The H cluster: A Light-Sensitive 6Fe Active Site in [FeFe]-Hydrogenases

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The hydrogen-activating cluster (H cluster) in [FeFe]-hydrogenases consists of two moieties. The $[2Fe]_H$ subcluster is a (L)(CO)(CN)Fe(μ-CO)(μ-RS_2)Fe(CO)(CN)(CysS) centre, where R is presumably a -CH_2-NH-CH_2- group. The Cys-bound Fe is called Fe1, the other iron Fe2. In active enzyme the $[2Fe]_H$ subcluster is directly linked to a [4Fe-4S] cluster, the $[4Fe\text{-}4S]_H$ subcluster, via the Cys-thiol group. The enzyme of *Desulfovibrio desulfuricans* is purified aerobically and is inactive. It can be activated by H_2 or artificial reductants, whereby the ligand L is removed and the midpoint potential of the $[4Fe\text{-}4S]_H$ cluster is decreased drastically. Once active, its activity is rapidly destroyed by oxygen.

The active H-cluster can exist in two redox states: H_{ox}, a S=1/2 system, and H_{red} which is diamagnetic. In both states the $[4Fe\text{-}4S]_H$ subcluster is oxidized. The stretch frequency of the terminally-bound CO on Fe1 is the same in both states, indicating that the n=1 valence change occurs on Fe2. This is consistent with the FTIR changes of the terminally-bound CO on Fe2. Extrinsic CO, a strong competitive inhibitor, binds to Fe2 in the H_{ox} state to form the H_{ox}-CO state. This makes a trivalent Fe2 unlikely. Hence, the H_{ox}/H_{red} transition is best described as a divalent/monovalent transition of Fe2, while we assign the S=1/2 system of H_{ox} to a monovalent state of Fe1. EPR spectra of ^{57}Fe-enriched enzyme are consistent with a delocalization of the unpaired spin onto the $[4Fe\text{-}4S]_H$ subcluster. This delocalization is weak in the H_{ox} state but quite strong in the H_{ox}-CO state. EPR spectra of H_{ox}-^{13}CO show that also Fe2 receives some spin density.

Contrary to the inactive enzyme, the active enzyme is highly sensitive to light. The $[2Fe]_H$ subcluster in some enzyme molecules looses CO by photolysis, while other molecules firmly bind the released CO to form the H_{ox}-CO state which is stable in light. The spectroscopic signatures of the H_{ox}-CO state created by this form of light-induced *cannibalism* can be recognized in nearly all reports on [FeFe]-hydrogenases published thus far. Although the enzyme in the H_{ox}-CO state is not destroyed by light, it is light sensitive at room temperature. Under ^{13}CO two of the intrinsic COs, both bound to Fe2, can be exchanged by ^{13}CO during illumination. EPR and FTIR spectra of the products will be shown.

At 30 K both the inhibiting extrinsic CO bound to Fe2 and the bridging CO of the H_{ox}-CO state can be photolyzed as demonstrated by FTIR. EPR spectra of the photolyzed product are consistent with a $3d^7$ system of monovalent Fe.