Insights into the Nitrogenase Mechanism

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Nitrogenase is the complex metalloenzyme that catalyzes the biological reduction (fixation) of N_2 to 2 NH_3, with an optimal reaction stoichiometry as shown in equation 1.

$$N_2 + 8\ e^- + 16\ MgATP + 8\ H^+ \rightarrow 2\ NH_3 + H_2 + 16\ MgADP + 16\ P_i \qquad \text{(eqn. 1)}$$

The Mo-dependent nitrogenase is composed of two separable component proteins called the Fe protein and the MoFe protein. The Fe protein functions as a MgATP-dependent reductant of the MoFe protein. The MoFe protein contains two unique metal clusters - an [8Fe-7S] (P-) cluster that likely acts as an electron transfer intermediary, receiving electrons from the Fe protein and passing electrons to the active site [7Fe-9S-Mo-X-homocitrate] (FeMo-) cofactor. One of the long-standing challenges in nitrogenase research has been elucidating the site of binding and the mechanism of reduction for N_2. Many models have been proposed for where and how N_2 might be reduced based on various levels of calculation and reactivity of small organometallic compounds, but little direct experimental evidence arising from studies on nitrogenase has addressed these questions. We have undertaken a combined genetic, kinetic, and spectroscopic approach in an attempt to shed light on the catalytic mechanism of nitrogenase. Using site-directed mutagenesis as a means to substitute amino acids within the MoFe protein that define the protein environment of FeMo-cofactor, freeze-quenching to trap intermediates, and EPR and ENDOR spectroscopies to characterized intermediates, several types of nitrogenase substrates have been trapped as reaction intermediates bound to FeMo-cofactor. Characterization of alkyne, proton, hydrazine, and N_2-reduction intermediates is underway. These studies taken together provide evidence for one FeS face of FeMo-cofactor (Fe atoms 2, 3, 6, and 7) as the site of interaction for all of these substrates. Further, characterization of these trapped substrate intermediates is providing details about the chemical state of the intermediates, providing insights into the reduction mechanism of nitrogenase.