NREL Highlights SCIENCE

Enzyme Fusions Optimize Photosynthetic Hydrogen Production in Algae

Research at NREL is demonstrating that engineering enzymes has the potential to improve efficiencies.

Photosynthesis uses the energy of sunlight to split water and capture CO_2 and, as a result, provides the biomass required for growth. In the absence of CO_2 and O_2 the photosynthetic circuit in green algae switches to split water and produce H_2 (summarized in Figure 1, top). Electrons from water-splitting are transferred to either CO_2 fixation or H_2 production by a series of carriers (e.g., ferredoxin) that form complete, parallel circuits. A central challenge to engineering photosynthetic organisms to produce more H_2 has been to find ways to divert most or all of the light-derived electrical potential from CO_2 fixation and other competing reactions to H_2 production. This is being addressed by identifying the factors that regulate competition, studying the protein interactions that compose electron transfer circuits, and engineering proteins to change the composition and divert more electrons to H_2. We have shown, *in vitro,* that under anaerobic conditions that support H_2 production, changing the normal H_2-producing enzyme, hydrogenase (H_2ase), into a Fd-H_2ase fusion protein alters the normal photosynthetic electron transfer circuit to produce more H_2 in the presence of the CO_2 fixation enzyme ferredoxin:NADP-oxidoreductase (FNR) (Figure 2, bottom). A model of the new fusion circuits are shown in boxes 1 and 2 in Figure 2; the reduced level of FNR activity is modeled as a third circuit in box 3. These new results suggest an engineering strategy to improve H_2 production *in vivo,* and a means to help resolve the fundamental mechanisms that regulate photosynthetic electron transport among competing pathways.

This research was a collaboration with a research team at the Massachusetts Institute of Technology (Iftach Yacoby and Shuguang Zhang).

Technical Contact: Paul King, *paul.king@nrel.gov*

Key Results

Achievement

NREL research demonstrated a process that improved the efficiency of hydrogen production by altering the normal photosynthetic electron transfer circuit.

Key Result

Under anaerobic conditions that support H_2 production, changing the normal H_2-producing enzyme, hydrogenase (H_2ase), into a Fd-H_2ase fusion protein produced more H_2 in the presence of the CO_2 fixation enzyme ferredoxin:NADP-oxidoreductase (FNR).

Potential Impact

The results suggest an approach to improve H_2 production *in vivo,* and a means to help resolve mechanisms that regulate photosynthetic electron transport among competing pathways.

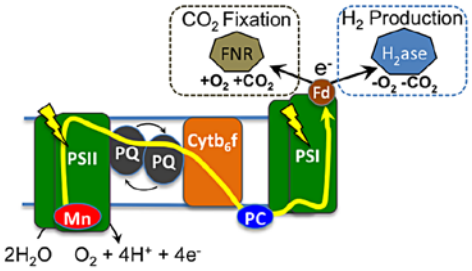

Figure 1. (top) Photosynthetic electron transport pathways that support CO_2 fixation and H_2 production. Light-activated PSII extracts electrons from water and transfers them to plastoquinone (PQ), Cytochrome b_6f, ($Cytb_6f$), plastocyanin (PC), and finally to PSI. A second light-activation of PSI moves electrons to Fd. Parallel circuits couple Fd to either FNR for CO_2 fixation, or H_2ase for H_2 production. During aerobic growth, H_2 production is absent due to: (i) oxygen formation by PSII, and (ii) CO fixation mediated by FNR. H_2 production occurs only in the absence of both O_2 and CO_2.

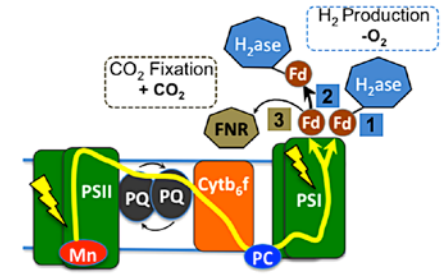

Figure 2. (bottom) Engineering the H_2-producing enzyme to an Fd-H_2ase fusion changes the H_2 production circuit to include a direct (box 1), along with an indirect (box 2) production mode. The CO_2 fixation circuit (box 3) remains open, but at a reduced level. These new electron-transfer modes that utilize Fd-H_2ase allow for the H_2 production reaction to compete with CO_2 fixation, improving the overall H_2 production efficiency.

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NATIONAL RENEWABLE ENERGY LABORATORY

15013 Denver West Parkway | Golden, CO 80401 | 303-275-3000 | www.nrel.gov

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