ABSTRACT

STRUCTURAL AND FUNCTIONAL STUDIES OF PROTEINS INVOLVED IN MITOCHONDRIAL FUNCTION AND STRUCTURE

Yanfeng Zhang

The dynamics of continuous fission and fusion events maintain normal mitochondrial morphology and reduce the number of functional defects that could lead to a variety of diseases. DLP-1 and MFNs are essential protein components of human mitochondrial fission and fusion machineries, and functional and structural studies of these proteins would increase our understanding of the molecular mechanisms of mitochondrial dynamics, function, and structure.

In this thesis, the biochemical and structural properties of recombinant DLP-1 and selected mutants have been studied. The G350D and R365S mutants in the middle domain severely impair the GTPase activity, but have no significant impact on the protein's oligomeric state, indicating that these two mutations interrupted the intramolecular but not intermolecular interactions, and therefore, the middle domain of DLP-1 is important for the protein activity probably by facilitating appropriate connections between the GTPase domain and the GED. The DLP-1 and the isolated PHlike domain bound free phosphoinositides indicated that DLP-1 may interact with membranes directly by binding acidic phospholipids preferentially phosphoinositides, and the PH-like domain may be responsible for the interactions. Although GTPase activity is abolished, the APH bound to liposomes, which suggested that in addition to the PH-like domain, other regions of DLP-1 may function as lipids-interacting enhancer as well as scaffolds for orienting the PH-like domain into appropriate membrane targeting.

Structural studies of DLP-1 and MFNs by way of X-ray crystallography have been attempted. Molecular protein engineering was designed and performed to improve protein solubility and to increase the likelihood of protein crystallization.

The recently identified (pro)renin receptor ((P)RR) is an important protein molecule for the renin-angiotensin system (RAS), a mechanism regulating blood pressure and cardiovascular function. The (P)RR C-terminus including the cytoplasmic tail is involved in the assembly of the Vo portion of the vacuolar proton-translocating ATPase. The cytoplasmic tail is short, but functionally important for the pivotal roles of (P)RR in a number of signal transduction pathways that activated by binding of (pro)renin. Finally, the last 19 amino acids of the (P)RR corresponding to the cytoplasmic tail were fused into the C-terminus of E. coli maltose binding protein (MBP), and the chimera was expressed in E. coli and purified to homogeneity. Protein crystals, in the presence and absence of the MBP ligand maltose, were obtained, and X-ray diffraction data to 2.0 A resolution were collected. Despite significantly different unit-cell dimensions and molecular packing, two monomers of the MBP fusion protein were found in the asymmetric unit for both structures. Although the (P)RR cytoplasmic tail appeared as a relatively flexible loop without obvious secondary structural elements, it seemed responsible for the dimerization of MBP fusion protein in the asymmetric unit. The residues in the cytoplasmic tail, particularly the two tyrosines, dominate the interdimer interactions, suggesting a role of the cytoplasmic tail in protein oligomerization.