Interaction of High Molecular Weight Kininogen, Factor XII, and Fibrinogen in Plasma at Interfaces

By L. Vroman, A. L. Adams, G. C. Fischer, and P. C. Munoz

Using ellipsometry, anodized tantalum interference color, and Coomassie blue staining in conjunction with immunologic identification of proteins adsorbed at interfaces, we have previously found that fibrinogen is the main constituent deposited by plasma onto many man-made surfaces. However, the fibrinogen deposited from normal plasma onto glass and similar wettable materials is rapidly modified during contact activation until it can no longer be identified antigenically. In earlier publications, we have called this modification of the fibrinogen layer “conversion,” to indicate a process of unknown nature. Conversion of adsorbed fibrinogen by the plasma was not accompanied by marked change in film thickness, so that we presumed that this fibrinogen was not covered but replaced by other protein. Conversion is now shown to be markedly delayed in plasma lacking high molecular weight kininogen, slightly delayed in plasma lacking factor XII, and normal in plasmas that lack factor XI or prekallikrein. We conclude that intact plasma will quickly replace the fibrinogen it has deposited on glass-like surfaces by high molecular weight kininogen and, to a smaller extent, by factor XII. Platelets adhere preferentially to fibrinogen-coated surfaces: human platelets adhere to hydrophobic nonactivating surfaces, since on these, adsorbed fibrinogen is not exchanged by the plasma. The adsorbed fibrinogen will be replaced on glass-like surfaces during surface activation of clotting, and platelets failing to find fibrinogen will not adhere.

The deposition of proteins by blood plasma onto flat surfaces of oxidized silicon crystal slices, glass, and anodized tantalum-sputtered glass can be studied by means of ellipsometry, Coomassie blue staining, and observation of interference colors, respectively. After exposure to plasma, exposure to antisera will result in deposition of antibodies that match the antigen substrate; the immune complexes can be observed by the same methods and allow identification of the proteins the plasma had deposited without the need of labeled reagents. With these techniques we found earlier that normal intact (i.e., not surface-activated) plasma, whether native, heparinized, or citrated, deposits fibrinogen onto most surfaces we studied, including the wettable ones listed above, within a few seconds. While this fibrinogen remains unaltered by the plasma when deposited on certain hydrophobic substrates, the fibrinogen deposited onto hydrophilic glass-like ones is altered by the plasma. This “change” is evident by a loss of the film’s ability to cause deposition of antibody from overlaid antisera to human fibrinogen. We did not know what process was involved in this loss of antigenicity, and therefore, had called it “conversion.” At room temperature in human blood, conversion occurs within 1 min on oxidized silicon crystals and within 10 min on glass or anodized tantalum. It is slightly delayed in factor-XII-deficient plasma. During conversion, the thickness of the film did not change measurably. We therefore presumed that the adsorbed fibrinogen loses its antigenicity as a result of replacement by some other materials present in intact plasma and that the fibrinogen molecules are not merely distorted or masked. Fibrinogen conversion affects platelet adhesion: platelets adhere preferentially where fibrinogen has been deposited and not where the film has been changed by the plasma. As a consequence, human blood deposits markedly more platelets on hydrophobic surfaces (such as glass rubbed with ferric stearate) where fibrinogen remains present, than on hydrophilic glass-like surfaces where the platelets arrived too late to interact with unaltered fibrinogen. Recently, we were able to study plasma from one patient lacking high molecular weight kininogen (HMK) and from another one lacking both HMK and low molecular weight kininogens. HMK is reported to have a high affinity for clot-activating surfaces, enabling it to carry factor XI to the interface where it is activated by factor XII—the first factor to be adsorbed and activated by the surface. On kaolin, the interactions between HMK and adsorbed factor XII now being studied appear complex, but suggest to us that HMK may be one agent responsible for altering preadsorbed fibrinogen.

Materials and Methods

High Molecular Weight Kininogen-Deficient Plasmas

Human plasma lacking in only this factor was obtained from George King Biochemical Inc., Overland Park, Kans. Plasma deficient in both high and low molecular weight kininogen (Williams trait) was generously provided by Dr. Robert W. Colman, Philadelphia, Pa. These and other plasmas were collected into 1 part of 3.8% sodium citrate/9 parts blood and stored at about -60°C. Purified human high molecular weight kininogen was generously

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provided by Dr. O. D. Ratnoff, Cleveland, Ohio (9.2 U/mg). Glass slides were obtained from A. H. Thomas, Philadelphia, Pa., Cat. 6685-Q-20. Tantalum-sputtered glass slides were kindly provided by Dr. N. Schwartz, Bell Telephone Labs., Murray Hill, N.J., and were anodized at about 20 V in 0.01% HNO₃. Polyvinylchloride slides (PV) came from A. H. Thomas, Cat. 6686-V15. Sparkleen was obtained from Fisher Sci. Co., Springfield, N.J., and Kimwipes from Kimberly-Clark Corp., Neenah, Wisc. For details regarding veronal buffer in saline (VS), see refs. 1 and 3; for those regarding Coomassie brilliant blue R, see ref. 2, however, the following is used without further dilution: 250 mg of the dye is dissolved into 50 ml methanol and 10 ml glacial acetic acid; bring volume to 100 ml with distilled water. Antisera to human fibrinogen came from Behring Diagnostics, Somerville, N.J., Cat. 14-513-301, and Schwarz-Mann, Division of Becton, Dickinson & Co., Orangeburg, N.Y., Cat. 904764.

Treatment of Surfaces

All slides were cleaned by brushing with concentrated Sparkleen, using Kimwipes wrapped around applicators; the slides were then rinsed with large amounts of distilled water, air-dried, and quickly passed over a colorless bunsen burner flame. They were used when cooled, within about 1 hr. To avoid plasma/air/solid interfaces, drops were deposited onto slides as follows: 0.1 ml of VS was placed on the center of a slide and spread to an approximately 1 x 1 inch area; 0.1 ml of plasma was placed into the VS and mixed without disturbing the solid/liquid interface. The slide was placed in a moist chamber and removed after 10 min. Then, 0.1 ml VS was placed near the free end of the slide and allowed to spread over the remaining 1 x 1 inch area of the surface. After merging with the area containing plasma, the 2 deposits were allowed to mix for about 10 sec, following which the entire slide was rinsed with VS, followed by water, and air-dried. Next, 0.1 ml VS was spread across part of both areas (the one where plasma had resided for 10 min and where it had stayed for about 10 sec only), and into this VS, antiserum to human fibrinogen was placed and mixed. This was rinsed off with VS and water 3 min later. The glass slides were stained with Coomassie blue for 3 min and rinsed with water; all slides were air-dried.

Evaluation of Results

The deep bronze first-order interference color of the anodized tantalum was shifted to red by adsorption of plasma proteins; where antibody to fibrinogen had been deposited on top of this film, the color shifted to purple or from purple to violet. On the glass slides, sites of deposited antibody stained a deeper blue than did the protein film, where it had not adsorbed antibody. Quantitative data could be obtained in several ways: (1) by measuring azimuth and ellipticity of light reflected by the film-bearing anodized tantalum surfaces in the ellipsometer; (2) by measuring the intensity of light transmitted by the treated glass slides after staining; and (3) simply by ranking colors by eye. All appeared reliable, but ranking was preferred. Data are listed (Table 1) as ranked differences in color between areas 'a' and 'b' (Fig. 1): site of antiserum to human fibrinogen on area previously exposed for 10 min (area 'a') versus 10 sec (area 'b') to plasma. Values from 0 to 4 were assigned for each slide as follows: (0) color identical for the two areas (no conversion); (1) area 'a' slightly lighter than area 'b'; (2) area 'a' lighter than area 'b' and clearly distinguishable from background protein film color 'c' (Fig. 1); (3) area 'a' much lighter than 'b' and faintly distinguishable from 'c'; (4) area 'a' much lighter than 'b' and indistinguishable from background color 'c' (complete conversion—no antibody to fibrinogen deposited).

Table 1. Alteration in Deposited Fibrinogen in Various Plasmas

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Anodized</th>
<th>Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4 4 4 4 4</td>
<td>4 4 4 4</td>
</tr>
<tr>
<td>Prekallikrein deficient</td>
<td>4 4 3 3</td>
<td>4 3 3 2</td>
</tr>
<tr>
<td>Factor XI deficient</td>
<td>3 2</td>
<td>2 2</td>
</tr>
<tr>
<td>Factor XII deficient</td>
<td>3 3 3 3</td>
<td>2 2 1 1</td>
</tr>
<tr>
<td>Fitzgerald</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Williams</td>
<td>1 0 0</td>
<td>1 1 0</td>
</tr>
<tr>
<td>Williams and HMK†</td>
<td>4 3</td>
<td></td>
</tr>
</tbody>
</table>

*Each entry, ranging from 4 to 0, represents ranking of a single experiment, with 4 indicating complete alteration and 0 indicating none at all (see text).
†0.1 ml Williams plasma premixed with 0.02 ml (about 0.02 µ) of purified high molecular weight kininogen.

RESULTS

Normal Plasma

On the area (See Fig. 1) where plasma resided for 10 sec only, antiserum to human fibrinogen placed onto area 'b' deposited additional material: area 'b' is darker than area 'd.' In contrast, where the normal plasma had resided for 10 min, the antiserum placed on area 'a' left only traces of material: areas 'c' and 'a' are about equally light. All samples of normal plasma tested on glass and on anodized tantalum showed this marked effect on the deposited fibrinogen (see Table 1).

Kininogen-Deficient Plasmas

A representative slide (see Fig. 1, Williams) is shown. Where plasma lacking high molecular weight kininogen, or both high and low molecular weight kininogens, had resided on glass for 10 min, fibrinogen remained: area 'a' left by the antiserum to fibrinogen is markedly darker than the background color 'c' left by the plasma after 10 min. In the sample shown, area 'a' is only slightly lighter than area 'b,' indicating that during the 10 min of contact, the plasma converted only a small amount of the deposited fibrinogen. Both Williams and Fitzgerald plasma samples were deficient in this activity (see Table 1). Plasma lacking factor XII was somewhat deficient, while plasmas lacking factor XI or prekallikrein did convert their fibrinogen deposits as did normal plasma.

DISCUSSION

Before we had begun to identify the proteins deposited by plasma, we had discovered that on a clot-
promoting surface, intact plasma deposits material and then removes some of it again in a process requiring intact factor XII. Later, we discovered that much of the material deposited initially is fibrinogen and that intact plasma destroys the antigenicity of this deposit in a temperature- and concentration-dependent reaction. We called this process “conversion.” Factor XII seemed less essential for conversion than for subsequent removal of material. Since removal followed, and was not part of the conversion process, we assumed that fibrinogen deposits were replaced by other material so that film thickness remained constant. Conversion and removal are both clearly evident in this study (see Fig. 1 and legend). Neither of these events takes place on hydrophobic substrates, such as polyvinylchloride or glass that had been polished with ferric stearate. On these surfaces, heparinized or citrated blood will deposit a dense layer of platelets within 10 min, but surface coagulation factors will not be activated, and the fibrinogen deposited by plasma will not be converted. The present study indicates that conversion is related to surface activation of clotting as follows. (A) Plasma deposits fibrinogen on the glass or glass-like surface. (B) Factor XII may displace some of the fibrinogen, but most is replaced by high molecular weight kininogen. (C) Removal of some product of activation follows. (D) Platelets will only adhere where fibrinogen remains on the surface.

One consequence of this apparent antagonism between the effects of surface properties on the activation of clotting versus the “stickiness” to platelets may very well be that a material that prevents both clotting and platelet adhesion will be hard to find.

REFERENCES


10. Ratnoff OD: Personal communication, 1979


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