Poster Presentation

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Observation of a metal-hydride in [NiFe] hydrogenase

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Hydrogenases catalyze the reversible hydrogen oxidation process by cleaving dihydrogen heterolytically.(1) For this reaction, the enzyme uses the transition metals Ni and Fe, which are abundant in Nature. Standard [NiFe] hydrogenaes are mainly composed of two subunits (total ~90 kDa) The [NiFe] active site is located in the center of the molecule. The active site of [NiFe] hydrogenase is composed of the dinuclear Ni-Fe center, where the Fe ion is coordinated by non-protein ligands (1CO and 2CN̄). Two thiolates of cysteine residues are bridging both metals. Furthermore, the Ni is coordinated to the two thiolates of cysteine residues in a terminal fashion. A third bridging ligand is found between the Ni and Fe atom, depending on the redox state.(1) In the inactive form, a third bridging ligand (O$\bar{\bar{H}}$) is found between Ni and Fe. Once the enzyme is activated, the bridging position is supposed to be vacant or bridged by a hydride. A previous X-ray crystallographic study at 1.4 Å resolution revealed that the bridging ligand (OH) is removed upon H2 reduction.(2) Electron paramagnetic resonance (EPR) spectroscopy showed that a hydride is located in the bridge between Ni and Fe, which is lost upon illumination at cryogenic temperature.(3) Here we present a crystallographic analysis of the fully reduced (Ni-R) state of [NiFe] hydrogenase from Desulfovibrio vulgaris Miyazaki F at 0.89 Å resolution. The ultra-high resolution analysis revealed the presence of the hydride bridge at the NiFe active site in the catalytically active state. Furthermore the CO and CN ligands could be identified and a protonated thiolate sulfur ligand of the Ni is postulated based on the electron density.

[1] *H. Ogata, W. Lubitz, Y. Higuchi, Dalton Trans, 2009, 7577-7587,* ***[2]*** *Y. Higuchi, H. Ogata, et al, Struct. Fold. Des., 1999, 7, 549-556,* ***[3]*** *M. Brecht, M. van Gastel et al, J. Am. Chem. Soc., 2003, 125, 13075-13083*

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