MOLECULAR PHYSIOLOGY AND BIOPHYSICS

EPR AND FLUORESCENCE STUDIES ON ERYTHROCYTE MEMBRANE SKELETAL PROTEINS: CDB3 AND ANKYRIN

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The protein complex composed of the cytoplasmic domain of band 3 (cdb3) and ankyrin forms one of the two major contact sites between the spectrin-based membrane skeleton and the lipid bilayer in human erythrocytes. This linkage is critical for maintaining the shape and viscoelastic properties of the membrane. Among the known membrane skeleton protein mutations, band 3 (anion exchanger 1 or AE1) variants account for about 20% of hereditary spherocytosis cases among Caucasians. The structure and function of cdb3 and ankyrin repeat domain 3-4 have been studied via site directed labeling methods in combination with conventional electron paramagnetic resonance (EPR), double electron electron resonance (DEER), and fluorescence spectroscopy. The central compact region (55-356) of the cdb3 dimer under physiological pH is indistinguishable from the crystal structure determined at pH 4.8. The N terminus (1-54) of cdb3 is dynamically disordered and capable of docking various cytoplasmic proteins. The similar disorder in the C terminus (357-359) of cdb3 is consistent with the weak motional coupling between the cytoplasmic domain and the transmembrane domain of band 3. The surface of the cdb3 peripheral domain that is opposite the dimerization

arm interacts with the ankyrin groove. The α-helix 2, α-helix 3 and β strands 6, 7 (hairpin) of cdb3 may be directly involved in ankyrin binding. The band 3 Tuscaloosa mutation (P327R), which is located at a loop turning point of the dimerization arm, does not dissociate the cdb3 dimer but does perturb the region adjacent to position 327 and the downstream C terminus, thereby slightly destabilizing the dimer structure. These spectroscopic studies establish a structural model for cdb3 and its binding partner ankyrin in solution at neutral pH, which provides an important platform to further characterize protein-protein interactions that stabilize the membrane and naturally occurring mutations that cause human diseases.

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