Contribution of Ankyrin-Band 3 Complexes to the Organization and Mechanical Properties of the Membrane Skeleton of Human Erythrocyte

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ABSTRACT

To understand the role of ankryin-band 3 complexes in the organization of the spectrin-based membrane skeleton and its contribution to the mechanical properties of human erythrocytes, intact skeletons and single-layered skeleton leaflets were prepared from intact and physically sheared membrane ghosts, expanded in low salt buffer, and examined by transmission electron microscopy.

While the structures of intact skeletons and single-layered skeleton leaflets shared many common features, including rigid junctional complexes of spectrin, actin, and band 4.1; short stretches (~50 Å) of flexible spectrin filaments; and globular masses of ankyrin-band 3 complexes situated close to the middle of the spectrin filaments, the defination of structural units in the intact skeleton is obscured by the superposition of the two layers, However, the spatial disposition of structural elements can be clearly defined in the images of the single-layered skeleton leaflets.

Partially expanded skeletal leaflets contain conglomerates of ankyrin-band 3 complexes arranged in a circular or clove-leaf configuration that straddles multiple strands of thick spectrin cables, presumbaly reflecting the association of ankyrin-band 3 complexes on neighboring spectrin tetramers as well as the lateral association of the spectrin filaments. Hyperexpansion of the skeleton leaflets led to dissociation of the conglomerates of ankyrin-band 3 complexes, full-extension of the spectrin tetramers, and separation of the individual strands of spectrin tetramers. Clearly defined strands of spectrin tetramers in the hyperexpanded single-layered skeletal leaflets often contained two sets of globular protein masses that divided the spectrin tetramers into three segments of approximately equal length.

These results suggest that the neighboring ankyrin-band 3 complexes form high-

molecular weight comglomerates in situ, and that the reversible association and dissociation of these conglomerates play an important role in the organization and function of the membrane skeleton of the human erythrocytes.

The membrane of THE human erythrocyte is reinforced at its cytoplasmic surface by a flexible filamentous skeleton which consists of spectrin, actin, and band 4.1 (2, 8, 13, 14, 37, 47). This network is durable, flexible, and elastic (17, 19, 26, 46). It can be isolated intact and still associated with a number of accessory proteins, notably ankyrin and band 3 (and its associated proteins), by dissolving away the lipid bilayer with Triton X-100 (36). Treatment of the Triton residual with molar salt solution removes the majority of the accessory proteins, leaving a basic skeleton of spectrin, actin, and band 4.1 (37).

Ultrastructural evidence has suggested that the skeleton is organized into an irregular polygonal lattice of short actin protofilaments interconnected by mutiple strands of spectrin tetramers (6, 20, 37). The association between actin and spectrin is strengthened by band 4.1 (10, 30). This skeletal network is attached to the membrane bilayer through the linkage between spectrin and band 3 via ankyrin (3-5, 15, 43) and through a second association of band 4.1 at the junctional complex to either glycophorin C (1, 27) or band 3 (32). The organization of the junctional complexes and their role in the integrity of the skeleton have been well-documented (reviewed in ref. 2), but little is known about the organization of the ankyrin-band 3 complexes in situ, and it is equally unclear whether the ankyrin-band 3 complexes play any role in the function of the skeleton in addition to being the major attachment site between the skeleton and the membrane bilayer.

In this study, we examined the structure of single-layered skeleton leaflets of erythrocyte membrane, with special attention to the spatial disposition of ankyrin-band 3 complexes in skeletons freshly released from the membrane bilayer and to changes in them accompanying the gradual expansion of the skeleton. Our results suggest that each

spectrin tetramer binds two molecules of ankyrin in situ and that ankyrin-band 3 complexes of neighboring spectrin tetramers form higher molecular weight conglomerates which dissociate upon expansion of the skeletal network. We suggest that the ankyrin-band 3 complexes may play important roles in the organization of the skeleton and that the reversible dissociation and association of the ankyrin-band 3 conglomerates may be the molecular basis underlying the mechanical properties of the skeletal network.

Materials and Methods

All chemicals were of reagent grade from Fisher Scientific Co. (Pittsburg, PA), Mallincrodt Inc. (St. Louis, MO), or J. T. Baker Chemical (Allentown, PA). Biochemicals were from Sigma Chemical Co. (St. Louis, MO). Electron microscopy supplies were from Ted Pella, Inc., Tustin, CA).

Preparation of physically sheared vesicles

Membranes (ghosts) were prepared as described (12) in 5 mM sodium phosphate at pH 8 (5p8) containing 2 mM $MgCl_2$. The ghosts were diluted by 4 volumes of 2 mM sodium phosphate at pH8 (2p8) and were physically sheared by forcing through a 27-gauge needle. The extent of shearing was monitored initially by phase-contrast light microscopy, but later on by the disappearance of color in the pallet of the Mg^{+2}-containing ghost solution. We found that three passages through a half-inch, 27-gauge needle is enough to fracture most (if not all) of the ghosts. Further shearing risks the dissociation of skeletal components. To avoid loss of components due to repetative shearing, the ghost solution was kept on ice throughout the shearing process. To test whether loss of skeletal components had occurred due to shearing, supernatant from the sheared ghost solution was centrifuged at 55,000 rpm for 15 min in a Beckman TL100 benchtop ultracentrifuge. Both supernatant and pellet from the ultracentrifugation were analyzed by SDS-PAGE.

Single-layered skeleton leaflets

Single-layered skeleton leaflets (SLSL) were prepared by incubating on ice for 10 min. 0.8 ml of sheared vesicles with 0.2 ml of 10% TX-100 in 2p8. The reaction mixtures was then diluted 20x by the same buffer containing 0.5 mM dithiothreitol and allowed to expand on ice for an hour.

Specimens for electron microscopy were prepared on fenestrated-carbon-film-coated copper grids accoding to our previous protocols (37). The specimens were examined in a JEOL 100CX electron microscope with a 200 μm and 45 μm condenser and objective aperture, respectively. The microscopic images were recorded on SO-163 electron image films (Eastman Kodak,Co., Rochester, NY) and were developed in D-19 developer.

Results

SDS-PAGE analysis showed that, with the exception of residual hemoglobin, the gel pattern of fragmented ghosts was indistinguishable from that of intact ghosts and that hemoglobin constituted the only detectable protein band in the supernatant of ultracentrifugation (data not shown). Both intact skeletons and SLSL prepared by the extraction of intact and fragmented ghosts, respectively, contained proteins essential to the integrity of the network (spectrin α and β subunits, band 4.1 and actin) as well as several accessory proteins (notably, band 2.1, a portion of band 3, band 4.2, and band 4.9).

Ultrastructure of the Intact Skeleton

We have previously shown that the skeletons freshly released from the membrane bilayer were highly condensed and lacked elongated filaments and that incubation of similar preparations for an hour at 0 °C in 2 mM NaPi (pH 7) containing 0.5 mM

dithiothreitol led to gradual expansion of the network and the appearance of short stubby junctional complexes among thin, flexible filaments of spectrin (see Fig. 1 and ref. 37). Globules and short thick filaments were the most common elements in the partially expanded reticulum, even on the faces of the associated lipid vesicles (e.g., Fig. 1, stars). In addition, thin linear filaments ran between compact clusters of granular features. The overall structural feature of the partially expanded skeleton was largely granular, and the maximum length of the spectrin filaments detected in those preparations seldom exceeded 70 nm, which is approximately one-third of the 200-nm length observed for spectrin tetramers in hyperexpaned skeletons (37) and in micrographs of purified spectrin tetramers in platimum-shadowed preparations (39). Moreover, the spectrin filaments in the image of the partially expanded skeletons invariably terminated in compact clusters of globular elements (see particles inside circles, Fig. 1).

Ultrastructure of Single-Layered Skeleton Leaflets

Even though short, rigid, actin protofilaments of uniform length and long, thin, flexible filaments of spectrin were easily identifiable in the intact skeletons that were expanded in buffer at low ionic strength, the spatial disposition of the basic structural units was obscured by the superposition of the top and bottom layers in the skeleton. We therefore examined SLSL prepared by the extraction of physically sheared ghosts. Because the size of the SLSL were much smaller than that of the intact skeleton, fenestrated carbon films with small holes were selected for EM specimen preparation, and the samples were not rinsed as extensively prior to staining with 1% uranyl acetate as those of intact skeletons. As a result, the micrographs of the leaflets tend to have higher backgound due to the presence of residual Triton X-100. However, the preparations were reproducibly uniform, and it was possible to identify single-layered leaflets at low

magnification by differences in the intensity of the negative stain over the holes of the fenestrated film.

Figures 2A and 2B are typical images of SLSL that were expanded at 0 °C for 1 h. Once again, the predominant features were short, rigid actin junctional complexes, flexible spectrin filaments, and small lipid plaques decorated with both intrinsic and peripherial membrane proteins. The lengths of the spectrin filaments in the SLSL preparations were comparable to those of the intact skeletons shown in Fig. 1. There seemed to be more open areas compared to the intact skeletons. We ruled out the loss of specific skeletal components as the reason for the decrease in surface concentration because SDS-PAGE patterns of the physically sheared ghosts were indistiguishable from those of the intact ghosts.

The image in Fig. 2B is particularly interesting, because it shows a number of short, rigid particles that can be identified as junctional complexes (arrowheads 1 to 6) interconnected by multiple strands of spectrin filaments that were held together close to their middle by globular protein aggregates in the shape of a circle or three-leaf clover (see asterisks in Fig. 2B). Judging from the abundance and position of those globules, they are likely to be conglomerates of ankyrin-band 3 complexes.

The maximum number of junctional complexes that the ankyrin-band 3 conglomerates were connected to in all images of SLSL was three (see asterisks in Fig. 2B), whereas the number of spectrin filaments at the junctional complexes was fewer than four, and the filaments were both thick and short compared to individual strands of spectrin tetramers in the hyperexpanded skeletons (see spectrin filaments in Fig. 4). The image of the circular conglomerate at the lower center of Fig. 2B (asterisk 3) shows that three bundles of spectrin cables (each with possibly two strands of spectrin tetramers) are

held together through the association of ankyrin-band 3 complexes at the center of a triangle defined by the junctional complexes at its vertices (arrowheads 3 to 5). The image in Fig. 2B also suggests that the six junctional complexes (arrowheads 1 to 6) may represent the vertices of four contiguous triangles, each sharing a common edge with one or two of its neighbors (see Fig. 2C and 2D). The two triangles with a conglomerate of ankyrin-band 3 complexes at their center were separated by the triangles without a protein conglomerate at the center. Based on this observation and the distribution of spectrin tetramers in a hexagonal lattice, we suggest that two of those neighboring triangles that share a common edge (one with and one without a ankyrin-band 3 conglomerate at the center) may represent the basic repeating unit (i.e., asymmetric unit) of the skeletal network. Thus, the four triangles in Fig. 2B represent two asymmeytric unit cells at the boundary of two overlapping hexagonal lattice that were ruptured during the fragmentation of the ghost (see Fig. 2C and 2D for graphic representation of part of the image in Fig. 2B, and its spatial disposition in the context of the two ruptured hexagons).

Hyperexpansion of the SLSL in low ionic strength buffer led to full extension of the spectrin tetramers and the separation of individual strands of spectrin filaments, as well as to the dissociation of the conglomerates of the ankyrin-band 3 complexes (Fig. 3). The junctional complexes which form the vertices of several overlapping irregular hexagons are now seen to be associated with five or six strands of spectrin filament that are approximately 200 nm in length (see Fig. 3A and its graphic reproduction in 3B). The width of the spectrin filaments is substantially thinner than those found in the partially expanded specimens. At places where the number of spectrin filaments at the junctional complex is less than six (see Fig. 3B arrowheads 5, 8, and 9), there are indications that

disruption of the network might have occurred.

Most of the well-defined spectrin tetramers in the hyperexpanded skeletal leaflets in Fig. 3A contain two globular masses which divide the spectrin tetramer into three segments of approximately 50-70 nm (for example, see the strands connecting junctional complexes 1 and 3, 1 and 4, 4 and 5, 3 and 6, 6 and 7 etc.). This suggests that both of the ankyrin-binding sites on the spectrin tetramer are occupied by an ankyrin molecule. The length of the segments between the distal ends of the spectrin (i.e., ends close to actin protofilaments) and the ankyrin-binding site and between the two anykyrin binding sites determined here are consistent with the value reported by Cohen et al.,(8) for the reconstituted spectrin-ankryin complexes.

Figure 3A also shows that two pairs of spectrin tetramers (brackets a, b, d, and e), each connecting three junctional complexes at the vertices of two different triangles, are held together close to their middle by globular complexes that are smaller in mass than the intact ankyrin-band 3 conglomerates. These smaller masses may represent an aggregation of fewer ankyrin-band 3 complexes, resulting from partial dissociation of an intact conglomerate of ankyrin-band 3 complexes (Fig. 3, half asterisks). The fact that spectrin tetramers a and b, and d and e are held together by partial conglomerates suggests that the strands opposing them, i.e., strands c and f, are likely to be their missing partners in the formation of the intact conglomerates. The putative association of strands a, b, and c and strands d, e, and f in the formation of the ankyrin-band 3 conglomerate and the pattern of alternating filled-and-empty triangles discussed earlier forms the basis of our assignments for the association of the rest of the ankyrin-band 3 complexes in the hyperexpanded SLSL network (see circles of ribbon in Fig. 3C).

Figure 4A is a simplified schematic of the structural model of the human

erythrocyte skeleton derived from the current study. In agreement with earlier reports, we believe that the skeleton is organized into junctional complexes interconnected by mutiple strands of spectrin tetramers. However, we have shown in earlier studies (38), as well as in the current investigation, that spectrin tetramers exist in a condensed state in situ. In this study we have also shown that neighboring spectrin tetramers are organized into groups of three through the association of the ankyrin-band 3 complexes. Thus, our model suggests that the skeleton is organized at two different loci, one at the level of the skeleton through the formation of a stable junctional complex and the other at the plan of the membrane bilayer and close to the middle of the spectrin tetramers through the association of the ankyrin-band 3 complexes. Each asymmetric unit of the lattice (parallelogram in Fig. 4a) consists of one junctional complex, three strands of spectrin tetramers, and a conglomerate of ankyrin-band 3 complexes. The fractional units of junctional complexes at three of the four corners of the asymmetric unit are interconnected by the three strands of spectrin tetramers that are held together close to their midpoint by the conglomerate of ankyrin-band 3 complexes. The formation of the ankyrin-band 3 conglomerates segregates the content to one end of the unit cell and reduces the rotational symmetry of the lattice, from C6 of an hexagon to C3 of a trigonal lattice (for a discussion of space group and symmetry operation, see ref. 42). Results from our current study also suggest that (a) the segments of the spectrin tetramers proximal to the junctional complexes are more likely to exist in an extended form and are likely to be involved in lateral interaction with its closest neighbor, and (b) the portions of spectrin close to its dimer-dimer interaction site are more likely to exist in a condensed state in situ and can only be extended upon dissociation of the ankrin-band 3 conglomerate.

DISCUSSION

Electron microscopic studies have showed that freshly released intact skeletons and SLSL share many common features. Both preparations contain short rigid junctional complexes amid an irregular network of flexible filaments and granular particles. The surface concentration of proteins in the SLSL is much lower than that in the intact skeleton because the removal of the overlapping layer, allowing the unambiqguous assignment of individual components and interconnecting structural units.

Similar to what we have previously reported for the intact skeleton (37), spectrin molecules in freshly released SLSL are highly condensed. The length of filamentous spectrins, except in the hyperexpanded specimens, seldom exceeds 70 nm. From the image of the SLSL, it appears that the distal ends of the spectrin tetramers exist more frequently in an extended form than the middle section of the molecule, and that the short segments of spectrin filaments in the partially expanded SLSL always connect to protein masses situated close to the middle of the spectrin filaments. A close re-examination of the images of the partially expanded network of intact skeleton shows not only that there are a fair number of circular conglomerates similar to those observed in the SLSL, but also that these conglomerates display certain substructural features (see asterisks in Fig. 1). Previously, we could not rule out the possibility that those clusters of granular features were the result of the juxtaposition of components in the upper and lower layers. However, the detection of the ankyrin-band 3 comglomerates and the identification of repeating units in the SLSL lends credibility to the existence of high-molecular-weight ankyrin-band 3 conglomerates in the freshly isolated intact skeleton. We cannot determine from the photomicrographs how many band 3 and ankyrin molecules are involved in the formation of each conglomerate. However, one can imagine

from the micrographs that there is an approximate threefold symmetry in the distribution of the protein masses. If both binding sites on the spectrin tetramer are occupied by an ankyrin-band 3 complex, there should be six ankyrins per conglomerate.

The number of band 3 molecules in the conglomerate depends on the oligomeric state of self-association of band 3 in the red cell membrane, which is at present an incompletely resolved issue. Results from different experimental techniques suggest that band 3 is dimeric (7, 11, 18, 33, 40, 41, 48), or tetrameric (25, 44), or in a monomer-dimer-tetramer equilibrium (28, 31, 34). Recently, Matayosh et al. (23, 24) suggested, on the basis of a comparison of the rotational diffusion of band 3 in intact cells and in ghosts both with and without mild trypsin treatment, that several classes of band 3 that differ greatly in mobility exist in ghosts and intact cells. These classes include freely rotating dimers, dimers that are linked to the skeleton, and higher molecular weight oligomeric band 3 complexes that are stabilized by hydrophobic interaction of the transmembrane domains. It is conceivable that freely rotating dimers were removed from our preparation by Triton X-100 extraction. However, we are not certain what proportion of the slow-rotating, high-molecular-weight band 3 complexes reported by Mayatoshi remain associated to the ankyrin-band 3 complexes in our skeleton and SLSL preparations. Based on the estimate of two molecules of ankryrin per spectrin tetramer (9) and an oligomeric state of dimer for band 3, the minimum number of protein molecules in an intact conglomerate of ankrin-band 3 complexes would be six copies of ankyrin and 12 copies of band 3. The molecular mass of such a conglomerate would be approximately twice that of the junctional complex, which on average contains 12 copies of G-actin, 6 copies of band 4.1, and two copies of membrane tropomyosin (37), not counting the ends of the six strands of spectrin tetramers emanating from it. It is difficult to estimate the

thickness of an irregular object in micrographs of negatively stained specimens, but if one assumes that the average thickness of the particles remains approximately the same, the area occupied by the putative ankyrin-band 3 conglomerates and the junctional complexes can easily account for the difference in protein masses. Considering the total number of band 3 in the ghost is five times higher than the number of ankrins (reef. 3), a ratio of two molecules of band 3 per ankyrin in the conglomerate is also consistent with our observation that approximately 40% of band 3 molecules remain associated with the TX 100 residues that were not treated with high concentration of NaCl (37).

We suggest, based on the current observations and results we previously published, that the membrane skeleton of human erythrocyte is organized at two different levels, one at the ends of spectrin tetramer through the formation of a stable spectrin-actin-protein 4.1 junctional complex, and the other through the association of the membrane attachment sites at close to the midpoint of the spectrin tetramers. While the organizational role of the junctional complexes is well-documented, the contribution of band 3 to the structure and function of the skeletal network has never been addressed. The reversible association and dissociation of the ankyrin-band 3 complexes close to the plane of the membrane bilayer not only provide an additional organization element, but also allow for rapid expansion and contraction of the skeleton through lateral diffusion of band 3, the major intrinsic membrane protein. In the absence of the conglomerate, the shear force experienced by the fragile membrane bilayer will only be transmitted to individual spectrin molecules through isolated band 3-ankrin-spectin cross bridges. With the participation of the conglomerate, there is a concerted effect on the transmittance of mechanical force and the extension of the spectrin tetramers which should lead to rapid transfer of physical stress from the fragile membrane bilayer to the more durable

membrane skeleton.

It is conceivable that the hydrophobic interactions of band 3 oligomers contribute significantly to the stability of the ankyrin-band 3 conglomerate; however, it is not yet clear whether the ankyrin molecules in the conglomerate are also involved in molecular interactions with each other or are the conglomerates stabilized mainly by interactions among the band 3 oligomers. Recently, Liu and coworkers showed that the deletion of eight hydrophilic amino acid residues from the cytoplasmic interface of band 3 molecules in patients with southeast asian ovalocytosis (SAO) is associated with (a) an increase in the affinity between ankyrin and the cytoplasmic domain of the abnormal band 3 molecule; (b) a decrease in the mobility of the abnormal band 3; and (c) an increase in the rigidity of the SAO red cells (16, 21). This, together with our observations, suggests that the association of band 3 may have a cooperative effect on the ankyrin-band 3 interactions as well as on the stability of the ankyrin-band 3 conglomerates.

Can our model reconcile the discrepencies that exist between the structure of spread skeleton and skeleton observed at the surface of intact ghost? Both electron microscopic study of freeze-fractured specimens (29) and theoretical treatments (44) have predicted a maximum length of 70 nm for spectrin tetramers, whereas EM of rotary shadowed specimens of isolated spectrin tretramers routinely demonstrate a contour length of 200 nm (39). Our model suggests that spectrin tetramers exist in a condensed form in situ which can be extended to a full length of 200 nm upon expansion of the skeletal network (37).

Recently, Ursitti et al. (45) studied the structure of the human erythrocyte cytoskeleton using quick-freeze, deep-etch, and rotary-shadowed specimens as well

as specimens that were fixed with tannic acid and oximium tetroxide. They found the membrane skeleton to be a dense network of intersecting straight filaments with an average length of 29 to 37 nm. Moreover, there were only 3 to 4 filaments at each intersection and about 400 intersections per square micrometer. Those observations are largely different from the six spectrin filaments per junctional complex and approximately 250 junctional complexes per square micrometer reported in the spread skeleton (6). In the current study, we observed that the ankyrin-band 3 complexes of neighboring spectrin filaments can undergo reversible association forming conglomerates with the appearance of a triskelion, or a three-pronged junction. Lateral association of neighboring spectrin tetramers in situ would also reduce the apparant number of filaments in connection with the junctional complexes. Both events should result in the appearance of intersections with three filaments, as observed by Ursitti et al. (45). Finally, the larger number of intersections detected in the quick-freeze, deep etch, and rotary-shadowed specimens may result from the difficulties in distinguishing junctional complexes from conglomerates of ankyrin-band 3 complexes.

In conclusion, we have prepared single-layered skeleton leaflets with minimum perturbation and revealed for the first time new roles for the spectrin-ankyrin-band 3 cross bridges in the organization and function of the membrane skeleton. The requirement of rapid dissociation of the conglomerates of ankyrin-band 3 complexes for the extension of spectrin and expansion of the skeleton implicates crosslinking of the ankyrin-band 3 conglomerates, either through

oxidative damage or interaction with denatured hemoglobin (22), as one of the major causes of the increased rigidity of aged red cells or red cells from patients with sickle cell anemia, which eventually lead to their removal from the circulation. We are current exploring conditions for the isolation of intact ankyrin-band 3 conglomerates for their characterization by other biochemical and biophysical means.

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FIGURE LEGEND

Figure 1. Photomicrograph of partially expanded intact skeleton.

Intact skeleton freshly released from ghosts in buffer containing 2 mM $MgCl_2$ were expanded at 0 °C for 1 h. in 20 volumes of 2p8, 0.5 mM DTT. The EM specimens were prepared according to method previously described (37) and examined in a JOEL 100CX elcectron microscope. Bar: 50 nm.

Figure 2. A and B, photomicrographs of single-layered skeletal leaflets after expansion for 1 h. at 0 °C in 2p8 containing 2 mM of dithiothreitol.

C, graphic reproduction of a portion of the image in panel B showing the six junctional complexes (arrowheads) and two complete and one partial ankyrin-band 3 conglomerates (asterisks) in the context of four contiguous triangles (dashed lines). The stars mark the presence of residual lipid vesicles held to the network through connection with ankyrin-band 3 complexes and/or band 4.1. D, schematic drawing of the same four contiguous triangles and their spatial disposition in the context of two overlapping hexagonal lattices. The regions enclosed by the dashlines are the portions of the network that were ruptured by sheared force.

Figure 3. A, photomicrograph of a hyperexpanded single-layered skeletal leaflet.

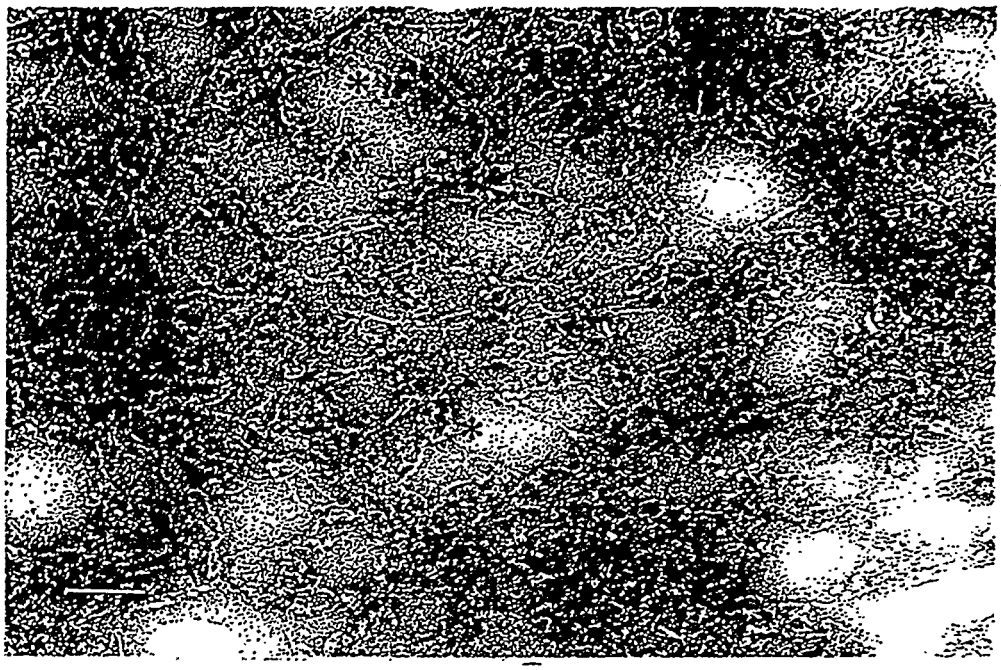
B, Graphic reproduction of image in A. Filled features represent junctional complexes, whereas open features represents ankyrin-band 3 complexes. The

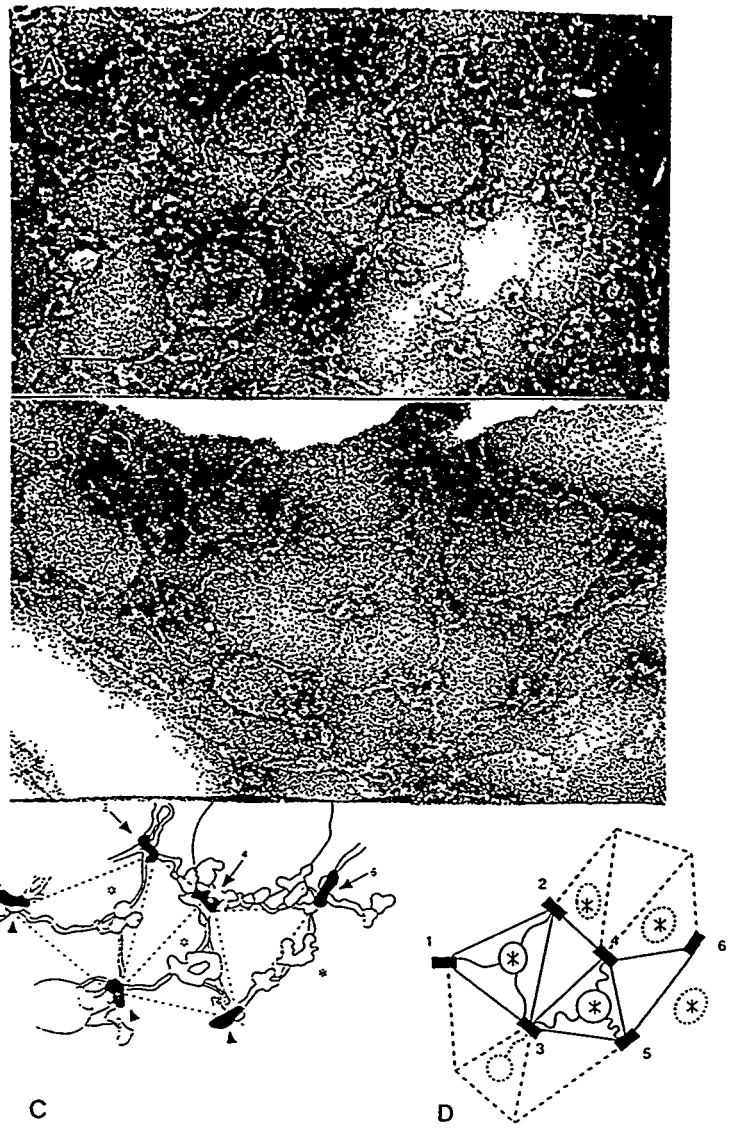
two pairs of spectrin tetramers, strands a and b and strands d and e, are held together by partial conglomerates of ankyrin-band 3 complexes. We suggest that tetramers c and f are likely the strands that were separated due to partial dissociation of the conglomerates. One of the two ankyrin-band 3 complexes of strand f is embedded in the lipid vesicle (star).

Junctional complexes 3, 4, 7, 8, 11, and 12 are associated with six strands of spectrin tetramers. There are at least two breakages in the context of the network both at the vicinity of junctional complex number 5, one between junctional complexes 5 and 8 and the other between 5 and 9 (question marks).

Figure 4. Schematic representation of an asymetric unit (dashed line) of the skeleton at the cytoplasmic surface of human erythrocyte membrane (panel a); an operational unit of the ankyrin-band 3 comglomerate (panel b) and its various stages of partial expansion (panels b to e).

Panels bm to em, photomicrographic equivalents of b to e. Arrows in micrographs denote images of actin protofilaments at the vertices of the hexagonal lattice.

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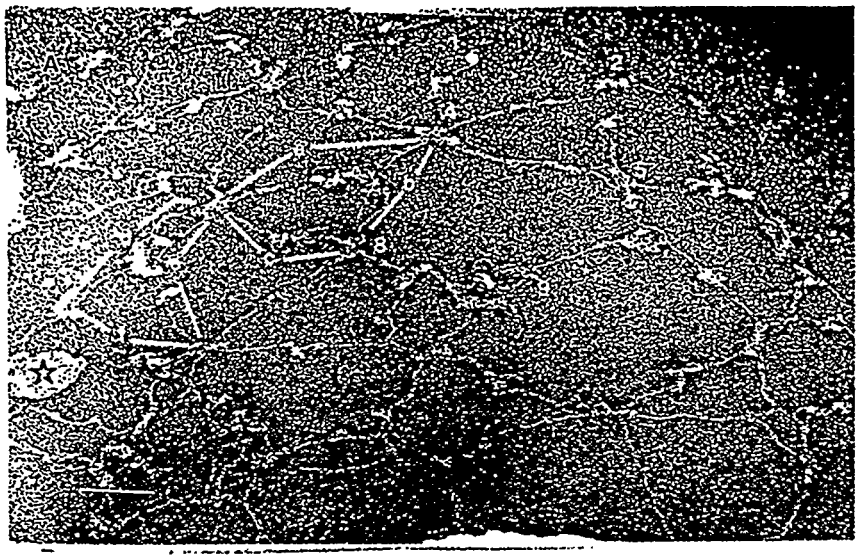

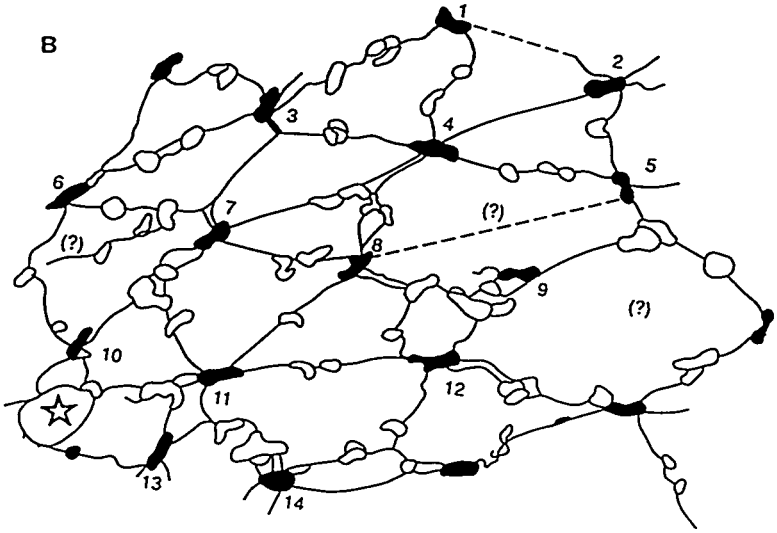
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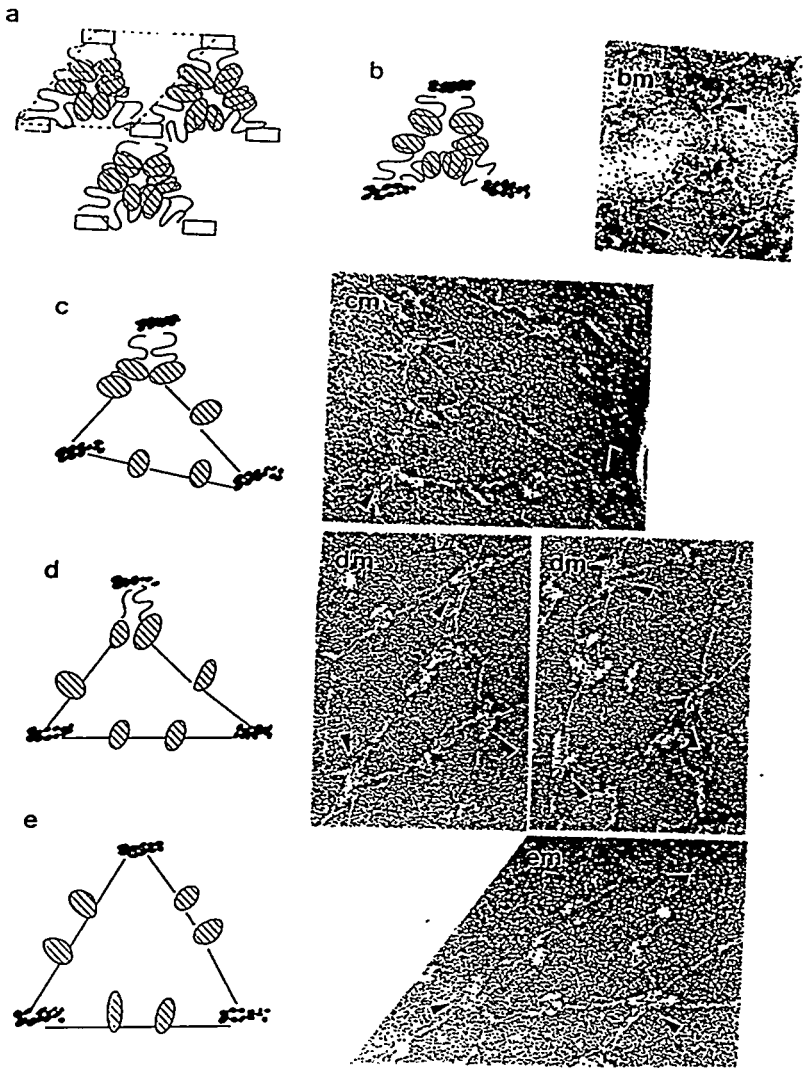
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