Characterization of Structural Dynamics of Thylakoid Membrane Transporter Protein Hcf106

Nimarsha Kamaradiwela Arachchige[1] and Carole Dabney-Smith[2]

1Department of Chemistry and Biochemistry, Miami University. 651 E High St, Oxford, OH.

kamarans@miamioh.edu.

Most chloroplast proteins are encoded by nuclear genes and synthesized in the cytosol as higher molecular weight precursors possessing N-terminal transit peptides which direct them to cross membranes through specific protein translocation systems to reach their final destinations. The chloroplast twin-arginine transport (cpTAT) complex consisting of three transmembrane proteins, Tha4 (cpTatA), Hcf106 (cpTatB), and cpTatC, is one such protein translocation system that transports fully folded proteins across ion-tight membranes. Hcf106, a single transmembrane protein, plays an important role in the process. However, it has been challenging to unravel structural and dynamic information for Hcf106 using conventional biophysical techniques. EPR spectroscopy is a rapidly growing technique to gain pertinent structural and dynamic properties of biological systems. Here, we use EPR spectroscopy to probe the biophysical properties of Hcf106 to gain insight into its role in transport.

Five single Cys-Hcf106 variants from the N-terminal transmembrane domain, amphipathic α-helix, and loosely structured C-terminus were generated using site-directed mutagenesis PCR. Purified Hcf106 variants were labeled with methyl methanesulfonothioate (MTSL) spin-label. The labeled Hcf106 was incorporated into 0.5% DPC detergent micelles, phosphatidylcholine (PC) liposomes, and thylakoid mimetic (ThML) liposomes. CW-EPR studies were conducted to report on the environment of the spin label. The CW-EPR spectral line shape analysis data suggested restricted motion of Hcf106 in lipid bilayer vesicles compared to detergent micelles. Moreover, spin label mobility data suggests that F5C, K37C, and A128C are solvent-exposed residues and V14C and L60C are transmembrane residues. In the future CW-EPR power saturation will be used to determine the relative membrane depth of regions of Hcf106 in proteoliposomes by calculating the depth parameter. This will allow us to identify the amino acid residues of Hcf106 that delineate the membrane boundaries as well as gain a more accurate understanding of Hcf106 conformation in the membrane.