Purification and characterization of Band 3 complexes from human erythrocyte membranes

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Band 3, also known as Anion Exchanger 1 (AE1) or Solute Carrier 4A1 (SLC4A1), is the predominant glycoprotein of the erythrocyte membrane. Functioning to exchange chloride ions for bicarbonate ions across the lipid bilayer, Band 3 is essential for efficient removal of carbon dioxide from tissues and delivery to the lungs. Band 3 also plays a structural role in erythrocytes, anchoring the actin-spectrin cytoskeleton *via* interactions that are important for maintaining erythrocyte integrity and function. Band 3 exists as a mixture of dimers and tetramers in the erythrocyte membrane. These oligomers form structural hubs around which the integral and peripheral membrane proteins of the erythrocyte are organized, giving rise to two major multi-protein complexes in the membrane, the ankyrin complex and the junctional complex. In the ankyrin complex, Band 3 tetramers are connected to the underlying membrane skeleton through interactions with ankyrin and protein 4.2.

In order to better understand the role of Band 3 in erythrocyte physiology, we are undertaking a structural characterization of intact Band 3 complexes isolated from human erythrocytes. As a start, we have examined the oligomeric state of Band 3 purified from detergent solubilized erythrocyte membranes, using size exclusion chromatography and blue-native PAGE. These experiments revealed that under certain extraction conditions Band 3 is predominantly tetrameric, and that tetramer formation is dependent on the type of detergent used and conditions of detergent solubilization. Current efforts are aimed at biochemical and biophysical characterization of Band 3 oligomerization, and electron cryomicroscopy analysis of Band 3 tetramers and higher-order Band 3 complexes from the red blood cell membrane.