

Exploring Novel Biocatalysts from Nature: O₂-tolerant [NiFe]hydrogenase

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Hydrogenases catalyze the cleavage and production of H₂ and have been purified and characterized from various microorganisms. Since Fontecilla-Camps *et al.* reported the first crystal structure of [NiFe]hydrogenase, hydrogenases and their inspired chemical catalysts are of interest in many areas of biological and technological applications. Thus [NiFe] hydrogenases are classified as either “O₂-sensitive” or “O₂-tolerant”, based on their catalytic ability in the presence of O₂. Both [NiFe] hydrogenases have the Ni-Fe active site for catalytic reaction and three Fe-S clusters for electron transfer. The O₂-sensitive [NiFe] hydrogenases do not display the catalytic activity in the presence of ambient O₂, forming Ni-A with a dioxygen ligand between two metals. In contrast, O₂-tolerant [NiFe] hydrogenases form only Ni-B or Ni-SR in the oxidized state, which can be immediately activated upon reduction with H₂. The prevention of the active site from producing Ni-A upon O₂-oxidation is associated with the O₂-tolerance. However, the mechanism of O₂-tolerance of hydrogenase has not been clearly understood.

We have isolated a new bacterium *Citrobacter* sp. S-77, containing a novel hydrogenase armed with high O₂-tolerance and H₂-activation. In this presentation, I will introduce our recent researches of a new O₂-tolerant [NiFe]hydrogenase being capable of high H₂-activation (1) and the crystal structure of O₂-tolerant [NiFe]hydrogenase previously reported (2). Our results confirmed that the overall structure and relative disposition of the metal centers of O₂-tolerant hydrogenase is similar to those of the O₂-sensitive hydrogenases, however, the proximal FeS cluster of O₂-tolerant hydrogenase is uniquely composed by [4Fe-3S]-6Cys, playing crucial roles not only in the electron transfer but also in preventing the formation of inactive state of the enzyme.

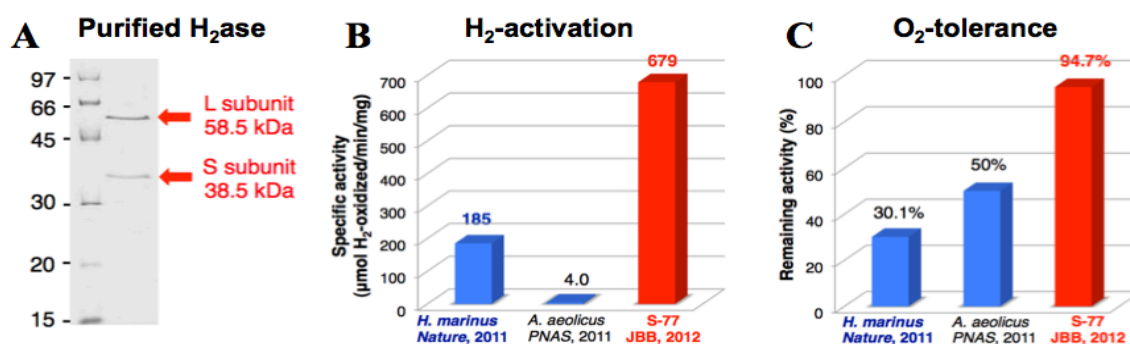


Fig. 1. Characterization of newly found [NiFe]hydrogenase from *Citrobacter* sp. S-77.

(A) SDS-PAGE analysis of S-77 hydrogenase, (B) Comparable H₂-activation and (C) O₂-tolerance of purified S-77 [NiFe]hydrogenase to those of other O₂-tolerant [NiFe]hydrogenases.

1. Eguchi S., Yoon K.S, Ogo S. O₂-stable membrane-bound [NiFe]hydrogenase from a newly isolated *Citrobacter* sp. S-77. *J. Biosci. Bioeng.* **114**, 479-484 (2012).
2. Shomura Y., Yoon K.S, Nishihara H., Higuchi Y. Structural basis for a [4Fe-3S] cluster in the oxygen-tolerant membrane-bound [NiFe]-hydrogenase. *Nature* **479**, 253-256 (2011).