Thrombasthenia With an Abnormal Platelet Membrane Glycoprotein IIb of Different Molecular Weight

By Stephanie M. Jung, Nobuhiko Yoshida, Nobuo Aoki, Kenjro Tanoue, Hiroh Yamazaki, and Masaaki Moroi

We describe an individual with abnormal platelet glycoprotein (GP) IIb of different molecular weight (mol wt), a defect that distinguishes this patient from previously reported thrombasthenics. The patient, a 21-year-old female, has a mild bleeding tendency; her platelets lack adenosine diphosphate (ADP) aggregation and have severely suppressed collagen aggregation but a normal response to ristocetin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of her platelets indicates that they contain two types of GPIIb molecules: one with an abnormal mol wt (122 kd, unreduced; 128 kd, reduced) and one with a normal mol wt (128 kd, unreduced; 118 kd, reduced). Relative to the amount of GPIIb in normal platelets, her platelets contain approximately 35% abnormal GPIIb and 20% normal GPIIb. Fibrinogen binding assays on the patient’s platelets indicated that they contained 25% of the normal amount of fibrinogen receptors. Crossed immunoelectrophoresis of the patient’s platelets demonstrated the formation of a GPIIb/Illa complex that was mainly composed of normal mol wt GPIIb and GPIIa. The patient’s father has decreased ADP aggregability, and his platelets also contained both abnormal and normal GPIIb (about 50% of the normal level and about 50% of the normal number of fibrinogen receptors); her mother has only normal GPIIb. These results indicate that the patient has heterozygous GPIIb molecules with an abnormality of GPIIb at the molecular level. Studies on this abnormal GPIIb should provide information about the function of GPIIb and the mechanism of its biosynthesis.

The major platelet membrane glycoproteins IIb and IIIa (GPIIb and GPIIIa) form calcium-dependent complexes that serve as fibrinogen receptors and are thereby requisite for aggregation.1 The abnormal glycoprotein content in thrombasthenic platelets was first reported by Nurden and Caen2 and later attributed to a deficiency in GPIIb and GPIIIa.3 Caen2 classified thrombasthenic individuals into two types according to the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of the platelet membrane glycoproteins: type I is characterized by the absence of platelet GPIIb and GPIIIa; type II platelets contain low levels of these glycoproteins.4 In addition to these patients with reduced amounts of GPIIb/IIIa, some reports have predicted the existence of thrombasthenic-like patients with normal amounts of GPIIb/IIIa, which suggests the presence of an abnormal GPIIb or GPIIIa.5-12 As yet, analyses have not been performed to determine how the structures of these abnormal GPIIb/IIIa molecules may differ from the normal ones. Here we report a patient with type II thrombasthenia according to the criterion of the absence of adenosine diphosphate (ADP) aggregation but normal clot retraction; this patient’s platelets contain a GPIIb of abnormal molecular weight (mol wt) in addition to normal—mol wt GPIIb; such a structurally different GPIIb molecule has not been reported to date in any thrombasthenic patient. We analyzed this abnormal molecule by several electrophoretic methods in conjunction with different staining techniques to verify that this molecule is indeed a GPIIb, and we have investigated the molecular basis of how this abnormal molecule differs from the normal one. MATERIALS AND METHODS Patient’s case history. The propositus (S.S.), a 21-year-old female, visited the outpatient department of Jichi Medical School Hospital to undergo a blood examination because of a bleeding tendency. The patient has bruised easily since childhood, had hypermenorrhea, and had experienced excessive bleeding after a tooth extraction when she was 19 years old. Coagulation studies showed a prolonged bleeding time (ten minutes as compared with three minutes for normal individuals), normal platelet count (21 x 10^5/μL), normal one-stage prothrombin time, and normal activated partial thromboplastin time. Although the whole blood clot retraction was normal (75%), the patient’s platelets showed no response to 10 μmol/L ADP. On the basis of these data, the patient was diagnosed as a type II thrombasthenic. For the delivery of her first child, the patient was transfused with 6 units of platelets to receive a cesarean section, which was chosen for better control of bleeding, and then transfused with 40 units of platelets during the six days after the operation. There was no intractable bleeding before or after the surgery. The propositus’s paternal great-grandmother and her maternal great-grandfather were siblings, but the family members have no history of thrombosis or abnormal bleeding. Her father underwent a laparotomy for a perforated duodenal ulcer and had tooth extractions without any signs of a bleeding tendency.

Platelet preparations. Whole blood was drawn from the cubital vein of the patient, the parents of the patient, and healthy volunteers into 0.1 vol of 3.8% sodium citrate. Platelet-rich plasma (PRP), prepared by centrifuging the whole blood at 700 g for five minutes, was used for the aggregation studies. For analyses of the platelet glycoproteins, the PRP was added with 0.1 vol of 0.5 mol/L EDTA solution, pH 7.4, and then sedimented by centrifugation at 900 g for ten minutes. The resultant platelet pellet was washed twice with a buffer consisting of 6.85 mmol/L citrate, 130 mmol/L NaCl, 4 mmol/L KCl, 5.5 mmol/L glucose, and 5 mmol/L EDTA (pH 6.5). The electrophoresis samples were prepared by dissolving the pellets in a solution of 2% SDS, 8 mol/L urea, and 10 mmol/L
N-ethylmaleimide (NEM) and then heating at 83°C for five minutes. Washed platelets were also fixed by paraformaldehyde according to the method of Plow and Marguerie14 and then used for binding studies.

Platelet aggregation, monitored by a Chronolog Lumiaggregometer (Chronolog Corp, Havertown, PA) at 37°C, was performed by stimulating aliquots of PRP (0.45 mL) with 50 μL of the following agonists (final concentrations in the aggregation mixture): 10 μmol/L ADP, 2 μg/mL collagen (from Hormon-Chemie, Munich), and 1.5 mg/mL ristocetin (from H. Lundbeck & Co, Copenhagen). The maximum aggregations of the patient’s platelets were compared with those of normal platelets.

Platelets were radioiodinated according to the method of Tuszyński et al15 with iodogen (Pierce Chemical Co, Rockford, IL). Washed platelets (4 x 10^8 cells) were reacted with 0.1 mCi Na^251 for 30 minutes in a 1.5-mL plastic tube that had been precoated with 100 μg of iodogen. Radiolabeled platelets were washed two times with Tyrode’s buffer without bovine serum albumin and then dissolved in 50 μL mL NEM. The dissolved platelets (10^11 cpm) were analyzed by two-dimensional, unreduced-reduced SDS-PAGE (described in the next section), and then the gel was dried and exposed to Fuji x-ray film (Fugi Photo Film Co, Ltd, Kanagawa, Japan) for about 1 week at –70°C.

Gel electrophoresis and related techniques. The Laemmli method16 was used for SDS-PAGE. Two-dimensional, unreduced-reduced gel electrophoresis was performed as described previously17,18: the unreduced sample was electrophoresed on a 5.5% Laemmli gel (first dimension) in a hematocrit tube (1.1 x 75 mm); this was followed by second-dimension electrophoresis on a 7.5% Laemmli minilab gel (8.6 x 7.0 x 0.1 cm) with a 0.8% agarose stacking gel layer containing 0.1 mOL dithiothreitol.

The proteins in the gels were stained by silver as described by Morrissey.19 After SDS-PAGE, the proteins were also electrophoretically transferred to nitrocellulose membranes (BA85, Schleicher and Schuell, Inc., Dassel, West Germany) and stained with antibodies to concanavalin A (Con A)-coupled horseradish peroxidase.17,18,20

The antiserum against platelet membranes were obtained from rabbits immunized with platelet membrane fractions isolated by the method of Barber and Jamieson.21 Monospecific antiserum against human platelet GPIIb was obtained by immunizing rabbits with GPIIb, which was purified from human platelets by the method of Carrell et al.22 PMI-1, a monoclonal antibody against GPIIb, has been described previously.22 Monoclonal antibodies against GPIIb (Tab)24 and the GPIIb/IIIa complex (10E5)25 were from Dr R.P. McEver, University of Texas Health Center, San Antonio, and Dr B.S. Collier, State University of New York, Stony Brook, respectively, and we would like to thank them for making them available for our binding studies.

Crossed immunoelectrophoresis was performed according to the method of Hagen et al.26 Washed platelets were dissolved in 38 mmol/L Tris, 0.1 mol/L Gly, 1% Triton X-100 (pH 8.6) at a concentration of 4 x 10^9 platelets/mL, and a 5-μL aliquot of each sample was used for the analyses. The second-dimension electrophoresis was run against gels containing anti-whole platelet protein antibody and anti-GP IIb/IIIa complex antibody. The latter antibody was made by injecting rabbits with washed agarose pieces that contained precipitin lines of GPIIb/IIIa. Compositions of the GPIIb/IIIa complex of the patient’s and normal platelets were analyzed by cutting out the precipitin lines of the complex in the agarose gels and then applying them to an SDS-PAGE gel after treatment with 1% SDS and 5% 2-mercaptoethanol.9

Quantitation of membrane glycoproteins. Different amounts of whole platelet proteins from the patient, her father, her mother, and a normal individual (control) were analyzed by SDS-PAGE, electrophoretically transferred to nitrocellulose sheets, and stained with anti–platelet membrane antibody. The stained glycoprotein bands of the control and samples that were run on the same gel were quantitated by densitometry, and the area under each peak (arbitrary units of area) of the control was plotted as a function of the protein applied to establish a standard curve for each protein. For each amount of applied sample, the amounts of GPIb, GPIIb (abnormal and normal), GPIIIa, and myosin contained in the platelets of the patient and her parents were calculated from the peak area of each of these proteins in these individuals by using the standard curve (normal individual) established for the respective protein and expressed as a percentage of the control. The values given in Table 2 are values of the mean ± SD obtained for each protein from three different amounts of applied sample.

Binding studies. The association of ^251-fibrinogen with fixed platelets was analyzed as previously described.17 Platelets (0.3 x 10^8 cells/mL) were stimulated with ADP (12.5 μmol/L) in the presence of ^251-fibrinogen (5 x 10^-8 to 2 x 10^-8 mol/L) and 1 mmol/L CaCl2. After 30 minutes’ incubation at room temperature, three 50-μL aliquots of the binding mixture were taken, and the platelets in each were separated by centrifugation through a 20% sucrose layer. The binding parameters were calculated by Scatchard analysis. The bindings of the monoclonal antibodies 10E5 and Tab were measured by using the respective ^251-antibodies at their saturating concentration (5 x 10^-4 mol/L) under the same conditions as used for fibrinogen binding. The nonspecific binding was obtained by binding labeled ligands in the presence of an excess amount of cold fibrinogen (1.5 x 10^-3 mol/L) or cold antibodies (2.5 x 10^-4 mol/L). The extents of antibody binding were calculated as percentages of the binding in normal platelets.

RESULTS

Aggregation studies. The patient’s PRP showed normal platelet aggregation in response to ristocetin, a very weak (less than 5% of normal) aggregation in response to collagen (2 μg/mL), and no aggregation in response to ADP (10 μg/mL) (Fig 1). ADP aggregation patterns of the patient’s father indicated that his platelets have a reduced response to this agonist, and at 10 μmol/L, his platelets form aggregates.
that dissociate during the late stage of aggregation (Fig 1).
The patient's mother had normal aggregation patterns in
response to all three agonists. These data suggest that the
patient's platelet abnormality is inherited from her father.

**Binding studies.** The level of GPIIb/IIIa complex expression on the patient's platelets was estimated by quanti-
tating the binding of fibrinogen and antibody against the
GPIIb/IIIa complex to her platelets. The fibrinogen binding
data given in Table 1 indicate that the number of fibrinogen
receptors was decreased to 25% of the control in the patient's
platelets and to 47% of the control in those of her father. The
patient and her father show normal binding affinities for
fibrinogen. The expression of antigenicities against the
GPIIb/IIIa complex and GPIIb on the patient's platelets
was measured from the extent of binding of the monoclonal
antibodies 10E5 and Tab, respectively, at their saturating
concentrations in comparison to the extent of binding in
normal platelets. The patient's platelets bound Tab at 32.2%
of the normal level and bound 10E5 at 36.0% of the normal
level. The bindings of the antibodies are consistent with the
view that the ability of the patient's platelets to bind fibrino-
gen is attributable to receptors composed of a complex of
GPIIIa and the normal GPIIb.

**Electrophoretic analysis.** Immunostaining by polyclonal
anti-GPIIb antibody, produced by immunizing rabbits
with purified GPIIb, indicates that the patient and her father
have qualitatively the same type of glycoprotein pattern,
with a quantitative difference in the amount of the normal
GPIIb band. The most striking characteristic of their patterns
is that both of these individuals show an extra band at a
position near the normal GPIIb band (the abnormal band is
indicated as GPIIb* in Fig 2). Relative to normal GPIIb, this
abnormal protein has a lower mol wt (122 kD against 129 kD
for normal GPIIb) in nonreduced conditions and shows a
higher mol wt (128 kD against 118 kD for normal GPIIb)
after the reduction of disulfide bonds. With this SDS-PAGE
immunostaining system, other thrombasthenic samples
(types I and II) show the absence or presence of a reduced
amount of normal GPIIb, but none contained a GPIIb band
of abnormal mol wt (data not shown). Immunostaining with
PMI-1, a monoclonal antibody against GPIIb, indicated that
the abnormal GPIIb does not have an epitope that reacts
with this antibody (data not shown).

The abnormal GPIIb also appears as a distinct spot in
nonreduced-reduced, two-dimensional SDS-PAGE (Fig 3).
In Fig 3, the spot corresponding to each glycoprotein was
identified by its mol wt, by its behavior after the reduction
of disulfide bonds (ie, whether it migrates above or below the
diagonal of proteins), and according to our previous studies
in which the characteristic position of each glycoprotein spot
in this system was established by lectin and antibody staining
patterns, surface labeling patterns, and the absence of a
specific spot in patterns of platelets from patients with known
glycoprotein defects (ie, types I and II thrombasthenics and
patients with Bernard-Soulier syndrome). When two-
dimensional SDS-PAGE was performed by using a 12.5%
gel as the second-dimension gel, the light chain of the normal
GPIIb separated as a discrete spot, but the patient's platelets
showed no extra spot. Even when two times as much patient's
sample was analyzed by SDS-PAGE, only a faint spot
corresponding to the β subunit of the normal GPIIb was
found, with no other spots near it, thereby suggesting that the
abnormal GPIIb is composed of a single polypeptide chain
(Fig 4).

The abilities of normal and abnormal GPIIb to interact
with the lectin Con A were also determined by staining
nitrocellulose blots of SDS-PAGE-separated proteins with
horseradish peroxidase-conjugated Con A. Con A reacted
equally with the normal and abnormal GPIIb, which sug-
gested that the carbohydrate moieties of these two proteins
are similar (data not shown).

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**Table 1. Binding of Fibrinogen to the Platelets From the Patient, Her Parents, and Normal Individuals**

<table>
<thead>
<tr>
<th>Number of Binding Sites per Platelet x 10⁴</th>
<th>kd x 10⁷ (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0.87 (1.15)</td>
</tr>
<tr>
<td></td>
<td>1.42</td>
</tr>
<tr>
<td>Father</td>
<td>3.04 (2.35)</td>
</tr>
<tr>
<td></td>
<td>1.65</td>
</tr>
<tr>
<td>Mother</td>
<td>5.63 (5.03)</td>
</tr>
<tr>
<td></td>
<td>4.43</td>
</tr>
<tr>
<td>Controls</td>
<td>4.58 (4.61 ± 1.39)</td>
</tr>
<tr>
<td></td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>4.98</td>
</tr>
<tr>
<td></td>
<td>2.65</td>
</tr>
<tr>
<td></td>
<td>5.85</td>
</tr>
</tbody>
</table>
Fig 3. Two-dimensional, nonreduced/reduced SDS-PAGE patterns of the patient and a normal individual. Three-microgram samples of solubilized whole platelets were analyzed by two-dimensional, nonreduced/reduced SDS-PAGE (first dimension, 5.5% acrylamide; second dimension, 7.5% acrylamide), electrophoretically transferred to nitrocellulose sheets, and then stained by anti-platelet membrane antibody. The abnormal GPllb (indicated by llb*) of the patient appears as a distinct spot that is not found in the pattern of the control platelets.

Quantitation of abnormal and normal GPIIb. To estimate the amount of abnormal GPIIb in the patient's platelets, GPIIb bands stained by anti-platelet membrane antibody were quantitated by densitometry as detailed in Materials and Methods (see Fig 5A through D, which shows the typical densitometric patterns obtained for a normal individual, the patient's mother, the patient's father, and the patient, respectively). The results (Table 2) indicate that the patient and her father have similar amounts of the abnormal GPIIb, and although they both have decreased levels of normal GPIIb, the patient shows a more marked deficiency. In addition, both the patient and her father show a suppressed level of GPIIIa, which may be a further abnormality present in these individuals. Both the patient and her father have more GPIIIa than normal GPIIb. Because there are a number of factors that may interfere with the quantitation of stained bands of proteins transferred to nitrocellulose sheets and because the separation of the normal and abnormal GPIIb is incomplete, the estimated value for the content of GPIIb would only be semiquantitative. However, because the estimations of the GPIb and myosin contents in the patient, her parents, and the control fall within the range of variations usually observed among individuals and because the values of the SD are 15% or less, our determinations of GPIIb should be a reasonable estimate of the actual contents.

Surface labeling of platelets. Normal and the patient's platelets were surface labeled with 125I by the lodogen method and analyzed by unreduced-reduced, two-dimensional SDS-PAGE. Autoradiograms of these gels (Fig 6)
albumin, and factor XIII but contained in the patient's platelets was calculated against antibody by crossed immunoelectrophoresis using rabbit antibody analyzed the patient and the control. The precipitin arcs of GPIIb/IIIa were dissolved in a buffer containing 1% Triton X-100 and a reduced amount of GPIIb/IIIa complex, and a normal level of clot retraction; however, her platelets contain almost the normal amount of fibrinogen and 20% to 30% of the GPIIb/IIIa complex present in normal individuals, which suggests that she has a milder deficiency than the so-far reported cases of type II thrombasthenia. Because the patient’s father has 50% of the normal level of GPIIb in addition to abnormal GPIIb and her mother has the normal amount of GPIIb, the patient would be classified as a heterozygote; however, her platelets showed a further decrease to 20% to 30% of the normal level of GPIIb, the cause of which is not known at present. Defining the primary defect of a genetic disorder such as type II thrombasthenia is difficult, and how type II thrombasthenia is inherited remains to be fully analyzed. Because our patient is the offspring of a consanguineous marriage, there is the possibility that the patient has inherited another defect that may have reduced the expression of normal GPIIb. By crossed immunoelectrophoresis, the amounts of GPIIb/IIIa complex in the platelets of two previously reported cases of type II thrombasthenia were determined to be 13% and 15% of the normal level. Although there may be some experimental differences in the GPIIb measurements performed by different laboratories, our data showing that the patient’s GPIIb/IIIa content is 20% to 30% of the normal level suggest that platelet aggregation requires the presence of at least 20% of the normal amounts of these glycoproteins. Her father has 56% of the normal GPIIb content and showed a slightly reduced aggregability, but he has no bleeding tendency; these observations are consistent with the previously reported data on heterozygotes of type I thrombasthenia.

The unique characteristic of the platelets from this patient and her father is that they contain a GPIIb of abnormal mol wt in addition to the normal GPIIb. The SDS-PAGE patterns indicate that the abnormal band has an apparently lower mol wt than normal GPIIb under nonreducing conditions, whereas the situation is reversed when the samples are reduced. Without knowledge about the sequence of these proteins, it is difficult to speculate on the exact reason for this.

**DISCUSSION**

The patient was classified as a type II thrombasthenic because she has a mild bleeding tendency, no platelet aggregation with 10 μmol/L ADP, a reduced amount of GPIIb/IIIa complex, and a normal level of clot retraction; however, her platelets contain almost the normal amount of fibrinogen and 20% to 30% of the GPIIb/IIIa complex present in normal individuals, which suggests that she has a milder deficiency than the so-far reported cases of type II thrombasthenia. Because the patient’s father has 50% of the normal level of GPIIb in addition to abnormal GPIIb and her mother has the normal amount of GPIIb, the patient would be classified as a heterozygote; however, her platelets showed a further decrease to 20% to 30% of the normal level of GPIIb, the cause of which is not known at present. Defining the primary defect of a genetic disorder such as type II thrombasthenia is difficult, and how type II thrombasthenia is inherited remains to be fully analyzed. Because our patient is the offspring of a consanguineous marriage, there is the possibility that the patient has inherited another defect that may have reduced the expression of normal GPIIb. By crossed immunoelectrophoresis, the amounts of GPIIb/IIIa complex in the platelets of two previously reported cases of type II thrombasthenia were determined to be 13% and 15% of the normal level. Although there may be some experimental differences in the GPIIb measurements performed by different laboratories, our data showing that the patient’s GPIIb/IIIa content is 20% to 30% of the normal level suggest that platelet aggregation requires the presence of at least 20% of the normal amounts of these glycoproteins. Her father has 56% of the normal GPIIb content and showed a slightly reduced aggregability, but he has no bleeding tendency; these observations are consistent with the previously reported data on heterozygotes of type I thrombasthenia.

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**Table 2. Quantitation of Platelet Proteins**

<table>
<thead>
<tr>
<th></th>
<th>Myosin (%)</th>
<th>GPIb (%)</th>
<th>GPIIb Normal (%)</th>
<th>GPIIb Abnormal (%)</th>
<th>GPIIIa (%)</th>
</tr>
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<tbody>
<tr>
<td>Mother</td>
<td>119 ± 15.4</td>
<td>89 ± 13.0</td>
<td>89 ± 14.1</td>
<td>0</td>
<td>104 ± 11.3</td>
</tr>
<tr>
<td>Father</td>
<td>123 ± 13.0</td>
<td>92 ± 8.1</td>
<td>56 ± 4.1</td>
<td>31 ± 5.8</td>
<td>78 ± 8.6</td>
</tr>
<tr>
<td>Patient</td>
<td>104 ± 11.2</td>
<td>106 ± 10.3</td>
<td>19 ± 1.7</td>
<td>34 ± 3.6</td>
<td>43 ± 5.0</td>
</tr>
</tbody>
</table>
unusual electrophoretic behavior; however, the two-dimensional, unreduced-reduced SDS patterns of the patient's platelets suggest some reasons for this behavior. In these patterns, the abnormal GPIIb migrates at a position slightly higher than the diagonal line of proteins, which suggests that this protein contains intrachain disulfide bonding. Furthermore, the abnormal molecule is apparently composed of a single polypeptide chain because no corresponding β subunit was observed in two-dimensional SDS-PAGE when 12.5% gels were used for the second-dimension (reduced) gel, whereas the normal GPIIb yielded a β subunit upon disulfide reduction.

The abnormal protein was verified to be GPIIb by the results of the staining experiments with monospecific polyclonal antibodies against GPIIb and the staining patterns obtained when using Con A, which is known to react with normal-mol wt GPIIb. PMI-1, a monoclonal antibody against GPIIb, did not, however, react with the abnormal protein. The lack of a reaction may be because PMI-1 recognizes a different epitope in the GPIIb molecule. Since the mol wt of the abnormal GPIIb is 1 to 2 kD different from that of normal GPIIb, the structure of this abnormal GPIIb may be sufficiently different so that it no longer contains the specific epitope recognizable by PMI-1. Crossed immunoelectrophoresis indicates that the GPIIb/IIIa complex in the patient's platelets is formed from normal GPIIb and GPIIIa. The platelets of the patient and her father contain GPIIIa at an excess, nonstoichiometric level relative to normal GPIIb, thereby suggesting that at least some part of the GPIIIa may be present as a noncomplexed form in their platelets. Furthermore, the data indicate that both the father and daughter have lower-than-normal levels of GPIIIa, the daughter having a 50% depressed level. Nurden et al.29 has reported the presence of nonequal amounts of GPIIb and GPIIIa in some thrombasthenic platelets, and most of the type I thrombasthenic patients showed the presence of significantly more GPIIIa than GPIIb. Presently, we do not know the reason why the patient's platelets contain more GPIIIa than GPIIb. Because GPIIb and GPIIIa are synthesized from different mRNAs30 and because most of these proteins are present as a complex in the normal platelets, it would be reasonable to assume that there is some mechanism that regulates the synthesis of each polypeptide; the presence of an abnormal GPIIb would affect this regulation by disturbing the balance of synthesis or degradation of these glycoproteins so that they might be present in unequal amounts. In our patient, the complex of normal GPIIb and GPIIIa is expressed at the cell surface as in normal platelets because fibrinogen binding, antibody (10E5) binding, and an assay of the amount of complex formation all gave similar values; however, the distributions of the abnormal GPIIb and the excess of GPIIIa in the platelets are still not known. The 125I-labeling experiment indicated that little or no radioactivity was incorporated into the abnormal GPIIb, whereas the normal GPIIb of the patient showed a considerable level of labeling if its lower amount relative to that of the abnormal GPIIb is taken into consideration. This suggests that the abnormal GPIIb is not exposed or exposed to a much lower extent to the cell surface.

Some reports have suggested the existence of dysfunctional platelet GPIIb/IIIa.31,12,33 The platelets of these
patients were all reported to have normal or subnormal amounts of GPIIb and GPIIIa but show platelet dysfunction similar to that of thrombasthenia: no ADP aggregation, no fibrinogen binding to platelets, no fibrinogen content, and no clot retraction. Two families could be classified as a subgroup of thrombasthenia not fitting types I and II. The platelets from these patients showed a normal level\(^\text{11}\) or more than 50\% of the normal level\(^\text{12}\) of the GPIIb/IIIa complex. The abnormality in one of the patients was suggested to be a deficiency in calcium-dependent exposure of the fibrinogen binding site for GPIIb/IIIa.\(^\text{12}\) The platelets of another of these patients showed the formation of a complex too unstable to support fibrinogen binding.\(^\text{11}\) One of the patients studied by Ginsberg et al\(^\text{12}\) was suggested to be homozygous because the binding of fibrinogen to his parents' platelets was reduced to about two thirds of the normal level. The abnormality in another patient\(^\text{11}\) was described as being sporadic, so it is not clear exactly what type of genetic abnormality the patient has. In our case, the patient is heterozygous and clearly has an abnormality of GPIIb at the molecular level as ascertained by SDS-PAGE, which clearly differentiates our case from previously reported ones. The abnormal GPIIb in our case has a higher mol wt than the \(\alpha\) chain of normal GPIIb, and no \(\beta\) chain was detected in this abnormal GPIIb. These data strongly suggest that the abnormality in this patient can be attributed to a defect in the intracellular processing of GPIIb that forms the mature two-chain GPIIb molecules. Recently, such a single-chain GPIIb was indicated as a precursor to two-chain GPIIb from a study using a cell-free synthetic system from HEL cells.\(^\text{20}\) Further studies on the structural abnormalities of this GPIIb of different mol wt will provide us with useful information about the function of GPIIb and the mechanism for the biosynthesis of this glycoprotein.

acknowledgment

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references

24. McEver RP, Bennet EM, Martin MN: Identification of the structurally and functionally distinct sites on human platelet mem-


29. Nurden AT, Didry D, Kieffer N, McEver RP: Residual amounts of glycoproteins IIb and IIa may be present in the platelets of most patients with Glanzmann's thrombasthenia. Blood 65:1021, 1985


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