A Platelet Membrane Protein Expressed during Platelet Activation and Secretion

STUDIES USING A MONOCLONAL ANTIBODY SPECIFIC FOR THROMBIN-ACTIVATED PLATELETS*

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To identify structures on the platelet surface which become expressed after platelet activation, we have prepared murine monoclonal antibodies specific for thrombin-activated platelets. Hybridomas were screened for clones producing antibodies which bound to thrombin-activated platelets but not to resting platelets. Clone KC4 was identified. The binding of purified 125I-labeled KC4 antibody, an IgG1κ, to thrombin-activated platelets was saturable. Minimal binding was observed to resting platelets. The interaction of antibody with thrombin-activated platelets was characterized by a binding constant, \( K_d \), of 7.2 ± 0.4 nM and revealed 13,400 ± 3,000 binding sites per platelet. The presence of Ca\(^{2+}\) or EDTA, a pH ranging from 4 to 10, or high ionic strength had no influence on antigen-antibody interaction. The KC4 antigen was expressed on the platelet surface after activation with ADP, collagen, epinephrine, or thrombin. The extent of \([^{14}C]\) serotonin release during activation was directly proportional to the availability of antigen on the platelet surface regardless of agonist or platelet aggregation. The antibody is directed against a single protein which migrated between GPIb and GPIa after sodium dodecyl sulfate gel electrophoresis. This protein was purified from platelet membranes by immunoaffinity chromatography using KC4 antibody-agarose and demonstrated an apparent molecular weight of 140,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis under both nonreducing and reducing conditions. Of the cells examined, only platelets contained this protein. These results indicate that platelet secretion is associated with the expression of an \( M_r = 140,000 \) integral membrane protein composed of a single polypeptide chain. This protein may be a component of the internal granule membrane which is fused with the plasma membrane during activation.

Platelets are anucleate cells which circulate in the blood in a resting inactive form. During the initiation of hemostasis these cells undergo major functional changes which can be observed biochemically and morphologically. For example, the cells assume a spheroidal shape, extend pseudopodia, and secrete the contents of internal granules (3, 4).

The changes on the platelet membrane that accompany platelet activation have been partially identified. Factor V (5), thrombomodulin (6), von Willebrand factor (7), and fibrinogen (8) are secreted from \( \alpha \)-granules and become associated with the platelet plasma membrane (9). The expression of the factor Xa receptor is directly related to the binding of factor V to the platelet surface (10). The fibrinogen receptor, which appears to be composed of glycoproteins IIb-IIIa, is expressed only in activated platelets (8, 11, 12). Studies comparing surface structures on resting and activated platelets have identified actin and an additional high molecular weight protein expressed on activated platelets (13).

We have initiated studies to identify important structures on the surface of activated platelets. In this report we describe a newly identified membrane protein expressed on the surface of activated platelets. This protein, identified and purified with a monoclonal antibody specific for activated platelets, is an integral membrane protein whose expression is secretion-dependent and agonist- and aggregation-independent.

EXPERIMENTAL PROCEDURES

Preparation of Gel-filtered Platelets—Blood was obtained from normal human donors and anticoagulated with Ware’s solution (0.13 M citrate buffer) at a 9:1 (v/v) ratio. Platelet-rich plasma, prepared by centrifugation of the citrated blood at 160 \( \times \) g for 15 min, was applied to a BSA1 discontinuous gradient, and the platelet concentrates were isolated (14). The platelets were further purified by gel filtration on a Sepharose 2B column equilibrated with HEPES buffer, pH 7.35. In order to preserve optimal platelet function for experiments in which platelet secretion was compared to antibody binding, gel-filtered platelets for these experiments were prepared omitting the discontinuous BSA gradient.

Thrombin-activated platelets were prepared by the addition of thrombin to a final concentration of 0.15 unit/ml to the gel-filtered platelet suspension and incubated without stirring for 2 min. Resting and thrombin-activated platelets were fixed by the addition of 3% glutaraldehyde. The suspension was stirred slowly for 30 min, washed twice with TBS (20 mM Tris-HCl, 0.15 M NaCl, pH 7.5), and stored at \(-70^\circ\) C in 60% (v/v) glycerol. Fixed resting platelets were initially prepared from adenosine- and acetylcholine-treated platelets. Once antibody-binding experiments demonstrated this to be unnecessary, resting platelets were prepared without these reagents.

Preparation of Anti-platelet Monoclonal Antibodies Specific for Activated Platelets—Balb/c mice were immunized intraperitoneally with 1–5 \( \times \) 10^8 thrombin-activated aggregated platelets suspended in 250

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1 The abbreviations used are: BSA, bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; HEPES buffer, 3.8 mM HEPES, 0.14 M NaCl, 3 mM KCl, 1 mM MgCl₂, 3.8 mM NaH₂PO₄, 0.1% dextrose, and 0.35% BSA; TBS, Tris-buffered saline; ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulfate.
were added and the plates incubated at 37 °C for 30 min. The microtiter wells were washed three times with TBS, 200 μl of TBS with 0.5% gelatin and 50 μg/ml of human IgG were added and the plates incubated at 37 °C for 30 min. The microtiter wells were washed three times with TBS, and 100 μl of hybridoma culture supernatant were added and incubated at 37 °C for 1 h. The microtiter wells were washed three times with TBS, 2 mM β-mercaptoethanol, 1.5 mM MgCl₂, and then 50 μl of sheep anti-mouse immunoglobulin conjugated with β-galactosidase (Bethesda Research Laboratories) were added and incubated at 22 °C for 2 h. After washing three times with TBS, 2 mM β-mercaptoethanol, 10 μl of β-galactosidase (100 μg/ml) in 0.05 M sodium phosphate, 1.5 mM MgCl₂, pH 7.2, were added. The release of p-nitrophenol over 30–60 min was monitored at 405 nm in a Dynatech MR580 MICROELISA Auto-Reader.

**Solution Phase Radioimmunoassay for Anti-platelet Antibody**—An indirect solution phase radioimmunoassay was used to evaluate antibody binding to resisting thrombin-activated platelets. Glutaraldehyde-fixed resting or thrombin-activated platelets (0.5 ml of a suspension of 1 x 10⁸ platelets/ml) were suspended in TBS containing 1% BSA and 50 μg/ml of human IgG, pH 7.5. After incubation at 22 °C for 15 min, the platelets were sedimented by centrifugation and the supernatant aspirated. The culture fluid (0.5 ml) from a cloned hybridoma cell line was added. The platelets were resuspended and incubated at 37 °C for 30 min, then sedimented by centrifugation, and washed once with 0.5 ml of TBS, 1% BSA, and human IgG (50 μg/ml). 125I-labeled F(ab)² of sheep anti-mouse immunoglobulin (100 μl) was added. The platelets were resuspended, incubated at 37 °C for 5 min, and sedimented by centrifugation. After washing three times with 0.5 ml of TBS-1% BSA containing 0.05% Tween 20, pH 7.5, the platelet pellet was assayed for 125I in a Beckman Gamma 8000 spectrometer.

A direct solution-phase radioimmunoassay was used to evaluate antibody binding to unfixed gel-filtered platelets (17). Unfixed gel-filtered platelets (100 μl, 1 x 10⁹/ml) in HEPES buffer, pH 7.35, were mixed with 100 μl of 125I-labeled KC4 monoclonal antibody at varying concentrations. After adding 50 μl of either thrombin (final concentration 0.1 unit/ml) or HEPES buffer, the platelet suspension was incubated at 22 °C for 15 min without stirring. The free and platelet-bound antibodies were separated using the oil method previously described (18). 125I-labeled KC4 antibody bound to the platelets was quantitated in a Beckman Gamma 8000 spectrometer. The data for direct binding experiments were analyzed using the method of Scatchard (19).

For experiments in which platelet secretion was compared with antibody binding, platelets were activated with a variety of agonists in the presence of 125I-labeled KC4 antibody (3.6 μg/ml). The platelets were sedimented by centrifugation, washed twice with TBS and 0.01% Tween 20, pH 7.5, and resedimented. The pellets were harvested by excising the bottoms of the microfuge tubes, and the 125I-associated with the platelet pellet was quantitated. Nonspecific trapping of labeled material was accessed using [3H]sorbitol. The percentage of [3H]sorbitol trapped in platelet pellets was 0.02% ± 0.005. Pellets from aggregated and nonaggregated platelets trapped equivalent quantities of sorbitol.

**KC4 Antibody Purification**—Hybrid cells producing KC4 antibody were fused intraperitoneally into Balb/c mice (18). The ascites that developed was recovered and diluted 1:1 with 0.1 mM sodium phosphate, pH 8.0, filtered, and applied to a column (0.8 x 14 cm) of protein A-Sepharose CL-4B (Pharmacia) using the method of Ey et al. (20). Antibody concentration was estimated using an ELISA plate reader. Antibody concentration was estimated using an ELISA plate reader.

**Immunoblotting**—The antigenic specificity of the KC4 antibody was determined by gel electrophoresis, electrophoretic transfer, and immunoblotting of platelet proteins (22). Gel-filtered platelets (5 x 10¹⁰/ml) were dissolved in 3% SDS. The platelet proteins were separated by electrophoresis in a 6% polyacrylamide gel containing SDS using the system of Laemmli and Farber (23). The proteins were then transferred to nitrocellulose paper in 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3, for 18 h at 150 mA (22). After transfer, the electrophoretic blots were incubated with TBS containing 3% BSA and nonimmune mouse IgG (50 μg/ml), pH 7.5, for 1 h at 22 °C. 125I-labeled KC4 antibody (1-5 x 10⁵ cpm in a total of 100 μl) was added and incubated at 22 °C for 1 h, and then the blots were washed four times in TBS, 0.01% Tween 20, pH 7.5, dried, and autoradiographed with Kodak X-Omat AR film for 1-2 days.

The antibody was isolated from agarose-precipitated KC4 antibody bound to the platelet surface (i.e. EDTA was not included in buffers), and adenosine and acetylsalicylic acid were used to protect platelets from activation. Human platelets were prepared from platelet-rich plasma by BSA discontinuous gradient centrifugation and gel filtration of platelet concentrates on Sepharose 2B. Purified platelets were activated with bovine thrombin, and the platelet aggregates were used as immunoassay. The thrombin-activated platelets and adenosine-treated resting

**RESULTS**

**Preparation of Monoclonal Antibodies**—Because of the difficulty in obtaining platelets which remain in their resting state, we expended considerable effort to prepare stable platelet preparations for use as antigens in immunoassays. Conditions were selected in which metal ions remained bound to the platelet surface (i.e. EDTA was not included in buffers), and adenosine and acetylsalicylic acid were used to protect platelets from activation. Human platelets were prepared from platelet-rich plasma by BSA discontinuous gradient centrifugation and gel filtration of platelet concentrates on Sepharose 2B. Purified platelets were activated with bovine thrombin, and the platelet aggregates were used as immunoassay. The thrombin-activated platelets and adenosine-treated resting
platelets were compared on the basis of morphological changes and [14C]serotonin release. Electron micrographs emphasized that the thrombin-activated platelets were degranulated and contained extensive pseudopodia. In contrast, the resting platelets maintained a discoid shape with numerous granules. The supernatant of serotonin-loaded resting gel-filtered platelets contained less than 2% of the total [14C]serotonin, whereas 60% of the [14C]serotonin was released from thrombin-activated platelets isolated on a BSA gradient followed by gel filtration.

Balb/c mice were immunized with thrombin-activated platelet aggregates, and their splenocytes were fused with sp2/0 plasma cells using standard methods (15). A single fusion experiment yielded 899 primary wells containing anti-platelet antibodies. Cells secreting anti-platelet antibodies were identified with an ELISA system. Parallel ELISAs were performed using either fixed thrombin-activated platelets or fixed adenosine-treated platelets bound to a solid phase. Hybrid cells, producing antibody which reacted preferentially with activated platelets compared to resting platelets, were cloned by limiting dilution. The most promising of these clones, KC4 and GF8, were maintained in continuous culture.

The interactions of monoclonal antibodies from these clones with resting and thrombin-activated platelets are described in Table I. The KC4 and GF8 antibodies bound preferentially to thrombin-activated platelets but bound minimally to resting platelets. By comparison, HG6 (a clone from the same fusion) produced antibodies that bound to thrombin-activated and resting platelets equivalently. In control experiments, supernatants from the parental sp2/0 plasma cell line and an anti-prothrombin-producing clone RLI.5 (35) showed minimal binding to either thrombin-activated or resting platelets.

**Antibody Binding to Fixed Thrombin-activated Platelets**—The binding of KC4 antibody to platelets was re-evaluated using a double antibody solution phase radioimmunoassay. In this assay, antibody in the cell culture supernatants of clone KC4 also displayed preferential binding to the activated platelets as compared to the resting platelets. These results suggested the specificity of the antibody for an antigen on the surface of the activated platelet, but detailed quantitation required the development of a solution phase radioimmunoassay in which purified antibody could be employed for direct measurement of its interaction with unfixed platelets.

**Purification of KC4 Antibody**—KC4 antibody was isolated from mouse ascites using protein A-Sepharose affinity chromatography. The bound immunoglobulin was eluted by 0.1 M sodium citrate, pH 6.0. This antibody preparation yielded a single band in SDS gels under nonreducing conditions and two bands, corresponding to the heavy and light chain, in SDS gels under reducing conditions. The purified antibody was an IgG1K, as determined by Ouchterlony immunodiffusion using type-specific antisera.

**Direct Binding of KC4 Antibody to Unfixed Activated and Resting Platelets**—Purified KC4 monoclonal antibody was labeled with 125I using chloramine-T. The interactions of this antibody with unfixed gel-filtered thrombin-activated platelets and unfixed gel-filtered resting platelets were studied in a solution phase radioimmunoassay. As shown in Fig. 1, the monoclonal antibody displayed marked preference for the activated platelets. The interaction of KC4 antibody with thrombin-activated platelets was saturable. However, the binding of the KC4 antibody to resting platelets was minimal. Untreated resting platelets as well as platelets treated with adenosine and acetylsalicylate yielded equivalent results. All further experiments were performed using resting platelets prepared without adenosine or acetylsalicylate.

The binding of KC4 antibody to thrombin-activated platelets was evaluated using a Scatchard analysis. Using representative data from experiment 4 in Table II, a plot of the bound antibody concentration divided by the free antibody concentration versus the concentration of bound antibody yielded a straight line (Fig. 2). These results indicate a single class of antibody-binding sites on the platelet surface. Based on the analysis of this experiment, the binding constant, $K_d$, for the interaction of antibody with thrombin-activated platelets was 6.9 nM. Each platelet contained 10,700 binding sites recognized by the KC4 antibody. These results further confirm the monoclonality of the antibody, manifested by homogeneity of the apparent binding constant measured.

The results of four independent experiments performed on platelets from four different donors are shown in Table II.

### TABLE I

**Binding of monoclonal antibodies to platelets**

| Culture supernatant (100 μl) was evaluated for platelet-binding antibodies using the solid phase ELISA. The amount of antibody bound was quantitated by the release of nitrophenol, monitored by the absorbance at 405 nm. |

| Antibody | Thrombin-activated platelets | Resting platelets |
|----------|------------------------------|------------------|
| KC4      | 0.323                        | 0.015            |
| GF8      | 0.396                        | 0.008            |
| HG6      | 1.196                        | 1.182            |
| SF2/0    | 0.012                        | 0.013            |
| RLI.5    | 0.053                        | 0.035            |

### TABLE II

**Binding of monoclonal antibody to thrombin-activated platelets**

Each experiment includes assays performed in duplicate at 10 separate antibody concentrations. The antibody concentrations varied between 10^{-10} M and 2 × 10^{-7} M.

| Experiment | $K_d$ (nM) | Binding sites/platelet | Correlation coefficient $r$ |
|------------|------------|------------------------|----------------------------|
| 1          | 7.4        | 16,374                 | 0.93                       |
| 2          | 7.5        | 12,160                 | 0.95                       |
| 3          | 6.8        | 14,087                 | 0.96                       |
| 4          | 6.9        | 10,716                 | 0.96                       |

| Average    | 7.2 ± 0.4  | 13,400 ± 3,000         | 0.96                       |
platelets, indicating that normal human plasma does not contain this platelet antigen. Buffers of high ionic strength, such as thrombospondin, that bind to the plasma contained this platelet antigen. Buffers of high ionic strength or EDTA on KC4 antibody-platelet interaction was evaluated. These results indicate that the KC4 antibody is not directed against a platelet antigen whose antigenic structure is stabilized by metal ions nor is this antigen associated with the platelet surface through the action of metal ions. Furthermore, human plasma did not inhibit antibody binding to platelets, indicating that normal human plasma does not contain this platelet antigen. Buffers of high ionic strength (Tris buffer containing 1 M NaCl) or buffers with a pH from 4 to 10 did not alter the binding of the KC4 antibody to platelets.

**Secretion-dependent Expression of the Platelet Antigen**—The interactions of KC4 antibody with thrombin-activated platelets and platelets activated with other agonists were compared. In preliminary experiments, the KC4 antibody bound to platelets that were activated and aggregated with collagen, ADP, epinephrine, or thrombin (Table III). This interaction was also observed in unstirred thrombin-activated gel-filtered platelets which did not aggregate. Therefore, the binding of KC4 antibody to platelets appeared to be independent of agonist and platelet aggregation. To evaluate whether the expression of the KC4 antigen was associated with secretion, platelets were loaded with [3H]serotonin. The release of [3H]serotonin from platelets upon activation by various agonists was compared to the binding of 125I-labeled KC4 antibody to these platelets. As shown in Fig. 4, antibody binding to the activated platelets correlated directly with secretion. Thrombin-activated platelets demonstrated maximal antibody binding and maximal secretion. Stimulation with ADP, epinephrine, or collagen resulted in lower levels of secretion and antibody binding. Platelets initially treated with acetylsalicylate (which impairs secretion) and activated with ADP, collagen, or epinephrine did not express the KC4 antigen. These results indicate that the expression of KC4 antigen

**TABLE III**

| Antibody bound | % | Thrombin (0.15 unit/ml) | 100 |
|----------------|---|------------------------|-----|
|                | % | ADP (10 μM)            | 46  |
|                | % | Epinephrine (10 μM)    | 66  |
|                | % | Collagen (0.45 mg/ml)  | 72  |
|                | % | No agonist             | 0   |

**Fig. 2.** Scatchard analysis of the interaction of KC4 monoclonal antibody with thrombin-activated platelets. $R$ is the molar concentration of antibody bound to platelets. $F$ is the free molar concentration of antibody.

**Fig. 3.** Effect of calcium and EDTA on KC4 antibody-platelet binding. The interactions of the KC4 antibody and thrombin-activated platelets or resting platelets were studied using the direct binding solution phase radioimmunoassay. The conditions were identical to those described in Fig. 1 except that CaCl$_2$ (4 mM) or EDTA (10 mM) was included in the incubation mixture. O, CaCl$_2$; △, EDTA; ●, △, thrombin-activated platelets; O, △, resting platelets.

There is excellent concordance of these data, with an average binding constant, $K_D$, of 7.2 ± 0.4 nM. The average number of binding sites per platelet was 13,400 ± 3,000.

Since platelet activation is associated with the secretion of proteins, such as thrombospondin, that bind to the plasma membrane in the presence of calcium ions, the effect of calcium or EDTA on KC4 antibody-platelet interaction was evaluated. As shown in Fig. 3, the binding curves of KC4 antibody-platelet interaction are unaltered by calcium ions or EDTA. These results indicate that the KC4 antibody is not directed against a platelet antigen whose antigenic structure is stabilized by metal ions nor is this antigen associated with the platelet surface through the action of metal ions. Furthermore, human plasma did not inhibit antibody binding to platelets, indicating that normal human plasma does not contain this platelet antigen. Buffers of high ionic strength (Tris buffer containing 1 M NaCl) or buffers with a pH from 4 to 10 did not alter the binding of the KC4 antibody to platelets.

**Fig. 4.** Comparison of secretory function and KC4 antigen expression in activated platelets. Fresh gel-filtered platelets were loaded with [3H]serotonin. The platelets were activated with various agonists and examined with 125I-labeled KC4 antibody for the expression of KC4 antigen. The amount of serotonin secreted is expressed on the y axis as the percentage of secreted serotonin compared to the total serotonin in the platelet. The binding of the KC4 antibody to platelets is expressed as the percentage binding relative to the binding to thrombin-activated platelets. All points represent duplicate determinations. Resting platelets, O, activated platelets, agonist: thrombin, ●, ADP, △, epinephrine, V; collagen, □. Platelets were (O, △, □, V, □) or were not (●, •, △, V, □) treated with acetylsalicylate. Platelet preparations that underwent aggregation are encircled.
is secretion-dependent, but agonist- and aggregation-independent.

**Antigen Specificity**—The specificity of the antibody for a platelet antigen was examined using the Western blot method. For purposes of comparison, platelet proteins from thrombin-activated platelets and resting platelets were solubilized in SDS and analyzed. As shown in Fig. 5, the KC4 antibody bound to a single band in the solubilized thrombin-activated platelets and resting platelets. This band migrated with an apparent molecular weight of 139,000. Platelets, surface-labeled with ^125_I using the lactoperoxidase method, were run for comparison. The characteristic band pattern of the ^125_I-labeled platelets showed GPIIb, GPIIa, and GPIII (25). The protein antigen of the KC4 antibody migrated between glycoproteins IIb and Ila. Red blood cells, neutrophils, monocytes, lymphocytes, GM4672 (a lymphocytoid cell line), and Alexander PLC/PRF/5 (a human hepatoma cell line) were solubilized in SDS and their proteins similarly examined for binding to the KC4 antibody using the Western blot method. None of these cells contained proteins which bound to this antibody.

**Purification of the KC4 Antigen**—The KC4 antigen was purified from crude platelet membranes by affinity chromatography. The proteins were extracted from the membranes using Triton X-100, and these proteins applied to an affinity column containing the KC4 antibody covalently coupled to agarose. The material applied to the column was heterogeneous (Fig. 6) and most of these proteins failed to bind to the KC4 antibody-agarose. The bound protein, eluted with diethylamine, migrated as a major diffuse band on SDS gels upon electrophoresis in nonreducing conditions (Fig. 6). Several minor high molecular weight contaminants were also observed. The dominant protein band corresponded to an apparent molecular weight of 140,000. The character of this band was unchanged in the presence of Ca^{++} or EDTA. In SDS gels run under reducing conditions, the purified KC4 protein migrated as a single narrow band also with a molecular weight of 140,000. These results indicate that the KC4 protein is composed of a single polypeptide chain.

**DISCUSSION**

Like the zymogens of the blood-clotting enzymes and the pro-cofactors which exist in the blood in a precursor form, platelets circulate in the blood as inert cells vis à vis their function in coagulation. Upon activation of blood coagulation, platelets undergo a metamorphosis which can be observed biochemically and morphologically. Despite considerable advances in the description of protein components of the platelet membrane (36), incomplete information is available concerning the exposure or expression of receptors and enzyme activities which are unique to the activated platelet membrane surface. To approach this problem, we have prepared monoclonal antibodies specific for activated platelets and characterized the antigen against which one of these antibodies is directed.

The KC4 clone was selected from among a large number of clones producing monoclonal anti-platelet antibodies on the basis of its ability to produce antibody which bound to activated platelets, but not to resting platelets. Because of the difficulty in getting reproducible numbers of platelets bound to the solid phase for the ELISA, we reserved this assay for screening purposes and relied on a solution phase radioimmunoassay to quantitate the reactivity of the KC4 antibodies with resting and activated platelets. Using unfixed gel-filtered platelets, we determined that the KC4 antibody bound to
thrombin-activated platelets with an average binding constant, $K_0$, of 7.2 nm. These antibodies bound less tightly to resting platelets, 100-fold more antibody being required with resting platelets to give binding comparable to that observed with the thrombin-activated platelets. On the basis of these experiments, it is not possible to distinguish between a pool of resting platelets which is contaminated with 1% activated platelets, platelets which allow partial entrance of antibody into the cell, or the partial expression of the KC4 antigen on the resting platelet surface to which the monoclonal antibody binds with lower affinity. Considerable efforts were taken to maintain the platelets in their resting state, including the use of adenosine and acetylsalicylate. However, KC4 antibody, at high concentration, bound to the resting platelets. Nonetheless, these antibodies demonstrated a marked preference for the activated platelet.

The accumulated data suggest that this is a platelet membrane protein that has not been previously purified. This conclusion is based upon the molecular weight of the protein, the number of KC4 antigens per platelet, and the distribution of the protein in resting and activated platelets. The protein migrated as a single band in SDS gels and yielded an apparent molecular weight of 140,000 under nonreducing and reducing conditions. This band reproducibly migrated between glycoprotein IIb and glycoprotein IIa. Approximately 13,000 KC4 antigens per platelet were observed. This value differs from the value estimated from the number of fibrinogen receptors (40,000 per platelet), a structure attributed to glycoproteins IIb-III (8). However, the recent report of McEver and Martin (37) indicates that their monoclonal antibody specific for thrombin-activated platelets recognizes about 9,000 sites per platelet, a value similar to our own. Further, the platelet antigen that they describe has a similar apparent molecular weight to the KC4 antigen. These results suggest that both the KC4 antibody and the antibody reported by McEver et al. may be directed against the same platelet antigen. A recent report by Gogstad et al. (38) describes a protein antigen, G18, present in the α-granule membranes but not in the plasma membrane of resting platelets. With a molecular weight of 130,000–135,000 in reduced and unreduced gels, this protein may be related to the KC4 antigen.

The expression of the KC4 protein on the platelet surface was independent of platelet aggregation or the agonist used to activate the platelets. Rather, expression was secretion-dependent, with the extent of KC4 protein expression directly correlated to the extent of platelet secretion. Acetylsalicylate, which inhibits secretion, also inhibited expression of this protein. However, this protein is distinct from other proteins secreted from the α-granules that thrombospondin, platelet factor four, and β-thromboglobulin are either released from the platelet into the surrounding environment or bind to the platelet plasma membrane through the action of calcium. KC4 antigen remains associated with the isolated platelet membrane. EDTA, solutions of high ionic strength, or solutions of low or high pH failed to dissociate the KC4 antigen from the platelet membrane. These results suggest that the KC4 antigen is an integral membrane protein in the activated platelet. Since KC4 antigen can be identified in solubilized resting platelets by the Western blot technique and can be isolated from a crude membrane fraction containing granule and plasma membranes, it may be a component of a granule membrane. A recent report of a monoclonal antibody, specific for activated platelets, that binds a subpopulation of internal granule membranes in platelets and macrophages is compatible with this hypothesis (39). However, the relation between the KC4 antibody and this antibody remains uncertain.

In summary, we have used an immunologic approach to identify a structure on the postsecretion plasma membrane surface of activated platelets. This platelet-specific protein is composed of a single chain with a molecular weight of about 140,000. Approximately 13,000 sites are expressed on the surface of normal platelets. This protein is an integral membrane protein of the activated plasma membrane. The complete characterization of this protein and the determination of its function should increase our understanding of platelet function and the role of the platelet membrane in hemostasis.

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