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 CO₂ and, as a result, provides the biomass required for growth. In the absence of CO₂ and O₂ the photosynthetic circuit in green algae switches to split water and produce H₂ (summarized in Figure 1, top). Electrons from water-splitting are transferred to either CO₂ fixation or H₂ production by a series of carriers (e.g., ferredoxin) that form complete, parallel circuits. A central challenge to engineering photosynthetic organisms to produce more H₂ has been to find ways to divert most or all of the light-derived electrical potential from CO₂ fixation and other competing reactions to H₂ 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 H₂. We have shown, in vitro, that under anaerobic conditions that support H₂ production, changing the normal H₂-producing enzyme, hydrogenase (H₂ase), into a Fd-H₂ase fusion protein alters the normal photosynthetic electron transfer circuit to produce more H₂ in the presence of the CO₂ 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 H₂ 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

Figure 1. (top) Photosynthetic electron transport pathways that support CO₂ fixation and H₂ production. Light-activated PSII extracts electrons from water and transfers them to plastoquinone (PQ), cytochrome b₆f (Cytb₆f), plastocyanin (PC), and finally to PSI. A second light-activation of PSI moves electrons to Fd. Parallel circuits couple Fd to either FNR for CO₂ fixation, or H₂ase for H₂ production. During aerobic growth, H₂ production is absent due to: (i) oxygen formation by PSI, and (ii) CO₂ fixation mediated by FNR. H₂ production occurs only in the absence of both O₂ and CO₂.

Figure 2. (bottom) Engineering the H₂-producing enzyme to an Fd-H₂ase fusion changes the H₂ production circuit to include a direct (box 1), along with an indirect (box 2) production mode. The CO₂ fixation circuit (box 3) remains open, but at a reduced level. These new electron-transfer modes that utilize Fd-H₂ase allow for the H₂ production reaction to compete with CO₂ fixation, improving the overall H₂ production efficiency.

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 H₂ production, changing the normal H₂-producing enzyme, hydrogenase (H₂ase), into a Fd-H₂ase fusion protein produced more H₂ in the presence of the CO₂ fixation enzyme ferredoxin:NADP-oxidoreductase (FNR).

Potential Impact
The results suggest an approach to improve H₂ production in vivo, and a means to help resolve mechanisms that regulate photosynthetic electron transport among competing pathways.