An Investigation Into the Role of Coagulation Factor XIII in ADP-Induced Aggregation and Fibrinogen Binding With Rabbit Platelets

By Elizabeth J. Harfenist, Gopa Raychaudhuri, Marian A. Packham, and J. Fraser Mustard

Because there was a possibility that activated factor XIII (factor XIIIa) might stabilize a platelet-fibrinogen aggregate through its crosslinking action, we have isolated plasma factor XIII, activated it, and studied the effect of factor XIIIa at a concentration of 3.3 µg/ml on aggregation and 125I-fibrinogen binding of rabbit platelets stimulated with 9 µM ADP. Factor XIIIa did not cause aggregation in the absence of ADP, nor did it enhance ADP-induced aggregation or substantially stabilize the platelet aggregate. The presence of factor XIIIa did not affect the amount of fibrinogen bound to platelets immediately after stimulation with ADP, but it appeared to cause a slow specific binding of 125I-fibrinogen to platelets whether or not they were stimulated with ADP. This binding, which was not inhibited by prostaglandin E1, did not lead to aggregation and was accompanied by crosslinking of fibrinogen through its Aα and γ chains, either to other fibrinogen molecules or to a platelet protein or proteins.

Factor XIII occurs as a circulating protein in plasma and platelets. In platelets it is located in the cytoplasm, and it is not released by collagen or thrombin. In the presence of calcium ions, factor XIII is converted by thrombin to activated factor XIII (factor XIIIa, fibrin stabilizing factor), a transglutaminase that introduces covalent bonds between fibrin molecules to form a stable crosslinked clot. Under certain conditions factor XIIIa also catalyzes the introduction of crosslinks between fibrinogen molecules without their prior conversion to fibrin, and between fibrinogen and fibrin molecules. The role of factor XIII in hemostasis is not well understood, although it has been established that a clot of crosslinked fibrin has greater mechanical strength and lower susceptibility to proteolysis than one of noncrosslinked fibrin. Congenital factor XIII deficiency can result in severe and prolonged bleeding, poor wound healing, or excessive scarring. However, not all symptoms can be related to the absence of crosslinks in clots, and it is possible that fibrin crosslinking is not the sole function of factor XIIIa. There have been many reports of the action of factor XIIIa on proteins other than fibrinogen and fibrin;1,5,8 crosslinks have been introduced into fibronectin, α2-antiplasmin, collagen, actin, myosin, and platelet membrane proteins, all of which are involved in hemostasis and thrombosis. Although platelets contain half the circulating factor XIII, the role of crosslinking in platelet function is not understood, particularly as platelet factor XIII is found in the cytoplasm and is not released during the platelet release reaction.9-11 Cohen et al.8 have reported that platelet factor XIII, activated when the intracellular Ca2+ concentration is raised, forms covalent bonds between membrane glycoproteins, which could lead to irreversible loss of membrane fluidity. Such an effect has been reported in erythrocytes.12

It has been established that rabbit platelets aggregate and bind fibrinogen when they are stimulated with adenosine diphosphate (ADP); they subsequently deaggregate and the fibrinogen dissociates.13,14 Since it is possible that factor XIIIa may stabilize a platelet aggregate or thrombus by its crosslinking action, we have isolated and characterized plasma factor XIIIa and studied its effect on ADP-induced aggregation and fibrinogen binding.

MATERIALS AND METHODS

Human fibrinogen (grade L) was from AB Kabi, Stockholm, Sweden. Human α-thrombin (lot H-1) was kindly supplied by Dr. D.L. Aronson, Bureau of Drugs and Biologics, FDA, Bethesda, Md.; it was dissolved at 75-100 U/ml in 50% glycerol and stored at −20°C. Prostaglandin E1 (PGE1) was a generous gift from the Upjohn Co., Kalamazoo, Mich. Creatine phosphate (CP), creatine phosphokinase (CPK), ADP, monodansylcadaverine, hirudin, heparin, and N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) were from Sigma Chemical Co., St. Louis, Mo. Bovine albumin (Pentex, fraction V) was from Miles Laboratories, Elkhart, Ind. Hammarsten casein was from BDH Chemicals, Toronto, Canada. DEAE cellulose was from Whatman Ltd., Maidenstone, Kent. Outdated plasma was obtained from the Canadian Red Cross, Toronto Blood Transfusion Service. Antithrombin III was prepared by the method of Miller-Andersson et al.15 Apyrase, prepared by the method of Molnar and Lorand,16 was dissolved in 0.15 M NaCl and stored at −20°C; at a concentration of 3 µl/ml it caused 75% conversion of 10 µM ADP to adenosine monophosphate in 2 min at 37°C; when it was used at 0.125 µl/ml we observed 17% degradation of 10 µM ADP in 10 min at 37°C, and it appeared to stabilize platelet suspensions over a long period (longer than 12 hr). Na125I (carrier free, NEZ 033L) and Na32CrO4 (specific activity 200-500 "From the Department of Biochemistry, University of Toronto, Toronto, Ontario, and the Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

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Address reprint requests to Dr. E.J. Harfenist, Department of Biochemistry, Medical Science Building, University of Toronto, Toronto, Ontario, Canada MSS 1A8.

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Ci/g of Cr, NEZ 030) were from New England Nuclear, Boston, Mass. 1-14C-Serotonin was obtained as 5-hydroxytryptamine-3-14C-creatinine sulfate (54 mCi/m mole) from Amersham Corp., Arlington Heights, Ill. 125I-albumin was from Charles E. Frost and Co., Montreal, Canada. All other chemicals were reagent grade. Reagents were dissolved in 0.15 M NaCl before use, and all concentrations are expressed as final values after all additions.

Suspensions of washed rabbit platelets were prepared essentially as described by Ardle et al.25,26 the final suspending medium was Tyrode solution containing 5 mM HEPES and albumin at 3.5 mg/ml (Tyrode-albumin). The platelet count was adjusted to 7 × 10^8/ml and antiparase was routinely added to the suspensions at 0.125 μl/ml. The platelets were labeled in the first washing fluid with 14C-serotonin and 125I to measure release and lysis. The methods used to study platelet aggregation and 125I-fibrinogen binding have already been described.25 Specific binding is defined as the amount of bound fibrinogen that was displaceable by an excess of unlabeled fibrinogen, and it was determined in all cases by subtracting nonspecific binding, measured in the presence of a 100-fold excess of unlabeled fibrinogen, from total binding. 125I-Albumin was used to measure trapped fluid in the platelet pellet.

Preparation of Factor XIII

Factor XIII was prepared from outdated human plasma using the method described by Curtis and Lorand.27 The product was obtained in a yield of 4.2 mg from 1 liter of plasma. It was characterized by polyacrylamide gel electrophoresis in dodecyl sulfate (SDS-PAGE) without reduction28 and found to consist of two components that gave bands in positions corresponding to molecular sizes of approximately 85,000 and 73,500 for the α and β subunits, in agreement with McDonagh and McDonagh.29 This factor XIII was activated with thrombin (4 U/ml) in the presence of 0.02 M CaCl2 and 0.025 M cysteine, and assayed by the amine incorporation method of Lorand and Gotoh30 using monodansylcadaverine. The results from SDS-PAGE and the dose-dependent incorporation of the amine into Hammarsten casein confirmed that the preparation was factor XIII.

Preparation of Factor-XIII-Free Fibrinogen

Factor XIII was removed from Kabi fibrinogen by a modification of the method of Lawrie et al.31 using DEAE-cellulose chromatography in calcium-containing buffers at 4°C. Fibrinogen was applied to the column in 0.05M Tris-HCl-0.045M NaCl-0.002M CaCl2, pH 8.6, and factor-XIII-free fibrinogen was eluted with 0.05M Tris-HCl-0.045M NaCl-0.002M CaCl2, pH 8.6. This product was clotted with thrombin (4 U/ml in the presence of CaCl2 and cysteine), and then reduced and analyzed by SDS-PAGE. No bands corresponding to the γ-chain dimers or α-chain polymers of fibrin were observed, confirming the absence of factor XIII in the fibrinogen preparation. Addition of an aliquot of the factor XIII preparation to the factor-XIII-free fibrinogen before clotting resulted in the appearance of bands corresponding to γ-chain dimers and α-chain polymers, reinforcing the identity of the factor XIII.

Studies on the Effects of Factor XIIIa on Platelet Aggregation and Fibrinogen Binding

Factor XIIIa was activated with thrombin (0.68 U/ml in the presence of 2 mM CaCl2) for 20 min at 22°C; cysteine was omitted because it had an inhibitory effect on platelet aggregation. The thrombin was inactivated either with hirudin (6.8 U/ml) or with antithrombin III and heparin (0.23 U/ml), since active thrombin would stimulate the platelets and also cause any added fibrinogen to clot. In preliminary experiments it was established that sufficient thrombin inhibitor was added to prevent both platelet aggregation and fibrinogen clotting by the corresponding thrombin-inhibitor mixture, and this was checked in each experiment. The effects of factor XIIIa (3.3 μg/ml) containing inactivated thrombin, or inactivated thrombin alone, on the characteristics of ADP-induced aggregation in the presence of added fibrinogen and of 125I-fibrinogen binding to platelets were studied. This concentration of factor XIIIa was chosen because it was not high enough to introduce crosslinks into fibrinogen over a period of 30 min at 37°C, but it did cause the introduction of crosslinks into 75% of the γ-chains in a fibrin clot formed by the action of thrombin (1.0 U/ml) on factor-XIII-free fibrinogen (0.9 mg/ml) for 10 min at 37°C; it also caused some γ-chain crosslinking when incubated with fibrinogen alone for 16 hr at 37°C. For the platelet studies, sufficient concentrations of amyrase (3 μl/ml) or CP/CPK (CP at 440 μg/ml and CPK at 0.47 U/ml) had been added to the platelet suspensions to cause deaggregation within 2–5 min in the absence of factor XIIIa, so that the rates of deaggregation and dissociation of bound fibrinogen could easily be compared. The effects of the inhibitor, PGE1, on aggregation and fibrinogen binding were measured by the method described previously.19

RESULTS

Platelet Aggregation

When factor XIIIa was added to a stirred platelet suspension at 37°C, no aggregation was observed up to 13 min (the longest period tested) unless ADP was added. The extent of ADP-induced platelet aggregation was not affected by the presence of factor XIIIa or either of the thrombin–inhibitor mixtures, but the times required for deaggregation were slightly prolonged (Fig. 1, Table 1). Incubation of the platelets with factor XIIIa for 13 min before adding the ADP did not affect the extent of aggregation or the rates of aggregation or deaggregation. The platelets deaggregated completely in the presence of factor XIIIa, and no subsequent aggregation was observed up to 14 min after the addition of ADP. The data shown in Table 1 were obtained by using amyrase at 3 μl/ml or CP/CPK to cause rapid deaggregation. When the rate of deaggregation was decreased by using a lower concentration of amyrase (0.125 μl/ml), factor XIIIa again had only a slight effect on the rate of deaggregation. With control platelets, the light transmission decreased to 50% of the maximum by 6 min, whereas with a suspension containing factor XIIIa the corresponding time was 6.8 min.

Experiments with thrombin–inhibitor mixtures in the platelet suspension produced similar aggregation and deaggregation patterns to those of control platelets, and no differences were observed between the effects of hirudin and antithrombin III–heparin as thrombin inhibitors, or between amyrase and CP/CPK as ADP scavengers. In later experiments, antithrombin III and heparin were used to inhibit thrombin, and amyrase at 3 μl/ml was used to remove ADP and deaggregate the platelets. Although the thrombin–
Table 1. Effect of Factor XIIIa on the Extent of Aggregation and on the Rate of Deaggregation of Washed Rabbit Platelets Stimulated With ADP in the Presence of Fibrinogen

<table>
<thead>
<tr>
<th>Additions to Platelet Suspension</th>
<th>Aggregation Peak Height* (% of Control)</th>
<th>Deaggregation Time† (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 M NaCl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Thrombin–inhibitor mixture</td>
<td>99 ± 8</td>
<td>110 ± 10‡</td>
</tr>
<tr>
<td>Factor XIIIa</td>
<td>98 ± 9</td>
<td>127 ± 23‡</td>
</tr>
</tbody>
</table>

*Aggregation peak height is defined as the maximum increase in light transmission of a platelet suspension measured from the minimum observed during shape change just after the addition of ADP.
†Deaggregation time was measured from the time of addition of ADP until the light transmission returned to the baseline.
‡These values are significantly different from each other and from those for control platelets (p < 0.05).

Final concentrations: ADP, 9 μM; fibrinogen, 60 μg/ml; factor XIIIa, 3.3 μg/ml; platelet count, 6 × 10⁵/μl; these results were obtained with apyrase at 3.3 μl/ml or CP at 440 μl/ml and CPK at 0.47 U/ml in the platelet suspensions in order to cause rapid deaggregation.

Values are means ± SD from 13 experiments.

(Table 2). Neither of the thrombin–inhibitor mixtures nor factor XIIIa caused platelet lysis or release of granule contents in excess of that observed with control platelets. PGE₁ (0.12 μM) when added 3 min before the ADP completely inhibited the aggregation of control platelets and of platelets in the presence of factor XIIIa.

**Fibrinogen Binding**

Specific binding of ¹²⁵I-fibrinogen to platelets 0.1 min after the addition of ADP was not affected by the presence of either of the thrombin–inhibitor mixtures or factor XIIIa (Table 3). However, even though the patterns of aggregation–deaggregation were similar and the platelets had completely deaggregated by 1–5 min, the rate of dissociation of ¹²⁵I-fibrinogen from platelets suspended with factor XIIIa was slower than from control platelets, and by 10 min after the addition of ADP the amount of ¹²⁵I-fibrinogen bound to the platelets was lower (Table 2). No synergism was observed between ADP and either addition to the platelet suspension

Inhibitor mixtures failed to cause either platelet aggregation or fibrinogen clotting. Aggregation experiments were done using low threshold concentrations of ADP to determine whether any synergism could be detected between these low concentrations of ADP and either the thrombin–antithrombin III–heparin mixture or factor XIIIa.
Characterization of $^{125}$I-Fibrinogen

To determine whether factor XIIIa had caused crosslinking of fibrinogen (to itself or to other proteins) during the platelet experiments, samples of $^{125}$I-fibrinogen that had been associated with the platelets for various times and under the different experimental conditions were reduced and studied by SDS-PAGE to detect the presence of Aα- or γ-chain crosslinks. For these experiments a low concentration of apyrase (0.125 μl/ml) was used to achieve the maximum possible $^{125}$I-fibrinogen binding to platelets for the longer time periods used. In a typical experiment, $^{125}$I-fibrinogen and platelets were incubated with the desired reagents for a specified time, the platelet suspension was centrifuged, and an aliquot of the supernatant, containing the $^{125}$I that had failed to dissociate from the platelets under these conditions, was examined by SDS-PAGE.

For clarity, the 3 types of $^{125}$I-fibrinogen characterized by SDS-PAGE are defined as follows.

**Fibrinogen-1:** the $^{125}$I-fibrinogen found in the supernatant of the initial platelet suspension after centrifugation.

**Fibrinogen-2:** the $^{125}$I-fibrinogen dissociated from platelets in Tyrode solution containing a high concentration of apyrase.

**Fibrinogen-3:** the $^{125}$I-fibrinogen obtained by solubilization of the platelet pellet in 10 M urea–2% SDS–2% β-mercaptoethanol.

Fibrinogen-1, which had been incubated with platelets for up to 10 min at 37°C, gave SDS-PAGE patterns similar to those of the starting material (bands corresponding to the Aα, Bβ, and γ chains of fibrinogen, Fig. 2A), regardless of the presence of thrombin-inhibitor mixture, factor XIIIa, or ADP. All

### Table 3. Effect of Factor XIIIa on the Specific Binding of $^{125}$I-Fibrinogen to Washed Rabbit Platelets

<table>
<thead>
<tr>
<th>Additions to Platelet Suspension</th>
<th>0.1</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>100</td>
<td>59 ± 19</td>
<td>38 ± 15</td>
<td>35 ± 15</td>
<td>46 ± 10</td>
</tr>
<tr>
<td>ADP + thrombin− inhibitor mixture</td>
<td>96 ± 8</td>
<td>66 ± 26</td>
<td>46 ± 13</td>
<td>47 ± 11</td>
<td>54 ± 11</td>
</tr>
<tr>
<td>ADP + factor XIIIa</td>
<td>96 ± 9</td>
<td>64 ± 14</td>
<td>61 ± 21</td>
<td>60 ± 26</td>
<td>84 ± 13</td>
</tr>
<tr>
<td>0.15 M NaCl</td>
<td>—</td>
<td>29 ± 7</td>
<td>27 ± 10</td>
<td>47 ± 26</td>
<td>62 ± 26</td>
</tr>
<tr>
<td>0.15 M NaCl + thrombin− inhibitor mixture</td>
<td>—</td>
<td>27 ± 14</td>
<td>33 ± 21</td>
<td>44 ± 20</td>
<td>59 ± 35</td>
</tr>
<tr>
<td>0.15 M NaCl + factor XIIIa</td>
<td>—</td>
<td>30 ± 11</td>
<td>26 ± 3</td>
<td>49 ± 32</td>
<td>94 ± 41</td>
</tr>
</tbody>
</table>

*Centrifugation time, the time at which the platelet suspension was removed from the aggregometer and placed in the centrifuge, was measured from the time of addition of ADP, or of 0.15 M NaCl in cases where no ADP was added.

†The amount of $^{125}$I-fibrinogen bound to control platelets 0.1 min after the addition of ADP was 2.01 ± 0.55% of the added $^{125}$I-fibrinogen (mean ± SD from 8 experiments).

These values are significantly greater than the values for $^{125}$I-fibrinogen bound to platelets at 10 min in the absence of factor XIIa ($p < 0.05$).

Final concentrations: ADP, 9z M; factor XIIIa, 3.3 g/ml; platelet count, 6 x 10⁵/μl; these results were obtained with apyrase at 3 μl/ml or CP at 440 μg/ml and CPK at 0.47 U/ml to produce conditions that would cause rapid deaggregation.

Values are means ± SD from 6 experiments.
samples of fibrinogen-2 gave results similar to those obtained with fibrinogen-1. This fibrinogen-2 consisted of approximately 50% that had been specifically bound to the platelets and approximately 50% nonspecifically bound and in trapped fluid. However, fibrinogen-3 showed a specific effect of factor XIIIa. In the presence of factor XIIIa high molecular weight radioactive material, some of which did not penetrate 7.5% gels, was observed; in contrast, fibrinogen-3 that had been incubated with platelets for 10 min at 37°C without factor XIIIa, in either the presence or absence of the thrombin–antithrombin III–heparin mixture, consisted of apparently unchanged Aα, Bβ, and γ chains. The high molecular weight material appeared in all samples of fibrinogen-3 in which the 125I-fibrinogen had been incubated with platelets in the presence of factor XIIIa for 5 min (Fig. 2B) or 10 min (Fig. 2C) after the addition of ADP, and in one case it was also observed after only 0.1-min incubation. 125I-Fibrinogen incubated with platelets and factor XIIIa for 10 min in the absence of ADP also gave high molecular weight bands upon electrophoresis, similar to those shown in Fig. 2, B and C. In several cases, one of the bands of radioactivity appeared in the position characteristic of the γ-chain dimer. For all 125I-fibrinogen samples, except fibrinogen-3 resulting from incubation with factor XIIIa, the ratios of the radioactivities, in the three bands corresponding to the Aα, Bβ, and γ chains of fibrinogen were similar (0.46 ± 0.05:1.00:0.32 ± 0.06, means ± SD of 13 samples; the radioactivity in the Bβ band has arbitrarily been assigned the value of 1.00). Difference in the ratios were observed for the samples of fibrinogen-3 that had been exposed to factor XIIIa; the radioactivities of both the Aα and γ chains were reduced with respect to that of the Bβ chain (0.28 ± 0.07:1.00:0.21 ± 0.02, means ± SD of 4 samples). These ratios could not be calculated for all samples because of insufficient separation between the Bβ and γ chains, but the ratios are significantly different (p < 0.05), and all the values calculated for the Aα and γ chains of fibrinogen-3 after incubation with factor XIIIa were lower than the corresponding values for any of the other samples.

**DISCUSSION**

The concentration of factor XIIIa was chosen so that it would not crosslink fibrinogen in solution under the conditions of our aggregation and binding experiments, but it was sufficient to introduce γ-chain cross-links into a fibrin clot, where the monomers are aligned in a favorable configuration, within 10 min. Thus, we were using conditions where no fibrinogen crosslinking was observed in the absence of platelets. It was possible.
that fibrinogen molecules that were bound to the platelet membrane might be aligned in a configuration that would enhance the crosslinking process, either between fibrinogen molecules or in linkages involving membrane glycoproteins, and such crosslinking might be expected to stabilize fibrinogen–platelet aggregates. However, we observed no enhancement or substantial stabilization of ADP-induced aggregation of rabbit platelets by factor XIIIa when it was added at this concentration, 3.3 μg/ml, which is approximately 11% of the maximum concentration that might be produced in normal human plasma.25 This is in contrast to the observations of Pastarova et al.,26 who reported that factor XIIIa, at approximately 125 μg/ml, enhances the aggregation of rat platelets induced by a mixture of ADP and thrombin. We also found that factor XIIIa does not affect the initial binding of 125I-fibrinogen to ADP-stimulated platelets. However, it does appear to cause a slow association of fibrinogen with platelets that is independent of ADP, is not inhibited by PGE1, and does not lead to aggregation. This apparently represents binding of fibrinogen to platelets rather than the appearance in the platelet pellet of fibrinogen polymers formed by the crosslinking action of factor XIIIa, since when 125I-fibrinogen and factor XIIIa were incubated together at 37°C for 13 min and then added to a platelet suspension that was centrifuged after 1 min, a much smaller amount of 125I-fibrinogen was found in the pellet, corresponding to the amount expected to be bound after 1 min in the absence of ADP. Specific effects of the particular thrombin inhibitor used were ruled out since hirudin and antithrombin III–heparin could be used interchangeably without affecting the results. Similarly, the replacement of apyrase as an ADP scavenger by CP/CPK had no effect. We assumed that the thrombin used to activate the factor XIII was completely inactivated, since neither of the thrombin–inhibitor mixtures nor the factor XIIIa caused platelet aggregation or fibrinogen clotting. However, a more sensitive test for traces of thrombin activity is synergism with low concentrations of ADP.27 A concentration of thrombin, too low to cause platelet aggregation, would be expected to enhance the aggregating potential of a low concentration of ADP, but when we tested the thrombin–inhibitor mixtures or factor XIIIa with 0.09 μM ADP, which caused no aggregation by itself, or with 0.22 μM ADP, which caused very slight aggregation, no enhancement was observed. Therefore, we are confident that the effects observed with factor XIIIa were not due to traces of active thrombin.

The argument for a specific effect of factor XIIIa on fibrinogen binding to rabbit platelets is strengthened by the characterization of the 125I-fibrinogen that had been associated with platelets. Control platelets, to which either 0.15M NaCl or the thrombin–antithrombin III–heparin mixture had been added, did not cause observable changes in 125I-fibrinogen during a 10-min exposure in the presence or absence of ADP. This was true even though there was an apparent increase in specific binding of 125I-fibrinogen to platelets by 10 min in all cases. However, the increase in binding to platelets in the presence of factor XIIIa at longer time intervals was significantly greater, and the 125I-fibrinogen that was separable from the platelets only by solubilization with detergent gave evidence of covalent crosslinking leading to high molecular size material. Apparently both the Aα and the γ chains of fibrinogen were involved in this process. We conclude that this covalent crosslinking of fibrinogen is related to the increase in fibrinogen binding to platelets after prolonged (10 min) incubation, but since we have measured only 125I, our observations do not allow us to distinguish between covalent attachment of fibrinogen to platelet membrane protein(s) and crosslinking between fibrinogen molecules leading to stabilization of their binding to platelets. The fact that thrombin inactivated with antithrombin III and heparin did not cause this crosslinking provides additional evidence that the phenomena observed with factor XIIIa cannot be due to traces of active thrombin.

In summary, factor XIIIa at the concentration used has no effect on the initial responses of rabbit platelets to ADP. Neither the extent of aggregation nor the binding of 125I-fibrinogen is enhanced. The rate of deaggregation is slightly reduced, but deaggregation is complete and the presence of factor XIIIa does not stabilize the platelet aggregates. However, factor XIIIa apparently causes a slow increase in specific binding of fibrinogen to platelets, which is independent of stimulation with ADP and does not lead to aggregation. This binding is accompanied by crosslinking of fibrinogen through its Aα and γ chains either to itself or to a platelet protein or proteins.

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