Structural Insights into FeMo Cofactor Biosynthesis

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Nitrogenase catalyzes the essential reaction in which atmospheric nitrogen is converted into a form accessible for metabolic consumption (recently reviewed in (*1*)). It has served as a focal point in the field of bioinorganic chemistry both because of its important role in fixing nitrogen under ambient conditions—the biological process accounts for ~ 50% of annual production and does not require the extremes of heat and pressure utilized by the industrial equivalent Haber-Bosch process—and because of the complexity of its associated metallo-clusters. Nitrogenase enzyme systems minimally consist of two metalloproteins, a catalytic component and a specific reductase, which, in the standard system, are referred to as the MoFe protein and the Fe protein. At the active site of the MoFe protein is a heterometallic cluster, the iron-molybdenum cofactor (FeMoco) (see *Fig. 1*, left). To better understand the function of FeMoco and to provide insight into a route for the chemical synthesis of a similar molecule, there is growing interest in understanding how FeMoco is synthesized *in vivo*.

Genetic studies have shown that FeMoco is assembled outside the MoFe protein in a stepwise process requiring several components including NifB-co, an iron- and sulfur-containing FeMoco precursor, and NifEN, an intermediary assembly protein where NifB-co is presumably converted to FeMoco (*2,3*). Extended x-ray absorption fine structure (EXAFS) spectroscopy experiments conducted at SSRL by M. Corbett, B. Hedman, and K. Hodgson, in collaboration with M. Ribbe and co-workers at UC Irvine, have recently been used to analyze the structure of a NifEN-bound FeMoco precursor. This protein-bound precursor is found to be a molybdenum-free analog of FeMoco (see *Fig. 1*, right), indicating that the iron core of FeMoco is completely assembled early in the biosynthetic pathway.

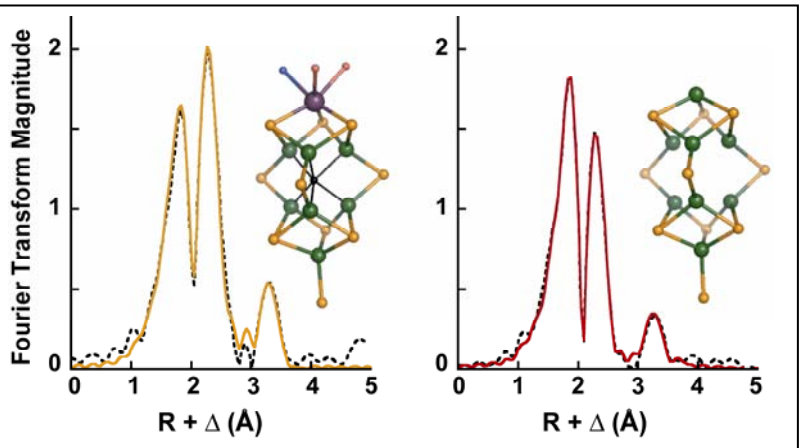

Fig. 1. Fourier transforms calculated over the *k*-range 2-16 Å^{-1} of EXAFS data (dotted) and fits (solid) from (*left*) MoFe protein-bound FeMoco and (*right*) the NifEN-bound FeMoco precursor. The EXAFS data were obtained, respectively, by subtraction of Δ*nifB* MoFe protein EXAFS from wild-type MoFe protein EXAFS and Δ*nifB* NifEN EXAFS from NifEN EXAFS.

The insets show the structure of FeMoco (*left*) and a structural model consistent with the XAS analysis of the NifEN-bound FeMoco precursor (*right*). Both structures were adapted from MoFe protein coordinates (*4*) and are colored by atom type: Mo (purple), Fe (green), S (orange), O (red), N (blue), and X (black), which may be carbon, nitrogen, or oxygen.

Both MoFe protein and NifEN contain two different types of iron-sulfur clusters. In order to isolate the Fe K-edge EXAFS signal from FeMoco or its precursor, EXAFS data were collected

from the wild-type proteins and from mutant proteins in which the cluster of interest is absent. Subtraction of the EXAFS signal of the mutant proteins from that of the wild-type proteins in ratios dependent on their respective metal contents produced data sets representative of either MoFe-protein bound FeMoco or the NifEN-bound precursor. Obtaining good data from this procedure required high resolution, low noise EXAFS data—the collection of which was made possible by the high flux and stability of focused wiggler beamline 9-3. The validity of this subtraction technique was verified by the agreement of the XAS results for MoFe-protein bound FeMoco with previous structural results (see, for example, *4*).

The XAS results for the NifEN-bound FeMoco precursor are consistent with an iron- and sulfur-containing cluster having at least seven iron atoms, the same long-range order as FeMoco, and a similar average iron site geometry as FeMoco. This precursor is formed in the absence of the several genes known to play a role in FeMoco assembly and MoFe protein maturation, which suggests a larger than expected role for the NifB protein in the assembly process. The most likely trajectory for FeMoco biosynthesis from this state would be loss of the non-sulfur-ligated terminal iron atom followed by incorporation of molybdenum in its place. This simple metal substitution reaction would be the most likely mechanism for the assembly of the alternative vanadium-containing nitrogenase cofactor as well.

These EXAFS results provide much needed physical evidence to support a mechanism for FeMoco biosynthesis and may prove instrumental in the development of a strategy for the chemical synthesis of FeMoco and future opportunities for a detailed analysis of this important yet enigmatic cluster.

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References

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