Mechanism of Transient Adsorption of Fibrinogen From Plasma to Solid Surfaces: Role of the Contact and Fibrinolytic Systems

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The transient detection of fibrinogen on surfaces has been described (Vroman effect) and high-mol-wt kininogen (HK) has been shown to play a role in this reaction. In this study, we attempted to identify the form of HK responsible for preventing detection of the fibrinogen initially adsorbed from plasma to various artificial surfaces and to determine if other plasma components were involved. We compared "*9" fibrinogen adsorption in the presence of normal plasma to plasma deficient in specific proteins. On all surfaces tested, we found that fibrinogen was displaced from the surface. The extent of displacement was greatly reduced, however, but not eliminated in HK-deficient plasma. Factor XII-deficient plasma also showed reduced fibrinogen displacement. These data indicate that HK can actually displace fibrinogen; however, factor XII, or a factor XII-mediated reaction also appears to be necessary for this displacement to occur. Furthermore, when normal plasma was first subjected to extensive contact activation by dextran sulfate, during which the HK was extensively degraded to components smaller than the light chain (as assessed by Western blotting), we observed greatly reduced displacement of fibrinogen. Extensive contact activation of Factor XI-deficient plasma failed to show low-mol-wt derivatives, however, and displacement of fibrinogen was similar to normal plasma that had not undergone extensive activation. These data indicate that HKα (active cofactor produced during contact activation by factor XIa or kallikrein) is primarily responsible for displacing fibrinogen, and that HK, (inactive cofactor generated by factor XIa) cannot displace fibrinogen. The fibrinogen from all plasma samples looked similar by Western blot analysis, suggesting that fibrinogenolysis was not a component of the Vroman effect. In addition, experiments performed with plasma prechromatographed on lysine agarose showed that a lysine-agarose adsorbable protein may be minimally involved in fibrinogen desorption and a synergism may exist between HK and that protein.

Although fibrinogen is rapidly adsorbed from plasma to solid surfaces, it is subsequently undetectable within a few minutes.1-7 This transient detection of fibrinogen has been called the Vroman effect4,4 in recognition of its discoverer, Leo Vroman.1,3 Fibrinogen that is initially adsorbed onto artificial surfaces appears to be displaced by other plasma constituents, since if the plasma is diluted, the fibrinogen remains detectable longer, presumably because the concentration of the other interacting components is decreased. Thus, the Vroman effect is manifested either as a maximum in fibrinogen adsorbed to a surface v time (at constant plasma concentration), or a maximum in fibrinogen adsorbed v plasma concentration (at constant time). The positions of the maxima are therefore dependent on the plasma concentration or the time of incubation, respectively.4,5

Vroman et al1,3 also reported that fibrinogen detection persists if Fitzgerald trait plasma, which is deficient in high mol wt kininogen (HK-deficient), or Williams trait plasma, which is deficient in both HK and low mol wt kininogen (HK-LK-deficient), is substituted for normal plasma in the surface adsorption experiments. Schmaier et al17 showed that HK, but not LK, normalized this fibrinogen-surface interaction, as measured by ellipsometry.

Contact activation of plasma proteins occurs when blood interacts with artificial surfaces. Glass and negatively charged surfaces promote autoactivation of factor XII, which can then activate additional plasma proteolytic enzymes. In contrast, siliconized glass and polyethylene induce contact activation more slowly. Once formed, activated factor XII cleaves prekallikrein and factor XI—each bound to the surface through a complex with HK.9,10 The cofactor of contact activation.11 HK, after it is activated by kallikrein (HKα), has an increased ability to bind to the surface with thezymogens, prekallikrein and factor XI.12 Factor XI, however, once activated to factor XIa, can proteolyze the light chain of HKα, resulting in a functionally inactive cofactor molecule (HKi).11

Experiments reported in this article were designed to evaluate the relationship of the different forms of HK to fibrinogen adsorption as well as to define the roles of the other contact proteins and plasminogen in surface–fibrinogen interactions during plasma–solid surface contact. A better understanding of the mechanism of blood and artificial surface interaction is essential for the design of less thrombogenic biomaterials.

MATERIALS AND METHODS

Surfaces. Three surface materials were used in the present study: pyrex glass, siliconized glass, and polyethylene. Pyrex glass tubing of 0.25 cm or 0.175 cm internal diameter was treated with...
chronic acid cleaning solution and then rinsed thoroughly with distilled, deionized water. Clean glass tubing was siliconized using Surfasil silicone compound (Pierce Chemical, Rockford, IL) according to the supplier's instructions. Polyethylene tubing was obtained from Clay Adams, Parsippany, NJ (Intramedic PE 320, internal diameter 0.27 cm) and was treated with methanol followed by distilled, deionized water. For adsorption studies, all tubes were equilibrated overnight in isotonic Tris buffer, pH 7.35 prior to introduction of plasma.

Plasmas. Human pooled normal plasma, prepared from blood anticoagulated with citrate, was obtained from the Canadian Red Cross, Ottawa. Fitzgerald trait (HK-deficient) plasma, and Hagen-Kubaft (“isohemors.”

Proteins. Human fibrinogen, obtained from Kabi Vitrum (Sweden), was dialyzed against isotonic Tris buffer, pH 7.35. It was as determined by coagulant and immunoassay. Factor XI-deficient plasma (obtained from a donor at Temple University Hospital, Philadelphia) contained <0.01 U/mL factor XI, where 1 U/mL is the amount in 1 mL normal pooled plasma from 20 donors. Williams trait plasma, deficient in both HK and LK (HK-LK deficient), was donated by Mrs. M. Williams, Philadelphia. Plasma deficient in plasminogen was prepared from freshly drawn, normal, single donor plasma, which was chromatographed by affinity adsorption on a lysine-agarose matrix according to the method of Deutsch and Mertz. Residual plasminogen was <5% of normal. Contact activation of plasma with dextran sulfate was performed as described by Kluit.

Western blot analysis of HK and fibrinogen in plasma. Samples (2.5 µL original plasma) were run on a 10% Laemmli SDS-PAGE minigel (80 x 60 mm) with β-mercaptoethanol prior to transfer to an Immobilon membrane. Transfer was accomplished with an ABN Polyblot unit (American Bioinetics, Emeryville, CA) for 1 hour at 130 mA at room temperature. The membrane was incubated in 5% nonfat dry milk in 10 mmol/L Tris-HCl, pH 7.5, + 0.15 mol/L NaCl for 30 minutes prior to being rinsed four times in the abovementioned buffer containing 0.05% Tween 20. The HK light chain antisera was diluted 1:500 in the buffer and incubated overnight. After four washes, the alkaline phosphatase-conjugated antisera (Boehringer Mannheim) was diluted 1:3000 and incubated for 2 hours. After four washes, the membrane was rinsed in 50 mmol/L sodium carbonate, 1 mmol/L MgCl2, pH 9.8. The blot was developed with BCIP/NBT in the carbonate buffer.

RESULTS

Effect of plasma concentration on fibrinogen adsorption to glass: Role of HK. After a 5-minute incubation of plasma was performed in a 25-cm glass tube (described under Methods) at various concentrations of normal plasma (either single donor heparinized, or pooled citrated), fibrinogen adsorption reached a maximum at ~1% plasma concentration and decreased as the concentration of plasma increased (Fig 1A). When a similar experiment was performed with HK-deficient plasma, we observed that more fibrinogen was initially adsorbed and less fibrinogen was subsequently displaced, as compared with normal plasma. When HK-deficient plasma was partially reconstituted by the addition of purified HK in the procofactor form (0.5 U/mL) prior to exposure to glass, the plasma concentration-dependent fibrinogen adsorption was partially normalized with respect to displacement. Because the shapes of such curves for normal plasma are dependent on the plasma sample used and vary in particular with respect to peak height, we normalized the data by plotting percentage of maximal fibrinogen adsorption v plasma concentration (Fig 1B). When we compared the normalized data, the normal plasma curves were similar whether citrate or heparin was used as anticoagulant, suggesting that free calcium ions are not important in this phenomenon. The normalized data for HK-deficient plasma (Fig 1B) revealed that the initial portion of the curve, representing diffusion-limited adsorption, was virtually identical to normal plasma, but the portion of the curve beyond the maximum showed relatively little decrease, even at the 10% plasma concentration. Thus,
Fig 1. A: Effect of plasma concentration on the adsorption of fibrinogen (5 minutes) from various plasmas to glass. Error bars show range of values for three independent experiments. Fitzgerald plasma, (HK deficient) (O); Fitzgerald plasma plus 0.5 U/mL HK (x); normal heparinized plasma (B); normal citrated plasma (C). B: Data plotted as percentage of maximum adsorption. HK-deficient (O) (n = 3); HK-deficient + 0.5 U/mL HK (x) (n = 2); normal citrated or heparinized plasma (B) (n = 9). Error bars indicate SEM.

the Vroman effect is greatly attenuated in HK-deficient plasma, ie, the ability of this plasma to displace adsorbed fibrinogen is reduced.

**Fibrinogen adsorption and desorption from polyethylene and siliconized glass.** Adsorption experiments with HK-LK-deficient plasma were performed using two other surfaces, namely polyethylene (Fig 2A) and siliconized glass (Fig 2B). The maximum adsorption of fibrinogen was again increased in plasma deficient in HK-LK, when compared with normal plasma, in both cases. With polyethylene, a surface on which fibrinogen is retained relatively strongly, the increase was very marked, but again there was still fibrinogen displacement at higher concentrations of HK-LK-deficient plasma. Siliconized glass, a surface on which rapid displacement of fibrinogen occurs with normal plasma, again showed an attenuation of the Vroman effect in HK-LK-deficient plasma. For this surface, the absolute difference between normal and HK-LK-deficient plasma was eliminated at higher plasma concentrations.

**Kinetics of fibrinogen adsorption to glass.** Fibrinogen adsorption kinetics from HK-LK-deficient plasma were studied using a glass surface at two concentrations of plasma. For HK-LK-deficient plasma at 0.5% concentration, the adsorption kinetics were essentially "classical"; ie, adsorption increased with time and eventually reached a constant, plateau value (Fig 3A) whereas for normal plasma, there was a distinct maximum at ~10 minutes and a subsequent decrease. The absolute fibrinogen adsorption from HK-LK-deficient plasma was again increased as in the previous experiments. At a higher concentration of plasma (2.5%), fibrinogen displacement was quite marked and the adsorption maximum occurred at a time less than 1 min (Fig 3B). The shape of the curve in the presence of HK-LK-deficient plasma was similar to normal plasma, but the absolute amount of fibrinogen adsorbed was increased. Clearly, there was some fibrinogen displacement activity at this plasma concentration, but even at 180 minutes, ~50% of the maximum adsorbed amount remained adsorbed whereas in normal plasma, by 180 minutes, adsorption had virtually decreased to zero. These kinetic data support the conclusion
that the ability to displace fibrinogen on various surfaces is dramatically decreased in HK-LK-deficient plasma.

Role of factor XII in fibrinogen desorption. The data presented thus far support the hypothesis that HK plays a major role in displacing adsorbed fibrinogen from artificial surfaces. Based on previous suggestions by Vroman et al. that factor XII might also be involved in this effect, and on our hypothesis that the Vroman effect is related to reactions occurring during the contact phase of blood coagulation, we determined fibrinogen adsorption to glass in factor XII-deficient plasma and compared it with Williams trait plasma (HK-LK-deficient). Both HK-LK-deficient and factor XII-deficient plasma showed similar behavior (Fig 4) which resulted in decreased fibrinogen desorption as compared to normal plasma, similar to Fig 1A. More fibrinogen desorption was evident in factor XII-deficient plasma than in HK-LK-deficient plasma, however. Thus, in agreement with Vroman et al., we find that both HK and factor XII play a role in the Vroman effect. The fibrinogen desorption in plasma deficient in HK-LK (Fig 4) as well as in plasma that is deficient only in HK (Figs 1A and 2) were virtually identical, supporting the observation of Schmaier et al. that LK is not involved in the Vroman effect.

Normal human plasma or factor XII-deficient plasma was diluted in either a series of plastic vessels or a series of glass vessels prior to the 5-minute incubation in the glass tube. An adsorption isotherm was performed on each sample, as in Fig 4. We observed that the normal plasma which was diluted in plastic, prior to glass exposure, displaced slightly more fibrinogen than the normal plasma samples which were diluted in glass. Factor XII-deficient plasma exhibited similar isotherms whether diluted in plastic or glass.

Effect of extensive proteolysis of HK on fibrinogen desorption. The effect of extensive contact activation of HK, which leads to the formation of HKi through proteolysis by factor XIa, was tested. Normal plasma and factor XI-deficient plasma were each treated with dextran sulfate, a potent contact phase activator, for 20 minutes prior to exposure to the 25-cm glass tube, and isotherm experiments were performed (Fig 5). The data for fibrinogen adsorption in dextran sulfate-treated factor XI-deficient plasma (or in untreated factor XI-deficient plasma, data not shown) was virtually identical to untreated normal plasma. Normal plasma exposed to dextran sulfate, however, which results in the formation of factor XIa, resembled the adsorption isotherm for HK-LK-deficient or factor XII-deficient plasma seen in Fig 4.

Western blot analysis of HK. The 10% samples from various isotherms were analyzed by Western blot analysis using a polyclonal antibody to HK light chain (Fig 6). The blot revealed that the HK in the factor XII-deficient plasma (lane 1) was slightly cleaved, explaining the slight displace-
Fig 5. Effect of minimal and extensive contact activation on the adsorption of fibrinogen to glass. Factor XII-deficient plasma was diluted in plastic or glass (○). Normal plasma was diluted in plastic (■) or glass (□). Normal plasma was pretreated with dextran sulfate (×). Factor XI-deficient plasma was pretreated with dextran sulfate (x→x). Adsorption isotherms were performed on each sample, as described in the Methods section. Error bars indicate SEM (three to ten determinations).

Fig 6. Western blot analysis of samples from Fig 5 for HK. Lane 1, factor XII-deficient plasma; lane 2, normal plasma diluted in plastic; lane 3, normal plasma diluted in glass; lane 4, normal plasma pretreated with dextran sulfate; lane 5, factor XI-deficient plasma pretreated with dextran sulfate; lane 6, HK-LK-deficient plasma. Intact, 120-kd parent molecule. HC (heavy chain), is apparently detected by the light chain antibody. LC, intact light chain. Cleavage products, degraded light chain.

Fig 7. Western blot analysis of samples from Fig 5 for fibrinogen. Lane 1, factor XII-deficient plasma diluted in plastic; lane 2, factor XII-deficient plasma diluted in glass; lane 3, normal plasma diluted in plastic; lane 4, normal plasma diluted in glass; lane 5, normal plasma pretreated with dextran sulfate; lane 6, factor XI-deficient plasma pretreated with dextran sulfate. α(α)n, α polymers; γ2, γ dimers; Aα, Bβ, and γ, normal fibrinogen subunits.
in normal plasma, preexposed to dextran sulfate (Fig 7, lane 5), we can see virtually intact $\alpha$, $\beta$, and $\gamma$ chains in each sample. The higher mol species correspond to cross-linked components.

To test the effect of plasmin on the Vroman effect, adsorption experiments were performed with normal plasma before or after passage over lysine-agarose to deplete plasminogen and plasminogen activators, and with the depleted plasma after reconstitution with purified plasminogen. HK levels were assayed before and after lysine-agarose treatment and were identical. The differences in the isotherms among these plasmas were small (Fig 8), but in replicate experiments the fibrinogen desorption in plasminogen-depleted plasma was consistently less than in normal plasma, suggesting that there was a small contribution of a lysine-agarose adsorbable protein to the Vroman effect. Because reconstitution of the depleted plasma with purified plasminogen did not restore the adsorption of fibrinogen to normal, we conclude that another protein(s), which is tightly adsorbed onto lysine-agarose, is required for the additional displacement of fibrinogen from glass.

Finally, the combined effects of HK and lysine-agarose depletion were investigated (Fig 9). HK-LK-decient plasma was chromatographed on lysine-agarose, as in Fig 8, and then the concentration-dependent fibrinogen adsorption was compared for this plasma and for untreated HK-LK-deficient plasma. The HK-LK-deficient plasma showed, similar to Fig 4, a relatively flat curve, with nonetheless a definite maximum at ~1.5%. The curve for lysine-agarose chromatographed HK-LK--deficient plasma showed no maximum, but rather a continuous increase in adsorption with an abrupt slope change at 1% to 1.5% plasma. This curve is similar to many adsorption isotherms seen in single protein systems or in simpler mixtures and showed no evidence of any Vroman effect. Thus, the small degree of fibrinogen desorption in HK-LK-deficient plasma may result from an unidentified protein that binds tightly to lysine-agarose.

**DISCUSSION**

The present article provides new information on the early protein adsorption and desorption events following plasma contact with foreign surfaces. Because the propensity towards thrombotic sequelae, including platelet adhesion and aggregation, may be determined by the identity of adsorbed proteins, these data are relevant to understanding the prothrombotic effects of artificial surfaces. Vroman's early demonstration that fibrinogen is the first protein to be detected after exposure to glass, indicates the specificity of plasma protein interactions. On the basis of abundance, albumin should be favored, whereas if coagulation is the predominant initial response of the blood to foreign surfaces contact coagulation proteins might be better candidates. Although the preference for fibrinogen adsorption is perhaps not fully understood, the relatively high surface activity of this protein is well known. For example, fibrinogen is strongly preferred in competition-adsorption with albumin and IgG. Nonetheless, an alteration in the structure of other proteins may increase their affinity for the surface, relative to fibrinogen. For example, fibrinogen will block the adsorption of procofactor HK to kaolin but will not prevent HKa from binding.

The new data we present concern the displacement of adsorbed fibrinogen. These data, obtained through experiments using radiolabeled fibrinogen, demonstrate that in the Vroman effect, fibrinogen is actually displaced from the surface rather than merely covered by HK and/or other proteins or reoriented so that it is no longer reactive to antifibrinogen antiserum. Furthermore, we conclude that fibrinogen displacement occurs primarily through the contact proteins with a small contribution from an as yet unidentified protein that binds tightly to lysine-agarose. Thus, in agreement with Vroman et al, we find that HK plays a major role in displacing fibrinogen. Our results are qualitatively similar to the results of Vroman et al, who used undiluted plasma and detected fibrinogen adsorption by means of antisera to fibrinogen, suggesting
that the Vroman effect can be appropriately studied even in a diluted-plasma environment. Factor XII also appears to be required, but our results indicate that its effect is indirect owing to its participation in kallikrein formation, which in turn converts HK to its “active” form.12 Similar to our observation that HKa (as opposed to HK) binds to kaolin in the presence of plasma concentrations of fibrinogen.12 Furthermore, factor XIa can directly activate HK.31 The slight displacement of fibrinogen by factor XII-deficient plasma is consistent with the fact that there was a small amount of cleaved HK in that plasma (Figs 4 and 5). Extensive contact activation by dextran sulfate (Fig 5) which resulted in an intense low-mol-wt derivative of HK (Fig 6, lane 4), destroyed most of the fibrinogen-displacing activity of normal plasma, but not of factor XI-deficient plasma, which did not demonstrate large amounts of degraded HK (Fig 6, lane 5). Scott et al13 previously concluded that factor XIa degrades the light chain of HK to a form that is inactive as a contact phase cofactor. Thus, this same degraded HK (HKi) appears unable to displace adsorbed fibrinogen. These data suggest a direct relationship between reactions occurring in the contact phase of coagulation and the loss of initially adsorbed fibrinogen from a surface.

The cleavage of the band at 120 kd (intact HK) produces HKa (Fig 6, lane 3) after exposure of plasma to glass, but with more extensive activation (Fig 6, lane 5) degradation occurs and results in products of <40 kd. This more extensive digestion requires factor XIa (Fig 6, lane 4). This conclusion is based on qualitative observations. Two factors are responsible for the inability to quantitate relative amounts of protein on a Western blot accurately. First, transfer times affect the amount of protein that leaves the gel. The lower mol-wt proteins may pass through the membrane before the higher mol-wt proteins are completely transferred. Second, the membranes have finite capacity to absorb protein; once the binding sites on the membrane are saturated, excess protein of that particular molecular weight fails to bind. To visualize the HK degradation products (Fig 6), we had to perform the transfer for a short time. With longer transfer times, we observed that the cleavage products <40 kd passed through the membrane and were found on membranes placed behind the primary transfer membrane (data not shown). The shorter transfer times resulted in incomplete transfer of proteins >40 kd, however, and these bands in the light chain (45 kd) region and above probably reflect partial transfer and/or saturation of the membrane. The 45-kd band visible in the normal plasma pretreated with dextran sulfate (Fig 6, lane 4) probably represents the total light chain remaining in that sample, whereas the 45-kd band of the other samples may represent only part of the light chain owing to saturation of the membrane. That the dense band of HK degradation products is visible in that sample (Fig 6, lane 4), however, indicates that extensive cleavage had occurred.

The data presented on the effect of plasminogen on fibrinogen displacement are less striking than those for HK. For plasma chromatographed on lysine-agarose, although there appeared to be a slight decrease in fibrinogen desorption, reconstitution with purified plasminogen did not normalize the isotherm, suggesting the involvement of another protein that binds tightly to the lysine-agarose and does not coelute with the plasminogen fraction.27 The role of this protein is confirmed since its depletion from HK-LK-deficient plasma appeared to eliminate the Vroman effect entirely (Fig 9). Additional data supporting a lack of involvement of plasmin in the Vroman effect comes from the failure of ω-aminocaproic acid to change the isotherm pattern (data not shown) as well as the lack of proteolysis of fibrinogen even in plasma subjected to extensive contact activation (Fig 7).

The Vroman effect appears to apply to a wide variety of surfaces interacting with plasma, but its characteristics, including the rate and extent of fibrinogen adsorption and displacement, vary considerably from surface to surface.44 Whether these variations are significant in relation to the thrombogenic potential of the surfaces remains to be ascertained. If the critical surface property were inertness toward activation of coagulation, and if displacement of fibrinogen leads to adsorption and activation of coagulation factors, then fibrinogen retention, ie, a minimal Vroman effect, may be desirable. If, on the other hand, inertness to platelets is the most important property for nonthrombogenicity, since adsorbed fibrinogen is generally believed to be platelet-reactive, fibrinogen displacement and a vigorous Vroman effect would be desirable.

The data required to test such hypotheses and to attempt correlations are incomplete, both with regard to measurements of fibrinogen desorption and the potential to activate the coagulation cascade for a wide spectrum of surfaces. Schmaier et al7 have observed that hydrophilic surfaces displace fibrinogen rapidly relative to hydrophobic, and that such surfaces are procoagulant. This broad classification of surfaces may be oversimplified, however, since polyethylene and siliconized glass are both relatively hydrophobic but have very different behavior with respect to the extent and rate of fibrinogen adsorption and desorption. In the case of siliconized glass, however, some loss of the silicone coating may occur so that this surface may develop a partial hydrophilic character as the experiment progresses. Thus, further studies will be required to establish correlations of the Vroman effect with properties of artificial surfaces.

A final comment on hydrodynamic conditions and their possible effects may be in order in relation to the present experiments. Flow is widely recognized to have a major influence on thrombus formation on both natural and artificial surfaces.32 As already described, the contact of plasma with surface in the present experiments occurred under static conditions, as is the case for all previous studies of the Vroman effect in various laboratories.17 For any phenomenon occurring at an interface, the reactants must first be transported to the interface before the surface reaction (in the present case, adsorption) can take place. In general, one of these two processes is faster than the other and the rate of the interfacial phenomenon is “controlled” by the slower of the two, ie, by transport or by adsorption. It is in the former case that flow may have an effect since fluid shear may augment transport by convection of reactants to the surface. This effect is particularly important in blood through local motions of RBCs across streamlines,32 which effectively increases the rate of transport of fluid constituents beyond
that expected for Brownian diffusion. Proteins are less affected in this regard than platelets. Moreover, in the absence of RBCs, flow-augmented transport is less important. Thus, for adsorption from solution, we have found that under conditions in which the surface is close to saturation, as in the present experiments, flow has no effect on fibrinogen adsorption to glass. In agreement, other researchers have shown that only the very early protein adsorption rates, when there is still a substantial fraction of uncovered surface, are transport controlled and thus subject to flow effects.

Although it would be unwise to state categorically without experimental evidence that flow would have no influence on the phenomena reported here, we believe that in a RBC-free system when the surface coverage of protein is substantial, as it should be at 5 minutes in these plasma systems, such flow effects would be relatively small.

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REFERENCES

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