IV.E Biological Production

IV.E.1 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Objectives

- Minimize, or truncate, the chlorophyll antenna size in green algae to maximize photobiological solar conversion efficiency and H_2 production.
- Demonstrate that a truncated Chl antenna size minimizes 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

This project addresses the following technical barriers from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

- X. Light Utilization Efficiency

Technical Targets

The Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan technical target for 2010 for this work was to reach a 15% utilization efficiency of absorbed light energy (out of a theoretical maximum of 30% possible) in unicellular green algae. Progress through this project has currently achieved a green alga utilization efficiency of absorbed light energy of about 15%.

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 green alga.

Accomplishments

- A new "truncated light-harvesting chlorophyll antenna size" mutant (*tlaX)* with a total of 195 Chl molecules in its photosynthetic apparatus (42% of the wild type) was isolated. The specific photosystem Chl antenna size measurements of the *tlaX* strain were PSII=80 and PSI=115 Chl molecules.
- A photon use efficiency of ~30% was measured for the *tlaX* photosynthesis. This translated into a ~15% utilization efficiency of absorbed light energy. Such achievement surpassed this year's 10% energy utilization efficiency target.
- Biophysical and biochemical analyses of the *tlaX* mutant were conducted. Chlorophyll antenna size, relative productivity, and light-harvesting Chl expression levels were measured.
- Molecular analyses of an earlier-isolated "truncated light-harvesting Chl antenna size" mutant, termed *tla1*, were performed. The DNA insertion site in the *tla1* mutant was mapped. Genomic, cDNA and protein sequences for the *Tla1* gene were obtained. A complementation analysis of the *tla1* mutant with the *Tla1* gene was conducted. Analysis of the complemented strains is in progress.
- Biochemical analyses of the *tla1* mutation were conducted. Antibodies against the Tla1 protein are currently being raised. The hydropathy plot of the Tla1 protein was measured. Sequence homologies for the Tla1 protein were completed.
- Functional analyses of the *Tla1* gene were conducted. Regulation of the chlorophyll antenna size by the *Tla1* gene was addressed, and a tentative model for the function of the *Tla1* gene was developed.

Future Directions

- Advance the biochemical and molecular characterization of the *tlaX* strain. Publish *tla1*- and *tlaX*-related analyses.
- Functionally characterize the corresponding *Tla1* and *TlaX* genes (how do they work?).
- 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.

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 (Kok 1953; Myers 1957; Radmer and Kok 1977). A cost-effective way to achieve this goal is to reduce the number of chlorophyll (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

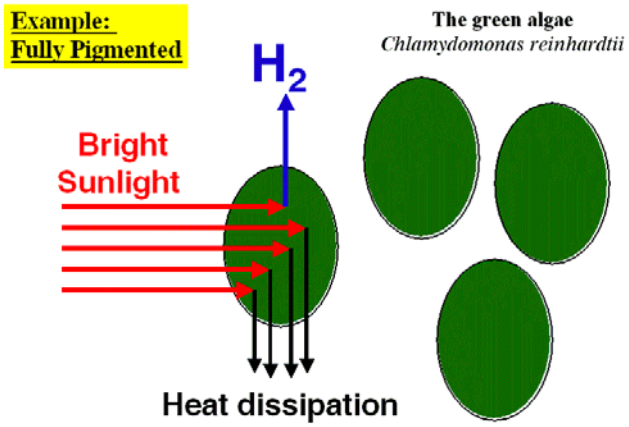

Figure 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis) and 'heat dissipate' most of it. Note that a high probability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

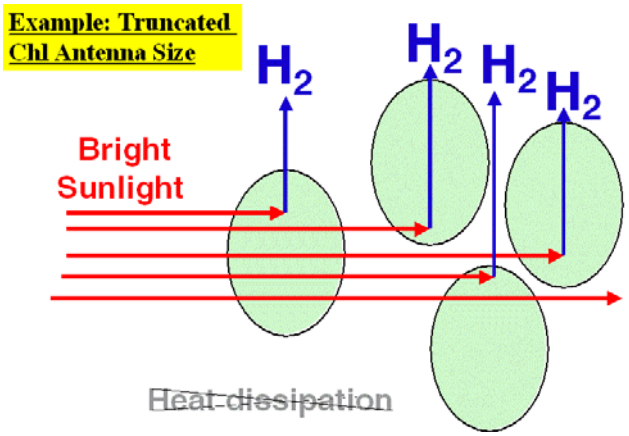

Figure 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2 production by cells deeper in the culture.

truncated Chl antenna size will enable about 3-4 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells (Melis et al. 1999).

Approach

An 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 Program. This objective is currently being approached through DNA insertional mutagenesis/screening and biochemical/molecular/genetic analyses of *Chlamydomonas reinhardtii* cells.

Results

Work described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*. This mutant has the smallest yet Chl antenna size known in green algae. This was achieved through 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 1.

Table 1. *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
Energy Utilization Efficiency	~5%	~10%	~15%	~30%

Work also focused on the molecular characterization of the *tla1* mutant, isolated during earlier research, and the cloning of the respective *Tla1* gene. Figure 3 shows the gene structure of the *tla1* mutant and wild type *C. reinhardtii* in the pJD67 plasmid insertion locus. Genomic and cDNA analysis of the *Tla1* gene (GenBank Accession Nos. AF534570 and AF534571) revealed the following wild type gene structure: 104 base pairs (bp) of 5'

Figure 3. Gene structure of the *tla1* mutant and wild type *C. reinhardtii* in the pJD67 plasmid insertion locus.

Figure 4. Alignment of Tla1-like proteins from different organisms. The alignment of the Tla1 deduced amino acid sequence of *C. reinhardtii* is compared to that of similar proteins of unknown function from *A. thaliana*, *Z. mays* and *H. sapiens* CGI 112 protein, and *D. melanogaster*. The alignment was done on the basis of a ClustalW analysis. Four protein domains with high sequence conservation can be deduced from this comparison.

untranslated region (UTR), a total coding region of 642 bp (codon 1 with 198 bp and codon 2 with 444 bp), a single intron of 108 bases and 1.26 kb of 3' UTR, encoding a protein of 213 amino acids. In the *tla1* mutant, the Adenine,Thymine,Guanine (ATG) start codon of the *Tla1* Opening Reading Frame (ORF) occurred immediately downstream of the 3' end of the plasmid insert. As a result of the plasmid insertion and genomic DNA deletion in the *tla1* mutant, the 3' end of the pJD67 DNA replaced the entire 5' UTR of the *Tla1* gene. Figure 4 shows the alignment of Tla1-like proteins from different organisms. The alignment of the Tla1 deduced amino acid sequence of *C. reinhardtii* is compared to that of similar proteins of as yet unknown function from *Arabidopsis thaliana*, *Zea mays* (maize) and *Homo sapiens* (human) CGI 112 protein, and *Drosophila melanogaster* (the fruit fly). Four protein domains with high sequence conservation can be deduced from this comparison, shown by the yellow-highlighted domains. The alignment was done on the basis of a ClustalW analysis in which asterisks **(*)** indicate identity, full colons **":"** indicate high similarity, and periods **"."** indicate low similarity.

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 not shown pending publication of these findings in a peer-reviewed journal).

FY 2005 Publications/Presentations

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2. Komine Y and Melis A (2004) *Chlamydomonas reinhardtii* thioredoxin-like protein mRNA, complete cds; nuclear gene. GenBank Accession Number AY762116.
3. Melis A, Seibert M and Happe T (2004) Genomics of green algal hydrogen research. Photosynth. Res. 82: 277-288.
4. Melis A (2005) Bioengineering of green algae to enhance photosynthesis and hydrogen production. Chapter 12 in *Artificial Photosynthesis: From Basic Biology to Industrial Application,* AF Collins and C Critchley (eds.), Wiley-Verlag & Co., In Press.
5. White A and Melis A (2004) Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures. Abstracts of the 13th Western Photosynthesis Conference, Asilomar Conference Center, Pacific Grove, CA. January 8-11, p. 33.

6. Melis A, Polle J and Kanakagiri S (2004) Genes for the regulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii*. Plant Biology 2004, Abstract #219 in the American Society of Plant Biologists 2004 Annual Meeting Program Book. p. 80.
7. Melis A, Polle JE and Kanakagiri S (2004) Genes for the regulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii.* Abstract #259 of the 13th International Congress of Photosynthesis. pp. 112-113.

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3. Myers J (1957) Algal culture. In: Kirk RE, Othmer DE (eds), Encyclopedia of chemical technology. Interscience, New York, NY, pp 649-668
4. Polle JEW, Kanakagiri S and Melis A (2003) *tla1,* a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. Planta 217: 49-59
5. Radmer R and Kok B (1977) Photosynthesis: Limited yields, unlimited dreams. Bioscience 29: 599-605