The Role of MgATP Binding in Nitrogenase Catalysis

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Nitrogenase is a complex metal-containing enzyme that catalyzes the conversion of nitrogen gas to ammonia. Nitrogenase is an ideal model system for the study of complex metal cluster mediated catalysis, electron transfer, complex metal cluster assembly, protein-protein interactions, and nucleotide-dependent protein conformational change. With regard to the latter, the involvement of MgATP in nitrogenase catalysis is similar to the role of nucleotides in a large class of nucleotide binding proteins that couple nucleotide binding and hydrolysis to protein conformational changes transduced within a macromolecular assembly. Over the past decade several structures of the nitrogenase have been determined representing defined states presumed to mimic conformations along the pathway of nitrogenase catalysis. Recently the structure of the L127 deletion variant of the Fe protein from *Azotobacter vinelandii* has been determined providing structural representation of what has been presumed to be a mimic of the MgATP bound "On State" of the Fe protein. The structure of the L127 deletion variant reveals a strikingly different protein conformation than observed previously for either the native state, MgADP bound state, or the Fe protein in the nitrogenase complex structures. These structures provide the basis for developing hypothesis for the conformational changes that the Fe protein undergoes during catalysis. One important question as we proceed with our studies is the true relationship between the structure of the L127 deletion variant and the structure of the MgATP bound state of the native Fe protein. Toward these ends, we are analyzing our current structural information, including the structure of the L127 deletion variant with bound MgATP, to ascertain how a similar conformation in the native Fe protein can be stabilized upon MgATP binding. In addition, we are currently evaluating this relationship using several limited-proteolysis and Small-Angle X-ray Scattering (SAXS) methods to probe protein conformational states and sulfur K-edge X-ray Absorption Spectroscopy (XAS) and Electron Paramagnetic Spectroscopy (EPR) to probe the [4Fe-4S] electronic structure in different conformational states.