The Ni-Fe Site of O_2-Tolerant Hydrogenases: Variations on Common Themes

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The aerobic ß-proteobacterium *Ralstonia eutrop*ha houses three Ni-Fe hydrogenases. The soluble, NAD-reducing (SH) and the membrane-bound (MBH) hydrogenases are involved in hydrogen conversion whereas the regulatory hydrogenase (RH) is a H_2-sensor. Although these hydrogenases are pronouncedly different regarding their overall organizations and cofactor complements, they are all fully functional in the presence of O_2. Hence, the *R. eutropha* hydrogenases are particularly promising for biotechnological and biomimetic applications.

We studied the Ni-Fe active site by X-ray absorption spectroscopy (XAS), EPR, and FTIR to derive its nuclear geometry and to track oxidation state changes during activation and catalysis. The Ni-Fe sites in all three enzymes [1,2,3] pronouncedly differ from that in the standard *D. gigas* Ni-Fe hydrogenase. The Ni and Fe atoms may carry additional CN ligands; not all of the thiols from the four conserved cysteines seem to coordinate the Ni; distinct structural changes occur upon H_2-binding; Ni(III) oxidation states (Ni-A,B,C states) seem not to be relevant for H_2 turnover; specific features may characterize H_2-converting and –sensor enzymes.

Structural models of the Ni-Fe sites in the three hydrogenases will be presented; mechanistic implications for hydrogen catalysis will be discussed. Unique strategies may be implemented to yield hydrogen-splitting and –sensing under aerobic conditions.

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