Sodium Borohydride Reduction of F_{430} Generates a New Cofactor Species : F_{330}

Mishtu Dey[1], Ryan C. Kunz[1], Yih-Chern Horng[1], Jennifer L. Craft[2], Thomas C. Brunold[2], and Stephen W. Ragsdale[1]

[1]*Department of Biochemistry, University of Nebraska Lincoln, and* [2]*Department of Chemistry, University of Wisconsin, Madison*

Methyl-coenzyme M reductase (MCR) from methanogenic archaea catalyzes the final step in the biological synthesis of methane. MCR contains an essential cofactor at the active site, which is a redox-active nickel tetrahydrocorphin, Coenzyme F_{430}. The active form of MCR contains Ni(I)-F_{430}. F_{430} is the most reduced tetrapyrrole in nature, containing only five double bonds. UV-visible, magnetic circular dichroism (MCD), mass spectroscopic, and ^{1}H and 2-dimensional NMR results demonstrate that the tetrapyrrole ring can undergo two-electron reduction to generate a species, called F_{330}, with an absorption peak at 330 nm. Two protons, one exchangeable and one non-exchangeable, are incorporated when F_{430} is reduced with sodium borohydride, while two deuteriums (one exchangeable and one non-exchangeable) are incorporated when sodium borodeuteride is the reducing agent. ^{1}H-NMR spectroscopy has been used to localize the position on the tetrapyrrole ring at which the hydride addition occurs. ^{1}H-NMR and MCD spectroscopic and computational results indicate F_{330} contains a low spin, most likely divalent Ni center. Thus, the conversion of F_{430} to F_{330} involves tetrapyrrole ring reduction but not metal-centered reduction. On the other hand, generation of the active Ni(I) form of the coenzyme with Ti(III) citrate, known as red1, involves a one-electron metal-centered reduction and not tetrapyrrole ring reduction. Computational work indicates that both ring reduction and metal center reduction cause similar shifts in the UV-visible spectra.