Studies on the Proteins of Human Platelet Membranes*

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SUMMARY

Isolated human platelet membranes which have been analyzed by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis have been shown to contain a heterogeneous group of polypeptides of different molecular weight classes ranging from 10,000 to over 250,000. Three major glycoproteins have been identified in the platelet membrane. Some of the architectural relationships of the platelet membrane polypeptides were probed by analysis of the residual protein gel patterns following incubation of the intact cells with proteolytic enzymes. The membrane polypeptides in their native state in intact cells were relatively resistant to low dose Pronase digestion in contrast to the high sensitivity of the isolated membrane fragments to enzyme digestion. The membrane glycoproteins in intact cells were particularly susceptible to trypsin and papain action. Acetylcholinesterase was a readily accessible membrane constituent which was rapidly inactivated by small amounts of Pronase. N-Ethylmaleimide and [3H]p-chloromercuribenzenesulfonate labeling of intact whole platelet revealed three major membrane polypeptides with reactive sulfhydryl groups. Isotopic labeling of one of these membrane polypeptides was partially blocked by prior incubation of washed platelets with anti-F(ab')2 thrombosthenin fragments.

Platelets are cells which show unique properties in their ability to adhere to surfaces and aggregate with each other (1). The origin of mammalian platelets is significantly different from that of nucleated cells. Platelets are formed by fragmentation of the cytoplasm of bone marrow megakaryocytes. A mature megakaryocyte may give rise to up to 3000 platelets (2). The mechanism by which the megakaryocyte undergoes a regulated intracellular fragmentation is not known; however, prior to platelet appearance, demarcation vesicles and tissures appear which fuse to form a network of intracellular membranes which eventually become the plasma membrane of the circulating platelet (3). Born has stated that "the morphogenesis of the platelet membrane is clearly an exceptional event in biology of which there are apparently no other similar examples" (2).

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plasma membranes as well as intracellular granule membranes
was washed two times in 20 to 30 volumes of buffered saline
(pH 7.4, 0.15 M NaCl-0.0175 M phosphate) and sedimented at
114,000 x g. The protease and cathepsin inhibitors were also
included in the washings in some experiments. In general 40
μg of membrane protein were obtained from 5 x 10⁸ cells.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate
(SDS)—The over-all approach was that of Leonard (8). The
membranes were dialyzed with repeated changes for 24 hours
against 5 mM EDTA-5 mM 2-mercaptoethanol (pH 7.5) at 4°C.
In some experiments the cathepsin and protease inhibitors were
included in the dialysis fluids. Sufficient 10% SDS was then
added to the contents of the dialysis bag to make a final SDS
centration of 2%, and the sample heated to 100°C for 3 min
to complete solubilation. SDS acrylamide gel electrophoresis
was performed in 1% SDS 0.1 M sodium phosphate buffer (pH
7.0). The gels were 5 mm in diameter and 6 mm long, 5% cross-
linked, and contained the same concentrations of SDS and so-
dium phosphate buffer as used in the medium. Gels were run
for 1 hour before application. Sucrose was added to the sample
to permit layering between the buffer and the top of the gel.
All samples applied to the gel contained 1 μl of 0.1% pyronin Y per
100 μl of sample as a tracking dye. The gels were run 10 ma per
gel approximately 2½ hours to bring the tracking dye to the end
of the gel. They were fixed in 50% trichloroacetic acid over-
night, stained for 24 hours in 0.25% Coomassie blue in 20% tri-
chloroacetic acid, and then extensively rinsed with 70% acetic
acid using a diffusion destainer (Bio-Rad, Rockville Center,
New York). Molecular weights were estimated using known
markers including purified γ-globulin (mol wt 160,000), bovine
γ-criconalbumin (mol wt 60,000), egg ovalbumin (mol wt 45,000),
beef pancreas chymotrypsinogen A (mol wt 25,000), and sperm
whale myoglobin (mol wt 17,800) (9). Separate gels were stained
for glycoproteins using a periodic acid-Schiff stain allowing am
the addition to the incubation medium of 2 mg per ml of soybean
trypsin inhibitor. The membranes were then separated from both
preparations and the protein patterns compared by gel electro-
phoresis.

Papain—Platelets were separated from a unit of fresh whole
blood as previously described and washed twice in buffered saline
and once in Krebs-Ringer solution, pH 7.0, containing 1 mM
cysteine and 2 mM EDTA (15). The cells were suspended with-
out clumping in 25 ml of the Krebs-Ringer solution and equili-
brated for 10 min at 37°C. Insoluble carboxymethylcellulose
papain (2 mg per ml) was added and the cells incubated with
shaking for 30 min at 37°C under 5% CO₂-95% O₂. The cells
were separated by centrifugation, washed four times in 1 mM
EDTA saline, and homogenized, and the membranes were sepa-
rated and analyzed.

Trypsin—Fresh platelets were separated as above and washed
twice in buffered saline and once in Krebs-Ringer solution, pH
8.0, without Ca⁺⁺ or Mg⁺⁺ containing 2 mM EDTA and 1 mg
per ml of glucose. The cells were incubated in the Krebs-Ringer
solution containing 2 mg per ml of soluble trypsin for 60 min at
37°C under 5% CO₂-95% O₂. The reaction was terminated by
the addition to the incubation medium of 2 mg per ml of soybean
trypsin inhibitor. The membranes were then separated from the
washed cells and analyzed. In some experiments, the incubating
platelets were exposed to insoluble carboxymethylcellulose trypsin
(1 mg per ml) for 30 min at 37°C and the cell membranes were sub-
sequently analyzed.

Acetylcholinesterase Assay—Platelets were harvested from
fresh whole blood washed twice in buffered saline and once in
"total" Tris. Cell suspensions containing 500,000 to 600,000
platelets per mm³ were exposed to varying concentrations of
Pronase for 10 min at 37°C. The reactions were partially inhibited
by the addition of EDTA to a final concentration of 15 mM
and the cells were washed and homogenized in the presence of tolue-
sulfonyl fluoride. The isolated membranes were assayed for
enzyme activity as described by Saba and Mason (16). Identical
experiments were performed in which the cells were exposed to F(ab')₂ fragments of anti-thrombostatin (see below) at a con-
centration of 2.0 mg per 5 x 10⁸ cells for 30 min at 37°C. The
cells were then washed and the membranes were isolated and
assayed for enzyme activity.

N-Ethyl[³H]maleimide Labeling of Intact Platelet Membrane—
The following studies were performed in order to determine the
distribution of the platelet membrane polypeptides containing
reactive sulfhydryl groups. Platelets were isolated from fresh
whole blood, washed once in buffered saline and once in Krebs-
Ringer bicarbonate solution, pH 6.8, without Ca⁺⁺ or Mg⁺⁺
containing 1 mM EDTA. Platelets from 1 unit of whole blood
varying in separate experiments from 5 to 8 x 10⁸ cells were equili-
ibrated in 25 ml of Krebs-Ringer solution for 10 min at 37°C.

The abbreviation used is: SDS, sodium dodecyl sulfate.
Radioactive labeling of the cell suspension was carried out at an N-ethylmaleimide concentration of 1 x 10^{-3} M. Fifty microcuries of N-ethyl[14C]maleimide (N-ethyl[2,3-14C]maleimide of specific activity 2.0 Ci per mole from Amersham) were added as 0.5 ml of 50% ethanol and the cell suspension was incubated for 20 min at 37°C. It was necessary to solubilize the reagent in ethanol for reproducible labeling. The cells were separated and washed four times in saline containing 1 mM EDTA and 5 mM nonradioactive N-ethylmaleimide. The membranes were separated from the washed cells and analyzed by acrylamide gel electrophoresis.

* N-ethyl[14C]maleimide Labeling of Isolated Platelet Membranes—Membranes were obtained from nonlabeled platelets derived from 1 unit of fresh whole blood. The isolated membranes were incubated in 1 ml of Krebs-Ringer solution, pH 6.8, in the presence of 1 uCi of N-ethyl[14C]maleimide of specific activity 2.0 Ci per mole (Amersham) for 20 min at 37°C. The membranes were washed four times in saline containing 1 mM EDTA and 5 mM nonradioactive N-ethylmaleimide, dialyzed extensively in 5 mM EDTA and 5 mM 2-mercaptoethanol, solubilized in SDS, and analyzed on acrylamide gels.

* [3H]p-Chloromercuribenzenesulfonate Labeling of Intact Cells—The following studies were performed in order to determine the distribution of the platelet membrane polypeptides containing reactive sulphydryl groups using a radioactive label which did not penetrate the platelet membrane (17). Platelets obtained from a fresh unit of whole blood were washed in buffered saline once and in Krebs-Ringer solution (pH 7.4, containing 1.29 x 10^{-3} M citrate). The cells were equilibrated for 30 min at 37°C in the Krebs-Ringer solution and labeled for 30 min with [3H]p-chloromercuribenzenesulfonate at a final concentration of 0.4 x 10^{-4} M (specific activity 136 Ci per mole, Amersham). The cells were washed eight times in saline containing 1 mM EDTA and 50 mM cold p-chloromercuribenzenesulfonate. The membranes were isolated and subsequently analyzed by acrylamide gel electrophoresis.

**Immunoinhibition of N-Ethyl[14C]maleimide Labeling of Platelet Protein**—These studies were designed to demonstrate specific inhibition of platelet membrane polypeptide sulphydryl labeling by prior incubation of cell suspensions with various antibody fragment preparations.

**Antiserum**—Anti-fibrinogen and anti-albumin were commercial preparations (Behringwerke, Certified Blood Donor Service, Woodbury, New York). Anti-thrombosthenin was prepared against partially purified thrombosthenin as previously described (4). The antiserum was absorbed with lyophilized platelet-free human plasma and was shown to have marked cross-reactivity with human smooth muscle (uterine) actomyosin (18). The antisera were absorbed three times with 100 mg of BaSO_4_ and heated to 60°C for 30 min to remove clotting factors. The y-globulin fractions were precipitated by the addition of an equal volume of 30% Na_2SO_4_. The precipitate was washed twice with 15% Na_2SO_4_ and solubilized in 1 ml of sodium acetate buffer (0.1 M, pH 4.1), then dialyzed versus the same overnight. One-tenth milliliter of a pepsin solution in acetate buffer was added at an enzyme to substrate protein concentration ratio of 1:50 and the antibody solution was incubated for 24 hours at 37°C. The pH at the end of the incubation was adjusted to 8.0 with 1 N NaOH and Na_2SO_4_ was added to a final concentration of 18%. The precipitate containing F(ab')_2_ fragments was solubilized and dialyzed in buffered saline.

Platelets obtained from 1 unit of fresh whole blood were washed once in buffered saline and once in Krebs-Ringer bicarbonate buffer, pH 6.8, containing 1 mM EDTA. The cells were suspended in 10 ml of the Krebs-Ringer solution and 0.25 ml of antibody solution (2.5 mg of protein per ml) was added. The cells were incubated for 30 min at 37°C, centrifuged, and washed once in buffer. The cell suspension exposed to antibody fragments was reconstituted in 25 ml of Krebs-Ringer bicarbonate solution, pH 6.8, without Ca^{2+} or Mg^{2+} containing 1 mM EDTA and radioabeled with N-ethyl[14C]maleimide as described above.

**Chemical Assay**—Protein was measured by the method of Lowry et al. (19). Chloroform method extraction for lipid analysis was performed as described by Folch et al. (20).

**Thin Layer Chromatography**—Samples of the concentrated lipid extract from the acrylamide gel slices were analyzed on separate thin layer plates for phospholipid, gangliosides, and neutral glycolipids using appropriate solvent systems (21). These studies were kindly performed by Dr. Aaron Marcus (Cornell University Medical School).

**RESULTS**

**Electrophoretic Fractionation of Platelet Membrane Proteins**—Electrophoresis of platelet membrane proteins in 5% polyacrylamide gels containing 1% SDS revealed polypeptide heterogeneity (Fig. 1). The multiple protein bands ranged in molecular weight from approximately 10,000 to over 200,000 (Fig. 2B). This protein pattern was remarkably reproducible and was observed in over 90 different whole unit platelet preparations. When stained for carbohydrate by the periodic acid-Schiff procedure, electrophoretic analysis of the membrane proteins revealed the pattern seen in Figs. 1 and 2A. Three major carbohydrate bands were observed in the molecular weight range of 70,000 to 150,000. A dense fast moving apparently low molecular weight periodic acid-Schiff-positive band was not visualized in the corresponding Coomassie blue-stained gel. A similar band has been observed on electrophoretic analysis of SDS-solubilized red blood cell membrane proteins (8, 22). Unstained slices of gel from this fast moving periodic acid-Schiff-positive region were homogenized and extracted for lipid. Thin layer chromatography of this concentrated extract using several different buffer systems revealed no evidence of phospholipid, ganglioside, or neutral glycolipids. The techniques used were sensitive to 0.1 μg of phospholipid phosphorus and 0.5 μg of sialic acid. The possibilit-

![Fig. 1. SDS acrylamide gel analysis of human platelet membrane protein. Bottom, Coomassie blue-stained gel containing 40 μg of membrane protein. Top, periodic acid-Schiff-stained gel containing 120 μg of membrane protein. The anode was at the right.](http://www.jbc.org/content/247/12/4470)

ity has to be considered that this fast moving periodic acid-Schiff-positive region represents a small glycopeptide which was split off a larger membrane protein during the process of preparing the membrane material.

Previous studies (22) have demonstrated that proteolytic degradation of erythrocyte membranes due to associated cellular proteases may alter membrane protein patterns even in 1% SDS solutions. Accordingly, in some experiments the cells were homogenized and the membrane separated in the presence of the protease inhibitors N-carbenzoyl-L-glutamyl-L-tyrosine and toluenesulfonyl fluoride. No major membrane protein differences were seen in the preparations separated in the presence of these inhibitors.

**Sialic Acid Distribution**—The sialic acid distribution of the separated membrane proteins was determined by applying a modified assay of the Warren procedure (11) to sequential slices of the gel. The sialic acid was primarily confined to the periodic acid-Schiff-positive band region (Fig. 3). The slowest moving presumably highest molecular weight glycoprotein appeared to contain the largest amount of sialic acid. The middle band appeared to also contain a significant portion of sialic acid. The lowest molecular weight fastest moving periodic acid-Schiff-positive protein band had little or no associated sialic acid.

**Enzymatic Probing of Platelet Surface Relationships**—In order to determine the relative “availability” of the several membrane proteins to enzyme action, cells were incubated with various enzyme preparations for a given time period; the membranes were then separated and the membrane protein analyzed. Platelets derived from a unit of blood were gently digested with Pronase (0.2 mg per ml for 10 min at 37°). The enzymatic reaction was partially inhibited by the addition of EDTA and the cells were separated and washed. One aliquot of cells was homogenized in sucrose while an equal aliquot was homogenized in su-


**FIG. 4. The effect of Pronase on human platelet membrane protein.**  A, Coomassie blue-stained acrylamide gel containing 40 μg of membrane protein from cells incubated with 0.2 mg per ml of Pronase and homogenized in the absence of protease inhibitor.  B, Coomassie blue-stained acrylamide gel containing 40 μg of membrane protein from cells incubated with 0.2 mg per ml of Pronase and homogenized in the presence of protease inhibitor.
Other enzyme preparations were utilized to probe the steric availability of the membrane proteins. Cell suspensions were incubated with insoluble papain (2 mg of carboxycellulose papain at 37° for 30 min) and the membrane protein pattern was analyzed. In contrast to the action of Pronase, papain digestion was associated with significant degradation of the two larger glycoproteins. Minimal changes were observed in the Coomassie blue-stained membrane protein gel following papain action. Similar membrane protein changes were observed following the incubation of washed cells with trypsin solutions. Of interest was the relatively large amount of fast moving periodic acid-Schiff material behind the dye front in the membranes derived from the papain-treated cells. Insolubilized carboxymethylcellulose trypsin produced no significant changes in the membrane protein pattern probably due to the relatively low enzymatic activity of the preparation. Incubation of cell suspensions with soluble trypsin (2 mg per ml at 37° and 30 min) produced marked changes in the carbohydrate-containing bands.

Acetylcholinesterase Inactivation Studies—Previous studies have demonstrated cholinesterase activity in human platelet membranes (16). The following studies were designed to determine the effect of specific alterations of the platelet membrane on platelet cholinesterase activity.

Cell suspensions in Tris buffer were exposed to Pronase at varying concentrations for 10 min at 37°. The reactions were partially inhibited by the addition of EDTA, the cells were washed and homogenized in the presence of toluenesulfon fluoride, and the isolated membranes were assayed for enzyme activity. At low concentrations of Pronase, a relatively small amount of enzyme activity was inhibited (Fig. 5). At 0.2 mg per ml, a concentration at which no significant membrane protein changes were noted on gel electrophoresis (Fig. 4B), approximately 25% of the enzyme activity was blocked. The results suggest that the enzyme may be near the surface. At higher Pronase concentrations, marked inhibition of enzyme activity was observed. At these concentrations, membrane protein degradation was detected on acrylamide gel electrophoresis.

Immunoinhibition studies were performed by incubating aliquots of washed platelets with F(ab')2 fragments of rabbit anti-human thrombosthenin, rabbit anti-human fibrinogen, and rabbit anti-human albumin. No inhibition of membrane acetylcholinesterase activity was detected following these incubation studies.

N-Ethyl[14C]maleimide Labeling of Membrane Proteins—For these studies, platelets were isolated from a unit of fresh whole blood, washed and incubated with the radioactive label for 10 min at 37° in Krebs-Ringer solution. The cells were then isolated, washed, and homogenized. The isolated membranes obtained by sucrose density gradient ultracentrifugation were solubilized in SDS and the membrane proteins were analyzed. Separate gels were stained for carbohydrate and protein, while an identical gel was sliced and the radioactivity of the individual cuts was monitored. The results of a typical experiment are shown in Fig. 6. Three major N-ethyl[14C]maleimide-labeled membrane protein peaks were detected. Two minor fast moving isotopically labeled peaks in the molecular weight range of 10,000 to 20,000 were seen in some runs (Fig. 6A). However, this latter pattern was quite variable. In some runs only one minor peak labeled while in other runs a broadened nonpeaked area of radioactivity was observed in this region of the gel. The cause of this variability was not determined. The possibility has to be considered that the N-ethylmaleimide alcohol diluent used in these incubation experiments was associated with some of the variability in isotopic labeling. N-Ethyl[14C]maleimide labeling of the whole cells did not lead to any significant alteration in the periodic acid-Schiff or Coomassie blue staining of the membrane proteins. The three major N-ethyl[14C]maleimide-labeled peaks

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**Fig. 5.** Acetylcholinesterase activity of membranes obtained from digested cells.
TABLE I

<table>
<thead>
<tr>
<th>Subcellular distribution of N-ethyl[14C]maleimide</th>
<th>Radioactivity incorporated %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble</td>
<td>61</td>
</tr>
<tr>
<td>Membrane</td>
<td>26</td>
</tr>
<tr>
<td>Granules</td>
<td>13</td>
</tr>
</tbody>
</table>

A similar pattern of N-ethyl[14C]maleimide labeling was observed when isolated purified membranes were incubated with [35S]p-Chloromercuribenzenesulfonate. It would appear from these studies that the fragmented membrane vesicles as well as the intact platelet plasma membrane have a similar distribution of N-ethyl[14C]maleimide binding sites. Studies by others have demonstrated that this reagent penetrates cell membranes and is distributed in intracellular sites (23).

Studies by others have demonstrated that this reagent penetrates cell membranes and is distributed in intracellular sites (23). Table I demonstrates the subcellular distribution of label in a typical experiment. For this study, a unit of platelets was labeled with the isotope and the distribution of radioactivity determined in the separate subcellular compartments.

In order to evaluate the possibility that the N-ethyl[14C]maleimide-labeling pattern of whole cells in fact represented intracellular labeling of the "inside" of the membrane due to the interiorization of the radioactive label, additional incubation studies were performed. [35S]p-Chloromercuribenzenesulfonate, a reagent which also binds to sulfhydryl groups but does not penetrate the cell membrane, was utilized (17).

[35S]p-Chloromercuribenzenesulfonate labeling of whole cells was repeated and a labeling pattern generally similar to that observed with N-ethyl[14C]maleimide (Fig. 7). Two peaks of labeling using the [35S]p-Chloromercuribenzenesulfonate label were repeatedly observed in the high molecular peak (200,000 to 300,000) while only one peak was detected in this region using the N-ethyl[14C]maleimide label. The significance of this difference remains to be explored.


Immunoinhibition of Sulphydryl Labeling—For these studies, washed platelets were incubated in Krebs-Ringer bicarbonate buffer with anti-thrombosthenin F(ab')2 fragments or nonimmune F(ab')2 fragments and then incubated with N-ethyl[14C]maleimide (88 μg of membrane protein on each gel). B, Coomassie blue-stained acrylamide gel of 38 μg of membrane protein from cells incubated with anti-thrombosthenin F(ab')2 fragments and the N-ethyl[14C]maleimide.

[35S]p-Chloromercuribenzenesulfonate label were repeatedly observed in the high molecular peak (200,000 to 300,000) while only one peak was detected in this region using the N-ethyl[14C]maleimide label. The significance of this difference remains to be explored.

Fig. 8. Immunoinhibition of N-ethyl[14C]maleimide labeling of human platelet membrane protein. A, isotopic labeling pattern of cells incubated with anti-thrombosthenin F(ab')2 fragments or nonimmune F(ab')2 fragments and then incubated with N-ethyl[14C]maleimide (38 μg of membrane protein on each gel). B, Coomassie blue-stained acrylamide gel of 38 μg of membrane protein from cells incubated with anti-thrombosthenin F(ab')2 fragments and the N-ethyl[14C]maleimide.

DISCUSSION

Heterogeneity of Membrane Polypeptides—The membranes of human platelets are known to contain a variety of proteins which can be fractionated according to their molecular weight in SDS polyacrylamide gels (24). The protein in the membrane is heterogeneous and consists of different molecular weight classes of...
polypeptide chains ranging from 10,000 to over 250,000. The presence of very high molecular weight polypeptide chains in the platelet membrane is in agreement with similar observations by others on erythrocyte membranes (8) and confirms Gwynne and Tanford's prediction that these high molecular weight polypeptide chains may be constituents of other cellular membranes (25). The function of these unusual polypeptides in cell membranes is not yet defined; however, it is of interest that Baenziger et al. have recently described the presence of a thrombin-sensitive protein with a molecular weight of 190,000, in a crude preparation of human platelet membranes (26). Three major glycoproteins with apparent molecular weight ranges from 70,000 to 150,000 were detected in the human membrane preparation. In view of the previously reported anomalous electrophoretic behavior of glycoproteins in SDS gels, these molecular weights should be regarded as approximate (27). The bulk of the membrane sialic acid was associated with two of these more slowly moving glycoproteins. Pepper and Jamieson have previously demonstrated that platelet membrane glycoproteins contain heterosaccharide units significantly different from those isolated from the erythrocyte membrane (28).

Previous authors have stressed the potential danger of proteolytic degradation producing artifacts in sample material analyzed by electrophoresis in SDS gels; these molecular weights should be regarded as approximate (27). The bulk of the membrane sialic acid was associated with two of these more slowly moving glycoproteins. Pepper and Jamieson have previously demonstrated that platelet membrane glycoproteins contain heterosaccharide units significantly different from those isolated from the erythrocyte membrane (28).

Previous studies have shown that N-ethylmaleimide binds to the platelet contractile protein system thrombosthenin and is capable of inhibiting both super precipitation and ATPase activity of the isolated contractile protein (23). We have previously demonstrated that at least part of the thrombosthenin of the human platelet was associated with the subcellular membrane fraction (4). Recent studies by Bovsey et al. using an immunohistochemical stain have demonstrated thrombosthenin on the surface of platelets (36). These findings suggested the possibility that one of the membrane polypeptides containing reactive sulfhydryl groups might be surface thrombosthenin. To test this possibility, immunoinhibition of radioactive sulfhydryl labeling by thrombosthenin antibody fragments was performed. Thrombosthenin F(ab')2 fragments were used in these studies to avoid aggregation-induced damage of washed cells by the intact antibody (37). In multiple studies, prior incubation of platelets with thrombosthenin antibody fragments...
was associated with a significant decrease in the N-ethyl[14C]-maleimide labeling of a membrane protein in the molecular weight range of 80,000. Nonimmune serum as well as other antibody F(ab')2 fragments did not alter the membrane labeling pattern. These observations suggest that one of the N-ethyl[14C]maleimide-labeled membrane polypeptides is related to the contractile thrombosthenin system. Recent studies suggest that this system is composed of several molecular species including platelet myosin, platelet actin, platelet myosin rod, platelet myosin head, and probably platelet tropomyosin (38). The exact chemical nature of the N-ethyl[14C]maleimide-labeled membrane polypeptide awaits further study.

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