HYDROGENASES

Air-stable [FeFe] hydrogenases

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Credit: American Chemical Society

Hydrogenases are appealing catalysts for fuel cells, as these highly efficient H₂-oxidation enzymes do not contain expensive precious metals and operate under very mild and safe conditions. Unfortunately, [FeFe] hydrogenases — the most active class — are irreversibly deactivated by O₂. This activity loss is associated with access of O₂ to the active centre, which displays an open coordination site at its FeS cluster.

Now, Olaf Rüdiger, James Birrell and co-workers report a simple protocol that allows handling oxygen-sensitive hydrogenases in the presence of air without activity loss by reversible coordination of a presumed HS[−] ligand to the open coordination site of the FeS cluster. The approach is simple, merely requiring chemical treatment of the enzyme with oxidizing agent hexaammineruthenium(III) chloride (HAR) and Na₂S.

Based on spectroscopic and electrochemical experiments as well as previous literature reports, the authors propose a mechanism for this reversible protection. Excess of Na₂S in solution provides H₂S. H₂S binds to the free coordination site of oxidized hydrogenase (H_{ox}). Reduction of the enzyme from excess of sulfide and concomitant release of the H₂S

ligand is prevented by the oxidizing agent HAR. The amine functionality of the FeS cluster then deprotonates the coordinated H₂S moiety yielding the HS[−] ligand. Subsequently, the enzyme goes through a transition state by intramolecular electron transfer coupled to deprotonation of the cluster's amine group. Finally, an oxidation step by HAR or air yields the oxygen-stable H_{ox}^{air} state.

The oxygen-stable enzyme can then be handled in the presence of air, which has the potential to alleviate the preparation of [FeFe]-hydrogenase-employing fuel cells and other biocatalytic devices. The native active H_{ox} state can be restored, for example, by treatment with Na₂S₂O₄ and subsequent reoxidation with HAR. Importantly, a redox hydrogel has been previously reported allowing the protection of [FeFe] hydrogenases from O₂ and high-potential inactivation in fuel cells. The provided simple protocol for handling [FeFe] hydrogenases in the presence of air makes their implementation in biotechnological applications more feasible and will probably motivate more researchers to work with this appealing enzyme class.

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