Advanced Paramagnetic Resonance Studies of the Fe-only Hydrogenase I from *Clostridium pasteurianum (Cp*I*)*

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Hydrogenases (H_2ase) comprise a class of metalloenzymes that catalyze perhaps the most fundamental of chemical reactions: $H_{2(g)} \leftrightarrow 2H^+_{(aq)} + 2e^-$. The best known hydrogenases contain a Ni ion at their multi-metal cluster active site, however, other hydrogenases contain only Fe at the active site (1). Examples of both Ni and Fe-only H_2ases have been structurally characterized by x-ray crystallography. The Fe-only active site, "H-cluster", is notable that it consists of a $[Fe_4S_4]$ cluster covalently linked to a di-Fe center, in which each Fe is coordinated by a cyano and a carbonyl ligand, with a bridging CO. The current proposal for the oxidation state of these two Fe ions that in the EPR-inactive, air ("super")oxidized form, H_{ox}^{air}, the H-cluster contains two Fe(II) ions (each LS $3d^6$, $S = 0$); in the EPR-active oxidized form, H_{ox}, one Fe becomes Fe(I) (LS $3d^7$, $S = 1/2$), and in the reduced form, H_{red}, the H-cluster consists of anti-ferromagnetically coupled Fe(I) ions, giving an $S_{total} = 0$ spin ground state.

We describe here 35 GHz continous wave (CW) and pulsed electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) studies on the H_{ox} state of the Fe-only H2ase I from *Clostridium pasteurianum* (CpI), for which there is an x-ray structure of the H_{ox}^{air} state (1). The H_{ox} enzyme has been studied in several forms: in the naturally found form with natural isotopic abundances and with an extrinsic, bound CO molecule (CO-terminal form, with both ^{12}CO and ^{13}CO) and in a form in which extrinsic (terminal) ^{13}CO exchanges with the endogenous, bridging CO to give an isotopolog that contains bridging ^{13}CO.

The CO-terminal and CO-bridging (natural H_{ox}) states exhibit different EPR spectra and more importantly, very different ^{13}C ENDOR spectra for the bridging versus terminal ^{13}CO ligands: the former exhibits very weak hyperfine coupling; the latter very strong. These results will be discussed in terms of structural and computational studies of model compounds.

1) "A Novel FeS Cluster in Fe-only Hydrogenases" Nicolet, Y.; Lemon, B. J.; Fontecilla-Camps, J. C.; Peters, J. W.; *Trends in Biochemical Sciences* **2000**, *25*, 138-144.