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p-Chloromercuribenzoate-induced dissociation of cytoskeletal proteins in red blood cells of rats

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Effects of *p*-chloromercuribenzoate (PCMB) on the cytoskeletal organization of rat red blood cells were studied. Upon incubation with 50 μM PCMB in 10 mM Tris-HCl (pH 7.4) at 37 °C for 30 min, 80% of actin and 45% of spectrin were released from the ghosts, resulting in the fragmentation of ghost membranes. Addition of 2 mM Mg^{2+} or 0.1 M KCl, or lowering incubation temperature to 0 °C substantially inhibited the solubilization of the cytoskeletal proteins and the fragmentation of ghost membranes, which enable to examine the effects of PCMB on the interaction between transmembrane proteins and the peripheral cytoskeletal network. Decreased recoveries of transmembrane proteins, such as band 3 and glycophorin, in Triton shell fraction were observed in the ghosts incubated with PCMB either in the presence of Mg^{2+} or at 0 °C. PCMB also inhibited the in vitro association of purified spectrin with spectrin-depleted inside-out vesicles through interaction with proteins in the vesicle, such as bands 2.1 and 3. In the PCMB-treated ghosts, intramembrane particles were highly aggregated, which further supports the PCMB-induced dissociation of the transmembrane proteins from the cytoskeletal network. The decreased recovery of glycophorin in the Triton shell fraction also observed in intact red blood cells upon incubation with PCMB. These results suggest that the main action of PCMB on red cell membranes under physiological condition, at higher ionic strength and in the presence of Mg^{2+}, is to dissociate transmembrane proteins from the peripheral cytoskeletal network, which may modify functions of these proteins.

Introduction

Evidence has been accumulated that in red blood cells the interaction of peripheral cytoskeletons with bilayer membranes, which may be dynamic through band 2.1 and other connecting proteins, plays an important role in maintaining cell shape [1–4]. Through these interactions, the cytoskeletal network composed of bands 1, 2 (spectrin), 5 (actin) and 4.1 provides elasticity to the cell membrane and prevents the fragmentation of cell membranes [3].

p-Chloromercuribenzoate (PCMB), a sulfhydryl-reactive compound, is known to be a potent effector of protein–protein interactions and to solubilize spectrin and actin from red cell membranes, resulting in the membrane fragmentation [5–7]. Recently, Chetrite et al. [8] showed that PCMB in the micromolar range induced drastic disorganization of red cell membranes without

Abbreviations: PCMB, *p*-chloromercuribenzoate; PCMBS, *p*-chloromercuribenzenesulfonate; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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appreciable release of spectrin and actin, suggesting that the action of PCMB on red cell membranes may consist in weakening some of the links between oligomeric actin, band 3 and other components of the red cell cytoskeleton. These studies, however, were carried out under conditions of low ionic strength in the absence of divalent cations. It is of importance to clarify the precise actions of PCMB on the red cell cytoskeleton under physiological conditions, at higher ionic strength and in the presence of Mg^{2+}. In the present study, we examined the action of PCMB on rat red cell ghosts in the presence of $MgCl_2$ and/or KCl at physiological concentrations, focusing on the interaction of transmembrane proteins, such as band 3 and glycophorin, with the cytoskeletal network, in addition to the organization of spectrin and actin.

In this report, we demonstrate that PCMB dissociates transmembrane proteins from the cytoskeletal network in the presence of Mg^{2+} at 2 mM, resulting in the redistribution of intramembrane particles.

Materials and Methods

Preparation and incubation of red cell ghosts

Red cell ghosts were prepared from blood samples obtained from male Jcl : Wistar rats (440–470 g, CLEA-Japan Co., Tokyo) as described before [9]. The washed ghosts were suspended in 5 vols. of 10 mM Tris-HCl (pH 7.4) (0.65 mg ghost protein/ml) containing PCMB, $MgCl_2$ and KCl at the concentrations indicated subsequently and incubated at 0 or 37 °C for up to 60 min. In some experiments, [*carboxy*-^{14}C]PCMB diluted to 8 μCi/μmol (Commissariat a l'Energie Atomique, Cedex, France) was supplemented. Then the incubation mixtures were centrifuged at $22\,000 \times g$ for 20 min at 2 °C and the resulting supernatants were subjected to the determination of released proteins. The pelleted ghosts were washed once with 10 vol of 10 mM Tris-HCl (pH 7.4) and used for the preparation of Triton shells.

Ghosts were also prepared from red blood cells treated with PCMB (0–4 mM) in 50 mM glycylglycine (pH 7.4), containing 5 mM KCl, 116 mM NaCl, 11.1 mM glucose, 0.54 mM adenine and 12.7 mM inosine for 30 min at 37 °C and subjected to the preparation of Triton shells.

Preparation of Triton shells from red cell ghosts

Red cell ghosts were suspended in 10 vol of 0.5% (v/v) Triton X-100 solution containing 0.1 M KCl, 10 mM Tris-HCl (pH 7.4) and 20 μg/ml phenylmethylsulfonyl fluoride (solution A) and stayed at 2 °C. After 10 min, Triton shells (Triton-insoluble residues) were pelleted by centrifugation at $22\,000 \times g$ for 20 min at 2 °C. In some cases, the mixture was applied on to linear sucrose gradient (10%–70% in 10 mM Tris-HCl (pH 7.4) and 0.1 M KCl) and centrifuged at $100\,000 \times g$ for 90 min at 2 °C, and then visible bands corresponding to Triton shells were recovered.

SDS-polyacrylamide gel electrophoresis

Electrophoresis in 10% polyacrylamide gel containing 0.1% SDS was performed according to the method of Laemmli [10]. In case of [^{14}C]PCMB-supplement, samples were prepared in the absence of 2-mercaptoethanol. Gels were stained with Coomassie brilliant blue R-250 or periodic acid-Schiff reagent as described by Fairbanks et al. [11]. Autoradiogram of stained and dried gels was obtained as described before [12]. Stained gels and autoradiogram were scanned in a densitometer (CS-930, Shimadzu Corp., Kyoto, Japan). The area of each peak was integrated with a digitizer.

Electron microscopic observation

The ghosts incubated with PCMB were washed and suspended with solution A. One droplet of this suspension was placed on to a 200-mesh copper grid with formvar film prepared according to the method of Byers and Branton [13]. Triton shells on the grid then were stained with 2% uranyl acetate and examined at 80 kV in an electron microscope (JEM-100C, JEOL, Tokyo, Japan).

A part of incubated ghosts were pelleted at 30 °C, mixed with 10 vols. of 1% glutaraldehyde in 12 mM Hepes (pH 7.4) and 2 mM $MgCl_2$ and fixed for 2 h at room temperature. The fixed ghosts were subjected to freeze-fracture electron microscopy as described before [14].

Reassociation of spectrin with spectrin-depleted inside-out vesicles

Spectrin and spectrin-depleted inside-out

vesicles prepared from rat red cell ghosts were incubated at the concentrations of 80 and 220 μg protein/ml, respectively, in 0.7 mM sodium phosphate (pH 7.6) containing 20 mM KCl, 1 mM $MgCl_2$ and PCMB (0–100 μM) for 90 min at 0 °C as described by Bennett and Branton [15]. After removal of unbound spectrin, the amount of spectrin associated with the vesicles was determined by SDS-polyacrylamide gel electrophoresis and densitometry.

Other methods

Microscopic photographs of ghosts were obtained in a dark-field microscope (Optiphoto, Nikon, Tokyo, Japan). The protein content was estimated by the method of Lowry et al. [16].

Results

Solubilization of spectrin and actin from red cell ghosts induced by PCMB

When red cell ghosts were incubated with PCMB (0–100 μM) in 10 mM Tris-HCl (pH 7.4) for 30 min at 37 °C, the amount of spectrin and actin solubilized increased in a dose-dependent manner and reached a plateau at 50 μM, where 80% of actin and 45% of spectrin were solubilized (Fig. 1a). The solubilization of both proteins was inhibited by lowering the incubation temperature and that of spectrin was completely inhibited at 0 °C (Fig. 1a). This PCMB-induced solubilization of cytoskeletal proteins at 37 °C completed within 30 min (Fig. 1b).

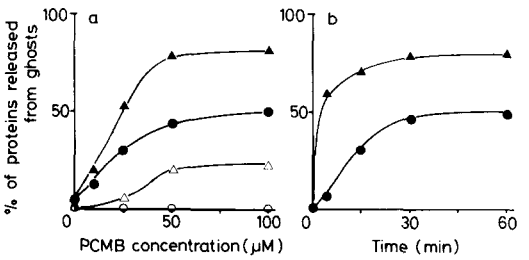


Fig. 1. Effects of PCMB concentration, incubation period and temperature on the solubilization of spectrin and actin. (a) Ghosts were incubated at 37 (closed symbols) or 0 °C (open symbols) for 30 min in the presence of 0–100 μM PCMB and the amounts of solubilized spectrin (●○) and actin (▲△) were determined as described in Materials and Methods. (b) Ghosts were incubated with 50 μM PCMB at 37 °C for up to 60 min and the amounts of solubilized spectrin (●) and actin (▲) were determined.

Effect of Mg^{2+} on the PCMB-induced solubilization of spectrin and actin

The PCMB-induced solubilization of spectrin and actin from ghosts was significantly inhibited by $MgCl_2$ (Fig. 2). The solubilization of spectrin was completely inhibited by Mg^{2+} at 0.5 mM and that of actin was decreased to 20% at 2 mM, a concentration equal to that in the red cell cytosol. Ca^{2+} at 2 mM or KCl at 0.1 M also showed inhibitory effects on the PCMB-induced solubilization to the same extent as Mg^{2+} at 2 mM (data not shown). The treatment with 50 μM PCMB induced a severe fragmentation of ghost membranes (Fig. 3b), which did not develop in the presence of Mg^{2+} at 2 mM (Fig. 3d). Even in the presence of Mg^{2+}, however, PCMB altered the shape of ghosts from discoidal to somewhat spherical (Figs. 3c and 3d).

Binding of [^{14}C]PCMB to ghost proteins

Red cell ghosts were incubated with [^{14}C]PCMB in 10 mM Tris-HCl (pH 7.4) for 30 min at 37 °C and the amount of [^{14}C]PCMB bound to each ghost protein was estimated by autoradiography after separation with SDS-polyacrylamide gel electrophoresis. As shown in Fig. 4a, PCMB was bound to most of ghost proteins, especially to bands 3, 4.2 and 5 (actin), and the binding reached a maximum at 50 μM and completed within 5 min (Fig. 4c).

Moreover, the binding was not affected by the

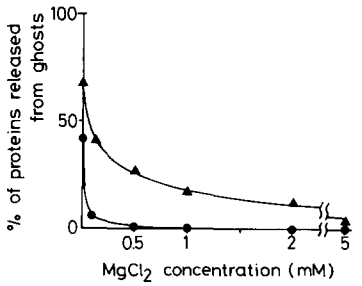


Fig. 2. Effect of Mg^{2+} on the PCMB-induced solubilization of spectrin and actin. Ghosts were incubated with 50 μM PCMB in the presence of 0–5 mM Mg^{2+} for 30 min at 37 °C and the amount of solubilized spectrin (●) and actin (▲) was determined as described in Materials and Methods.

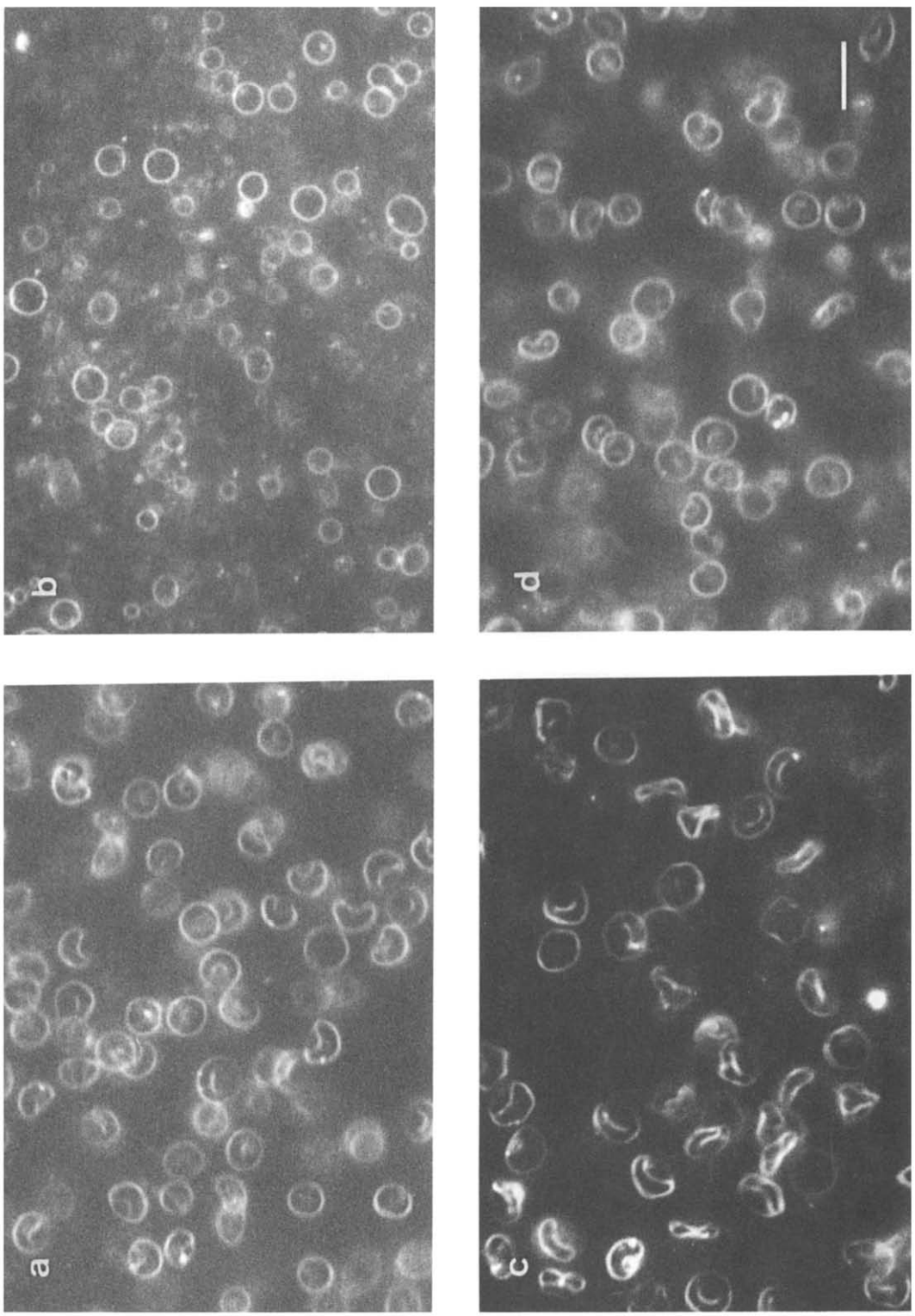


Fig. 3. Effects of PCMB and Mg^{2+} on the shape of ghosts. Ghosts were incubated with (b, d) or without 50 μM PCMB (a, c) in the presence (c, d) or absence of 2 mM Mg^{2+} (a, b) for 30 min at 37 °C, and were photographed in a dark-field microscope. Scale bar, 10 μm.

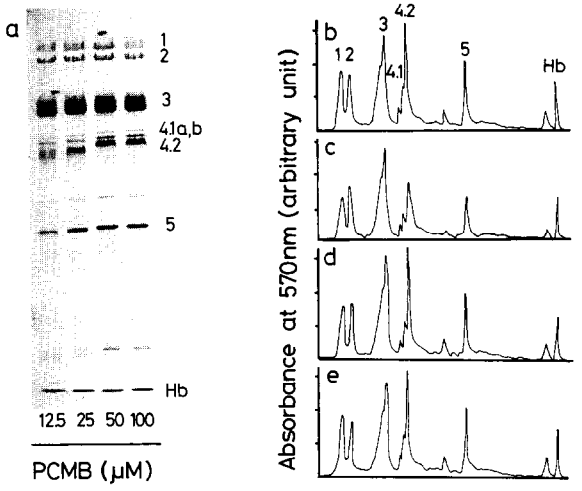


Fig. 4. Binding of [^{14}C]PCMB to ghost proteins. Ghosts were incubated with 12.5–100 μM PCMB containing [^{14}C]PCMB in the absence of Mg^{2+} for 30 min at 37 °C (a) or with 50 μM PCMB in the presence (c, e) or absence of 2 mM Mg^{2+} (b, d) for 5 (c) or 30 min (b, d, e) at 0 (d) or 37 °C (b, c, e). After separation by SDS-polyacrylamide gel electrophoresis, proteins binding [^{14}C]PCMB were visualized by autoradiography. The obtained autoradiogram (a) was scanned in a densitometer (b–e).

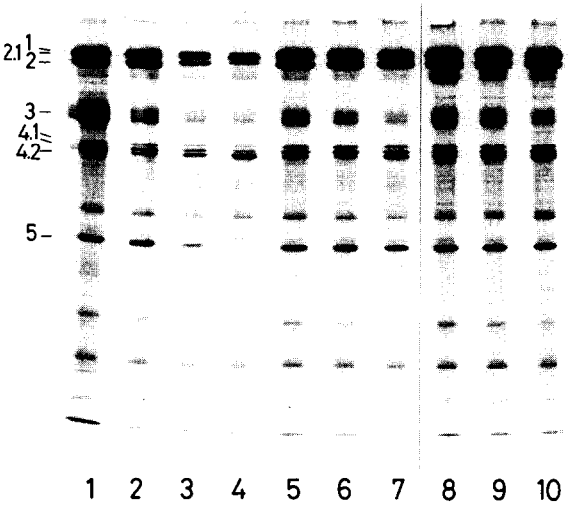


Fig. 5. SDS-polyacrylamide gel electrophoretogram of Triton shells prepared from ghosts treated with PCMB. Ghosts were incubated with 0 (lanes 2, 5, 8), 25 (lanes 3, 6, 9) or 50 μM PCMB (lanes 4, 7, 10) in the absence (lanes 2–7) or presence of 2 mM Mg^{2+} (lanes 8–10) for 30 min at 0 (lanes 5–7) or 37 °C (lanes 2–4, 8–10). Untreated ghosts (lane 1) and Triton shells prepared from those ghosts were subjected to SDS-polyacrylamide gel electrophoresis.

presence of Mg^{2+} at 2 mM or lowering the incubation temperature to 0 °C (Figs. 4e and 4d).

Protein composition and shape of Triton shells prepared from PCMB-treated ghosts

Fig. 5 shows the electrophoretogram of Triton-insoluble residues (Triton shells) obtained from the ghosts incubated with PCMB (0–50 μM) in the absence or presence of 2 mM Mg^{2+}, at 37 or 0 °C for 30 min. Triton shells prepared by either simple centrifugation or sucrose linear gradient centrifugation gave essentially the same pattern (data not shown). The incubation with PCMB at 37 °C in the absence of Mg^{2+} resulted in a decreased recovery of most ghost proteins into the Triton shell fraction except bands 2.1 and 4.2, which increased in this fraction in a dose-dependent manner (Fig. 5, lanes 2–4). The increased recovery of band 4.2 resulted from PCMB-induced denaturation and precipitation of this protein, since band 4.2 was specifically precipitated from Triton-soluble fraction obtained from untreated ghosts upon incubation with PCMB at 37 °C (data not shown). The incubation with PCMB at 0 °C or in the presence of Mg^{2+} at 2 mM decreased the recovery of band 3 into the Triton shell fraction (Fig. 5, lanes 5–7 and 8–10). The increased recovery of band 4.2 was also observed in the presence of Mg^{2+} (Fig. 5, lanes 8–10) but not at 0 °C (lanes 5–7), suggesting its temperature depend-

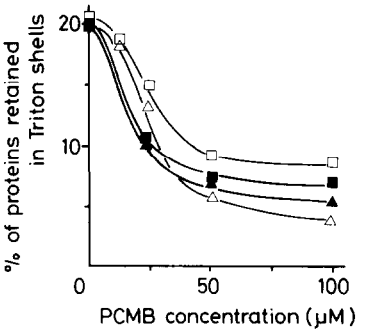
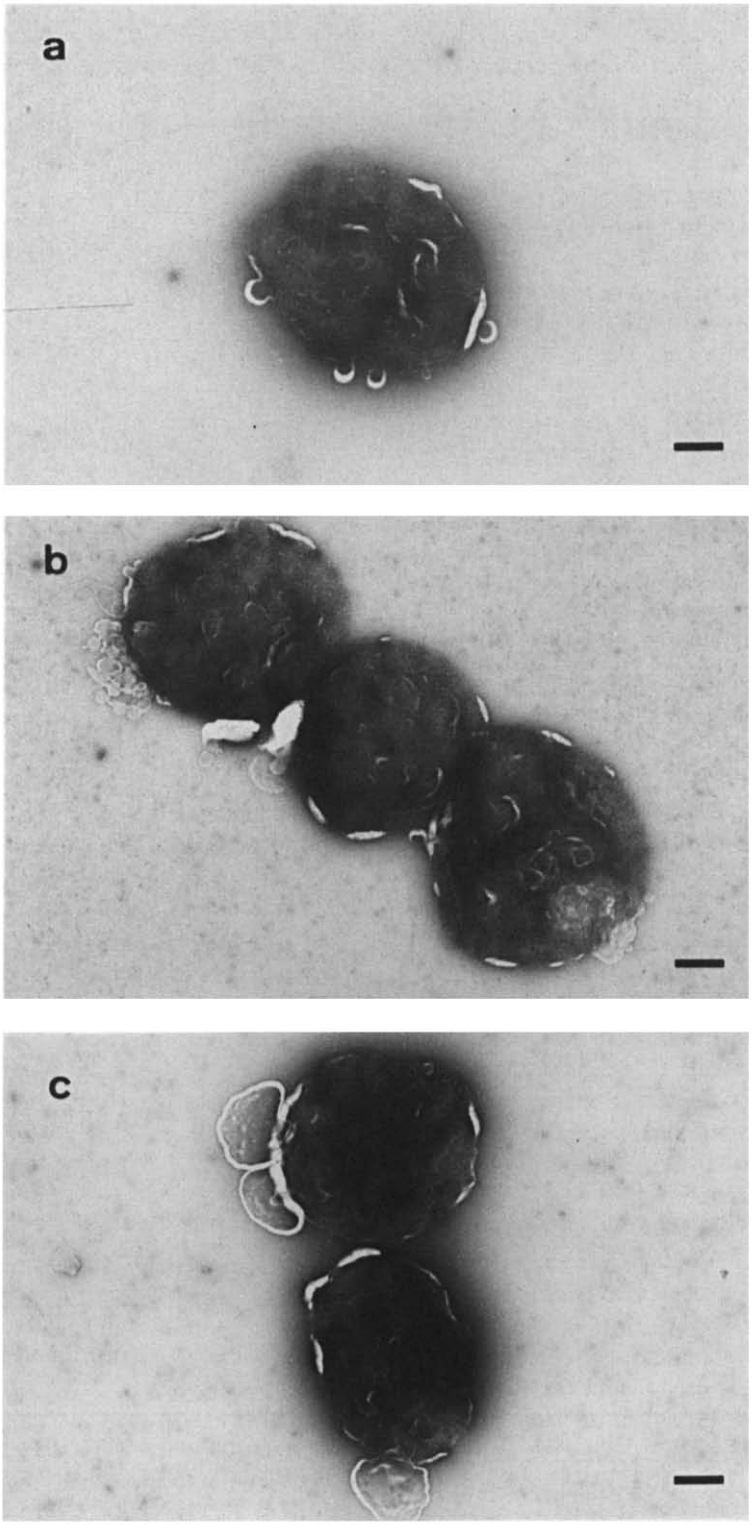


Fig. 6. Decreased recovery of transmembrane proteins in Triton shell fractions prepared from PCMB-treated ghosts. Ghosts were incubated with 0–100 μM PCMB in the presence of 2 mM Mg^{2+} at 37 °C (open symbols) or in the absence of Mg^{2+} at 0 °C (closed symbols) for 30 min. The amount of bands 3 (■□) and glycophorin (▲△) in Triton shell fractions was determined as described in Materials and Methods.


a
b
c

ency. In addition to band 3, the amount of recovered glycophorin (periodic acid-Schiff reagent reactive protein, molecular weight about 40000) also decreased in a dose-dependent manner under the both incubation conditions and reached a plateau at 50 μM (Fig. 6).

The shape of Triton shells was examined in a transmission electron microscope after staining with uranyl acetate. Triton shells obtained from ghosts incubated in the presence of Mg^{2+} at 2 mM either without or with PCMB (25 or 50 μM) exhibited essentially the same shape (Fig. 7).

Inhibitory effect of PCMB on the association of spectrin with spectrin-depleted inside-out vesicles

To clarify the action of PCMB on the interaction of transmembrane proteins with the peripheral cytoskeletal network, the effect of PCMB on the association of purified spectrin (Fig. 8a, lane 4) with spectrin-depleted inside-out vesicles (Fig. 8a, lane 2) was examined. As shown in Fig. 8b, PCMB inhibited the association of spectrin in a dose-dependent manner. Since the spectrin purified from PCMB-treated ghosts associated with the vesicles to the same extent as the spectrin from untreated ghosts did (data not shown), the inhibitory effect of PCMB could be attributed to its action on the proteins in the vesicles, such as bands 2.1, 3 and 4.2 and glycophorin.

Effect of PCMB on the distribution of intramembrane particles in ghost membranes

It has been shown that the distribution of intramembrane particles in red cell membranes is significantly affected by the treatments that modify the organization of spectrin networks, such as isoelectric precipitation, low ionic strength extraction, and incubation with polycations [17–19]. In the ghost membrane treated with PCMB (50 μM) at 37 °C in the presence of 2 mM Mg^{2+}, intramembrane particles were highly aggregated (Fig. 9b), while those in the ghosts incubated in the absence of PCMB were distributed almost evenly (Fig. 9a).

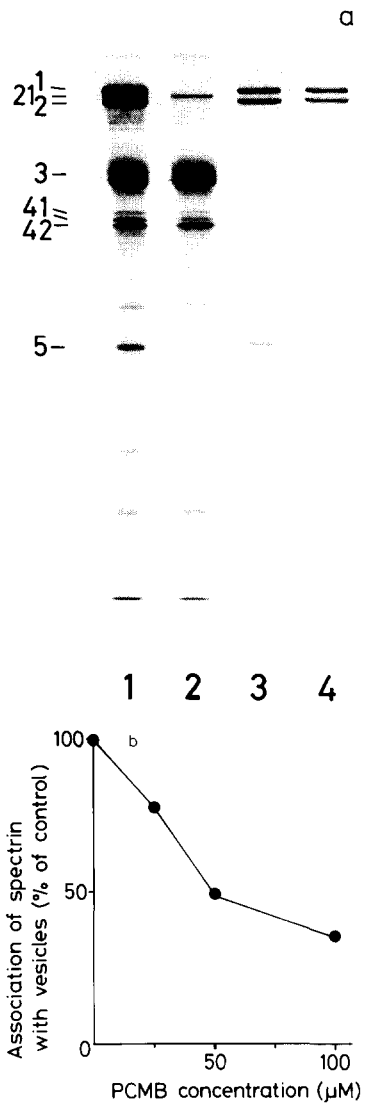


Fig. 8. Effect of PCMB on the association of spectrin with spectrin-depleted inside-out vesicles. Purified spectrin (a, lane 4) was incubated with spectrin-depleted inside-out vesicles (a, lane 2) in the presence of 0–100 μM PCMB. The amount of associated spectrin was determined as described in Materials and Methods (b). Electrophoretograms of ghosts (a, lane 1) and crude spectrin (a, lane 3) were also shown.

Fig. 7. Electron micrographs of negatively-stained Triton shells. Ghosts were incubated with 0 (a), 25 (b) or 50 μM PCMB (c) in the presence of 2 mM Mg^{2+} at 37 °C for 30 min. Triton shells prepared from the ghosts were stained with uranyl acetate and photographed in an electron microscope. Scale bar, 1 μm.

Decreased recovery of glycophorin in the Triton shells prepared from red blood cells treated with PCMB

It is of interest to know whether the dissoci-

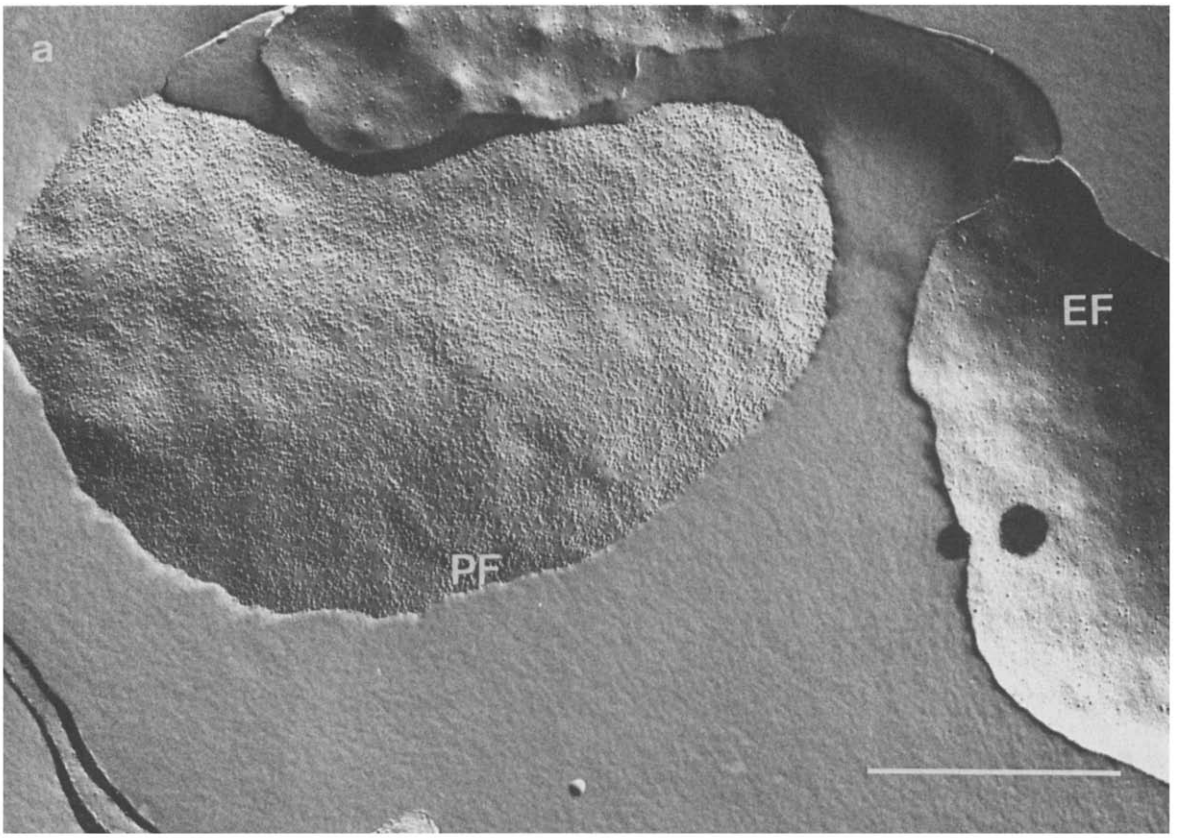


Fig. 9. Freeze-fracture electron micrographs of PCMB-treated and untreated ghosts. Ghosts were incubated with (b) or without 50 μM PCMB (a) in the presence of 2 mM Mg^{2+} at 37° C for 30 min and photographed as described in Materials and Methods. EF, external fracture face; PF, protoplasmic fracture face. Scale bar, 1 μm.

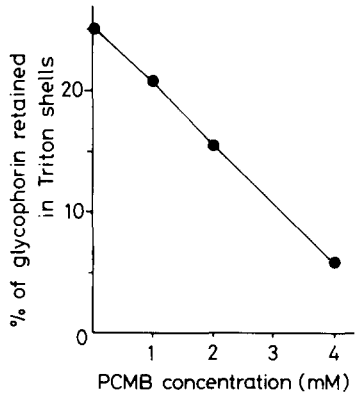


Fig. 10. Decreased recovery of glycophorin in Triton shell fractions prepared from PCMB-treated red blood cells. Ghosts were prepared from red blood cells incubated with 0–4 mM PCMB at 37° C for 30 min. The amount of glycophorin in Triton shell fractions from the ghosts was determined as described in Materials and Methods.

ation of integral proteins from the peripheral cytoskeletal network as observed in PCMB-treated ghosts also occurs in intact red blood cells upon incubation with PCMB. Ghosts were prepared from red blood cells after incubation with PCMB (0–4 mM) for 30 min at 37° C, which induced no significant hemolysis (data not shown). The amount of glycophorin recovered in Triton shell fraction decreased depending on the concentration of PCMB (Fig. 10).

Discussion

The present study indicates that PCMB displays at least two separable actions on rat red cell ghosts. The first is a well-known action to solubilize peripheral cytoskeletal proteins, such as spectrin and actin, which produces the fragmentation

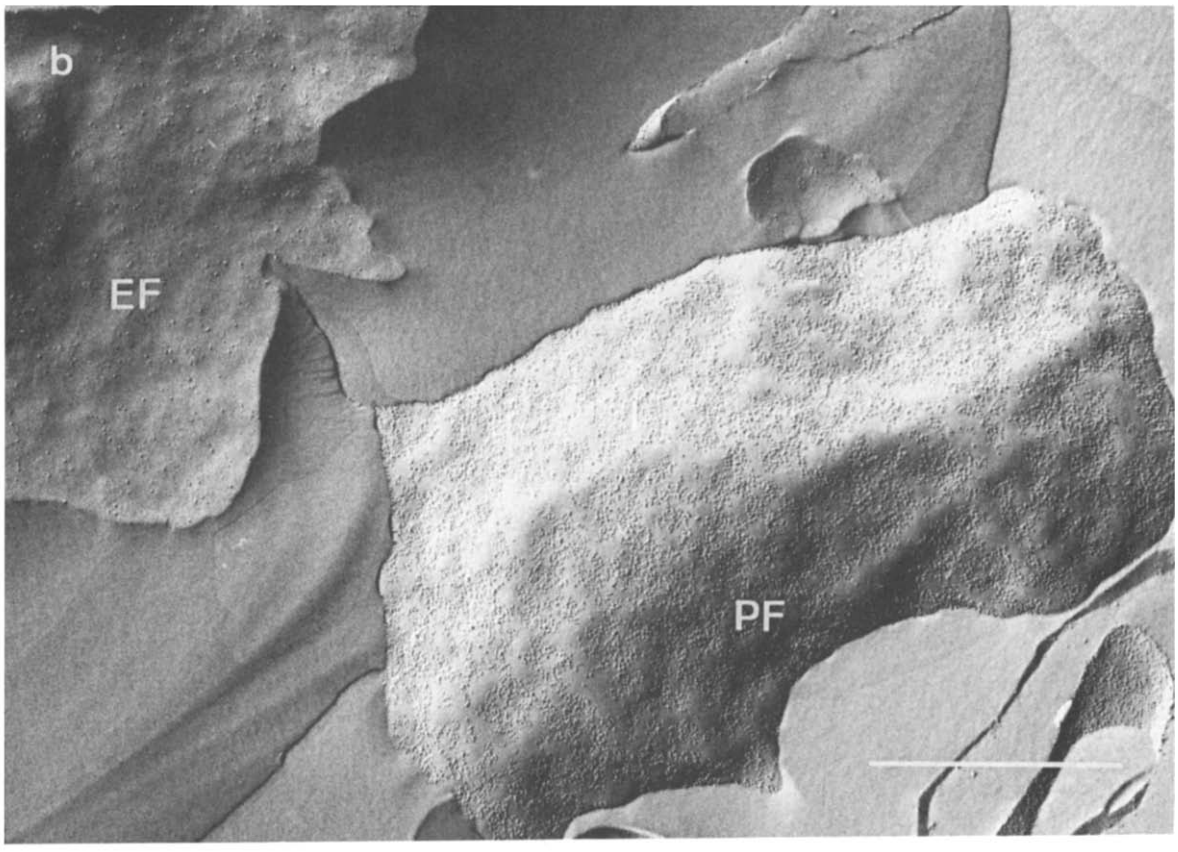


Fig. 9. Continued.

of ghost membranes. The second is to dissociate transmembrane proteins, such as band 3 and glycophorin, from the peripheral cytoskeletal network, which can be observed even after inhibiting the solubilization of spectrin and actin by either lowering the incubation temperature to 0 °C, raising ionic strength or adding divalent cations, such as Mg^{2+} and Ca^{2+}. Similar results were obtained using human red cell ghosts under the same conditions but at lower PCMB concentrations (unpublished results).

It is well documented that spectrin and actin are solubilized from the ghosts by the treatment with low concentrations (< 0.1 mM) of PCMB or *p*-chloromercuribenzenesulfonate (PCMBS) [6,7]. The solubilization of spectrin indicates that interactions between spectrin and transmembrane proteins via bands 2.1 or 4.1 are weakened either by modification of spectrin, band 2.1, band 4.1 or combination of these proteins. The solubilization of actin results from depolymerization of actin or weakening of the interaction of actin with spectrin-band 4.1 complex. The preferential reaction of [^{14}C]PCMB with actin (Fig. 4) strongly suggests a causal role of modification of actin. At higher concentrations (0.5–1.0 mM), bands 2.1, 4.2 and 6 were solubilized in addition to spectrin and actin [6,7], suggesting a weakening of the interaction of these proteins with the cytoplasmic domain of band 3. This result and the preferential binding of [^{14}C]PCMB to band 3 (Fig. 4) support a causal role of band 3 modification in the solubilization of spectrin. Decreased recovery of band 3 in Triton shells, which were preapred from ghosts treated with PCMB in the absence of the solubilization of spectrin and actin (Fig. 6), provides evidence for weakening of the interaction of band 3 and band 2.1. PCMB also inhibited the association of spectrin with spectrin-depleted inside-out vesicles (Fig. 8) through interaction with vesicle proteins but

not with spectrin. This result is consistent with the observation of Bennett [20] that *N*-ethylmaleimide, another well-known thiol-reactive compound, inhibited the binding of spectrin with the vesicles through interaction with band 2.1. At present it cannot be decided whether weakening of the interaction of band 3 and band 2.1 causes secondary changes of band 2.1 structure resulting in the decrease of spectrin binding or it results from minor modification of band 2.1 by PCMB as suggested by Chetrite et al. [8]. Nevertheless, band 2.1 still remains as a candidate for a primary acting site of PCMB. The observed solubilization of glycophorin from Triton shells preapred from ghosts or red blood cells treated with PCMB in the absence of the solubilization of spectrin and actin (Fig. 6 and 10) suggests a weakening of interaction of band 4.1 and glycophorin. This interpretation is supported by the fact that band 4.1 was solubilized from the ghosts at still higher concentration (5 mM) of PCMB or PCMBS [6,7]. Although unequivocal demonstration must await further investigations on the binding of PCMB to these connecting proteins, the results represent evidence for PCMB-induced weakening of the interaction of transmembrane proteins with the spectrin-actin network.

The inhibitory effects of Ca^{2+} and Mg^{2+} on the PCMB-induced solubilization of spectrin and actin were not due to the inhibition of PCMB binding because neither of the modified incubation conditions affected the binding of [^{14}C]PCMB to ghost protein (Fig. 4). It is, therefore, likely that their effect was provoked by inhibiting some conformational changes of the proteins which are necessary for the dissociation of the spectrin-actin network as suggested by the effects of divalent cations on the solubilization at low ionic strength [9,19]. The solubilization of actin still occurred partially under those conditions. It seems, however, not to be crucial for the organization of the cytoskeletal network itself because Triton shells prepared from ghosts incubated in the presence of Mg^{2+} either without or with PCMB exhibited essentially the same shape (Fig. 7). Nevertheless the ghost shape was altered from discoidal to somewhat spherical by the incubation with PCMB in the presence of Mg^{2+} (Fig. 3). It is, therefore, conceivable that the alteration of ghost shape is produced by the dissociation of transmembrane proteins from the cytoskeletal network.

The distribution of intramembrane particles, consisting of transmembrane proteins, can be altered by various treatments, most of which affect the spectrin-actin network, resulting in the modification of the interaction between transmembrane proteins and the cytoskeletal network [17–19]. The incubation of red cell ghosts with PCMB also produced an aggregation of the intramembrane particles (Fig. 9), which also evidences that PCMB dissociates transmembrane proteins from the spectrin-actin network.

Band 4.2 in rat red blood cells bound [^{14}C]PCMB preferentially (Fig. 3) and was easily precipitated upon incubation at 37 °C (Fig. 5). The increased recovery of band 4.2 in Triton shell fraction was also observed in the ghosts treated with Cd or Zn, thiol-reactive metals [9]. These results suggest that band 4.2 in rat red blood cells is sensitive to thiol-reactive compounds-induced denaturation.

In conclusion, main action of PCMB on red cell membrane under the physiological conditions, at higher ionic strength and in the presence of Mg^{2+}, is to dissociate transmembrane proteins from the peripheral cytoskeletal network, which may modify functions of the proteins.

Acknowledgements

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References

1 Branton, D., Cohen, C.M. and Tyler, J. (1981) Cell 24, 24–32
2 Gratzer, W.B. (1981) Biochem. J. 198, 1–8
3 Haest, C.W.M. (1982) Biochim. Biophys. Acta 694, 331–352
4 Bennett, V. (1985) Annu. Rev. Biochem. 54, 273–304
5 Carter, J.R. (1973) Biochemistry 12, 171–176
6 Steck, T.L. and Yu, J. (1973) J. Supramol. Struct. 1, 220–232
7 Ralson, G.B. and Crisp, E.A. (1981) Biochim. Biophys. Acta 649, 98–104

8 Chetrite, G., Dubreuil, Y.L. and Cassoly, R. (1983) Biochim. Biophys. Acta 731, 16–22
9 Kunimoto, M. and Miura, T. (1985) Biochim. Biophys. Acta 816, 37–45
10 Laemmli, U.K. (1970) Nature 227, 680–685
11 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2616
12 Aoki, Y., Kunimoto, M., Shibata, Y. and Suzuki, K.T. (1986) Anal. Biochem. 157, 117–122
13 Byers, T.J. and Branton, D. (1985) Proc. Natl. Acad. Sci. USA 82, 6153–6157
14 Kunimoto, M., Miyasaka, K. and Miura, T. (1986) J. Biochem. 99, 397–406
15 Bennett, V. and Branton, D. (1977) J. Biol. Chem. 252, 2753–2763
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
17 Pinto da Silva, P. (1972) J. Cell Biol. 53, 777–787
18 Elgsaeter, A. and Branton, D. (1974) J. Cell Biol. 63, 1018–1030
19 Elgsaeter, A., Shotton, D.M. and Branton, D. (1976) Biochim. Biophys. Acta 426, 101–122
20 Bennett, V. (1978) J. Biol. Chem. 253, 2292–2299