Absence of High-Affinity Band 4.1 Binding Sites From Membranes of Glycophorin C- and D-Deficient (Leach Phenotype) Erythrocytes

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We investigated the role of glycophorins C and D in the association of band 4.1 with the erythrocyte membrane by measuring the binding of band 4.1 to erythrocyte inside-out vesicles stripped of endogenous band 4.1. Vesicles were prepared from either normal erythrocytes or erythrocytes completely lacking glycophorins C and D (Leach phenotype). Band 4.1 binding to vesicles from normal erythrocytes gave rise to a nonlinear Scatchard plot, indicative of two classes of binding sites: a low-capacity, high-affinity class of sites (about 10% of the total) and a high-capacity, low-affinity class of sites. Vesicles prepared from Leach erythrocytes had a binding capacity for band 4.1 that was, on average, 32% lower than that of vesicles from normal erythrocytes. This difference was caused by the complete absence of the high-affinity binding sites as well as by a decrease in the number of low-affinity binding sites.

The erythrocyte membrane skeleton has a great influence on the shape of the erythrocyte and the deformability of its membrane, and is composed of a self-assembled network of spectrin tetramers, short filaments of actin, and band 4.1. The membrane skeleton is anchored to the lipid bilayer via band 3 and the glycophorins, the major erythrocyte integral membrane proteins. Two linkage proteins are involved in the attachment of the skeletal network to the bilayer: ankyrin, which links spectrin to the cytoplasmic domain of band 3, and band 4.1, which binds to the cytoplasmic domains of band 3 and glycophorins. Whereas the role of the band 4.1-band 3 association remains unclear, the binding of band 4.1 to glycophorins has been suggested to be essential in the interaction of the cytoskeleton with the bilayer.

Four glycophorins (A, B, C, and D) have been identified in the human erythrocyte membrane. Glycophorin C, although a minor glycophorin (50,000 to 100,000 copies per cell), has been proposed to be the principal attachment site for cytoskeletal band 4.1. Glycophorin C is the only glycophorin that remains bound to the cytoskeleton after detergent extraction of erythrocyte membranes, and is absent from cytoskeletons of band 4.1-deficient erythrocytes. Moreover, band 4.1 regulates membrane glycophorin C content. Finally, glycophorin C deficiency results in elliptocytic erythrocytes and in decreased membrane deformability and mechanical stability. By contrast, glycophorin A deficiency fails to induce such alterations.

However, other studies suggest that glycophorin A, which is the major glycophorin (500,000 to 1,000,000 copies per cell), may also be involved in the association of band 4.1 with the membrane. Although glycophorin A does not seem to interact with the cytoskeleton in its native state, band 4.1 can be cross-linked to glycophorin A as well as to glycophorin C in native membranes. The binding of ligands such as lectins or antibodies to the extracellular domain of glycophorin A induces dramatic changes in the interactions of its cytoplasmic domain with the cytoskeleton. Moreover, antibodies raised against the cytoplasmic domain of glycophorin A have been shown to block partially the binding of exogenous band 4.1 to erythrocyte membrane stripped of their cytoskeletal proteins.

In vitro, interaction between glycophorin A and band 4.1 has been shown to be strictly dependent on membrane phosphatidylinositol 4,5-bisphosphate (PIP$_2$) which is tightly bound to glycophorin A. As observed in nucleated cells, human erythrocytes show an active turnover of PIP$_2$ that is catalyzed by a specific kinase and a specific phosphomonoesterase. Only a fraction (60%) of PIP$_2$ is accessible to these enzymes and to phosphoinositidase C, the enzyme responsible for PIP$_2$ hydrolysis. We have recently shown that this fraction of PIP$_2$ is the only one involved in the regulation of band 4.1 association with the membrane.

Here, we have used Leach phenotype human erythrocytes (referred to as Leach erythrocytes in the following), which are totally deficient in glycophorins C and D, to evaluate the involvement of glycophorins in the association of band 4.1 with the membrane. Our results show that lack of glycophorin C and D results in the absence of high-affinity membrane binding sites and in a decrease in low-affinity membrane binding sites for band 4.1. Our results show also that glycophorins C and D are not required for the formation of PIP$_2$-sensitive band 4.1 binding sites.
MATERIALS AND METHODS

Adenine (A9126), calcium ionophore A23187 (C7522), diisopropyl fluorophosphate (DF0879), inosine (I4125), iodoacetamide (I6125), leupeptin (L2884), phenylmethylsulfonyl fluoride (PMSF; P7626), sodium azide (S2002), and Triton X-100 were obtained from Sigma (St Louis, MO). Dithiothreitol (DTT) was from US Biochemicals (Cleveland, OH). Bovine serum albumin (BSA), fraction V (12659) was from Calbiochem (San Diego, CA). All other reagents used were of analytical grade. Silica gel 60 precoated plates were from Whatman (Maidstone, UK). Tween 20 and reagents for gel electrophoresis were obtained from Bio-Rad (Richmond, CA). Methanol (M6909), potassium iodide (KI) or alkaline pH as described previously, with minor modifications. 26 SDS-PAGE and immunoblotting. Membrane proteins of normal or Leach erythrocytes (60 µg protein) were analyzed by SDS-PAGE using 9% acrylamide according to the method of Laemmli. 27 In some experiments, after electrophoresis, proteins were transferred onto nitrocellulose paper for detection of glycoporphin C or of band 3 by specific antibodies. The following steps were performed at room temperature. Blots were first stained with a 3% trichloroacetic acid solution containing 0.1% Red Ponceau to monitor the efficiency of the transfer. After complete destaining in water, they were blocked in 150 mmol/L NaCl, 5 mmol/L sodium phosphate, pH 7.2, 0.5 g/L sodium azide (medium A) containing 5% BSA for 1 hour. They were subsequently incubated with the glycoporphin C or the band 3 antisera diluted 1:3000. 28 Band 4.1, used at the concentration indicated in the figures, was incubated with 18 µg/mL BSA for 1 hour at 22°C. The vesicles were centrifuged at 20,000 rpm for 20 minutes in a Beckman 42.2 Ti rotor (Beckman Instruments, Palo Alto, CA). The supernatant dissolved in dimethylsulfoxide as a 2 mmol/L stock solution. The incubation was performed for 20 minutes and terminated by the addition of 20 vol of ice-cold 150 mmol/L NaCl, 5 mmol/L sodium phosphate, pH 8.0, 0.15 mmol/L MgCl2, 10 mmol/L HEPES, pH 7.4, 10 mmol/L glucose, 20 mmol/L inosine and resuspended in this medium at a hematocrit of 10%. The cell suspension was divided into two samples, which were preincubated at 37°C for 10 minutes in the presence of EGTA or CaCl2 (1 mmol/L) before the addition of calcium ionophore A23187 (5 mmol/L)
was carefully aspirated and the $^{125}$I in the pellet was measured in a Gamma counter. To account for nonspecific band 4.1 sedimentation or sticking to tubes, control samples containing all of the components except vesicles were treated identically, and the $^{125}$I counts of these samples were subtracted from the corresponding samples with vesicles. Each data point represents the mean of duplicate determinations. Control experiments showed that binding equilibrium was reached after 1 hour of incubation (data not shown).

Data analysis. All binding data were analyzed using the Enzfit computer program (Elsevier-Biosoft), using either a one- or a two-site binding model.

Antiserum. Rabbit polyclonal antiserum against an hexadecapeptide (residues 113-128) of the cytoplasmic domain of glycophorin C$^{15}$ was a generous gift from Dr Philip S. Low and Dr Christian R. Lombardo (Purdue University, West Lafayette, IN). Rabbit polyclonal antiserum was raised against the 43-kD cytoplasmic domain of band 3 prepared as previously described.$^{32}$

Other procedures. Protein concentration was determined by the method of Bradford,$^{33}$ using BSA as a standard.

RESULTS

Glycophorin C has been suggested to be the principal attachment site of band 4.1 to the erythrocyte membrane.$^{2,8,13-15}$ However, other studies suggest that glycophorin A may also be involved in this association.$^{2,4,7,8-11}$ To determine the importance of glycophorin C in the binding of band 4.1 to the erythrocyte membrane, we compared the binding of band 4.1 to sI0Vs prepared from either control or Leach erythrocytes.

Comparison of Band 4.1 Binding to Control and Leach Vesicles

Previous studies have shown that glycophorin C migrates on SDS gels with an apparent molecular mass of 31 to 34 kD$^{14}$ and that the Leach phenotype patient studied here lacks glycophorin C.$^{15}$ A more recent study$^{34}$ has shown that this patient lacks both glycophorins C and D because of a gene defect that deletes the entire transmembrane and cytoplasmic domains of these glycophorins. We confirmed by immunoblotting that glycophorin C was absent from the erythrocyte membranes of the Leach patient J.C. Whereas control membranes showed two reactive bands of approximately 35 and 25 kD, Leach membranes showed neither of these bands (data not shown).

Figure 1 shows that the binding capacity of vesicles from Leach membranes was 26% lower than that of control vesicles. Scatchard analysis (inset) of the binding data showed several differences between Leach and control vesicles. (1) Leach vesicles showed a complete absence of a class of high-affinity binding sites detected in control vesicles (Fig 1, inset), which, in this experiment, contributed 5% of the total band 4.1 binding capacity of vesicles (see legend to Fig 1). (2) Compared with normal vesicles, Leach vesicles had a 22% reduction in the binding capacity of low-affinity band 4.1 binding sites (the only class of sites distinguished on Leach vesicles). Also, in four separate experiments, the Scatchard plot of band 4.1 binding to Leach vesicles was convex. One possible explanation for a convex Scatchard plot is positive cooperativity in band 4.1 binding, but we have no independent evidence to support this interpretation.

The binding capacity of Leach vesicles for band 4.1 was found to be reduced by an average of 32% ± 15% relative to that of normal vesicles in four independent experiments. The high-affinity binding sites, which accounted for an average of 10% of the total number of binding sites on normal vesicles, were absent from Leach vesicles in all experiments. Because of the small contribution of the high-affinity sites to the total binding capacity of the vesicles, most of the difference in band 4.1 binding capacity between Leach and normal vesicles was caused by the smaller number of low-affinity sites in Leach vesicles. In addition to this difference in binding capacity, the apparent $K_d$ of the low-affinity binding sites was twofold higher in Leach vesicles than in control vesicles in the three other experiments not shown here.

Effects of PIP2 Depletion on Band 4.1 Binding Capacity of Control and Leach Vesicles

PIP$_2$ has been shown to be involved in the regulation of band 4.1 association with some of its membrane receptors.$^{6,10,11}$ To rule out the possibility that the lower band 4.1 binding capacity of Leach vesicles was caused by a lower PIP$_2$ content, we assayed membrane PIP$_2$ in control and Leach membranes. The results showed that the amounts of membrane PIP$_2$ were similar in both types of cells (4.05 and 5.35 nmol PIP$_2$/mg membrane protein in controls and 4.05 and 4.70 nmol PIP$_2$/mg membrane protein in Leach; results of two independent experiments) and were in accordance with previous studies.$^{35,36}$ Thus, the lower band 4.1 binding capacity of Leach vesicles did not result from a lower PIP$_2$ content.

To investigate further the role of PIP$_2$ in regulating the association of band 4.1 with glycophorin C and D, we determined the effects of PIP$_2$ depletion on band 4.1 binding to control and Leach vesicles. Two methods were used to alter membrane PIP$_2$ content of erythrocytes: ATP depletion or activation of the endogenous phosphoinositidase C in intact cells. ATP depletion alters phosphoinositide content by inhibition of phosphoinositide kinases.$^{36}$ displacing the metabolic equilibrium from PIP$_2$ to phosphatidylinositol 4-monophosphate and phosphatidylinositol. Activation of phosphoinositidase C, achieved by incubating erythrocytes with Ca$^{2+}$ in the presence of the ionophore A23187, induces hydrolysis of PIP$_2$. The PIP$_2$ depletions obtained after ATP depletion or activation of phosphoinositidase C in control cells were in accordance with previous studies.$^{6,26,35-37}$ However, the depletion was always enhanced in Leach erythrocytes compared with control erythrocytes (Table 1).

Figure 2 shows that depletion of PIP$_2$ by activation of phosphoinositidase C resulted in decreased band 4.1 binding to vesicles from normal erythrocytes, as previously reported$^{6,11}$ (see Table 1). Scatchard analysis (Fig 2, inset) showed that the PIP$_2$-depleted vesicles had a decreased band 4.1 binding capacity caused principally by a reduction in the number of low-affinity binding sites. The binding of band 4.1 to the high-affinity sites was not significantly altered by PIP$_2$ depletion, although the $K_d$ was increased by fourfold. This increase in the $K_d$ of the high-affinity site was seen in three other experiments.

Vesicles prepared from Leach erythrocytes also showed a
decrease in their band 4.1 binding capacity after phosphoinositidase C-induced PIP_2 depletion (Fig 3 and Table 1). PIP_2 depletion resulted in a decrease in the binding capacity of the single class of binding sites identified in the Leach vesicles without any significant alteration of their apparent kd (Fig 3, inset). The Scatchard plot still showed evidence of convexity in the low band 4.1 region of the curve (more evident in the three other experiments not shown here). These results show that band 4.1 binding sites other than those on glycophorin C or D are regulated by PIP_2.

To rule out the possibility that the decrease in band 4.1 binding capacity after activation of the phosphoinositidase C might have resulted from a degradation of band 3 and/or glycophorin molecules, we monitored these proteins by Western blotting. The results clearly showed that the treatment did not induce any detectable proteolysis of band 3 in both types of cells or of glycophorin C in control cells (data not shown). The results of the phosphoinositidase C experiment, presented in Figs 2 and 3 and in Table 1, were confirmed in two other independent experiments in which PIP_2 reduction was induced by ATP depletion of intact cells (Table 1). The ATP depletion was performed in the presence of EGTA to prevent metabolic depletion-induced increase in cytosolic Ca^{2+}, which might have adversely affected the protein composition of the resulting vesicles.

**DISCUSSION**

Our data show that membrane vesicles prepared from erythrocytes of the Leach phenotype have a binding capacity for band 4.1 that is on average 32% less than that of vesicles from normal erythrocytes. This lower binding capacity is reflected in the complete absence of a class of high-affinity binding sites as well as in a 30% (average) deficit of low-affinity binding sites. The results suggest that all of the high-affinity sites and about 30% of the low-affinity sites are composed exclusively of glycophorin C or D or require glycophorin C or D as a component.

Even though membrane vesicles from Leach erythrocytes lack glycophorin C and D, 20% to 40% of their band 4.1 binding sites are PIP_2 sensitive. This is nearly the same range of PIP_2-sensitive sites as is found in vesicles from normal cells. In vesicles from normal cells the effect of PIP_2 depletion on the properties of the high-affinity band 4.1 binding site (which must involve glycophorin C or D) is a fourfold increase in kd with no effect on the binding capacity. We conclude that, whereas the PIP_2-induced change in the kd of

![Graph](image-url)

**Fig 1.** Comparison of ^125^I-band 4.1 binding to control or Leach KI sIOVs. The indicated concentrations of ^125^I-band 4.1 were incubated with 18 μg/mL vesicles prepared from (a) control or (b) Leach erythrocytes. Procedures of vesicle preparation and band 4.1 binding measurement are described under Methods. Inset, Scatchard plot of binding of band 4.1 to vesicles prepared from control (a, kd, 2.3 ± 5.9 × 10^{-8} mol/L, capacity = 20 ± 10 μg/mg vesicle protein; kd_2 = 1.9 ± 0.3 × 10^{-7} mol/L, capacity_2 = 325 ± 10 μg/mg vesicle protein) or Leach (b, kd = 2.0 ± 0.4 × 10^{-7} mol/L, capacity = 255 ± 15 μg/mg vesicle protein) erythrocytes. When fitting the binding data for the Leach vesicles (b) the lowest three data points were omitted because of the appearance of positive cooperativity.

| Table 1. Changes in the PIP_2 Content and in the Band 4.1 Binding Capacity of Control or Leach Vesicles on Phosphoinositidase C Activation or ATP Depletion |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|                                  | Control Vesicles                 | Leach Vesicles                   |                                  |                                  |
| Phosphoinositidase C activation  |                                  |                                  |                                  |                                  |
| in intact cells                  | 57*                              | 36*                              | 75*                              | 41*                              |
| ATP depletion of intact cells (2 h) | 58                               | 27                               | 72                               | 28                               |
|                                  | 58                               | 40                               | 81                               | 19                               |

Values shown are the percent decrease. PIP_2 membrane content was altered by phosphoinositidase C activation in intact cells or by 2 hours of ATP depletion of intact cells as described in Methods. Phospholipid radioactivity and band 4.1 binding to vesicles prepared from untreated or treated control and Leach cells were measured as described under Methods. The results shown are from three independent experiments.

* Results of the experiment presented in Figs 2 and 3.
the high-affinity site may be of some importance, glycophorins C and D do not play a major role in the PIP_2-sensitive changes in band 4.1 binding capacity.

What are the PIP_2-sensitive low-affinity binding sites? The two likely candidates are band 3 and glycophorin A. In Gascard et al., we show that band 4.1 binding to band 3 (which accounts for approximately 65% of band 4.1 binding sites) is unlikely to be sensitive to PIP_2. This is because removal of the cytoplasmic domain of band 3 by trypsinization of stripped vesicles hardly changes the number of PIP_2-sensitive sites on the vesicles. Thus, the PIP_2-sensitive low-affinity sites must involve other membrane components. Glycophorin A is the most logical candidate because PIP_2-dependent binding of band 4.1 to glycophorin A has been shown previously.

A novel feature of the results shown here is that the Scatchard plots are biphasic, a feature not detected in our previous work, but suggested in one other previous study. A possible explanation for this difference is that in this study we added several binding measurements at very low concentrations of band 4.1 to increase the resolution of our Scatchard plots. It should also be noted that the band 4.1 binding capacities of the vesicles in the present study were considerably lower than those reported in our previous study but similar to those reported in Danilov et al. Possibly related to these points is the fact that the range of reduction in band 4.1 binding caused by PIP_2 depletion was higher in the present study (27% to 40%) than in our previous work (5% to 22%). We considered the possibility that the conditions used to prepare the stripped vesicles (e.g., pH 11

Fig 2. Effect of phosphoinositidase C activation in intact cells on 125I-band 4.1 binding to control KI sIOVs. The indicated concentrations of 125I-band 4.1 were incubated with 18 μg/mL vesicles prepared from untreated erythrocytes or vesicles prepared from erythrocytes in which phosphoinositidase C had been activated. Procedures of vesicle preparation, of phosphoinositidase C activation in intact cells, and of band 4.1 binding measurement are described under Methods. Inset, Scatchard plot of binding of band 4.1 to vesicles prepared untreated erythrocytes (open circles) or vesicles prepared from erythrocytes in which phosphoinositidase C had been activated (closed circles). The indicated concentrations of phosphoinositidase C activation in intact cells, and of band 4.1 binding measurement are described under Methods. Inset, Scatchard plot of binding of band 4.1 to vesicles (open circle) or vesicles prepared from erythrocytes in which phosphoinositidase C had been activated (closed circles) and phosphoinositidase C activation in intact cells, and of band 4.1 binding measurement are described under Methods. To obtain the best fit, the Scatchard plots were fitted to the equation: 

\[ \frac{B}{f} = \frac{k}{r} - \frac{f}{r} \]

where B is the bound ligand, f is the free ligand, and r is the total ligand concentration. The slopes of the linear regression lines are depicted for each concentration of band 4.1 binding capacity.

Fig 3. Effect of phosphoinositidase C activation in intact cells on 125I-band 4.1 binding to Leach KI sIOVs. The indicated concentrations of 125I-band 4.1 were incubated with 18 μg/mL vesicles prepared from untreated Leach erythrocytes (open circles) or from Leach erythrocytes in which phosphoinositidase C had been activated (closed circles). Procedures of vesicle preparation, of phosphoinositidase C activation in intact cells, and of band 4.1 binding measurement are described under Methods. Inset, Scatchard plot of binding of band 4.1 to vesicles prepared untreated Leach erythrocytes (open circles) or vesicles prepared from Leach erythrocytes in which phosphoinositidase C had been activated (closed circles). The indicated concentrations of phosphoinositidase C activation in intact cells, and of band 4.1 binding measurement are described under Methods. To obtain the best fit, the Scatchard plots were fitted to the equation: 

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where B is the bound ligand, f is the free ligand, and r is the total ligand concentration. The slopes of the linear regression lines are depicted for each concentration of band 4.1 binding capacity.
treatment) were responsible for the different binding capacities in the several studies. However, because pH 11 treatment was used in some of the experiments reported here and in all of the studies reported in Danilov et al. it is unlikely that this treatment is responsible for the different binding capacities found in the several studies. Moreover, we found no consistent differences in the binding characteristics of vesicles prepared by pH 11 extraction and those prepared by KI extraction.

We previously proposed (see Gascard et al. for further discussion) that differences in band 4.1 binding capacity could result from changes in the intermolecular or intramolecular associations of band 3 and the glycophorins, leading to exposure of more or fewer sites on both molecules in different experiments. This hypothesis is supported by the fact that the proportion of trypsin-sensitive (largely or exclusively band 3 associated) to trypsin-insensitive sites (including glycophorins) was always similar, regardless of the total band 4.1 binding capacity in a particular experiment. Several lines of evidence suggest that some band 3 and glycophorin molecules may be associated in the membrane. Such associations could account for the observations that some antisera to band 3 or glycophorins block a higher percentage of membrane binding sites than would be expected from the separate contribution of these proteins to vesicle binding capacity.

Erythrocytes deficient in glycophorin C and D have a nearly normal morphology, although approximately 10% of the cells have been reported to be elliptocytic. However, membranes from glycophorin C- and D-deficient erythrocytes have been reported to have a 50% reduced mechanical stability and a 40% reduced deformability. Several lines of evidence suggest that the principal function of glycophorin C is to anchor some fraction of membrane skeletal band 4.1 to the membrane. It has also been proposed that the abnormal mechanical properties of Leach erythrocytes arise from defective or reduced skeletal-membrane linkages. If these hypotheses are true, then the mechanical abnormalities of Leach membranes could be caused by (1) the absence of the high-affinity band 4.1 binding sites, (2) the deficit in the low-affinity binding sites and consequent reduction in total membrane band 4.1 binding capacity, or (3) a combination of (1) and (2).

Recent results (A.H. Chishti, personal communication, June 1993) indicate that membranes from Leach erythrocytes have about 20% less band 4.1 than do membranes from normal erythrocytes. It is possible that this deficit could account for the abnormal mechanical properties of the Leach membranes cited above. It is also tempting to relate this observation to the lower than normal band 4.1 binding capacity of Leach vesicles found here. However, such comparisons must be made with caution because in this and previous studies the band 4.1 binding capacity of stripped vesicles (from both normal and Leach erythrocytes) was found to be greater than the endogenous band 4.1 content of ghosts. For example, we calculate that a binding capacity of 340 µg band 4.1/mg stripped vesicle protein (Fig 1) is equivalent to approximately 120 µg band 4.1/mg ghost protein, or about 2.4 times the endogenous band 4.1 content of ghosts (see Gascard et al. for further discussion).

In summary, our results show that glycophorin C and/or D is involved in the formation of high-affinity low copy number band 4.1 binding sites in normal erythrocyte membranes. These glycophorins also contribute to the formation of more numerous low-affinity band 4.1 binding sites. Our data show that membrane components other than glycophorin C or D (likely glycophorin A) can bind band 4.1 in a PIP_2-sensitive manner, and suggest that, except for a twofold to threefold alteration in k_d, binding of band 4.1 to glycoporphorin C and D may be independent of PIP_2. Regulation of band 4.1-membrane associations is important not only in the erythrocyte, but also in nonerythroid cells that contain analogues of both band 4.1 and glycophorin C (the only glycophorin species found in nonerythroid cells). Our results support the idea that membrane skeletal linkages involving band 4.1-like proteins in nonerythroid cells may be remodeled by agonist-induced changes in membrane PIP_2, but leave open the question of the identity of the band 4.1 binding sites involved.

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REFERENCES

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