Molecular Basis for Red Cell Membrane Properties

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Abstract

Encouraging recent developments have begun to bridge the gap between what we know about membrane biochemistry and our knowledge of *in situ* physical properties of the red cell membrane, a prototypical biological membrane. This review provides a contemporary view of the relationship between red cell membrane molecular architecture and membrane material properties, emphasizing new developments that underscore the subtleties of soft cell interfaces.

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The red cell membrane exhibits complex material behavior. It is highly elastic (100-fold softer than latex rubber of comparable thickness), responds rapidly to applied stresses (time constants in the range of 100 milliseconds) and is capable of undergoing large membrane extensions without fragmentation. These unusual material properties are the consequence of evolution-driven engineering which evolved a composite structure in which a plasma membrane envelope composed of amphiphilic surfactant molecules is anchored to a network of skeletal proteins through tethering sites (transmembrane proteins) in the bilayer. Whereas the lipid bilayer is a condensed fluid surface, the skeletal network can exhibit varying degrees of extensional rigidity. The roles for these two structural components appear to be as follows: the lipid bilayer (plus transmembrane proteins) chemically isolates and regulates the cell interior and the skeletal network provides rigid support and stability to the bilayer interface. Though much is known regarding the red cell membrane, the essential question is how to relate material properties of the red cell to the molecular composition and architecture of the membrane. To address this question, we begin the discussion with a brief overview of the biochemical composition and the structural organization of the red cell membrane and note the insights gained from studies of red cell membrane pathologies. Then in the context of these molecular features, we can discuss the current working model for the structural basis of red cell membrane material properties.

Biochemical Composition and Structural Organization of the Red Cell Membrane

The red cell membrane has been very well characterized biochemically and the structural organization of the various lipid and protein components is continuing to evolve. About 52% of the membrane mass is protein, 40% is lipid and 8% is carbohydrate. The major lipid components of the membrane bilayer are unesterified cholesterol and phospholipids which are present in nearly equimolar quantities. The principal components of the skeletal network, spectrin, actin, protein 4.1, adducin, tropomyosin and tropomodulin, form an hexagonal lattice of junctional complexes through specific protein-protein interactions (Mohandas and Evans, 1994). The physical linkage of membrane skeleton to the lipid bilayer is mediated by a number of transmembrane proteins including band 3 and glycophorin C (Mohandas and Evans, 1994).

Much of our understanding of the structural organization of the red cell membrane has been derived from carefully designed biochemical studies using purified components. However, recently developed biophysical approaches have enabled the documentation of the various membrane component interactions in intact membranes (Discher et al., 1994; Discher and Mohandas, 1996). For example, studies on normal red cells and pathologic red cells with deficiencies in specific membrane components using the newly developed technique of Fluorescence-Imaged Microdeformation has enabled the unequivocal documentation of *in situ* linkages between band 3 and the membrane skeleton mediated by ankyrin and between glycophorin C and the membrane skeleton mediated by protein 4.1 (Discher et al., 1994; Gimm et al., 1997). Additional linkages that have not been previously recognized have also been identified and these include the association of the Rh complex with the membrane skeleton.

Effect of Bilayer-Skeletal Protein Network Linkages on Membrane Cohesion

The importance of bilayer-skeletal protein network linkages on membrane cohesion is documented by studies using red cells that are totally deficient in either band 3 or ankyrin (Gimm et al., 1997; Peters et al., 1996). Reductions in the number of linkages between the bilayer and the membrane skeleton due to absence of either band 3 or ankyrin results in decreased cohesion between the bilayer and membrane skeleton resulting in membrane vesiculation and loss of membrane surface area (Gimm et al., 1997; Peters et al., 1996). Decreased membrane spectrin content, which also leads to reductions in the number of linkages between the bilayer and membrane skeleton, results in loss of membrane surface area (Chasis et al., 1988). Thus a critical number of linkages between the bilayer and the membrane skeleton are required for maintaining membrane cohesion.

Effect of Bilayer-Skeletal Protein Network Linkages on Membrane Rigidity

Physical association of the network to the bilayer is also an important determinant of the elastic resilience of the red cell membrane. While a minimum number of linkages between the bilayer and the membrane skeleton are needed for membrane cohesion, a large increase in the number of linkages will hinder the ability of the spectrin molecules to undergo the conformational rearrangements needed for deformation. The marked decrease in the ability of the hereditary ovalocytic red cells to undergo membrane deformation is an excellent example of the importance of this mechanism in regulating membrane deformation. Increased interaction of the mutant band 3 with the membrane skeleton in the ovalocytic red cells has been shown to account for marked increases in membrane rigidity of these membranes (Mohandas et al., 1992). Changes in the extents of association of the cytoplasmic domains of bilayer spanning proteins with the membrane skeleton can regulate membrane rigidity.

Effect of Lateral Skeletal Protein Linkages on Membrane Mechanical Stability

A critical role for lateral protein-protein linkages (spectrin-spectrin interaction and spectrin-actin-protein 4.1 interaction) in regulating membrane mechanical stability has been documented by studies using red cells with defects in spectrin and protein 4.1 (Chasis and Mohandas, 1986; Mohandas and Chasis, 1993). Weakening or disruption of either spectrin-spectrin interaction or spectrin-actin-protein 4.1 interaction in the spectrin-based membrane skeleton results in loss of membrane mechanical integrity and leads to cell fragmentation (Chasis and Mohandas, 1986; Mohandas and Chasis, 1993). Qualitative defects in protein 4.1 and alpha- and beta-spectrin that result in the weakening of lateral skeletal protein linkages have been shown to result in decreased mechanical stability (Chasis and Mohandas, 1986; Mohandas and Chasis, 1993; Mohandas and Evans, 1994). Thus lateral protein associations in the membrane skeleton play a key role in regulating membrane mechanical integrity and cohesion.

This concise summary of red cell membrane physiology illustrates some new insights into the origins of material behavior of red cell membrane and also illustrates how studies of red cell membrane pathologies and genetic structural mutations provide exceptional opportunities to test critically hypothesis for the origin of material behavior at the molecular level. It is hoped that our improved understanding of a simple biological membrane at the molecular level will further our abilities to unravel mysteries of biomolecular design.

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