

Regulation of H₂ and CO₂ Metabolism: Factors Involved in Partitioning of Photosynthetic Reductant in Green Algae

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Objectives

The overall objective of this project is to develop fundamental understanding about the regulation of the partitioning of photosynthetic reductant between the H₂-production and the CO₂-fixation pathways. Specific objectives are to: (a) identify protein factors whose expression may be required for optimal hydrogenase expression; (b) identify active promoter regions and transcriptional elements for the two algal hydrogenases; and (c) determine whether the recently identified FIXL homologs in *C. reinhardtii* play a role in O₂-sensing mechanism and mediate components of the anoxic regulatory response that leads to hydrogenase expression.

Abstract

Photobiological H₂ production from water is a clean, non-polluting and renewable technology that could play a significant role in a future hydrogen economy. The potential light conversion efficiency to H₂ by biological organisms is theoretically about 10%. One of the limitations to meeting this efficiency is the low availability of reductant to the hydrogenase, due to the existence of competing metabolic pathways, such as CO₂ fixation. This project aims at understanding how photosynthetic reductant is partitioned between different metabolic pathways at the level of ferredoxin. In particular, the project continues to investigate the mechanism that underlies the anoxic regulation of hydrogenase in *C. reinhardtii* at the transcriptional and post-transcriptional levels. These goals are being pursued by (a) using high-quality sensors developed by Dr. Mathew Posewitz at Colorado School of Mines to identify and characterize candidate mutants displaying altered hydrogen production from insertional libraries; (b) fusing the truncated promoters of HYDA1 and HYDA2 to the SNAP reporter gene lacking its own promoter, to identify the DNA region required for anaerobic gene expression; (c) investigating the O₂ and CO-binding affinities of the truncated FXL1

and FXL5 homologues from *C. reinhardtii* expressed in *E. coli* and further studying their role in hydrogenase signal transduction; (c) probing the physiological and metabolic differences between structurally different algal [FeFe]-hydrogenases.

Progress Report

- To define the role of individual hydrogenases in green algae, we screened a *Chlamydomonas hyda2* mutant background (generated under DOE's BER funding) for mutants lacking all hydrogenase activity. A double *hyda2hyda1* mutant has been identified; it is being further characterized and is being used as the background strain for expression of recombinant [FeFe]-hydrogenases in *Chlamydomonas* by DOE's FCT Program.
- To identify active promoter regions for the two algal hydrogenases, we fused the truncated promoters of HYDA1 and HYDA2 to the SNAP reporter gene. Transformants expressing the SNAP protein with truncated promoters were shown to be transcribed only under anaerobic condition. The results indicate that region between position -144 and -1 for HYDA1 and -149 to -1 for HYDA2 with respect to the transcription start site is required for anaerobic specific gene expression.
- To determine whether the recently identified FIXL homologs in *C. reinhardtii* play a role in O₂-sensing mechanism and mediate components of the anoxic regulatory response that leads to hydrogenase expression, we cloned the PAS domains of FXL1 and FXL5 from *C. reinhardtii*, overexpressed them in *Escherichia coli* and purified the ~13 kDa proteins. The recombinant FXL1 and FXL5 domain polypeptides both stained positively for heme, while ΔFXL1 and ΔFXL5, mutated in the conserved, ligand binding histidine residue resulted in loss of heme binding. Met-FXL1 and Met-FXL5 had their Soret absorption maximum around 415 nm followed by several weaker absorptions at longer wavelengths, 530/560 nm, which are characteristic of protein-bound heme, and are very similar to absorption spectra of *Rhizobium* FixL. Ligand binding measurements in varying partial pressures of O₂ and CO showed that FXL1 and FXL5 both bind O₂ and CO with high affinity. This indicates that the *Chlamydomonas* FXL proteins may use an O₂-sensing mechanism analogous to that reported in nitrogen-fixing bacteria, and thereby mediate components of the anoxic regulatory responses observed in this metabolically versatile alga. The present study unequivocally indicates the discovery of putative FXL-like proteins

in *Chlamydomonas reinhardtii* and demonstrates their heme and ligand binding properties.

- To probe the physiological and metabolic differences between two structurally different algal [FeFe]-hydrogenases, we assessed H₂ production in the trebouxiophyte *Chlorella* NC64A, which is the first alga known to encode both the H and F-clusters of hydrogenases. We show for the first time that F-cluster-containing hydrogenases are coupled to both anoxic photosynthetic electron transport and dark fermentation in a green alga. Hydrogen photoproduction in *Chlorella* NC64A is as sensitive to O₂ inactivation as in *C. reinhardtii*. Phylogenetic analysis indicates that all known algal HYDA enzymes are monophyletic, suggesting that they emerged once within the algae. Furthermore, phylogenetic reconstruction indicates that the multiple *HYDA* copies in the algal taxa are the result of gene duplication events that occurred independently in each algal lineage, and that the ancestor of the Trebouxiophyceae and Chlorophyceae likely encoded a single, H and F-cluster-containing HYDA.

Future Directions

- Candidate transcription factor proteins for hydrogenase will be identified, over-expressed in *E. coli* and purified. We will perform direct interaction of transcription factor-hydrogenase promoter DNA binding assays. To understand the involvement of other downstream proteins with transcription factor, the latter will be tagged (streptavidin or histidine tag) and used to search for interacting proteins.
- Selected *C. reinhardtii* mutants showing aberrant H₂ production will be further analyzed with respect to HYDA1 and HYDA2 transcript and protein levels. Complementation of the the phenotype with the transformed gene will be performed and physiological studies will be carried out to determine the role of the disrupted gene in algal metabolism.
- To understand the involvement (if any) of FIXL proteins in signal transduction in response to O₂, promising FIXL candidates will be tagged (streptavidin or histidine tag) and over-expressed in *C. reinhardtii*. Tagged FIXL proteins will be used to search for interacting proteins involved in the signal transduction pathway.
- To understand the mechanism that underlies the anoxic regulation of hydrogenase, we will express SNAP proteins under the regulation of the HYDA1 and HYDA2 promoters into *hydA1/hydA2* single or double mutants. Additionally, we will perform DNA binding assays to identify the transcription factors binding to the promoter regions of each hydrogenase.

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