Regulation of Plasma Factor XIII Binding to Fibrin In Vitro

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The binding of plasma factor XIII to fibrinogen or fibrin has been chemically or enzymatically induced to polymerize was studied. Factor XIII binding was assayed using a \( ^3H \)-putrescine incorporation assay and an \( ^{125}I \)-plasma factor XIII binding assay. More than 80% of the native and radiolabeled plasma factor XIII was bound to fibrin I formed by reptilase in EDTA, citrate, or heparin anticoagulated plasma. Plasma factor XIII and \( ^{125}I \)-factor XIII was bound (89.6% to 92.5%) to fibrin II formed by thrombin or in either citrate or EDTA anticoagulated plasma. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of \(^{125}I\)-plasma factor XIII bound to fibrin I or fibrin II formed by reptilase or thrombin in the presence of EDTA demonstrated the B-subunit remained bound to the a-chains or thrombin-cleaved a-chains. In the presence of calcium chloride and thrombin, the B2-subunit dissociated and factor XIIIa was bound. Protamine sulfate caused fibrinogen polymerization in the absence of divalent cations and reduced both plasma factor XIII and immunologic fibrinogen levels. Fibrinogen polymerized by protamine sulfate bound plasma factor XIII and the a2-subunit of \( ^{125}I \)-platelet factor XIII. Plasma factor XIII was also bound to sonicated non-cross-linked fibrin II in either normal plasma or afibrinogenemic plasma. Plasma levels of several coagulation proteins were unchanged after the addition of reptilase, protamine sulfate, or sonicated fibrin to plasma. These results demonstrate that a specific binding site for the a2-subunit of plasma factor XIII is present on polymerized fibrinogen, fibrin I, and fibrin II. Furthermore, the presence of divergent cations, thrombin-cleavage of plasma factor XIII, and release of fibrinopeptides A or B are not required for plasma factor XIII binding to polymerized fibrinogen and fibrin.

PLASMA factor XIII circulates as a complex composed of two a-chains and two b-chains noncovalently associated.1 Factor XIIIa, the fibrin stabilizing enzyme, is formed during clotting in two steps. In the first step, thrombin cleaves a 4,000 dalton activation peptide from each a-chain of plasma factor XIII.2 After the release of the activation peptides, an inactive intermediate is formed, designated factor XIII'. In the second step, factor XIII' in the presence of calcium undergoes a conformational change which causes the B2-subunit to dissociate.3 The active site sulfhydryl group is then exposed on the a-chains producing Factor XIIIa.4 Fibrinogen lowers the calcium concentration required to dissociate the B2-subunit from factor XIII'.5 In addition, addition lowers the thrombin concentration required to release the activation peptides from factor XIII'.6

Soluble fibrinogen assembles into a fibrin gel following proteolysis of fibrinopeptides A and B by thrombin. After fibrinopeptide A is removed, fibrin monomers form protofibrils, which contain overlapping fibrin molecules aligned end-to-end. After the protofibrils reach a critical size, they associate laterally to form thick fibrin fibers.7,8 Fibrinopeptide B is released after fibrinopeptide A,9,10 and formation of fibrin protofibrils enhances the release of fibrinopeptide B.11 Fibrin I is formed by reptilase cleavage of fibrinopeptides A from fibrinogen.9 In contrast, fibrin II is formed after thrombin cleavage of fibrinopeptides A and B.9

During blood coagulation, factor XIIIa catalyzes formation of \( \gamma \)-glutamyl-c-lysyl peptide bonds between polymerizing fibrin molecules.12-15 Factor XIIIa-modified fibrin is required for normal hemostasis since factor XIII-deficient individuals have a serious lifelong bleeding disorder.16,17 Cross-linked fibrin has been shown to be mechanically stronger18,19 and more resistant to lysis by plasmin than non-cross-linked fibrin.20,21 In addition, factor XIIIa-catalyzed cross-linking of \( \alpha2 \) plasmin inhibitor to fibrin was correlated with increased resistance of cross-linked fibrin to plasmin lysis.22,23 These characteristics explained in part the importance of cross-linked fibrin for normal hemostasis. Factor XIIIa was reported to increase the adherence of platelets to fibrin24,25 and the subendothelium by cross-linking fibronectin to either fibrin26 or collagen.27 Factor XIIIa must be bound to fibrin in order for these reactions to occur.

Factor XIIIa binding to fibrin was demonstrated by studies in which functional28-30 and immunologic factor XIII levels31,32 were reduced in serum compared to plasma. In addition, thrombin-cleaved a-chains of factor XIIIa were bound to fibrin in vitro.33,34 Triantaphyllopoulos has reported that fibrin monomers reduced the levels of factor XIII in plasma after the fibrin gel formed.35 The role of fibrin polymerization and release of fibrinopeptides A and B in regulating plasma factor XIII binding to fibrin has not been studied in detail.

In this study, plasma factor XIII binding to polymerized fibrinogen and fibrin in a plasma milieu was characterized. Plasma factor XIII bound to fibrinogen or fibrin that was enzymatically or chemically induced to polymerize. Plasma factor XIII binding to the polymerized fibrinogen was mediated through the a2-subunit of factor XIII and divalent cations were not required.

MATERIALS AND METHODS

Human plasma collected from normal donors was anticoagulated in either 3.2% citrate (one part citrate to nine parts blood). 10
mmol/L of EDTA or 10 U/mL of heparin. Platelet-poor plasma prepared by centrifuging blood at 2,500 \( \times \) g for ten minutes was stored at \(-70^\circ\) C. A fibrinogeneminc plasma (containing less than 30 mg/dL immunologic fibrinogen) was purchased from George King Biomedical Corporation, Overland, Kan. Human \( \alpha \)-thrombin (8,160 NIH U/mL) was supplied by Dr J. W. Fenton, II (NY State Department of Health, Albany, NY). Plasma and platelet factor XIII were purified and stored as described.34 Factor XIII and immunoglobulin G (Sigma, St Louis) were radioiodinated by the lactoperoxidase method.34 Factor XIIIa was prepared by incubating platelet factor XIII (20 \( \mu \)g/mL) with thrombin (2.5 \( \mu \)U/mL) in 0.02 mol/L of Tris-HCl, 0.13 mol/L of NaCl, pH 7.4 (Tris buffered saline [TBS]) buffer) for 30 minutes at 37 \( \circ \) C. Thrombin was inhibited by adding 0.1 mmol/L of p-nitrophenyl-p-guanidino- benzoate (PNPGB, Sigma) dissolved in \( \pi \pi \) dimethylformamide.

Measurement of plasma factor XIII levels. Plasma factor XIII was assayed in plasma as described.30 All assays were performed in triplicate, and results were reported as the mean \( \pm \) SD. Factor XIII levels were calculated as a percentage of the total plasma factor XIII activity.

Preparation of fibrinogen. Human fibrinogen grade L purchased from Kabi Diagnostica (Stockholm, Sweden) was incubated with Lysine-Sepharose (Sigma) and gelatin-agarose (Biorad, Richmond, Calif) to remove plasminogen and fibronectin, respectively.36 Factor XIII was inactivated by incubating the fibrinogen in 3.3 mol/L of urea for 16 hours at 22 \( \circ \) C.37 The factor XIII-free fibrinogen was exhaustively dialyzed in TBS buffer containing 3 mmol/L of EDTA.

Plasma factor XIII binding to fibrin I. Reptilase (Atroxin, Sigma) suspended in distilled, deionized water (0.2 \( \mu \)g/mL) was added to plasma. After 30 minutes at 37 \( \circ \) C, the fibrin was compressed and serum-assayed for unbound factor XIII. Reptilase (0.2 \( \mu \)g/mL) incubated with 10 \( \mu \)g of either purified platelet or plasma factor XIII in 1 mL of TBS for 60 minutes at 37 \( \circ \) C did not generate factor XIII activity. Fibrin I formed by reptilase in heparinized, EDTA, and citrated plasma was washed in 10 mmol/L of EDTA and solubilized in 4% SDS, 6 mol/L of urea, 2% \( \beta \)-mercaptoethanol; then 30 \( \mu \)g of solubilized fibrin was separated by SDS-PAGE.38 No \( \gamma \)-chain cross-linking was detected. Reptilase clotted 95% of the fibrinogen in citrate, heparin, and EDTA-plasma in 30 minutes at 37 \( \circ \) C.

Plasma factor XIII binding to polymerized fibrinogen. Protamine sulfate type X (10 mg/mL) (Sigma) in TBS buffer was added to either citrated normal or fibrinogenemic plasma (0.2 mL). After 30 minutes at 4 \( \circ \) C, samples were centrifuged at 10,000 \( \times \) g for ten minutes in a Beckman microfuge, and the supernatant was assayed for unbound factor XIII. Protamine sulfate (4.0 mg/mL) had no effect on the plasma factor XIII level in fibrinogenemic plasma.

Plasma factor XIII binding to fibrinogen. Polymerized fibrinogen, fibrin I, and fibrin II. Fibrinogen polymerized with protamine sulfate (4 mg/mL). After 30 minutes at 4 \( \circ \) C, the mixture was centrifuged, and bound \( ^{125}I \)-factor XIII was determined by measuring the radioactivity in the supernatant. This value was subtracted from the total radioactivity in the incubation mixture, and the percentage of bound \( ^{125}I \)-Factor XIII was calculated. All assays were performed in triplicate, and results were expressed as the mean \( \pm \) SD.

Preparation of fibrin II. Thrombin (10 U/mL) was incubated with 1 mL of fibrinogen (2.0 mg/mL) for one hour at 37 \( \circ \) C. The fibrin was washed, centrifuged, and thrombin-inhibited by 5 mmol/L of phenylmethanesulfonfonylfluoride. The fibrin was sonicated in 0.2 mL of 0.02 mol/L of Tris-HCl, 0.13 mol/L of NaCl, 1 mmol/L of EDTA pH 7.4 for three separate 15-second bursts at power setting 5, using a W-220 sonicator with a microtip from Heat Systems, Ultrasonics Inc. The sonicated fibrin was centrifuged and resuspended in 0.2 mL of TBS. The fibrin was resuspended by a ten-second burst prior to use in the factor XIII binding assay.

Characterization of \( ^{125}I \)-factor XIII bound to polymerized fibrinogen, fibrin I, and fibrin II. Polymerized fibrinogen, fibrin I, or fibrin II containing bound \( ^{125}I \)-factor XIII, was washed at 4 \( \circ \) C in TBS buffer containing 5 mmol/L of EDTA, 1% (vol/vol) protamine sulfate and solubilized in 4% SDS, 6 mol/L of urea, 0.0125 mol/L of Tris-HCl, pH 6.8 at 56 \( \circ \) C for 18 hours. The solubilized material (52,000–115,000 cpm) was separated by 10% SDS-PAGE.39 Gels were dried, and autoradiography was performed using Kodak \( \cdot \) X-2 film. Plasma factor XIII was identified by the characteristic mobility of the a-chains and b-chains in nonreduced SDS-polycrylamide gels.36 \( ^{125}I \)-factor XIII contained 40% of the radiolabel in the a-chains and 51% in the b-chain areas when radioactivity was quantitated after separation by SDS-PAGE.34 If the bound and unbound material had a similar percent of radioactivity in the a-chain and b-chain area, \( ^{125}I \)-factor XIII was bound. Factor XIIa binding to fibrin was identified by the more rapid migration of the thrombin-cleaved a-chain, designated a', as compared with the native a-chain in nonreduced SDS-polycrylamide gels.31 Furthermore, the b-subunit was dissociated from factor XIIa.31

Plasma factor XIII binding to fibrin II. Citrated plasma (10% vol/vol) was incubated in TBS buffer containing 5 mmol/L of EDTA, 1.5 mmol/L of Gly-Pro-Arg-Pro and fibrin II (2.0 mg/mL) in a total volume of 0.1 mL. Incubations were performed at 4 \( \circ \) C, 22 \( \circ \) C, and 37 \( \circ \) C for various times. The incubation mixture was then centrifuged for two minutes at 10,000 rpm in a Beckman microcentrifuge, and the supernatant was assayed for factor XIII. Similar experiments were performed using either normal or fibrinogenemic plasma (10% vol/vol) in TBS buffer containing 5 mmol/L of EDTA, 1.5 mmol/L of Gly-Pro-Arg-Pro, and incubating for 15 minutes at 37 \( \circ \) C with various fibrin II concentrations.

Measurement of antithrombin III, fibrinogen, \( \alpha \)-macroglobulin, \( \alpha \)-plasmin inhibitor, and ristocetin cofactor levels. Immunological levels of fibrinogen, antithrombin III, plasma, and \( \alpha \)-macroglobulin were determined using radial immunodiffusion kits supplied by Calbiochem, La Jolla, Calif. \( \alpha \)-Plasmin inhibitor levels were determined by methylamine treating plasma to inhibit \( \alpha \)-macroglobulin and the antiplasmin activity measured with the chromogenic substrate S-2251.37 Factor VIII ristocetin-cofactor measurements were performed using the commercial von Willebrand's Factor assay kit supplied by General Diagnostics, Morris Plains, NJ.

RESULTS

Plasma factor XIII binding to fibrin I. Reptilase converted fibrinogen to fibrin I without activating plasma factor XIII.3 Similar amounts of native plasma factor XIII bound
to fibrin formed in plasma anticoagulated by either heparin, citrate, or EDTA (81.5% to 84.6%).

\[ 125I\text{-Plasma factor XIII binding to fibrin I provided direct evidence that the reduction in plasma factor XIII levels was due to plasma factor XIII binding and not to inhibition of factor XIIIa. A similar percentage of } 125I\text{-plasma factor XIII (80.5% to 88.3%)} \text{ was bound to fibrin formed by reptilase in plasma anticoagulated by either citrate, EDTA, or heparin.} \]

The effect of divalent cations on plasma factor XIII binding to fibrin I was studied using heparinized plasma. Calcium chloride, magnesium chloride, manganese chloride, and EDTA did not modify factor XIII binding to fibrin I in heparinized plasma (82.9% ± 3.8%). In addition, heparin (10 U/mL) did not modify plasma factor XIII binding to fibrin I formed by reptilase in EDTA treated plasma.

\text{Plasma factor XIII binding to polymerized fibrinogen.} \text{ Because plasma factor XIII was bound to fibrin I, and because we have found that plasma factor XIII is bound reversibly to fibrinogen,}^{26} \text{ we investigated to discover if plasma factor XIII bound to polymerized fibrinogen. Fibrinogen polymerized by protamine sulfate forms a fibrin-like structure when examined by electron microscopy.}^{38} \text{ Protamine sulfate caused a concentration-dependent reduction in the immunologic fibrinogen and factor XIII levels in citrated plasma (Fig 1). Protamine sulfate had no effect on plasma factor XIII levels when added to afibrinogenemic plasma alone. Similar results were obtained with plasma containing 10 mmol/L of EDTA.} \]

\text{Effect of fibrinogen on plasma factor XIII binding to fibrin I and polymerized fibrinogen.} \text{ The fibrinogen concentrations required for maximal and half-maximal factor XIII binding to fibrin I (Fig 2A) or polymerized fibrinogen (Fig 2B) were similar. When either fibrin I or polymerized fibrinogen was studied, maximal factor XIII binding occurred at 1 mg/mL of fibrinogen, and 50% of the plasma factor XIII was bound at 0.3 mg/mL fibrinogen.} \]

\text{Characterization of } 125I\text{-plasma factor XIII bound to fibrin I and fibrin II.} \text{ 121I-Plasma factor XIII bound to fibrin I (71.8% ± 8.5%) and the b2-subunits did not dissociate (Fig 3, lanes A and B). This indicates that divalent cations were not required for plasma factor XIII binding to fibrin I. SDS-PAGE of the fibrin confirmed that } \gamma \text{ chain cross-linking was absent.} \]

\text{125I-Plasma factor XIII was added to EDTA-anticoagulated plasma and fibrin II formed by thrombin. SDS-PAGE of the bound and unbound } 125I\text{-factor XIII demonstrated the presence of thrombin-cleaved a-chains, and b-chains (Fig 3, lanes C and D). The bound and unbound factor XIII contained a similar percentage of radioactivity in the b-chain area. Therefore, the b2-subunit remained associated with the thrombin-cleaved a-chains and did not modify thrombin-cleaved factor XIII binding to fibrin II.} \]

\text{Calcium-mediated dissociation of the b2-subunit from thrombin-cleaved plasma factor XIII was also studied (Fig 4). SDS-PAGE of the } 125I\text{-plasma factor XIII bound to fibrin in the presence of calcium electrophoresed in a position (Fig 4, lane A) similar to thrombin-cleaved } 125I\text{-platelet factor XIII (Fig 4, lane C) and ahead of } 125I\text{-platelet factor XIII (Fig 4, lane D).} \]

\text{Because the b2-subunit remained associated with thrombin-cleaved Factor XIII bound to fibrin, we studied platelet factor XIII binding to fibrin. Reptilase caused 76% ± 8.5% of the platelet factor XIII to bind fibrin I. Because platelet factor XIII only contained the a2-subunit, this demonstrated}
Characterization of $^{125}$I-plasma factor XIII bound to fibrin I and fibrin II in the absence of divalent cations. $^{125}$I-plasma factor XIII (1.0 µg; 2950 cpm/ng) was added to 1.0 mL of EDTA plasma and clotted by adding reptilase (0.2 µg) or thrombin (1.0 U/mL) for 30 minutes at 37 °C. The bound (lane A) and unbound (lane B) materials were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Upper band, intact a-chains; lower band, b-chains. The bound (lane C) and unbound (lane D) $^{125}$I-plasma factor XIII from fibrin formed by thrombin was analyzed.

that the a₂-subunit of factor XIII regulated binding to fibrin I.

Characterization of $^{125}$I-plasma factor XIII and $^{125}$I-platelet factor XIII bound to polymerized fibrinogen. Protamine sulfate caused 70% ± 8.5% of $^{125}$I-plasma factor XIII to bind to polymerized fibrinogen in citrated plasma. SDS-PAGE of the bound $^{125}$I-plasma factor XIII demonstrated the presence of both the a-chains and b-chains in a percentage similar to the starting material. This demonstrated that plasma factor XIII was bound to polymerized fibrinogen.

Evidence that the a₂-subunit of plasma factor XIII regulated binding to fibrin I. Protamine sulfate caused 70% ± 8.5% of $^{125}$I-plasma factor XIII to bind to polymerized fibrinogen in citrated plasma. Under conditions in which 85% of the $^{125}$I-platelet factor XIII was bound to polymerized fibrinogen, the bound (Fig 5, lane C) and unbound factor XIII (Fig 5, lane D) migrated in the original a-chain position.

Plasma factor XIII binding to fibrin II. Plasma factor XIII binding to sonicated fibrin II was studied since experiments with intact clots were technically difficult to perform. In addition, plasma factor XIII may require time to diffuse into the fibrin gel. Similar techniques have been used by others to study plasminogen binding to fibrin. Plasm factor XIII rapidly bound to fibrin II added to citrated a fibrinogenemic plasma at 4 °C, 22 °C, and 37 °C (Fig 6A). After 120 seconds, 200 seconds and 270 seconds, 50% of the plasma factor XIII was bound to fibrin II at 4 °C, 22 °C, and 37 °C, respectively (Fig 6B). Similar results were obtained using EDTA anticoagulated plasma. Gly-Pro-Arg-Pro (1.5 to 5.0 mmol/L) did not inhibit plasma factor XIII and factor XIIIa binding to fibrin II in fibrinogenemic plasma. A similar amount of plasma factor XIII was bound to polymerized fibrin added to normal plasma (Fig 6B), demonstrating that soluble fibrinogen did not inhibit plasma factor XIII binding to fibrin II. SDS-PAGE of $^{125}$I-plasma factor XIII bound to fibrin II demonstrated that both the a-chains and b-chains were bound and present in amounts similar to the original $^{125}$I-plasma factor XIII. Thrombin cleavage of the a-chains was not detected. The fibrin II concentration required to cause optimal and 50% factor XIII binding was similar to the concentrations required for factor XIII binding to fibrin I and polymerized fibrinogen (Figure 2A and B).

Specificity of plasma factor XIII binding to polymerized fibrinogen fibrin I and fibrin II. The specificity of plasma factor XIII binding to polymerized fibrinogen, fibrin I, and fibrin II was studied by assaying the supernatant from protamine-treated plasma, reptilase clotted plasma, and plasma incubated with fibrin II. The levels of antithrombin III, plasminogen, factor VIII ristocetin cofactor, α₂-macroglobulin, and α₂-plasmin inhibitor in plasma were within 18% of the original plasma concentration.
A B C D

Fig 5. Identification of $^{125}$I-platelet factor XIII bound to polymerized fibrinogen. $^{125}$I-platelet factor XIII (1.0 μg) was added to 1.0 ml of TBS buffer, pH 7.4 containing fibrinogen (2.5 mg/ml) and protamine sulfate (4.0 mg/ml) for 30 minutes at 4 °C. The bound (lane C) and unbound $^{125}$I-platelet factor XIII (lane D) were electrophoresed. $^{125}$I-Platelet factor XIII (lane A) and thrombin-activated $^{125}$I-platelet factor (lane B) serve as markers. More than 85% of the $^{125}$I-platelet factor XIII bound to polymerized fibrinogen in this experiment.

Plasma factor XIII was not nonspecifically trapped in the fibrin gel since less than 5% of the $^{125}$I-immunoglobulin G was bound to polymerized fibrinogen, fibrin I, or fibrin II.

DISCUSSION

Lorand and Dickenman were the first to demonstrate that serum factor XIII levels are lower than plasma levels.28 Since then, immunological studies have demonstrated that the α-chains of plasma factor XIII are reduced as compared with serum levels,31,32 and others have confirmed that factor XIII levels in serum are lower than plasma levels.29,30

Using a functional factor XIII assay and $^{125}$I-plasma factor XIII, we have studied plasma factor XIII binding to polymerized fibrinogen, fibrin I, and fibrin II in platelet-poor plasma. We found that plasma factor XIII composed of α₂-subunit and β₂-subunit bound to polymerized fibrinogen, fibrin I, and fibrin II. The β₂-subunit did not modify plasma factor XIII binding. The binding of plasma factor XIII to polymerized fibrinogen, fibrin I, and fibrin II did not require divalent cations. However, in the presence of calcium chloride, the β₂-subunit dissociated from thrombin-cleaved plasma factor XIII.

$^{125}$I-platelet Factor XIII that contained only the α₂-subunit was also bound to polymerized fibrinogen. These data suggested that polymerization of fibrinogen or fibrin was sufficient to localize plasma factor XIII to the fibrinogen polymer. Furthermore, the release of the fibrinopeptides A and B from fibrinogen was not necessary for plasma factor XIII binding. In summary, plasma and platelet factor XIII binding to polymerized fibrinogen and fibrin was regulated by a site on the α₂-subunit and did not require divalent cations.

Our results confirm and extend the studies of Triantaphyllopoulos demonstrating that fibrin monomer reduces plasma factor XIII levels after fibrin gelation.35 We have demonstrated that plasma factor XIII was rapidly and selectively bound to fibrin in a plasma milieu, indicating that other plasma proteins did not compete for plasma factor XIII binding. The results of studies with fibrin II demonstrated that plasma factor XIII was bound to non-cross-linked fibrin protofibrils after they assembled to form fibrin fibers.

Janus et al6 found that the rate of thrombin-cleavage of plasma factor XIII is increased by fibrinogen and occurs soon after the release of fibrinopeptide A. We have recently reported that thrombin cleavage of fibrinogen and factor XIII are closely coordinated events during the clotting of platelet-rich plasma, and that thrombin cleavage of factor
XIII correlates with fibrin polymerization. Furthermore, inhibition of fibrin polymerization by Gly-Pro-Arg-Pro delays thrombin cleavage of factor XIII. We also found that plasma factor XIII binds reversibly to fibrinogen through the $\alpha_2$-subunit and, based on the binding constant, $(1 \times 10^{-4} \text{ mol/L})$, >90% of plasma factor XIII is complexed to fibrinogen. We therefore propose that plasma factor XIII reversibly binds to fibrinogen in plasma through its $\alpha_2$-subunit and remains associated with des-A-fibrinogen after the release of fibrinopeptide A from fibrinogen. Plasma factor XIII bound to des-A-fibrinogen is cleaved by thrombin during formation of fibrin I polymers. After plasma factor XIII is cleaved by thrombin, the $\beta_2$-subunit dissociates in the presence of calcium to form factor XIIIa. The factor XIIIa then catalyzes the formation of $\gamma$-glutamyl-$\epsilon$-lysyl bonds of the fibrin I polymers. Consistent with this model are findings that des-A-fibrinogen and des A,B-fibrinogen increase the rate of thrombin-catalyzed Factor XIIIa formation in plasma when they polymerize.

Plasma factor XIII binding to fibrinogen and fibrin may serve to regulate factor XIIIa formation and to prevent factor XIIIa from circulating in plasma. As a result of these interactions, plasma factor XIII and factor XIIIa are localized to sites of fibrin formation. Studies are in progress to characterize the region on the factor XIII $\alpha_2$-subunit that is bound to fibrin. Clinical disorders of fibrin stabilization may result from abnormal factor XIII binding to fibrin or defects in fibrin polymerization.

REFERENCES

33. Folk JE, Chung SI: Blood coagulation factor XIII: Relationship of some biological properties to subunit structure in proteases
and biological control, E Reich, DB Rifkin and E Shaw (eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1975


1985 66: 1028-1034

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