II.H.3 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Contract Number: DE-FG36-05GO15041

Start Date: January 1, 2005
Projected End Date: December 31, 2008

Objectives

- Minimize, or truncate, the chlorophyll (Chl) antenna size in green algae to maximize photobiological solar conversion efficiency and H_2-production.
- Demonstrate that a truncated Chl antenna size would minimize absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity by the green alga mass culture.

Technical Barriers

(AG) Light Utilization Efficiency

Technical Targets

- The Hydrogen, Fuel Cells and Infrastructure Technologies Multiyear Program Plan technical target for 2005 for this project was to reach a 10% utilization efficiency of absorbed light energy (out of a theoretical maximum of 30% possible) in unicellular green. Progress through this project has currently achieved a green alga utilization efficiency of absorbed light energy of about 15% (please see Table 1).

TABLE 1. Progress Achieved *vs* the DOE targets: Utilization Efficiency of Incident Solar Light Energy, E_0xE_1, %

Year	2000	2003	2007	2010	2015
Program Targets	3%	10%*		15%	20%
Actual Progress Achieved	3%	10%	15%		

* Target adjusted upward to match ahead-of-schedule progress achieved.

Approach

- Employ DNA insertional mutagenesis, screening, biochemical and molecular genetic analyses for the isolation of "truncated Chl antenna size" mutants in the green alga *Chlamydomonas reinhardtii.*
- Clone and characterize the gene(s) that affect the "Chl antenna size" property in *Chlamydomonas reinhardtii*.
- Apply such genes to generate a "truncated Chl antenna size" in this and other green algae.

Accomplishment

1. Completed the molecular and genetic analysis of the *tla1* mutant.
2. Completed the functional analysis of the *Tla1* gene.
3. Published manuscript on the utility of the *Tla1* gene in conferring a truncated chlorophyll antenna size and on the mechanism by which it maximizes light utilization efficiency and hydrogen production in microalgal cultures.
4. Isolated a new "truncated chlorophyll antenna size" mutant (*tlaX)* with a total of 195 Chl molecules in its photosynthetic apparatus (42% of the wild type). The specific photosystem Chl antenna size measurements of the *tlaX* strain were PSII=80 and PSI=115 Chl molecules.
5. An ~15% utilization efficiency of absorbed light energy was achieved (see Table 1).

Introduction

The goal of the research is to generate green algal strains with enhanced photosynthetic productivity and H_2-production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells [1,3,5]. A cost-effective way to achieve this goal is to

reduce the number of Chl molecules that function in the photosystems of photosynthesis. Thus, efforts are under way to isolate microalga mutants with a truncated chlorophyll antenna size.

The rationale for this R&D is that a truncated light-harvesting Chl antenna size in green algae will prevent individual cells at the surface of the culture from over-absorbing sunlight and wastefully dissipating most of it (Figure 1). A truncated Chl antenna size will permit sunlight to penetrate deeper into the culture, thus enabling many more cells to contribute to useful photosynthesis and H_2-production (Figure 2). It has been shown that a truncated Chl antenna size will enable about three to four times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells [2].

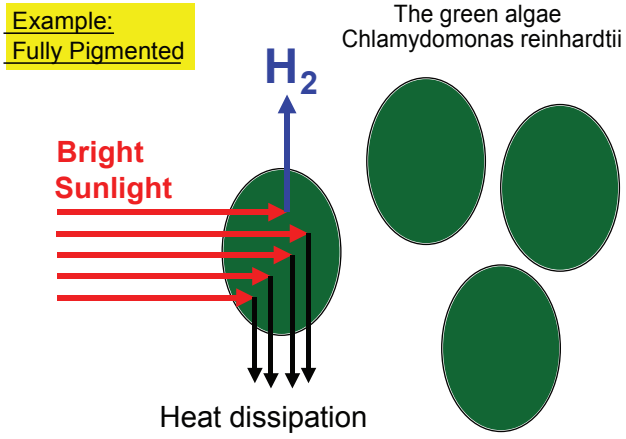

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability of absorption by the first layer of cells would cause shading of cells deeper in the culture.

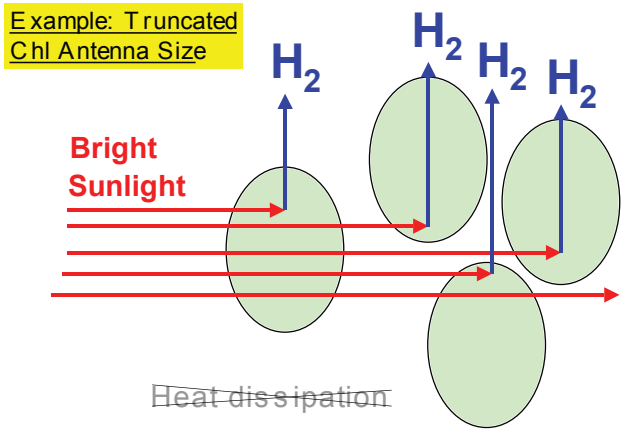

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2-production by cells deeper in the culture.

Approach

The immediate objective of the research is to identify genes that control the Chl antenna size of photosynthesis and, further, to manipulate such genes so as to confer a truncated Chl antenna size in the model green alga *Chlamydomonas reinhardtii.* Identification of such genes in Chlamydomonas will permit a subsequent transfer of this property, i.e., "truncated Chl antenna size", to other microalgae of interest to the DOE Hydrogen, Fuel Cells and Infrastructure Technologies Program. This objective is currently being approached through deoxyribonucleic acid (DNA) insertional mutagenesis/screening and biochemical/molecular/genetic analyses of *Chlamydomonas reinhardtii* cells.

Results

The *Chlamydomonas reinhardtii* mutant *tla1* (truncated light-harvesting chlorophyll antenna size) was earlier generated upon DNA insertional mutagenesis and shown to specifically possess a smaller than wild type chlorophyll antenna size in both photosystems. This strain exhibited a 10% utilization efficiency of incident solar light energy, i.e., substantially greater than the 3% utilization efficiency of the wild type. Molecular and genetic analysis revealed that the exogenous plasmid DNA was inserted at the end of the 5' untranslated region (UTR) and just prior to the Adenine-Thymine-Guanine (ATG) start codon of a hitherto unknown gene (termed *Tla1)*, which encodes a protein of 213 amino acids. The *Tla1* gene in the mutant is transcribed with a new 5' UTR sequence, derived from the 3' end of the transforming plasmid. This replacement of the 5' UTR resulted in enhanced transcription of the *tla1* gene in the mutant but inhibition in the translation of the respective *tla1* mRNA. These results provided evidence that down-regulation of the Tla1 expression is necessary and sufficient to truncate the chlorophyll antenna size and to improve solar utilization efficiencies in a green algal mass culture. Specific applications of the *Tla1* gene in H_2-production were discussed.

Work further described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tlaX,* having a **t**runcated **l**ight-harvesting chlorophyll **a**ntenna size. This mutant has the smallest yet Chl antenna size known in green algae. This was achieved upon generating and screening an additional 4,500 transformants for this purpose, following the protocol of Polle et al. (2003). Properties of the *tlaX* putative "truncated Chl antenna size" strain are summarized in Table 2.

TABLE 2. *Chlamydomonas reinhardtii* cellular chlorophyll content, photosystem chlorophyll antenna size and energy utilization efficiency in wild type, *tla1* and *tlaX* mutant strains, as determined by spectrophotometric kinetic analysis (n = 5, ±SD).

	Wild Type	*tla1*	*tlaX*	Long-Term Goal
Chl/cell mol x10^{-15}	2.4 ±0.5	0.9 ±0.06	0.93 ±0.1	
Chl-PSII	222±26	115±36	80±30	37
Chl-PSI	240±4	160±12	115±10	95
Light Utilization Efficiency	~3%	~10%	~15%	~30%

Conclusions

- Significant, ahead-of-schedule progress was achieved in terms of acquiring "truncated Chl antenna size" mutants. *This demonstrates feasibility of the approach chosen and success of the methods employed.*
- A truncated light-harvesting chlorophyll antenna size in the *tla-type* mutants leads to enhanced solar conversion efficiencies and greater photosynthetic productivity of the algae under bright sunlight conditions.
- Insights on the molecular mechanism for the regulation of the Chl antenna size by the *Tla1* gene were obtained (results published by Tetali et al. 2007, Planta 225: 813-829).

Future Directions

- Advance the biochemical and molecular characterization of the *tlaX* strain.
- Establish transformation (sense and antisense) protocols with *Tla*-type genes to enhance the down-regulation of the Chl antenna size in *Chlamydomonas reinhardtii.*
- Perform comparative green-alga light utilization efficiency and photosynthetic productivity measurements under mass culture conditions in wild type and *tla*-type mutants.
- Isolate additional *tla*-type mutants.
- Perform genetic crosses to combine different *tla*-type properties.

References

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2. Melis A, Chen H-C (2007) Modulation of sulfate permease for photosynthetic hydrogen production. United States Patent 7,176,005 (issued 13-Feb-2007).

3. Tetali SD, Mitra M, Melis A (2007) Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *Tla1* gene. Planta 225: 813-829.

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