FEMO COFACTOR: THE CHARACTERIZATION OF NATURE'S CARBIDE

Reported by Marshall R. Brennan November 15, 2012

INTRODUCTION

Nitrogen "fixation," the conversion of N_2 to NH_3 at ambient temperatures and pressures, is a key part of the biosynthesis of amino acids and other nitrogen-containing biomolecules.[1] This is accomplished by the nitrogenase enzyme, found most notably in the bacteria *Clostridium pasteurianum, Azotobacter agilis,* and, *Azotobacter chroococcum.*[2] While the mechanism of enzymatic nitrogen fixation is still a subject of intense research, the past 35 years have seen major progress in the characterization of the active components of nitrogenase. Most importantly, the structure of the redox-active site has been a source of controversy only recently resolved as an extremely rare fully-ionized carbide (C^{4-}) at the center of the metalloenzyme's active site; the experiments leading to this assignment constitute the subject of this seminar.

EARLY DISCOVERY & CHARACTERIZATION OF FEMOCO

Although the nitrogenase enzyme was isolated from *Clostridium pasteurianum* in 1960,[4] it was not until 1992 that Rees successfully deduced the structure of FeMoCo at 2.7 Å resolution.[5] Figure I shows the structure elucidated by Rees, in which a [4Fe-3S] subcluster is bridged by three μ_2-sulfides to a [Mo-3Fe-3S] subcluster, with *a central fully-ionized light atom bound to all six iron nuclei.* In Rees' initial 2.7 Å structure, the identity of this central bridging atom could not be identified, though the following year a refined 2.2 Å structure narrowed the possible identities to C, N, O, or S.[6]

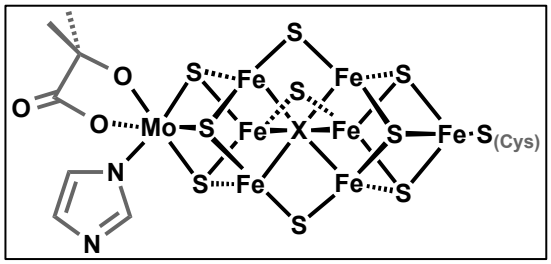

Figure I. Schematic representation of FeMoCo. X represents bridging ligand with light electron density.

ON THE IDENTITY OF THE BRIDGING LIGAND, X

The identity of this bridging ligand proved controversial. As the fixation mechanism is not known, it was postulated that this ligand could offer insight into this process.[7] Rees originally proposed that the atom is a nitride, postulating that the ligand cavity is involved in substrate binding.[6] Further refinements to the structure by Lawson[8] (1.6 Å) and Rees[9] (1.16 Å) did little to support this hypothesis. In the subsequent decade following structure elucidation, many researchers attempted to identify this interstitial atom, through computational and physical methods (Fig. II).

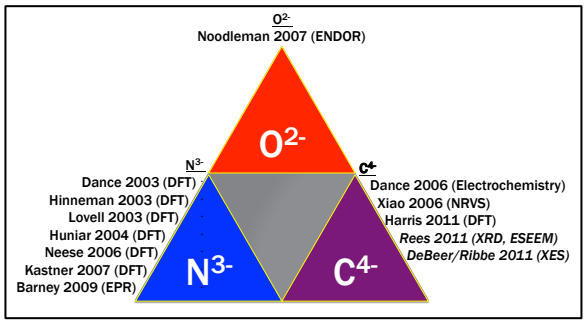

Figure II. Selected experiments identifying the bridging ligand (Refs: C^{4-}: 10a-e, N^{3-}: 11a-g, O^{2-}: 12)

In 2011 two concurrent papers in *Science*—by DeBeer & Ribbe and Rees—served to conclusively identify the carbide. In DeBeer's report, X-ray Emission Spectroscopy (XES) was used to probe the probability of each nucleus type.[10e] Correlations were drawn between experimental and theoretical data that implicated a carbide ligand. Concurrently, Rees published a 1.0 Å crystal structure with sufficiently high-resolution to identify the carbide in conjunction with resonance spectroscopy.[10d]

Earlier this fall, work by Ribbe and coworkers sought to identify the biosynthetic origins of the carbide ligand.[13] In this study, the researchers identified *S*-adenosyl methionine (SAM) as a potential carbon source. Using a novel fusion protein of the biosynthetic cluster, it was identified that SAM incubated in the presence of the protein yielded FeMoCo, *S*-adenosyl homocysteine, and 5'-deoxyadenosine. Through radiolabeling experiments, it was shown that the methyl group on SAM is radically cleaved by the forming cluster and subsequently deprotonated to form the C^{4-} ligand.

SUMMARY

Through the acquisition of high-resolution crystallographic data and state-of-the-art spectroscopic measurements, the structure of the FeMo Cofactor was conclusively identified, including the central light atom. The controversy surrounding the identity of this atom underscores the importance of N_2 fixation as a technology for the production of ammonia. While the mechanism of this enzyme's redox activity is not yet known, work has already begun on the identification of the source of the carbide, its role in the cofactor, and how the cofactor can influence small molecule catalyst mimics.

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