Fibrinogen-Binding Properties of the Human Platelet Glycoprotein IIb–IIIa Complex: A Study Using Crossed-Radioimmunoelectrophoresis

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Fibrinogen-binding platelet proteins have been examined by crossed-immunoelectrophoresis of solubilized, washed platelets followed by the incubation of the immunoplates with $^{125}$I-fibrinogen and exposure to $x$-ray films. Incubation with 0.1 mg/ml of $^{125}$I-fibrinogen revealed the binding of fibrinogen to the immunoplates, representing the glycoprotein IIb–IIIa complex, factor XIIIa chain, a granule membrane protein termed G4, fibrinogen, and albumin. Only the glycoprotein IIb–IIIa precipitate and the fibrinogen precipitate showed significant binding when the concentration of $^{125}$I-fibrinogen was lowered to 0.01 mg/ml. The binding to all the immunoplates was completely abolished in the presence of a 20-fold excess of unlabeled fibrinogen, but not when unlabeled fibrinogen was replaced by the same amounts of bovine serum albumin or human hemoglobin. This indicates that the binding of fibrinogen is specific. Furthermore, $^{125}$I-fibronecin and $^{125}$I-thrombin did not bind to the glycoprotein IIb–IIIa precipitate. The binding of $^{125}$I-fibrinogen to the precipitates representing the glycoprotein IIb–IIIa complex, the factor XIIIa chain, and G4, but not to the albumin precipitate, was significantly lowered in the presence of EDTA. This effect of EDTA increased with increasing pH, with no binding at pH 8.7. Immunoprecipitates of dissociated glycoprotein IIb and IIIa did not bind $^{125}$I-fibrinogen. Binding of $^{125}$I-fibrinogen to the glycoprotein IIb–IIIa precipitate was dependent on the presence of either Ca$^{2+}$ or Mg$^{2+}$. The binding to the factor XIIIa chain required Ca$^{2+}$, and the binding to G4 required Mg$^{2+}$. The results indicate that the glycoprotein IIb–IIIa complex, but not the separate glycoproteins IIb and IIIa, can act as Ca$^{2+}$ or Mg$^{2+}$-dependent fibrinogen receptor, under proper physiologic conditions.

PLATELET AGGREGATION is a central event in hemostasis. ADP-induced platelet aggregation was early reported to be dependent on fibrinogen. Recently, receptor-kinetic studies have revealed that ADP induces fibrinogen receptors on the platelet surface and that the binding of fibrinogen to the platelet surface correlates with platelet aggregability. The identification of the fibrinogen receptor, the elucidation of the mechanism of receptor induction, and the molecular relationship between fibrinogen binding and platelet aggregation may therefore be central to the understanding of platelet behavior. The molecular arrangements that binds platelets together is still unknown, but some studies indicate that the dimeric fibrinogen molecule may form a bridge between fibrinogen receptors on adjacent platelets.

More observations link the major membrane glycoproteins IIb and IIIa to the binding of fibrinogen to the platelet surface. In the hereditary disease, Glanzmann's thrombasthenia, the platelets exhibit a severely decreased fibrinogen-binding capacity, and they are not aggregated by ADP. The main molecular defects in this disease are the lack of glycoprotein IIb and IIIa and α-granule-located fibrinogen. Antibodies specific to glycoprotein IIb/IIIa inhibit platelet fibrinogen binding as well as platelet aggregation. Recently, Nachman and Leung have reported that a mixture of the glycoproteins IIb and IIIa isolated by lectin affinity chromatography formed complexes with fibrinogen, indicating that these glycoproteins together constitute a fibrinogen-binding entity. Using crossed-immunoelectrophoresis, Hagen et al. showed that glycoproteins IIb and IIIa were present as a complex after solubilization of platelets by Triton X-100. This has been confirmed by Kunicki et al.

The glycoprotein IIb–IIIa complex seems to be kept together by divalent cations. Polley et al. suggested from ultrastructural studies that thrombin stimulation initiates the specific formation of the glycoprotein IIb–IIIa macromolecular complex.

In the present study, we demonstrate that the glycoprotein IIb–IIIa complex, but not the separated glycoproteins IIb and IIIa, binds fibrinogen in the presence of Ca$^{2+}$ and Mg$^{2+}$. Furthermore, this glycoprotein complex appears to be the only surface-located, fibrinogen-binding entity in platelets that are detectable in our system and therefore probably represent the fibrinogen receptor involved in platelet aggregation.

MATERIALS AND METHODS

Agarose, type HSA, was from Litex, Glostrup, Denmark. Bovine serum albumin, human hemoglobin, ADP, apyrase, and Triton X-100 was from Sigma Chemical Company, St. Louis, Mo. Rabbit antibodies to whole human platelets were prepared as described by Hagen et al. Platelets were isolated as described elsewhere.
Platelet membranes were isolated as described previously. The membranes were washed with 0.27 M sucrose prior to use.

Crossed immunoelectrophoresis was performed as described by Hagen et al. using membranes or platelets solubilized in 0.038 M Tris-0.1 M glycine buffer (pH 8.7) containing Triton X-100. The antibodies used were against whole platelets.

Dissociation of the glycoprotein IIb-IIIa complex was performed by solubilization of platelet membranes in 0.038 M Tris-0.1 M glycine buffer (pH 8.7) containing 1% Triton X-100 and 2 M EDTA.

125I-labeled human fibrinogen was prepared as follows. Fibrinogen (“Kabi,” Stockholm, Sweden) was dialyzed against 0.05 M Tris-HCl buffer (pH 7.4) at 4°C and chromatographed on gelatin-Sepharose 4B in order to remove contaminating fibrinogen and fibrolyzed, carboxymethylated subunit chains demonstrated normal chains apart from a slight heterogeneity in the Aα-chains. Clotting ability was 89%. 125I-Fibrinogen was stored in plastic vials in aliquots of 0.5 ml (10 mg/ml) at -70°C until used.

Crossed-radiimmunoelectrophoresis was performed according to the technique introduced by Grimme et al. for thrombin. After crossed-immunoelectrophoresis, the agarose gels were washed 3 times by pressing and swelling in 0.154 M NaCl to remove nonprecipitated proteins and buffer ions. The agarose was finally dried and exposed on Kodak X-Omat x-ray films for 3-14 days.

For examination of cation-specificity in binding of fibrinogen to immunoprecipitates, the immunoplates were incubated with 20 mM Tris-HCl buffer (pH 7.4)-0.154 M NaCl-20 mM Tris-HCl buffer (pH 7.4) containing 125I-fibrinogen at 0.01-0.5 mg/ml. The optimal conditions for fibrinogen binding were in the presence of 1 mM Ca2+ and 1 mM Mg2+. In some experiments, 2 mM EDTA was added. The immunoplates were incubated for 18 hr at 20°C followed by removal of excess 125I-fibrinogen by washing in 0.154 M NaCl for 6 hr with 3 shifts of solution (3 x 200 ml/immunoplate). The washed immunoplates were finally dried and exposed on Kodak X-Omat x-ray films for 3-14 days.

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RESULTS

Fibrinogen-Binding Platelet Proteins

Fibrinogen-binding platelet proteins were examined by crossed-radiimmunoelectrophoresis using a medium of physiologic ionic strength and pH 7.4 containing Ca2+ and Mg2+ in addition to radiolabeled fibrinogen. Variations in pH between 7 and 9 did not significantly alter the results described below. Using 0.1 mg/ml of 125I-fibrinogen in the incubation media, 5 immunoprecipitates were labeled (Fig. 1). No additional fibrinogen-binding precipitates could be seen when the concentration of 125I-fibrinogen was increased to 0.5 mg/ml. As concluded from previous identifications of the antigens corresponding to the various immunoprecipitates, those labeled with 125I-fibrinogen represented fibrinogen, the glycoprotein IIb-IIIa complex, an α-granule protein termed G4, factor XIIIa chain, and albumin. (Fig. 1, A and B). The strong binding to the fibrinogen immunoprecipitate was probably due to unoccupied binding sites on the fibrinogen antibodies or exchange of precipitated fibrinogen. When the concentration of the 125I-fibrinogen in the incubation medium was lowered to 0.01 mg/ml, only the precipitates representing fibrinogen and the glycoprotein IIb-IIIa complex were significantly labeled (Fig. 1C). Dilution of the 125I-fibrinogen with unlabeled fibrinogen above 2 mg/ml, i.e., a 20-fold excess of unlabeled fibrinogen, completely abolished the labeling of all the immunoprecipitates mentioned. When unlabeled fibrinogen was replaced by the same amounts of bovine serum albumin or human hemoglobin, no similar decrease in the binding of 125I-fibrinogen to the immunoprecipitates was seen, except for the binding to the albumin precipitate (data not shown). This strongly indicates that the binding of fibrinogen to the glycoprotein IIb-IIIa complex, the factor XIIIa chain and G4 represent specific binding phenomena and also indicate that the binding to the albumin precipitate represents a general tendency of this protein to bind to other proteins. Similar studies using 125I-fibronectin and 125I-thrombin (unpublished results) show that none of these proteins bind specifically to the glycoprotein IIb-IIIa precipitate. This provides further evidence for the specificity of the binding of fibrinogen to the glycoprotein IIb-IIIa complex. This binding was not altered by preincubation of the immunoplates with ADP or asparagine, nor by the inclusion of either of these substances in the incubation medium.

Dependence of Complex Formation Between
Glycoprotein IIb and IIIa

In order to dissociate the glycoprotein IIb–IIIa complex, washed platelet membranes were solubilized in Tris-glycine buffer (pH 8.7) containing 1% Triton X-100 and 2 mM EDTA. Crossed-immunoelectrophoresis showed that dissociation had occurred by the disappearance of the glycoprotein IIb–IIIa precipitate and the concomitant appearance of two new immunoprecipitates representing glycoprotein IIb and glycoprotein IIIa, respectively (Fig. 2). Immunoplates obtained in parallel to those shown in Fig. 2 were
Fig. 1. Crossed-immunoelectrophoresis of solubilized platelets against antibodies to whole platelets followed by incubation of the immunoplates with $^{125}$I-fibrinogen. (A) Stained immunoplate (B) Autoradiography of immunoplate incubated with 0.1 mg $^{125}$I-fibrinogen/ml (C) Autoradiography of immunoplate incubated with 0.01 mg $^{125}$I-fibrinogen/ml. The platelets were solubilized in 0.038 M Tris·0.1 M glycine buffer (pH 8.7) containing 1% Triton X-100 and aliquots of about 60 μg protein were applied to each agarose gel. Electrophoresis in the first dimension was performed at 10 V/cm for 1 hr. An antibody-free intermediate gel was inserted between the first-dimension gel and the second-dimension gel containing antibodies at 400–600 μg protein/ml. The second-dimension electrophoresis was performed overnight at 2 V/cm. Electrode buffers contained Tris·glycine buffer (pH 8.7) and all agarose gels contained the same buffer and 0.5% Triton X-100. After electrophoresis, the gels were pressed and swelled in 0.154 M NaCl 3 times and finally dried. The immunoplates were either stained with Coomassie Brilliant Blue or incubated with $^{125}$I-fibrinogen in 10 ml Tris-HCl buffer (pH 7.4)–0.154 M NaCl-1 mM CaCl$_2$-1 mM MgCl$_2$ for 18 hr at 20°C. After incubation, excess of $^{125}$I-fibrinogen was removed by washing in 0.154 M NaCl. The immunoplates were finally dried and exposed to x-ray films for (B) 3 days or (C) 5 days. Note the confluence between the precipitate lines formed with the glyco-protein IIb–IIIa complex and fibrinogen in A. This represents an occasionally appearing phenomena. The arrow in C indicates that a similar confluence also is present in the autoradiograms.
Fig. 2. Crossed-immunoelectrophoresis of isolated platelet membranes solubilized at pH 8.7 in the presence of EDTA in order to dissociate the glycoprotein IIb-IIIa complex. The membranes were suspended in 0.038 M Tris-0.1 M glycine buffer (pH 8.7)-2 mM EDTA to about 0.5 mg protein/ml and solubilized by the addition of 1% Triton X-100. A control without EDTA showed an immunoprecipitate representing the intact glycoprotein IIb-IIIa complex. Other experimental conditions as in Fig. 1.

incubated with $^{125}$I-fibrinogen under optimal conditions for binding, but none of the two immunoprecipitates representing the glycoproteins IIb and IIIa bound $^{125}$I-fibrinogen significantly, even when 0.5 mg/ml of $^{125}$I-fibrinogen were used and the immunoplates were exposed to the x-ray films for a prolonged period of time (autoradiograms not shown). The other immunoprecipitates usually labeled showed significant labeling under these conditions, and also, the glycoprotein IIb-IIIa precipitate obtained after crossed-immunoelectrophoresis of platelet membranes showed labeling.

**Effect of EDTA**

Divalent cations are necessary for the maintenance of the glycoprotein IIb-IIIa complex, and these cations can be removed by EDTA or EGTA provided the pH is above 8.0, resulting in a dissociation of the complex. No similar effect is obtained at pH 7.4, and the phenomenon is probably due to a pH-dependent increase in the chelating capacity of EDTA and EGTA as well as a decreased capacity of the proteins to bind the actual ions. To investigate the effect of EDTA on the binding of fibrinogen to the glycoprotein IIb-IIIa precipitate, immunoplates obtained after crossed-immunoelectrophoresis of whole platelets against antiplatelet antibodies were incubated with $^{125}$I-fibrinogen (0.1 mg/ml), 2 mM EDTA, and no addition of divalent cations in media at various pH. At pH 7.4, the presence of EDTA in the incubation medium caused only moderately reduced $^{125}$I-fibrinogen binding to the immunoprecipitates representing the glycoprotein IIb-IIIa complex, the factor XIIIa chain, and G4 compared to the binding obtained in a medium containing Ca$^{2+}$, Mg$^{2+}$, and no EDTA (Fig. 3A compared to Fig. 1B). However, the binding of $^{125}$I-fibrinogen to these precipitates decreased with increasing pH in the presence of EDTA (Fig. 3B and C), whereas no significant differences were observed between the immunoplates after Coomassie staining (not shown). At pH 8.7, there was no detectable binding of $^{125}$I-fibrinogen to the glycoprotein IIb-IIIa precipitate. The binding to the albumin precipitate appeared to be unaltered by the replacement of the divalent cations with EDTA at all the pH values tested. Increase of pH to 8.7 in the absence of EDTA had very little effect on the binding of fibrinogen to the immunoprecipitates (data not shown).

**Specificity of Divalent Cations**

Previous reports have demonstrated the involvement of divalent cations in the maintenance of the glycoprotein IIb-IIIa complex. We have also presented results in the preceding section that indicate a participation of divalent cations in the binding of fibrinogen to the glycoprotein IIb-IIIa complex. The pH-dependent ability of EDTA to chelate protein-bound divalent cations has been utilized in the present study to investigate divalent cation specificity in the fibrinogen binding to the various platelet proteins. At pH 8.7, EDTA appears to bind the divalent cations more strongly than glycoprotein IIb-IIIa, whereas at pH 7.4, the binding to the proteins seems to be the stronger. Immunoplates obtained after crossed-immunoelectrophoresis of solubilized platelets against antiplatelet antibodies were used. Divalent cations were removed from the proteins by preincubation of the immunoplates with EDTA at pH 8.7. The immunoplates were then incubated with 2 mM EDTA in Tris-HCl buffer at pH 7.4 and $^{125}$I-fibrinogen (0.1 mg/ml) was added. To one set of immunoplates no further additions were performed. To a second set, 1 mM Ca$^{2+}$ was added. To a third set, 1 mM Mg$^{2+}$ was added, and to a fourth set 0.5 mM Ca$^{2+}$ and 0.5 mM Mg$^{2+}$ were added. Since the addition of buffer without
Fig. 3. The effect of EDTA on binding of $^{125}$I-fibrinogen to platelet proteins. Autoradiographs of immunoplates (whole platelets against antplatelet antibodies) incubated with 0.1 mg $^{125}$I-fibrinogen/ml at varying pH in the presence of EDTA and no divalent cations added. (A) pH 7.4. (B) pH 8.0. (C) pH 8.7. Fig. 1B represents a control without EDTA but containing 1 mM CaCl$_2$ and 1 mM MgCl$_2$. The immunoplates were prepared as in Fig. 1 and incubated with 0.1 mg $^{125}$I-fibrinogen/ml in 0.02 M Tris-HCl buffer-0.154 M NaCl-2 mM EDTA at the pH indicated. The x-ray films were exposed for 3 days. Other experimental conditions were as in Fig. 1.
EDTA often restored the fibrinogen-binding capacity of the glycoprotein IIb-IIIa complex completely, an excess of EDTA was present to ensure specificity in the effects of the divalent cations added. Buffers without EDTA will probably contain small amounts of divalent cations that may be sufficient to restore glycoprotein IIb-IIIa metal ion complexes.19 Control immunoplates not pretreated with EDTA at pH 8.7, but in all other respects treated identical to the other immunoplates at pH 7.4, showed binding of 125I-fibrinogen to the immunoprecipitates as in Fig. 1B. A regular control without the addition of divalent cations showed only a very weak binding to the glycoprotein IIb-IIIa precipitate and to the factor XIIIa chain (Fig. 4A). When Ca2+ was present, a clearly significant binding to the glycoprotein IIb-IIIa precipitate and the factor XIIIa chain, but not to G4, was seen (Fig. 4B). When Mg2+ was added, a binding to the glycoprotein IIb-IIIa precipitate and G4, but not to the factor XIIIa chain, was seen (Fig. 4C). In the presence of both Ca2+ and Mg2+, all of the three protein precipitates were significantly labeled (Fig. 4D). The binding to the albumin precipitate appeared to be constant and not influenced by alterations in the ionic conditions. There were no significant differences between the immunoplates after Coomassie staining (not shown). The results indicate that the various platelet proteins have a different requirement for divalent cations in order to express their fibrinogen-binding properties. Similar results were obtained when EDTA was replaced by EGTA or when the concentrations of both these chelators were raised to 10 mM (date not shown).

**DISCUSSION**

Crossed-radioimmunoelectrophoresis as used in the present study is based on the principle that proteins to some extent maintain their binding properties after complex formation with immunoglobulins and therefore also may be accessible for specific binding even when present in immunoprecipitates.21 This principle will only work for those proteins that are complexed in such a way that sites critical for binding are not altered or covered by the immunoglobulins. Polyclonal antibodies as used here will probably block some binding sites, but generally a certain fraction of the protein antigens in an immunoprecipitate will be able to bind ligands or other proteins. However, since blocking of such binding occurs to a different extent, the binding of the ligands will not offer an adequate measure of the relative binding capacity of the various antigens. Thus, the amount of fibrinogen bound to the glycoprotein IIb-IIIa precipitate does not appear to be as large as that bound to the factor XIIIa chain as judged from the x-ray films versus the stained immunoplates. We have reasons to believe that the glycoprotein IIb-IIIa complexes available for fibrinogen binding are easily saturated in the present system, since the fibrinogen concentration may be decreased tenfold with little reduction in fibrinogen binding to the glycoprotein IIb-IIIa precipitate, whereas the binding to the factor XIIIa chain and G4 is not detectable under such conditions. It has also been noticed that the glycoprotein IIb-IIIa complex seems to contain more antigenic determinants than its dissociated subunits, glycoprotein IIb and glycoprotein IIIa. The structures corresponding to the antigenic determinants appearing with complex formation may also be critical for the fibrinogen binding, since this occurs only with the complex.

The specificity of the binding of fibrinogen to the glycoprotein IIb-IIIa complex is indicated by inhibition of binding of labeled fibrinogen in the presence of an excess of unlabeled fibrinogen. Furthermore, such inhibition did not occur when unlabeled fibrinogen was replaced by the same amounts of two other proteins. Specificity is further indicated by the finding that two other labeled proteins with connection to platelet function, i.e., fibronectin and thrombin, did not bind to the glycoprotein IIb-IIIa complex. Lastly, specificity is indicated by the dependence of divalent cations.

The presence of divalent cations seems to be a prerequisite for the formation and maintenance of the glycoprotein IIb-IIIa complex.16-19 These divalent cations can be chelated by EDTA or EGTA provided the pH exceeds 8.0.19 Conditions similar to those leading to the dissociation of the glycoprotein IIb-IIIa complex will also lead to abolished binding of fibrinogen to the corresponding immunoprecipitate. In both cases the original states may be reestablished by the addition of Ca2+ or Mg2+ (present results and ref. 19). It is also demonstrated here that the immunoprecipitates representing the separate glycoproteins IIb and IIIa do not bind fibrinogen. The results therefore indicate that it is the divalent cation-dependent formation of the glycoprotein IIb-IIIa complex that induces the fibrinogen-binding properties of the glycoproteins. However, it cannot be excluded that divalent cations, in addition to those keeping the glycoprotein IIb-IIIa complex together, are required for the induction of the fibrinogen-binding properties of this preformed glycoprotein complex. If so, the present data indicate that Mg2+ as well as Ca2+ can be used on both sites. Furthermore, it will be expected that the cations involved in glycoprotein IIb-IIIa complex formation as well as those involved in fibrinogen binding to the preformed glycoprotein complex, bind more strongly to the proteins than to EDTA at pH 7.4 and that removal of both types of divalent cations only can be achieved
Fig. 4. Dependence of divalent cations on the binding of fibrinogen to platelet proteins. Autoradiographs of immunoplates preincubated with EDTA at pH 8.7 followed by incubation with $^{125}$I-fibrinogen and divalent cations with an excess of EDTA at pH 7.4. (A) Control $^{125}$I-fibrinogen and 2 mM EDTA. (B) Incubated with $^{125}$I-fibrinogen, 2 mM EDTA, and 1 mM CaCl$_2$. (C) Incubated with $^{125}$I-fibrinogen, 2 mM EDTA, 1 mM MgCl$_2$. Immunoplates were prepared by crossed-immunoelectrophoresis of solubilized platelets against antibodies to whole platelets. The immunoplates were incubated with 2 mM EDTA at pH 8.7 to remove the divalent cations. Then the immunoplates were transferred to solutions containing 2 mM EDTA at pH 7.4. The amounts of divalent cations indicated and 0.1 mg $^{125}$I-fibrinogen/ml were added and the immunoplates were processed further as described in Fig. 1.
with EDTA at higher pH. Nachman and Leung\(^1\) reported that Ca\(^{2+}\) had to be present to obtain complex formation between the glycoproteins IIb and IIIa, and fibrinogen, but did not discuss whether the complex between the glycoproteins was preformed or not. Using available data on Ca\(^{2+}\) binding to the fibrinogen molecule,\(^2\) it can be verified that the conditions used in one of the present experiments, i.e., an excess EDTA over divalent cations at pH 7.4, almost completely depletes the fibrinogen molecules of their Ca\(^{2+}\). Since full binding of fibrinogen still appears, it may be concluded that the divalent cations on the fibrinogen molecule as such are not critical for the binding of fibrinogen to the glycoprotein IIb–IIIa complex. The specificity of Ca\(^{2+}\) or Mg\(^{2+}\) for the binding of fibrinogen to the glycoprotein IIb–IIIa complex by the present methods is in agreement with the cation requirement observed for the inducible fibrinogen receptor on the platelet surface.\(^3\)\(^4\)\(^5\)\(^6\)\(^7\) The binding of fibrinogen to the factor XIIIa chain in a Ca\(^{2+}\)-dependent manner corresponds to the available data on the behavior of factor XIII\(^2\)\(^9\) and thus serves as a good control of cation specificity in our experiments. The Mg\(^{2+}\)-dependent binding to the \(\alpha\)-granule protein G4\(^3\)\(^0\)\(^1\)\(^2\) is unexplained at present.

Absence of the platelet glycoproteins IIb and IIIa in Glanzmann’s thrombasthenia type I\(^1\)\(^0\)\(^1\)\(^1\)\(^2\) is accompanied by a strongly reduced platelet fibrinogen binding and the absence of platelet aggregation.\(^8\)\(^9\) This strongly indicates that these glycoproteins are involved in platelet fibrinogen binding in some way. The work performed by Nachman and Leung\(^1\)\(^4\) and the present work show that a divalent cation-dependent glycoprotein IIb–IIIa complex can bind fibrinogen and therefore may constitute the receptor. Furthermore, it is shown that these two glycoproteins do not bind fibrinogen separately. Polley et al.\(^2\)\(^0\) have suggested that the glycoproteins IIb and IIIa are separate entities in the membranes of the unstimulated platelets but that they rapidly form complexes upon activation of the platelets by thrombin. We have previously demonstrated that the dissociation of the glycoprotein IIb–IIIa complex may be reversed in isolated membranes,\(^1\)\(^9\)\(^1\) Kunicki et al.\(^1\)\(^4\) showed a similar reassociation in detergent extracts of platelets. This also shows that the separate glycoprotein IIb and IIIa molecules are able to form such a complex and that such complex formation may be the mechanism by which the platelet fibrinogen receptor is induced.

Since no alteration occurred in the binding of fibrinogen to the glycoprotein IIb–IIIa complex after addition of ADP or removal of ADP by apyrase, it is suggested that the function of ADP on the platelet surface is to induce the complex formation between the glycoproteins IIb and IIIa and not to support the binding directly. The mechanism controlling this process of receptor induction remains to be investigated.

There has been some controversy as to whether there are one or two categories of fibrinogen receptors on the platelet surface. Some investigators have presented data that correlate well with the presence of a single class of fibrinogen receptors,\(^5\)\(^8\)\(^3\)\(^3\)\(^3\)\(^3\) while others have presented data indicating two classes of receptors, one high affinity receptor that is present also in platelets from patients with Glanzmann’s thrombasthenia and one low affinity receptor absent in this disease.\(^8\)\(^3\)\(^6\) Peerschke et al.\(^7\) have pointed to the fact that the curvilinear plot obtained in Scatchard analyses could be due to negative cooperativity within a single class of receptors as well as the presence of two classes of receptors. The present results would support the idea of a chemically homogeneous class of receptors, since the glycoprotein IIb–IIIa complex is the only surface-located entity that is shown to bind fibrinogen in our system.

REFERENCES

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