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INTEGRAL MEMBRANE PROTEIN INTERACTION WITH TRITON CYTOSKELETONS OF ERYTHROCYTES

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Summary

The organization of erythrocyte membrane lipids and proteins has been studied following the release of cytoplasmic components with the non-ionic detergent Triton X-100. After detergent extraction, a detergent-resistant complex called the erythrocyte cytoskeleton is separated from detergent, solubilized lipid and protein by sucrose buoyant density sedimentation. In cytoskeletons prepared under isotonic conditions all of the major erythrocyte membrane proteins are retained except for the integral protein, glycophorin, which is quantitatively solubilized and another integral glycoprotein, band 3, which is only 60% removed. When cytoskeletons are prepared in hypertonic KCl solutions, band 3 is fully solubilized along with bands 2.1 and 4.2 and several minor components. The resulting cytoskeletons have the same morphology as those prepared in isotonic buffer but they are composed of only three major peripheral proteins, spectrin, actin and band 4.1. We have designated this peripheral protein complex the 'shell' of the erythrocyte membrane, and have shown that the attachment of band 3 to the shell satisfies the criteria for a specific interaction. Although Triton did affect erythrocyte shape, cytoskeleton lipid content and the activity of membrane proteases, there was no indication that Triton altered the attachment of band 3 to the shell. We suggest that band 3 attaches to the shell as part of a ternary complex of bands 2.1, 3 and 4.2.

Introduction

Non-ionic detergents have been used in a variety of membrane systems to solubilize lipids and integral membrane proteins without causing the loss of

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DFP, diisopropylphosphofluoridate.

enzymatic function or otherwise denaturing the proteins [1,2]. As a logical extension of those studies, investigators have studied the attachment of integral membrane proteins to peripheral proteins of the cell cytoskeleton by using non-ionic detergents to remove non-interacting membrane lipids and proteins [3,4]. It has been reasoned that since many cellular processes involve communication between the cell surface glycoproteins and the cell cytoskeleton, there may exist physical contacts between the components which would be insensitive to non-ionic detergents. The human erythrocyte provides a well-characterized cell system in which the effects of detergents on the attachment of cell surface proteins to the cell cytoskeleton can be investigated.

Our previous studies indicated that a major erythrocyte membrane glycoprotien, band 3, copurified with erythrocyte 'cytoskeletons' prepared by Triton extraction of intact cells [5]. Whole cells were extracted so as to avoid altering the ionic environment of the proteins and consequently affecting the component interactions. Because an intact cell system was extracted, additional detergent-induced artifacts may possibly have been introduced. We have, therefore, studied the process of Triton extraction of erythrocytes to determine if the detergent altered the component interactions. Of particular interest are the specificity of and ionic requirements for the coisolation of integral membrane proteins with the erythrocyte cytoskeleton.

Materials and Methods

Human erythrocytes were obtained from healthy donors and used within 2 days. Citrate (10 mM final concentration) was used as an anticoagulant. Blood was centrifuged (1500 $\times g$ for 10 min), the supernatant and buffy coat were aspirated, and cells were suspended in an isotonic Hepes/Ringer solution. This procedure was repeated once and then the suspended cells (50% hematocrit) were incubated with 2 mM diisopropylphosphofluoridate (DFP) in Hepes/Ringer for 30 min at 37°C. After DFP treatment, cells were pelleted and resuspended in Tris-buffered saline (140 mM NaCl, 24 mM Tris, pH 7.4) twice. A 40% suspension of red cells in Tris-buffered saline was mixed on ice with an equal volume of a Triton X-100 suspension in the extraction buffer (140 mM KCl, 24 mM Hepes, 1.0 mM $MgCl_2$, 0.5 mM EGTA, 0.05 mM $CaCl_2$, and 2 mM reduced glutathione (pH 7.0)). The mixture was applied to a linear sucrose gradient (10—60%) in the extraction buffer without Triton and centrifuged 100 000 $\times g$ for 1.5 h. The light-scattering band in the gradient was isolated by puncturing the bottom of the tube and collecting 1 ml fractions. The most protein was found in the fraction with the most light scattering. The desnity of the cytoskeletons was determined by measuring the refractive index of the fraction with maximal light scattering. After visual examination, the fractions containing light-scattering material (normally 3 ml) were pooled, diluted ten fold and centrifuged at 40 000 $\times g$ for 20 min. A portion of the diluted material was counted in a Petroff-Hausser bacteria counter using dark-field illumination to determine the total number of cytoskeletons. After pelleting, the cytoskeletons were resuspended in Tris-buffered saline and the total protein content of the cytoskeletons was measured using the Lowry method [6] with bovine serum albumin as a standard.

Light microscopy. Cytoskeletons were fixed by mixing 1 : 1 with a 2% solution of glutaraldehyde in phosphate-buffered saline (145 mM NaCl, 5 mM phosphate, pH 7.4). Cytoskeletons were viewed by darkfield illumination in an Olympus BH microscope.

Lipid analysis. Lipids were extracted from the cytoskeleton by the procedure of Alan and Michell [7] and concentrated by vacuum evaporation. Samples were exchanged into $CHCl_3$ by successive evaporations and applied to thin-layer plates (Silica Gel G, Merck). Plates were developed in $CHCl_3/CH_3OH/$ conc. NH_4OH (90 : 51 : 11) and visualized by I_2 vapors. Spots corresponding to the various lipid classes were scraped into 5-ml tubes, digested with 10 N H_2SO_4, and the phosphate was assayed by the micromethod of Bartlett [8]. Erythrocyte ghosts were run as a control and values were obtained which agreed with published figures.

Extraction of erythrocyte ghosts. Washed red cells at 50% hematocrit were lysed 1 : 100 in 10 mM Tris, pH 7.4, or 5 mM phosphate, pH 7.4, and pelleted by centrifuging at 25 000 × *g* for 10 min. The pellet was washed twice with the lysis buffer. The white membranes were suspended at 1.2 mg/ml in 140 mM KCl, 24 mM Hepes (pH 7.0) either with or without 1 mM Mg^{2+}, 1 mM ATP and 0.2 mM EGTA. Membranes with MgATP were incubated at 37°C while those without were kept on ice for 15 min. The membranes were then mixed with a 1% Triton extraction solution and the normal extraction procedure was followed.

SDS gel electrophoresis. Samples were solubilized by the addition of 2% SDS, 5% β-mercaptoethanol and by incubation at 100°C for 2—3 min. The procedure of Fairbanks et al. [9] was used for the gel electrophoresis with 3.25% or 5.6% polyacrylamide gels.

Results

Triton extraction

When erythrocytes were treated with lytic concentrations of Triton X-100 under isotonic conditions, a complex of protein, lipid and detergent was isolated by buoyant density sedimentation in a linear sucrose gradient. These complexes, termed 'cytoskeletons' [5], were prepared with different detergent concentrations to determine the effects of detergent. Triton X-100 concentrations were expressed as mg of detergent/1 ml of packed cells (approx. 10^{10} cells/ml) because the properties of the cytoskeletons correlated with this parameter and not with the percent of detergent in the extraction solution. With increasing amounts of detergent the buoyant density of the cytoskeletons increased radically (Fig. 1). This was the equilibrium buoyant density since the cytoskeletons migrated no further into the gradient upon continued centrifugation. Both the detergent and lipid are lower in density than protein; therefore, the loss of either component could explain the rise in density. When the detergent content of the cytoskeletons was measured, however, only 1—1.5% of the cytoskeleton mass was found to be detergent (Table I). On the other hand, the lipid content of the cytoskeletons was as high as 35% and decreased with increasing detergent concentration (Fig. 1). Increasing the detergent concentration removes larger amounts of membrane phospholipid

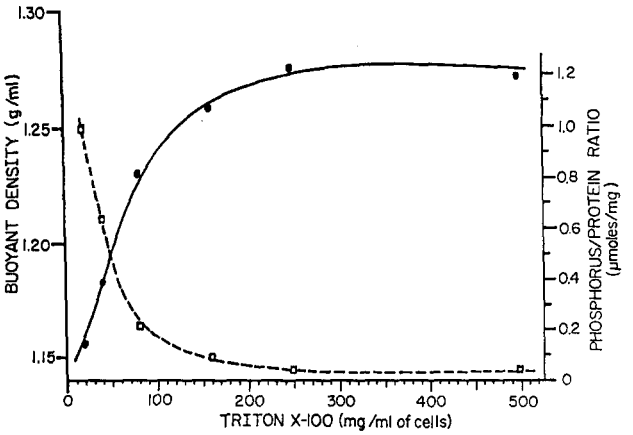


Fig. 1. The buoyant density and the phosphate content of the cytoskeletons are plotted versus the ratio of Triton X-100 to cells (mg of Triton X-100/ml of cells).

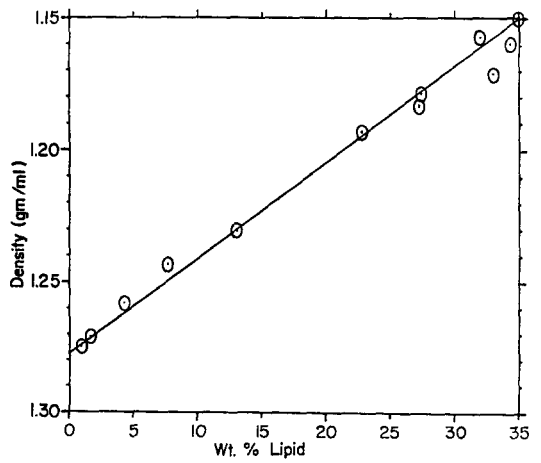


Fig. 2. The wt% of phospholipids is plotted versus the density of the cytoskeleton. The wt% of phospholipid was the quotient of the mass of phospholipid (a molecular weight of 740 was assumed) and the combined mass of phospholipid and protein/cytoskeleton.

TABLE I

Determined using [^{3}H]Triton X-100. Phospholipid was computed from lipid phosphorus content of the shells.

Triton concentration	Cytoskeletal protein/particle (mg)	Buoyant density (g/ml)	Triton (mg/mg protein)	Phospholipid (mg/mg protein)
0 (Tris ghosts)	$5.0 \pm 0.4 \cdot 10^{-10}$	1.15 ± 0.01	0	0.66 ± 0.06
30 mg/10^{10} cells	$2.7 \pm 0.4 \cdot 10^{-10}$	1.17 ± 0.01	0.02 ± 0.005	0.50 ± 0.05
300 mg/10^{10} cells	$1.5 \pm 0.2 \cdot 10^{-10}$	1.27 ± 0.01	0.01 ± 0.003	0.03 ± 0.02

and in the limit over 95% of the phospholipid can be removed. If the density is primarily determined by the phospholipids and protein, then the density of the cytoskeletons should be linearly related to the wt% of phospholipids. As seen in Fig. 2, the linear relationship holds. From Fig. 2 it is possible to determine the phospholipid content if the cytoskeleton density is known.

During the process of Triton extraction the membrane may undergo dramatic shape changes which could alter membrane protein interactions. Since the changes would occur too rapidly after mixing to be observed, the process was experimentally slowed by either mixing the cells with increasing concentrations of detergent or by fusing a drop of cells with a drop of detergent solution under a coverslip where cells could be observed as detergent diffused to them. With increasing detergent concentration the cells would initially crenate, then higher concentrations would reverse the shape to a cupped or invaginated form, but upon lysis the membranes would recover a disc-like morphology. As the membrane lipids are extracted with higher detergent concentrations, the membranes shrink in area without observable crenation or cupping. The extracted membranes or cytoskeletons reach a limiting size of 2.5—3.0 μm in diameter regardless of whether the Triton extraction was performed at 0°C or at 25°C, in 0.02—0.6 M KCl, with 0—2 mM EDTA or 0—5 mM Mg^{2+}, or at pH 6.5 or 8.5. The limiting size was also not a function of the phospholipid content (Fig. 3a and b). Thus the cytoskeleton size is remarkably uniform but the particle has 25% or less of the surface area of the original membrane.

When pelleted cytoskeletons were analyzed in the electron microscope after embedding and sectioning, morphological differences between the 'lipid-containing' (approx. 60% lipid present) and the 'lipid-free' (less than 5% lipid present) cytoskeletons were evident (Fig. 3c and d). The membranous shell contains bilayer-like structures when lipid is present but has a fuzzy appearance when lipid is removed.

The number of cytoskeletons recovered from the gradient is normally 50—60% of the number of cells applied. Significant amounts of material are lost through adherance to the walls of the tube which accounts for most of the lost cytoskeletons.

The amount of protein/cytoskeleton is not constant with the removal of lipids but decreases (Table I). Although Triton does affect the Lowry assay, the change cannot be accounted for by detergent. Another possibility is that specific proteins are removed along with the lipid. As seen in Fig. 4 there is only a slight change with lipid removal in the Coomassie blue-staining pattern of the cytoskeletons electrophoresed on SDS gels. Components 3 and 4.2 do decrease in staining intensity but only by 10—30% which would account for 3—5% of the total protein. Likewise the extraction of glycophorin could not account for a significant loss of protein. Although the analyses of protein concentration throughout the gradient have revealed that the majority of the membrane proteins are in the single light-scattering band, with higher detergent concentrations there is more protein in the low density fractions. Only large complexes with high sedimentation coefficients such as the cytoskeletons will reach their equilibrium density during the centrifugation whereas small fragments which bud off from the cytoskeletons will be found in lower density fractions. In Fig. 3 the micrograph of the cytoskeletons which contain lipid

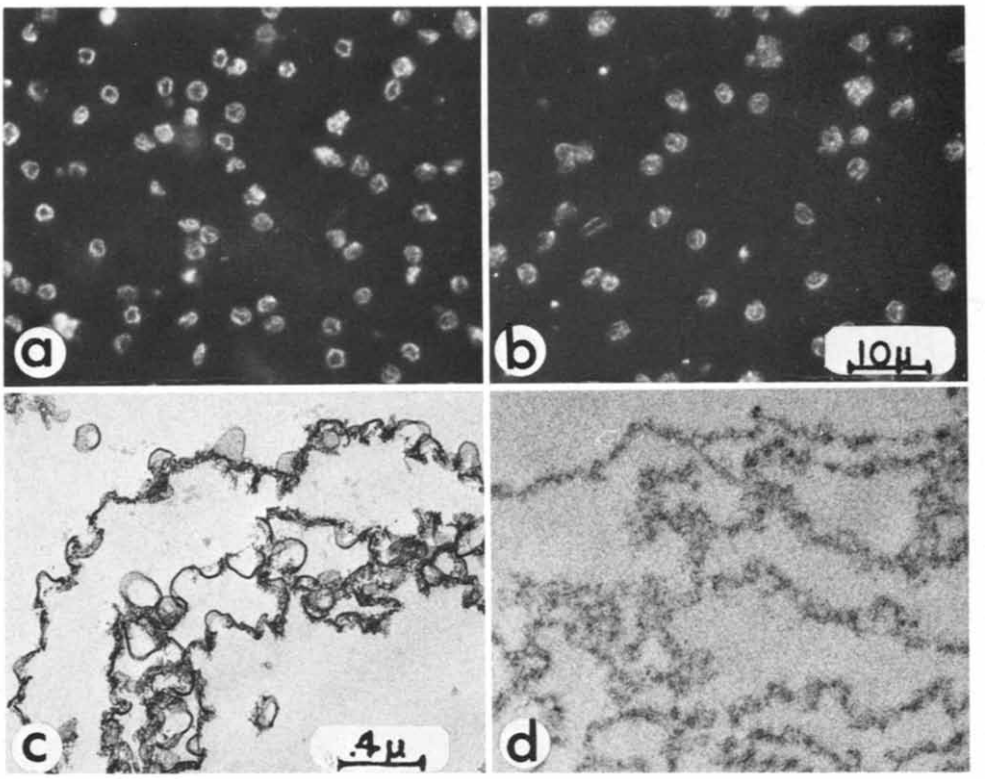


Fig. 3. Darkfield light (a and b) and electron (c and d) micrographs of cytoskeletons prepared with 30 mg (a and c) or 300 mg (b and d) of Triton/ml of cells show that the limiting size of the cytoskeletons is invariant with phospholipid content but the bilayer structure is lost with lipid removal.

(Fig. 3c) clearly shows regions which appear as buds that may pinch off with further lipid removal. The loss of protein from the cytoskeletons with lipid extraction is nearly random and can be explained as the result of loss of cytoskeleton fragments.

From the densitometer scans of Coomassie blue-stained gels an estimate of the percentage of the various polypeptides in the cytoskeletons can be made by integration. In Fig. 4 the percentage of staining in the various fractions is noted below the peaks. When the percentage protein in a given band is multiplied by the amount of protein/cytoskeleton, an estimate of the amount of that protein/cytoskeleton can be made. In cytoskeletons prepared with 30 mg of Triton/ml of cells the amount of spectrin/cytoskeleton was the same as the amount of spectrin/ghost. With higher concentrations of Triton the recovery of spectrin diminished. Of the Coomassie blue-staining components spectrin, actin and 4.1 were preferentially retained, whereas band 3 was extracted to the greatest extent.

Identification of band 3

Up to this point band 3 has been identified only by its mobility on SDS-polyacrylamide gels but we have verified that the transmembrane glycoprotein

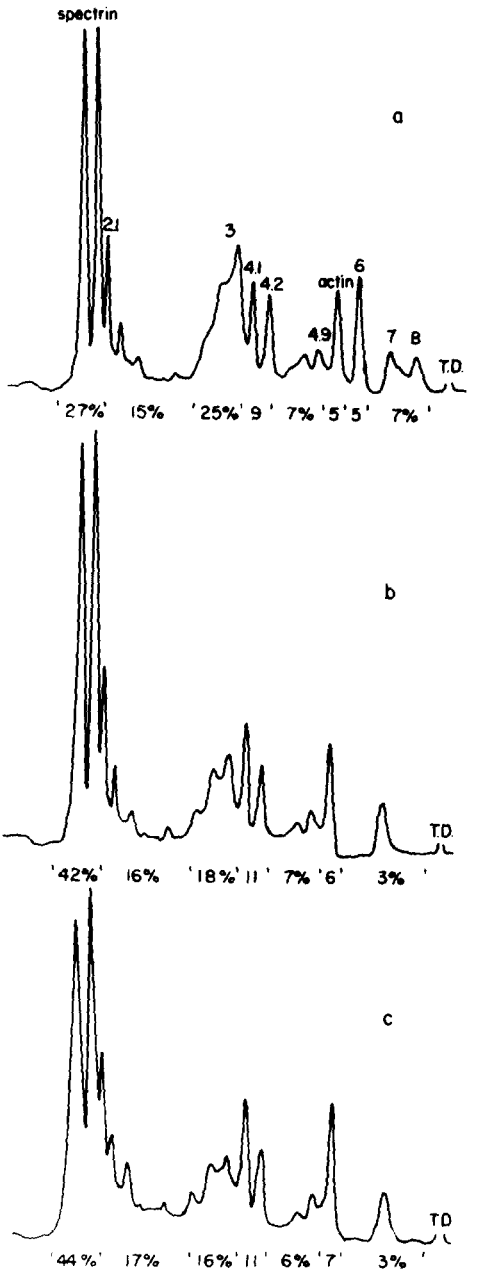
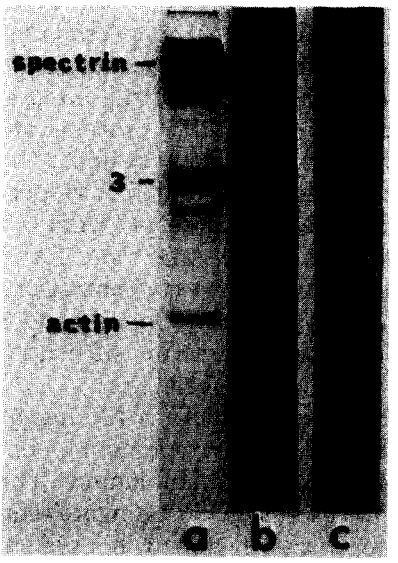


Fig. 4. Densitometer scans (550 nm) of SDS-polyacrylamide gels (3.25% acrylamide) of (a) erythrocyte membranes prepared with 10 mM Tris (pH 7.4), and cytoskeletons prepared with (b) 30 mg, or (c) 300 mg of Triton X-100/ml of cells.

is present using specific antibodies and proteolytic cleavage. In Fig. 5 we see that component 3 binds antibodies directed against the 88 000 dalton transmembrane glycoprotein. The lower molecular weight proteins which react with the anti-band 3 antibodies are also found in freshly prepared erythrocyte



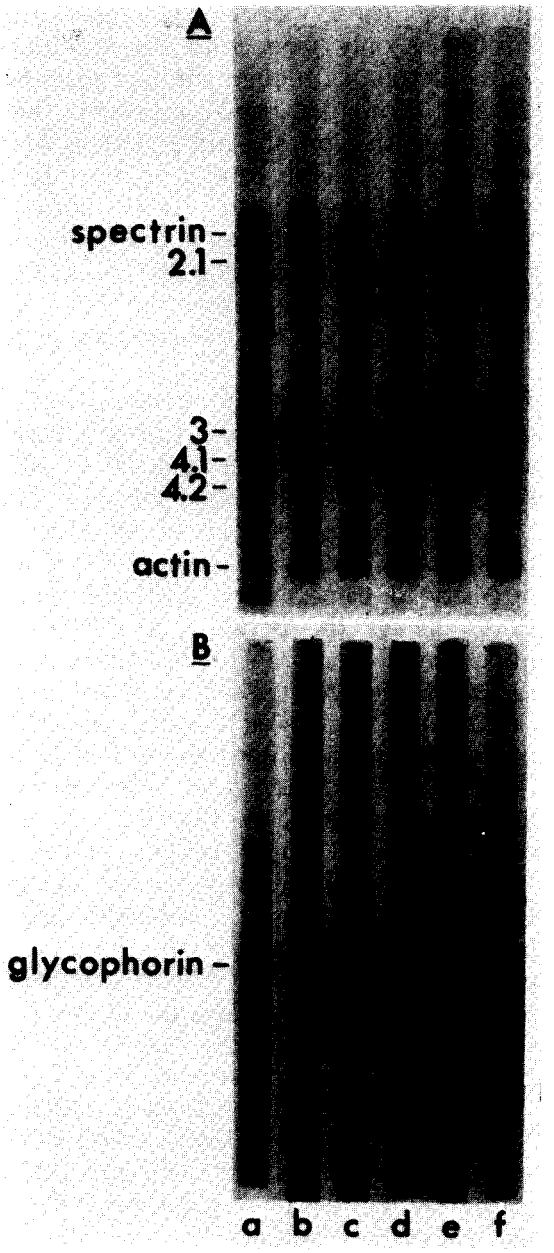


Fig. 5. SDS-polyacrylamide gels of cytoskeletons (300 mg of Triton X-100/ml of cells) stained with (a) Coomassie blue, and autoradiograms of gels of the stained with (b) anti-band 3 antibody of Fukida et al. [25] or (c) antispectrin antibody. The procedure of Burridge [26] was used to identify the antigens in the gel except that ^{125}I-labeled *Staphylococcus aureus* protein was used to tag antigen-antibody complexes in the gel.

Fig. 6. SDS-polyacrylamide gels (3.25% acrylamide) stained with Coomassie blue (A) or periodic acid-Schiff (PAS) (B) of ghosts (a) or cytoskeletons prepared with 5 mg (b), 15 mg (c), 30 mg (d), 100 mg (e) or 300 mg (f) of Triton X-100/ml of cells. In (A) 20 μg and in (B) 100 μg of protein was applied to each gel.

ghosts (Marchesi, V., personal communication). On the other hand anti-spectrin antibodies only react with the 220 000—250 000 dalton components in the cytoskeletons. Although the fact that the antibody binds to the cytoskeleton material shows that band 3 is present, the antibody-staining technique does not allow quantitation of band 3 content of the cytoskeletons. Chymotrypsin has been used to cleave band 3 in the intact cell and after chymotrypsinization, there is no staining in the 88 000 dalton region. A band appears upon chymotrypsinization with an apparent molecular weight of approx. 60 000 and the intensity of staining of the 60 000 dalton protein is as great as that of band 3. Therefore band 3 is present in the cytoskeletons and the majority of the staining intensity at 88 000 daltons is from band 3.

Extraction of lipids and integral proteins

If integral proteins were randomly distributed in the lipid bilayer as has been suggested [10], then the removal of 50% of the lipid might be expected to remove 50% of the integral proteins. Therefore, we have analyzed cytoskeletons having different lipid contents for the two major integral membrane proteins, band 3 and glycophorin. In Fig. 6 we see that the Coomassie blue-staining intensity of band 3 stays relatively constant with decreasing lipid concentration whereas the periodic acid-Schiff-staining glycophorin is almost completely removed. Extractability has been quantitated by comparing the percentage of the integral protein with the percentage of lipid retained in the cytoskeletons. The extraction of component 3 and glycophorin greatly exceeds that of the lipid (Fig. 7) until about 40% of the lipid is removed. Extraction of the remaining 60% of the lipid only removes an additional 4—8% of the integral membrane proteins. It, therefore, appears that the extractable proteins are easily extracted but a portion of the protein remains tightly bound. The tightly bound fractions of band 3 and glycophorin are 40% and 5%, respectively, of the polypeptide originally present. When we varied the pH from 6.5 to 8.5, the ionic strength from 0.05 to 0.20, divalent cation (Mg^{2+} and Ca^{2+}) concentrations from 10^{-9} to 10^{-3} M and ATP and glutathione concentrations from 0 to 2 mM, there was no change in the amount of tightly bound band 3 or glycophorin. Therefore, there is a relatively constant interaction of band 3 but only a negligible interaction of glycophorin with the cytoskeletons.

Analysis of the chloroform/methanol extract of cytoskeletons (30 mg of Triton/ml of cells) revealed that 70% of the phospholipids were sphingomyelin, 20% were phosphatidylcholine and 10% were phosphatidylethanolamine. The solvent system used for quantitation did not allow resolution of sphingomyelin and phosphatidylserine. However, when the plates were developed in a system which resolved these two lipids (chloroform/acetone/methanol/acetic acid/water, 5 : 2 : 1 : 1 : 0.5), phosphatidylserine was found to be present in only trace amounts.

Removal of band 3 from cytoskeletons

Certain conditions were found to remove band 3 from the cytoskeletons but in all cases the loss of band 3 was coupled with the loss of components 2.1, 4.2 and numerous minor bands. Further, in all cases the morphology of the cytoskeletons was unaltered by the removal of band 3 and the other proteins.

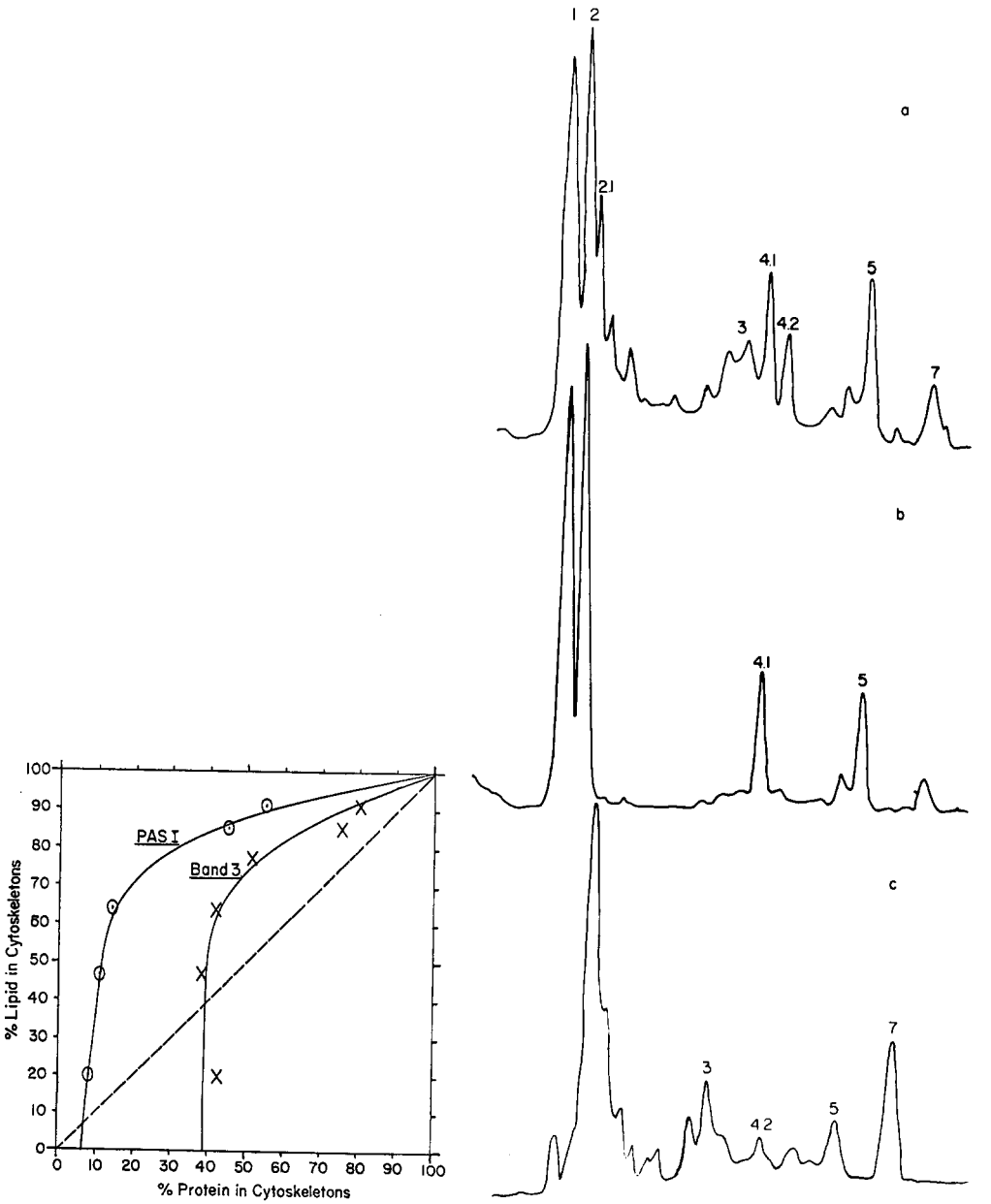


Fig. 7. Percentage of lipid remaining/cytoskeleton is plotted versus the percentage of band 3 and glycophorin retained/cytoskeleton. The percentage of glycophorin was computed from the periodic acid-Schiff-staining (PAS) intensity/mg of protein whereas the ratio of band 3 to spectrin staining intensity was used to calculate the percent of band 3.

Fig. 8. Densitometer scans (550 nm) of Coomassie blue-stained 3.25% polyacrylamide gels of (a) cytoskeletons (300 mg Triton X-100/ml of cells); (b) the pellet after extraction of cytoskeletons with 0.6 M KCl, 0.5 mM ATP (pH 7.5), and centrifugation at 40 000 X *g* for 10 min, and (c) the supernatant of that extraction.

Hypertonic treatment (greater than 0.3 M KCl) of intact cells during the Triton extraction produced cytoskeletons which contained spectrin, actin, 4.1 and only trace amounts of other proteins. The density of the hypertonic cytoskeletons which lacked bands 3, 2.1, 4.2, etc. was not greater than the density of isotonic cytoskeletons except at detergent concentrations greater than 200 mg Triton/ml of cells. When lipid-free isotonic cytoskeletons were treated with 0.6 M KCl immediately after isolation from the sucrose gradient, components 2.1, 3, 4.2, etc. were solubilized but in aged cytoskeletons those components were less extractable. As seen in Fig. 8 the solubilized components were recovered in the supernatant which indicated that the components were not lost through proteolysis.

If erythrocyte membranes are prepared by hypotonic lysis and washing in 10 mM Tris, then Triton extraction will also remove 2.1, 3, and 4.2 from the cytoskeleton. After the membranes are exposed to 10 mM Tris for 1—2 h, the ionic conditions and membrane shape can be altered to approximate those of the intact cell without altering the composition of the Triton cytoskeleton. On the other hand, if membranes are prepared with 5 mM phosphate (pH 7.4), then a significant fraction of band 3 is retained in the Triton cytoskeletons.

Discussion

Definition of the shell

An important result of these studies is the definition of the basic structural complex of the cytoskeletons which we have designated the shell [11]. The shell is composed of spectrin, actin, bands 4.1 and 4.9 and has the same morphology as the cytoskeleton in the light microscope. Notably lacking in the shell is the presumed membrane binding site for spectrin, component 2.1 [12], and the integral protein, band 3. Since the shell structure is lost when either spectrin [13] or actin [14] alone are solubilized but the loss of 2.1 and 3 have no affect on the structure, the attachment of spectrin to its binding site and the integral protein, band 3, are not required for the structural integrity of the shell. The lipids also appear not to be part of the shell structure because their removal has no effect on the morphology of the shell. The shell, therefore, is a peripheral protein complex which we suggest is as extensive as and tightly bound to the membrane bilayer in vivo. In this study we have found that a portion of the integral protein, band 3, is bound to the shell upon Triton extraction of intact erythrocytes.

Possible detergent artifacts

Although a specific integral protein copurifies with the shells, the addition of Triton may have altered the in vivo interaction. Considerable inferential evidence, however, has been collected which indicates that Triton X-100 does not alter protein-protein complexes in the erythrocyte cytoskeleton [1,2,4,5,15, 16]. When whole cells are treated with detergents, however, there are additional detergent effects which can alter membrane shape and can release or activate degradative enzymes. The shape changes that follow the addition of Triton to erythrocytes are explicable as effects of amphipathic compounds on the relative membrane surface areas [17,18], but the cell morphology does not

correlate with the binding of band 3 to the shell. On the other hand, proteolysis was activated by Triton but no other degradative activity was observed during cytoskeleton preparation. DFP pretreatment blocked the proteolysis which was noted particularly in component 2.1. No evidence was found which indicated that the current procedure for cytoskeleton preparation altered protein-protein interactions in the erythrocyte membrane.

Integral protein binding to the shell

These Triton extraction studies provide a definition of the binding of integral proteins to the shell. Because 90% of glycophorin and 60% of band 3 are solubilized with 40% of the lipids, the interactions of these components with the shell appear weak. A strong interaction of 40% of band 3 with the shell is found and this corresponds to an approximate stoichiometry of 1 mol of band 3 dimer/mol of spectrin dimer. Further studies are underway to determine if certain minor glycoproteins or certain fractions of band 3 are retained in the cytoskeletons.

The attachment of 3 to the shell is dissociated by hypertonic solutions and by hypotonic Tris solutions. In both of those cases components 2.1 and 4.2 are also removed from the cytoskeletons. Bennett and coworkers have shown that component 2.1 forms a binding site for spectrin on inside out vesicles [12,19]. An interpretation consist with these findings is that components 3, 4.2 and 2.1 form a complex which is attached to the shell by an interaction between spectrin and component 2.1. This may be an oversimplified view since in our earlier studies when component 2.1 was proteolysed, component 3 was still retained in the cytoskeletons [5].

Erythrocyte membrane structure

These findings support a more defined model of the erythrocyte membrane [11] in which the membrane is composed of three major structural components: (1) the membrane bilayer of lipids and integral proteins; (2) the peripheral protein shell, and (3) linkage proteins which serve to connect the other two components. Several pieces of evidence support the view that the bilayer and the shell are distinct entities. When intact cells are extracted with Triton most bilayer components are readily solubilized and separated from the shell. In a similar manner ATP depletion of erythrocytes causes the shedding of integral proteins and lipids in microvesicles which contain no shell proteins [21]. The specific retention of the lipid, sphingomyelin, in the cytoskeleton need not imply a protein-lipid interaction, but may be a result of the presence of a high percentage of saturated fatty acids in sphingomyelin [1,20]. Conversely, hypotonic extraction of erythrocyte membranes solubilizes the major shell components, spectrin and actin, and leaves the bilayer. When the shell components are solubilized the bilayer fragments into small vesicles and when the bilayer is removed, the shell contracts to one-fourth of its original area. We suggest that the bilayer and shell are not continuously joined but that there are a discrete number of contact points formed by linkage proteins such as component 2.1. The linkage proteins may or may not be involved in controlling the two-dimensional distribution of membrane glycoproteins.

There is considerable interest in the mechanism by which shell components,

specifically spectrin, control the lateral distribution of glycoproteins in erythrocyte membranes [22,23]. Cherry et al. [24] have found that the rotational motion of band 3 is unaffected by spectrin removal. They suggest, therefore, that spectrin must indirectly control the lateral distribution of band 3. In these studies we also find no evidence of extensive attachment of glycoproteins to spectrin but all the studies cannot be directly compared because different conditions were used.

In conclusion, we wish to emphasize that the Triton extraction procedure will remove components which are only weakly attached to the shell through detergent action or dilution on the sucrose gradient. Yet we observe that a significant fraction of the integral protein band 3 is attached to the shell even after treatment with solutions containing 5% Triton X-100. The attachment of band 3 depends upon the presence of bands 2.1 and 4.2 which we suggest form linkages between the bilayer glycoproteins and the shell. These linkages possibly provide a direct means of communicating changes in band 3 cross-linking by lectins to shell components involved in erythrocyte membrane shape changes [13]. In addition, the linkage will obviously stabilize the association between the shell and the bilayer and prevent separation of the two which leads to bilayer vesiculation and condensation of the shell.

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References

1 Tanford, C. and Reynolds, J.A. (1976) Biochim. Biophys. Acta 457, 133—153
2 Helenious, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79
3 Koch, G.L.E. and Smith, M.F. (1978) Nature 273, 274—278
4 Yu, J., Fischman, D. and Steck, T.L. (1973) J. Supramol. Struct. 1, 220—233
5 Sheetz, M.P. and Sawyer, D. (1978) J. Supramol. Struct. 8, 399—412
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
7 Alan, D. and Michell, R.H. (1975) Biochem. J. 148, 471—478
8 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
9 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606—2617
10 Singer, S.J. and Nicolson, G.L. (1972) Science 175, 720—725
11 Sheetz, M.P. (1979) in Erythrocyte Mechanics and In Vivo Flow, Kroc Symposia, Vol. 12, Alan Liss, Inc., New York, in the press
12 Bennett, V. and Stenbuck, P.J. (1979) J. Biol. Chem. 254, 2533—2541
13 Sheetz, M.P., Sawyer, D. and Jackowski, S. (1978) in The Red Cell (Brewer, G., ed.), pp. 431—450, Alan R. Liss, Inc., New York
14 Sheetz, M.P. (1979) J. Cell Biol. 81, 266—270
15 Wolosin, J.M., Ginsbury, H. and Cabantchik, A.I. (1977) J. Biol. Chem. 252, 2419—2427
16 Tilney, L.G. and Detmers, P. (1975) J. Cell Biol. 66, 508—520
17 Sheetz, M.P. and Singer, S.J. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4457—4461
18 Sheetz, M.P., Painter, R.G. and Singer, S.J. (1976) J. Cell Biol. 70, 193—203
19 Bennett, V. (1978) J. Biol. Chem. 253, 2292—2299
20 Dodge, J.T. and Phillips, G.B. (1967) J. Lipid Res. 8, 667—675
21 Lutz, H., Lieu, S. and Palek, J. (1977) J. Cell Biol. 73, 548—560
22 Nicolson, G.L. and Painter, R.G. (1973) J. Cell Biol. 59, 395—406
23 Shotton, D., Thompson, K., Wofsy, L. and Branton, D. (1978) J. Cell Biol. 76, 512—531
24 Cherry, R.J., Burkli, A., Busslinger, M., Schneider, G. and Parish, G.R. (1976) Nature 263, 389—393
25 Fukida, M., Eshdat, Y., Farone, G. and Marchesi, V.T. (1978) J. Biol. Chem. 253, 2419—2428
26 Burridge, K. (1978) Proc. Natl. Acad. Sci. U.S. 73, 4457—4461