Does the Conformation of Adsorbed Fibrinogen Dictate Platelet Interactions With Artificial Surfaces?

By Jack N. Lindon, Gerald McManama, Leslie Kushner, Edward W. Merrill, and Edwin W. Salzman

Platelet activation by polymer surfaces is thought to require preliminary adsorption of fibrinogen and perhaps changes in fibrinogen conformation. We measured fibrinogen adsorption by a series of polymers by two methods, using either 125I-labeled fibrinogen or 125I-labeled antifibrinogen antibodies, and correlated the results with platelet reactivity ( retention and secretion) in columns of beads coated with the polymers. For polyalkyl methacrylates with 1 to 4 carbon side chains, platelet reactivity varied directly with increasing length of the alkyl side chain and with the quantity of bound fibrinogen recognizable by antifibrinogen antibody but not with the total quantity of fibrinogen adsorbed. The same pattern of results was seen with five antibody preparations, including affinity-purified Fab fragments against the D or E domain of fibrinogen. Tests of platelet retention and fibrinogen binding to four polyalkyl acrylates and to three unrelated polymers (polystyrene, polymethyl methacrylate, and a polyether polyurethane) indicated that platelet retention correlated positively with both total fibrinogen binding and with the amount of antibody-recognizable fibrinogen bound. Drugs that block platelet aggregation, but not adhesion, did not alter the hierarchy of platelet retention to the polyalkyl methacrylates. These data suggest that, contrary to previous views, platelet adhesion to artificial surfaces increases with increasing surface coverage of adsorbed fibrinogen if the bound fibrinogen maintains a conformation such that its functional domains remain recognizable by antibody probes.

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When a synthetic polymer such as a prosthetic arterial graft or dialysis membrane is exposed to a layer of adsorbed plasma components composed primarily of proteins, since the high concentration and diffusivity of interactions of platelets and other blood cells with the artificial surface are preceded by formation of a layer of adsorbed plasma components composed primarily of proteins, since the high concentration and diffusivity of plasma proteins, compared with the much larger blood cells, bring the proteins to the surface first. Interactions of plasma proteins in the fluid phase with the adsorbed protein film can also lead to activation of the coagulation, complement, and fibrinolytic systems. The composition of this protein film and the molecular configurations of its constituents, as influenced by the nature of the underlying polymer surface, are, therefore, of particular interest.

Packham et al and Zucker and Vroman reported almost 20 years ago that fibrinogen potentiates the adhesion of platelets to glass surfaces. Notwithstanding the more recently described participation of other "adhesive proteins" (eg, factor VIII:VWF and fibronectin) in adhesion of platelets to subendothelial connective tissue, reports from numerous laboratories have shown that for many surfaces the adsorption of fibrinogen rather than other plasma proteins correlates with the ability of the surfaces to promote platelet adhesion. Many surfaces when exposed to mixtures of purified proteins or to whole plasma preferentially adsorb fibrinogen, sometimes with subsequent desorption, which Vroman and coworkers have reported to require high molecular weight kininogens. It has been suggested that changes in the conformation of adsorbed fibrinogen molecules induced by interaction with the surface may lead to their recognition by platelets as "foreign" and may be responsible for platelet adhesion and spreading on the surface, and ultimately to secretion of the contents of storage granules and aggregation of additional platelets.

Experiments presented here were designed to test the hypothesis that variations in platelet interaction with surfaces exposed to whole blood correlate with the extent of fibrinogen adsorption. As a model system, we studied a series of closely related polymers—the polyalkyl methacrylates (Fig 1)—which consist of linear carbon backbones on which every second carbon possesses both a methyl group and a carboxylic acid group in ester linkage with an alkyl alcohol (eg, polybutyl methacrylate contains carboxylic acid groups complexed by ester linkage to n-butyl alcohol). The length of the alkyl side chains determines many of the polymers’ physical and chemical properties.

We have previously tested platelet reactivity of the polyalkyl methacrylates using an in vitro system in which whole blood, maintained at 37 °C, was passed through polymer-coated glass bead columns, and platelet retention by the columns and secretion of 14C-serotonin and beta thromboglobulin were measured. Platelet reactivity (ie, platelet retention on the columns due to adhesion to the surfaces and platelet aggregation and secretion of platelet contents) was found to increase in parallel with the length of the alkyl side chain. Platelet retention in the columns varied from 30% for polymethyl methacrylate (PMMA) to approximately 70% for polybutyl methacrylate (PBMA). Secretion of beta thromboglobulin by platelets exposed to these polymers varied from 2 µg/mL for PMMA to 5 µg/mL for PBMA; serotonin secretion paralleled beta thromboglobulin secretion. We now report that these polymers do not vary in their capacity to adsorb fibrinogen from a solution of the purified protein or from whole blood plasma, contrary to the expected result, but that the platelet reactivity of the polyalkyl methacrylates appears to correlate with their ability to bind fibrinogen in its "native" or "unaltered" conformation (defined as the ability of the fibrinogen molecule to be recognized by antifibrinogen antibodies).
MATERIALS AND METHODS

Polymers. Polyalkyl methacrylates were synthesized by free radical polymerization initiated by exposing alkyl methacrylate monomers to high doses (~10 MeV) of ionizing radiation using a Van de Graaff accelerator. The monomers (methyl, ethyl, n-propyl, and n-butyl methacrylate; Polysciences, Warrington, Pa) were placed in 20 mL glass vials and irradiated in an argon environment. After polymerization was allowed to proceed for several hours at room temperature, the polymers were separated from unreacted monomer by precipitating in methyl alcohol (Fisher Chemical Co, Boston, Mass) and redissolving in chloroform (Fisher Chemical Co). Polymers (mol wt 300,000; Pressure Chemical Co, Pittsburgh, Pa) and Biomer (lot no. BSROI7A; Ethicon, Inc, Somerville, NJ) were used as received from the manufacturers. Polymers were then passed through a gelatin-Sepharose column to be bead columns and glass cover slips.

Fibrinogen. Fibrinogen was purified from human plasma using a modification of the glycine/lysin precipitation technique described by Wang. An additional step was added: the fibrinogen preparation was passed through a gelatin-Sepharose column to remove contaminating fibronecetin. The resultant fibrinogen was >95% clottable by thrombin and showed, on SDS-PAGE, less than 1% contamination by either fibrinectin or von Willebrand factor. Purified fibrinogen stocks were adjusted to 10 mg/mL with 0.3 mol/L NaCl and stored in aliquots at ~60 °C.

Fibrinogen was labeled with 125I using the “Iodogen” technique described above. After labeling, antibodies were separated from unbound 125I by chromatography on G-25 Sephadex columns pre-conditioned with PBS.

Platelet retention testing. Protocols for platelet retention testing in polymer-coated glass bead columns have been described in detail. Briefly, polyethylene columns (0.8 cm x 20.5 cm) were filled with acid-washed, 0.3 mm diameter glass beads (total surface area, ~200 cm²) and coated with polymer by deposition from a 2% chloroform solution. Polymer-coated columns were washed with saline and then exposed to whole blood (maintained at 37 °C) at a flow rate of 0.25 mL/min. Platelet counts were determined for four 1 mL effluent fractions of undiluted blood and for the blood prior to column exposure, and the percentage of platelet retention was calculated from the ratio of the average platelet count in the effluent fractions to the whole blood platelet count.

Statistical analysis. Comparisons among data for different polymers were made using 3-way analysis of variance and the Duncan’s multiple range test for grouped, parametric populations. Data organization and analysis were performed on the Prophet system, a national computer resource sponsored by the Chemical/Biological Information Handling Program, National Institutes of Health.

independent of the specific activities of the fibrinogen solutions employed.

Antibodies. Two commercial antihuman fibrinogen antibodies (IgG fractions; Atlantic Antibodies, Scarboro, Me and U.S. Biochemical Corp, Cleveland, Oh) and antihuman IgG antibody (IgG fraction; Sigma Chemical Co, St. Louis, Mo) were used without further purification. In addition, Fab fragments of affinity purified antifibrinogen, anti-domain D and anti-domain E antibodies (kindly supplied by Drs Jack Hawiger and Marek Kluczewiak) were prepared as previously described. Briefly, serum from rabbits immunized against purified human fibrinogen was affinity purified using a fibrinogen-Sepharose column. The antifibrinogen antibodies were then passed through D-domain-Sepharose or E-domain-Sepharose affinity columns, and the anti-D-domain and anti-E-domain antibodies were eluted with 0.2 mol/L glycine, pH 2.4. Finally, Fab fragments of each of these three antibody preparations were prepared by papain digestion.

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For measurement of total fibrinogen binding, cover slips were immersed in a solution of purified fibrinogen containing 125I-fibrinogen (concentration, 250 µg/mL; specific activity, ~1000 cpm/µg) or in whole or diluted plasma spiked with 125I-fibrinogen (specific activity, ~3000 cpm/ng) and submersed for up to one hour under static conditions. The cover slips were then removed and washed for 15 seconds in PBS and the bound radioactivity was counted. For measurement of fibrinogen binding using labeled antifibrinogen antibodies (Fg-Ab*), the cover slips, after exposure to the fibrinogen solution (250 µg/mL) or to whole or diluted plasma, were washed in PBS and placed in 15 mm x 75 mm test tubes containing 0.5 mL of a solution containing bovine serum albumin (3 mg/mL; Sigma Chemical Co) and Fg-Ab*. The slips were incubated for one hour, with continuous shaking, and were then washed with PBS and counted.

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RESULTS

Total fibrinogen binding. Fibrinogen adsorption was measured by dipping polymer-coated cover slips into buffered fibrinogen solutions or into diluted or undiluted blood plasma, all containing radiolabeled fibrinogen. One objection to the use of a "dipping" technique for measuring protein adsorption is that the dipped surface may become coated with denatured protein as it passes through the air-solution interface (the Langmuir-Blodgett effect). Figure 2 illustrates the binding isotherms obtained when polybutyl methacrylate-coated coverslips were exposed for eight minutes to various concentrations of purified fibrinogen solutions labeled with $^{125}$I-fibrinogen. Identical isotherms were obtained by dipping polymer-coated cover slips into fibrinogen solutions or by adding fibrinogen to buffer in which the coverslips were already immersed, indicating that denatured fibrinogen at the air-solution interface did not affect the measurement of fibrinogen binding in this system. Binding of fibrinogen to PBMA increased markedly as the fibrinogen concentration was increased to approximately 50 $\mu$g/mL. Above 50 $\mu$g/mL, binding increased only marginally with increasing fibrinogen concentration. However, a saturation plateau was not obtained even with fibrinogen concentrations as high as 2 mg/mL. Binding isotherms were similar for each of the polyalkyl methacrylates.

Measurement of the time course of $^{125}$I-fibrinogen binding to the four polyalkyl methacrylates indicated that the bulk of the binding occurred within the first 30 seconds, with a slow, progressive increase in binding occurring thereafter (Fig 3A). Exposure of the polyalkyl methacrylate polymers to 250 $\mu$g/mL fibrinogen solutions for one hour resulted in the binding of approximately 0.3 $\mu$g/cm$^2$ and did not differ significantly among the four polymers ($P > 0.10$, n = 9). Fibrinogen adsorption in this range probably represents a monolayer of bound protein (see Discussion).

When the polyalkyl methacrylates were incubated in plasma for up to 60 minutes, a more gradual increase in binding occurred (Fig 3B), with total binding after 60 minutes approaching 0.2 $\mu$g/cm$^2$ for each of the methacrylates. However, Vroman et al. have shown that, for surfaces exposed to plasma, competition with other plasma proteins (especially high molecular weight kininogen) can quickly and markedly reduce the amount of fibrinogen bound. Brash et al. have shown that these early adsorption and desorption events occur more slowly and can be more readily examined if the plasma is diluted. When the polymers were exposed to 20% plasma (1 part plasma diluted with 4 parts PBS), fibrinogen binding was greatest at the earliest time point measured (15 seconds) and then decreased quickly to an apparent plateau at approximately 0.16 $\mu$g/cm$^2$ (Fig 3C). When the methacrylate polymers were exposed to 1% plasma, fibrinogen-binding kinetics showed a maximum at one to three minutes followed by a decrease in bound fibrinogen to an apparent plateau at less than 0.1 $\mu$g/cm$^2$ (Fig 3D). For undiluted plasma and 20% plasma, the time course and amounts of fibrinogen binding were indistinguishable for the four methacrylate surfaces. For 1% plasma, the binding kinetics were similar for the four polymers and the levels of fibrinogen bound to the polyethyl, propyl, and butyl methacrylates were not significantly different ($P > 0.10$, n = 3). However, for incubations of up to eight minutes in 1% plasma, fibrinogen binding to polymethyl methacrylate was slightly, but significantly ($P < 0.05$, n = 3) less than to the other three polymers.

Fibrinogen binding assayed with antifibrinogen antibodies. Surface-bound fibrinogen was also assessed using polyclonal antibodies raised against human fibrinogen and labeled with $^{125}$I. Preliminary experiments (data not shown) indicated that binding of antifibrinogen antibodies to fibrinogen-coated polyalkyl methacrylates was essentially complete in one hour. Antifibrinogen antibody adsorption to polyalkyl methacrylates that had first been exposed to solutions of purified fibrinogen (250 $\mu$g/mL) for up to 60 minutes is shown in Fig 3E. Binding ranged from 1.26 ng/cm$^2$ for PMMA to 3.11 ng/cm$^2$ for PBMA, after the 60-minute exposure, and was significantly different for each of the polymers at each time point tested ($P < 0.05$, n = 9). These low levels of antibody binding were due to the low concentration of labeled antibody solution employed. When the antibody concentration was increased, antibody binding increased to levels similar to that of the bound fibrinogen and the differences in antibody binding to the four polymers were no longer observed (Table 1).

When the polyalkyl methacrylates were first exposed to plasma for up to 60 minutes and then to a low concentration of antifibrinogen antibody (Fig 3F), antibody binding among the four methacrylates was not significantly different after preexposure to plasma for 8 or 60 minutes; but, after short preexposures (15 seconds, 1 minute, or 2.5 minutes), small but statistically significant differences in antibody binding to each of the four methacrylates ($P < 0.05$, n = 14) were observed. When 20% plasma was employed for preexposure (Fig 3G), the differences in antibody binding to the four methacrylates at the early time points were larger, but still disappeared after eight minutes of preexposure. When the polymers were preexposed to 1% plasma (Fig 3H), the subsequent differences in antibody binding were large and statistically significant ($P < 0.05$, n = 6) at all time points tested. When antifibrinogen antibody binding was assessed.
concomitantly for methacrylates preexposed either to a fibrinogen solution or to plasma, comparable binding was observed. For example, preexposure of the polymers for one minute to either undiluted plasma, 1% plasma, or fibrinogen (250 μg/mL) followed by incubation with labeled antibody (100 ng/mL) led to antibody binding ranging from 0.58 to 0.81 ng/cm² for undiluted plasma, 0.87 to 2.6 ng/cm² for 1% plasma, and 1.5 to 2.2 ng/cm² for fibrinogen solution. As shown in Fig 4, there are statistically significant positive correlations between antifibrinogen antibody binding and both platelet retention ($R = 0.984, P < 0.02$) and beta thromboglobulin release ($R = 0.978, P < 0.03$) induced by the polyalkyl methacrylates. These results suggest that platelet reactivity on these polymer surfaces may be dictated by the amount of antibody-recognizable ("native") fibrinogen on the surface rather than by total fibrinogen adsorption.

Differences in antifibrinogen antibody binding to polymer surfaces coated with nearly identical amounts of bound fibrinogen might result from variations in the orientation of the fibrinogen molecules on the different polymers or from variation in the extent of the conformational alterations induced in fibrinogen by the energy of adsorption. If the different methacrylates bind fibrinogen with no particular orientation but with the induction of progressively severe conformational alterations, then a variety of antibodies should show the same hierarchy of affinity for the surface-bound fibrinogen molecules. If, in the other hand, the different methacrylates do not induce conformational

**Table 1. Binding of Antifibrinogen Antibody (Fg-Ab) to Polyalkyl Methacrylate Polymers Previously Exposed to Fibrinogen**

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<tr>
<th>Polymer</th>
<th>Fg-Ab Bound (ng/cm²)</th>
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<tr>
<td></td>
<td>0.3 μg/mL †</td>
</tr>
<tr>
<td>PMMA</td>
<td>1.26 ± .12 ‡</td>
</tr>
<tr>
<td>PEMA</td>
<td>2.09 ± .12</td>
</tr>
<tr>
<td>PPMA</td>
<td>2.94 ± .05</td>
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<tr>
<td>PBMA</td>
<td>3.07 ± .07</td>
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†Values shown are the concentrations of antifibrinogen antibody solutions to which polymers were exposed following initial fibrinogen exposure.

‡Data represent means ± standard deviations: for the 0.3 μg/mL column, n = 9; for the 1, 10, and 200 μg/mL columns, n = 3.
changes but simply adsorb the fibrinogen with different orientations, then antibodies that recognize different parts of the fibrinogen molecule should show different hierarchies of antibody binding.

In order to test these two possibilities, experiments were conducted using three different antifibrinogen antibody preparations (two whole IgG fractions and one Fab fragment) and also Fab fragments of antibodies prepared against the D-domain (the terminal region) and the E-domain (the central region) of fibrinogen. Although the extent of the binding varied significantly from one antibody preparation to another, the rank order of antibody binding to the polyalkyl methacrylates was the same for each antibody (Fig 5). As a control, binding of an anti-IgG antibody preparation to the fibrinogen-coated surface was 10- to 100-fold less than that of the antifibrinogen antibodies and showed no material-to-material variation. For each of these polyclonal antibody preparations (even the anti-D and anti-E-region antibodies), binding to surface-bound fibrinogen molecules must be a complicated function of interactions within numerous epitope-antibody systems. However, these results suggest that the rank order of antifibrinogen antibody binding to the polyalkyl methacrylates was due to the degree to which each of these surfaces altered the conformation of bound fibrinogen molecules rather than to differences in the orientation of the bound fibrinogen molecules on the surfaces.

Platelet retention and fibrinogen binding by other polymers. In order to test the generality of the finding that platelet activation on polyalkyl methacrylates correlates with the binding of unaltered fibrinogen, platelet retention tests and fibrinogen binding assays were performed with two additional groups of polymers, the polyalkyl acrylates and a selected group of unrelated polymers. Polyalkyl acrylates have a structure similar to that of the polyalkyl methacrylates except for the replacement with hydrogen atoms of the methyl groups on the carbon backbone. A previous study showed that platelet activation in glass bead columns coated with various polyalkyl acrylates increased as the length of the polymer side chain increased from one carbon (polymethyl acrylate, PMA) to four carbons (polybutyl acrylate, PBA).

As shown in Fig 6A, variations in both total fibrinogen binding and antifibrinogen antibody binding correlated with the increase in platelet retention seen with the longer side chain acrylates.

Direct comparisons of platelet retention and fibrinogen binding were also made using two other polymers, polysty-
Study of polyalkyl methacrylates (Fig 4) is due to the fact that antibody binding correlated with the differences in platelet variations in both total fibrinogen binding and antifibrinogen changes. Data analysis of the same experiment. Platelet retention ranged from 27% for Biomer to 52% for PMMA and 71% for polystyrene. With this group of polymers, as with the polyalkyl acrylates, variations in both total fibrinogen binding and antifibrinogen antibody binding correlated with the differences in platelet retention (Fig 6B). The apparent discrepancy in platelet retention values for PMMA in this study and in the previous study of polyalkyl methacrylates (Fig 4) is due to the fact that different blood donors were used for the two studies and that there may be substantial donor-to-donor variability in the platelet retention test. For this reason, comparisons of platelet retention by different materials are only made for groups of materials tested simultaneously using the same blood donors.

Effect of platelet aggregation inhibitors on platelet retention. The results presented above indicate that the binding of "unaltered" fibrinogen to polymer surfaces correlates with platelet retention on the surfaces, even when the total amount of fibrinogen bound does not. However, platelet retention in polymer-coated glass bead columns results from both platelet adhesion to the surfaces and subsequent formation of platelet aggregates. In order to determine whether the binding of unaltered fibrinogen to artificial surfaces is involved in platelet adhesion, platelet retention on polyalkyl methacrylate-coated columns was studied using whole blood with or without a platelet aggregation inhibitor. In vitro addition of indomethacin, or aspirin ingestion two hours prior to blood donation, decreased the extent of platelet retention by the three polyalkyl methacrylates tested but did not change the rank order (Fig 7). Analysis of variance indicated that aspirin produced an insignificant drop (P = 0.25) in platelet retention and that indomethacin produced a significant drop (P < 0.05) in platelet retention that appeared, from examination of the bead columns by scanning electron microscopy, to be due to the nearly complete elimination of platelet aggregates on the polymer surfaces. Data analysis by Duncan’s multiple range test showed significant differences (P < 0.05) among the polymers both before and after drug treatment. Thus, the correlation noted above between "native" fibrinogen binding and platelet retention also holds for platelet adhesion to the polyalkyl methacrylates. It is possible, therefore, that platelet adhesion to polymer surfaces is mediated by unaltered, surface-bound fibrinogen.

DISCUSSION

The apparent requirement of platelet adhesion to surfaces for "native" adsorbed fibrinogen (ie, fibrinogen recognizable by antifibrinogen antibody and therefore presumably only minimally altered in conformation by adsorption onto the surface) is reminiscent of the essential role fibrinogen plays in platelet aggregation. In plasma, fibrinogen does not induce platelet aggregation but acts as a cofactor, binding to platelets only after platelet stimulation by agonists such as ADP which induce the expression of glycoprotein receptors for fibrinogen on the platelet surface. The bound fibrinogen may then act, possibly in concert with von Willebrand factor, as a bridge between contiguous platelets.

An analogous phenomenon may be involved in adhesion of platelets to artificial surfaces. The traditional view is that of Chiu et al.[14] who suggested that platelet interaction with surface-bound fibrinogen depends on the conformational alteration of the protein, induced by the energy of adsorption. Numerous studies[15,16] have shown that surfaces can induce conformational changes in adsorbed fibrinogen and other proteins. It has become widely accepted that surfaces that bind and denature fibrinogen most extensively are the ones most likely to induce platelet adhesion and activation. However, evidence presented here indicates that the reactivity of platelets with at least some artificial surfaces depends on the ability of the surfaces to adsorb a protein film containing fibrinogen with its functional domains maintained in a recognizable, minimally altered conformation.

For each of the three groups of polymers tested, platelet retention correlated positively with the amount of bound fibrinogen that remained recognizable by antifibrinogen antibodies. For the polyalkyl acrylates and for a group of unrelated polymers (PMMA, Biomer, and polystyrene) total fibrinogen binding also correlated with platelet retention. But for the polyalkyl methacrylates a positive correlation between platelet retention and antifibrinogen antibody binding obtained even though total fibrinogen binding kinetics were virtually indistinguishable for the four materials. Thus, the maintenance of fibrinogen’s native conformation may be important in platelet-surface interactions, even though for some polymers total fibrinogen binding is also correlated with platelet retention. In addition, the fact that the hierarchy of platelet interaction with the polyalkyl methacrylates was maintained even when platelet aggregation was blocked argues that this correlation is likely due to the direct mediation of platelet adhesion by "unaltered" fibrinogen on the surfaces.

The fibrinogen adsorption kinetics for methacrylate polymers exposed to whole or diluted plasma are similar to those reported by Vroman et al[17] for glass surfaces and by Brash et al[18] for glass, siliconized glass, and polyethylene. These kinetics indicate that some of the fibrinogen, bound after short incubation times, is removed from the surface, possibly via competition with other plasma proteins. Vroman et al[17] have presented evidence that high molecular weight kini-
nogen is responsible for this effect. This apparent binding and removal of fibrinogen from the methacrylate polymers is also reflected in the measurements of antifibrinogen antibody binding.

The hierarchy of antifibrinogen antibody binding to the fibrinogen-coated methacrylate polymers was only observed when the fibrinogen-coated polymers were exposed to low concentrations of the antibody. As the antibody concentration was increased, antibody binding increased to -0.25 μg/cm² (similar to the level of bound fibrinogen) and was indistinguishable for the four methacrylates. These results indicate that the differences in antibody binding on different polymers are likely due to differences in binding affinities of the antibody for the adsorbed fibrinogen molecules rather than to differences in the number of antibody-recognizable binding sites. Identical hierarchies of antibody binding to the fibrinogen-coated methacrylates were seen with antibodies against whole fibrinogen and also with anti-D region and anti-E region antibody preparations, suggesting that the different methacrylate polymers were not binding fibrinogen molecules in different orientations. Similar experiments using monoclonal antibodies may provide more definitive answers regarding the relative contributions of molecular orientation in conformational alteration to the antibody binding capacities of surface-bound fibrinogens. However, these observations could be explained if the different methacrylate polymers were inducing subtle, but distinguishable (by the antibodies) changes in the overall conformation of the bound fibrinogen molecules.

Chuang et al. examined two commonly used hemodialysis membranes, cuprophan and polycrylonitrile, and found that platelet retention by these two surfaces was negatively correlated with total fibrinogen binding but positively correlated with the amount of surface-bound fibrinogen detected with antifibrinogen antibodies. Although Chuang and associates did not draw the same conclusions we have reached regarding the importance of fibrinogen's native conformation in the reactivity of the adsorbed protein with platelets, their data are compatible with this hypothesis.

If adsorbed fibrinogen does mediate platelet adhesion to surfaces, is prior activation of the platelet (perhaps by ADP) required or is the interaction of the platelet with adsorbed fibrinogen itself sufficient to induce activation? Red cell hemolysis or mechanical effects on platelets could produce low levels of ADP near the blood/artificial surface interface, but in that case one must ask why platelet fibrinogen receptors exposed by this ADP would not already be occupied by soluble plasma fibrinogen. If platelet reactivity with artificial surfaces results from the interaction of nonactivated platelets with "native" adsorbed fibrinogen, how does such an interaction occur? For all of the polymers tested, total fibrinogen binding ranged between 0.2 μg/cm² and 0.5 μg/cm². Assuming dimensions of 60 A by 450 A for a rod-shaped fibrinogen molecule, close-packed monolayers would range from 0.2 μg/cm² for side-on attachment to 1.6 μg/cm² for end-on attachment. At saturation the concentration of fibrinogen in this surface layer would approach 350 mg/mL. Such close-packing of fibrinogen, perhaps with a particular spatial distribution of "native" reactive sites, could explain the reactivity of these surfaces with platelets.

Such a bound array might behave as a multivalent ligand with the fixed fibrinogen molecules acting to crosslink fibrinogen receptors in the platelet membrane and inducing platelet activation in a manner similar to the "mobile receptor" hypothesis of Jacobs and Cuatrecasas. Low fibrinogen binding affinities of nonactivated platelet binding sites would not preclude such a model. Hornick and Karush have shown that anti-DNP (2,4-dinitro-phenyl) antibodies, with a monovalent association constant of 6 x 10⁸ (mol/L)⁻¹, bind to DNP-coated bacteriophage with an association constant of 3.5 x 10¹⁳ (mol/L)⁻¹. Such effects of polyvalency have been treated theoretically by Crothers and Metzger and similar mechanisms have been proposed by Santoro and Cunningham to explain collagen-induced platelet aggregation and by Luscher et al. and Ganguly et al. to explain platelet activation by large molecules with repeating structures and by thrombin.

If surface-bound fibrinogen molecules do act as multivalent ligands to bind and activate platelets, then efforts to develop nonthrombogenic materials must strive to produce surfaces that, when in contact with plasma, will adsorb a protein film notable for its lack of fibrinogen maintained in a conformationally unaltered state.

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Does the conformation of adsorbed fibrinogen dictate platelet interactions with artificial surfaces?

JN Lindon, G McManama, L Kushner, EW Merrill and EW Salzman