Covalent Crosslinking of von Willebrand Factor to Fibrin

By Masao Hada, Marek Kaminski, Paula Bockenstedt, and Jan McDonagh

Factor XIlla crosslinks a limited number of substrates via ϵ(γ-glutamyl)-lysyl bond formation. It crosslinks fibrin to itself, α2-plasmin inhibitor and fibronectin to fibrin, and fibronectin to collagen. Results presented here show that plasma von Willebrand factor (vWF) is a substrate for factor XIlla and can be crosslinked to fibrin during gel formation. vWF-fibrin crosslinking was studied in purified systems and in plasma with 125I-vWF and 131I-fibrinogen. vWF incorporation into fibrin increased with time or increasing factor XIlla. After electrophoresis of dissolved clots, distribution of 125I and 131I was measured and showed that vWF was crosslinked to the α chain of fibrin and chains of fibrin during the process of fibrin gel formation. This reaction can occur in plasma and requires only that the gelation process be long.

MATERIALS AND METHODS

von Willebrand factor purification and radiolabeling. vWF was purified from human plasma (obtained from the American Red Cross Blood Services, Northeast Region) by a modification of the procedure of Kao and colleagues, as previously described. Protein concentrations were determined by the method of Lowry and co-workers and by electrophoresis on SDS gels. Fibrinogen preparations. Fibrinogen, in particular, and also fibronectin, bind to the platelet glycoprotein Ib-IIIa receptor, which raises the possibility of specific vWF interaction with these proteins that also facilitate platelet adhesion and aggregation. Purification of each of these proteins from plasma is hampered by contamination with the other two. Fibrinogen is necessary for the cryoprecipitation of vWF, and both fibrinogen and fibronectin are required for heparin-induced precipitation of vWF. vWF also precipitates in the presence of fibrin-fibronectin complexes. The conditions under which vWF forms aggregates with fibrinogen—fibronectin are nonphysiologic, and it is difficult to determine if such aggregate formation has physiological relevance. However, it is possible that similar interactions between fibrin and vWF may occur at sites of vessel wall injury where glycosaminoglycans on damaged endothelial surface or in the matrix may be available.

It has previously been reported that serum from hemophilia A with long clotting times contains less vWF than do the corresponding plasma samples. This was only observed in in vitro clotting systems when the clotting time was prolonged. However, it was also observed in normal samples when the clotting time was long. From this it was hypothesized that a time-dependent interaction between vWF and fibrin could occur during clotting under appropriate conditions. Further studies with factor XIII-deficient or factor XIII-supplemented plasma indicated that this interaction might involve covalent crosslinking of vWF to fibrin.

In the present study, the interaction of vWF with fibrin in purified test systems and in plasma clotting systems has been characterized. Evidence is presented that vWF is a substrate for the transglutaminase reaction catalyzed by factor XIIIa. Monomeric vWF can covalently crosslink to itself, but the polymeric forms do not. However, under appropriate conditions vWF polymers can be covalently crosslinked to the α chain of fibrin during the process of fibrin gel formation. This reaction can occur in plasma and requires only that the gelation process be long.

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Submitted Aug 5, 1985; accepted Feb 17, 1986.

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0006-4971/86/6801-0014$03.00/0
In some experiments, this was used as a source of vWF. In experiments using radiolabeled fibrinogen, the fibrinogen preparation was freed of vWF by affinity chromatography on fibrin monomer Sepharose after labeling.\textsuperscript{22} vWF was eluted by 50 mmol/L of Tris-H$_2$PO$_4$·0.1 mol/L of NaCl-25 mmol/L EACA, pH 7.6. Fibrinogen was eluted by Tris-H$_2$PO$_4$·EACA buffer containing 1 mol/L of NaCl, pH 4.1. The thrombin inhibitor, d-phenylalanine-1-phenylalnine-arginine chloromethyl ketone (PPACK) (Calbiochem-Behring, La Jolla, Calif), was added to the sample and to the elution buffers. After elution, fibrinogen was dialyzed v Tris-saline, pH 7.4, and concentrated by precipitation with polyethylene glycol. This fibrinogen was labeled with $^{131}$I by the solid-phase lactoperoxidase-glucose-oxidase procedure using Enzymobeads (Bio-Rad, Richmond, Calif). The specific radioactivity was 0.025 to 0.035 $\mu$Ci/$\mu$g of protein.

Factor XIII. Factor XIII was purified from human plasma or placental concentrate as previously described.\textsuperscript{23} Placental concentrate (Fibrogammin) was kindly provided by Behringwerke A.G.; it contained the intracellular form of factor XIII (equivalent to platelet factor XIII, $\alpha_2$) and primarily albumin. Because in these studies the same results were obtained with purified factor XIII and with the concentrate, the concentrate was routinely used without further purification. In the concentrate, 1 U is equal to the amount of factor XIII in 1 mL of normal plasma. Factor XIII activity was assayed by the $^{14}$C-putrescine incorporation assay with activation of the zymogen by thrombin and CaCl$_2$.\textsuperscript{24} In experiments in which factor XIIIa was added directly to the test system, the zymogen was treated with 1 U/mL of thrombin and 10 mmol/L of CaCl$_2$ for 30 minutes at room temperature. After cleavage of the factor XIII A chain, thrombin was inhibited by addition of PPACK or hirudin, which did not interfere with factor XIII $\alpha_2$ enzyme activity.

Putrescine incorporation into vWF. The substrate suitability of vWF for the crosslinking reaction catalyzed by factor XIIIa was determined by measuring the covalent incorporation of $^{14}$H-putrescine or $^{14}$C-putrescine (New England Nuclear, Boston) into purified vWF in the presence of factor XIIIa. The following experimental conditions were used: 0.99 $\mu$mol/L of vWF, 50 mmol/L of putrescine, 16.7 $\mu$mol/L of dithiothreitol, 6.7 $\mu$mol/L of CaCl$_2$, and 21 U/mL of thrombin. Factor XIIIa was varied from 0 to 5 U/mL. In some experiments hirudin (83 U/mL) was added after factor XIII activation and before addition of vWF to the test system. Incorporation of putrescine into vWF was allowed to proceed at room temperature for 2 minutes to 18 hours. The reaction was terminated by spotting samples on filter paper and immediately immersing them in cold 10% trichloroacetic acid (TCA) for 20 minutes. Filter papers were transferred to 5% TCA and then washed three times each in ethanol-acetone (1:1) and 100% acetone. Samples were air dried and counted in a scintillation counter. The recovery of vWF by this technique was 97.8% to 100%.

Covalent incorporation of putrescine into vWF was also analyzed by SDS gel electrophoresis. Samples were similarly prepared (50 mmol/L of $^{14}$H-putrescine, 0.99 $\mu$mol/L of vWF, 6.7 $\mu$mol/L of CaCl$_2$, 21 U/mL of thrombin, 0 to 5 U/mL factor XIIIa); incorporation was allowed to proceed for 18 hours at 4 C; it was terminated by addition of SDS sample buffer containing dithiothreitol, followed by incubation at 100°C for 5 minutes. SDS gel electrophoresis was carried out on 4% cylindrical gels under reducing conditions. Gels were sliced in 1-mm sections and dissolved in 30% H$_2$O$_2$ overnight at 56°C, and total radioactivity was measured.

Covalent crosslinking of vWF to fibrin in a purified system. For these experiments factor XIII (0 to 50 U/mL) was preactivated with thrombin (5 U/mL) and CaCl$_2$ (10 to 100 mmol/L) for 30 minutes at 37°C. In the second step, the activation mixture was diluted fivefold to tenfold with $^{125}$I-fibrinogen and $^{125}$I-vWF, and clotting was allowed to proceed for 2 minutes to 18 hours. Fibrinogen, which had no factor XIII activity, was 1.4 to 1.9 mg/mL; vWF was 48 to 56 $\mu$g/mL for the test samples and 0 in the controls. Test systems contained 10 $\mu$L $^{125}$I-fibrinogen (78,300 cpm/$\mu$L) and 10 $\mu$L $^{125}$I-vWF (10,700 cpm/$\mu$L). At various time intervals, clots were separated, compressed to remove the fluid, and washed with Tris-saline containing 50 mmol/L of EDTA. Total radioactivity in the clots and supernatants was measured. Supernatants were also assayed for vWF antigen by electroimmunoassay. Washed clots were dissolved in urea-SDS-DTT sample buffer and analyzed by SDS gel.\textsuperscript{25} After electrophoresis, the gels were sliced in 1-mm sections and counted in a $\gamma$-spectrometer.

Covalent crosslinking of vWF to fibrin in a plasma system. Normal plasma (fibrinogen, 2.4 to 2.6 mg/mL; vWF antigen 10 to 15 $\mu$g/mL) and plasma from a patient with severe hemophilia A (fibrinogen, 2.6 mg/mL; vWF antigen, 20 $\mu$g/mL) were allowed to clot by addition of CaCl$_2$ (10 mmol/L). In some experiments, clotting times were adjusted by adding hirudin to normal plasma or thrombin to factor VIII-deficient plasma. In some experiments, trace amounts of $^{131}$I-fibrinogen and $^{125}$I-vWF were added to the plasmas before clotting. Clots were allowed to form for 3 hours at 37°C and overnight at 4°C. Clots and sera were then reanalyzed for vWF and fibrin as in the test system, using purified reagents.

Electroimmunoassay. vWF antigen was quantitated by Laurell immunoelectrophoresis.\textsuperscript{26} Electrophoresis was performed in 0.9% agarose gels, containing 0.65% monospecific rabbit antiserum to human vWF (Calbiochem-Behring, Marburg, FRG), in Tris-barbital buffer (pH 8.6, ionic strength 0.03) at constant current (1 mA/cm) at 4°C for 18 hours. Gels were stained with Coomassie blue and destained with glacial acetic acid–methanol–water. There were no changes in vWF concentration when factor XIII was added to the mixture.

RESULTS

Measurement of the covalent incorporation of a lysine analogue into a protein as a function of factor XIII activity is a straightforward way to determine if the protein contains glutamine(s) that may be suitable substrate(s) for transglutaminase catalyzed crosslinking. Figure 1 shows the results of such an experiment for purified, monomeric vWF. In the presence of factor XIIIa, vWF covalently incorporated putrescine in the expected time-dependent and concentration-dependent manner. At 1 hour, 0.97 $\mu$ mole of putrescine

![Fig 1. Covalent incorporation of $^{14}$H-putrescine into von Willebrand factor by factor XIIIa. Factor XIIIa was activated with thrombin and CaCl$_2$ plus DTT, and then hirudin was added. vWF (0.495 mg/mL) was added to factor XIIIa and $^{14}$H-putrescine. At various times, aliquots were placed on filter paper, washed with TCA, ethanol-acetone, and acetone, and counted; (-----) 1 U/mL of factor XIII; (-----) 5 U/mL of factor XIII.](image-url)
was covalently bound per µmole of vWF monomer when vWF was incubated with 1 U/mL of factor XIIIa. Increasing the factor XIIIa to 5 U/mL increased the incorporation at 1 hour to 1.55 µmoles of putrescine incorporated per micromole of vWF monomer. These results clearly indicate that under certain experimental conditions factor XIIIa readily incorporates putrescine into glutamine residues of monomeric vWF. Further studies to define the conditions for this reaction showed that both thrombin and reducing agent enhanced the rate of incorporation, indicating that thrombin proteolysis and reduction exposed additional glutamines that could participate in crosslinking. After 18 hours of incubation, there was ~20% enhancement in the total putrescine incorporation in the presence of thrombin.

Further investigation of vWF crosslinking showed that when purified vWF was fully reduced and incubated with factor XIIIa, not only did monomeric vWF incorporate putrescine, but it also covalently crosslinked to itself (Fig 2A). In contrast, incubation of vWF polymer with factor XIIIa did not result in vWF crosslinking to itself (Fig 2B). As shown in Fig 2A, vWF was fully reduced by addition of dithiothreitol to the incubation mixture. After 18 hours, samples were subjected to SDS gel electrophoresis in reducing buffer; gels were then sliced into 1-mm sections and radioactivity was measured. The results show radioactive bands migrating in positions corresponding to vWF mono-mer, dimer, and trimer, as well as higher polymer formation. This indicates that vWF monomer has both glutamine and lysine groups that can participate in the crosslinking reaction. However, incubation of purified vWF polymer with factor XIIIa resulted in incorporation of putrescine but not in covalent crosslinking of vWF with itself, indicating that in the polymeric state only glutamines, not lysines, were available for crosslinking.

Covalent crosslinking of vWF to fibrin was investigated in test systems containing purified vWF (trace-labeled with 125I) with purified fibrinogen (trace-labeled with 131I). Factor XIII was activated with thrombin and CaCl2, and this mixture was added to the fibrinogen–vWF mixture to initiate clotting and crosslinking. All the clotting reactions were carried out in the absence of reducing agents, that is, with intact fibrinogen and vWF polymer. In some studies, after termination of the reaction, the reaction products were reduced and analyzed on reduced SDS gels. Figure 3A shows the covalent crosslinking of vWF to fibrin at various factor XIIIa concentrations. In this experiment, the molar ratio of fibrinogen to vWF monomeric unit was 30:1; at the highest

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**Fig 2.** Crosslinking of von Willebrand factor by factor XIIIa. (A) Monomeric vWF was incubated with factor XIIIa and 3H-putrescine as in Fig 1 with 1 U/mL of factor XIII. The crosslinking reaction was terminated by mixing samples with sodium dodecyl sulfate (SDS) reduced gel electrophoresis buffer, and electrophoresis was carried out in cylindrical gels. Gels were cut in 1-mm transverse sections, dissolved in H2O2, and counted. (B) Polymeric 125I-vWF plus CaCl2 was incubated in the presence (□) or absence (□□) of factor XIIIa for 4 hours and then analyzed by reduced SDS gel electrophoresis. Gels were cut in 1-mm sections and counted.

**Fig 3.** Incorporation of vWF into fibrin gels. (A) Factor XIIIa dependence. Fibrinogen (factor XIII-free) plus vWF (molar ratio 30:1) was clotted with thrombin and CaCl2 and varying concentrations of factor XIII. Factor XIII concentration is shown at the right of each curve. At various times, clots were separated from fluid, washed in EDTA, and vWF was measured by electroimmunoassay. (B) Effect of vWF on fibrin incorporation. Samples were similarly prepared except that trace 125I-fibrinogen was added. Fibrin in the washed clots was measured by radioactivity in the presence (□) or absence (□□) of vWF.
factor XIIIa concentration tested, 80% of the vWF was covalently bound to fibrin. Analysis of the material in fibrin-vWF clots showed that the presence of vWF had a small effect of the rate of the fibrin gel formation (Fig 3B). At 2 minutes, 58% of the fibrinogen in the test system was in the clot in the sample without vWF, and 48% was in the clot in the sample with vWF. At 15 minutes, the values were nearly the same (87% without vWF and 85% with vWF); by 2 hours, there was no difference in the fibrin content of the two clotting systems.

Composition of the fibrin-vWF gels was analyzed by reduced SDS gel electrophoresis. Figure 4 shows the radioactivity profile of vWF and the fibrin polypeptide chains for fibrin formed in the presence or absence of vWF. At the earliest time point (2 minutes) fibrin γ chain content was decreased and γ-γ dimers were prominent. Fibrin α chains were also somewhat decreased, and small oligomers of α chains were visible, as well as some high-mol-wt α polymer. At this point, a small amount of vWF-α chain dimers and some larger components were seen, but there was no vWF in the α polymer fraction. In this assay, monomeric vWF, derived from vWF polypeptides that had been disulfide-bonded to other polypeptide chains that had become crosslinked to fibrin, was also observed. A principal effect of vWF appeared to be to decrease the rate of high-mol-wt α polymer formation. Similar results were observed at 15 minutes, at which time some vWF had appeared in the α polymer fraction and the sample without vWF still contained more α polymer than the sample with vWF. By 4 hours, most of the vWF had become crosslinked into the α polymer fraction, with residual vWF monomers and vWF-α chain dimers remaining.

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Fig 4. Effect of vWF on fibrin crosslinking. Samples were prepared as follows: (A) 125I-fibrinogen and 125I-vWF were mixed together to show the control proteins; (B through D) samples were prepared in three ways—125I-fibrinogen + vWD, fibrinogen + 125I-vWF, and 125I-fibrinogen. In each case with vWF, the molar ratio was 30 fibrinogen:1 vWF. In all cases, the samples were clotted with thrombin, CaCl2, and 5 U/mL of factor XIII. At intervals, the crosslinking reaction was stopped: samples were analyzed by sodium dodecyl sulfate gel electrophoresis, followed by measurement of radioactivity in the gel segments as in Fig 2B to determine the crosslinking patterns of fibrin without vWF (—), fibrin with vWF (— ), and vWF (——). (A) Control gel showing starting fibrinogen and vWF: no reaction with factor XIII. (B) Reaction time, 2 minutes. Several fibrin peaks can be identified: from right to left, these represent γ chain (mol wt 48,000), β chain (mol wt 54,000), α chain (mol wt 64,000), γ dimer (mol wt 96,000), and α dimer (mol wt 139,000). A trimer (~mol wt 215,000 to 231,000). Larger oligomers cannot be identified with certainty. High-mol-wt α polymer is indicated by the rising counts at the left side (top) of the gel. (C) Reaction time, 15 minutes; (D) reaction time, 240 minutes. In C and D, free γ chain has disappeared, but other lower mol wt peaks are the same as in B.
Table 1. Incorporation of vWF Into Fibrin During Clotting of Normal Plasma and Hemophilia A Plasma

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Hemophilia A</th>
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</thead>
<tbody>
<tr>
<td>Recalcification time</td>
<td>10 min</td>
<td>69 min</td>
</tr>
<tr>
<td>Plasma fibrinogen</td>
<td>7.3 μmol/L</td>
<td>7.9 μmol/L</td>
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<tr>
<td>Fibrin in gel</td>
<td>92%</td>
<td>76%</td>
</tr>
<tr>
<td>Plasma vWF</td>
<td>0.06 μmol/L</td>
<td>0.08 μmol/L</td>
</tr>
<tr>
<td>vWF in gel</td>
<td>3%</td>
<td>20%</td>
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Covalent crosslinking of vWF to fibrin was also investigated in a plasma test system. Table 1 shows that upon recalcification of normal plasma little vWF became incorporated into the fibrin gel. However, recalcification of plasma from a patient with severe hemophilia A resulted in incorporation of 20% of the plasma vWF. The same results were also obtained with factor IX-deficient plasma, indicating that this was not a special property of hemophilic vWF. In these studies, plasma levels of fibrinogen, vWF, and factor XIII were normal, and the only significant differences were the long recalcification times and increased fibrin-vWF crosslinking in the deficient plasmas. This observation was further investigated by adjusting normal and factor VIII-deficient plasmas to the same clotting times and measuring vWF incorporation in the fibrin gels (Fig 5). Normal plasma was clotted with 10 mmol/L of CaCl₂ and dilutions of hirudin and deficient plasma with CaCl₂ and dilutions of thrombin so that clotting times of the two plasmas were approximately equal. The results showed that when the clotting times were the same, the amount of vWF incorporation into fibrin was also the same. The distribution of vWF in the plasma clots was examined on reduced SDS gels. In the normal plasma clot (recalcification time 10 minutes), it can be seen that very little vWF was incorporated (Fig 6A). The expected distribution of crosslinked fibrin components was also seen, with predominantly α chain, γ-γ dimer, and high-mol-wt α polymer. Smaller amounts of α monomer and α chain oligomers were also observed. With the factor VIII-deficient clots (recalcification time 69 minutes) vWF was found in the α polymer fraction (Fig 6B). Monomeric vWF and vWF-α chain dimer could also be clearly identified on the gels. The content of α polymer was decreased, and the amount of intermediate α chain oligomers increased. Thus, in factor VIII-deficient plasma or in normal plasma in which the clotting time was prolonged, vWF was covalently bound to fibrin in a manner identical to that observed with purified reagents.

**DISCUSSION**

The product of the crosslinking reaction catalyzed by factor XIIIa is ε-(γ-glutamyl)lysine, which results from formation of a peptide bond between the γ-carboxamide of peptide-bound glutamine and the ε-amino of lysine, thus forming a covalent bridge which links two proteins together. Factor XIIIa appears to have significant substrate specificity requirements, and only certain glutamine and lysine residues in certain proteins are suitable substrates. These include the crosslinking of fibrin γ chains to form γ-γ dimers and fibrin α chains to high-mol-wt α polymers. Fibronectin and α2-antiplasmin are crosslinked to fibrin α chains, and fibronectin can also crosslink to collagen. The studies described here show clearly that both monomeric and polymeric forms of von Willebrand factor also act as substrates for factor XIII activity by providing appropriate glutamine residues for the crosslinking reaction. Crosslinking of vWF to fibrin α chains can occur readily during clotting.

vWF is present in normal plasma as a series of disulfide-bonded polymers that range in molecular size from 800,000 to >12,000,000. In these studies, several forms were used: purified vWF monomers, purified polymers with the size distribution of plasma vWF, and the array of polymers distributed in plasma. Purified monomers and polymers, as
well as plasma vWF, could participate in crosslinking; but only monomers could clearly crosslink to other monomers to form vWF dimers, trimers, and higher polymers (Fig 2A). Disulfide-bonded polymers of vWF were not crosslinked by factor XIIIa to form larger aggregates (Fig 2B). However, vWF polymers were readily crosslinked to fibrin. In these experiments, it was not ascertained whether the size distribution of polymers which crosslinked to fibrin was random or whether a particular fraction was preferentially involved.

Crosslinking of fibrin \( \gamma \) chains to form \( \gamma-\gamma \) dimer occurs at the protofibril stage of fibrin gelation.\(^1\) It is rapid, occurs with very low factor XIIIa levels, and is difficult to inhibit.\(^2\) This may be partly because fibrin monomer units are already aligned in the protofibril so that the \( \gamma \) chain crosslinking sites are properly oriented. In contrast, crosslinking of fibrin \( \alpha \) chains is slow and highly dependent on the factor XIIIa concentration, with much of the \( \alpha \) polymer being formed after gelation.\(^3\) Crosslinking of both fibronectin and vWF to \( \alpha \) chains is similar to \( \alpha \) polymer formation. In purified test systems with fibronectin, \(~50\%~\) is crosslinked to fibrin before visible gelation occurs when the thrombin concentration is in the range of \(0.05 \text{ to } 2.0 \text{ U/mL}\), and the remaining fibronectin crosslinking occurs much more slowly.\(^4\) Similar results were observed with vWF-fibrin crosslinking in purified systems, in which prolonged incubation resulted in increased vWF crosslinking after the gel had formed (Fig 4). However, in the plasma system, increased incorporation of vWF polymers was observed when the gelation time was increased (Fig 5), but it was not observed with prolonged incubation after the gel had formed relatively rapidly (Fig 6). The requirement for a long gelation time in plasma may explain why vWF may not be observed as a substrate for factor XIIIa when plasma is clotted rapidly.\(^5\)

Participation of vWF as a substrate for crosslinking by factor XIIIa is similar to fibronectin crosslinking. Each protein can crosslink to itself only in the reduced forms. Both proteins crosslink to the \( \alpha \) chain of fibrin and, in so doing, decrease the rate of \( \alpha \) polymer formation. However, crosslinking of one protein to \( \alpha \) chain does not inhibit the crosslinking of the other. This is similar to the previously described observation that fibronectin and \( \alpha_2 \)-antiplasmin do not compete for crosslinking sites in \( \alpha \) chains of fibrin.\(^6\) It is known that \( \alpha \) chains can form at least three covalent crosslinks per polypeptide chain, but this number is not normally obtained in \( \alpha \) polymer formation. It appears that some of the lysine crosslinking sites are more readily occupied by glutamines in other proteins than by other \( \alpha \) chains.\(^7\)

It is unlikely that more than one other protein would be crosslinked to a given \( \alpha \) chain because the concentration of \( \alpha \) chains in the gel is so much greater than that of other proteins. The principal glutamine crosslinking sites in fibronectin\(^8\) and \( \alpha_2 \)-antiplasmin\(^9\) are located very close to the amino-termini, well suited for their interdigitation within the \( \alpha \)-polymer. A crosslinking site in vWF has not yet been localized.

The observation that vWF polymer formation crosslink to fibrin during plasma clotting (Figs 5 and 6) may explain the decreased level of serum vWF sometimes observed when clotting of hemophilia plasma was prolonged. This was previously ascribed to an alteration in serum vWF.\(^1\) Other investigators have also observed decreased \( \alpha \) polymer formation in plasma clots of patients on coumarin or with severe factor VIII deficiency.\(^2\) We interpret the data presented here, as well as the observations of others, to indicate that when the clotting of plasma is prolonged, other proteins, including vWF, can be covalently bound to fibrin \( \alpha \) chains by factor XIIIa, with a concomitant decrease in the rate of \( \alpha \) polymer formation. It seems likely that this reaction might also be expected to occur in vivo when thrombin generation is limited.

Although the significance of vWF crosslinking to fibrin is unclear, several possibilities can be considered. One possibility involves the contact sites of the fibrin clot with vascular subendothelium. We have obtained evidence that vWF can also crosslink to collagen.\(^3\) Hence, the possibility exists for vWF to function as a covalently held bridge between the fibrin clot and collagen in the subendothelium. The large molecular size of the vWF polymer would be appropriate for such a function. vWF is relatively resistant to plasmin digestion,\(^4\) and its presence in the fibrin clot might also increase resistance of the clot to plasmin degradation. Recent evidence has also been presented concerning the function of vWF in mediating the interaction between platelets and polymerizing fibrin.\(^5\) Covalent crosslinking of a small fraction of vWF to fibrin as it polymerizes would stabilize this interaction. Thrombospordin is also a substrate for factor XIIIa,\(^6\) and it too could have a role in crosslinking reactions involving vWF on the platelet surface.

ACKNOWLEDGMENT

We wish to thank Drs K. Fukutake, M. Fujimaki, and S. Ikematsu, Department of Clinical Pathology, Tokyo Medical College, for their support and cooperation. We also wish to thank Behringwerke A.G. for the Fibrogammin used in these studies.

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F XIIIa CROSSLINKING OF vWF TO FIBRIN


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