Interactions between Protein 4.1 and Band 3
AN ALTERNATIVE BINDING SITE FOR AN ELEMENT OF THE MEMBRANE SKELETON*

(Received for publication, August 30, 1984)

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Protein 4.1 from human erythrocytes formed a complex with band 3 in inside-out erythrocyte membrane vesicles and with soluble peptides derived from the cytoplasmic domain of band 3. Protein 4.1 labeled metabolically with 32P bound saturably to vesicles depleted of endogenous protein 4.1. The soluble cytoplasmic domain of band 3 (43K) competitively displaced approximately 60% of bound 32P-protein 4.1 from reconstituted membrane vesicles. Pretreatment of vesicles with anti-43K similarly inhibited the re-binding of protein 4.1. In solution, 43K-43K formed a complex with protein 4.1 that saturated at 1:1 stoichiometry and migrated as a discrete band when analyzed by nondenaturing polyacrylamide gel electrophoresis. In rate-zonal sedimentation in isotonic salt solutions, protein 4.1 and 43K sedimedated as a sharp peak at 4.4 S. In experiments aimed at exploring the role of the protein 4.1-band 3 interaction in the organization of the membrane skeleton, the effect of spectrin was investigated. Spectrin and protein 4.1 formed a complex which co-sedimented in sucrose gradients, but the addition of 43K to preformed spectrin-protein 4.1 complexes resulted in disruption of the complex and co-sedimentation of most of the protein 4.1 with 43K. These results suggest that protein 4.1 can associate with band 3 in the erythrocyte membrane and that this association may modulate the attachment of the membrane skeleton to the membrane.

The membrane skeletons of vertebrate red blood cells are attached to the lipid bilayer indirectly through a class of proteins which are in turn bound to the integral membrane protein 4.1, a 210,000-Da protein present in erythrocytes and other cell types (1-4). Ankyrin, a 210,000-Da protein present in erythrocytes and other cell types (5, 6, 26), is the best characterized example of this class; in erythrocytes, it binds to band 3 with one of its two domains and to spectrin with the other (7). Protein 4.1 also appears to be a member of this linking class. It binds to both spectrin and actin (8-10) and can form a high affinity association with the cytoplasmic segment of glycophorin A (11). Ankyrin and protein 4.1 associate with spectrin at different sites. Ankyrin binds to the β subunit at a site close to the oligomer binding region (12), while protein 4.1 seems to be attached to the opposite end of spectrin, possibly in complex with actin oligomers (2, 8-10). These findings have led to the suggestion that the spectrin-actin network is attached to the overlying membrane at at least two different sites and that these sites are presumably regulated by different factors.

Band 3 plays a number of other roles in addition to that of the ankyrin-binding site. Approximately 1.2 × 106 copies of this integral membrane protein are present in each human erythrocyte, existing in dimeric and tetrameric forms of the 95,000-Da monomer (3, 13). Band 3 serves as the major anion channel of the erythrocyte (14) and is also a binding site for hemoglobin, protein 4.2, and several glycolytic enzymes (3, 15-17). In the present paper we report a direct association between band 3 and protein 4.1. We find that band 3 and protein 4.1 form a complex in reconstituted erythrocyte vesicles, that the isolated cytoplasmic domain of band 3 retains this function, and that formation of a band 3-protein 4.1 complex modulates the interaction of protein 4.1 with spectrin.

EXPERIMENTAL PROCEDURES

Materials—Fresh human blood was obtained from volunteer human donors and anticoagulated with acid citrate dextrose. Bovine pancreatic chymotrypsin was obtained from Millipore Corp. aldolase and glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle were obtained from Sigma in the highest grade available. Polyacrylamide and other electrophoresis reagents were from Bio-Rad. 125I-Bolton-Hunter reagent and [γ-32P]ATP were from New England Nuclear.

Preparation of Erythrocyte Proteins—Two units of whole blood were washed and hypotonically lysed as described (26). Following spectrin extraction (12), protein 4.1 was extracted in 1 M KCl and further purified in modification of the method of Tyler et al. (8, 30). The cytoplasmic domain of band 3 was prepared from high-salt extracted vesicles as described (5) by cleavage with α-chymotrypsin followed by chromatography on DEAE-cellulose (Whatman) and gel filtration on Ultragel AcA 44 resin (LKB). Material prepared by this method is referred to as 43K peptides although such preparations appear more heterogeneous on SDS gels. The additional bands represent smaller forms of the same band 3 domain. In all purifications, fractions were analyzed by SDS-PAGE on 10% gels (27), and purified pools were concentrated by vacuum dialysis against isotonic KCl buffer. Protein concentrations were determined by the method of Lowry et al. (28) using a bovine serum albumin standard (Sigma). Amino acid analyses performed on a Durrum analyzer were used to correct the Lowry values to precise concentrations in determinations of molecular stoichiometry. The correction factors were 1.06 for 43K and 0.8 for protein 4.1.

Electrophoresis—Nondenaturing 2-4% polyacrylamide slab gels were prepared and run at 4°C for 18-24 h at 50 V according to the method of Morrow et al. (29). Samples were prepared in a final volume of 100 μl in isotonic KCl buffer. Immediately prior to electro-

1 The abbreviations used are: 43K, the cytoplasmic domain of band 3; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HEPES, 4-(2-hydroxyethyl)-1-piperizineethanesulfonic acid.

2 D. W. Speicher, personal communication.
phoresis, 25 μl of 1 mM sucrose containing bromphenol blue were added, and 100-μl aliquots were loaded onto the gel. SDS-PAGE was performed on 10% gels using the Laemmli system (27). Gels were stained in Coomassie Brilliant Blue. Autoradiography of dried gels was performed using Kodak X-Omat film and intensification screens with exposure at −70 °C. The films were processed in a Kodak X-Omat processor.

**Ultracentrifugation**—12.4-ml sucrose gradients were constructed in the indicated buffers utilizing an Auto-Densofoc (Haeke-Buchler). Gradients were layered over a 350-μl 50% sucrose cushion in the same buffer. Samples were applied in volumes of 100–300 μl. After centrifugation at 200,000 × g in a Beckman SW 40 rotor, gradients were fractionated into 0.35-ml fractions from top to bottom using the Auto-Densifoc. Optical density at 280 nm was continuously monitored with a UA-5 monitor (Isco). 100-μl aliquots of alternate fractions were analyzed by SDS-PAGE. Gradients were calibrated with standards of cytochrome c, bovine serum albumin, and aldolase, all obtained from Sigma.

**Radioactive Labeling**—43K was labeled with 32P-labeled Bolton Hunter reagent as described (6, 7) to an initial specific activity of 9620 Ci/mol. Band 4.1 was metabolically labeled with [γ-32P]ATP in a cyclic AMP-dependent fashion as described (30).

**Vesicle Binding Studies**—KCl-urea stripped vesicles were prepared from 10-μl aliquots of freshly drawn human blood as described (5, 7) to an initial specific activity of 200,000 cpm/μg protein. Vesicles were fractionated into 0.35-ml fractions from top to bottom using the Auto-Densifoc. Optical density at 280 nm was continuously monitored with a UA-5 monitor (Isco). 100-μl aliquots of alternate fractions were analyzed by SDS-PAGE. Gradients were calibrated with standards of cytochrome c, bovine serum albumin, and aldolase, all obtained from Sigma.

**Results**

Protein 4.1 binds to band 3 in erythrocyte membrane vesicles. In one series of experiments, protein 4.1 was competitively displaced from its membrane binding site when the soluble cytoplasmic domain of band 3 in the form of a 43K peptide was added to vesicles containing bound protein 4.1. A second series of experiments showed that the binding of protein 4.1 to vesicles was diminished if the vesicles were pretreated with antibody to the cytoplasmic domain of band 3. Fig. 1 shows a qualitative demonstration of these findings. In these experiments, equal amounts of 32P-protein 4.1 were added to stripped inside-out erythrocyte membrane vesicles treated as noted in the figure legend, and the samples were then separated into pellet and supernatant fractions and analyzed by SDS-polyacrylamide gel electrophoresis. With no additional treatments, the majority of protein 4.1 sedimented with the membrane fraction. When vesicles bearing bound protein 4.1 were exposed to an approximately 10-fold molar excess of 43K respect to protein 4.1, the distribution of protein 4.1 changed dramatically, with the majority appearing in the supernatant. Similarly, pretreatment of the vesicles with affinity-purified antibody to the cytoplasmic domain of band 3 also reduced the amount of protein 4.1 bound to the membrane vesicles. In all instances, the distribution of the 32P-labeled material demonstrated by autoradiography corresponded to the distribution of the Coomassie-staining material, thus validating the 32P-labeled material for use in quantitating the association of protein 4.1 with vesicles.

As a test of the specificity of the interaction, the binding of aldolase and glyceraldehyde-3-phosphate dehydrogenase was examined in this system. Although these enzymes bind to the cytoplasmic face of erythrocyte membranes under conditions of low ionic strength (16, 17), no appreciable association was observed in the isotonic conditions employed in this system.

The amount of protein 4.1 bound to the reconstituted vesicles was significantly greater than the amount of protein 4.1 normally present in erythrocyte ghosts (Fig. 1). In contrast, the amount of protein 4.1 remaining in the vesicles after competition by 43K or inhibition by antibody approximated the normal level of protein 4.1. This result was examined quantitatively by measuring the amount of 32P-labeled protein 4.1 bound and in the presence or absence of antibody pretreatment and by measuring the fraction of bound protein 4.1 after the addition of varying amounts of 43K.

Table 1 shows that anti-43K pretreatment inhibits protein 4.1 re-binding by approximately 65%. Antibody to glycoporphin A inhibits by 30%, and antibody to all glycoporphins inhibits by 8%, suggesting that glycoporphins B and C may also serve as binding sites as previously proposed (11, 36). When these antibodies were combined, statistically significant increments of inhibition were observed, suggesting two classes of receptors were affected by this treatment. Inhibition by antilycoporphin and by anti-43K are not additive; this may reflect steric hindrance. Anti-spectrin had a minimal effect, thereby excluding a nonspecific property of immunoglobulin.

The binding isotherm of protein 4.1 associating with membrane vesicles is shown in Fig. 2. Two striking features are evident: the binding is saturable, and it seems to be composed of two components, one of high affinity with an approximate Kd of 2 × 10^{-5}, and one of lower affinity, with an approximate Kd of 5 × 10^{-7}. The suggestion that there may be two binding sites for protein 4.1 is further born out by examination of the competition curve, shown in Fig. 3. This curve shows that a maximum of approximately 65% of bound protein 4.1 could be displaced from the vesicles when a large molar excess of 43K was added. These data are consistent with the distribution of protein 4.1 between the high and low affinity sites of the membrane, as well as with the binding isotherm.
Protein 4.1 Binds to Band 3 in Red Cell Membranes

FIG. 1. Reconstitution of membrane vesicles with protein 4.1. Membrane vesicles (30 μg) were reconstituted by incubation at 4°C for 45 min with 35 μg of 32P-protein 4.1 (4 × 10⁶ cpm). 43K (194 μg) or an equal volume of buffer were then added and the mixture incubated at 37°C for 30 min. In a second experiment, vesicles were preincubated at 4°C for 15 min with anti-43K (100 μg). Protein 4.1 was added, and the vesicles were incubated for an additional 45 min at 4°C. In a final experiment, vesicles were incubated at 4°C for 45 min with aldolase (30 μg) or glyceraldehyde-3-phosphate dehydrogenase (30 μg). The final volume of all samples was 150 μl in HEPES-buffered isotonic KCl. Following incubation, pellets and supernatants were separated by centrifugation and analyzed by SDS-PAGE. Pellets are shown in odd-numbered lanes; the adjacent even-numbered lanes show the corresponding supernatant.

Lanes 1 and 2, vesicles + protein 4.1; Lanes 3 and 4, vesicles + protein 4.1 + 43K; Lanes 5 and 6, vesicles + anti-43K + protein 4.1; Lanes 7 and 8, vesicles + aldolase; Lanes 9 and 10, vesicles + glyceraldehyde-3-phosphate dehydrogenase; Lane G, erythrocyte ghosts. Panel A shows the Coomassie-stained 10% gel, and panel B shows the corresponding autoradiograph.

TABLE I

Inhibition of protein 4.1 rebinding by antibody

| Treatment          | Protein 4.1 bound/10V protein | Inhibition |
|--------------------|-------------------------------|------------|
|                    | μg/mg                         | %          |
| None               | 237 ± 11.7                    | 65.1       |
| Anti-43K           | 82.7 ± 2.1*                   | 65.1       |
| Anti-CB2           | 167 ± 12.8*                   | 29.5       |
| Anti-glycoporphins | 121 ± 4.5*                    | 48.9       |
| Anti-43K + anti-CB2| 62.5 ± 2.5*                   | 73.6       |
| Anti-43K + anti-glycoporphins | 61.2 ± 4.1* | 74.2       |

* P < 0.001.
* P < 0.05.
* Range of duplicates.

and can be blocked by peptides derived from the cytoplasmic domain of band 3, while the high affinity site is affected by neither of these treatments. These findings suggest that band 3 is the low affinity binding site for protein 4.1. The saturation
were incubated for 30 min at 37 °C with 43K. Following incubation, pellets and supernatants were separated by centrifugation and analyzed by liquid scintillation counting. Data were analyzed by the method of Dixon (38).

levels seen here are greater than those reported in a recent study (34), which measured protein 4.1 rebinding to vesicles over a more limited range.

Protein 4.1 and 43K Associate in Solution—The interaction of protein 4.1 with the isolated cytoplasmic domain of band 3 was also demonstrated by nondenaturing polyacrylamide gel electrophoresis and rate-zonal sedimentation. Fig. 4 shows the 43K and protein 4.1 formed a complex with an electrophoretic mobility slightly greater than that of protein 4.1 alone. This complex was in equilibrium with the free species, since the addition of a 3.6-fold molar excess of unlabeled 43K to a preformed complex resulted in displacement of the labeled material from the complex. Fig. 5 represents a similar experiment, in which increasing amounts of 43K were added to a fixed amount of protein 4.1. Duplicate lanes were slices from other gels and run in a second dimension of SDS-PAGE. Panels C and D show that the new band seen on nondenaturing electrophoresis contains both protein 4.1 and 43K and that the stoichiometry of the complex is similar when the interacting species are mixed at initial ratios of 43K to protein 4.1 of 1.3 and 5.3. Finally, the portions of second-dimension gels containing 43K were cut out, divided into bound and free segments, and counted in a γ counter. Table II shows that the complex saturates at a molar ratio which approaches 1:1.

The complex between 43K and protein 4.1 was also resolved by rate-zonal sedimentation in isotonic sucrose gradients. Under these conditions, protein 4.1 sedimented near the top of the gradient as a sharp peak at 3.0 S. Similarly, 43K alone formed a sharp peak at 4.2 S, a sedimentation coefficient most consistent with the noncovalent dimeric form reported by other workers (18). The combination of the two proteins sedimented as a single discrete complex at 4.4 S. The complex required the native structure of protein 4.1. When protein 4.1 was reacted with N-ethylmaleimide, it failed to associate with 43K (Fig. 6).

43K Disrupts the Spectrin-Protein 4.1 Complex—Protein 4.1 is thought to link the membrane skeleton to the lipid bilayer through interactions with spectrin and integral membrane proteins (2, 8–11). The role of band 3 in this linkage was investigated by using rate-zonal sedimentation to determine the stability of the protein 4.1-spectrin complex in the presence of 43K. The incomplete association of protein 4.1 and spectrin in isotonic sucrose gradients (data not shown) can be enhanced in low ionic strength buffer (Fig. 7). The addition of excess 43K to preformed spectrin-protein 4.1 complexes displaced the majority of the protein 4.1 from its association with spectrin. Instead, it sedimented largely in association with 43K. This result suggests that 43K modulates the interaction of spectrin and protein 4.1, possibly through competition for a common binding site or through an allosteric mechanism.

DISCUSSION

These findings define a new interaction of an integral membrane protein with an element of the membrane skeleton. Previous models recognized the interaction of band 3 with ankyrin and spectrin in serving to attach the membrane skeleton to the lipid bilayer; the attachment of the opposite end of the spectrin molecule is less well understood. Protein 4.1 has been shown to bind with high affinity to the cytoplasmic segment of glycophorin A (11); the other glycophorins (11, 36) and phosphatidylserine (37) have been proposed as membrane attachment sites for protein 4.1. The results described here show that band 3 is a second class of integral membrane protein to which protein 4.1 can bind. The interaction occurs in solution with purified components, and glycophorins are not required for protein 4.1 to bind to band 3. Moreover, the isolated cytoplasmic domain of band 3 can displace approximately 65% of bound protein 4.1 from eryth-
Protein 4.1 binds to Band 3 in Red Cell Membranes

Protein 4.1 and 43K form a saturable complex with discrete stoichiometry; analysis by nondenaturing gel electrophoresis and SDS-PAGE. Protein 4.1 (20 μg) was incubated with 125I-43K for 30 min at 4 °C and electrophoresed. Panel A shows a Coomassie-stained nondenaturing gel, and B is the corresponding autoradiogram. Lane 1, protein 4.1 alone; Lane 2, 43K alone; Lanes 3–6, contain protein 4.1 plus 5, 11, 22, or 44 μg of 43K. Lane 1 contains no radioactivity and is not seen in the autoradiogram. The corrected molar ratios of 43K to protein 4.1 in these samples were 0.6, 1.4, 2.7, and 5.4. Panels C and D show analyses of lanes 4 and 6, respectively, by SDS-PAGE in a second dimension.

Table II

Stoichiometry of the protein 4.1-43K complex

| 43K added/protein 4.1 mol/mol | 43K bound μg | 43K bound/protein 4.1 mol/mol |
|-------------------------------|--------------|-------------------------------|
| 0.6                           | 3.55 ± 0.05  | 0.41                          |
| 1.4                           | 6.82 ± 0.07  | 0.80                          |
| 2.7                           | 7.70 ± 0.24  | 0.90                          |
| 5.4                           | 7.50 ± 0.22  | 0.87                          |

Ratios of 43K to protein 4.1 in these samples were 0.6, 1.4, 2.7, and 5.4. Anti-43K alone inhibits protein 4.1 rebinding by 65%, and in combination with anti-glycophorin, by 74%. This suggests that at least 74% of rebound protein 4.1 is attached to specific membrane proteins; the true figure is probably higher since the lack of additivity of the antibody effects may represent steric hindrance with resultant incomplete inhibition. Thus, protein 4.1 is a component of the membrane skeleton which possesses the novel ability to bind alternatively and independently to two different attachment sites in the lipid bilayer.

An appreciation of the functional significance of the two binding sites for protein 4.1 requires a dynamic view of the membrane skeleton. The deformability and shape of the erythrocyte membrane vesicles, indicating that protein 4.1 is not bound to both band 3 and glycophorins simultaneously. Studies with antibodies to 43K and glycophorin support these conclusions.
Protein 4.1 or N-ethylmaleimide-4.1 (100 μg) was incubated for 15 min at 4 °C in the presence and absence of 43K (200 μg) in 150 μl of isotonic KCl. The samples were loaded onto 5–20% sucrose gradients in the same buffer; absorbance is shown in the upper right-hand panel. The arrow indicates the direction of sedimentation. Panels A–E show analysis of alternate fractions by SDS-PAGE. A, 43K, ---; B, 4.1, ---; C, 4.1 + 43K, ---; D, N-ethylmaleimide-4.1, ---; E, N-ethylmaleimide 4.1 + 43K, ·····.

erythrocyte, and perhaps other cells, appear to be related to metabolic events. For example, changes in the levels of 2,3-diphosphoglycerate and phosphatidylinositol 4,5-diphosphate cause alterations in the membrane skeleton, presumably as an appropriate response of the cell to its environment (32). The precise molecular details whereby these metabolites exert their effects are presently obscure, but the findings described here suggest some ways by which metabolic changes inside red cells may influence these membrane processes.

Although there are approximately five times as many potential binding sites for protein 4.1 on band 3 than there are on glycophorin A, based simply on the relative amounts of the two proteins in the membrane, it is likely that most of the 4.1 would be bound to the higher affinity sites on glycophorin, but only if the glycophorins are complexed to metabolically labile polyphosphoinositides. Factors that modify the states of phosphorylation of this phospholipid might alter this balance, allowing protein 4.1 to shift from glycophorin to band 3 sites.

The binding of specific ligands to the external segments of glycophorin could provide a stimulus for the shift of 4.1 from one site to another if dephosphorylation of polyphosphoinositides occurs as a consequence of ligand binding, and this could have pronounced effects on the membrane skeleton. One recent study seems to imply that some sort of functional linkage exists between glycophorin A and band 3 as hypothesized above. When glycophorin was cross-linked in erythrocyte ghosts by antibody to its extracellular segment, the rotational diffusion of band 3 decreased (19). This finding was taken to demonstrate a direct association of glycophorin A with band 3 in the membrane. Since treatment of leaky ghosts with anti-spectrin antibody failed to affect this process, mediation of this phenomenon by the membrane skeleton was considered unlikely. The present findings suggest another interpretation; the reduced mobility of band 3 may have been related to the binding of 4.1 molecules that dissociated from

\[^3\] R. A. Anderson and V. T. Marchesi, manuscript in preparation.
glycophorin sites as a consequence of antibody attachment to their external segments. The lack of an effect of anti-spectrin antibody would be predicted from the observation that the protein 4.1-band 3 complex does not bind spectrin.

This work has examined a simplified model of cytoskeletal organization, characterizing three components first as interacting pairs and then as an ensemble. In native erythrocytes, the situation is more complex, since actin associates with spectrin and protein 4.1 in a manner still to be defined. It is likely that actin may further modulate the spectrin-band 3 protein 4.1 interaction described above.

Recent studies have demonstrated that a variety of non-erythroid cells contain analogs of the proteins which comprise the erythrocyte membrane skeleton. Protein 4.1 (2, 20), band 3 (21), and spectrin (22-25) all have their counterparts in a growing and functionally diverse list of cells. The studies reported here may reflect the potential complexity that is characteristic of the interactions of the membrane skeleton with the integral membrane proteins of the lipid bilayer. These complex interactions are likely to be regulated by metabolic events within the cells, some of which may be triggered by stimulation of surface receptors. The simplified membrane of the erythrocyte may, in this instance, highlight the functions as well as the structures that are to be found in other cells.
Acknowledgments—We wish to thank Drs. Daniel Weaver, Spyridon Georgatos, Isabel Correas, and William Horne for helpful discussions. Verna Sowell provided excellent technical assistance. Marcia Bologna ably assisted in the preparation of the illustrations as did Catherine Haynes and Beth Auerbach in the typing of the manuscript.

REFERENCES
1. Branton, D., Cohen, C. M., and Tyler, J. (1981) Cell 24, 24-32
2. Cohen, C. M. (1983) Semin. Hematol. 20, 141-158
3. Haest, C. W. M. (1982) Biochim. Biophys. Acta 694, 331-352
4. Marchesi, V. T. (1983) Blood 61, 1-11
5. Bennett, V., and Stenbuck, P. J. (1980) J. Biol. Chem. 255, 6424-6432
6. Hargreaves, W. R., Giedd, K. N., Verkleij, A., and Branton, D. (1980) J. Biol. Chem. 255, 11965-11972
7. Weaver, D. C., Pasternack, G. R., and Marchesi, V. T. (1984) J. Biol. Chem. 259, 6170-6175
8. Tyler, J. M., Hargreaves, W. R, and Branton, D. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5192-5196
9. Ungewickell, E., Bennett, P. M., Calvert, R., Ohanian, V., and Gratzer, W. B. (1979) Nature (Lond.) 280, 811-814
10. Cohen, C. M., and Foley, S. F. (1982) Biochim. Biophys. Acta 688, 691-701
11. Anderson, R. A., and Lovrien, R. E. (1984) Nature (Lond.) 307, 655-658
12. Morrow, J. S., Speicher, D. W., Knowles, W. J., Hau, C. J., and Marchesi, V. T. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6592-6596
13. Steck, T. L. (1972) J. Mol. Biol. 66, 298-305
14. Knauf, P. A. (1979) Curr. Top. Membr. Transp. 12, 249-363
15. Casoly, R., and Salany, J. M. (1983) Biochim. Biophys. Acta 745, 134-139
16. Yu, J., and Steck, T. L. (1975) J. Biol. Chem. 250, 9176-9184
17. Murthy, S. N. P., Liu, T., Kaul, R. K., Kohler, H., and Steck, T. L. (1981) J. Biol. Chem. 256, 11203-11208
18. Appel, K. C., and Low, P. S. (1981) J. Biol. Chem. 256, 11104-11111
19. Nigg, E. A., Bron, C., Girardet, M., and Cherry, R. J. (1980) Biochemistry 19, 1887-1893
20. Cohen, C., Foley, S., and Kongsren, C. (1982) Nature (Lond.) 299, 248-250
21. Kay, M. M., Tracey, C. M., Goodman, J. R., Cone, J. C., and Bassel, P. S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6882-6886
22. Levine, J., and Willard, M. (1981) J. Cell Biol. 90, 631-643
23. Glenney, J., Glenney, P., and Weber, K. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4002-4005
24. Bennett, V., Davis, J., and Fowler, W. (1982) Nature (Lond.) 299, 126-131
25. Repasky, E., Granger, B., and Lazarides, E. (1983) Cell 29, 821-835
26. Bennett, V. (1979) Nature (Lond.) 281, 597-599
27. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
29. Morrow, J., Haigh, W., and Marchesi, V. T. (1981) J. Supramol. Struct. 17, 275-287
30. Leto, T. L., and Marchesi, V. T. (1984) J. Biol. Chem. 259, 4603-4608
31. Tarone, G., Hamasaki, N., Fukuda, M., and Marchesi, V. T. (1979) J. Membr. Biol. 48, 1-12
32. Sheetz, M. P. (1980) Semin. Hematol. 20, 175-188
33. Caturecasa, P. (1970) J. Biol. Chem. 245, 3059-3065
34. Shiffer, K. A., and Goodman, R. S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4404-4408
35. Cotmore, S. F., Furthmayr, H., and Marchesi, V. T. (1977) J. Mol. Biol. 113, 559-555
36. Mueller, T. J., and Morrison, M. (1981) in Erythrocyte Membranes: Recent Clinical and Experimental Advances (Krukenberg, W. C., Eaton, J. W., and Brewer, G. J., eds) pp. 95-112, Alan R. Liss, New York
37. Sato, S. B., and Ohnishi, S. (1983) Eur. J. Biochem. 130, 19-26
38. Dixon, M. (1983) Biochim. J. 255, 170-171