REGULAR ARTICLE

Reactivity of Human Platelets With Immobilized Fibrinogen is Dictated by the Chemical Character of the Surface

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Abstract

Quiescent platelets readily adhere to surface-immobilized fibrinogen. In contrast, platelets exposed to soluble fibrinogen do not demonstrate such activity. As part of an effort to characterize this phenomenon, a solid-phase reagent was prepared by adsorbing human fibrinogen to polystyrene-divinylbenzene microparticles. Using a suspension of human platelets, phase-contrast microscopy was used to quantitate directly platelets bound to fibrinogen-coated beads. This method is fast, straightforward, and requires minimal amounts of reagents and sample. An existing turbidimetric assay was modified to monitor optically the rate and extent of platelet–fibrinogen binding. When platelet-rich plasma was added to a stirred suspension of fibrinogen-coated beads, the rate of aggregation was related directly to the concentration of fibrinogen on the bead surface. This response could not be mitigated by the thrombin inhibitor, hirudin, indicating that any thrombin generated in the reaction has no role in bead aggregation. Conversely, the $\alpha_{IIb}\beta_3$ antagonist, abciximab (ReoPro), completely prevented aggregation, implicating specific fibrinogen–$\alpha_{IIb}\beta_3$ interactions as responsible for the observed effect. Beads coated with either albumin or a densely packed, pure film of the neutral phospholipid, phosphatidylcholine (lecithin), do not aggregate under identical conditions, nor do fibrinogen-coated beads aggregate when platelet-depleted plasma is added. When fibrinogen was coated to beads as a mixed film with lecithin, a striking increase in reactivity toward platelets was demonstrated, compared to unmodified beads. These studies indicate that the observed adhesion of platelets to beads is a direct result of platelet–fibrinogen interactions and platelets respond differently to fibrinogen when presented as a mixed film with lipid, compared to the protein alone at an interface. © 2001 Elsevier Science Ltd. All rights reserved.

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Circulating platelets have no thrombogenic interactions with soluble components of blood, including fibrinogen, the main protein of the clot matrix. When activated using any number of agonists, for example, collagen or adenosine diphosphate (ADP), platelets aggregate and secrete a variety of bioactive molecules. Platelet aggregation is dependent on plasma fibrinogen, which serves as a protein

tether between apposed platelets via the cell membrane receptor, $\alpha_{IIb}\beta_3$ (GP IIb/IIIa) [1]. The glycoprotein $\alpha_{IIb}\beta_3$ binds to the dodecapeptide sequence located at the carboxyl terminus of the γ-chain of the fibrinogen molecule [2], of which the last four amino acids (Ala-Gly-Val-Asp) are essential [3]. The activated form of the $\alpha_{IIb}\beta_3$ receptor is only minimally expressed on quiescent platelets [4,5] and is likely a means of regulating platelet reactivity. Platelets respond to newly generated thrombogenic surfaces, for example, exposed subendothelium, by first adhering to the nascent surface. Adhesion of platelets to bioactive surfaces, as with aggregation, requires specific platelet membrane glycoproteins and plasma proteins, von Willebrand factor, and fibrinogen being most recognized. The same agonists that promote aggregation also induce platelet adhesion.

In the absence of agonists, platelets can also be activated when exposed to certain reactive surfaces, for example, fibrinogen immobilized at an interface [6,7]. This adhesion cannot be prevented/stopped by apyrase or PGE_1, indicating that released ADP or typical platelet activation is not responsible for this reaction [8,9]. Solution-phase fibrinogen constitutes a secondary ligand for platelets, as ADP-activated platelets preferentially bind immobilized fibrinogen, even in the face of many-fold excess soluble fibrinogen [10]. Platelet activity can be associated with unwanted outcomes, an important example being the thrombotic consequences following an atherosclerotic plaque fissure. Plaque rupture exposes the hydrophobic core cholesteryl esters to blood [11,12]. Fibrinogen is not only required for platelet adhesion to this new 'foreign' surface but has also been recognized as a risk factor in predicting complications of atherosclerosis [13]. Certainly, a greater understanding of the differential response of platelets to certain biologic and artificial surfaces will promote more rational management of thrombotic disorders.

Various models have been devised to investigate the reactions involving platelets, the fibrinogen film, and the adsorbing surface. A convenient means to study biologically relevant reactions involving immobilized fibrinogen employs latex microparticles [14–16]. The bead model offers a significant surface/volume ratio

and, importantly, allows rigorous quantitation of admixtures of proteins (fibrinogen and albumin) and lipids (phospholipids and cholesterol), creating a model readily adaptable to the study of a spectrum of physiologically meaningful surfaces [17–20]. Predictably, there exists a direct relationship between platelet adherence to a surface and the fibrinogen density, where platelets have greater reactivity with a maximally packed film of fibrinogen than with a surface otherwise equivalent but having less fibrinogen [21]. Others have reported that the conformation of adsorbed fibrinogen is different from solution-phase fibrinogen [6,22], a densely packed film of fibrinogen having a conformation more like soluble fibrinogen. This suggests that the character of the fibrinogen film might be as important as the surface density. Further, the morphology of platelets adhering to fibrinogen at low density is different from platelets bound to high-density fibrinogen [21], suggesting that the nature of the fibrinogen film is critical in dictating the platelet response.

In this report, I characterize the adherence of platelets to fibrinogen when the protein is spread as a pure film or as a mixed film with the neutral phospholipid, lecithin. A standard platelet aggregometer was used to develop a sensitive assay that measures the rate and extent of platelet binding to solid-phase fibrinogen. The method directly assesses the binding of fibrinogen by platelets and is specific for known biologic interactions. Platelet binding to fibrinogen-coated microparticles was also assessed directly using phase-contrast microscopy to enumerate bound platelets. By both methods, platelets were more reactive with a mixed film of lecithin and fibrinogen than with a pure film of the protein. These disparities are not explained solely by the amount of fibrinogen on the surfaces and suggest a different molecular orientation of the protein when bound to surfaces of different hydrophobicities. The orientation of fibrinogen at an interface appears as an important determinant in the reactivity of the protein with platelets. One goal of these studies is a more complete characterization of platelet–fibrinogen interactions at surfaces. Results of these studies should be of interest to those investigators concerned with platelet adhesion to surfaces, both biologic and

synthetic, and the thrombotic complications involving immobilized fibrinogen.

1. Materials and Methods

1.1. Reagents and Chemicals

Fibrinogen was obtained from Enzyme Research Laboratories, South Bend, IN. After thorough dialysis against 10 mM 4(2-hydroxylethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.40, aliquots of the protein were stored at -70 °C. Fibrinogen concentration was determined using the molar absorptivity of the protein at 280 nm of 5.12×10^5 M^{-1} cm^{-1} [23]. Radiolabeled (^{125}I) human fibrinogen was purchased from ICN Biochemicals, Irvine, CA. Specific activity was typically $\sim 5.0 \times 10^{16}$ Bq mol^{-1}. L-α-lecithin (phosphatidylcholine) from egg yolk was from Avanti Polar Lipids, Birmingham, AL. Following dissolution in ethanol, lecithin was stored under nitrogen at -70 °C. Polystyrene-divinylbenzene particles, nominal diameter 6.4 ± 1.9 μm, were purchased from Seradyn, Indianapolis, IN and were prepared for experiments using an existing protocol [24]. The $\alpha_{IIb}\beta_3$ antagonist, abciximab (ReoPro), was a gift from Centocor, Malvern, PA. The following proteins and chemicals were from Sigma, St. Louis, MO: albumin, fraction V, fatty acid free; the peptide, Gly-Pro-Arg-Pro amide; hirudin, from leeches, 1000 U mg^{-1}; and human thrombin, 3000 U mg^{-1}. All buffers and salts were of the highest commercial quality. Organic solvents were HPLC grade. Water was purified using a commercial apparatus (Milli-Q, Millipore, Bedford, MA).

1.2. Preparation of Fibrinogen-Coated Beads

Fibrinogen was adsorbed to polystyrene-divinylbenzene beads either as a pure film of the protein or as a mixed film with the neutral phospholipid, lecithin, according to an established procedure [14]. The nonsaturating film of lecithin was at a nominal density of 160 Å^2/molecule. Beads, with or without lipid, avidly bind fibrinogen, but beads saturated with lecithin bind no protein [17,25,26]. When the inter-face is maximally packed with fibrinogen, lecithin-modified beads of the diameter used in these studies adsorb the protein to a surface concentration of 0.97 μmol cm^{-2}. After washing away unbound protein, beads were resuspended in bovine albumin (1.0 mg ml^{-1} in water). Lecithin/fibrinogen-coated beads prepared in this manner are routinely used in experiments within 6 h, even though fibrinogen desorbs from beads only very slowly, with a half-life of several hundred hours [14,27].

1.3. Preparation of Platelet Suspensions

The protocol for this study was approved by the Human Subjects Committee of the Marquette University Institutional Review Board, and informed consent was obtained from volunteers prior to blood donation. Blood was drawn from healthy donors into 0.129 M sodium citrate, then centrifuged at $125 \times g$ for 12 min to obtain platelet-rich plasma. For some experiments, platelets were used directly. For other experiments, platelets were isolated from plasma proteins using gel filtration according to an established procedure [28]. Prior to filtering, the platelet-containing plasma was treated with prostaglandin E$_1$ (300 nM) to inhibit platelet activation during processing.

1.4. Direct Quantitation of Platelet Adherence to Fibrinogen-Coated Beads Using Phase-Contrast Microscopy

Platelets (~ 2 μm diameter) are of noticeably smaller diameter than PSDVB beads (6.4 μm). The two can be readily distinguished using standard bright-field microscopy under high power ($\times 400$ magnification). However, a preferred method is to use phase-contrast microscopy to facilitate rapid and accurate discrimination of beads and platelets. The number of bound platelets per bead serves as a direct reflection of the affinity of platelets for the test surface. As controls for these experiments, beads were coated with either a maximally packed film of lecithin or were saturated with the hemostatically inactive protein, albumin. A typical assay was performed as follows: fibrinogen-coated beads were resuspended to a nominal concentration of 6.5×10^9 l^{-1}. A volume of this bead suspension was

combined with an equal volume of platelet-rich plasma that had been standardized to 2.50×10^{11} l^{-1}, the mixture blended by repipetting with low-energy sonication for 10 s. An aliquot of the test mixture was transferred immediately to a silanized glass slide and covered with a plastic coverslip. After allowing the beads/platelets to settle (~ 15 s), platelets that were adherent to beads were enumerated and the count normalized to platelets bound per 100 beads. In these experiments, platelets were not separated from plasma proteins. Studies using ^{125}I-fibrinogen indicated no desorption of fibrinogen from these beads, and no secondary fibrinogen adsorption from plasma, at all surface densities of fibrinogen used.

1.5. Turbidimetric Assay to Quantitate Platelet Adherence to Fibrinogen-Coated Microparticles

Aggregated particles scatter less light than do monodisperse particles. Aggregation can be monitored turbidimetrically using any spectrophotometer equipped with a stirred cell device and linear recorder or, as a convenient alternative, using a standard platelet aggregometer (Chronolog, Havertown, PA). An existing method [29] was adapted to quantitate the rate at which platelets and beads aggregate when combined. This technique of monitoring platelet–bead aggregation can be adapted to study any number of biologically relevant interactions involving surfaces. Following preparation, beads were resuspended in BSA to a concentration that yielded an apparent absorbance (500 nm) of 1.00. This photooptical method provides a convenient means of standardizing bead suspensions to a concentration that is equivalent to 6.50×10^9 beads l^{-1} [17]. A typical aggregation assay was performed using 500 μl of bead suspension in a 0.5 ml cuvette. Bead suspensions to be tested were prewarmed at 37 °C for 2 min. The suspension was then stirred at 1000 rpm for 30 s, after which 20 μl of platelet suspension was added. For these trials, platelets were concentrated to 1.50×10^{12} platelets l^{-1}. The signal corresponding to the difference between monodisperse and completely aggregated beads and platelets was arbitrarily designated as a relative absorbance unit of 1.00. A suspension of beads of diameter 0.93 ± 0.01 μm was used as a refer-

ence solution after diluting in water to an apparent absorbance of 1.20. Conveniently, these smaller beads do not settle out of solution, even after 6 h. Aggregations were typically complete after 8–10 min.

1.6. Inhibition of Platelet–Bead Aggregation

Several approaches were employed to further elucidate the mechanism of the observed aggregation, speculated to be due solely to bead–platelet adhesion via fibrinogen bridging. The commercial antagonist, abciximab (ReoPro), was used to block fibrinogen binding to the platelet receptor. This reagent has been shown to occupy $\alpha_{IIb}\beta_3$ receptors even in unactivated platelets [5]. PRP was prepared as above. To this was added abciximab (200 nM) and the mixture incubated with gentle rocking at 25 °C for 10 min. The antagonist-treated platelets were then reacted with a suspension of fibrinogen-coated beads, supplemented with abciximab to the same concentration as the PRP. In another experiment, the peptide, Gly-Pro-Arg-Pro, a recognized inhibitor of fibrin dimerization [30], was used to treat fibrinogen-coated beads to test the proposal that platelet–bead aggregation is a consequence of interbead fibrin dimerization. This tetrapeptide inhibits fibrin dimerization that occurs in bead–bead aggregation [29]. Prior to analysis, the bead suspensions were supplemented with Gly-Pro-Arg-Pro to 200 μM. The fibrinogen-coated beads were then reacted with PRP in a manner identical to that described above. A different trial used hirudin, the potent thrombin inhibitor, to negate any thrombin activity that might be generated by the activation of platelets in the presence of surface-immobilized fibrinogen. Hirudin was added to PRP in amounts ranging from 0 to 1.00 U per assay. Immediately after mixing, the treated PRP was added to the stirred bead suspension to initiate the assay. By definition, 1.00 U of hirudin inhibits 1.00 U of thrombin. Thus, this amount of hirudin should far exceed the thrombin that could be reasonably expected to be produced by the amount of plasma factors in the 20 μl volume of PRP and in the time frame of the assay.

1.7. Adenosine Triphosphate (ATP) Secretion as an Indicator of Platelet Activation by Immobilized Fibrinogen

Platelet activation results in the release of bioactive factors from platelet granules, including ATP [31]. ATP secretion can be conveniently monitored with a bioluminescent luciferin/luciferase assay [32,33]. Five-hundred microliters of PRP were warmed at 37 °C for 5 min prior to adding 50 μl Chronolume reagent. Following an additional 2 min at 37 °C with stirring, 50 μl bead suspension was added to the platelet suspension, after which aggregation and luminescence were monitored simultaneously. ATP in the sample was calculated by comparing to a 2.0 nmol standard.

2. Results

2.1. Platelet-to-Fibrinogen Binding at an Interface is Dependent on the Nature of the Fibrinogen Film

In striking contrast to soluble fibrinogen, and in the absence of agonists, platelets readily adhere to immobilized fibrinogen (Fig. 1). This adhesion occurs when platelets are suspended in plasma or buffers that contain albumin as the sole protein. The number of adherent platelets is related to the density of fibrinogen at the interface, that is, maximally packed films of the protein attract more platelets than do more loosely packed films [21]. To initially characterize the binding of platelets to the fibrinogen films used in these studies, latex beads were exposed to concentrations of fibrinogen ranging from 0 to 1.0 mg ml^{-1} (~ 3.0 μM), achieving a range of surface densities of fibrinogen, up to a saturating film (~ 0.39 μg cm^{-2} or ~ 1.15 pmol cm^{-2}). This surface density of fibrinogen is consistent with previous work [25] and reports from others [10]. Some experiments compared beads prepared in this fashion with beads that were coated with a nonsaturating film of lecithin prior to the fibrinogen. Beads pretreated with lecithin adsorb fibrinogen to a maximum density of ~ 0.33 μg cm^{-2} (0.97 pmol cm^{-2}). When platelets were exposed to beads coated with a pure film of fibrinogen, the number of platelets bound was related linearly to the solution concentration of fibrinogen used to coat the beads over the entire range of concentrations tested ($r^2 = .9729$; Fig. 2). In contrast, a mixed film of lecithin and fibrinogen bound platelets in a saturable fashion. With this model system, a solution concentration of 10 μg ml^{-1} fibrinogen yields surface densities of 0.013 and 0.011 μg cm^{-2} for unmodified and lipid-modified beads, respectively. Both bead treatments supported only minimal platelet adhesion at this fibrinogen density. These data suggest that for lipid-modified beads, exposure to 20 μg ml^{-1} fibrinogen is essentially equivalent to a saturated film, in terms of number of plate-

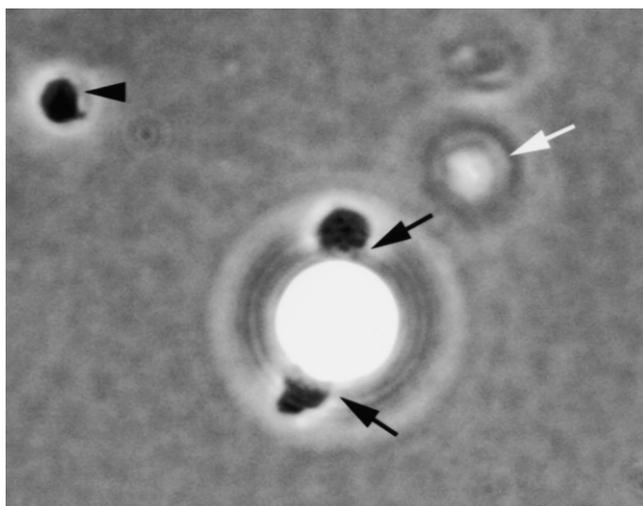Fig. 1. Photomicrograph demonstrating typical platelet adhesion to latex beads (6.4 μm diameter) saturated with a monolayer of fibrinogen ($\times 1000$ magnification). Arrows point to adherent platelets (black arrows), a free platelet (black arrowhead), and a bead out of the plane of focus (white arrow).
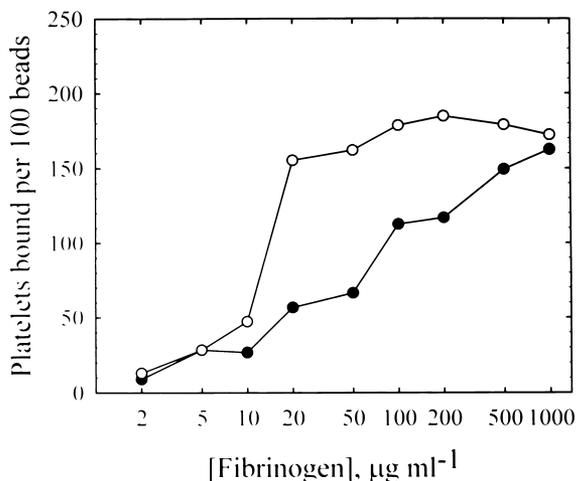
Fig. 2. Platelet adsorption to fibrinogen-coated latex beads as a function of solution concentration of fibrinogen, for lipid-modified beads (open circles) and unmodified beads (solid circles). Counts were normalized to platelets bound per 100 beads.

lets adhering to the protein-coated surface. More intriguing, this minimally packed film of fibrinogen on the lipid-modified beads is as platelet reactive as a saturated film of the protein on unmodified beads.

2.2. Platelet–Bead Interactions can be Monitored Using a Turbidimetric Method

When exposed to thrombin, beads coated with fibrinogen aggregate as a consequence of fibrin formation and interbead fibrin dimerization [17]. Since aggregated beads scatter less light than do monodisperse beads, bead aggregation can be monitored turbidimetrically. This phenomenon, and this technique of monitoring bead–bead interactions, has been used extensively in previous studies [17,18,25,29] and is amenable to the study of many interactions involving adsorbed proteins. Preliminary studies in this laboratory and reports from others [34] had indicated that this technique might prove useful in monitoring platelet–bead adhesion. Indeed, Fig. 3 depicts a typical aggregation profile after platelets are added to a stirred suspension of fibrinogen-coated beads. Platelets promoted aggregation of fibrinogen-coated beads, whether the platelets were suspended in native plasma or in buffer after gel filtration. Visual inspection of aggregates by microscopy revealed platelet–bead clus-

ters of varying sizes; however, there was no evidence of platelet–platelet aggregation nor bead–bead aggregation. Beads coated with albumin or a densely packed, pure film of lecithin do not aggregate under these conditions. Further, fibrinogen-coated beads do not aggregate when platelet-depleted plasma is added, and bead–bead aggregation does not occur in the presence of platelets (data not shown). When platelets
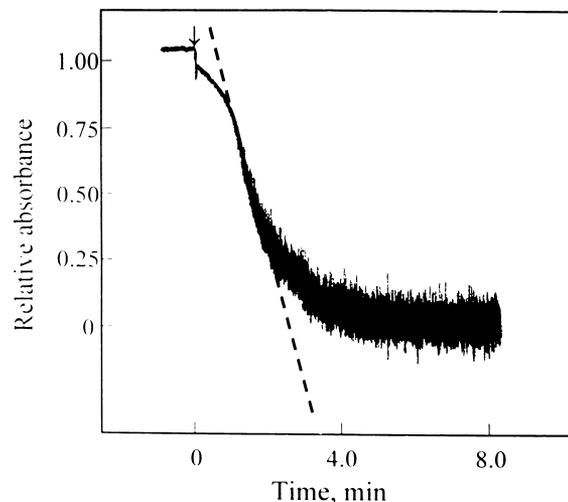

Fig. 3. Representative tracing depicting aggregation of fibrinogen-coated beads upon exposure to platelets. Straight line corresponds to maximum rate of aggregation.
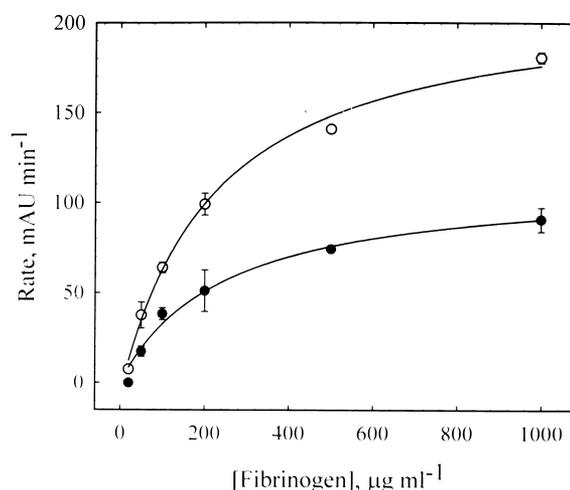

Fig. 4. Rates of aggregation of fibrinogen-coated beads that were exposed to platelets, as a function of solution concentration of fibrinogen. Solid lines represent curves from an equation described in Results. Data points for lipid-modified beads (open circles) and unmodified beads (solid circles) represent mean of three replicates. Error bars are 1.0 S.D.

were exposed to either a pure film of fibrinogen or the mixed lipid/fibrinogen film, an apparent saturable process was observed (Fig. 4). Data were fitted to an equation of the form $v = V_{max} \times F/(A + F)$, where v is the observed rate of aggregation, V_{max} is the maximal rate of aggregation, F is the solution concentration of fibrinogen (in μg ml^{-1}) used to coat the beads, and A is the concentration of fibrinogen that yields half-maximal aggregation. Applying this equation to experimental data yields maximal rates of 112.7 and 222.9 mAU min^{-1} for unmodified and lipid-modified beads, respectively. Interestingly, the solution concentration of fibrinogen that yielded a half-maximal rate of aggregation was not different for the two bead treatments (243.9 and 257.3 μg ml^{-1} for unmodified and lipid-modified beads, respectively). If this latter statistic can be equated to an apparent affinity constant of platelets for the protein-coated surfaces, the data suggest no difference in the "strength" of platelet–fibrinogen interactions on these surfaces, even though fibrinogen on the lipid-treated beads supports a markedly greater degree of platelet adhesion.

Aggregation also occurs when gel-filtered platelets are added to fibrinogen-coated beads that are suspended in 1.0 mg ml^{-1} (~3 μM) fibrinogen, albeit at a faster rate than platelets in buffer alone. This, taken with the concentration-dependent rate of aggregation, suggests that (1) both solution- and solid-phase fibrinogen are active in the aggregation process, but (2) solid phase is a more important determinant of the initial rate of platelet activation in this system. It is interesting to note the twofold rate increase in the presence of solution-phase fibrinogen, even though the molar ratio of solution-phase/solid-phase fibrinogen is greater than 300 under these conditions. These findings support the notion that solution-phase fibrinogen plays a major role in the aggregation process, but the initial activation rate of platelets is dependent on surface-immobilized fibrinogen.

2.3. Platelet–Bead Aggregation Involves $\alpha_{IIb}\beta_3$ but not Direct Fibrinogen–Fibrinogen Contacts Between Beads

It was reasoned that the observed aggregation of platelet–bead mixtures could be due to two independent, but possibly coexistent, mechanisms: (1) specific binding of platelets to bead-bound fibrinogen via $\alpha_{IIb}\beta_3$ and/or (2) direct fibrinogen-to-fibrinogen contacts between beads, with platelets only being trapped in the growing aggregate. To ascertain the mechanisms that account for the observed aggregation, the $\alpha_{IIb}\beta_3$ antagonist, abciximab (ReoPro), was incubated with the platelet suspension prior to addition to the bead suspension. At a concentration of 10 μg ml^{-1} abciximab ($\sim 2 \times 10^{-7}$ M), aggregation was prevented completely (Fig. 5), with intermediate concentrations accomplishing partial inhibition (data not shown). This finding strongly supports the notion that specific platelet-to-bead-bound fibrinogen contacts are occurring. If direct fibrinogen-to-fibrinogen binding plays a role in this process, the peptide Gly-Pro-Arg-Pro should be an effective antagonist of fibrin(ogen) dimerization. When the bead suspension was fortified with 200 mM Gly-Pro-Arg-Pro, addition of platelets still prompted aggregation at an equivalent rate. This amount of the peptide had previously been shown to completely eliminate bead aggregation via fibrin(ogen)-dependent mechanisms [29]. Platelet ATP assays indicated ~70 pmol of ATP released in the typical binding experiments. Because these experiments are carried out using platelets suspended in plasma, it is possible that some amount of thrombin is generated in the course of these experiments. When the thrombin
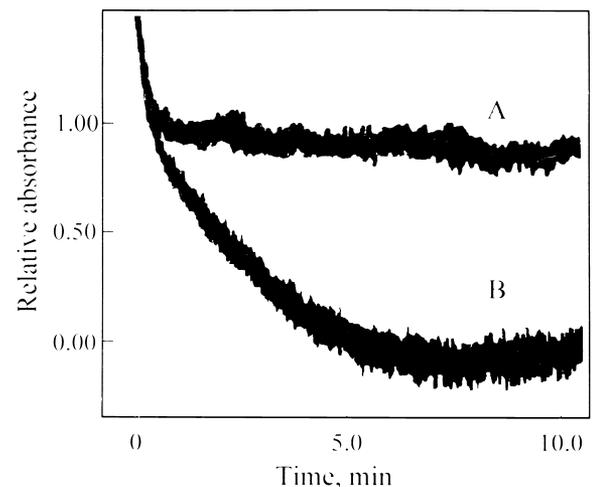

Fig. 5. Comparison of aggregation profiles of fibrinogen-coated beads stirred with platelets, either with (A) or without (B) the platelet $\alpha_{IIb}\beta_3$ antagonist, abciximab, added.

inhibitor, hirudin, was included in the assay mixture (0.2 U per assay), there was no effect on the observed aggregation. This finding is consistent with no thrombin being generated in the course of the assay and further justifies the use of PRP as equivalent to isolated platelets for these studies.

3. Discussion

Fibrinogen has long been recognized as an essential cofactor in platelet adhesion and aggregation. Since both platelets and fibrinogen are critical in the biologic processing of new surfaces, artificial or biologic, focused characterization of the molecular properties of interfacial fibrinogen and mechanisms of platelet adherence are essential in gaining a greater understanding of these processes in thrombosis. A proven model system was used to investigate the reactivity of different fibrinogen films with platelets. Not only were platelets found to be more reactive with higher surface densities of fibrinogen but also a marked difference in platelet reactivity was demonstrated toward beads coated with a mixed film of the neutral phospholipid, lecithin, and fibrinogen compared to a pure film of the protein. This altered reactivity was predicted from previous work [17,26] and provides dramatic evidence of molecular differences when fibrinogen is in these different microenvironments.

When fibrinogen adsorbs to a low surface concentration on an unmodified hydrophobic surface, a minor fraction of fibrinogen, nonelutable by detergents, is more dominant than when the protein adsorbs to a lecithin-modified surface at the same concentration [25]. This nonelutable species of fibrinogen is not as reactive as the major, elutable fraction in biologically relevant events, for example, fibrin dimerization. One explanation for this difference in reactivity is that, with increasing density, a dominant fraction of the protein exists in a near-native state and proportionately less as the nonnative species. It was reasoned that this minor species should also be less reactive with cellular elements, such as platelets, and surfaces that allow a greater proportion of the elutable fraction should react more readily. Indeed, platelets were more reactive with fibrinogen films of greater surface density, and a mixed film of fibrinogen and lecithin induced a greater rate of platelet aggregation than a pure film of the protein. The amount of protein on each surface does not account for these differences, as there is $\sim 20\%$ less fibrinogen on the lecithin-modified beads at saturating conditions, an effect attributable to exclusion of protein from the surface by the polar head group of the phospholipid. Rather, a reasonable explanation for this enhanced reactivity is a more native tertiary structure of fibrinogen when present as an admixture with lipid.

The solution concentration dictates the molecular orientation of fibrinogen at an interface. Molecular orientation of the adsorbed protein may also contribute to the observed alterations in function. When spread to a low surface density, fibrinogen is horizontally elongated at the interface, with the protein becoming increasingly normal on the surface as surface density increases [10,35]. While the structure of adsorbed fibrinogen differs from that of soluble fibrinogen when the protein is adsorbed from dilute solution [36], higher concentrations induce a 'solution-like' character to the adsorbed protein film [37]. As an extension of this model, a lipid-modified surface may induce a similar ordering of the protein film, promoting a 'solution-like' phase at a concentration lower than would occur on an unmodified hydrophobic surface.

These investigations rely heavily on a sensitive assay for quantitating the affinity of fibrinogen-coated beads for platelets. When monitoring the turbidity of stirred solutions of fibrin(ogen)-coated microparticles, the rate and extent of aggregation is a function of the solution concentration of fibrinogen used to coat the surfaces and, thus, the reactivity of the protein film with platelets. The aggregation method also provides a convenient means to quantitate low levels of solution-phase fibrinogen [38] and offers these attributes: (1) the method is adequately precise, with coefficients of variation of 10.2% and 2.4% for surfaces coated from 50 and 1000 $\mu g\ ml^{-1}$ solutions of fibrinogen, respectively; (2) the method is rapid, with duplicate trials completed in under 8 min; and (3) since the test surface mimics a biologic interface, the components are studied in a near-native state. The method can be

used to systematically evaluate functional differences in the fibrinogen film adsorbed to surfaces of various hydrophobicities. This simple assay should be of use to anyone interested in measuring the reactive properties of adsorbed fibrinogen and interactions of protein films with platelets.

This report provides further evidence that the reactivity of fibrinogen with cellular elements of blood can vary, depending on the chemical properties of the surface to which the protein is adsorbed. These findings might contribute to a more complete, mechanistic characterization of platelet–fibrinogen interactions and toward the logical design of synthetic polymers for blood-contacting roles.

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