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

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Hydrogenases (H$\text{}_2$ase) comprise a class of metalloenzymes that catalyze perhaps the most fundamental of chemical reactions: $\text{H}_2(g) \leftrightarrow 2\text{H}^+(aq) + 2e^-$. 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$\text{}_2$ases have been structurally characterized by x-ray crystallography. The Fe-only active site, “H-cluster”, is notable that it consists of a [Fe$_4$S$_4$] 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$_\text{ox}^{\text{air}}$, the H-cluster contains two Fe(II) ions (each LS 3d$^6$, $S = 0$); in the EPR-active oxidized form, H$_\text{ox}$, one Fe becomes Fe(I) (LS 3d$^7$, $S = 1/2$), and in the reduced form, H$_\text{red}$, the H-cluster consists of anti-ferromagnetically coupled Fe(I) ions, giving an $S_{\text{total}} = 0$ spin ground state.

We describe here 35 GHz continuous wave (CW) and pulsed electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) studies on the H$_\text{ox}$ state of the Fe-only H$\text{}_2$ase I from *Clostridium pasteurianum (CpI)*, for which there is an x-ray structure of the H$_\text{ox}$ state (1). The H$_\text{ox}$ 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$_\text{ox}$) 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.