fibrinogen. Yet, the value of (f T max ) = 0.2 corresponding to k 2 k 1 = 3 (Equation 1) remained the same at high fibrinogen concentrations in either Na + or Ch + as it was with low fibrinogen concentrations in Na +.

rWT and Mutant Y225P Thrombins—As would be expected and observed in three experiments with dilute fibrinogen (data not shown), the Y225P mutant thrombin was as slow in Na + as in Ch +, rWT was as slow as Y225P in Ch +, and rWT was much faster than Y225P in Na +. The comparisons of the rWT versus Y225P with dilute fibrinogen in Na + resembled the comparisons of plasma thrombin in Na + versus Ch +. More importantly, the differences between rWT and Y225P in Na + narrowed to the point of nearly vanishing (Fig. 5) in reactions with concentrated fibrinogen as also was observed with plasma thrombin.

Thrombin Is Not Potentiated by Peak II Fibrinogen—Peak II fibrinogen, a minor component of normal plasma fibrinogen (~15% of the fibrinogen), has a C-terminal extension on one of the two γ-chains, which among other functions possesses a site for high affinity non-active site binding of thrombin (17). As shown in Fig. 6, the differences in rates of fibrin formation of this fibrinogen at high concentration (11 mg/ml) in Na + versus Ch + were almost as large as that observed with unfractionated fibrinogen at a low concentration (Fig. 1). This lack of effect of concentrated peak II fibrinogen on the slow → fast transition in Ch + can be attributed to its tight binding of thrombin at a non-catalytic site retarding the catalytic interaction (17).

Purified α-Fibrin Is Not a Better Substrate Than Fibrinogen—Pursuant to the basis for the fast release of the second FPA in thrombin/fibrinogen reactions, we questioned whether the α-fibrinogen might just be a better substrate than fibrinogen. However, high pressure liquid chromatography determinations of FPA release indicated that the specificity constant (k cat/K m) for the release of FPA by the fast form of thrombin acting on fibrinogen-free α-fibrin was approximately 20% lower than that from fibrinogen (Table 1). No difference was observed in the reactions with slow thrombin. The FPA measurements with fast thrombin conformed with four comparisons (4 × 6 reaction periods) by GPRphoresis showing that conversion of
the purified α-profibrin at a concentration twice that of fibrinogen to equalize FPA substrate concentrations consistently lagged behind the conversion of the fibrinogen to α-profibrin.

DISCUSSION

It is well known that the active site of thrombin is altered in its binding to fibrinogen, and the low affinity thrombin binding sites in the N-terminal domains of the Aa-chains of fibrinogen are critical to the efficient release of the fibrinopeptides (24). The fibrinogen binding sites of thrombin, which function in its orientation for efficient release of FPA, are not exposed in its orientation for efficient release of FPA, are not exposed in slow thrombin but become exposed in the slow → fast allosteric transition that is induced by Na⁺ binding. The results of this study demonstrate that only the fast form of thrombin releases the second FPA at an apparent rate faster than it releases the first FPA, and moderate to high concentrations of fibrinogen transform slow thrombin to a fast acting form in a manner analogous to the transformation induced by sodium binding. The latter observation confirms an earlier prediction that fibrinogen may stabilize the slow → fast transition (25). The transformation by fibrinogen occurs independent of the Na⁺ binding site, because the mutant Y225P thrombin is almost as fast as rWT with high fibrinogen concentrations in Na⁺, whereas the mutant is normally slow in both Na⁺ and Ch⁺ as shown from prior analyses of FPA release (16) and confirmed here in our measurements of α-profibrin and fibrin formation.

The plateau levels of α-profibrin, measured here by GPR-phoresis and immunoprobing, in the reactions with fast thrombin were consistently lower by half of the level that would be expected for the independent release of the first FPA (producing the α-profibrin) and the second FPA (transforming the α-profibrin to α-fibrin). The low plateau level of α-profibrin observed here agrees with that determined earlier using a different method to assess α-profibrin levels (5), the difference between FPA release measured by high pressure liquid chromatography and the transformation of 125I-labeled fibrinogen to fibrin measured by phosphorimaging scans. The high plateau level of α-profibrin observed with slow thrombin indicates that this allosteric form releases the two FPA independently at equal rates. The thrombin-like venom enzyme, ancrod, also releases the two FPA independently observed with slow thrombin indicates that this allosteric form releases the two FPA independently at equal rates. The thrombin-like venom enzyme, ancrod, also releases the two FPA independently (22, 26). But when fast thrombin releases the second FPA independently of the release of the first FPA, as observed in its reaction with the purified α-profibrin where the second is the only FPA in place, it releases that FPA no faster than it releases any FPA. Thus, there must be some coupling of the release of the second FPA with release of the first to explain the faster release of the second FPA. In the reactions between fast thrombin and fibrinogen with both peptides in place. The conversion of fibrinogen to fibrin involves two modes of FPA release as parallel processes: 1) sequential FPA release producing α-profibrin, which must independently be converted to fibrin; and 2) a coupled or joint release of both FPA resulting in the direct conversion of fibrinogen to fibrin.

The fast release of the second FPA is unrelated to the accel-
erated release of FPB that accompanies aggregation of α-fibrin (11), because the fast release of the second FPA is an intramolecular event. We know from ongoing ultracentrifuge studies that purified α-fibrinogen forms dimers and tetramers. However, as found in this study, the release of FPA from purified α-fibrinogen is not faster than its release from fibrinogen. The fast release of the second FPA accordingly is unrelated to any aggregation and is an intramolecular phenomenon.

Equation 1, which portrays \( p_{\text{max}}/k_T \) for a sequential reaction involving independent release of the first and second FPA, inadequately assumes that fibrin production can be represented with a single rate constant \( (k_2) \) involving only the conversion of α-fibrinogen to fibrin. Because of the indication of a partial coupling of release of the first and second FPA, the overall reaction probably consists of three concurrent reactions involving: 1) the direct conversion of fibrinogen to α-fibrinogen and release of the first FPA (Equation 3), 2) secondary conversion of α-fibrinogen to fibrin with independent release of its FPA (Equation 4), and 3) direct conversion of fibrinogen to fibrin because of coupled release of both FPA (Equation 5). Equation 5 could be written with an intervening step \( (E\phi \rightarrow E\phi + A) \) prior to the conversion of the substrate complex to fibrin, but the form of the overall rate equation would still have the form of a first-order reaction with a single rate constant. These reactions are represented in the combined model (Equation 6). The model is simply a rational explanation of our findings. As modeled, the relative values of the rate constants \( k_{1-1} \) and \( k_{2-2} \) represent the probabilities of release of either one or both FPA in each encounter between the thrombin and fibrinogen. In this reaction scheme, α-fibrinogen production can be viewed as arising because the coupling of the two FPA leading to direct conversion of fibrinogen to fibrin is imperfect.

\[
\begin{align*}
E + \phi & \rightarrow E\phi \rightarrow p + A & \text{(Eq. 3)} \\
E + p & \rightarrow E\phi \rightarrow f + A & \text{(Eq. 4)} \\
E + \phi & \rightarrow E\phi \rightarrow f + 2A & \text{(Eq. 5)}
\end{align*}
\]

The coupling of release of the FPA is certainly associated with the exosite binding by fast thrombin. Intramolecular reseating of the thrombin is one possibility, because the parallel orientation of the two Aα-chains (27) in the N-terminal domain of fibrinogen would make it unnecessary for thrombin to completely reorient itself for the second clip. However, recent crystal structure determinations of chicken fibrinogen conform with the proposition that thrombin would not have to leave its binding site to release the second FPA along with the first FPA (28). Studies on thrombin reactions with rabbit fibrinogen indicate that the release of the second FPA is tightly coupled to the first, because very little α-fibrinogen is produced in the course of coagulation of that species of fibrinogen by either bovine or human thrombin (23, 29).

The almost concurrent release of the two FPA from rabbit fibrinogen was suggested to underlie the high susceptibility of rabbits to disseminated intravascular coagulation, and conversely, the fully independent release of the two FPA by atroixin may underlie its utility as a defibrinating agent, producing large quantities of α-fibrinogen prior to inducing coagulation (23). Thus, the degree of coupling of the first and second FPA release has physiologic significance. It is seemingly better to have partial rather than either full or negligible coupling of the release of the two FPA.

The steady-state kinetics of FPA release by Na+-free thrombin differ for dilute and for concentrated fibrinogen. No other thrombin substrates induce a similar concentration-dependent change. As noted, slow thrombin is potentiated to a fast acting form by fibrinogen itself, but the potentiation becomes clearly evident only in concentrated fibrinogen solutions. To explain the differing steady-state kinetics of Na+-free thrombin in dilute versus concentrated fibrinogen, we suggest that the potentiated thrombin reverts to its slow acting form in the interim among encounters with fibrinogen molecules in dilute solution, whereas at high fibrinogen concentration, the potentiated thrombin encounters other molecules before reverting to the slow acting form.

Why is it that the potentiation of thrombin by fibrinogen does not lead to a fast release of the second FPA in dilute fibrinogen? One possibility might be that the initial seating of thrombin has to occur with it in the fast form for the coupled release to occur. If the thrombin is not initially oriented by exosite binding, it would not be in the proper orientation for rapid release of the second FPA. As noted previously (16), the efficient docking of fast thrombin lowers the energy barrier and diffusion-controlled nature for efficient cleavages of FPA. The cleavage of FPA may be the stimulus for exposing the exosite binding domains that are normally masked in slow thrombin transiently transforming it to fast thrombin, but the initial seating in the fast form may be critical for the coupled release of the second FPA. More sensitive methods for measuring α-fibrinogen production will be needed to address these possibilities conclusively.

The potentiation of the mutant Y225P thrombin to a transiently fast acting form by high concentrations of fibrinogen makes it more of a procoagulant than anticipated, but we do not know to what degree this would occur in plasma or in whole blood. There is 20 times more protein than fibrinogen in plasma. As observed with purified Peak II fibrinogen, the binding of thrombin through its non-catalytic exosite substantially blunted the potentiating effect observed with unfractionated fibrinogen. We anticipate that the binding of thrombin by other components of blood may cause much of the transiently potentiated thrombin to revert to its slow form among its encounters with fibrinogen molecules.

Because of the plasma sodium, it is unlikely that the potentiation of normal thrombin by fibrinogen has much physiologic significance, possibly with the exception for fish such as sharks, which have low sodium but high amine salt concentrations in their blood. The significant aspect of the potentiation is that it demonstrates a slow → fast transition of slow thrombin albeit short-lived in its interaction with fibrinogen as suggested earlier (16). The principal value of this study is the demonstration of a partial coupling of the release of the second FPA with release of the first FPA. We believe that the partial rather than full or negligible coupling has substantial physiologic significance in determining a healthy balance between susceptibility to disseminated intravascular coagulation and hemorrhagic diathesis.

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