Activation Affects Access to the Platelet Receptor for Adhesive Glycoproteins

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Abstract. Blood platelets have a receptor for macromolecular adhesive glycoproteins, located on a heteroduplex membrane glycoprotein complex (GPIIb/IIIa) that only becomes "exposed" when platelets are activated. Binding of the adhesive glycoproteins, in particular fibrinogen, to the receptor is required for platelet aggregation, which in turn is required to arrest bleeding. A murine monoclonal antibody whose rate of binding to the receptor is affected by platelet activation was both cross-linked and fragmented to assess the effects of changes in molecular size on its rate of binding to unactivated and activated platelets. The results indicate that small molecules can bind more rapidly to the receptors on unactivated platelets than can large molecules and that activation involves a conformational and/or microenvironmental change that permits the large molecules to bind more rapidly.

THE activation response of human blood platelets represents a remarkable evolutionary adaptation. Under normal conditions, platelets must circulate in isolation to avoid vascular occlusion; at sites of vascular injury, however, they must aggregate one with another nearly instantaneously in order to minimize hemorrhage. It is now clear that this is accomplished by a mechanism wherein any one of several agonists (ADP, epinephrine, thrombin, collagen, or thromboxane A_2) interacts with platelets so as to "expose" a receptor for fibrinogen; this receptor is only minimally expressed or entirely cryptic on unactivated platelets (2). The subsequent binding of fibrinogen to platelets correlates closely with aggregate formation, perhaps because the fibrinogen acts as a bridging molecule between platelets (fibrinogen has a bivalent structure and molecular dimensions suitable for such a function), although additional post-fibrinogen binding steps may also be involved (20, 24). Compelling evidence exists that a calcium-dependent complex made up of two glycoproteins (GPIIb, a 2-chain, disulfide-bonded glycoprotein of M_r 143,000, and GPIIIa, a single-chain glycoprotein of M_r 88,000) is the site to which fibrinogen binds (2, 3, 15). In fact, recent data indicate that other high molecular weight adhesive glycoproteins (fibronectin, von Willebrand factor, and perhaps thrombospondin) may also bind to or near the GPIIb/IIIa complex upon appropriate activation (13, 26, 28), but the physiologic roles for these interactions remain to be clarified. At present, all platelet aggregation induced by agonists present in vivo seems to rely on this receptor mechanism; thus, it occupies a crucial role in hemostasis and thrombosis.

The mechanism by which the GPIIb/IIIa receptor becomes exposed is poorly understood. Both glycoproteins can be labeled on unactivated platelets by membrane-impermeant probes and so they must be present on the surface of the platelets even when they cannot bind fibrinogen (3). Because the two glycoproteins are held together by calcium, it was proposed that the activation mechanism might involve increased availability of calcium and the joining together of the glycoproteins (15). However, evidence derived from several different immunologic and biochemical approaches indicates that GPIIb and GPIIIa probably exist as a complex even on unactivated platelets (5, 9, 15, 31). Thus, it can be inferred that the exposure mechanism must involve a change in the GPIIb/IIIa complex itself and/or its microenvironment.

Studies to define the exposure mechanism have been hampered by the lack of a probe that can report on such a change in the receptor, and so they have been confined to what can be inferred from the binding of fibrinogen, its fragments, or the other ligands themselves. I recently reported a new murine monoclonal antibody (designated 7E3) directed against the GPIIb/IIIa receptor whose rate of binding to platelets was significantly increased by activation with ADP or several other agonists. This increase indicated preferential or exclusive reactivity with the activated form of the complex (4). The availability of the antibody permitted the present study, which is designed to test the hypothesis that the exposure mechanism is size-selective; that is, fibrinogen and the other ligands are excluded or restricted from binding to unactivated receptors by virtue of their large size, and activation results in greater access of such large molecules to the binding site on the receptor. I reasoned that if this were true, smaller fragments ($F[ab']_2$ and Fab') of the 7E3 antibody might bind more rapidly to unactivated platelets than the intact antibody and show less enhancement of their binding with platelet activation. Conversely, cross-linked aggregates of the intact antibody (dimers, trimers, and tetramers), which approach the molecular dimensions of fibrinogen, would be expected to bind less rapidly than the intact anti-

body to unactivated platelets and show a more dramatic increase in binding rate with activation.

Materials and Methods

Purification and Iodination of 7E3 and Its Fragments

Intact antibody 7E3 was purified from ascites fluid by ion-exchange chromatography (DE-52; Whatman Chemical Separation Inc., Clifton, NJ) using a linear gradient from 0–0.15 M NaCl in 5 mM Tris/Cl. It was then either further purified by affinity chromatography on protein A–agarose (Boehringer Mannheim Diagnostics, Houston, TX) and iodinated with iodogen (Pierce Chemical Co., Rockford, IL; 25 μg coated on a glass scintillation vial in dichloromethane; 0.26 mg protein in 1 ml; 1 mCi ^{125}I; 10 min; 22°C) or fragmented into $F(ab')_2$ with pepsin (2% wt/wt pepsin A [Worthington Biochemical Co., Freehold, NJ] for 17 h at 37°C) and purified by chromatography on Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, NJ). The $F(ab')_2$ fragment was then iodinated and either used directly or after conversion into Fab' by reduction (50 mM 2-mercaptoethanol, 60 min, 22°C) and alkylation (60 mM iodoacetamide [pH 8.3] 30 min, 22°C). Unlabeled, purified 7E3 and the three radiolabeled preparations were denatured in SDS and electrophoresed in a 7% polyacrylamide gel as previously described (4). Another IgG_1 monoclonal antibody produced in our laboratory (6D1) that is directed against platelet glycoprotein Ib (5) served as a control. It was purified, fragmented, and iodinated as above, with only minor variations.

Cross-linking of 7E3 (30)

Intact 7E3 antibody was purified by protein A-agarose affinity chromatography using the Bio-Rad MAPS buffer system (Bio-Rad Laboratories, Richmond, CA) and then dialyzed against 0.2 M Tris/Cl (pH 8.53). The antibody was then concentrated to 57 mg/ml (0.2 ml) using a membrane filtration device (Centricon 30; Amicon Corp., Danvers, MA) that had been prerun with the 0.2 M Tris/Cl buffer to remove residual glycerol. A fivefold molar excess of dimethyl suberimidate (8.8 μl of a freshly prepared, 40-mM stock solution in the same buffer) was then added and allowed to react for 1 h at 22°C. The reaction was stopped by adding 0.8 ml of 0.2 M Tris/Cl, (pH 2.2) and placing the solution (final pH 7.3) on ice. The solution was then immediately applied to a 1 × 115-cm column of Sepharose 6B (Pharmacia Fine Chemicals) and eluted with 0.15 M NaCl, 0.01 M Tris/Cl, 0.05% sodium azide (pH 7.4) at 2 ml/h. Polyacrylamide gel electrophoresis showed that the column had separated intact 7E3 from 7E3 dimers and a combination of 7E3 trimers plus tetramers. 2 ml of the intact 7E3 was then digested with pepsin and a portion further reduced and alkylated as indicated above. The five different 7E3 preparations (Fab', $F[ab']_2$, intact 7E3, dimer, and trimer + tetramer) were then iodinated with ^{125}I using iodogen and electrophoresed in a 5.5% SDS polyacrylamide gel; after drying, the gel was subjected to radioautography.

Antibody Binding Studies

Platelet-rich plasma (PRP)[1] ($1.64–3.13 \times 10^{11}$ platelets/liter) was prepared from whole human blood anticoagulated with 0.01-vol of 40% sodium citrate. For the studies with intact antibody 7E3 and its fragments, 0.2 ml of the PRP was incubated for 30 s with either 22 μl of buffer (0.15 M NaCl, 0.01 M Hepes [pH 7.4]) or 50 μM ADP in the same buffer (final concentration 5 μM) and then aliquots of the radiolabeled antibody species were added to achieve final concentrations between 2.4 and 19 nM for each species. For studies with the cross-linked 7E3 and its fragments, PRP (3.22×10^{11} platelets/liter) was treated with buffer or ADP as above and then the radioactive species were added (1–7.5 μl; 3.1 nM final concentration for each species assuming a M_r of 480,000 for the trimer plus tetramer). At the indicated time points, duplicate 0.1-ml samples were layered over 0.1 ml of 30% sucrose, and platelet-bound antibody was separated from free antibody by centrifugation. The total active antibody concentration in each preparation was estimated by incubating a trace amount of the antibody with an excess of platelets for 1–2 h and determining the percentage bound (69–89% for the intact antibody and its fragments, and 81–93% for the cross-linked antibody and its fragments) (4). The binding rate was then calculated from the percentage of active antibody bound per minute per platelet, with normalization to 1 nM. Control experiments showed that nonspecific binding using an excess of intact 7E3 for each species was 0.5–3% of the total radioactivity. Equilibrium binding studies were performed as previously described (4) to determine the maximum number of 7E3 molecules of each species that could bind per platelet.

1. *Abbreviation used in this paper*: PRP, platelet-rich plasma.

Determination of Apparent Stokes Radii

The apparent Stokes radii of antibody 7E3 and its fragments were determined by chromatography on a 111 × 1-cm column of Sephacryl S-200 (Pharmacia Fine Chemicals) using a 0.5 M NaCl, 0.01 M Tris/Cl, 0.05% sodium azide, pH 7.4 buffer, and a flow rate of 2 ml/h. The void volume was determined with blue dextran and the column was calibrated with catalase (52.2 Å), aldolase (48.1 Å), bovine serum albumin (BSA) (35.5 Å), ovalbumin (30.5 Å), chymotrypsinogen A (20.9 Å), and ribonuclease A (16.4 Å) as recommended by the manufacturer (gel filtration calibration kit; Pharmacia Fine Chemicals) by plotting the $\sqrt{-\log(K_{av})}$ vs. the apparent Stokes radius. Radiolabeled 7E3 and its fragments were chromatographed twice, once with the standard protein with the closest M_r and then, for confirmation, with the standard proteins that appeared to elute just before and just after the 7E3 species being tested. In each case, the repeat chromatogram confirmed the relative elution position of the 7E3 species.

The apparent Stokes radii of the cross-linked 7E3 species (dimer and trimer plus tetramer) and fibrinogen (Cutter Laboratories, Berkeley, CA) were determined on a 115 × 1 cm column of Sepharose 6B (Pharmacia Fine Chemicals) using a 0.15 M NaCl, 0.01 M Tris/Cl, 0.05% azide, pH 7.4 buffer with a flow rate of 2 ml/h. The column was calibrated with thyroglobulin (85.0 Å), ferritin (61.0 Å), catalase, and aldolase as described above (gel filtration calibration kit; Pharmacia Fine Chemicals) and then fibrinogen and the 7E3 species were tested. Since the observed value for fibrinogen (79.5 Å) was considerably below the 107–110 Å predicted by its diffusion coefficient (17, 22), ^{125}I-labeled fibrinogen (a gift of Dr. Ellinor Peerschke, State University of New York at Stony Brook, Stony Brook, NY) and thyroglobulin were chromatographed together; this confirmed that fibrinogen eluted later than thyroglobulin. A similar difference between the predicted and observed Stokes radii for fibrinogen has been found in previous studies (17, 22) and one conceptual model advanced to explain this is that under certain circumstances asymmetric molecules may preferentially enter gel pores "end-on" (22). Since the 7E3 trimer plus tetramer has a M_r greater than fibrinogen, I decided to confirm its elution after fibrinogen by chromatographing a mixture of fibrinogen and radiolabeled trimer plus tetramer; the latter again eluted later than the former, reflecting fibrinogen's greater asymmetry.

Immunoelectrophoresis of the radiolabeled antibody species was performed as previously described (4). After electrophoresis, the arcs were developed with rabbit anti-mouse IgG (Sigma Chemical Co., St. Louis, MO). The intact antibody and the dimethyl suberimidate-treated dimeric and trimeric plus tetrameric antibody all had the same mobility, which was somewhat anodal to the insertion well. The $F(ab')_2$ and Fab' fragments both migrated to the same position, which was somewhat less anodal than the other species, but still anodal to the insertion well.

Results

7E3 and its $F(ab')_2$ and Fab' fragments were purified and radiolabeled (Fig. 1). Fig. 2 shows the binding of intact 7E3 and its fragments to platelets in PRP ($\sim 1.8 \times 10^{11}$ platelets/liter) as a function of time. The rates were nearly linear over the first minute and then decreased to 79 ± 1% (mean ± SD) of the 1-min value at 2 min. The percentage of added antibody that was bound at 1 min ranged from ~7% for the intact antibody without ADP to ~20% for the $F(ab')_2$ fragment with ADP, indicating that the free antibody concentration was not limiting at the early time points. The binding rate to unactivated platelets was lowest with the intact antibody, intermediate with the $F(ab')_2$ fragment, and greatest with the Fab' fragment. Pretreatment of platelets with ADP increased the rate of binding of the intact antibody most, had an intermediate effect on the $F(ab')_2$ fragment, and had the least effect on the binding of the Fab' fragment.

Table I gives the results from another series of experiments performed with a different preparation of 7E3 and its fragments, in which three different concentrations of antibody

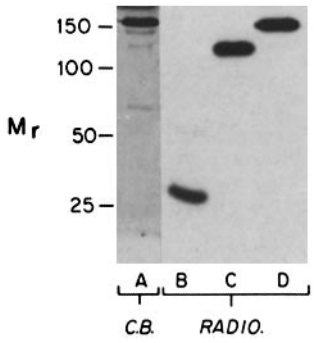

Figure 1. Analysis of intact antibody 7E3 and its fragments. Lane *A*, a Coomassie Blue stain of the intact 7E3. Lanes *B–D*, radioautographs of Fab′ 7E3, F(ab′)$_2$ 7E3, and intact 7E3. The Fab′ has an M_r of ~25 kD rather than 50 kD because the two ~25-kD chains are held together by noncovalent interactions that are disrupted by SDS.

were used, the platelet count was ~3.0 × 10^{11} platelets/liter, and the binding rates were derived from a 1-min incubation. The binding rates for the intact antibody and its fragments were essentially constant over the range of concentrations tested (2.4–19 nM) with even the fastest binding sample, (Fab′)$_2$ fragment binding to ADP-treated platelets, showing only a 10% difference. At the lowest concentration of antibody, the fraction of platelet GPIIb/IIIa molecules occupied by antibody at 1 min ranged from 1.1% for the intact antibody without ADP to 3.5% for the F(ab′)$_2$ fragment with ADP, indicating that the concentration of free GPIIb/IIIa molecules was not limiting. As in the studies shown in Fig. 2, the smaller fragments bound significantly more rapidly to unactivated platelets than did the intact antibody; the Fab′$_2$ fragment bound 63% faster than the intact antibody and the Fab′ fragment bound 83% faster than the intact antibody. When platelets were preactivated with ADP, antibody binding rate increased at all concentrations, with the intact antibody demonstrating the greatest increase in binding rate (166%), the Fab′$_2$ fragment having an intermediate increase (98%), and the Fab′ having the least (36%). The binding rates of the three species to ADP-activated platelets were also compared with each other; unlike the progressive increase in binding rates with smaller species that was observed with unactivated platelets, the intact antibody and Fab′ fragment had similar rates, whereas the binding rate of the F(ab′)$_2$ fragment was ~25% greater. Equilibrium-binding studies on the same samples showed that activation with ADP did not significantly alter the maximum number of molecules of each species that could bind per platelet, but the number of Fab′ molecules bound to both unactivated and activated platelets consistently exceeded the number of intact 7E3 or F(ab′)$_2$ molecules (Table II). This latter observation may be due to steric factors and/or the valence difference between the species.

Since the smaller fragments differ from the intact antibody in diffusion rate in addition to size, and the Fab′ fragment differs from the intact antibody and the F(ab′)$_2$ fragment in valence, control studies were performed with antibody 6D1, which reacts with GPIb. In one of three similar studies performed at 1 nM antibody using a 30-s time point, the F(ab′)$_2$ fragment of 6D1 bound 19% more rapidly than the intact antibody (1,989 vs. 1,665 molecules/platelet/min/nM), whereas the Fab′ fragment's rate (1,839) was less than that of the F(ab′)$_2$ fragment, and only 10% greater than that of the

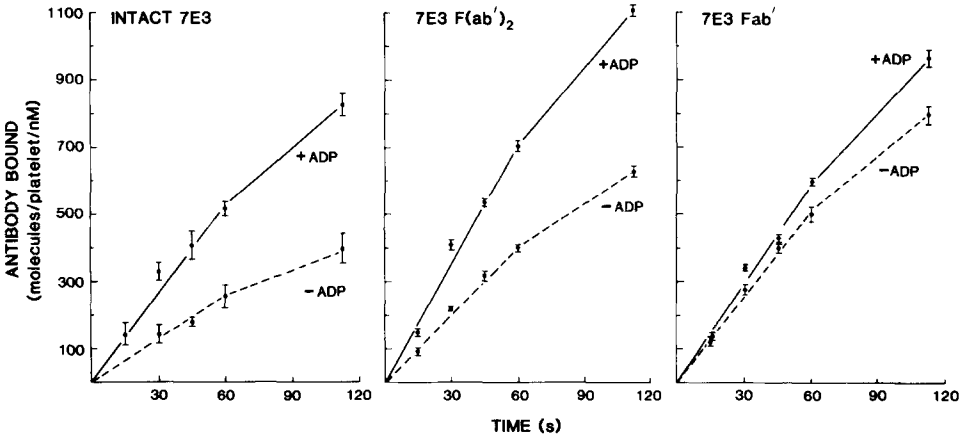

Figure 2. Time course of binding of intact 7E3 and 7E3 fragments to unactivated and activated platelets. PRP (0.2 ml; 1.64–1.89 × 10^{11} platelets/liter) was incubated with buffer or ADP for 30 s and then 1 μl of radiolabeled antibody (16 nM final concentration for all species) was added. Platelet-bound antibody was separated from free antibody at the indicated times. The points represent the means of four different experiments performed in duplicate. The bars are ± 1 SEM.

Table I. Rate of Binding of 7E3 and Its Fragments (molecules per platelet per minute per nanomolar)

	Intact 7E3		F(ab′)$_2$7E3		Fab′7E3	
Concentration	−ADP	+ADP	−ADP	+ADP	−ADP	+ADP
nm						
2.4 ± 0.5	199*	540	317	705	375	517
6.6 ± 1	209	546	375	662	381	511
19.0	211	569	319	634	383	515
	207 ± 6‡	551 ± 12(166%)	337 ± 14	666 ± 13(98%)	379 ± 6	515 ± 8(36%)

PRP (3.05–3.13 × 10^{11} platelets/liter) was incubated with buffer or ADP for 30 s and then 22.4 μl of radiolabeled antibody was added to achieve three different final concentrations for each species (2.4 ± 0.5, 6.6 ± 1.0, and 19 nM). After 1 min, free antibody was separated from bound antibody. Similar data were obtained in four other experiments, and two other preparations of 7E3 and its fragments gave similar results.

* Mean of three separate experiments.

‡ Mean ± SEM was obtained by pooling all the data from the three different experiments conducted at three different antibody concentrations. The numbers in parentheses are the percentage increases in binding rates with activated platelets compared with unactivated platelets.

Table II. Maximum Binding of 7E3 and Its Fragments to Platelets

	−ADP	+ADP
7E3	45,700 ± 1,100*	38,200 ± 5,400
$F(ab')_2$	45,500 ± 3,400	49,100 ± 2,100
Fab'	75,400 ± 4,900	93,200 ± 6,100

Equilibrium-binding studies were performed on the three PRP samples reported in Table I after adjustment to 10^{11} platelets/liter. The numbers of Fab' molecules bound per platelet, with or without ADP stimulation, were significantly different from the numbers of intact 7E3 and $F(ab')_2$ fragments bound per platelet ($P < 0.02$). Similar differences were observed in three other experiments. Differences between values for each of the species, with and without ADP stimulation, were not significant.
* Mean ± SEM

intact antibody. These data suggest that the $F(ab')_2$ may bind more rapidly than the intact antibody due to an increase in diffusion rate (although it is possible that steric considerations may also be involved in access to GPIb), but that any additional advantage in diffusion rate enjoyed by the Fab' fragment is neutralized by the decrease in valence from two to one. The results with 6D1 are very similar to those obtained when 7E3 bound to ADP-activated platelets (the $F[ab']_2$ bound more rapidly than the intact antibody, but the Fab' bound less rapidly than the $F[ab']_2$), but markedly different from those obtained when 7E3 bound to unactivated platelets. (The $F[ab']_2$ fragment of 7E3 bound 63% faster than the intact antibody whereas the $F(ab')_2$ fragment of 6D1 bound only 19% faster. The Fab' fragment of 7E3 bound 83% faster than the intact antibody whereas the Fab' fragment of 6D1 bound just 10% faster.) This suggests that a factor(s) other than bulk diffusion rate and valence affects the binding of 7E3 and its fragments to unactivated platelets, whereas the differential binding rates of 7E3 and its fragments for activated platelets are consistent with the differences in diffusion rates and valence among the species as judged by the data with 6D1.

Fig. 3 shows the characterization and binding data on 7E3 antibody treated with the cross-linking agent dimethyl suberimidate (30). In accord with the proposed hypothesis, the dimeric and trimeric plus tetrameric species bound less rapidly than the intact antibody to unactivated platelets and showed the greatest increment in binding rate with activation. Apparent Stokes radii determinations showed that even the 7E3 trimer plus tetramer had a smaller radius than fibrinogen (Table III). Fibrinogen, in fact, does show a greater difference in binding rates to unactivated and activated platelets than any of the species tested; very little fibrinogen binds to unactivated platelets despite prolonged incubation (2), whereas even the trimer plus tetramer showed a pattern of slow, but progressive binding with time.

Discussion

These data demonstrate that smaller M_r species of 7E3 bind more rapidly to unactivated platelets than do larger M_r species. In addition, the smaller species show less of an increase in binding rate when platelets are activated with ADP than do the larger ones. Although there are many potential explanations for these data, I believe the most consistent explanation is that there is only very limited access of large molecules to the binding site on the GPIIb/IIIa complex receptor on unactivated platelets and that platelet activation results in increased accessibility of ligands of large size to this binding site. Although the antibody species differ in diffusion rate, valence, and charge, these differences cannot account for the differential binding behavior with unactivated and activated platelets. If the increased diffusion rate of the smaller species were the sole factor responsible for the observed increased rate of binding to unactivated platelets, then one would also expect these same differentials in binding rates with activated platelets, in which case the binding rate ratios for activated and unactivated platelets would be identical for all of the species. The data in Table I and Figs. 2 and 3 show this is not the case, with Fig. 3 *C* graphically depicting a systematic change in the ratios of the rates. Additional evidence against differences in diffusion rate and/or valence explaining the binding behavior of 7E3 and its fragments to unactivated platelets comes from the studies showing that antibody 6D1 (directed against GPIb) and its fragments behaved very differently from 7E3 with unactivated platelets, but very similarly to 7E3 with activated platelets. Moreover, changes in valence between the species would be more likely to operate to minimize the effects of size; thus, the increased valence of the cross-linked species would more likely increase, rather than decrease, their binding rates with unactivated platelets, and the decreased valence of the Fab' fragment would more likely decrease, rather than increase, its binding rate with unactivated platelets. Finally, although there is a difference in charge between the $F(ab')_2$ and Fab' fragments on the one hand, and the intact antibody, the dimethyl suberimidate-treated dimeric antibody, and the trimeric plus tetrameric antibody on the other hand, this cannot account for the pattern of differences observed within each group of identical charge.

The recent demonstration that small peptides containing the amino acid sequence in the γ-chain of fibrinogen that is thought to be involved in binding to platelets bind equally well to unactivated and activated platelets (14), is also consistent with a model in which the GPIIb/IIIa exposure mechanism operates, at least in part, by modulating access on a size-dependent basis. Similarly, preliminary evidence indicates that a hepta-peptide containing the arginine-glycine-aspartic acid sequence that has been implicated in the binding of fibronectin, von Willebrand factor, and the Aα-chain of fibrinogen to platelets, also binds equally well to unactivated and activated platelets (16). Additional support comes from the discovery that an IgM monoclonal antibody directed against GPIIb/IIIa that is larger than any of the species tested in the current study, showed a greater discrimination between unactivated and activated platelets than does

Table III. Apparent Stokes Radii of Fibrinogen and Antibody 7E3 Species

	Å
Fibrinogen	79.5
7E3 Trimer plus tetramer	72.5
7E3 Dimer	62.0
7E3	40.9
7E3 $F(ab')_2$	33.5
7E3 Fab'	23.9

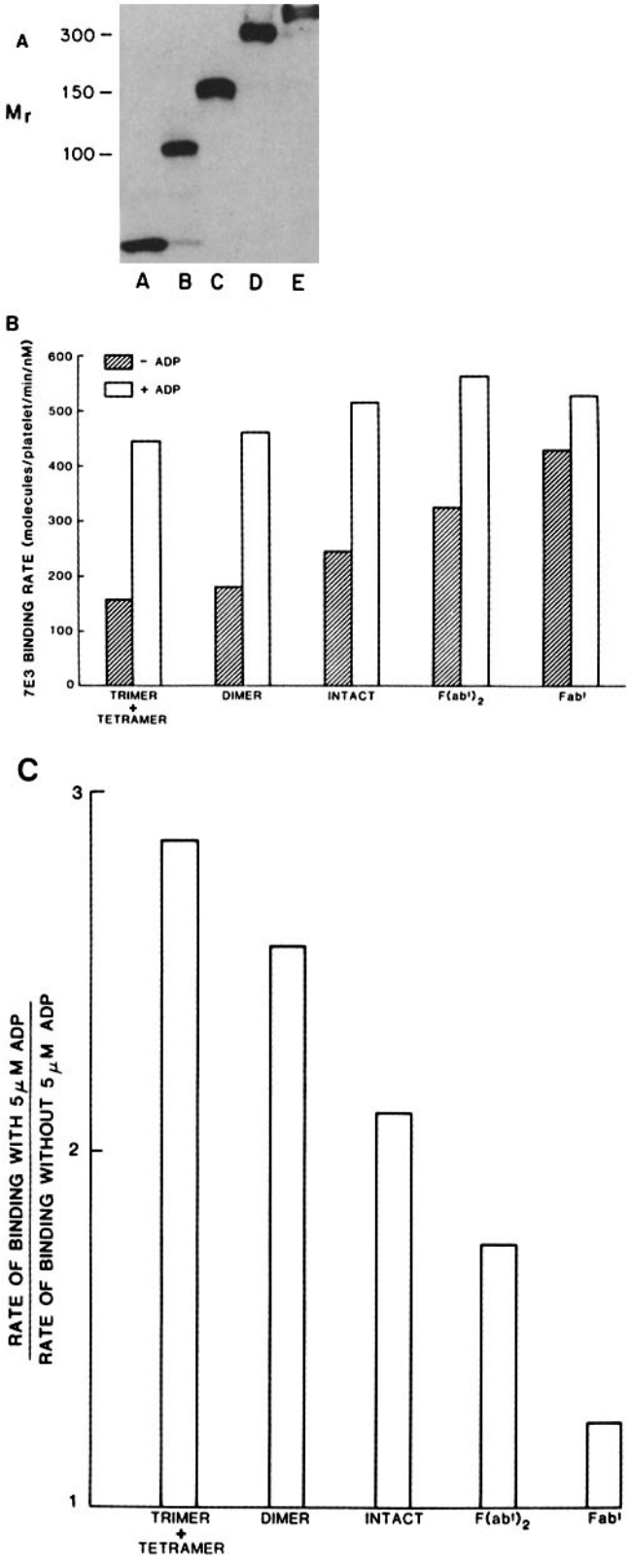

Figure 3. (*A*) Radioautographic analysis of cross-linked and digested 7E3 antibody. The radiolabeled antibody species were electrophoresed in a 5.5%-SDS polyacrylamide gel; after drying, the gel was subjected to radioautography. *A*, Fab' 7E3; *B*, $F(ab')_2$ 7E3; *C*, intact 7E3; *D*, 7E3 dimer; *E*, 7E3 trimer plus tetramer. (*B*) Binding of cross-linked, intact, and fragmented 7E3 species to platelets. Whereas the binding rates were determined from 2-min incubations and the data in Fig. 2 indicate that binding rates may begin to decrease after 1 min, the results may be slight underestimations of the actual values. However, since all the rates appear to decrease by the same percentage at 2 min (see Fig. 2), there should be no effect on the ratios shown in Fig. 3 *C*. Data shown are the means of two separate experiments in which duplicate determinations were made. (*C*) Enhancement of binding of cross-linked, intact, and fragmented 7E3 species. Data from the experiment reported in *B* are expressed as the ratio of the binding rate after 5-μM ADP activation to the rate without activation. Similar results were obtained in experiments using 1-, 2-, and 10-μM ADP stimulation.

7E3 (32). Since there was a continuum of binding rates for the different species of 7E3 rather than a threshold phenomenon, there may be considerable variation in the accessibility of different GPIIb/IIIa receptors. This is consistent with there being heterogeneity among these receptors. Moreover, since fibrinogen shows more of a threshold response than even the largest 7E3 aggregates, additional factors may operate to prevent its interaction with unactivated platelets.

Whether the ultimate control of the access of large molecules to the GPIIb/IIIa complex is mediated by a change in the receptor's conformation or its microenvironment remains to be determined. The observations that chymotrypsin digestion of platelets results in exposure of fibrinogen receptors even in the absence of agonist activation (18, 21) and that GPIIb/IIIa extracted from platelets retains the ability to bind at least some fibrinogen (1, 12, 19, 23) might seem to favor a model invoking a microenvironmental change, but additional direct data are required before this model can be accepted. Finally, there is reason to suspect that the interaction of adhesive glycoproteins with the platelet GPIIb/IIIa complex may have significance that transcends platelet physiology; hence, proteins immunologically related to GPIIb and GPIIIa have been identified in endothelial cells (8, 33) and endothelial cells have been reported to bind fibrinogen (7). Moreover, the arginine-glycine-aspartic acid sequence, which has been implicated in the binding of adhesive glycoproteins to platelets (10, 11, 16, 27, 29) and the binding of fibronectin to a variety of eukaryotic cells (25), is found in a substantial number of proteins that are involved in different cell adhesion phenomena (25). Additional studies will be required to assess whether comparable activation-dependent changes in access are involved in the interactions between these other cells and adhesive glycoproteins.

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