NifB-co, a Key FeMo-co Precursor, is a Fe_6S_9 Core with an Interstitial Light Atom

All life depends on the input of the element nitrogen into the biosphere by biological nitrogen fixation (the reduction of dinitrogen to ammonia, $N_2 \rightarrow 2NH_3$) performed only by diazotropic microorganisms. These microorganisms achieve N_2 fixation using a family of metalloenzymes, called nitrogenases (N_2ases). Biological N_2 fixation is responsible for about half of the protein available for human consumption. The other half is produced using natural gas in fertilizer factories by the Haber-Bosch process. A better understanding of N_2ases may have an impact on our ability to transition to a more sustainable energy economy.

The active site of Mo-N_2ase, which is contained within the so-called MoFe protein (one of the component proteins in Mo-N_2ase), is a remarkable [7Fe-9S-Mo-X-homocitrate] cluster, called the iron-molybdenum cofactor (FeMo-co). FeMo-co is regarded as one of the most complex Fe-S clusters found in biology (Fig. 1). The exact identity of the interstitial light atom X is unknown; it can be assigned to C, N, or O. Knowledge of the FeMo-co biosynthetic assembly process, and the nature of X, is critical to understanding the N_2ase catalytic mechanism.

Figure 1 shows the current understanding of the biosynthetic pathways for Mo-N_2ase [1]. A key metabolic intermediate in the biosynthesis of FeMo-co is NifB-co, a low-molecular weight Fe-S cluster. In addition to being a precursor of FeMo-co, NifB-co is also hypothesized to be the precursor of FeV-co and FeFe-co, suggesting that NifB-co forms the core portion that is common among all three N_2ase active site cofactors.

Recently, we used two synchrotron radiation techniques, namely *K*-edge extended X-ray absorption fine structure (EXAFS) and nuclear resonance vibrational spectroscopy (NRVS) to probe the structural and dynamics of the Fe sites of NifB-co bound to the small protein NifX (NifX:NifB-co) (**BL09XU**) [2]. EXAFS is a well-developed technique [3], capable of solving the local structure (typically within 5 Å) of the probed atom (in our case, Fe). NRVS is a novel vibrational spectroscopy [4]. The NRVS experiment involves scanning an extremely monochromatic X-ray beam ($\Delta E \sim 1$ meV) through a nuclear resonance. Apart from the 'zero phonon' (recoil-free) Mössbauer resonance, there are additional transitions that correspond to nuclear excitation plus excitation or de-excitation of vibrational modes.

Figure 2 shows the EXAFS and NRVS spectra of NifX:NifB-co and the simulations. Spectra for FeMo-co are also shown to help calibrate and interpret the NifX:NifB-co results. The EXAFS simulation of NifX:NifB-co reveals a set of S ligand at ~2.3 Å and a set of Fe next nearest neighbors at ~2.6 Å. A set of Fe-Fe interactions at 3.7 Å is also clear, which has so far been observed only in Fe-S centers for FeMo-co. The above features can be viewed as a strong indicator for the presence of the Fe_6S_9 core in NifB-co, similar with the trigonal prism core in a matured FeMo-co. Three different structural models (Fig. 3) have been used in the EXAFS simulation, the best fit was obtained by using the 6Fe model. Furthermore, a search profile in the simulation confirms a Fe-X interaction with ~1 atom at 2.06 Å, consistent with the same interaction in a maturated FeMo-co. This is the first time this interaction has been reported in NifB-co, and is highly significant for the understanding

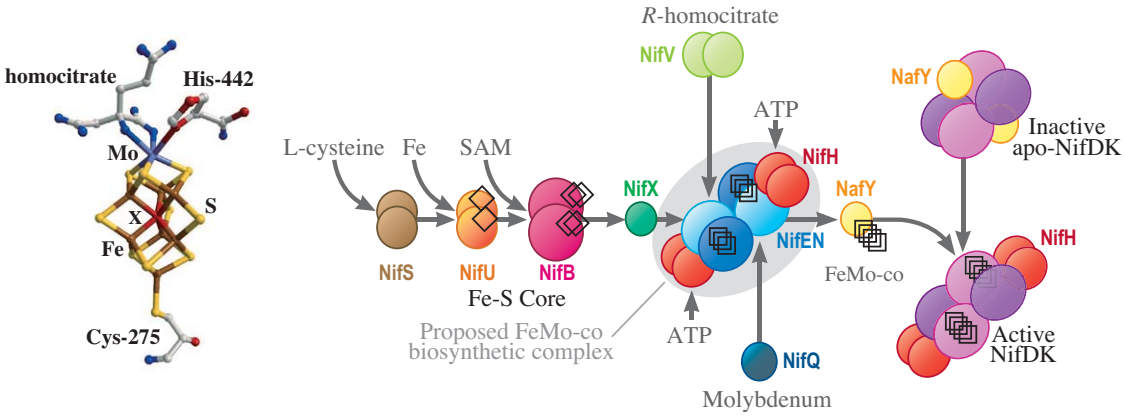

Fig. 1. Left: the structure of FeMo-co as determined by X-ray crystallography on the Mo-N_2ase enzyme (Protein Data Bank: 1M1N). Right: Proposed scheme for FeMo-co biosynthesis showing the proposed roles of NifB, NifX, NifH, NafY, and the MoFe protein (NifDK) N_2ase [1].

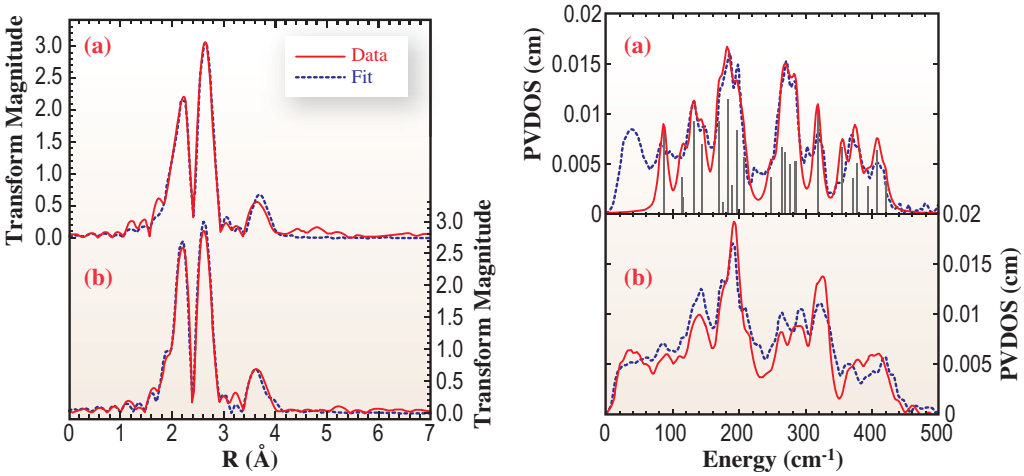

Fig. 2. Left: Fe *K*-edge Fourier transformed EXAFS of **(a)** NifX:NifB-co (---) with the 6Fe model fit (---), and **(b)** data (---) and fit (---) of NafY:FeMo-co. Right: **(a)** NRVS of NifX:NifB-co (---) and the 6Fe model fit (---); **(b)** NRVS of NafY:FeMo-co (---) and NMF:FeMo-co (---).

of FeMo-co maturation and the N_2ase catalytic mechanism. In our simulation, X is simulated as N atom, C, and O also can obtain similar results. In addition, measurements in the Mo *K*-edge region conclusively demonstrate the absence of Mo in NifB-co.

The NRVS spectrum of NifX:NifB-co shows similar spectral features as the NRVS of NafY:FeMoco (FeMo-co bound to the small protein NafY) and NMF:FeMoco (FeMo-co isolated in *N*-methylformamide (NMF)) (Fig. 2). Features above 250 cm^{-1} are primarily due to Fe-S stretching modes. The most striking feature is the intensity observed between 180 and 200 cm^{-1} in the NifX:NifB-co NRVS. This feature is considered to arise from the breathing modes of the Fe_6S_9 core of FeMo-co in the presence of the interstitial light atom X [5], indicating that the interstitial light atom has already been incorporated into the NifB-co cluster. A simulation using the models in Fig. 3 confirms this assignment, while a model without this interstitial atom results in the absence of this 180-200 cm^{-1} band.

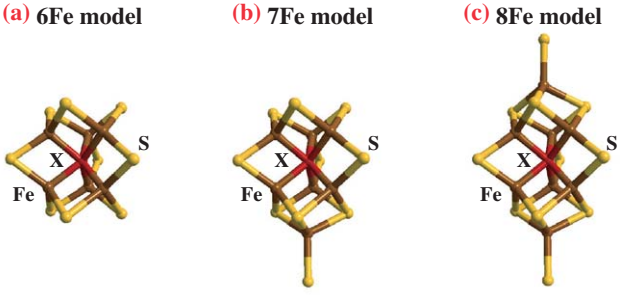

Fig. 3. Proposed models for NifB-co as described in the text. **(a)** 6Fe **(b)** 7Fe **(c)** 8Fe. Color code: Fe (brown), sulfur (yellow), interstitial atom X (red).

Taken together, the EXAFS and NRVS data show that NifB-co, the key FeMo-co biosynthetic intermediate, is most likely to consist of a Fe_6S_9 cage (Fig. 3). The presence of an interstitial atom in NifB-co is confirmed for the first time. This finding is significant because it rules out the possibility that the interstitial atom is incorporated in a later stage of the FeMo-co biosynthetic pathway - it implies that the function of the NifB enzyme is to perform the reaction of synthesizing this unique structure. This study demonstrates that EXAFS and NRVS are a powerful combination to reveal the structural information in the iron-containing metalloproteins.

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