Characterization of Human Platelet Glycoprotein Antigens Giving Rise to Individual Immunoprecipitates in Crossed-Immunoelectrophoresis

By Thomas J. Kunicki, Alan T. Nurden, Dominique Pidard, Nigel R. Russell, and Jacques P. Caen

Washed human platelets were labeled with 125I by the lactoperoxidase-catalyzed method and solubilized in 1% Triton X-100. The soluble proteins were analyzed by crossed-immunoelectrophoresis in 1% agarose, employing a rabbit antibody raised against whole human platelets. Analysis of autoradiograms developed from dried agarose gels led to the establishment of a normal reference pattern that was consistent for platelets obtained from more than 50 normal individuals. Six platelet membrane glycoprotein antigens contained in four distinguishable precipitates were identified. Each identification was based on direct sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of 125I-antigens contained in individually excised precipitates. These platelet antigens include major membrane glycoproteins previously designated Ia, Ib, Ila, IIb, Ila, and IIa. Glycoproteins IIb and Ila were shown to be contained in a single immunoprecipitate, while glycoproteins Ia and IIla were routinely detected in a single different immunoprecipitate. Analysis of soluble proteins from platelets of five patients with Glanzmann’s thrombasthenia demonstrated either a complete absence or a marked reduction of only one radiolabeled precipitate, that containing membrane glycoproteins IIb and Ila. Platelet samples from two patients with Bernard-Soulier syndrome were devoid of a different precipitate, that containing membrane glycoprotein Ib.

Crossed-immunoelectrophoresis has been used for the quantitative analysis of erythrocyte membrane proteins by Bjerrum and Bog-Hansen, and subsequently extended to structural and biosynthetic studies of several membrane systems.

Recently, Hagen et al. and Shulman and Karpatkin used crossed-immunoelectrophoresis to analyze the polypeptide components of normal human blood platelets and to compare normal platelets with those of patients with either Glanzmann’s thrombasthenia or the Bernard-Soulier syndrome, two clinically different hereditary disorders of platelet function. While Hagen et al. observed different abnormal precipitin profiles in platelets from patients with each disorder, Shulman and Karpatkin noted a great deal of similarity between the respective abnormal profiles and suggested that the antigenic differences observed in both groups of patients may have reflected differences in endogenous membrane proteolysis.

Since crossed-immunoelectrophoresis represents a relatively new approach to the identification and characterization of platelet proteins and glycoproteins, conclusions concerning the electrophoretic behavior of individual precipitates in agarose gels (e.g., shifts in mobility, changes in precipitate height, etc.) would be more meaningful were the identity of the antigens giving rise to individual precipitates known. Considering this to be a prerequisite to any valid comparisons between precipitate profiles of normal and functionally abnormal platelets, we began an extensive analysis of antigens present in a majority of the precipitates routinely generated by crossed-immunoelectrophoresis of Triton X-100 soluble platelet protein from several normal donors. This report is concerned solely with the identification of 125I-antigens labeled by the lactoperoxidase-catalyzed iodination of intact platelets.

Our data demonstrate the presence of 125I-antigens representing virtually all of the major membrane glycoproteins of human platelets currently identifiable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Having established the identities of human platelet 125I-antigens present in major immunoprecipitates consistently observed by crossed-immunoelectrophoresis of platelet proteins from a large number of normal donors, the profiles derived from platelets of five patients with Glanzmann’s thrombasthenia and two patients with the Bernard-Soulier syndrome were analyzed.

Materials and Methods

The following reagents were obtained commercially: acrylamide, bisacrylamide, SDS-PAGE high molecular weight and low molecular weight protein standards, SDS, N,N,N',N'-tetramethylethylenediamine (TEMED) (Bio Rad, Richmond, Calif.); Freund’s incomplete adjuvant (DIFCO, Detroit, Mich.); agarose-type HSA (Litex, Glostrup, Denmark); Na125I (New England Nuclear, Boston, Mass.); aprotonin, lactoperoxidase, Triton X-100 (Sigma Chemical Co., St. Louis, Mo.); DE 52-DEAE cellulose (Whatman Ltd., Maidstone, England).
Preparation of Rabbit Polyspecific Anti-Platelet Antibodies

Antisera against whole human platelets were prepared in rabbits as previously described. Platelets from 8 normal healthy donors were isolated, washed extensively in 0.01 M Tris, 0.145 M NaCl, 0.001 M EDTA, pH 7.4 (Tris-EDTA), pooled and frozen at −70°C in aliquots of 1 ml containing 10⁶ platelets (2.1–2.2 mg protein). Prior to each sensitization, an aliquot of platelets was thawed and emulsified in an equal volume of complete Freund’s adjuvant. Equal portions of the platelet plus adjuvant emulsion were injected subcutaneously into the backs of each of 12 New Zealand white rabbits, each rabbit receiving approximately 180 μg of total platelet protein. Injections were given at 2-wk intervals for the first 3 sensitizations, and rabbits were subsequently given booster injections at intervals of 4 wk. Commencing 3 mo after the first injection, the rabbits were routinely bled 2 wk after each booster immunization. Sera collected during 3-mo period were pooled, and IgG was isolated by ammonium sulfate precipitation and DEAE cellulose chromatography, as described by Harboe and Ingold. The purity of IgG fractions was determined by immunoelectrophoresis against goat anti-rabbit IgG (gamma chain) and by SDS-polyacrylamide gel electrophoresis. Further purification by gel exclusion chromatography (Sephadex G200; Pharmacia, Inc.) was performed initially, but was found to be unnecessary for the purposes of these experiments. The protein concentration of purified IgG was based on absorbance at 280 nm using the value of E 1% 1 cm = 15.0 for rabbit IgG.

Preparations of Rabbit Polyspecific Anti-Platelet Antibodies were used in this study and have been designated: “pool I,” obtained 6 mo after the initial sensitization; and “pool II,” obtained 9 mo after the initial sensitization.

Platelets

PRP was collected from normal donors and patients with Glanzmann’s thrombasthenia as described. Platelets were washed in Tris-EDTA three times and resuspended in Tris-glycine. The platelet concentration was determined by phase contrast microscopy. Erythrocyte or leukocyte contamination was determined to be routinely less than or equal to 1/2000 platelets.

PRP was obtained from the blood of Bernard-Soulier patients by an adaptation of the method of Solum et al. Whole blood was layered over a mixture of 1 volume 32.8% (w/v) sodium metrizoate and 2 volumes 4% (w/v) dextran T500 (d = 1.200), resulting in the sedimentation of erythrocytes. Leukocytes were then pelleted from the resultant platelet-rich plasma (PRP) by repeated slow centrifugation. The platelets were washed 3 times in Tris-EDTA, and the washed platelets finally resuspended in Tris-glycine. Leukocyte contamination was routinely less than 1/500 platelets; erythrocyte contamination, less than 1/1000 platelets.

Crossed-Immunoelectrophoresis

Nine volumes of the washed platelet suspension in Tris-glycine (6 × 10⁷ platelets/ml) were mixed with 1 volume of 10% (v/v) Triton X-100 in Tris-glycine. The mixture was agitated at 4°C for 30 min, then centrifuged at 80,000 G for 1 hr at 4°C. Supernatants containing soluble protein were aspirated, divided into 50–100 aliquots, and stored at −80°C, if not used immediately. Samples stored for up to 1 mo in this manner could be thawed and analyzed by crossed-immunoelectrophoresis with little, if any, modifications of resultant immunoprecipitate profiles. Profiles derived from samples frozen and thawed more than once were routinely distorted, therefore, repeated freeze-thawing was avoided. Previous studies demonstrated that the inclusion of proteolytic inhibitors in stored samples was unnecessary. Protein concentrations of soluble preparations, as determined by the method of Markwell et al., ranged from 8 to 10 mg/ml. The yield of total protein in Triton X-100 soluble preparations was 91% ± 3% (mean ± SD; n = 15).

One-hundred micrograms (10–12 μl) of Triton X-100-soluble protein was electrophoresed at 10 V/cm for approximately 60 min in first-dimension gels consisting of 1% agarose, 0.5% (v/v) Triton X-100 in Tris-glycine. The exact duration of electrophoresis was determined by the concomittant migration of a standard hemoglobin solution (red cell lysate) to a distance of 1.5 cm from the sample well. Electrophoresis in the second dimension was performed at 2 V/cm for 18 hr into a biphasic gel system that consisted of (1) an intermediate gel containing 1% agarose, 0.5% (v/v) Triton X-100 in Tris-glycine alone, and (2) an upper gel containing 1% agarose, 0.5% (v/v) Triton X-100 in Tris-glycine mixed with the rabbit antiplatelet antibody preparation (750 μg/sq cm). During electrophoresis, gels were maintained at 15°C using a temperature-regulated circulating water bath (Haake, Model N112). Following electrophoresis, gels were washed, dried, and when desired, stained with Coomassie blue R.

SDS-Polyacrylamide Gel Electrophoresis of Immunoprecipitates

In order to provide sufficient material for subsequent SDS-polyacrylamide gel electrophoresis, a minimum of five identical platelet samples were subjected to crossed-immunoelectrophoresis under identical conditions. A single representative gel was washed and stained with Coomassie blue R to serve both as a template for the location of poorly visible precipitates on the remaining unstained gels, and a permanent reference pattern for the correlation of crossed-immunoelectrophoresis and SDS-polyacrylamide gel electrophoresis. The remaining gels were washed extensively (up to 5 or 6 washes) but not stained. The peak of the precipitate to be analyzed was then excised from the plate with a clean scalpel and deposited into a 11 × 75 mm glass tube. Routinely, identical precipitates from at least four gels were pooled into a single tube. An arbitrary volume of H₂O was then added to each tube and the volume of the agarose-H₂O mixture was determined. Appropriate volumes of Tris-EDTA containing 0.05 M N-ethylmaleimide (pH 7.0) and a solution of 10% (w/v) SDS in H₂O were then added to achieve a final concentration of 0.005 M N-ethylmaleimide and 2% (w/v) SDS. Tubes were then stopped, briefly agitated, heated in a boiling water bath for 5 min, and transferred to a 56°C water bath to prevent agarose gelation. The contents of a given tube was divided equally between two additional tubes. To the first tube of each pair was added one-tenth volume of 2-mercaptoethanol; to the second tube, one-tenth volume of H₂O. Stopped tubes were then placed in a boiling water bath for 5 min, and finally returned to a 56°C water bath, until used. The entire contents of a given tube was transferred to an individual sample well of the polyacrylamide slab gel.

Electrophoresis was performed in 7.0%–12.0% exponential gradient acrylamide slab gels as described by Laemmli. The ratio (gg) of bisacrylamide to acrylamide was maintained at 0.027 throughout the gradient. Gels were electrophoresed at 25 V (constant voltage) for 15–16 hr, until a bromophenol blue tracking solution had migrated to within 0.5 or 1.0 cm of the gel edge. Gels were fixed and stained with Coomassie blue R by the method of Fairbanks et al. or with periodic acid Schiff reagent by the method of Zacharias et al. The following purified molecular weight standards (Bio Rad) were electrophoresed in the presence of 10% 2-mercaptoethanol in a separate sample well: myosin (mol wt 200,000), β-galactosidase (mol wt 116,500), phosphorylase-B (mol

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Table 1. Apparent Molecular Weights and $^{125}$I-Labeling Characteristics of Selected Major Platelet Membrane Glycoproteins

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>Apparent mol wt†</th>
<th>Relative Incorporation of $^{125}$I</th>
</tr>
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<tbody>
<tr>
<td>Ia</td>
<td>153,000</td>
<td>+</td>
</tr>
<tr>
<td>Ib</td>
<td>170,000</td>
<td>+</td>
</tr>
<tr>
<td>Iib</td>
<td>142,000</td>
<td>+</td>
</tr>
<tr>
<td>IIa</td>
<td>138,000</td>
<td>+</td>
</tr>
<tr>
<td>IIb</td>
<td>170,000</td>
<td>+</td>
</tr>
</tbody>
</table>

†Based on the results of bidimensional SDS-PAGE of protein samples from normal platelets labeled with $^{125}$I by the lactoperoxidase-catalyzed method of Phillips and Poh Agin. The glycoprotein nomenclature used by these authors is adopted in the Table with the exception that glycoproteins III and IV are here designated IIa and IIb, respectively.

‡Reduced apparent molecular weights were obtained in the presence of 10% 2-mercaptoethanol.

§Glycoproteins Ib and IIb are separated into subunits in the presence of 10% 2-mercaptoethanol. In each case, the larger subunit is designated $\gamma$, the smaller subunit, $\beta$. Adapted from Phillips and Poh Agin. The glycoprotein nomenclature proposed by these authors is adopted in the Table with the exception that glycoproteins III and IV are here designated IIa and IIb, respectively.

RESULTS

$^{125}$I-Antigen Profiles of Normal Platelet Proteins

An $^{125}$I-antigen profile (autoradiograph) typical of those obtained by crossed-immunoelectrophoresis of Triton X-100 soluble platelet protein from 28 normal individuals against rabbit antibody pool I is shown in Fig. 1A. The most densely labeled precipitate has previously been shown to contain a complex of glycoproteins IIb and IIIa. Other precipitates (labeled 1, 2, and 3 in Fig. 1) are routinely detected, while a fifth precipitate is often observed. Although the fifth precipitate was detectable on most gels analyzed, it is poorly reproduced on photographs of the same gels. To circumvent this technical problem, the position occupied by the peak of this precipitate is indicated by an open arrow in all figures of this article.

A profile typical of those obtained by electrophoresis of Triton X-100-soluble platelet protein from 28 normal individuals against rabbit antibody pool II is shown in Fig. 1B. Profiles derived from electrophoresis against either antibody pool were qualitatively similar. Although the height of individual precipitates in profiles obtained using antibody pool II differed somewhat in comparison to those in profiles obtained using antibody pool I, these differences in precipitate height were due solely to changes in the characteristics of antibodies (affinity, specificity) in the respective pools, since the same differences were observed when identical platelet protein preparations were electrophoresed against either pool.

As indicated in Table 1, membrane glycoproteins show very different degrees of iodination when labeling of intact platelets is performed by the lactoperoxidase-catalyzed method. The incorporation of $^{125}$I is used in this study solely to localize selected glycoproteins in crossed-immunoelectrophoresis profiles and to facilitate the identification of these glycoproteins in subsequent SDS-polyacrylamide gel electrophoretic analyses.

$^{125}$I-Antigen Profiles of Patients With Glanzmann’s Thrombasthenia

Based on precipitate position and form, analyses of $^{125}$I-antigens present in protein preparations from patients with type I (Fig. 2) or type II (Fig. 3) Glanzmann’s thrombasthenia demonstrated an absence of (type I) or marked decrease in (type II) only the precipitate known to contain a complex of glycoproteins IIb and IIIa, confirming the previous observation of Hagen et al. In either case, those precipitates in normal profiles designated 1 through 3 were consistently detected in thrombasthenic profiles. The results
Fig. 1. Crossed-immunoelectrophoresis of Triton X-100-soluble proteins from 125I-labeled normal platelets against (A) rabbit antibody pool I or (B) rabbit antibody pool II. Platelets were labeled with 125I by the lactoperoxidase-catalyzed method, washed, then solubilized by addition of 1% Triton X-100. Soluble platelet proteins (100 µg) were electrophoresed against purified rabbit IgG (750 µg/sq cm) containing multispecific anti-human platelet antibody. No antibodies were incorporated into the intermediate gel. Electrophoresis in the first dimension was performed at 10 V/cm for 1 hr (left to right); in the second dimension, at 2 V/cm for 18 hr (bottom to top). Following electrophoresis, gels were washed, dried, and exposed to Kodak X-Omat MA film. Autoradiographs developed from such gels are shown. The immunoprecipitate is so indicated. Additional prominent immunoprecipitates are arbitrarily designated nos. 1–3. A fifth precipitate (open arrow) is indicated, the intensity and height of which varied substantially from one normal platelet protein preparation to the next. Bar represents 1 cm.

In normal platelet 125I-antigen profiles, such as those depicted in Fig. 1A and B, the most prominent immunoprecipitate is that given by glycoproteins IIb and IIIa. Consequently, an analysis of the 125I-antigens present in immunoprecipitates 1, 2, and 3 derived from normal platelet samples is not as straightforward as would be desired due to the inevitable “contamination” of extracts of these immunoprecipitates by trace amounts of glycoproteins IIb and IIIa. To more clearly identify those 125I-antigens present in immunoprecipitates 1, 2, and 3, soluble 125I-antigens derived from platelets of patients with type I Glanzmann’s thrombasthenia, which lack glycoproteins IIb and IIIa (Fig. 2), were used as source material. SDS-polyacrylamide gel electrophoresis of the contents of agarose gel excised from the regions encompassing the peaks of immunoprecipitates 1, 2, and 3 is shown in Fig. 4. Precipitate 1 contains an 125I-antigen with electrophoretic mobilities in the presence or absence of 2-mercaptoethanol identical to those previously ascribed to glycoprotein Ib.14 Two 125I-antigens with electrophoretic mobilities characteristic of glycoproteins Ia and IIa14 were detected in the extracts of precipitate 2. Finally, a single 125I-antigen with the electrophoretic mobilities...
Ilb and IIIa, Ia and IIa, and IIIb were consistently detected in profiles derived from platelets of this patient. Similar results were observed in studies of protein preparations from a second Bernard-Soulier patient, N.V. Patient N.V. was that patient studied by Hagen et al.4

DISCUSSION

By crossed-immunoelectrophoresis of Triton X-100-soluble proteins from 125I-labeled normal platelets against a polyspecific rabbit anti-platelet antibody preparation, several 125I-antigens are routinely detected. Based on their characteristic mobilities in SDS-polyacrylamide gel electrophoresis in the presence or absence of the reducing agent, 2-mercaptoethanol, these antigens are identified as major membrane glycoproteins previously designated Ia, Ib, IIa, IIb, IIIa, and IIIb. These results confirm and extend previous observations by Hagen et al.4 who identified precipitates in crossed-immunoelectrophoretic gels given by glycoproteins Ib, IIb, and IIIa. Despite the fact that the studies reported here were performed on Triton X-100-soluble protein from 125I-labeled whole platelets, there is no doubt that the 125I-antigens in question represent surface exposed plasma membrane glycoproteins. This conclusion is supported by the observation that mobilities characteristic of glycoprotein IIIb14 was detected in the extracts of precipitate 3.

SDS-polyacrylamide gel analyses of 125I-antigens contained in precipitates 1, 2, and 3 obtained by crossed-immunoelectrophoresis of normal platelet proteins (not shown) gave identical results, excluding the contamination with glycoproteins IIb and IIIa caused by the overlap of the immunoprecipitate previously shown to contain these glycoproteins.4,7

Attempts to identify the 125I-antigen(s) present in the fifth immunoprecipitate (see open arrow in Figs. 1, 2, 3, and 5) were unsuccessful due to the variable intensity of this immunoprecipitate from one preparation to another and the relatively weak labeling of the antigen(s) involved by lacto peroxidase-catalyzed iodination.

125I-Antigen Profiles of Patients With Bernard-Soulier Syndrome

Again based on precipitate position and form, crossed-immunoelectrophoretic analyses of 125I-antigens present in protein preparations from a patient (W.B.) with the Bernard-Soulier syndrome demonstrated a complete absence of the glycoprotein Ib precipitate (Fig. 5), confirming the previous observation of Hagen et al.,4 who studied a different Bernard-Soulier patient. Precipitates containing glycoproteins IIb and IIIa were consistently detected in profiles derived from platelets of this patient. Similar results were observed in studies of protein preparations from a second Bernard-Soulier patient, N.V. Patient N.V. was that patient studied by Hagen et al.4

Fig. 2. Crossed-immunoelectrophoresis of Triton X-100-soluble proteins from 125I-labeled type I thrombasthenic platelets against rabbit antibody pool II. Legend as in Fig. 1. Note the absence of the immunoprecipitate given by glycoproteins IIb and IIIa (Fig. 1B).
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Fig. 4. Identification of $^{125}$I-antigens contained in immunoprecipitates numbered 1 through 3. The regions of agarose gel encompassing the peaks of precipitates numbered 1 through 3 produced by crossed-immunoelectrophoresis of type I thrombasthenic platelet protein (see Fig. 2) were individually excised, solubilized in SDS, and analyzed by SDS-polyacrylamide gel electrophoresis in the absence of (NR) or presence of (R) 2-mercaptoethanol. An autoradiogram developed from a dried acrylamide gel is shown. Each of the $^{125}$I-labeled bands present in the autoradiograph shown in this figure corresponded to bands on the original gel which were also stained by periodic acid Schiff reagent. $^{125}$I-antigens present in each precipitate were identified by comparing the relative mobilities of $^{125}$I-bands derived from each precipitate with previously established electrophoretic mobilities of the major membrane glycoproteins (see Table 1). In this manner, it was shown that precipitate 1 is given by glycoprotein Ib; precipitate 2, by glycoproteins Ia and Ila; and precipitate 3, by glycoprotein IIIb. Relative molecular weights are shown at left. df. Dye front.

Fig. 5. Crossed-immunoelectrophoresis of Triton X-100-soluble proteins from $^{125}$I-labeled Bernard-Soulier platelets against rabbit antibody pool II. Legend as in Fig. 1. Note the absence of the immunoprecipitate previously designated 1 (Fig. 1) and shown to be given by glycoprotein Ib (Fig. 4).
specific radioactively labeled surface glycoproteins suggest that (1) the disintegration of the membrane during the solubilization process is reproducible and effectively complete, and (2) that, barring molecular interactions between different antigens in the nonionic detergent suspension, each precipitate observed in the immunoelectrophoresis profiles corresponds to a single glycoprotein entity. Because crossed-immunoelectrophoresis is performed under nondenaturing conditions, however, it is not surprising that the occurrence of glycoprotein complexes has been reported. Studying Lubrol W and sodium deoxycholate solubilized rat liver microsome and plasma membrane proteins, Blomberg and Raftel identified multienzyme complexes that gave rise to individual immunoprecipitates. Recently, Golovtchenko-Matsumoto and Osawa demonstrated that human erythrocyte band 3 protein, although pure by the criterion of polyacrylamide gel electrophoresis and located in a single immunoprecipitate following crossed-immunoelectrophoresis of Triton X-100-soluble erythrocyte membrane proteins, can be resolved into three components by crossed-immunoafinoelectrophoresis employing concanavalin A in the first-dimension gel. In each of the preceding studies, the association of individual antigens into complexes was shown to be a reflection of true intramembrane organizations and not to be the result of artificial interactions of the proteins with detergent micelles.

In this regard, the existence of a platelet membrane glycoprotein complex has been demonstrated by crossed-immunoelectrophoresis. Although Hagen and coworkers and Shulman and Karpatkin provided immunoelectrophoretic evidence that an intramembrane complex of glycoproteins IIb and IIIa probably exists, the recent study of Kunicki et al., using crossed-immunelectrophoresis, provided direct proof for the existence of this complex and established a molecular basis for the formation of the complex. In that study, it was shown that in the presence of $\text{Ca}^{++}$, glycoproteins IIb and IIIa exist in a complex that can be dissociated by chelation of $\text{Ca}^{++}$. Because of the crucial role of $\text{Ca}^{++}$ and fibrinogen in platelet–platelet adhesion and previous reports implicating glycoproteins IIb and IIIa as the receptor(s) for fibrinogen, it was suggested that the reversible $\text{Ca}^{++}$-dependent association of glycoproteins IIb and IIIa might represent a key step in the process of platelet aggregation.

The fact that two other membrane glycoproteins, those designated Ibb and IIIb, are consistently found as the individual components of two distinct immunoprecipitates lends credence to the conclusion that the presence of multiple antigens, such as glycoproteins IIb and IIIa, within a single immunoprecipitate is a reflection of true intramembrane organization. The question arises, then, whether or not glycoproteins Ia and IIa, routinely found in a single immunoprecipitate, are also associated within the intact membrane. Based on the preceding discussion, this would seem to be a distinct possibility. Nevertheless, further studies are necessary to prove the existence of an intramembranous complex of glycoproteins Ia and IIa. To date, we have not observed either of these glycoproteins to exist in individual immunoprecipitates, regardless of the conditions of crossed-immunelectrophoresis employed (e.g., in the presence or absence of $\text{Ca}^{++}$ and $\text{Mg}^{++}$, concanavalin A, or wheat germ agglutinin, or after the substitution of other nonionic detergents for Triton X-100).

In an attempt to avoid a confusing and, in our opinion, unnecessary system of nomenclature for immunoprecipitates, we have not conformed to those already reported, but will identify glycoprotein antigens contained in individual immunoprecipitates with reference to the nomenclature for such glycoproteins established by SDS-polyacrylamide gel techniques. For the information of the reader, however, it appears that the immunoprecipitates defined in our electrophoretic system would correspond to those defined by previous reports, as follows: glycoproteins IIb/IIIa—proteins 16, 17, and 18; glycoproteins Ia/IIa—protein 17; glycoprotein lb—protein 13; and glycoprotein IIIb—none. By systematic SDS-polyacrylamide gel electrophoretic analysis of protein components within excised sections encompassing the entire area of second-dimension agarose gels, we can state with confidence that each of the glycoproteins identified in this report is located solely within the region defined by the respective immunoprecipitate (T.J. Kunicki, unpublished observations).

As a result of these studies, we have been able to identify those immunoprecipitates in crossed-immunelectrophoretic profiles of Triton X-100-soluble human platelet proteins that contain major membrane glycoproteins, some of which are the subjects of intensive investigations. It is hoped that these observations will provide a basis for further studies concerning the structure and function of these glycoproteins and their molecular interrelationships with each other and other protein and nonprotein components of the human platelet plasma membrane.

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