Structural, Functional, and Integration Studies of Solar-Driven, Bio-Hybrid, H_2-Producing Systems

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Objectives

The overall goals of the project are to develop an understanding of [FeFe]-hydrogenases (H_2ase) as model hydrogen (H_2) activation catalysts, and to elucidate the parameters that control hydrogen production by H_2ases in photoelectrochemical cells and molecular photocatalytic complexes. The research objectives include: (i) developing theoretical models of H_2ase to understand electron/proton-transfer (ET and PT) and catalysis; (ii) characterizing redox-induced changes in H_2ase catalytic site structures using mutagenesis and infra-red (FTIR) spectroscopy; (iii) optical and electronic studies of H_2ase-carbon nanotube and nanoparticle complexes; and (iv) electrochemical characterization of native and structurally minimized H_2ases on Au-SAM electrodes using electrochemical STM. These efforts will provide fundamental knowledge how H_2, and how to functionally integrate them as components of artificial hydrogen production schemes.

Technical Barriers

Developing efficient solar hydrogen production requires robust and efficient catalysts, and the understanding of how to integrate them with photoactive materials into efficient artificial systems. The H_2ases possess many characteristics of an ideal catalyst, and elucidation their basic structure-function properties can help to guide efforts to develop synthetic catalysts. A fundamental challenge is to resolve how the metallo-clusters, essential ligands and the surrounding peptide function together to achieve an overall efficient catalytic process. Another is how to control molecular interactions between H_2ases and photoactive molecules to achieve self-assembly into bio-hybrid photocatalytic complexes for hydrogen production.

Abstract

We seek to advance the understanding of [FeFe]-hydrogenases (H_2ase) structure-function, and the parameters that control assembly, charge-transfer and catalytic efficiencies of H_2ases as model catalysts in bio-hybrid, solar H_2-production systems. The knowledge that is gained will help to elucidate the biochemistry of H_2 activation, and support design and development of more efficient solar-to-H_2 conversion by artificial photosynthetic schemes. H_2ases are expressed using a recombinant expression system, and purified enzymes are characterized using a combination of biochemical, electrochemical (electrochemical Scanning Tunneling Microscopy, STM) and spectroscopic (FTIR) techniques. The assembly and characterization of purified H_2ases in bio-hybrids is being studied using solution-phase, photoactive nanoparticles, and as an electrocatalyst in photoelectrochemical cells. Together with the experimental studies, theoretical models are being developed to investigate the H_2ase catalytic site, electron-transfer [FeS]-clusters, substrate transfer pathways, and bio-hybrid complexes. The latest progress in these respective research areas will be summarized.

Progress Report

(i) Computational chemistry studies of H-cluster models with perturbed diatomic ligands have revealed a potential role of the bridging ligand in buffering charge upon enzyme reduction during catalysis.

(ii) A quantum chemical H_2ase model encompassing the H-cluster, accessory [4Fe-4S]-clusters and surrounding protein was constructed. Custom computational methods allow for breaking the spin symmetry within accessory [4Fe-4S]-clusters, and integration of the initial gas-phase calculation into a QM/MM model of the complete H_2ase. The free energies along PT pathways were investigated using QM/MM and umbrella sampling techniques. Several important residues were identified and pK_a values estimated by thermodynamics integration method.

(iii) Brownian dynamics and molecular dynamics simulation techniques predicted the binding structures between H_2ase and SWNTs or bulk carbon surfaces, and ET rates. ET appears to be at least a 100-fold faster for SWNTs than for a bulk carbon surface, and independent of SWNT diameter.

(iv) The native H_2ase has been successfully adsorbed to Au electrodes bearing self-assembled thiol-based monolayers (SAMs) and retains activity. Binding is via interactions between positively charged patches on the enzyme and carboxylate groups on the SAM.

Single-molecule images have been obtained in an electrochemical STM and suggest tunneling currents increase under an applied bias. Removal of adsorbed H_2ase from the STM surface helped confirm that the features observed with the STM are H_2ase.

(v) Electrostatically guided assemblies of H_2ase with mercaptopropionic acid capped CdTe nanocrystals (NC) and CdS nanorods have been successfully formed in solution. Both types of complexes were isolated by gel electrophoresis and showed evidence of compositional heterogeneity. Photoluminescence and hydrogen evolution studies suggest that competition of electron transfer with internal relaxation pathways is favored for 1:1 molecular ratios. We have preliminary Transient Absorption kinetics for H_2ase:CdS complexes that shows the estimated electron-transfer step from CdS to H_2ase occurs at a timescale of ~10^2 nsec, whereas internal relaxation of CdS occurs at ~10 nsec.

Future Directions

- Tunneling currents will be examined between accessory [4Fe-4S]-clusters and the H-cluster in the H_2ase model. Dynamics trajectories will also be analyzed to determine protein conformational features correlating with large couplings, as approximated by empirical pathways analysis. The free energies along the PT pathways will be further refined based on the *pKa* calculation results.
- We will continue to investigate the binding free energies for H_2ase with SWNTs and bulk carbon surfaces using MD simulation techniques, and develop these models to predict binding modes, orientations and ET processes.
- We will continue to characterize redox-induced changes in catalytic site structures of H_2ases using FTIR spectroscopy to define ligand conformations of oxidized and reduced states, and use mutagenesis to characterize the role of the peptide environment.
- Single-molecule electrochemistry of immobilized H_2ase on Au-electrodes will continue to be developed to characterize the binding interaction, tunneling currents and the operating potentials of the enzyme. Mutated H_2ases lacking individual accessory iron-sulfur clusters functioning in electron-transfer will be studied in order to learn more about the conductive path through the protein.
- We will develop and apply FTIR spectroscopy to characterize the binding and charge-transfer reactions between H_2ases and SWNTs both in solution and in films, to identify how the SWNT intrinsic electronic properties affect redox active modes of H_2ase.
- Characterize redox-induced changes in H_2ase-NC complexes under photoexcitation using steady-state and time-resolved optical and FTIR techniques.

Publication list (including patents) acknowledging the DOE grant or contract

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