X-ray Emission Spectroscopy Evidences a Central Carbon in the Nitrogenase Iron-Molybdenum Cofactor

The availability of reduced (fixed) nitrogen species is one of the basic requirements for life on earth. Nitrogen fixation involves cleavage of the strongest homodinuclear chemical bond. Natural nitrogen fixation proceeds primarily through bacterial nitrogenase enzymes, whose iron-molybdenum cofactors (FeMoco) are the primary catalysts for this remarkable feat. FeMoco consists of a molybdenum-containing 7-iron, 9-sulfide cluster [Figure 1]. A high-resolution nitrogenase crystal structure reported by Einsle and co-workers in 2002 revealed an unidentifiable light atom, "X," at the center of this cluster.[1] Since then, electron paramagnetic resonance and x-ray absorption spectroscopies combined with several computational approaches have failed to unambiguously identify X.

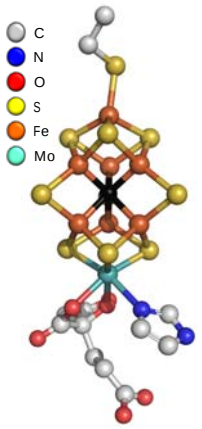

Figure 1. 1.19 Å x-ray structure of FeMoco, with "X" pictured as a black atom in the center.

Using valence-to-core (V2C) iron Kß x-ray emission spectroscopy (XES) and computational modeling, a team led by Serena DeBeer (jointly of Cornell University and the Max-Planck-Institute for Bioinorganic Chemistry) has shown that X is a carbon atom. V2C XES comprises transitions from occupied ligand-centered molecular orbitals to a Fe 1s core-hole initially generated during x-ray photoexcitation. These transitions are very weakly allowed due to Fe 4p admixture.[2,3] In essence, V2C XES uses the photoabsorber as a window into the electronic structure of directly coordinated atoms.

V2C XES features two regions: the $K\beta_{2,5}$ and Kß". The $K\beta_{2,5}$ consists of transitions from ligand n*p* orbitals. These MOs can feature substantial metal-ligand covalency and as such are difficult to predict *a priori*. However, the lower-energy Kß" features are more readily rationalized. They monitor transitions from ligand n*s* orbitals, which are minimally involved in bonding. As such, Kß" transitions can be directly correlated to the n*s* ionization potential of bound ligands. Calibrations with various model coordination complexes and iron materials show that Kß" features from different light atoms (B, C, N, O, F) are separated by ~2-4 eV. Thus, V2C XES can discriminate between neighboring atoms on the periodic table.[4] This marks a substantial advantage over extended x-ray absorption fine structure, which has an error ~ Z = 1.

With these calibrations guiding them, the team recorded V2C XES spectra at Stanford Synchrotron Radiation Lightsource Beam Line 6-2 from fully constituted Mo-nitrogenase, isolated FeMoco, and closely related iron-sulfur "P clusters" [Figure 2]. All of the biological samples were prepared in the lab of Markus Ribbe (University of California, Irvine). These spectra were correlated to calibrated computations from the group of Frank Neese (University of Bonn and Max-Planck-Institute for Bioinorganic Chemistry). Features corresponding to a central N or O atom were decidedly absent, while increased intensity consistent with a central C could be clearly observed. Thus, the team has declared "X = C."

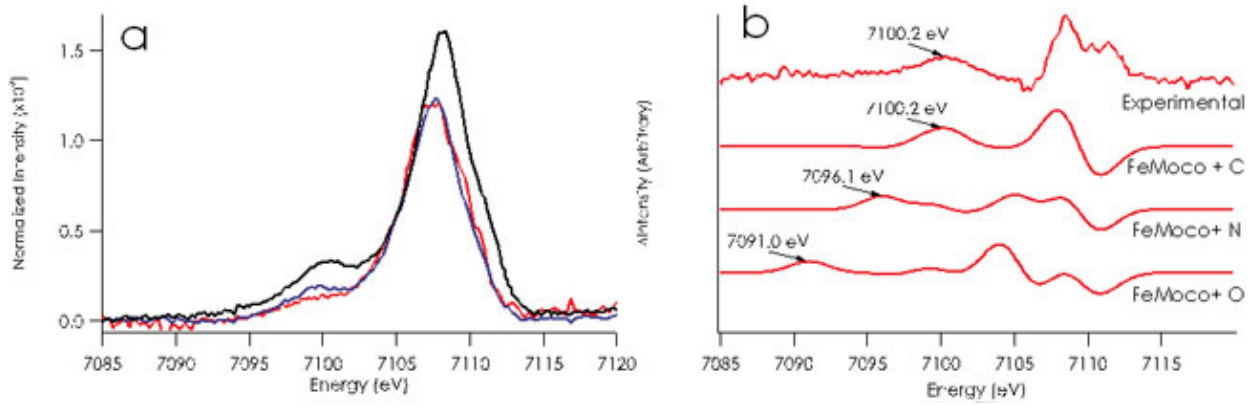

Figure 2. a) V2C XES spectra of isolated FeMoco (black), MoFe protein (blue), and of the P-clusters (red). b) Difference spectra from subtraction of P-cluster V2C XES from experimental FeMoco data, as well as subtractions using XES calculated for FeMoco with different central light atoms.

DeBeer and co-workers will return to SSRL to pursue further questions related to biological nitrogen fixation.

References

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Contact

Serena DeBeer, serena.debeer@mpi-mail.mpg.de

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