DFT investigation of the Ni-A inactive state of the [NiFe]-hydrogenases: inactivation mechanism under aerobic conditions

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Introduction: Dihydrogen is considered as a future non polluting energy vector. In this context Hydrogenase enzymes are of fundamental interest for their capability to catalyse the reversible interconversion of protons and reducing equivalents into molecular hydrogen. While the [FeFe]-H₂ases are irreversibly inactivated by O₂, the oxidized [NiFe]-hydrogenases can be reactivated by one-electron reduction and protonation. This property, in addition to the high catalytic efficiency and the absence of expensive metals in their active site makes the [NiFe]-H₂ases a very promising target for reverse engineering studies aimed at the development of bioinspired catalysts.

Aim of the work: To better characterize the inactive oxidized Ni-A state of this enzyme, quantum mechanics calculations have been carried out in the framework of the Density Functional Theory (DFT) on a very large model of the active site. Mechanisms of oxidation of active forms of the enzyme to the Ni-A and Ni-B states were investigated under aerobic conditions.

Ni-A state:
The Ni-A state, described as Ni⁵⁺Fe⁰ with an oxygenic bridging ligand, is an EPR active oxidized state of the [NiFe]-hydrogenases. Based on crystallographic analyses, different O₂-reduction products have been proposed to bind to the Ni-A state but its exact nature is still not clear.

A recent crystallographic structure shows a bridging monoatomic oxygen species and the Cys64 oxidized to its sulfenated form[1].

**Ni-A structures proposed by previous crystallographic and DFT studies, were also investigated.**

Relative energies and comparison of experimental and predicted IR-frequencies are in agreement with the assignment of Ni-A to a species containing a bridging OH⁻ and oxidized Cys64.

Oxidation mechanism:

Aerobic oxidation of O₂-sensitive [NiFe]-Hydrogenases converts the enzyme into two chemically distinct inactive forms; Ni-A and Ni-B. If the [NiFe]-H₂ase is sufficiently reduced, its treatment with O₂ leads to the formation of a pure Ni-B oxidized state. When a less reduced enzyme is exposed to O₂ the Ni-A is formed in large amounts[2].

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References