A Minimal Model Hydrogenase

Start Time: Saturday, May 2, 2020

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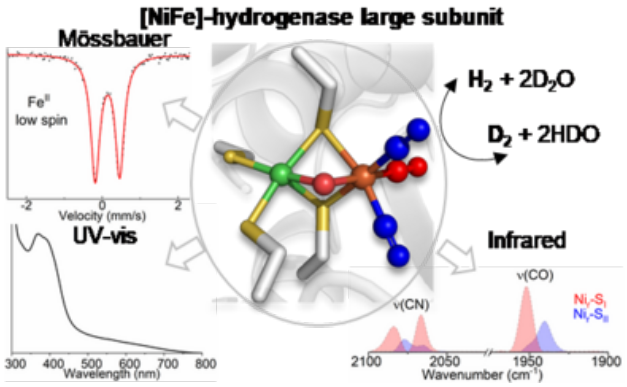

The large subunit of the regulatory [NiFe]-hydrogenase from Ralstonia eutropha **– a minimal hydrogenase?**

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The UniSysCat research groups of Christian Limberg, Peter Hildebrandt, Ingo Zebger, Oliver Lenz, in collaboration with the Einstein Visiting Fellow Stephen P. Cramer and the former UniCat researcher Claudio Greco characterized the isolated large subunit of a bipartite [NiFe]-hydrogenase, providing insight into the core biochemical and spectroscopic characteristics of the catalytic center.

Chemically synthesized compounds that are capable of facilitating the reversible splitting of dihydrogen into protons and electrons are rare in chemists' portfolio. The corresponding biocatalysts – hydrogenases – are, however, abundant in the microbial world. [NiFe]-hydrogenases represent a major subclass and display a bipartite architecture, composed of a large subunit, hosting the catalytic NiFe(CO)$(CN)_2$ cofactor, and a small subunit whose iron–sulfur clusters are responsible for electron transfer. To analyze in detail the catalytic competence of the large subunit without its smaller counterpart, we purified the large subunit HoxC of the regulatory [NiFe]-hydrogenase of the model H_2 oxidizer *Ralstonia eutropha* to homogeneity. Metal determination and infrared spectroscopy revealed a stoichiometric loading of the metal cofactor. This enabled for the first time the determination of the UV-visible extinction coefficient of the NiFe(CO)$(CN)_2$ cofactor. Moreover, the absence of disturbing iron–sulfur clusters allowed an unbiased look into the low-spin Fe^{2+} of the active site by

Mössbauer spectroscopy. Isolated HoxC was active in catalytic hydrogen–deuterium exchange, demonstrating its capacity to activate H_2. Its catalytic activity was drastically lower than that of the bipartite holoenzyme. This was consistent with infrared and electron paramagnetic resonance spectroscopic observations, suggesting that the bridging position between the active site nickel and iron ions is predominantly occupied by water-derived ligands, even under reducing conditions. In fact, the presence of water-derived ligands bound to low-spin Ni^{2+} was reflected by the absorption bands occurring in the corresponding UV-vis spectra, as revealed by time-dependent density functional theory calculations conducted on appropriate in silico models. Thus, the isolated large subunits indeed represent simple [NiFe]-hydrogenase models, which could serve as blueprints for chemically synthesized mimics. Furthermore, our data point to a fundamental role of the small subunit in preventing water access to the catalytic center, which significantly increases the H_2 splitting capacity of the enzyme.

For more information, click here (DOI).