Immunoinhibition of Ristocetin-Induced Platelet Aggregation

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ABSTRACT Human platelets washed and fixed in paraformaldehyde aggregate in the presence of the antibiotic ristocetin and normal plasma. This aggregation response is abolished after digestion of the fixed platelets with chymotrypsin. Antisera to fixed washed platelets were produced in rabbits and absorbed with chymotrypsin-treated, fixed washed platelets. Monovalent Fab fragments obtained from the isolated γ-globulin fractions of the antisera blocked ristocetin-induced aggregation of fixed washed platelets in buffer and normal platelets in platelet-rich plasma. By double-antibody immunoprecipitation, it was shown that the antibody which blocked the ristocetin reaction interacted with a platelet membrane surface protein of mol wt 155,000. The results suggest that the glycoprotein I complex on the surface of the human platelet mediates ristocetin-induced von Willebrand factor-dependent platelet aggregation.

INTRODUCTION

Platelet membrane glycoproteins have been implicated in von Willebrand factor-dependent ristocetin-induced platelet aggregation (1–3). Three major membrane glycoproteins with apparent mol wt of 155,000 (glycoprotein I), 135,000 (glycoprotein II), and 100,000 (glycoprotein III) are present on the surface of the human platelet (2, 4–6). Recent studies suggest that glycoprotein II can be resolved further into two separate components IIa and IIb (1). Platelets from patients with Glanzmann's thrombasthenia have decreased amounts of glycoprotein IIb (3, 7). Platelets from patients with the Bernard-Soulier syndrome show decreased adhesion to rabbit aorta subendothelium, and fail to aggregate with the antibiotic ristocetin in the presence of plasma von Willebrand factor (8, 9). Several recent studies have demonstrated an abnormality in the membrane glycoproteins of Bernard-Soulier platelets (1, 3). These giant platelets contain diminished amounts of sialic acid, and have decreased amounts and (or) molecularly altered forms of glycoproteins I–III (1, 3, 10). Nurden and Caen's studies suggest that the major membrane lesion in the Bernard-Soulier syndrome involved a marked reduction in glycoprotein I (mol wt 155,000); references 3 and 10. This surface glycoprotein, which is high in sialic acid, may serve as a potential receptor for a component(s) of the subendothelium during the platelet's interaction with this structure (1, 2). The possibility has also been raised that this 155,000 mol wt surface glycoprotein serves as a receptor for the plasma von Willebrand factor during aggregation induced by ristocetin (2, 11). It has previously been demonstrated that chymotrypsin-treated normal platelets have decreased membrane glycoproteins and aggregate poorly in the presence of von Willebrand factor and ristocetin (1). Similarly, trypsin-treated, formalin-fixed platelets do not bind von Willebrand factor and do not aggregate in the presence of plasma and ristocetin whereas nonenzyme-treated fixed cells do (12).

These observations suggested that selective proteolytic digestion of platelets might allow analysis of the surface protein(s) which interact directly with the von Willebrand factor and/or ristocetin. For this purpose, antibodies were produced in rabbits to paraformaldehyde-fixed washed platelets. The antibodies were absorbed with chymotrypsin-treated, fixed platelets and studied for the ability to induce a Bernard-Soulier-like abnormality in normal platelets. The results suggest that antibodies with an apparent specificity for surface glycoprotein I block ristocetin-induced aggregation of normal platelets.

METHODS

Formalin-fixed washed platelets. Platelet-rich plasma was prepared as previously described (13). The platelets were separated from plasma and washed three times using the Ardlie buffer system (14). The washed platelet button was suspended in 0.154 M NaCl, 0.01 M phosphate buffer, pH 7.4 (PBS)[1] containing 2% paraformaldehyde and 0.1 mg/ml

Dr. Jaffe is the recipient of an NIH Research Career Development Award 1 K04 HL 00237 and a Career Scientist Award from the Irma T. Hirschl Trust.

Received for publication 2 July 1976.

[1] *Abbreviations used in this paper*: AHF, antihemophilic factor; FWP, fixed washed platelets; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; VWF, von Willebrand factor.

apyrase (Sigma Chemical Co., St. Louis, Mo.). The platelet suspension was incubated at 4°C for 18 h, washed twice with PBS, resuspended at 200,000 platelets/μl in PBS containing 0.01% sodium azide. The fixed washed platelets were stored at 4°C for up to 4 wk.

Enzyme treatment of fixed washed platelets. 1 ml of fixed washed platelets (200,000/μl) was incubated at 37°C with 100 μg chymotrypsin for 15 min. 0.5 mM Phenyl methylsulfonyl fluoride (Sigma Chemical Co.) was added to the suspension that was incubated for 5 min at 23°C. The platelets were removed by centrifugation, washed twice in PBS, and resuspended in PBS at the original cell count. In other experiments, fixed washed platelets were similarly treated with 30 μg/ml trypsin or 4 U/ml neuraminidase (Worthington Biochemical Corp., Freehold, N. J.). The neuraminidase experiments were performed in 0.05 M sodium acetate buffer, pH 5.0.

Aggregation studies. For aggregation studies, venous blood drawn into 0.1 vol 3.2% sodium citrate was centrifuged at 225 *g* for 10 min to yield platelet-rich plasma. For some experiments, the platelet-rich plasma was labeled with 0.45 μM [^{14}C]serotonin (57 mC/mmol 5-hydroxy-[2-^{14}C]tryptamine creatinine sulfate, Amersham/Searle, Corp., Arlington Heights, Ill.) by incubation at 37°C for 15 min. 0.4-ml aliquots of platelet-rich plasma were stirred for 1 min at 37°C in siliconized cuvettes in a Payton aggregation module (Payton Associates, Buffalo, N. Y.) and aggregating agents were added. The responses to ADP, arachidonic acid (Analabs, Inc., North Haven, Conn.), collagen (Sigma Chemical Co.), thrombin (Parke, Davis, & Co., Detroit, Mich.), and ristocetin (Abbott Laboratories Diagnostics Div., South Pasadena, Calif., lot 791126) were recorded on a Riken Denshi (Payton Associates) linear recorder. Arachidonic acid was evaporated to dryness under nitrogen and dissolved in 0.1 M Na_2CO_3 1 h before use. Release of [^{14}C]serotonin was measured by liquid scintillation counting of supernate from aggregated samples rapidly chilled in ice and centrifuged. The fixed washed platelets were studied at concentrations of 100,000–150,000)/μl. In the experiments with fixed platelets, the sensitivity of the recording was increased by setting 100% transmission with a dilute platelet suspension (half the concentration of the test platelet suspension) rather than with buffer.

Antisera. Antisera to fixed washed platelets were produced in rabbits. 2×10^8 fixed washed platelets were washed twice in PBS, emulsified with an equal volume of complete Freunds' adjuvant, and injected into multiple intradermal sites on the rabbit's shaved back. The animals were reimmunized with intradermal injections at 2 and 4 wk, and with an intramuscular injection (without adjuvant) at 2 mo. Antiserum was obtained 2 wk after the final injection. The antisera were absorbed four times with chymotrypsin-treated, fixed washed platelets; 1 ml of antiserum was incubated with 2×10^9 washed chymotrypsin-treated, fixed platelets for 1 h at 37°C and 2 h at 4°C. The absorbed antisera (anti-FWP (fixed washed platelets)) did not agglutinate the chymotrypsin-treated, fixed platelets in buffer but did agglutinate nonenzyme-treated, fixed washed platelets at dilutions of 1:10–1:20. The absorbed antisera were further absorbed for 1 h at 37°C with human factor VIII concentrate (5 U factor VIII/ml antisera) (Profilate, Abbott Laboratories, Diagnostics Div.). The absorbed antisera were absorbed once with 0.1 vol aluminum hydroxide gel (Accurate Chemical & Scientific Corp., Hicksville, N. Y.) and heated to 56°C for 30 min to remove residual coagulation proteins. Anti-FWP had no inhibitory effect on VIII:AHF (antihemophilic factor, clotting activity) in human plasma as compared to nonimmune rabbit serum (15). The inhibition of VIII:AHF in human plasma by anti-FWP was assayed after equal volumes of antibody and plasma were incubated for 2 h at 37°C. The mixture was assayed for VIII:AHF by a one-stage method using VIII:AHF-deficient plasma (16). Commercial antifibrinogen, antialbumin, anti-IgG globulin, anti-IgM globulin, anti-α_2-macroglobulin, anti-C3, and anti-factor VIII were obtained from Behring Diagnostics, American Hoechst Corp., Somerville, N. J. Antibody to platelet myosin was prepared by the method of Pollard et al. (17) and characterized as previously described (18).

γ-Globulin fractions. γ-Globulin fractions of the antisera were prepared as described by Kolb et al. (19). The antiserum was dialyzed for 24 h at 4°C against 500 vol of sodium phosphate buffer (0.01 M phosphate, pH 7). After removal of insoluble protein by centrifugation at 3,000 *g* for 30 min, the dialyzed sample was applied to a 1.5 × 28-cm TEAE-cellulose column equilibrated with the dialysis buffer. The break-through protein was collected and concentrated by precipitation at 0°C with 50% ammonium sulfate, then dissolved in and dialyzed against 0.15 M NaCl. $F(ab')_2$ fragments were prepared by dialyzing the IgG fractions (10 mg/ml) against 0.1 M acetate, pH 4.5, overnight at 4°C, and digesting the protein for 7 h at 37°C with 0.4 mg pepsin (Worthington Biochemical Corp.) per 100 mg of IgG. The pepsin was then inactivated by raising the pH of the mixture to 7.5 using 1 M Tris buffer. The $F(ab')_2$ fragments were separated from the Fc fragments by gel filtration on a Bio-gel A 0.5-m column (Bio-Rad Laboratories, Richmond, Calif.) using 0.05 M Tris, 0.01 M citrate, 0.154 M NaCl, pH 8.0 buffer. The $F(ab')_2$ peak was concentrated by ultrafiltration using a ProDiCon ultrafiltration apparatus (Bio-Molecular Dynamics, Beaverton, Oreg.), and reduced for 6 h at 37°C in 0.01 M cysteine. Sodium dodecyl sulfate (SDS)-gel electrophoresis of the Fab fragments in the unreduced state revealed a single mol wt species of 50,000 which moved with a mol wt of 25,000 after reduction with dithiothreitol.

SDS-gel electrophoretic analysis of iodinated washed platelets. This was performed essentially as described by Jenkins et al. (1). Washed human platelets were prepared as described above, except that the final wash was in 0.01 M Tris, 1 mM EDTA, pH 7.4. 1.2 mCi of ^{125}I (Amersham/Searle Corp.) was added to a stirred 1-ml suspension of 10^9 washed platelets in the Tris EDTA buffer followed by 10 μl 0.1 M sodium phosphate, pH 7.4, containing 30 μg lactoperoxidase (Calbiochem, San Diego, Calif., purified grade 100 IU/mg). Four 10-μl aliquots of freshly prepared hydrogen peroxide solution (1 mM in 0.154 M NaCl) were then added at 10-s intervals. 3 ml of the Tris EDTA buffer was then added and the platelets sedimented and washed in the same buffer. The final platelet pellet was resuspended in 0.4 ml water, 0.2 ml 10% SDS was added, and the pellet solubilized by boiling at 100°C for 5 min. Portions of the SDS-solubilized platelets were reduced immediately before electrophoresis by boiling for 3 min, with equal volumes of a mixture containing 2% 2-mercaptoethanol, 8 M urea, and 2% SDS in 0.02 M sodium phosphate buffer, pH 7.35. The samples were analyzed by SDS-polyacrylamide gel electrophoresis as described by Weber and Osborn (20) using 5% gels. Radioactivity labeling patterns were determined by slicing the gels and assaying the radioactivity of the individual fractions in a gamma spectrometer. Gel slices were 2.0 mm in thickness.

Immunoprecipitation studies of solubilized iodinated platelets. For these studies the iodinated platelets were washed and solubilized in Lubrol PX (Sigma Chemical Co.) as previously described (21). 1×10^9 iodinated washed platelets were incubated for 18 h at 4°C in 0.5 ml PBS containing 0.1% Lubrol, 0.4 mM *N*-CBZ-α-L-glutamyl L-tyrosine, 250 μg/ml soybean trypsin inhibitor (Worthington Biochemical Corp.), 0.5 mM phenyl methylsulfonyl fluoride,

and Trasylol 100 U/ml (FBA Pharmaceuticals, New York). The soluble protein was separated from the residual insoluble material by ultracentrifugation at 100,000 *g* for 1 h at 4°C. Soluble human platelet membrane protein was prepared using Lubrol PX as previously described (21).

Double-antibody immunoprecipitation studies were performed as follows: 50 μl Lubrol-solubilized, iodinated platelet protein solution (1.6 mg/ml) and 20 μl of nonradioactive (cold carrier) Lubrol-solubilized, platelet membrane protein (2.0 mg/ml) were incubated with 50 μl of anti-FWP γ-globulin (2 mg/ml) for 1 h at 37°C and 18 h at 4°C. 175 μl of a γ-globulin fraction (3 mg/ml) of goat anti-rabbit γ-globulin was then added and the mixture was again incubated for 1 h at 37°C and overnight at 4°C. The resulting immunoprecipitate was sedimented at 12,000 *g* for 20 min at 4°C and washed five times with cold PBS. Control experiments were performed using normal rabbit serum γ-globulin. The washed immunoprecipitates were solubilized in 100 μl of a solution containing 8 M urea, 2% SDS, and 14 mM dithiothreitol by boiling for 5 min and analyzed by SDS-polyacrylamide gel electrophoresis. The gel slices were analyzed for radioactivity as described above.

Molecular weight markers included phosphorylase *a*, mol wt 94,000 (Sigma Chemical Co.); ovalbumin, mol wt 43,000 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.); chymotrypsin, mol wt 25,000 (Calbiochem); and α-2-macroglobulin subunit, mol wt 185,000 (kindly provided by Dr. Peter Harpel, Cornell Medical Center, New York).

RESULTS

Inhibition of ristocetin-induced aggregtion by chymotrypsin treatment of fixed washed platelets. Ristocetin alone did not aggregate fixed washed platelets in buffer. In the presence of a small amount of plasma, marked aggregation occurred with the addition of ristocetin to a final concentration of 1 mg/ml (Fig. 1*a*). Digestion of the fixed washed platelets with chymotrypsin abolished this aggregation (Fig. 1*b*).

Immunoinhibition of ristocetin-induced platelet aggregation. Nonprecipitating antibodies raised in rabbits to fixed washed platelets were absorbed extensively with chymotrypsin-treated, fixed washed platelets and human factor VIII concentrate. The absorbed intact antiserum did not agglutinate chymotrypsin-treated, fixed washed platelets but did agglutinate nonenzyme-treated, fixed washed platelets in buffer, and normal platelets in platelet-rich plasma. The absorbed antiserum had no inhibitory effect on the factor VIII procoagulant activity in normal plasma. Monovalent Fab fragments of the isolated γ-globulin fraction of this antiserum were utilized to study the ristocetin reaction. Anti-FWP Fab fragments blocked ristocetin-induced aggregation of fixed washed platelets (Fig. 2*a*). Control Fab fragments of anti-fibrinogen had no effect (Fig. 2*b*). In addition, intact γ-globulin preparations from the following antisera were noninhibitory: antialbumin, anti-C3, anti-α-2-macroglobulin, anti-IgM, anti-IgG, and antiplatelet myosin. Anti-factor VIII γ-globulin-inhibited ristocetin-induced aggregation of the fixed washed platelets. This inhibition was abolished by absorption of the anti-factor VIII with human factor VIII concentrate.

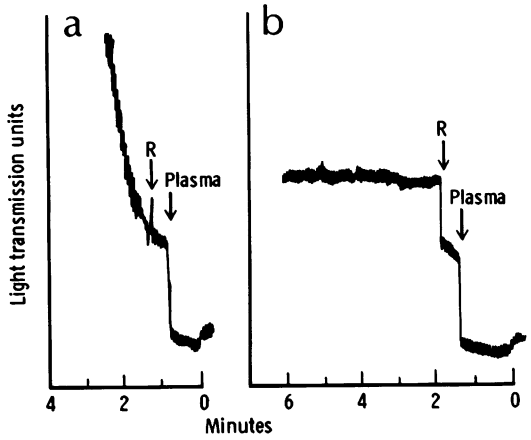

FIGURE 1 Inhibition of ristocetin-induced aggregation by chymotrypsin treatment of fixed washed platelets. (*a*) Fixed washed platelets (120,000/μl in 0.4 ml PBS) plus plasma (50 μl) plus ristocetin (50 μl, final concentration-1 mg/ml). (*b*) Chymotrypsin-treated, fixed washed platelets (120,000/μl in 0.4 ml PBS) plus plasma (50 μl) plus ristocetin (50 μl, final concentration-1 mg/ml). R, ristocetin.

Anti-FWP Fab fragments completely abolished ristocetin-induced aggregation in platelet-rich plasma (Fig. 3*a*). Antifibrinogen Fab fragments had no effect (Fig. 3*b*).

Specificity of anti-FWP. Aggregation studies were performed to determine whether the inhibition by anti-FWP was specific for ristocetin-induced aggregation. Anti-FWP Fab fragments inhibited ristocetin-induced platelet aggregation and serotonin release in platelet-rich plasma (Table I). In contrast, the antibody fragments had no effect on the platelet aggregation or serotonin release in platelet-rich plasma induced by arachidonic acid, thrombin, collagen, or ADP.

Immunoprecipitation of iodinated platelet protein by anti-FWP. These studies were performed to deter-

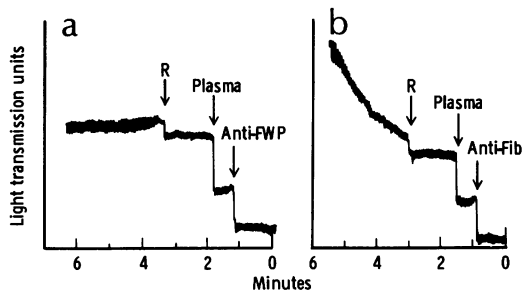

FIGURE 2 The effect of anti-FWP Fab fragments on the ristocetin-induced aggregation of fixed washed platelets. (*a*) Fixed washed platelets (110,000/μl in 0.4 ml PBS) plus anti-FWP Fab (25 μl, 2 mg/ml) plus plasma (50 μl) plus ristocetin (25 μl, final concentration-1 mg/ml). (*b*) same as (*a*) except that antifibrinogen Fab fragments were used.

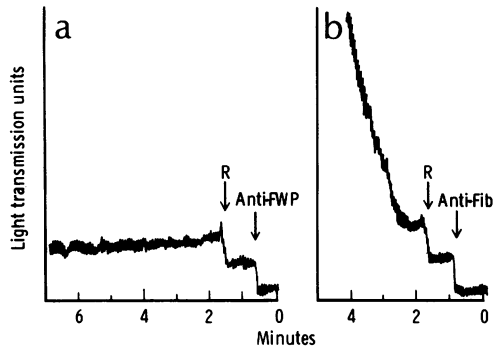

FIGURE 3 The effect of anti-FWP Fab fragments on the ristocetin-induced aggregation of platelet-rich plasma. (*a*) Platelets (300,000/μl, 0.4 ml) plus anti-FWP Fab (25μl, 2 mg/ml) plus ristocetin (75 μl, final concentration-1 mg/ml). (*b*) Same as (*a*) except that antifibrinogen Fab fragments were used.

mine whether the antibody which blocked the ristocetin reaction also interacted with a specific membrane protein(s). For this purpose, the platelet surface proteins were iodinated, the cells solubilized in Lubrol, and the radioactively labeled proteins subjected to double-antibody immunoprecipitation. The iodination pattern of the platelet surface proteins as solubilized by SDS is shown in Fig. 4*a*. This pattern resembles that previously described (1, 22) and serves as a reference marker. The iodination pattern of the platelet surface proteins as solubilized by Lubrol and then analyzed by SDS-polyacrylamide gel electrophoresis is shown in Fig. 4*b*. Approximately 70% of the cell protein was solubilized by treatment with Lubrol. The iodination profile (Fig. 4*b*) of the Lubrol-solubilized platelets appeared to contain all the surface proteins seen in Fig. 4*a* though the proportions were different. Double-antibody immunoprecipitation of the Lubrol-solubilized surface iodinated platelets was performed using γ-globulin fractions of rabbit anti-FWP and then goat anti-rabbit γ-globulin. SDS-polyacrylamide gel electrophoresis of the resulting immunoprecipitate (Fig. 4*c*) revealed a peak with an apparent mol wt of 155,000. Double-antibody immunoprecipitation using γ-globulin fractions of normal rabbit serum revealed no radioactive peaks.

DISCUSSION

Normal platelets contain surface-bound as well as internally stored von Willebrand factor, a protein synthesized by endothelial cells which is necessary for normal platelet function (21, 23, 24). Recent studies from several laboratories suggest the possibility that the platelet possesses a surface receptor that binds the plasma von Willebrand factor (1–3, 7–9, 11). We have previously demonstrated that a platelet membrane protein soluble in Lubrol PX binds purified human factor VIII (25). Okumura and Jamieson (11) have isolated a platelet glycoprotein, glycocalicin, which blocks ristocetin-induced platelet aggregation presumably by competitive inhibition. These studies taken together suggest that surface glycoproteins are involved in ristocetin-induced platelet aggregation.

Abnormalities which involve the interaction between platelets and von Willebrand factor have been reported in two well-defined clinical states. Von Willebrand's disease is characterized by a quantitative or qualitative deficiency in the plasma von Willebrand factor (VIII:VWF), one of the three described properties of human plasma factor VIII (23). The deficiency is functionally characterized in vivo by an abnormally long bleeding time or in vitro by an abnormality of assays of platelet function (defective ristocetin-induced platelet aggregation, poor retention of platelets in glass bead columns, and decreased adhesion of platelets to blood vessel subendothelium). The Bernard-Soulier syndrome is a hereditary hemorrhagic disorder characterized by a long bleeding time, thrombocytopenia, and giant, morphologically abnormal platelets. Despite the presence of normal plasma von Willebrand factor, platelets from patients with Bernard-Soulier syndrome are not aggregated by ristocetin. Several recent studies have suggested the possibility that the lesion in this disorder is related to an abnormality in platelet membrane glycoprotein, in particular, a reduction in a 155,000 mol wt surface protein referred to as glycoprotein I (1–3, 7).

It is not clear how ristocetin supports the von Willebrand factor-dependent aggregation of platelets. Zucker et al. have shown that von Willebrand factor binds to fixed platelets in the presence of ristocetin (12). We have previously suggested that ristocetin may alter platelet surface VIII:VWF so as to sterically reorient unavailable or masked sites (21). The fact that

TABLE I
The Effect of Anti-FWP Fab Fragments on Platelet Aggregation Induced by Various Agents

Aggregating agent	Aggregation*	[^{14}C]Serotonin release*
Ristocetin	12.2‡	3.4
Arachidonic acid	100	101.3
Thrombin	100	92.6
Collagen	96.6	78.7
ADP	96.7	80.0

* Percent of value without anti-FWP Fab.

‡ Values represent the mean of three experiments using platelet-rich plasma from different normal donors. Platelet-rich plasma (0.4 ml) was preincubated with anti-FWP (Fab) fragments (50 μl of 2 mg/ml). Control samples were preincubated with buffer in place of anti-FWP. Ristocetin was used at 1.6 mg/ml, arachidonic acid at 0.5 mM, thrombin at 1 U/ml, collagen at 6 mg/ml, and ADP at 5 μM (final concentrations).

bovine factor VIII aggregates human platelets in the absence of ristocetin (26) suggests that the mechanism of action of ristocetin may be related to its ability to convert the human VIII system to a more bovine form. One of the major differences between bovine and human factor VIII is in their carbohydrate content (27). Studies on bovine factor VIII have indicated that the integrity of carbohydrate side chains on the VIII molecule is necessary for subsequent aggregation responses (28, 29). The importance of carbohydrate in this system has been strengthened by the recent demonstration that, in some patients with von Willebrand's disease, the VIII:VWF molecule differs from normal VIII:VWF in the carbohydrate portion (30). Although ristocetin-induced aggregation is clearly a nonphysiological phenomenon, it has proven to be a useful probe of the initial events of primary hemostasis.

Our studies were designed to analyze the protein(s) on the platelet surface, in addition to VIII:VWF, which are necessary for the ristocetin-mediated aggregation response. We utilized, as immunogens, paraformaldehyde-fixed platelets which serve as inert, metabolically inactive particles in this reaction (31). The fact that fixed platelets aggregate in the presence of ristocetin and VIII:VWF is an argument for the surface localization on paraformaldehyde-fixed platelets of the platelet component involved in the response. Chymotrypsin-treated normal platelets (1) as well as chymotrypsin-treated, fixed platelets (Fig. 1) do not aggregate in the ristocetin system. It would appear logical from these observations that enzymatic degradation releases or destroys the surface protein necessary for the aggregation reaction. Antisera to fixed washed platelets were absorbed with chymotrypsin-treated, fixed washed platelets to obtain a reagent specific for those surface components involved in ristocetin aggregation. It was necessary to absorb the antisera with plasma factor VIII to rule out a direct anti-VIII:VWF effect. The fully absorbed antisera had no demonstrable anti-VIII:AHF (clotting) activity. Because platelets have a membrane Fc receptor, immunoinhibition studies were performed with nonagglutinating Fab fragments (32). The nonagglutinating monovalent Fab fragments blocked the ristocetin-induced aggregation of normal platelets and fixed washed platelets (Fig. 2) while bivalent intact anti-FWP γ-globulin agglutinated normal platelets and fixed washed platelets. These observations argue for a surface specificity of the antisera. The fact that anti-FWP Fab fragments inhibited ristocetin-induced aggregation of normal platelets in plasma but had no effect on arachidonic acid, thrombin, collagen, or ADP-induced platelet aggregation indicated a significant degree of restricted membrane specificity (Table I).

The double antibody immunoprecipitation studies demonstrate that anti-FWP reacted with a Lubrol-solubilized radiolabeled surface component(s) of

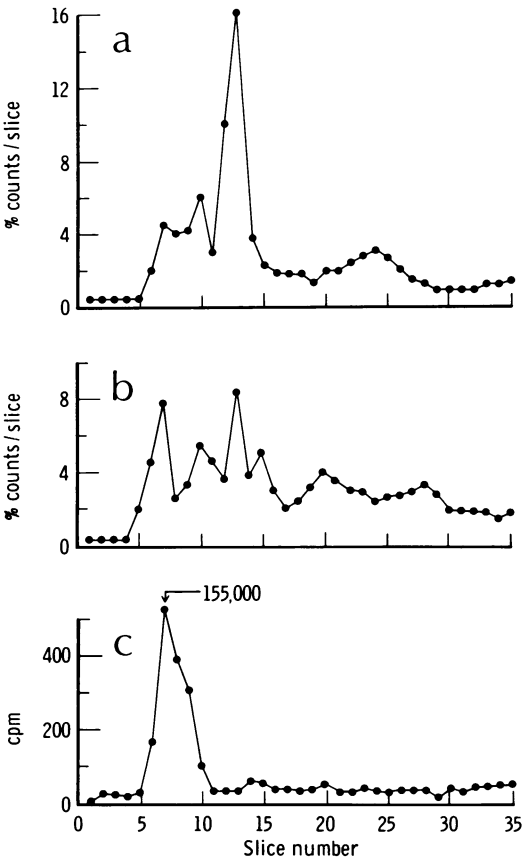

FIGURE 4 Immunoprecipitation of iodinated platelet surface protein by anti-FWP. (*a*) ^{125}I-labeling pattern of the exposed surface membrane polypeptides of normal washed platelets solubilized in SDS. 40 μg of platelet protein on a 5% SDS-polyacrylamide gel. (*b*) ^{125}I-labeling pattern of exposed surface membrane polypeptides of normal washed platelets solubilized in Lubrol. 35 μg of platelet protein on a 5% SDS-polyacrylamide gel. (*c*) Solubilized immunoprecipitate obtained from double-antibody immunoprecipitation reaction using anti-FWP performed on Lubrol solution of iodinated platelets shown in (*b*). 5% SDS-polyacrylamide gel.

155,000 mol wt. It should be noted that Lubrol may not have solubilized all of the membrane surface components. Thus it is possible that one or more other membrane constituents, not detectable by these techniques, also participate in the ristocetin-induced VIII:VWF platelet response. In sum, our results suggest that anti-FWP blocked a platelet membrane protein of the same molecular weight as the surface glycoprotein I which is deficient in Bernard-Soulier disease and which may interact with von Willebrand factor on the surface of the platelet. It is not possible from our studies to determine whether glycoprotein I on the platelet surface acts as a true receptor for VIII:VWF or functions as one part of a larger macromolecular complex which leads to platelet aggregation in the presence of ristocetin and von Willebrand factor by processes yet to be determined.

ACKNOWLEDGMENT

This work was supported by grants from the National Institute of Health (HL 18828) and The Arnold R. Krakower Hematology Foundation.

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