EPR and ENDOR Characterization of Nitrogenase Enzymatic Intermediates

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Nitrogenase catalyzes the reduction of N_2 to NH_3 on the most complicated metalloenzyme active site, the molybdenum-iron cofactor (FeMo-cofactor). This talk will describe our progress in trapping intermediates during substrate reduction by nitrogenase, and our use of electron paramagnetic resonance (EPR) and electron-nuclear double resonance (ENDOR) spectroscopies to characterize them.[1] We have now: *(i)* trapped an intermediate that forms during the reduction of propargyl alcohol ((HC≡C-CH_2OH; PA) and used a novel set of *quantitative* ^{1,2}H ENDOR measurements, plus ^{13}C ENDOR measurements, to show that it contains a novel bio-organometallic structure: a product complex of allyl alcohol (H_2C=CH-CH_2OH bound as a ferracyclopropane to Fe2 of the cofactor;[2] *(ii)* characterized an intermediate formed during proton reduction which is suggested by preliminary analysis most likely containing two hydrides bound to the cofactor;[3] *(iii)* trapped at least two intermediates in which N_2 and/or its reduced forms are bound to the cofactor,[4] and initiated their ENDOR characterization. As time permits, we will discuss the metal-ion valencies of cofactor turnover intermediates,[5] and also update our efforts[6] to identify 'X', the thing in the middle of the cofactor.

(1) Dos Santos, P. C. *et al*. *Acc. Chem. Res.* **2005**.
(2) Lee, H.-I. *et al*. *J. Am. Chem. Soc.* **2004**, *126*, 9563-9569.
(3) Igarashi, R. Y. *et al*. *J. Am. Chem. Soc.* **2005**, *In Press*.
(4) Barney, B. M. *et al*. *Biochemistry* **2005**, *In Press*.
(5) Lee, H.-I. *et al*. *J. Am. Chem. Soc.* **2005**, *Submitted.*
(6) Lee, H.-I. *et al*. *J. Am. Chem. Soc.* **2003**, *125*, 5604-5605.