A Monoclonal Antibody to a Membrane Glycoprotein Binds Only to Activated Platelets

Rodger P. McEver and Mary N. Martin

From the Division of Hematology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284

Rearrangements of membrane glycoproteins are believed to occur during platelet activation, but these changes have not been well defined. We have developed a monoclonal antibody, named S12, which demonstrates dramatically enhanced binding to platelets after thrombin activation. Unstimulated gel-filtered platelets from 12 normal individuals bound only 800 ± 470 (S.D.) 125I-S12 molecules/cell, while platelets stimulated with 0.5 unit/ml of thrombin bound 9,600 ± 2,600 molecules/cell (Kd = 1.5 nM). Increasing thrombin concentrations produced similar increases in platelet 125I-S12 binding and [14C]serotonin secretion. S12 binding was not dependent on divalent cations. ADP and epinephrine, which caused no [14C]serotonin secretion, had little or no effect on S12 binding. We isolated the S12 binding protein by affinity chromatography of Lubrol PX-solubilized human platelet membranes on S12-agarose. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the isolated protein stains with both periodic acid-Schiff and Coomassie Blue and has an apparent molecular weight of 138,000 (unreduced) and 148,000 (reduced). After radioiodination of intact platelets the protein was also labeled, with apparently equal intensity in both control and thrombin-stimulated cells. The protein's staining, radiolabeling properties, and mobility on sodium dodecyl sulfate gels relative to glycoprotein Ib-IIa fit previously defined criteria for membrane glycoprotein Ila. Our studies provide further evidence for alterations in membrane glycoproteins after platelet stimulation and suggest that S12 may serve as a useful probe of in vivo platelet activation.

When activated, blood platelets undergo a rapid series of morphological and biochemical changes that promote their physiologic role in the cessation of bleeding. The major result of these changes is that platelets immediately become involved in cell surface contact interactions with exposed subendothelial fibers as well as with adjacent platelets (1). Cellular changes accompanying these interactions include the conversion from a disc shape to a spiny sphere with long extended pseudopods and the secretion of granule contents. The molecular events that accompany and facilitate contact interactions are not fully defined. Some cytoplasmic changes are well documented, including actin polymerization, protein phosphorylation, hydrolysis of cytoplasmic proteins by a calcium-activated protease, and cytoskeletal protein interactions with the membrane (2). However, less is known about the molecular rearrangements on the plasma membrane surface that account for the transition from a circulating to an adherent cell.

The initial platelet interaction with subendothelium is facilitated by the association of membrane glycoprotein Ib with von Willebrand factor immobilized on the surface of exposed collagen or microfibrils (1, 3–5). It is not clear that this interaction requires an altered platelet surface. Surface change does appear to be required for the induction of the fibrinogen receptor that mediates platelet-platelet contact interactions (6–10). Several complementary studies indicate that this receptor is membrane glycoprotein Ib-Ila (11–19). We (12) and others (13) have produced monoclonal antibodies that react with glycoproteins Ila and Ila only when the two polypeptides are associated. Platelet-binding studies with these antibodies suggest that Ila and Ila exist as subunits of a single molecule in unstimulated platelets. Since platelet activation is required for fibrinogen binding, our results suggest that the membrane association of Ila and Ila is not sufficient for the expression of fibrinogen receptor activity. An additional conformational change must occur after platelet stimulation. Studies employing immunocytochemistry suggest that clusters of Ila-Ila molecules also form in the membrane after platelet activation with thrombin (20).

We now report the development of a new monoclonal antibody, named S12, that recognizes a membrane glycoprotein antigen that is exposed on the cell surface only after platelet activation. Our studies provide further insight into membrane glycoprotein rearrangements after platelet stimulation. A preliminary report of this work has been published (21).

EXPERIMENTAL PROCEDURES

Hindin, DFP, PMSF, prostaglandin E, Sepharose CL-2B, Sephadex G-20, cyanogen bromide-activated Sepharose 4B, Type I a agarose (M, ~ 9.0–10.15), Lubrol PX, ADP, and epinephrine were obtained from Sigma. Nonimmune mouse IgG, goat IgG directed against mouse IgG, affinity-purified rabbit IgG directed against mouse IgG, and rabbit antiserum against mouse (Fab'2) and Fc fragments were from N. L. Cappel Laboratories Inc., Cochranville, PA. IgG of protein A-containing Staphylococcus aureus was prepared from The Enzyme Center, Boston, MA. Purified human thrombin was a gift from Dr. J. W. Fenton, II, Albany, NY. [14C]Serotonin and Na121 were from Amerham Corp. Aquasol was from New England Nuclear. Enzymo-beads were from Bio-Rad.

The abbreviations used are: DFP, diisopropylphosphorofluoridate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethysulfonyl fluoride.

* This work was supported by a grant-in-aid from the American Heart Association with funds contributed in part by the American Heart Association, Texas Affiliate, and by Research Grant 113.81 from the North Atlantic Treaty Organization. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Platelets—Blood from aspirin-free normal volunteers was anticoagulated with 0.1 volume of 3.8% sodium citrate. Platelets were gel filtered in Tyrode's buffer (0.138 M NaCl, 0.029 M KCl, 0.012 M NaHCO3, 0.036 M Na2HPO4·H2O, pH 7.4) as described (12). Binding studies were performed within 1 h after gel filtration. For some binding experiments, platelet-rich plasma was stimulated with ADP or epinephrine, platelets were washed in the presence of apyrase and calcium (22), since we found platelets prepared in this fashion to be more sensitive to stimulation with ADP and epinephrine as measured by platelet aggregation studies. Platelets used for immunoprecipitation studies or affinity chromatography were prepared by differential centrifugation (25).

Monoclonal Antibody Production—Hybridomas producing antiplatelet antibodies were generated by fusion and screening procedures described previously in detail (12, 24). Monoclonal IgG from one clonal line, S12, was purified from mouse ascites by ammonium sulfate precipitation and DEAE-cellulose chromatography (24). Fab fragments were prepared from mouse IgG by papain digestion (25). The Fab fragments were separated from Fc fragments and undigested IgG by DEAE-cellulose chromatography (25). When analyzed by SDS-PAGE, the Fab fragments were free of (Fab')2 fragments and intact IgG. In immunodiffusion experiments the Fab fragments acted with antisera against mouse Fab fragments but not with antisera to mouse Fc fragments.

Protein iodination—Monoclonal IgG or Fab fragments were radio-labeled with Na125I using a commercial product, Enzymobeads. Radioiodinated protein was separated from free 125I by gel filtration on a Sephadex G-25 column. The specific activity of the iodination was 200–400 cpnmg, and greater than 98% of the radioactivity was precipitated by 8% trichloroacetic acid.

Platelet Secretion and Binding Assays—Gel-filtered platelets were used for cell-binding assays except where other isolation methods are specified. Direct binding of 125I-monoclonal IgG or Fab fragments was performed as described previously (12, 24) except that some platelet samples were also activated with thrombin, ADP, or epinephrine. Unless otherwise stated, the agonist was added to the reaction mixture last, after addition of radiolabeled antibody.

Platelet secretion was assessed by measuring the release of [14C]serotonin per ml of platelet-rich plasma for 15 min at 37°C. After gel filtration or washing, total platelet [14C]serotonin content was determined by adding 50 μl of the platelet reaction mixture to 4 ml of Aquasol, followed by liquid scintillation counting in a Beckman LS7000 counter. After centrifugation of the remaining platelet pellet, [14C]serotonin was measured by counting 50 μl of the supernatant in 4 ml of Aquasol. The per cent of secretion was taken as the ratio of 14C counts in the supernatant relative to total 14C counts in the platelet suspension. "Background" secretion averaged 10% in unstimulated gel-filtered platelets and 5% in washed platelets (22) and was subtracted. Maximum secretion with thrombin stimulation from one of these cloned lines, named S12, was in turn applied to the S12-Sepharose column. Both columns were washed sequentially with 20 ml of the following buffers: 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX; 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX; and 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX. Bound protein was eluted with 0.1 M glycine NaOH, pH 11.5, 0.5 mM PMSF, 0.1% Lubrol. The eluate was neutralized with 1 M Tris-HCl, pH 7.4, 0.1% Lubrol PX, and dialyzed at 4°C against 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5 mM PMSF, 0.1% Lubrol PX.

The isolated proteins were analyzed on 7–12% gradient polyacrylamide–SDS-containing slab gels by the method of Laemmli (26). Reduced samples contained 5% 2-mercaptoethanol.

Immunoprecipitation—Washed platelets were prepared from EDTA-anticoagulated blood and solubilized at 10 cells/ml in 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 1 mM EDTA. The platelets were divided into two 2-ml aliquots, one of which was incubated with 1 unit/ml of thrombin for 5 min, followed by 2 units/ml of hirudin for 5 min. Surface proteins of both control and thrombin-treated samples were then radioiodinated with Na125I as described by Norden et al. (27). After washing, total [125I]label in each sample was reduced to 0.1%. The flow-through fraction was in turn applied to the S12-Sepharose column. Both columns were washed sequentially with 20 ml of the following buffers: 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5 mM PMSF, 1 mM EDTA, 1% Lubrol PX (immunoprecipitation buffer) and centrifuged at 12,000 X g for 5 min in an Eppendorf microfuge. Then, 0.2-ml portions of the supernatants were incubated with 20 μg of either S12 or nonimmune mouse IgG for 1 h at room temperature, followed by a 1-h incubation with 80 μg of affinity-purified rabbit IgG directed against mouse IgG. The immune complex was precipitated by addition of 100 μl of 10% Ig GSor followed by a 15-min incubation at 37°C and centrifugation for 10 X g at 12,000 X g. The pellet was washed three times with 0.5 ml of immunoprecipitation buffer, and bound protein was dissociated by a 1-h incubation with 50 μl of 0.0625 M Tris-HCl, pH 6.8, 2% SDS. After centrifugation, dissociated proteins were fractionated by SDS-PAGE. The gels were stained with Coomassie Blue R and dried. Radioactive bands were visualized by exposing the dried gels to Kodak X-Omat AR film in a cassette containing a DuPont Cronex Lightning Plus intensifying screen.

The same immunoprecipitation experiment was also performed with radioiodinated platelets from a patient with Glanzmann's thrombasthenia, except that platelets were not treated with thrombin. The patient, J.K., has severe mucocutaneous bleeding, a prolonged bleeding time, and absent platelet aggregation in response to physiologic agonists. Blood from Dr. Marc Shuman in San Francisco, shipped by overnight express to the Medical Center Hospital Blood Bank, San Antonio, TX. The platelets were isolated by differential centrifugation (23) and resuspended in 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.4 mM PMSF at 20 X 10^8 platelets/ml, and stored at 70°C. Approximately 10 X 10^7 platelets were thawed, sonicated (four times at 48 watts for 15 s at 4°C), and centrifuged at 2,000 X g for 15 min. The pellet was discarded and the supernatant then centrifuged at 100,000 X g for 60 min at 4°C. The resultant pellet, representing a crude platelet membrane fraction, was solubilized in 30 ml of 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 2 mM DFP, 1% Lubrol PX, and centrifuged again at 100,000 X g for 60 min at 4°C. The supernatant was first applied to a 10-ml column of unactivated Sepharose 4B equilibrated in the above buffer except that the Lubrol PX concentration was reduced to 0.1%. The flow-through from this column, which contained <5% of the material initially nonspecifically bound to Sepharose, was then applied to the T10-Sepharose column equilibrated in the same buffer. The T10-Sepharose flow-through fraction was in turn applied to the S12-Sepharose column. Both columns were washed sequentially with 20 ml of the following buffers: 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX; 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX; and 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.1% Lubrol PX. Bound protein was eluted with 0.1 M glycine NaOH, pH 11.5, 0.5 mM PMSF, 0.1% Lubrol. The eluate was neutralized with 1 M Tris-HCl, pH 7.4, 0.1% Lubrol PX, and dialyzed at 4°C against 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5 mM PMSF, 0.1% Lubrol PX.

The isolated proteins were analyzed by 7–12% gradient polyacrylamide–SDS-containing slab gels by the method of Laemmli (26). Reduced samples contained 5% 2-mercaptoethanol.


doi:10.1074/jbc.261.1.9800

RESULTS

Binding of the Monoclonal Antibody S12 to Platelets—As described in previous reports (12, 24), we used a screening radioimmunobinding assay to select hybridoma cells producing IgG apparently specific for platelets. Monoclonal IgG from one of these cloned lines, named S12, was purified for further study. In preliminary experiments we found that S12 IgG did not inhibit the binding to platelets of either of our radioiodinated monoclonal antibodies, Tab and T10 (12, 24), directed against glycoprotein IIb-IIIa. We then prepared 125I-labeled S12 IgG and 125I-labeled monovalent S12 Fab fragments for use in direct platelet-binding assays. Time course studies indicated that binding of either S12 preparation was greater than 90% complete at 10 min and complete by 30 min (not
shown). Therefore, subsequent studies were performed using a 30-min incubation with radiolabeled ligand. As shown in Fig. 1, very few $^{125}$I-S12 Fab molecules bound to unstimulated platelets even at high ligand concentrations. In contrast, when platelets were activated with thrombin, a dramatic increase in S12 Fab binding was observed. Nonspecific binding was negligible because greater than 99.5% of bound $^{125}$I-labeled S12 Fab was displaced when a 100-fold molar excess of unlabeled S12 IgG or Fab fragments was added simultaneously. Binding was reversible and saturable. Scatchard analysis (Fig. 2) indicated that $^{125}$I-S12 Fab fragments bound with high affinity to a single class of binding sites on thrombin-stimulated platelets. In this experiment the dissociation constant for $^{125}$I-S12 Fab was 6.2 nM with 8900 binding sites/platelet (0.067 µg/10$^9$ platelets). After stimulation with 0.5 unit/ml of thrombin, platelets from six normal donors bound 10,500 ± 1,690 (S.D.) $^{125}$I-S12 Fab molecules/cell. Binding studies with intact S12 IgG produced similar results, except that in some experiments data points plotted by Scatchard analysis were scattered with no consistent pattern (not shown). Gel-filtered unstimulated platelets from 12 normal donors bound only 800 ± 470 $^{125}$I-S12 IgG molecules/platelet. After treatment with 0.5 unit/ml of thrombin, the same platelets bound 9,600 ± 2,600 S12 IgG molecules/platelet at saturation with a mean $K_d$ of 1.5 nM.

We then investigated in greater detail the relationship between thrombin activation of platelets and S12 binding. Preincubation of platelets with 5 mM EDTA had no effect on the level of S12 bound (not shown). Since platelets, even when unstirred, sometimes aggregated after thrombin addition, most binding assays were performed in the presence of 1 mM EDTA. Thrombin inactivated with DFP failed to mobilize S12-binding sites, suggesting that platelet activation, not thrombin binding per se, was required. To confirm that the effect of thrombin was on platelets rather than the antibody itself, we inactivated thrombin with hirudin, a specific inhibitor of the proteolytic action of thrombin, either before or after adding platelets to the reaction mixture. As shown in Table I, preincubation of $^{125}$I-S12 Fab fragments with thrombin followed by hirudin did not increase S12 binding over the control without added thrombin. In contrast, preincubation of platelets with thrombin followed by hirudin mobilized S12 binding sites similar to the reaction mixture containing no hirudin. Thus, thrombin exposed S12 binding sites by activating platelets rather than by a direct effect on S12 itself.

The relationship between the thrombin dose required to support platelet S12 binding and to induce [$^3$H] serotonin secretion is shown in Fig. 3. Increasing doses of thrombin stimulated [$^3$H] serotonin secretion and $^{125}$I-S12 Fab binding in a similar fashion, although S12 Fab binding tended to increase at slightly lower concentrations of thrombin than did serotonin secretion. Since secretion of $\alpha$-granule proteins may occur at lower thrombin concentrations than secretion of dense granule substances (30), it is possible that the degree of S12 binding in response to thrombin is more closely related to platelet activation.

**TABLE I**

| Reaction mixture | $^{125}$I-S12 Fab bound molecules/platelet |
|------------------|------------------------------------------|
| 1. S12 + thrombin (then hirudin, then platelets) | 426 |
| 2. Platelets + thrombin (then hirudin, then S12) | 6460 |
| 3. Control, activated platelets (platelets + S12 + thrombin) | 7299 |
| 4. Control, unactivated platelets (platelets + S12) | 509 |

The reaction mixtures as numbered in the table were as follows: 1) $^{125}$I-S12 Fab + thrombin → 10 min hirudin → 10 min platelets. Results are means of duplicate determinations. The range of the duplicates was within 5% of the mean.
to α-granule rather than dense granule i.e., [14C]serotonin release. However, we were unable to measure simultaneous release of α-granule proteins by radioimmunoassay because of the presence of 125I-labeled S12.

We also studied the ability of two other platelet agonists, ADP and epinephrine, to mobilize S12-binding sites. As shown in Table II, washed platelets stimulated with 20 μM epinephrine showed no change in S12 binding. Stimulation with 20 μM ADP produced only a minimal increase in binding sites. Neither agonist caused detectable [14C]serotonin secretion. Higher concentrations of ADP did not mobilize additional S12-binding sites.

Identification of the Platelet Antigen—Several approaches were used to characterize the platelet protein recognized by the S12 antibody. We first employed crossed immunoelectrophoresis, a technique previously used by us to identify the areas on glycoprotein IIb-IIIa to which the monoclonal antibodies Tab and T10 bind (12). Fig. 4A illustrates the Coomassie Blue-stained pattern obtained when solubilized platelets were electrophoresed against polyclonal rabbit anti-platelet antibodies. The predominant immunoprecipitate seen represents the glycoprotein IIb-IIIa complex (31, 32). In this experiment, 125I-S12 IgG was incorporated in the intermediate gel. The corresponding autoradiograph (Fig. 4B) demonstrates that 125I-S12 bound to a single immunoprecipitate distinct from glycoprotein IIb-IIIa. The appearance of the immunoprecipitate and the binding of 125I-S12 were identical when platelets were solubilized in the presence or absence of 5 mM EDTA (not shown).

We next isolated the protein to which S12 binds by affinity chromatography of Lubrol PX-solubilized platelet membranes on S12-agarose, as described under “Experimental Procedures.” Fig. 5 shows a Coomassie Blue-stained SDS-polyacrylamide gel of the bound protein eluted from the S12-agarose column. For comparison, glycoprotein IIb-IIIa purified on a T10 (12) affinity column was applied to adjacent lanes of the gel. The unreduced protein has an apparent molecular weight of 138,000. After disulfide bond reduction, the protein migrated more slowly with an apparent molecular weight of 148,000. A less prominent band with M, greater than 200,000, seen in the unreduced sample, is not present after disulfide reduction. This may represent disulfide-linked dimers of the protein and may be analogous to disulfide-related aggregates of glycoprotein IIb-IIIa in apparent association with actin, sometimes noted after protein purification (24). Other faintly staining bands of lower molecular weight, not seen in every preparation, probably represent minor contaminants or degradation products of the major polypeptide. The purified protein also stains prominently with periodic acid-Schiff (not shown). Its mobility on SDS gels relative to glycoprotein IIb-IIIa is identical to that previously described for glycoprotein IIa (33).

When intact platelets are subjected to lactoperoxidase-catalyzed 125I-labeling, glycoprotein IIa is among the surface proteins which are radioiodinated (27, 33). To see if the protein recognized by S12 can also be radioiodinated, we immunoprecipitated the protein from platelets that were first surface labeled and then solubilized in nonionic detergent. Fig. 6 demonstrates an autoradiograph of the radiolabeled platelet proteins and the immunoprecipitated material after electrophoresis on an SDS-polyacrylamide gel. All the major glycoproteins radiolabeled by the lactoperoxidase method (27, 33) are seen in the platelet sample. The protein immunoprecipitated by S12 is also radiolabeled, comigrates with glycoprotein IIa from whole platelets, and has the same apparent molecular weight as that seen in the intact sample. This suggests that the protein recognized by the S12 antibody is a single type IIa collagenase.
molecular weights, reduced and nonreduced, as the purified Coomassie Blue-stained material (Fig. 5). In a parallel experiment as described under "Experimental Procedures," platelets were first stimulated with thrombin in the presence of EDTA, then radiolabeled, solubilized, and subjected to immunoprecipitation. Both the radiolabeling pattern of intact platelets and the intensity of the immunoprecipitated radioactive band were similar to those of unstimulated platelets (not shown). The same radiolabeled protein was also immunoprecipitated from platelets from a patient with Glanzmann's thrombasthenia who had no detectable labeled IIb or IIIa (not shown).

**DISCUSSION**

In this study we have described a monoclonal antibody, S12, that binds minimally to unstimulated platelets but shows marked enhancement of binding after platelet activation with thrombin. We employed affinity chromatography to isolate the protein recognized by S12 and further characterized it by immunoprecipitation from radiolabeled platelets and by crossed immunoelectrophoresis. The isolated molecule fits the criteria established for platelet membrane glycoprotein IIa. 1) Its apparent molecular weight of 138,000 (unreduced) and 148,000 (reduced) and its mobility on SDS gels relative to glycoproteins Ia, Ib, and IIIa are identical to those described for IIa (27, 33). 2) The protein stains prominently with both Coomassie Blue and periodic acid-Schiff (33). 3) The protein is accessible to radiiodination on unstimulated platelets (27, 33). It is, of course, difficult to define a protein solely on the basis of structural properties. Unlike platelet glycoproteins Ib and IIb-IIIa (1, 34), glycoprotein IIa cannot yet be defined either by functional criteria or by its deficiency in a specific disorder. Moreover, we have not been able to immunoprecipitate all the radiolabeled material migrating at the IIa position (not shown). Thus we cannot rule out the possibility that more than one protein has the staining, labeling, and mobility characteristics of IIa. However, since no other criteria are available, we propose that the protein we have isolated with a specific monoclonal antibody be defined as glycoprotein IIa.

Gel-filtered platelets bind ~800 S12 molecules/cell, and platelets isolated by differential centrifugation bind ~1800 molecules/cell. When platelets are gel filtered in the presence of prostaglandin E₁, dibutyryl CAMP, and EDTA, S12 binding is sometimes reduced to less than 100 molecules/platelet (not shown). This suggests that the low level binding in "unstimulated" platelets may be due in part to partial activation during the isolation procedure.

When platelets are activated with thrombin, ~10,000 S12...
IgG or Fab molecules bind per cell. Given a 1:1 binding of antibody to protein, we estimate that there are ~10,000 molecules of IIa present on each platelet. Although substantial, this number of copies is less than the 40,000 IIb-IIIa (12, 24) or 25,000 IIb (35) molecules present per cell. We have also recovered less IIa than IIb-IIIa when both glycoproteins are isolated by sequential affinity chromatography from the same platelet preparation.

Platelet activation by thrombin is clearly required for the increased expression of S12 binding sites, since thrombin inactivated by hirudin or DFP had no effect on S12 binding. The thrombin-induced increase in S12 binding appeared to occur nearly concurrently with [3H]serotonin secretion. In contrast to thrombin, epinephrine had no effect on S12 binding and ADP produced only a modest increase in binding sites. Although neither ADP nor epinephrine caused detectable [3H]serotonin secretion, we cannot exclude the possibility that the slight increase in S12 binding seen with ADP was associated with α-granule secretion which was not measured in our assay. Since ADP and epinephrine may induce secretion by “aggregation-dependent” mechanisms (36), it is possible that additional S12-binding sites would be mobilized with these agonists in a stirred system containing Ca²⁺ and fibrinogen which would promote platelet aggregation. However, we have not been able to address this question because of the difficulties in performing binding assays with aggregated platelets. Thus, while S12 binding and secretion appear analogous to the Ca²⁺-dependent binding of released α-granule proteins such as thrombospondin, fibronectin, and von Willebrand factor (37-40).

The thrombin-induced molecular rearrangements responsible for increased platelet S12 binding remain to be defined. One possibility is that glycoprotein IIa might be a granule membrane component. During the release reaction, the granule membrane would fuse with the plasma membrane, where IIa would then be accessible to antibody binding. If this were the case, however, IIa should be radiolabeled more strongly after platelet activation with thrombin. We find no difference in labeling intensity. In addition, gray platelets, which are markedly deficient in α-granules and their packaged proteins, are in progress to localize the glycoprotein in resting and stimulated platelet preparation. Although gray platelets, which are noted no inhibition by S12 IgG of platelet aggregation induced by ADP or thrombin (not shown). Whatever the functional significance of IIa, the S12-binding studies provide additional support for rearrangements of membrane glycoproteins during platelet activation. Our data also suggest that S12 may serve as a useful marker of platelets activated in vivo in various pathological conditions. Studies are in progress to address this issue.

Acknowledgments—We thank Dr. Marc Shuman for providing blood from a patient with thrombasthenia, Dr. James N. George for many helpful discussions, and Judi Skinner for her preparation of the manuscript.

REFERENCES

1. George, J. N., Nurden, A. T., and Phillips, D. R. (1984) N. Engl. J. Med. in press
2. Fox, J. E. B., and Phillips, D. R. (1983) Semin Hematol. 20, 243-257
3. Tobelom, G., Levy-Toledano, S., Bredoux, R., Michel, M., Nurden, A., Caen, J. P., and Degos, L. (1976) Nature (Lond.) 263, 427-429
4. Saksenena, K. S., Bollis, P. A., and Siams, J. J. (1979) Nature (Lond.) 270, 636-638
5. Fawel, F., Granz, M. E., Legrand, Y. J., Souchon, H., Tobelom, G., Jackson, D. S., and Caen, J. P. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 551-554
6. Bennett, J. S., and Vilaire, G. (1979) J. Clin. Invest. 64, 1395-1401
7. McEver, R. P., Plow, E. F., and Elgintoglu, T. S. (1979) J. Biol. Chem. 254, 5307-5385
8. Hawiger, J., Parkinson, S., and fitschon, S. (1980) Nature (Lond.) 283, 197-199
9. Peerschke, E. I., Zucker, M. B., Grant, R. A., Egan, J. T., and Johnson, M. M. (1980) Blood 55, 841-847
10. Korencki, E., Newnawari, S., Morinelli, T. A., and Kloczewiak, M. (1981) J. Biol. Chem. 256, 5596-5601
11. George, J. N., and Phillips, D. R. (1980) Blood 55, 841-847
12. McEver, R. P., Bennett, E. M., and Martin, M. N. (1983) J. Biol. Chem. 258, 5269-5275
13. Fawel, F., Montgomery, R. B., Bennett, J. S., and Kunicki, T. J. (1983) J. Biol. Chem. 258, 12852-12856
14. Diminino, G., Thagorasen, P., Perussi, B., Martinez, J., Shapiro, S., Traynor, G., and Brown, S. (1985) Blood 61, 140-148
15. Celler, B. S., Peerschke, E. I., Scudder, L. E., and Sullivan, C. A. (1983) J. Clin. Invest. 72, 329-338
16. Nathan, R. M., and Leung, L. K. L. (1982) J. Clin. Invest. 69, 263-269
17. Bennett, J. S., Vilaire, G., and Cines, D. B. (1982) J. Biol. Chem. 257, 18544-18554
18. Lee, H., Nurden, A. T., Thomaidis, A., and Caen, J. P. (1981) Br. J. Haematol. 47, 47-52
19. Castel, G. O., Brostad, F., Kruzzes, M.-B., Hagen, I., and Solum, N. O. (1982) Blood 60, 662-671
20. Polley, M. J., Leung, L. K. L., Clark, F. Y., and Nathan, R. M. (1981) J. Biol. Chem. 256, 1058-1064
21. McEver, R. P., and Martin, M. N. (1985) Blood 66, Suppl. 1, 262a (abstr.)
22. Patscheke, H. (1981) Haemostasis 30, 14-27
23. Tollefsen, D. M., Feagler, J. R., and Majerus, P. W. (1974) J. Biol. Chem. 249, 2646-2651
24. Good, A. H., Wofley, L., Kimura, J., and Henry, C. (1980) In Selected Methods in Cellular Immunology (Matshel, B. H., and Shig, S. M., eds) pp. 284-286. W. H. Freeman, San Francisco, CA
25. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
26. Nurden, A. T., Dupuis, D., Kunicki, T. J., and Caen, J. P. (1981) J. Clin. Invest. 67, 1431-1440
27. Markwell, M. A., Haas, S. M., Tierke, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206-210
28. Tittle, I. R., and Donsahue, H. (1968) Methods Immunochemical. Ed, pp. 343-346 Academic Press, New York
29. Witte, L. D., Kasell, K. L., Nossal, H. L., Lages, B. A., Weiss, H. I., and Goodman, D. W. S. (1978) Circ. Res. 42, 402-409
30. Hagen, I., Nurden, A., Bjerrum, O. J., Solum, N. O., and Caen, J. (1980) J. Biol. Chem. 255, 722-733
31. Kunicki, T. J., Feagler, J. R., and Majerus, P. W. (1981) Blood 58, 268-278
32. Phillips, D. R., and Agin, P. P. (1977) J. Biol. Chem. 252, 2121-2126
33. McEver, R. P., and Majerus, P. W. (1983) In The Metabolic Basis of Inherited Disease (Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L., and Brown, M. S., eds) 5th Ed, pp. 1561-1572, McGraw-Hill, New York
34. Celler, B. S., Peerschke, E. I., Scudder, L. E., and Sullivan, C. A. (1983) Blood 66, 99-109
35. Charo, I. F., Feinman, R. D., and Detwiler, T. C. (1977) J. Clin. Invest. 60, 763-773
36. George, J. N., Lyona, R. M., and Morgan, R. K. (1980) J. Clin. Invest. 66, 1-10
37. George, J. N., and Onofre, A. R. (1982) Blood 59, 194-197
38. Ginsberg, M. H., Painter, R. G., Forsyth, J., Birdwell, C., and Plow, E. F. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1049-1058
39. Phillips, D. R., Jennings, L. K., and Prasanna, H. R. (1980) J. Biol. Chem. 255, 11629-11632
40. Nurden, A. T., Kunicki, T. J., Dupuis, D., Soria, C., and Caen, J. P. (1982) Blood 59, 708-718
41. Gros, J. M., Phillips, D. R., Rao, G. H. R., Plow, E. F., Watz, D. A., Ross, R. Harker, L. A., and Johnson, J. G. (1980) J. Clin. Invest. 66, 102-109
A monoclonal antibody to a membrane glycoprotein binds only to activated platelets.
R P McEver and M N Martin

J. Biol. Chem. 1984, 259:9799-9804.

Access the most updated version of this article at http://www.jbc.org/content/259/15/9799

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/15/9799.full.html#ref-list-1