BESH2024 Mechanistic Investigations on Hydrogen Catalysis by [Fefe]-Hydrogenase

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Objectives

The long-term objective of this project is to advance the understanding of mechanisms of redox enzymes that function in photosynthetic energy transduction networks. One of these enzymes, [FeFe]-hydrogenase, catalyzes the evolution of H_2 by coupling to the reducing potential generated by photosynthetic water oxidation as a response to dark-to-light transitions in the cell. This reaction is fundamental to microbial energy conservation, and H_2 as a whole plays a critical role in the regulation of cellular energetics. [FeFe]-hydrogenases operate by unique proton-coupled electron-transfer chemistry (PCET), and we are investigating the composition of active site intermediates, mechanisms of proton-transfer and electron-transfer, thermodynamics of individual reaction steps, and electron-injection from external redox networks. An overarching aim of this work is to elucidate the mechanistic principles for biological transformation of photochemical potential into chemical bonds with an emphasis on how it is accomplished by specialized protein cofactors tailored for electron-transfer and catalysis.

Technical Barriers

[FeFe]-hydrogenases couple electrochemical potential to the reversible chemical transformation of H_2 and protons; however, the reaction mechanism and composition of intermediates are not fully understood. Fast PCET and oxygen sensitivity has challenged research efforts to define the biophysical properties of the functional intermediates of catalysis, especially key iron-hydride intermediates. The aim of this project is to use the algal [FeFe]-hydrogenase from *Chlamydomonas reinhardtii* to determine the mechanism and role of hydride intermediates in H_2 catalysis.

Abstract

Algal H_2 production is a model pathway for coupling water oxidation to renewable production of H_2. [FeFe]-hydrogenases catalyze the reaction $2e^- + 2H^+ \rightleftarrows H_2$ by means of a unique active-site cofactor, the H-cluster. The H-cluster is carefully positioned within the protein framework to optimize proton- and electron-transfer reactions required by the reaction. Developing a unified model of H_2 catalysis is challenging due to the structural diversity of enzymes, the inherently fast exchange reaction between H_2, protons and electrons, and the complexity of redox intermediates. Using integrated spectroscopy, we have been investigating the mechanism of catalysis with the [FeFe]-hydrogenase from *Chlamydomonas reinhardtii*. We also have been using the CaI [FeFe]-hydrogenase from *Clostridium acetobutylicum* to probe the mechanisms of electron-injection and electron-transfer to the H-cluster. The outcome of this work will help to reveal how H_2 metabolism is controlled during adaptive transitions of photosynthetic energy transduction, to advance the understanding of PCET in H_2 activation, and to inspire the design of more efficient organometallic complexes for artificial photosynthetic systems and fuel cells.

Progress Report

Identification of an Iron-Hydride [FeFe]-Hydrogenase Intermediate

Using a variant of the [FeFe]-hydrogenase from *Chlamydomonas reinhardtii* (CrHydA1), we characterized a short-lived catalytic Fe-hydride H-cluster intermediate (H_{hyd}) (Figure 1). The altered proton-transfer kinetics of the CrHydA1 (C169S) variant, where the primary proton donor (Cys residue 169) to the H-cluster is changed to a Ser residue, made it possible to enrich for the otherwise fast-lived hydride intermediate. Initial hydrogen-deuterium isotope exchange experiments with Fourier transform infrared (FTIR) spectroscopy and density functional theory (DFT) analysis suggested the presence of a terminal hydride bound to the distal Fe site of the 2Fe subcluster ($[2Fe]_H$) of the H-cluster. Further analysis by infrared and Mössbauer spectroscopy, together with computational modeling, revealed details of the electronic and geometric structure of the Fe–H^- bond present in H_{hyd} formed during reversible, heterolytic H_2 activation. Redox titrations showed that the reduction potential of H_{hyd} resides at the 1-electron to 2-electron transition in the catalytic cycle leading to formation of H_2. A significant shift in the midpoint potential of the H_{ox} H-cluster state compared to the wild-type enzyme demonstrates the role of the protein environment in tuning the active site for catalytic activity. The determination of the primary properties of the H-cluster-hydride intermediate reveals the inner workings for how the H-cluster enables facile PCET through positioning of the electron rich hydride proximal to a proton-exchangeable group and an electron relay [4Fe-4S] cluster.

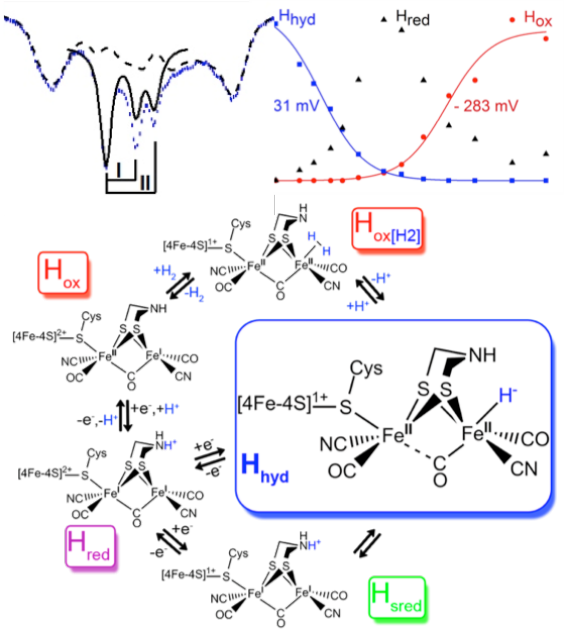

Figure 1. Mechanistic model, Mössbauer spectroscopic signatures, and electron paramagnetic resonance redox properties of H_{hyd}, the H-cluster hydride intermediate in the reversible H_2 activation reaction catalyzed by [FeFe]-hydrogenase.

Thermodynamic Analysis Of H-Cluster Redox Transitions using Photocatalytic Complexes

Photocatalytic complexes consisting of clostridial [FeFe]-hydrogenase Ca1and CdSe nanocrystals were used to study the mechanism of electron injection into [FeFe]-hydrogenase and the activation thermodynamics of the initial step of proton reduction (Figure 2). Compared to the algal CrHydA1 enzyme, Ca1 contains additional FeS clusters (F-clusters) that function in electron-transfer to the H-cluster. With previous collaborative studies with Professor Dukovic's group at University of Colorado Boulder, we showed evidence that nanocrystals interact with the ferredoxin binding site near the surface localized [4Fe-4S] F-cluster, the presumed entry point for electrons. Here, we combined light-driven experiments on the photocatalytic complexes with rapid-scan infrared (IR) and steady-state electron paramagnetic resonance (EPR) detection to probe both the pathway for electron-transfer and thermodynamics of active-site chemistry. The rapid scan IR made it possible to detect in real-time the formation of H-cluster redox intermediates during catalytic proton reduction, while the EPR followed the reduction to the F-cluster conduit. Together, the results show how the F-clusters function both as a conduit and a reservoir to drive catalysis. The results also reveal that the initial transition of the H-cluster from oxidized to reduced forms and the first step of catalytic proton reduction proceeds by concerted PCET.

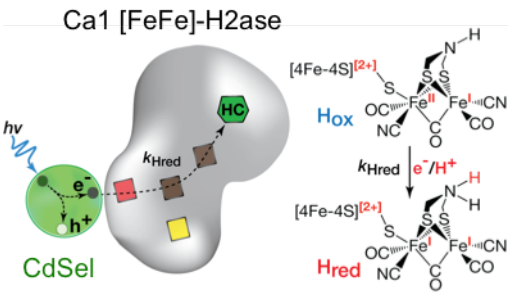

Figure 2. Photocatalytic CdSel:Ca1 complex and H-cluster reduction scheme for the initial proton-reduction step of H_2 catalysis.

Future Directions

- Utilize nuclear resonance vibrational spectroscopy (NVRS), in collaboration with the Cramer group at University of California Davis, to measure the properties of the Fe-hydride H-cluster intermediate trapped in C169S CrHydA1. Hydrogen-deuterium isotope exchange will be used to identify hydride modes by the NRVS technique, and the studies will be integrated with DFT to develop structural models for reduced H-cluster intermediates.
- Develop mechanistic models that describe the individual PCET transitions and protonation states of the H-cluster. The specific order of protonation and electron-transfer steps remains largely unknown and assumed for currently catalytic models. Cryogenic IR spectroscopy, along with hydrogen-deuterium exchange and mutagenesis, will be used to identify CO and CN^- signatures that can be ascribed to different H-cluster intermediates.
- Explore the universality aspect of the enzymatic activation of H_2. It remains an open question as to whether all [FeFe]-hydrogenase operate by a similar mechanism or employ the same set of intermediates. Mössbauer spectroscopy, in collaboration with Professor Yisong Guo at Carnegie Mellon University, will be used in combination with EPR and IR spectroscopy on the CrHydA1 enzyme to examine the formation of H-cluster intermediates at poised potentials and compared to properties of other bacterial [FeFe]-hydrogenases.

Selected Publications

1. Mulder D.W., Guo Y., Ratzloff M.W., King P.W. "Identification of a catalytic iron-hydride at the H-cluster of [FeFe]-hydrogenase." *J. Am. Chem. Soc.* **2017**, *139*, 83–86.
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4. Swanson K.D., Ratzloff M.W., Mulder D.W., Artz J.H., Ghose S., Hoffman A., White S., Zadvornyy O.A., Broderick J.B., Bothner B., King P.W., Peters, J.W. "[FeFe]-hydrogenase oxygen inactivation is initiated at the H-cluster 2Fe subcluster." *J. Am. Chem. Soc.* **2015**, *137***:** 1809-1816.
5. Wilker, M.B., Shiopoulos, K., Brown, K.A., Mulder, D.W., King, P.W., and Dukovic, G. "Electron transfer kinetics in CdS Nanorod-[FeFe]-hydrogenase complexes and implications for photochemical H_2 generation." *J. Am. Chem. Soc.* **2014,** *136*:4316.
6. Mulder, D.W., Ratzloff, M.W., Bruschi, M., Greco, C., Koonce, E., Peters, J.W., King, P.W. "Investigations on the role of proton-coupled electron transfer in hydrogen activation by [FeFe]-hydrogenase." *J. Am. Chem. Soc.* **2014**, *136*:15394.
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