Inhibition by CN^- provides insight into the catalytic mechanism of [FeFe] hydrogenases

Maria Alessandra Martini,[a] Konstantin Bikbaev,[bc] Patricia Rodríguez-Maciá,[d] Serena DeBeer, [a] Ingrid Span,[bc] James A. Birrell[a]

[a] Department of Inorganic Spectroscopy, Max Planck Institute for Chemical Energy Conversion, Mülheim an der Ruhr, Germany
[b] Institut für Physikalische Biologie, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[c] Department of Chemistry and Pharmacy, Bioinorganic Chemistry, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany
[d] Inorganic Chemistry Laboratory, Department of Chemistry, University of Oxford, Oxford, UK
maria.martini@cec.mpg.de

[FeFe] hydrogenases are highly active enzymes that catalyze the interconversion of protons and electrons with molecular hydrogen. The cofactor at the active site (the H-cluster) is composed by a canonical [4Fe-4S] cluster ($[4Fe\text{-}4S]_H$) linked to a unique di-iron subcluster ($[2Fe]_H$) by a cysteine. In $[2Fe]_H$ the two Fe ions are further coordinated by a bridging 2-azapropane-1,3-dithiolate (ADT) ligand, three CO (one bridging) and two CN^- ligands, leaving an open coordination site on one Fe where H_2 activation or formation occurs. The strong-field CO and CN^- ligands are thought to stabilize low-valent, low-spin Fe(II) and Fe(I) oxidation states in $[2Fe]_H$ e.g. Fe(II)Fe(I) in the active oxidized (H_{ox}) state. Conversion to the reduced Fe(I)Fe(I) state is triggered by protonation of the ADT ligand, meanwhile the overoxidized Fe(II)Fe(II) state is observed in the crucial iron-hydride containing H_{hyd} state. Additionally, two oxygen-stable forms of the H-cluster with an overoxidized $[2Fe]_H$, called H_{trans} and H_{inact}, can be generated by binding of exogenous sulfide at the open site of $[2Fe]_H$ under oxidizing conditions. Here, we investigate H_{trans}-like and H_{inact}-like states that accumulate in [FeFe] hydrogenase mutants where the cysteine in the proton-transfer pathway leading to and from the active site is mutated to alanine. Our data suggest that in these two states a third CN^- ligand is bound to the apical position of $[2Fe]_H$. These states can be generated both by "cannibalization" of CN^- from damaged $[2Fe]_H$ subclusters as well as by addition of exogenous KCN. To our knowledge this is the first reported interaction of exogenous CN^- with [FeFe] hydrogenases. Similar CN^--bound states can also be generated in wild-type hydrogenases, but do not form as readily as with the Cys → Ala mutants. These results help us to understand how the fine balance between charge, σ-donation and π-backdonation contributions from the strong-field ligands CO and CN^- affect the electronic structure of the H-cluster, and we discuss the implications for catalysis.