

# ISOLATION OF HUMAN PLATELET PLASMA MEMBRANES WITH POLYLYSINE BEADS

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## ABSTRACT

Human platelet plasma membranes were isolated with polylysine beads according to the technique developed by Jacobson and Branton (1977, *Science* [Wash. D. C.] **195**:302-304). Lactoperoxidase-catalyzed surface iodination revealed that ninefold greater  $^{125}\text{I}$  specific activity was associated with the membranes isolated on beads than with whole platelets. Enrichment in the bead membrane preparation of the activities of membrane marker enzymes, bis(*p*-nitrophenyl)phosphate phosphodiesterase and Na,K-ATPase, was 8.0 and 4.4, respectively. Contamination with enzymes of other organelles, cytochrome oxidase and  $\beta$ -glucuronidase, was relatively low as compared with membranes isolated by sucrose gradient centrifugation. Analysis by SDS polyacrylamide gel electrophoresis showed that a full complement of surface glycoproteins was present on the membranes isolated with polylysine beads. The polylysine bead technique is a rapid, reproducible and efficient method for the preparation of relatively pure platelet plasma membranes.

**KEY WORDS** platelet · plasma membrane · membrane isolation · polylysine beads

Platelet plasma membrane surface glycoproteins play major roles in mediating the interactions of platelets in physiological hemostasis. Platelet adhesion to subendothelium as well as platelet aggregation is dependent to a significant extent on plasma membrane integrity as evidenced by the fact that two inherited platelet functional disorders, Bernard-Soulier syndrome and Glanzmann's thrombasthenia, are characterized by abnormalities in membrane glycoproteins (21, 22). The platelet plasma membrane also appears to possess specific surface receptors for certain physiologic stimulatory agents such as ADP (17), epinephrine (20), thrombin (33), and prostaglandins (16) which markedly influence platelet function. Much of the recent knowledge of the importance of the platelet membrane in hemostasis has come from the availability of techniques to separate reasonably clean

fractions of isolated membranes from subcellular components such as mitochondria and granules. Recently, Jacobson and Branton (10) developed a novel method using polylysine-coated beads for the isolation of erythrocyte membranes and HeLa cell membranes (5). With this method, surface membranes of cells attach to the polylysine beads and, after cell disruption, relatively pure preparations of plasma membranes remain attached to the beads. Theoretically, this technique provides an effective method to separate plasma membranes from subcellular organelles and intracellular membranes.

We have applied this method of isolation to washed human platelets. The highly purified surface membranes have been partially characterized.

## MATERIALS AND METHODS

### *Materials*

Poly-L-lysine (approximate mol wt 80,000), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl (EDC), im-

ipramine, lactoperoxidase, neuraminidase (*Cl. perfringens*), cytochrome *c* (horse heart), and bis(*p*-nitrophenyl)phosphate were obtained from Sigma Chemical Co. (St. Louis, Mo.). Galactose oxidase (55 U/mg) was obtained from Worthington Biochemical Corp. (Freehold, N. J.) and polyacrylamide beads Bio Gel P-2 (200–400 mesh) from Bio-Rad Laboratories (Richmond, Calif.). Carrier-free  $^{125}\text{I}$ -Na,  $^3\text{H}$ NaBH<sub>4</sub> (7 Ci/mmol), and  $^{14}\text{C}$ serotonin (58 mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, Ill.).

### Platelets

Fresh human platelet-rich plasma was obtained from The New York Blood Center. After removal of residual erythrocytes and leukocytes by centrifugation at 210 *g* for 10 min, platelets were sedimented by centrifugation at 1,700 *g* for 10 min and washed three times with a buffer containing 75 mM Tris-HCl, 100 mM KCl, and 12 mM Na citrate, pH 6.3 (4). These procedures were carried out at room temperature.

### Isolation of Platelet Membranes with Polylysine Beads

Attachment of platelets to polylysine beads and subsequent separation of membranes on the beads were performed following the method of Cohen et al. (5). Polylysine beads were prepared with polyacrylamide beads as described by Jacobson and Branton (10). 1 ml of packed washed platelets was washed in sucrose-acetate buffer (mixture of 7 vol of 310 mM sucrose and 3 vol of 310 mM Na acetate, pH 5.2) containing 1 mM EDTA and suspended in the same buffer to 50% vol/vol. To this suspension, 4 ml packed volume of polylysine beads prewashed in sucrose-acetate buffer, pH 5.2, and suspended to 50% vol/vol, were added dropwise with gentle stirring. After incubation at room temperature for 10 min, 20 ml of ice-cold sucrose-acetate buffer was added, and the beads were suspended by gently inverting the tube several times. The tube was then kept standing in ice for 10–15 min to allow the beads to settle. The supernate was aspirated and discarded. This procedure to remove unbound platelets was repeated five times and monitored with phase-contrast microscopy. The beads with attached platelets were then suspended in 2 vol of cold 10 mM Tris-HCl, pH 7.4, and subjected to two bursts of sonication, each for 15 s at 50 W with a sonicator (model W 200 R, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.), followed by several washings, and the beads were suspended to 50% vol/vol in the Tris buffer.

### Electron Microscopy

PREPARATION FOR SCANNING ELECTRON MICROSCOPY: Samples of whole platelets and sonicated platelets on polylysine beads (~0.1 ml) were suspended in 1 ml of sucrose-acetate buffer, pH 5.2. Samples

were fixed for 30 min by adding 1 ml of 4% glutaraldehyde in sucrose-acetate buffer and washed several times in the same buffer. They were then postfixed for 30 min by adding 2% osmium tetroxide in distilled water to an equal volume of polylysine beads in sucrose-acetate buffer, and then washed several times with distilled water. Beads from each sample in a concentration of 50 mg/ml were allowed to settle for 10 min onto squares of gelatin-coated aluminum foil covered by 8% glutaraldehyde in distilled water (11). Gelatin-coated foil was then dipped in distilled water to remove excess beads and dehydrated through a series of solutions of ethanol and water, 10–100%, in gradations of 10%. Foil squares covered with beads were critical-point dried in a Bomar SPC-1500 critical-point dryer (The Bomar Co., Tacoma, Wash.), attached to aluminum stubs with carbon paint, coated with gold-palladium, and examined in an ETEC autoscanning electron microscope.

PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPY: Sonicated platelets on polylysine beads were fixed by adding 1 ml of 4% glutaraldehyde in 10 mM Tris-HCl, pH 7.4, to 1-ml suspension of sample for 30 min and washed in cold Tris buffer. The sample was then postfixed by adding 2% osmium tetroxide in distilled water in an equal part to suspension in buffer, fixed for 30 min, and washed with cold 0.1 M cacodylate buffer. The sample was then suspended into 5-mm diameter wells cut in cooled agar in a petri dish. The petri dish was placed on ice to allow the agar-bead suspension to cool and solidify. Blocks containing the sample were cut from the petri dish in 2 × 2 × 1 mm squares and dehydrated through 50, 70, 80, and 95% alcohol and, in addition, six times in 100% ethanol. Blocks were embedded in Epon standard techniques and polymerized in flat molds at 60°C for 96 h. Thin sections on copper grids were stained with uranyl acetate and poststained with Reynolds' lead citrate. They were examined and photographed in a Philips 301 transmission electron microscope.

### Platelet Release Reaction

Platelet-rich plasma prepared from normal donors' blood anticoagulated with 3.8% Na citrate (ratio 9 vol:1 vol) was incubated with 1.5  $\mu\text{Ci}$   $^{14}\text{C}$ serotonin/ml at 37°C for 10 min, and the labeled platelets were washed as described above. After another wash with sucrose-acetate buffer, pH 5.2, containing 1 mM EDTA and 1  $\mu\text{M}$  imipramine, the platelets were suspended to 8 × 10<sup>9</sup> cells/ml in the same buffer. To 0.1 ml of this suspension, 0.2 ml of 50% vol/vol polylysine bead suspension in the same buffer was added. After mixing gently at room temperature for 10 min, 1.8 ml of the cold sucrose-acetate buffer was added and the suspension was centrifuged. The radioactivity of the supernate was counted. As a control, 0.1 ml of the sucrose-acetate buffer was used instead of the polylysine bead suspension, and the amount of  $^{14}\text{C}$ serotonin released was estimated by calculation as described previously (12).

### *Iodination of Platelets*

Surface labeling of platelets with  $^{125}\text{I}$  was performed by the method using lactoperoxidase and  $\text{H}_2\text{O}_2$  (24). To a 6-ml suspension of washed platelets ( $10^{10}$  cells) in phosphate-buffered saline (PBS, 10 mM Na phosphate and 145 mM NaCl, pH 7.4), 2 mCi of carrier-free  $^{125}\text{I}$ -Na and 120  $\mu\text{l}$  of 1 mg/ml lactoperoxidase were added, and six 50- $\mu\text{l}$  aliquots of freshly prepared 1 mM  $\text{H}_2\text{O}_2$  were then added at 15-s intervals with constant stirring. Finally, 6 ml of 0.1 mM Na metabisulfite in PBS was added, and the platelets were sedimented and washed three times with PBS. When labeling membranes on beads with  $^{125}\text{I}$ , the same procedures were employed, except that 4 ml of suspension containing 1 ml of membrane-bound beads and one-half the amount of each reagent to be added were used.

### *Tritiation of Membrane Glycoproteins with $[^3\text{H}]\text{NaBH}_4$*

The method devised by Gahmberg and Hakomori (6) for cell surface labeling with  $[^3\text{H}]\text{NaBH}_4$  was employed. Neuraminidase was purified according to Hutton and Regoezi (9), and galactose oxidase dissolved in PBS was heated at 50°C for 30 min before use (6). These enzymes prepared as described had no detectable protease activity as measured by use of RBB-hide (hide powder azure, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) as substrate (28). 3 ml of a washed platelet suspension ( $5 \times 10^9$  cells) in a buffer consisting of 10 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, and 1 mg/ml glucose, pH 7.4, was incubated with 1 mg/ml neuraminidase and 3.3 U/ml galactose oxidase at 37°C for 15 min. After the platelets were washed and resuspended in 3 ml of the same buffer, they were further incubated with 1 mCi  $[^3\text{H}]\text{NaBH}_4$  at room temperature for 20 min, and the platelets were washed three times in 10 mM Tris-HCl, pH 7.4, containing 145 mM NaCl.

To label membranes on beads, 1 ml of the beads suspended in 3 ml of the buffer was processed in the same way.

### *Protein Determination*

As described by Cohen et al. (5), the protein of membranes on beads was extracted by 3% SDS in  $\text{H}_2\text{O}$  and determined by Lowry's method without the use of  $\text{CuSO}_4$ , using crystalline bovine serum albumin as standard.

### *Enzymatic Assays*

Assays for enzyme activities of membranes isolated on beads were performed principally by the procedures of Cohen et al. (5). In a plastic tube with a cap, 0.2–0.3 ml of the beads was incubated in 0.7–0.8 ml of an individual reaction medium while constantly inverting the tube with a rotating apparatus in a dry incubator at 37°C. The reaction was stopped by chilling the mixture

in ice, and after sedimentation of the beads by a brief centrifugation, the supernate was assayed for the product of enzymatic reaction. Bis(*p*-nitrophenyl)phosphate phosphodiesterase was determined as described by Taylor and Crawford (32). The assay methods for Na,K-ATPase and cytochrome oxidase were those described by Cohen et al. (5).  $\beta$ -Glucuronidase was measured according to the method of Stahl and Touster (30). All the incubation media contained 0.1% Triton X-100. For comparison, whole platelet homogenate and membranes isolated by sucrose gradient centrifugation (14) were assayed in the same manner. The nitrogen decompression technique (3) was used to homogenize platelets in this preparation.

### *SDS Polyacrylamide Slab Gel Electrophoresis*

Samples for electrophoresis were prepared from membranes on beads as follows: 1 ml of the beads was vigorously vortexed with an equal volume of 3% SDS in  $\text{H}_2\text{O}$ , and after a brief centrifugation the supernate was removed. The extraction was repeated twice, and the combined supernates were heated at 100°C for 5 min and then dialyzed against 1 liter of  $\text{H}_2\text{O}$  containing 0.1% SDS at 4°C for 24 h with two changes of the dialyzing medium. After lyophilization, the extracted materials were dissolved in 40–50  $\mu\text{l}$  of a solution containing 62.5 mM Tris-HCl, pH 6.8, 2% dithiothreitol, 5% glycerol, and 0.001% bromophenol blue, and heated again at 100°C for 3 min. Compositions of gel and electrode buffer were those of Laemmli (13). Gels of 8% acrylamide with an overlaid stacking gel containing 4% acrylamide, 1.2 mm in thickness, were prepared in a Bio-Rad model 220 slab gel electrophoresis cell, and samples were electrophoresed at 6 mA/slab while the tracking dye was migrating in stacking gel, and at 12 mA thereafter. For fluorography, the gel in which tritiated samples had been electrophoresed was treated as described by Bonner and Laskey (3). Molecular weight standards used were myosin heavy chain (200,000), phosphorylase *a* (94,000), bovine serum albumin (68,000) and its dimer (136,000), actin (43,000), and  $\alpha$ -chymotrypsinogen (25,700).

## RESULTS

### *Morphology*

After polylysine beads were mixed with platelets and washed as described in Materials and Methods, the beads were observed by phase-contrast microscopy to be extensively covered by platelets. After sonication, no cells were visibly attached to the beads. The scanning electron micrograph in Fig. 1*a* shows a platelet-covered polylysine bead. Small vacant areas on the bead surface, on close observation, revealed attachment of cellular fragments. These fragments probably represent pieces

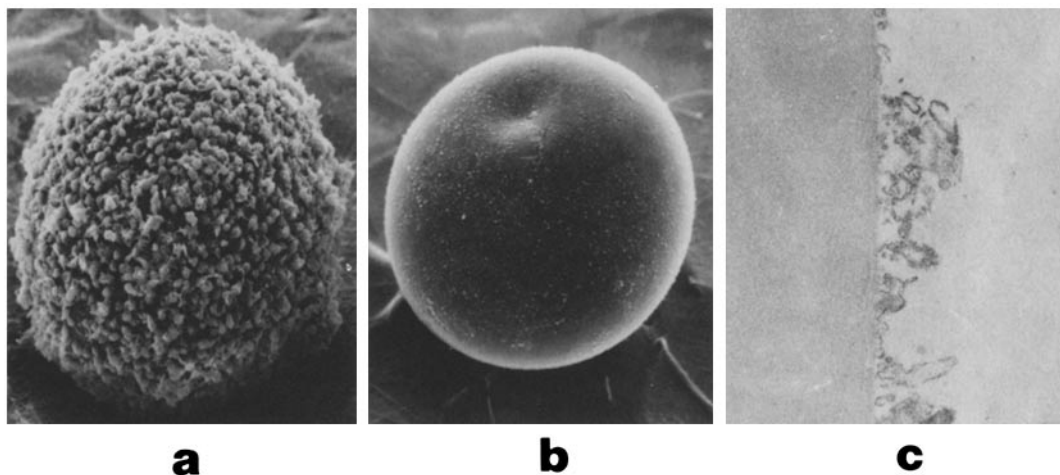


FIGURE 1 Electron micrographs of platelets and their membranes attached on polylysine bead. (a) Scanning micrograph of polylysine bead before sonication. The surface of the bead is coated with platelets.  $\times 800$ . (b) Scanning micrograph of polylysine bead and platelets after sonication. Intact platelets are absent and fragments of membrane adhere to the surface.  $\times 955$ . (c) Transmission micrograph of polylysine bead after sonication. Note fragments of membrane on surface.  $\times 17,350$ .

of surface membrane left from partially detached platelets. Membrane fragments were seen on the sonicated bead in a scanning electron micrograph (Fig. 1b). By transmission electron microscopy, characteristic vesicular structures of membranes were observed on the bead (Fig. 1c).

#### Platelet Release Reaction

Under some circumstances, soluble polylysine may induce platelet release of intracellular constituents (8, 15). To determine whether the polylysine beads induce the release reaction under the conditions utilized for the isolation of membranes, [ $^{14}\text{C}$ ]serotonin release was studied (Table I). No intracellular release was detected.

#### Purity of the Membrane Preparation

**IODINATION STUDIES:** Platelet surface proteins were labeled by lactoperoxidase-catalyzed iodination, and the specific radioactivity in membranes isolated on beads was compared with that of whole platelets to estimate the degree of purification. Although the labeling efficiency varied from experiment to experiment, the enrichment in the membranes was approximately ninefold in four separate experiments (Table II). This compares to a sixfold enrichment that we obtained previously in the membranes isolated by sucrose gradient centrifugation (18).

**ENZYMATIC STUDIES:** To evaluate further

TABLE I  
Platelet Release of [ $^{14}\text{C}$ ]Serotonin by Polylysine Beads

	cpm/ $10^7$ Cells
Counts in washed platelets	3,672
Counts in platelets in SAB*	3,857 (100%)
Counts released by mixing with polylysine beads	9 (0.2%)

\* SAB, sucrose-acetate buffer.

TABLE II  
Enrichment of  $^{125}\text{I}$  Label in Membranes Isolated on Polylysine Beads

Exp	sp act		Enrichment
	Membranes	Whole Cells	
	cpm/mg protein $\times 10^{-3}$		
1	727	77	9.3
2	305	38	7.9
3	918	103	8.9
4	1,558	170	9.3

the purity of the membrane fraction, an enzyme profile analysis was performed. Bis(*p*-nitrophenyl)phosphate phosphodiesterase (2, 31) and Na,K-ATPase (2) were utilized as markers for plasma membrane. Cytochrome oxidase and  $\beta$ -glucuronidase activities were assayed as markers of mitochondria and granules (lysosomes), respectively. These enzyme activities in bead prepara-

TABLE III  
Enzyme Activities of Platelet Membranes Isolated on Polylysine Beads and Comparison with Those of Whole Cell Homogenate and Membranes Prepared by Sucrose Gradient Centrifugation

Enzymes	sp act			Ratio	
	A Membranes on beads	B Whole cell ho- mogenate	C Membranes by SGC**	A/B beads	C/B SGC**
	<i>nmol/mg protein/h</i>				
BpND* phosphodiesterase	184	23	170	8.0	7.3
Na,K-ATPase	364	82	296	4.4	3.6
Cytochrome oxidase	27	424	61	0.06	0.14
$\beta$ -Glucuronidase	8	78	27	0.10	0.34

\* BpND, bis(*p*-nitrophenyl)phosphate.

\*\* SGC, sucrose gradient centrifugation.

tions were compared with enzyme activities in sucrose gradient isolated membranes. Table III presents the means of three determinations with the preparations from different batches of platelets. The purification of membranes with the bead technique using bis(*p*-nitrophenyl)phosphate phosphodiesterase as a marker was comparable to that obtained using  $^{125}\text{I}$  as a surface marker (Table II). Membrane enrichment was less using Na,K-ATPase activity as a membrane marker. The specific activities of the mitochondrial marker cytochrome oxidase and the granule marker  $\beta$ -glucuronidase were less in the membrane bead preparations compared to the membranes obtained by sucrose gradient centrifugation (Table III). The sucrose density gradient membranes contained twice as much contaminating mitochondria enzyme and three times as much granule enzyme.

It should be noted that these data provide only a rough estimation of purity in view of the fact that enzymatic functions in membranes attached on polylysine beads may be different from those in free membranes (5).

#### Protein Analysis

The protein content of membranes on beads was in the range of 250–300  $\mu\text{g}/\text{ml}$  of packed beads. The beads with attached intact platelets before sonication contained 12–14 mg protein/ml, thus membranes remaining on the beads represented 2–2.5% of the attached whole platelet protein.

The proteins of membranes on beads were extracted by SDS and the concentrated sample, reduced with dithiothreitol, electrophoresed in SDS polyacrylamide slab gels. The myosin (200,000 daltons) and prominent actin (43,000 daltons) bands were easily identified (Fig. 2B). With pe-

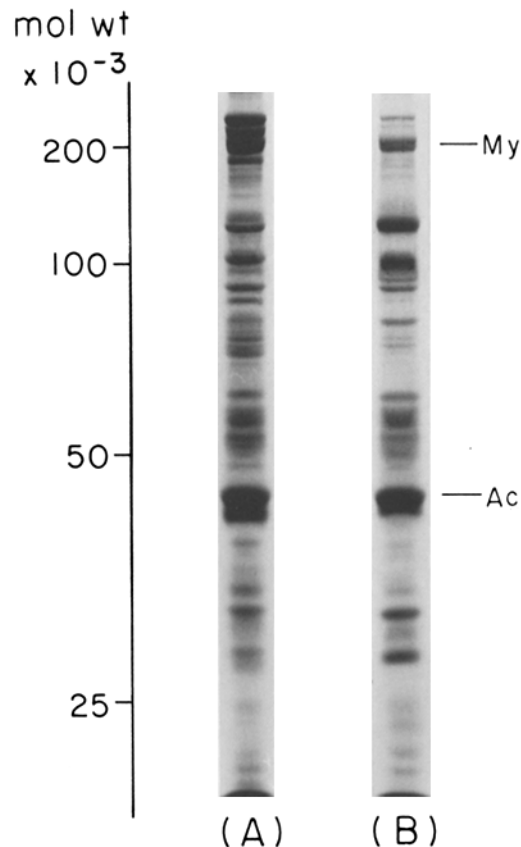


FIGURE 2 SDS polyacrylamide slab gel electrophoresis of whole platelets (A) and membranes isolated with polylysine beads (B). Both were stained with Coomassie Blue. The sample for Fig. 2A was prepared by solubilizing a pellet of washed platelets in 10 vol of a solution consisting of 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 2% dithiothreitol, 5% glycerol, and 0.001% bromophenol blue, which was immediately followed by heating at 100°C for 5 min. My, myosin, Ac, actin.

riodic acid-Schiff (PAS) staining, six major membrane glycoproteins were identified (Fig. 3B): three intense bands of apparent mol wt 125,000 (GP 125), 100,000 (GP 100), and 85,000 (GP 85), and three less intense bands of mol wt 220,000 (GP 220), 175,000 (GP 175), and 155,000 (GP 155). GP 125 was constantly located at the position of a band strongly stained by Coomassie Blue. This glycoprotein has been referred to previously as GP IIb (7, 25).

The autoradiograph of separated proteins of membranes isolated on beads from surface-iodinated platelets in Fig. 4B shows a pattern similar to that previously obtained with membranes prepared by sucrose gradient centrifugation (18). An intensely labeled band with apparent mol wt 100,000 and several less intensely labeled polypeptides in the higher molecular weight range corresponded nicely to PAS-stained bands in a parallel electrophoretogram (Fig. 4A). Attempts were made to correlate the designations of the surface glycoproteins of the membrane isolated on beads

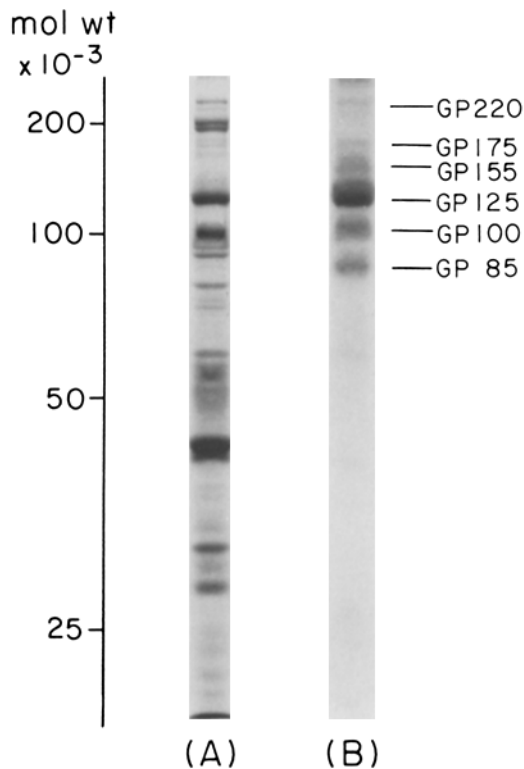


FIGURE 3 SDS polyacrylamide slab gel electrophoresis of membranes isolated with polylysine beads. Staining was with Coomassie Blue (A) and with PAS for carbohydrates (B).

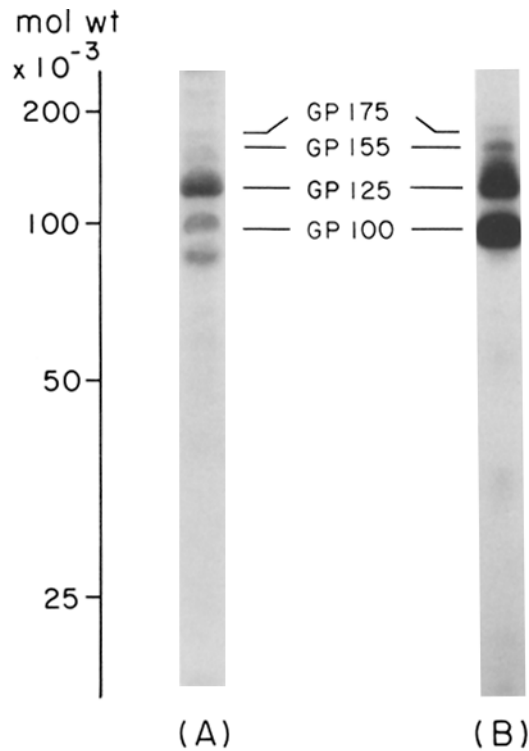


FIGURE 4 PAS staining (A) and autoradiography (B) of slab gel in which membranes isolated with polylysine beads from  $^{125}\text{I}$ -labeled platelets were electrophoresed.

with the proteins previously described by us as well as others in membranes prepared by other techniques. GP 100 is the platelet major glycoprotein (18) or GP III. GP 125 is probably GP IIb as noted by its association with a distinct Coomassie Blue stain as described above, and GP 85 is probably GP IV according to Phillips' and Agin's nomenclature (27). GP 155 may be GP Ib, but at present it is not possible to correlate clearly GP 155 as well as the GP 175 band with surface glycoproteins previously described by others. GP 220 may correspond to the 210,000-dalton glycoprotein that we have observed to be present together with 150,000-dalton glycoprotein in the GPI preparation purified by wheat germ agglutinin affinity chromatography (19). GP I (150,000 daltons) has been observed previously to be intensely stained by PAS (7, 23, 24). This contrasts with GP 155 and GP 175 bands in Fig. 3B. It is possible that glycocalicin which belongs to the GP I complex and is readily released by sonication (23) was lost during the procedure of membrane isolation on beads. The surface glycoproteins of

membranes isolated on beads were also studied by the tritiated borohydride method (6). For these studies, membranes were isolated from [ $^3\text{H}$ ]NaBH $_4$  surface-labeled platelets, electrophoresed in polyacrylamide slabs, and analyzed by fluorography. The labeling patterns of the isolated membrane proteins and whole cell homogenate proteins were almost identical, and resembled the  $^3\text{H}$ -labeling patterns reported by Phillips and Agin (26) and Anderson and Gahmberg (1). By this method, at least 10 surface glycoproteins were identified (Fig. 5).

These studies suggest that the membranes isolated on polylysine beads contain all of the species of glycoproteins (with the possible exception of glyocalicin) known to be part of the platelet surface.

#### Labeling of Isolated Membranes

To test the accessibility of the proteins in the isolated membranes on beads to lactoperoxidase-catalyzed iodination and neuraminidase-galactose oxidase-dependent tritiation, the labeling patterns of the membranes on beads were compared with those of intact platelets. After iodination (Fig. 6), more proteins were labeled in membranes on

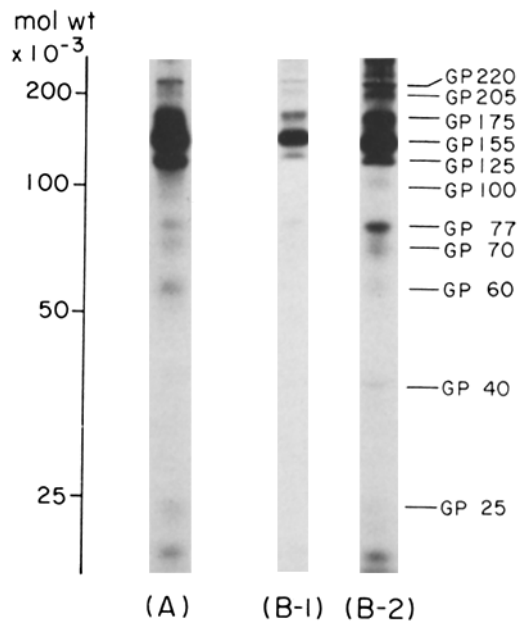


FIGURE 5 Fluorography of slab gel in which whole platelets labeled with [ $^3\text{H}$ ]NaBH $_4$  (A) and membranes isolated with polylysine beads from them (B-1 and B-2) were electrophoresed. In B-1 and B-2 different amounts of the membrane sample were used.

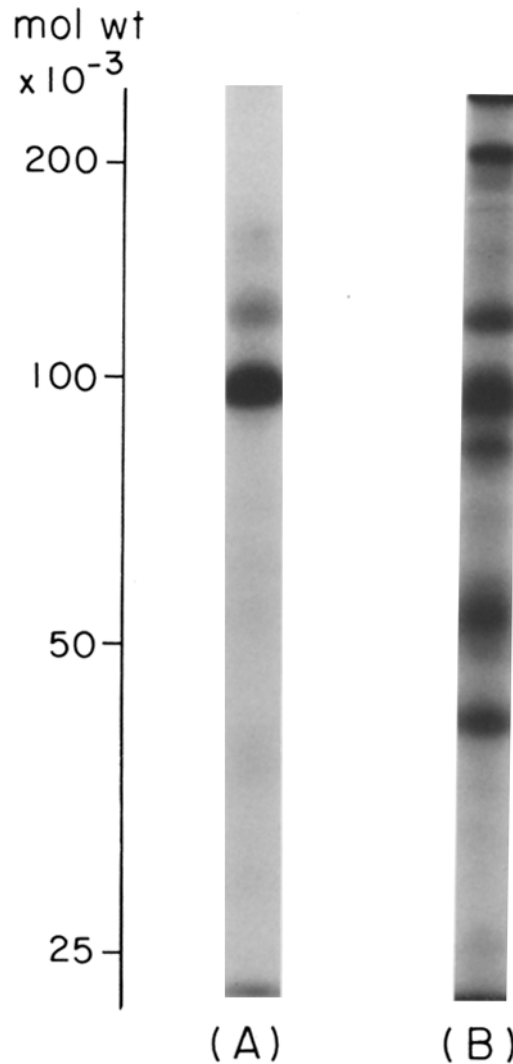


FIGURE 6 Comparison of autoradiographic patterns in slab gel electrophoresis of membranes isolated from  $^{125}\text{I}$ -labeled platelets (A) and membranes labeled with  $^{125}\text{I}$  after isolation on polylysine beads (B).

beads than in intact platelets, indicating greater protein exposure in the former. The intense labeling of the 100,000 mol wt band (GP III) in the bead preparation suggests that this protein is as freely available in vesicles as in the intact cell. This raises the possibility that this protein may have a transmembrane exposure. This may apply also to GP 125. A similar finding had been obtained with membranes isolated by sucrose gradient centrifugation (18). In contrast, when membranes on beads were  $^3\text{H}$ -labeled after enzyme treatment, some proteins incorporated less  $^3\text{H}$  (Fig. 7). This probably

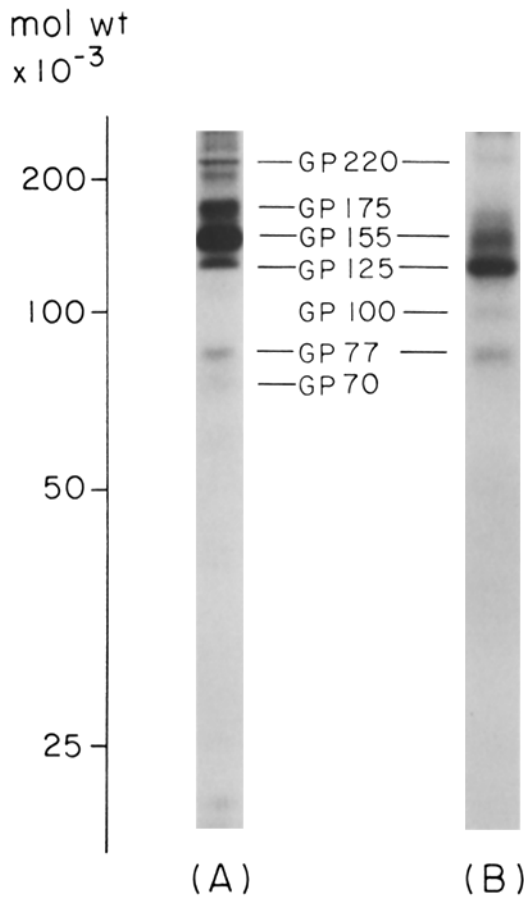


FIGURE 7 Comparison of fluorographic patterns of membranes isolated from platelets labeled with  $[^3\text{H}]$   $\text{NaBH}_4$  (A) and membranes labeled after isolation on polylysine beads (B).

reflects the decreased availability of glycoprotein sialic acid residues in membrane vesicles, some of which may be attached in an inside-out configuration, thus leading to the blockage in the attachment of the tritium label.

#### DISCUSSION

The present studies were performed to improve the conventional methods now available for the isolation of human platelet surface membranes. Several methods currently in use by most laboratories include sucrose density ultracentrifugation of washed cell homogenates or sonicates (29) and centrifugal separation of membrane vesicles obtained from glycerol osmotic lysis of washed whole platelets (2). One of the major difficulties with the present methodology is the relatively high degree

of contamination of the surface plasma membranes with intracellular membranes. Recently, Jacobson and Branton (10) reported a new technique for the isolation of surface membranes using polylysine beads. The technique which has been utilized successfully for the isolation of erythrocyte membranes and HeLa cell membranes (5) is based upon the electrostatic binding of positively charged polylysine beads and negatively charged whole cells, leading to the firm attachment of large areas of surface membrane which remain bound to the beads after washing and sonic disruption. The ionic environment in which cell attachment takes place is obviously an important variable which greatly influences the degree of membrane sticking. By light and scanning electron microscopy, we determined that maximum platelet bead attachment took place in a sucrose-acetate buffer, pH 5.2.<sup>1</sup>

Two major considerations prompted the development of the polylysine bead system for the isolation of platelet membranes: speed and purity. The entire separation procedure from the start to finish was easily accomplished within 1 d, generally taking 4–5 h. By avoiding osmotic lysis, sonication, and/or homogenation until the beads are effectively saturated with platelet surface membrane, it was possible to obtain a higher purity yield of surface membrane free of intracellular, mitochondrial, and/or granule membrane. The enzyme enrichment studies (Table III) demonstrate a reasonable degree of membrane purification. Of importance is the significantly smaller degree of intracellular organelle contamination of the membrane bead preparation compared to those obtained using sucrose gradient centrifugation of homogenates. The degree of purification of platelet membranes as assessed by surface iodination labeling was also higher than that by sucrose gradient ultracentrifugation (Table II).

By SDS gel electrophoretic studies it was possible to resolve a full complement of membrane surface glycoproteins, although some glycoproteins (GP 175 and GP 155) did not correspond completely to previously described membrane surface glycoproteins. The relationship among the several surface glycoproteins remains to be elucidated.

One of the questions which arises from these studies is whether polylysine beads may alter the structural integrity of the platelet membrane. By

<sup>1</sup> Kinoshita, T. Unpublished observations.



scanning electron microscopy (Fig. 1a), the beads were observed to be covered primarily with monolayers of adherent platelets; very few platelet aggregates were seen. No platelet release reaction took place under the conditions used during these studies (Table I). It is therefore probable that there was no significant induced alteration in the native platelet plasma membrane.

We conclude that polylysine beads provide a rapid, reproducible, and efficient technique for preparation of relatively pure platelet surface membranes. The method should be useful for the analysis of the surface organization of the plasma membrane of platelets.

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