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MAXIMIZING PHOTOSYNTHETIC PRODUCTIVITY AND SOLAR CONVERSION EFFICIENCY IN MICROALGAE BY MINIMIZING THE LIGHT-HARVESTING CHLOROPHYLL ANTENNA SIZE OF THE PHOTOSYSTEMS

Anastasios Melis*, John Neidhardt and John R. Benemann
Department of Plant and Microbial Biology, 411 Koshland Hall, University of California, Berkeley, CA 94720-3102

Abstract

The solar conversion efficiency and productivity of photosynthesis in light-acclimated *Dunaliella salina* (green algae) were analyzed. Cells were grown under continuous low-light (LL; 100 μmol photons m^{-2} s^{-1}) or high-light (HL; 2,000 μmol photons m^{-2} s^{-1}) conditions. HL-grown cells exhibited signs of chronic photo-inhibition, i.e., a lower pigment content, a highly truncated chlorophyll (Chl) antenna size for the photosystems, and accumulation of photodamaged photosystem-II (PSII) reaction centers in the chloroplast thylakoids. In spite of these deficiencies, high-light-grown cells showed photosynthetic productivity (300 mmol O_2 (mol Chl)$^{-1}$ s^{-1}) that was ~3 times greater than that of the normally pigmented LL-grown cells (~100 mmol O_2 (mol Chl)$^{-1}$ s^{-1}). Recovery from photoinhibition in the HL-grown cells, induced in the absence of a light-harvesting Chl antenna size enlargement, increased photosynthetic productivity further to ~650 mmol O_2 (mol Chl)$^{-1}$ s^{-1}. It is shown that, under moderate to high light conditions, *D. salina* with a highly truncated Chl antenna size will display superior photosynthetic productivity, solar conversion efficiency and H_2 production when compared to the normally pigmented control cells. Estimates of H_2 production in mass culture suggest an average of 220 L H_2 m^{-2} d^{-1} for the cells with the truncated Chl antenna, and less than 50 L H_2 m^{-2} d^{-1} for the normally pigmented cells.

Introduction

Microalgal cultures growing under full sunlight are believed to have lower solar-to-biomass energy conversion efficiencies than when growing under low light intensities. The reason for this inefficiency is that, at moderate to high photon flux densities, the rate of photon absorption by the antenna chlorophylls far exceeds the maximal rate of photosynthesis, resulting in dissipation of the excess photon energy as fluorescence or heat. More than 80% of absorbed photons could thus be wasted, reducing solar conversion efficiencies by these cells and culture productivity to unacceptably low levels. Under bright incident sunlight, fully pigmented cells are also subject to photoinhibition [Powles 1984, Melis 1991, Baroli and Melis 1998], further lowering solar conversion efficiencies and photosynthetic productivity. To make the situation worse, cells deeper in the algal culture are deprived of much needed sun-light as this is strongly attenuated due to filtering by the first layer of cells in the culture container [Naus and Melis 1991, Neidhardt et al. 1998].

Variation in the level of irradiance during plant growth brings about reversible structural and functional adjustments in their photosynthetic apparatus [Anderson 1986, Melis 1991]. It has been demonstrated that the Chl antenna size of green algae such as *Chlorella vulgaris* [Ley and Mauzerall 1982], *Dunaliella tertiolecta* [Sukenik et al. 1988], *Dunaliella salina* [Smith et al. 1990] and *Chlamydomonas reinhardtii* [Neale and Melis 1986, Melis et al. 1996] changes in response to the level of irradiance during cell growth. In general, LL promotes larger Chl antenna size for the photosystems (up to 500 Chl molecules for photosystem-II). HL elicits a smaller Chl antenna size (as low as 60 Chl molecules for this photosystem) [Smith et al. 1990, Melis 1996].

Exposure of plants and algae to high irradiance may cause photoinhibition of photosynthesis [Powles 1984, Barber and Andersson 1992]. When grown under continuous illumination of high intensity (HL=2,000 μmol photons m^{-2} s^{-1}) in the presence of $NaHCO_3$ as the sole carbon source, *D. salina* chloroplasts assemble ~7% of the PSI complexes and ~65% of the PSII complexes compared to low-light (LL=100 μmol photons m^{-2} s^{-1}) controls. However, of the PSII present in the thylakoid of HL-grown cells, only about 20-25% were photochemically competent, the rest occurring as photodamaged centers containing an irreversibly inactive PSII reaction center (D1) protein [Smith et al. 1990, Vasilikiotis and Melis 1994]. Thus, in HL-acclimated, $NaHCO_3$-grown *D. salina*, photosynthesis and growth depend solely on ~7% of the PSI and ~15% of the PSII centers that are operational in LL-grown cells.

Theoretically, a truncated chlorophyll (Chl) antenna size of the photosystems (PS) in the chloroplast of microalgae could alleviate these difficulties because it will minimize absorbance of bright incident sunlight and wasteful dissipation of excitation energy, prevent photoinhibition, diminish mutual cell shading, permit for greater transmittance of light through the culture and, thus, result in a more uniform illumination of the cells. Overall, this would result in greater solar conversion efficiencies and photosynthetic productivity in mass microalgal cultures.

It is predicted that, under bright light intensities, a smaller Chl antenna size will result in a relatively higher light intensity for the saturation of photosynthesis in individual cells but, concomitantly, in a much greater solar conversion efficiency and cellular productivity on a per Chl basis. These theoretical considerations may appear to be a paradox and their validity has not yet been tested in the laboratory. This manuscript presents the results of a feasibility study and experimental demonstration of these concepts. The work in this paper builds upon recent research in this laboratory [Smith et al. 1990, Kim et al. 1993, Neidhardt et al. 1998, Melis et al. 1998]. It presents a comparative analysis of the photosynthetic productivity and solar conversion efficiency of normally pigmented and Chl antenna deficient *D. salina*. The results support the notion that, in mass culture, cells with a highly truncated Chl antenna size will exhibit superior photosynthetic productivity and solar conversion efficiencies compared to that of normally pigmented control cells.

Materials and Methods

Growth of Dunaliella salina cultures

The unicellular green alga *Dunaliella salina* was grown in a hypersaline medium containing 1.5 M NaCl, 0.2 M Tris-HCl (pH 7.5), 0.1 M KNO_3, 0.1 M $MgSO_4$, 6 mM $CaCl_2$, 2 mM KH_2PO_4, 40 μM $FeCl_3$ dissolved in 400 μM EDTA [Pick et al. 1986]. Bicarbonate was added to the medium as the ever present carbon source to a concentration of 25 mM. Supplemental CO_2 was provided by bubbling the culture with a mixture of 3% CO_2 in air. The medium also contained a mixture of micronutrients in the following concentrations: 150 μM H_3BO_3, 10 μM $MnCl_2$, 2 μM Na_2MoO_4, 2 μM $NaVO_3$, 0.8 μM $ZnCl_2$, 0.3 μM $CuCl_2$, 0.2 μM $CoCl_2$.

Growth media were inoculated with several ml of a stock suspension of *D. salina* cells and were cultivated in flat bottles (about 4 cm thick) at a temperature between 26 and 29°C. The cells grew exponentially in the density range between 0.15-1.5x10^6 cells/ml [Naus and Melis 1991]. Measurements were performed with cultures having a cell density between 0.8-1.3x10^6 cells/ml.

The cultures were grown under either low light (incident irradiance of ~100 μmol photons m^{-2} s^{-1}) or high light conditions (irradiance of ~2,000 μmol photons m^{-2} s^{-1}). The incident irradiance was measured with a LI-COR, Model LI-185B radiometer. Shaking of the cultures along with the use of light reflectors ensured a uniform illumination of the cells.

Cell count and chlorophyll quantitation

The cell density in the cultures was obtained upon counting with a Hemacytometer (improved Neubauer chamber) and by use of an Olympus BH-2 compound microscope at a magnification of x100. For the counting, cells were

immobilized and stained by addition of several µl of Utermoehl oil to 0.25-1 ml aliquot of the culture.

Chlorophyll concentrations were measured upon pigment extraction in 80% acetone after removal of cell debris by centrifugation, and by measuring the absorbance of the solutions at 663 and 645 nm. The amount of chlorophyll was calculated by use of Arnon's equations [1949].

Photosynthesis measurements

Photosynthetic activity of the cells was measured by a Clark-type oxygen electrode. An aliquot of 5 ml cell suspension was applied to the oxygen electrode chamber. In order to compare the relative quantum yield of photosynthesis between the different samples, about the same Chl concentration (2-3 mM) was loaded in the oxygen electrode. To ensure that oxygen evolution was not limited by the carbon source available to the cells, 100 µl of a 0.5 M sodium bicarbonate solution (pH 7.4) was added prior to the oxygen evolution measurements. Samples were illuminated with increasing light intensities under stirring and at a temperature of 25°C. The rate of oxygen evolution under each of these light intensities was recorded continuously for a period of 2.5 min. The results were plotted to show the light saturation curves of photosynthesis either on a per chlorophyll or on a per cell basis.

The concentration of the photosystems in thylakoid membranes was estimated spectrophotometrically from the amplitude of the light *minus* dark difference at 700 nm (P700) for PSI, and 320 nm (Q_A) for PSII [Melis 1989]. The light-harvesting Chl antenna size of PSI and PSII was measured from the kinetics of P700 photo-oxidation and Q_A photoreduction, respectively [Melis 1989].

Results and Discussion

Information about the efficiency and productivity of photosynthesis can be obtained directly from the light-saturation curve of photosynthesis (the so-called "P vs I" curve) in which the rate of O_2 evolution, or CO_2 assimilation, is measured and plotted as a function of the actinic light intensity. In such a presentation, the rate of photosynthesis first increases linearly with light intensity and then levels off as the saturating light intensity (I_s) is approached. The slope of the initial, linear, increase defines the quantum yield of photosynthesis (O_2 evolved per photon absorbed) [Björkman and Demmig 1987, Neale et al. 1993]. The rate of photosynthesis is saturated at light intensities higher than I_s. This light-saturated rate (P_{max}) provides a measure of the capacity of photosynthesis for the particular sample [Powles and Critchley 1980]. The three parameters (quantum yield, I_s, and P_{max}), measured with dilute cultures under conditions of little mutual shading, define the vital signs and photosynthetic properties of the algal cells.

It was of interest to compare the optical properties and performance characteristics of LL and HL-acclimated *D. salina*. The objective of this work was to assess the organization and function of the photosynthetic apparatus and to test for the hypothesis that a truncated Chl antenna size would actually help cells to achieve a higher *per chlorophyll* capacity of photosynthesis and greater solar energy conversion efficiencies under moderate to high light intensities.

Optical properties, photosynthetic apparatus organization and performance in LL and HL-grown Dunaliella salina

Cells grown under continuous LL or HL in the presence of 25 mM $NaHCO_3$ as the sole carbon source, had similar doubling times (8-8.5 h, Table 1). However, compared to the LL-, HL-grown cells had only 25% of the cellular Chl content, a much higher Chl *a*/Chl *b* ratio and a substantially truncated Chl antenna size for both photosystem-I (PSI) and photosystem-II (PSII) in their chloroplast (Table 1).

Table 1. Effect of growth irradiance on pigment content, photosystem Chl antenna size, and rate of photosynthesis in *Dunaliella salina*. Cells were grown at low-light (100 µmol photons m^{-2} s^{-1}) or at high-light (2,000 µmol photons m^{-2} s^{-1}).

	Low-light grown	**High-light grown**
Cell doubling time (h)	8.0	8.5
Chl/cell (molecules/cell)	$0.41*10^9$	$0.10*10^9$
Chl *a*/Chl *b* (mol:mol)	4.5:1	12:1
N_{PSI}	230	105
N_α	560 (65%)	---
N_β	140 (35%)	130 (05%)
N_{core}	---	60 (95%)
P_{max} mmol O_2 (mol Chl)$^{-1}$ s^{-1}	100	320
Quantum yield, rel. units	1.0	0.37

The number of Chl (*a* and *b*) molecules specifically associated with PSI (N_{PSI}) was lowered from 230 in LL to 105 in HL. In LL-cells, about 65% of the functional PSII centers were $PSII_\alpha$ with an antenna size N_α of approximately 500 Chl (*a* and *b*)

molecules. The remaining 35% of the functional PSII were of the PSIIβ-type with an antenna size N_β of ~140 Chl (*a* and *b*) molecules. This well-known PSII α–β antenna heterogeneity [Melis 1991] was essentially absent in the HL-cells. In the latter, 95% of all functional PSII centers possessed a small antenna composed of ~60 Chl molecules. These results are consistent with the notion that HL-cells grown in the presence of $NaHCO_3$ exist in a state of chronic irradiance stress as photosynthesis is limited by the slow carbon supply [Smith et al. 1990, Vasilikiotis and Melis 1994, Baroli and Melis 1996]. Such alterations in the pigment and photosystem content of the cells brought about significant changes in the optical properties of the respective cultures. Figure 1 shows the transmittance of light through a LL-grown and a HL-grown *D. salina* culture. For the same cell density 10^6 cells/ml) , it is evident that transmittance of light is attenuated strongly as a function of distance from the surface in the fully pigmented cells. At 5 cm below surface, the level of irradiance is less that 20% of that incident to the culture (Fig. 1). On the contrary, the HL-grown cells with the truncated Chl antenna size permit more than 70% of the incident irradiance to reach the 5 cm mark below the culture surface. It is evident from these considerations that illumination of the culture will be more uniform in cells with a truncated Chl antenna size.

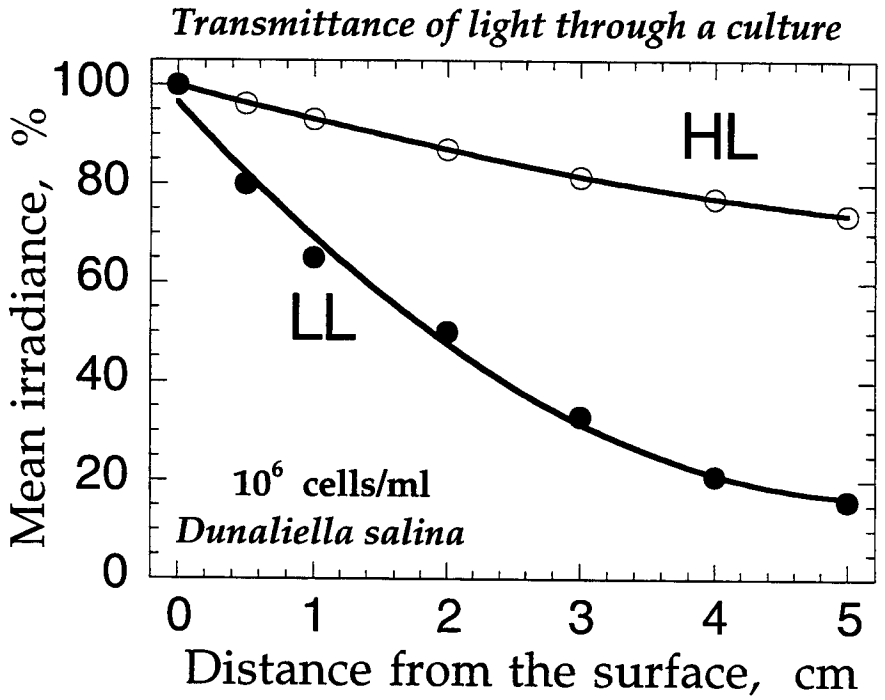

Figure 1. Mean irradiance as a function of distance from the surface in *Dunaliella salina* cultures. Fully pigmented cells were grown under low-light (LL). Cells with a truncated Chl antenna size were grown under high irradiance (HL).

The effect of the truncated Chl antenna size and chronic photoinhibition status on the quantum yield and rate of photosynthesis were assessed. Figure 2A shows the light-saturation curves of LL and HL $NaHCO_3$-grown *D. salina*. It is

evident that LL-grown cells, in which the rate of light absorption limits photosynthesis, have a light-saturated rate (P_{max}) of ~100 mmol O_2 (mol Chl)$^{-1}$ s^{-1}. The HL-cells with the truncated Chl antenna size and chronic photoinhibition condition reached a light-saturated rate of photosynthesis (P_{max} =~320 mmol O_2 (mol Chl)$^{-1}$ s^{-1}) that is ~3 times greater than that of the normally pigmented cells. This difference is attributed to the much smaller Chl antenna size for the HL-grown cells, translating into higher per Chl productivity. Consistent with this interpretation is also the difference in the I_s values which is 8-10 times greater for the HL grown than for the LL-grown cells, suggesting an average 8-10 times greater effective Chl antenna size for the latter (Table 1, [Herron and Mauzerall 1972]).

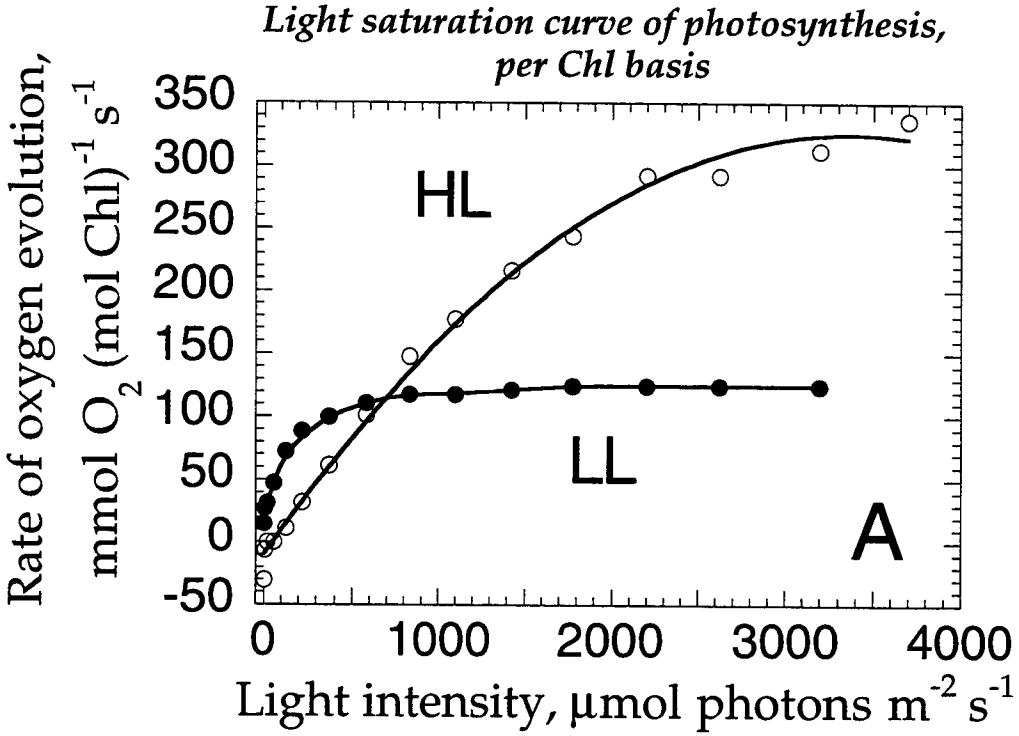

Figure 2A. The light-saturation curve of photosynthesis in $NaHCO_3$-grown *Dunaliella salina*. Cells were grown either at ~100 µmol photons m^{-2} s^{-1} (LL) or at ~2,000 µmol photons m^{-2} s^{-1} (HL). Rates of oxygen evolution on a *per chlorophyll* basis were measured as a function of incident intensity to the cell suspension.

The same results, plotted on a per cell basis (Fig. 2B), show a greater cellular productivity for the LL-grown cells (~75 pmol O_2 10^{-6} cells s^{-1}), compared with the HL-grown cells in which the cell productivity was at ~55 pmol O_2 10^{-6} cells s^{-1}. Again, this difference underscores the chronic photoinhibition status of the HL-grown cells where, in addition to the truncated Chl antenna size, a significant fraction of PSII centers are photochemically inert due to photodamage [Vasilikiotis and Melis 1994]. This configuration of the photosynthetic apparatus in the HL-cells resulted in about similar growth rates with the LL-cells (Table 1). Clearly, however, both rates of growth are below those that can be achieved under optimal growth conditions [Smith et al. 1990, Baroli and Melis 1996].

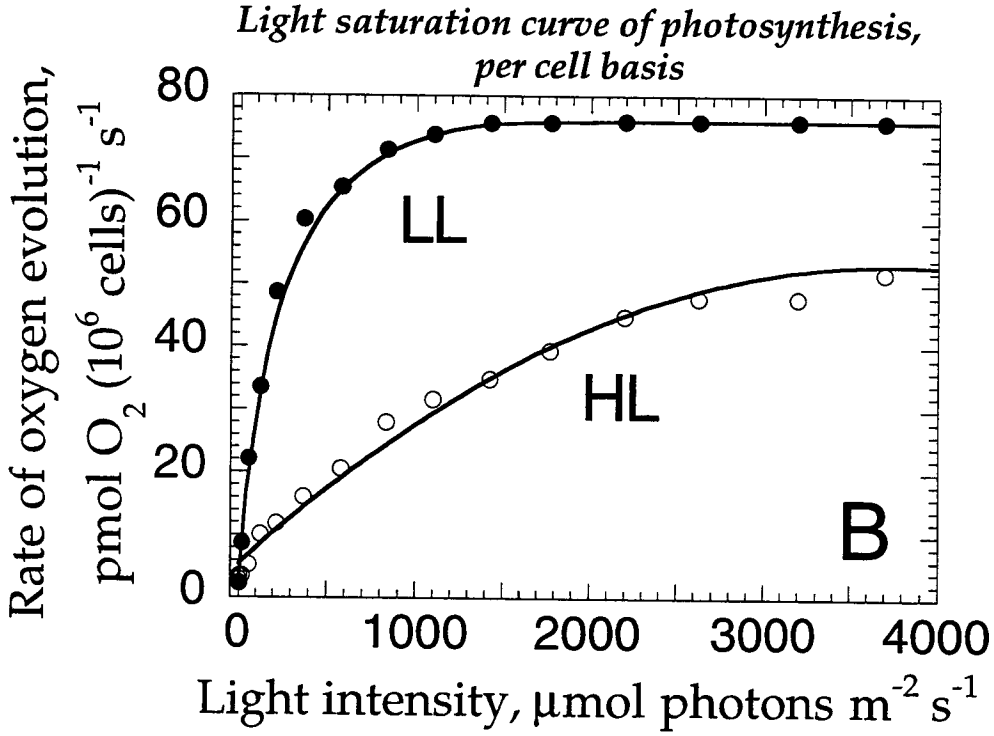

Figure 2B. The light-saturation curve of photosynthesis in $NaHCO_3$-grown *Dunaliella salina.* Cells were grown either at ~100 µmol photons m^{-2} s^{-1} (LL) or at ~2,000 µmol photons m^{-2} s^{-1} (HL). Rates of oxygen evolution on a *per cell* basis were measured as a function of incident intensity to the cell suspension.

It is also evident from the results of Fig. 2A that the initial slopes of the light-saturation curves (which provide a measure of the quantum yield of photosynthesis) are different for the two samples. This is better shown in Fig. 3, which shows the initial portions of the curves in Fig. 2A. We estimated that the initial slope of the light-saturation curve (quantum yield of photosynthesis) of the LL-grown cells (=0.42 rel. units) was steeper than that of the HL-grown cells (=0.15 rel. units). This difference shows that not all Chl molecules are photochemically competent in the HL-grown cells due to the chronic photoinhibition of photosynthesis that prevails in these cells [Smith et al. 1990, Kim et al. 1993, Baroli and Melis 1996]. On the basis of the relative quantum yield in these two measurements (Table 1), it would appear that only about 37% of the Chl molecules are photochemically competent in the HL, the rest being photochemically inert due to the accumulation of photodamaged and, therefore, inactive PSII centers in the HL-thylakoids. In principle then, the P_{max}=~300 mmol O_2 (mol Chl)$^{-1}$ s^{-1}) and the cellular productivity of the HL-grown *D. salina* with a truncated Chl antenna size could be even higher (by a factor of about 3) if there was a way to repair the photodamaged PSII centers while preserving the small Chl antenna size of the HL-grown samples.

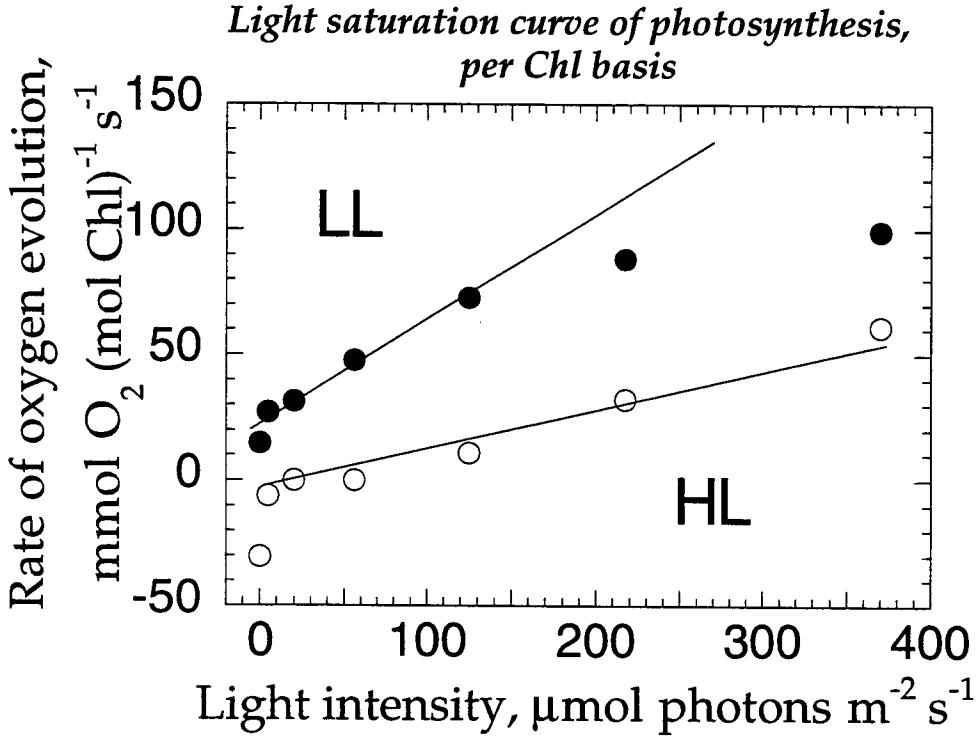

Figure 3. The light-saturation curve of photosynthesis in $NaHCO_3$-grown *Dunaliella salina*. Cells were grown either at ~100 µmol photons m^{-2} s^{-1} (LL) or at ~2,000 µmol photons m^{-2} s^{-1} (HL). The initial slope of the rate of photosynthesis *versus* irradiance (relative quantum yield on a Chl basis) in the LL-and HL-grown cells.

These results suggested that a truncated Chl antenna size for the photosystems will enhance the solar conversion efficiency and productivity of photosynthesis. To further illustrate the effect of Chl antenna deficiency, it was important to correct for the effect of chronic photoinhibition on the cellular photosynthesis. To this end, we devised an experimental approach that would promote the repair of photodamaged PSII centers without the induction of a concomitant significant increase in the Chl antenna size of the photosystems. We performed "light shift" experiments in which HL-grown cultures, with cells in the exponential phase of growth, were shifted to LL-growth conditions. We reasoned that upon a HL--->LL transition, both recovery from photoinhibition and an increase in the Chl antenna size will occur. However, the PSII repair from photoinhibition reportedly occurs with a half-time of about 60 min [Vasilikiotis and Melis 1994, Baroli and Melis 1996], whereas the increase in the Chl antenna size occurs with slower kinetics having a half time of ~4 hours. Thus, in the early stages of a HL--->LL shift, one would encounter a situation where significant recovery from photoinhibition would have occurred with only a minimal increase in the Chl antenna size of the photosystems.

Light shift experiments

Figure 4A shows the adjustment of the light-saturated rate of photosynthesis (P_{max}) in $NaHCO_3$-grown cells following a HL--->LL transition. It is evident that P_{max} increases promptly as a function of time upon the HL--->LL transition, from ~310 mmol O_2 (mol Chl)$^{-1}$ s^{-1}, measured at zero time, to a transient maximum of ~475 mmol O_2 (mol Chl)$^{-1}$ s^{-1}, attained within the first 2 h under LL conditions. This change reflects chloroplast recovery from photoinhibition, i.e., the repair of photodamaged PSII centers and the *de novo* biosynthesis/assembly of PSI centers, both of which bring about a greater capacity for photosynthetic electron transport in the thylakoid membrane [Neidhardt et al. 1998]. Subsequent incubation (> 2h) under LL-conditions caused a gradual decline in the value of P_{max}, reflecting the significant accumulation of Chl in the chloroplast, and an increase in the light-harvesting Chl antenna size which resulted in a lower per Chl P_{max} value for the cells.

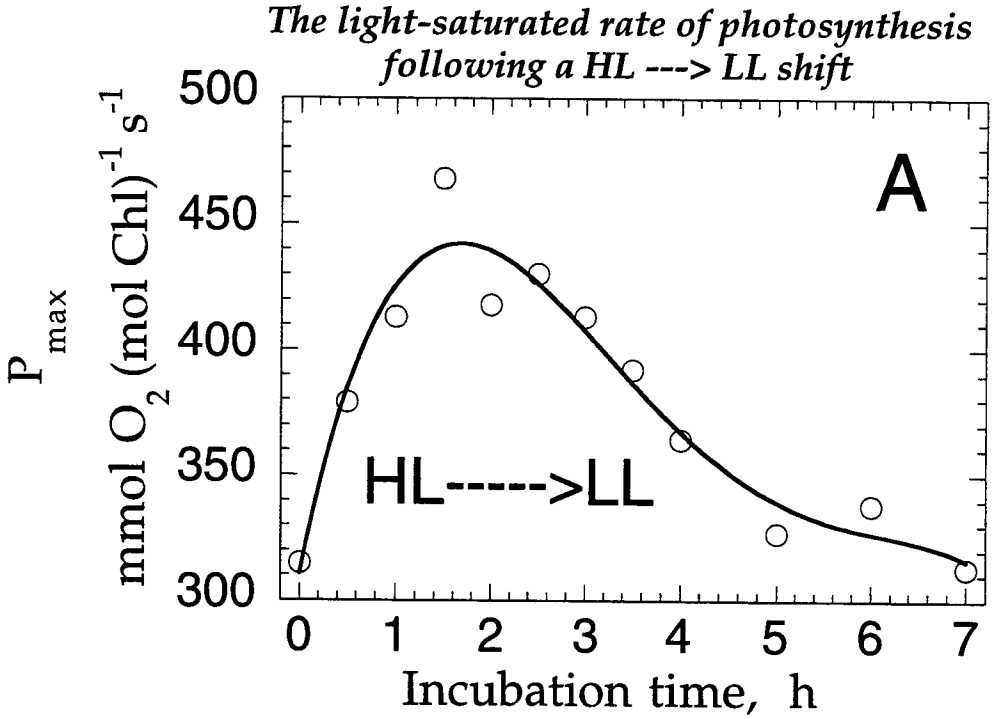

Figure 4A. Changes in the light-saturated rate of cellular photosynthesis (P_{max}) in *D. salina* following a switch of HL-grown cells to LL-growth conditions. The switch in growth irradiance occurred at zero time.

Figure 4B shows the change in the Chl/cell ratio following a HL--->LL transition. Within 7 hours, the Chl/cell ratio increased from less than 4 to about $9x10^{-16}$ mol cell^{-1}. Concomitantly, the Chl *a*/Chl *b* ratio of the cells decreased from ~12/1 to a low value of ~6/1 over the same time period (not shown). The lowering of the Chl *a*/Chl *b* ratio reflects accumulation of Chl *b* and the ensuing increase in the auxiliary light-harvesting chlorophyll antenna size of the photosystems. Both

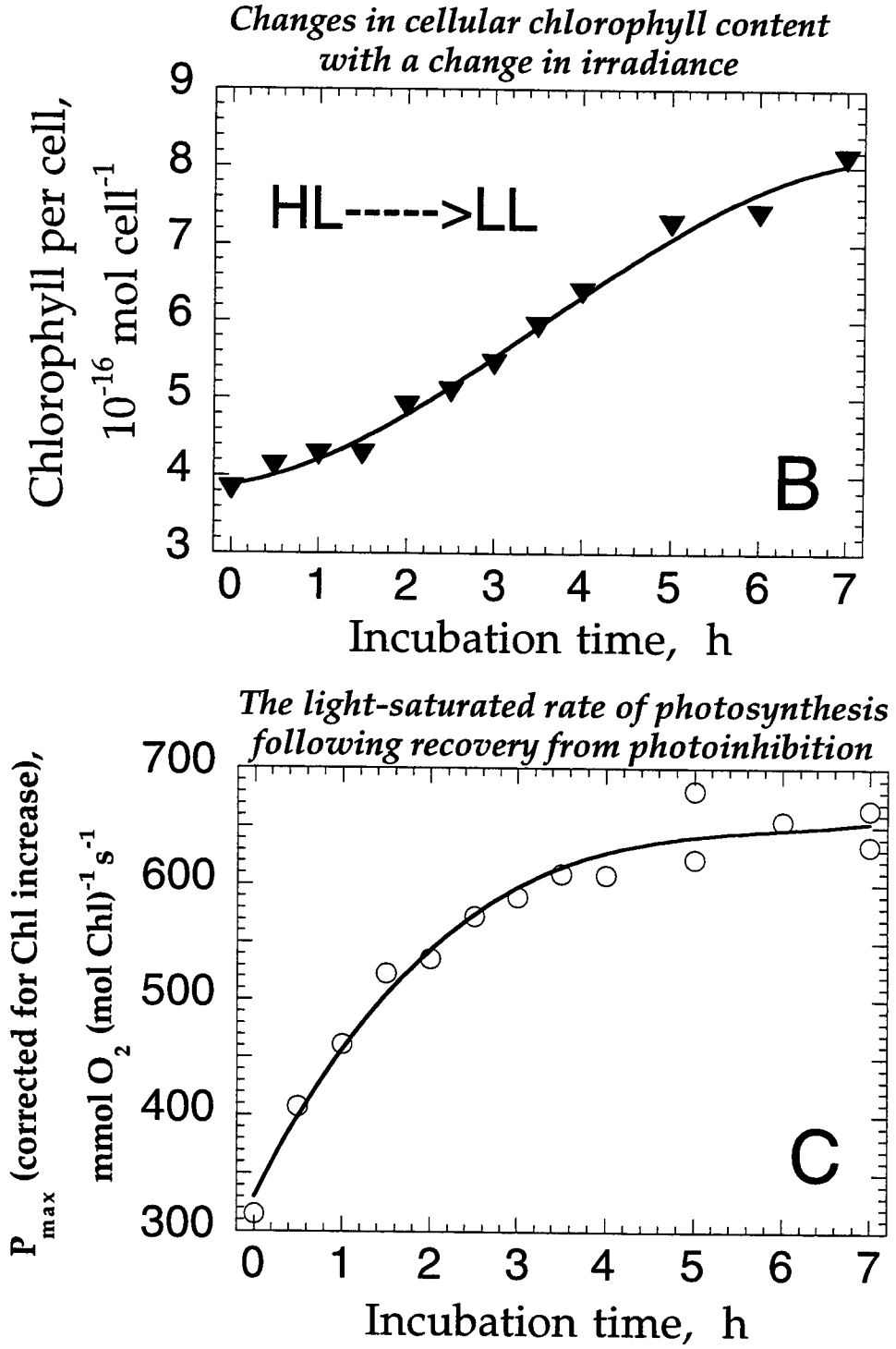

Figure 4. **(B)** Changes in Chl/cell ratio in $NaHCO_3$-grown *D. salina* following a switch of HL-grown cells to LL-growth conditions. The switch in growth irradiance occurred at zero time. **(C)** Light-saturated rates of photosynthesis (P_{max}), corrected for the Chl/cell increase of (B), as a function of incubation under LL. Note the 2.5-fold increased capacity of photosynthesis upon recovery from photoinhibition.

changes are consistent with earlier measurements of the Chl antenna size increase upon a HL--->LL transition [Kim et al. 1993].

Figure 4C shows estimated values of P_{max} as a function of incubation time following a HL--->LL transition. This presentation depicts the P_{max} values that would have been attained upon recovery from photoinhibition in the absence of a concomitant Chl antenna size increase. Results in Fig. 4C were obtained from those of Fig. 4A by correcting for the Chl/cell increase shown in Fig. 4B. Fig. 4C shows that, following recovery from photoinhibition, P_{max} in HL-grown *D. salina* would have increased from ~310 to over 650 mmol O_2 $(mol\ Chl)^{-1}\ s^{-1}$. The exponential increase in the value of P_{max} following the HL--->LL transition mainly reflects the kinetics of the repair of photodamaged PSII centers. The measured half time of ~1 h (Fig. 4C) is consistent with earlier findings on the half time of the PSII repair from photodamage [Vasilikiotis and Melis 1994, Baroli and Melis 1996].

In principle then, in the absence of photoinhibition, the photosynthetic performance of *D. salina* with a truncated light-harvesting antenna size, would be greater by a factor of ~2.5 than that shown in Fig. 2A (HL). Fig. 5 compares the light-saturation curve of photosynthesis of LL-grown cells with the calculated light-saturation curve of HL-grown cells, after the correction of the latter for the effect of photoinhibition. It is evident from the results of Fig. 5 that photosynthetic productivity, on a per Chl basis, would be greater by a factor of ~6 in the cells with a

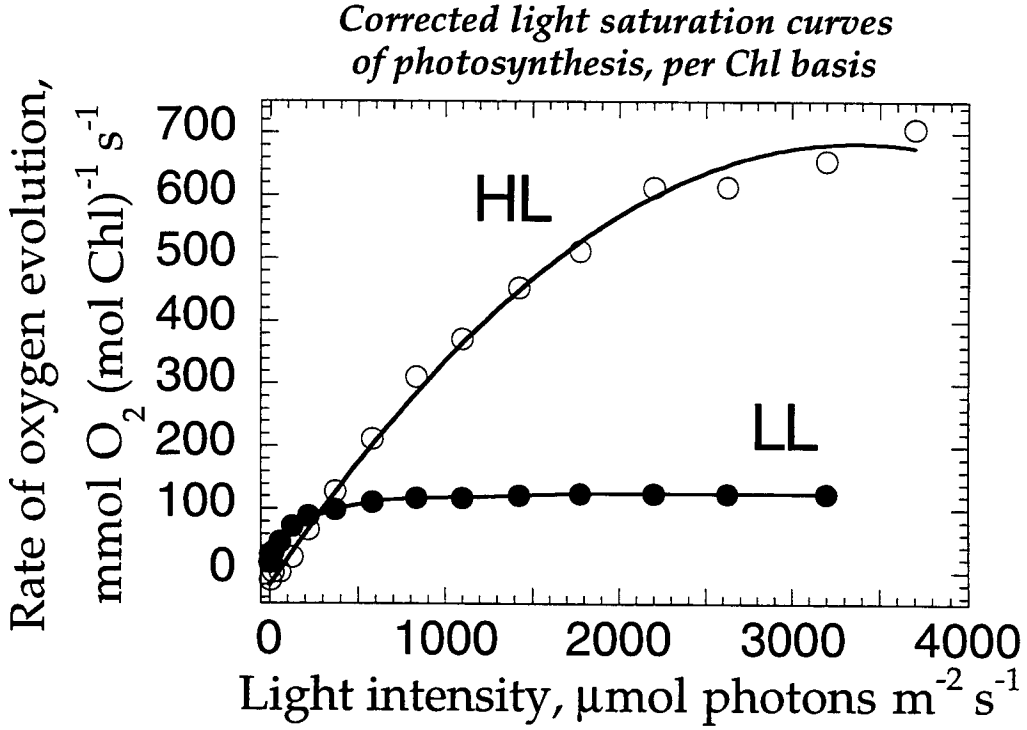

Figure 5. Light-saturation curves of photosynthesis in $NaHCO_3$-grown *D. salina*. (LL) Rates of oxygen evolution on a *per chlorophyll* basis were measured as a function of incident intensity to the suspension of low-light-grown cells. (HL) Rates of oxygen evolution on a *per chlorophyll* basis were estimated from the results of Fig. 2 (HL) upon correction for the effect of photoinhibition (Fig. 4C).

truncated Chl antenna (HL) than in the normally pigmented cells (LL). Moreover, the initial slopes of the light-saturation curves among the two cell types were similar, suggesting that under light-limiting conditions, the quantum yield of photosynthesis would be about the same in the normally pigmented cells (LL) and in the cells with a truncated Chl antenna size (HL).

A significant difference between the two cell types, however, is the fact that photosynthesis in the LL-grown cells saturates at an intensity of about 400 μmol photons m^{-2} s^{-1}, whereas photosynthesis in the cells with the truncated Chl antenna does not quite saturate even at 3,500 μmol photons m^{-2} s^{-1}. This difference has important implications for the solar conversion efficiency in the two cell types. It is predicted that both cell types will absorb sunlight in direct proportion to the incident intensity. However, only the cells with the truncated Chl antenna size will be able to sustain high solar conversion efficiencies at all irradiances. The normally pigmented cells will be unable to utilize intensities exceeding 400 μmol photons m^{-2} s^{-1}. Thus, under bright sunlight conditions, LL-grown cells will dissipate as heat the majority of the absorbed irradiance. A quantitative analysis of the solar conversion efficiency of normally pigmented and Chl antenna deficient cells is given below.

Solar conversion efficiencies and estimates of hydrogen production

It is possible to estimate solar conversion efficiencies in the fully pigmented cells (LL) versus that of the truncated Chl antenna cells (HL). This derivation is based on the observation that the quantum yield of photochemistry, measured under light-limiting conditions, is greater than 0.8 in vascular plants and green algae of diverse origins [Avron and Ben-Hayyim 1969, Sun and Sauer 1971, Thielen and Van Gorkom 1981, Ley and Mauzerall 1982, Bjorkman and Demmig 1987]. Fig. 6 shows photosynthetic solar conversion efficiencies as a function of incident irradiance in normally pigmented (LL) and Chl antenna deficient cells (HL). It is evident that, at low intensities (less than 200 μmol photons m^{-2} s^{-1}), both cell types would perform with a relatively high solar conversion efficiency (normalized to 0.85 for both cell types at 0 μmol photons m^{-2} s^{-1}). At higher incident intensities, however, solar conversion efficiencies for the fully pigmented cells declined sharply, reaching a value of ~0.05 (5%) at full sunlight (2,500 μmol photons m^{-2} s^{-1}). The Chl antenna deficient cells (Fig. 6, HL) also exhibited a decline in solar conversion efficiency with incident irradiance. However, this was noticeable only at intensities greater than 500 μmol photons m^{-2} s^{-1}, reaching a solar conversion efficiency of ~0.45 at full sunlight. From the results of Fig. 6, it was estimated that the integrated solar conversion efficiency over the entire physiological light intensity range (0-2,500 μmol photons m^{-2} s^{-1}) was 65% for the Chl antenna deficient (HL) and only 17% for the normally pigmented cells (LL).

It is evident from the above considerations that overall photosynthetic solar conversion efficiency in green algal cultures will strongly depend on the Chl antenna size of the photosystems and on the level of the solar intensity in the course of the day. Fig. 7 shows the profile of the daily solar radiation received at mid-latitudes during the spring in the North Hemisphere [Bjorkman and Ludlow

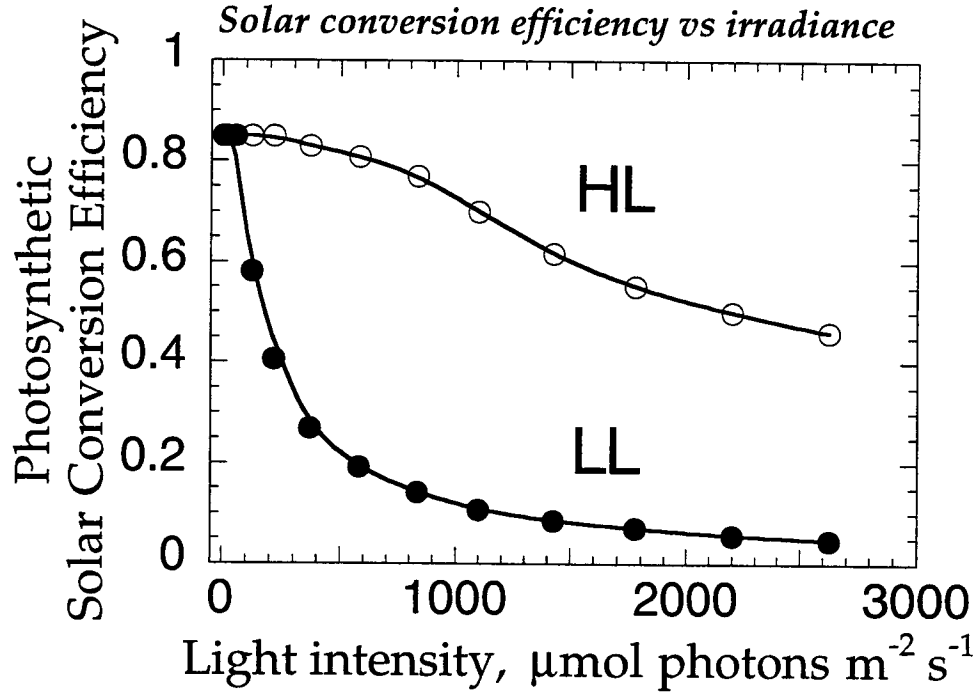

Figure 6. Photosynthetic solar conversion efficiency as a function of incident light intensity in normally pigmented (LL) and Chl antenna deficient (HL) *D. salina.*

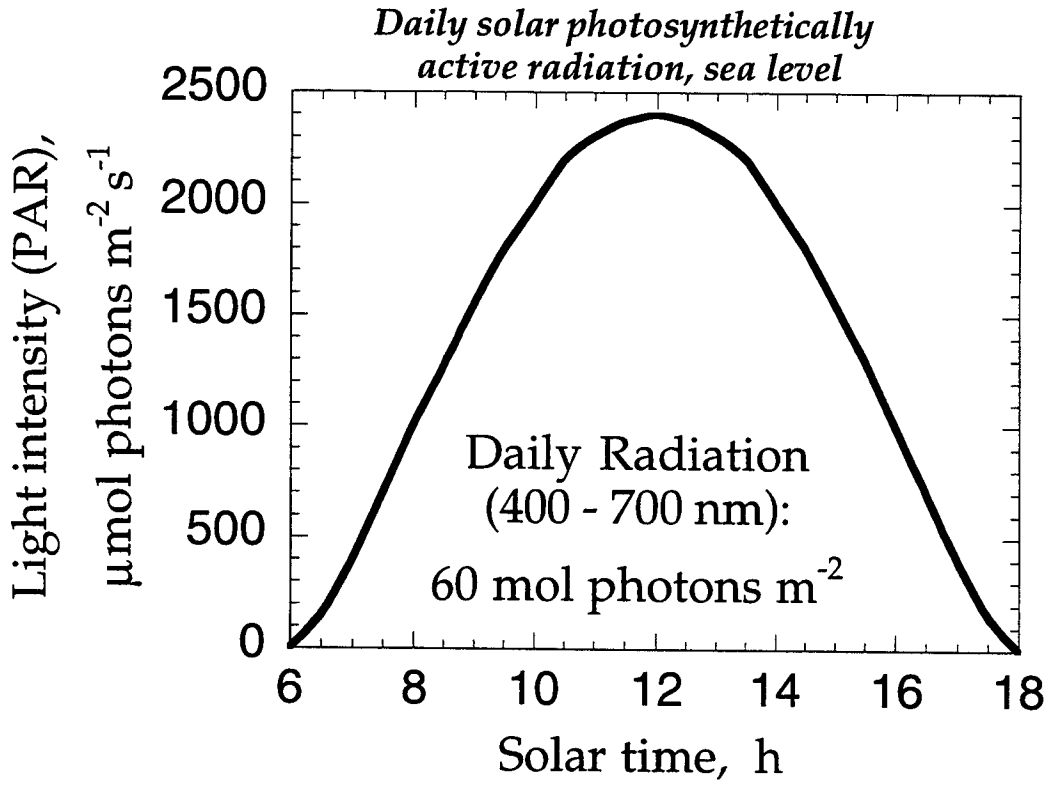

Figure 7. Profile of the daily solar photosynthetically active radiation at sea level.

1972, Kirk 1983]. The integrated area under the daily radiation curve indicated a total daily dosage of about 60 mol photons m^{-2}. This daily radiation could be slightly higher during the summer months and lower during the winter months in the north hemisphere. Moreover, it could be significantly attenuated by cloud cover and other weather conditions that affect solar luminosity. Important in the daily solar radiation profile (Fig. 7) is the observation that an intensity of 500 μmol photons m^{-2} s^{-1} was exceeded soon after 7:00 h. The solar intensity did not recede to that level until just before 17:00 h. Thus, for a period of time equal to about 10 hours, photosynthetic solar conversion efficiency in the fully pigmented cells would be less than 20% (Fig. 6, LL) whereas Chl antenna deficient cells will operate with a conversion efficiency in the range between 85-45% (Fig. 6, HL).

On the basis of these considerations, it is possible to calculate the upper limit of hydrogen production by fully pigmented and Chl antenna deficient cells grown in a photobioreactor under mass culture conditions. This calculation assumes that 60 mol photons m^{-2} (Fig. 7) will be received and absorbed by the respective green algal cultures and that electrons in the photosynthetic apparatus will quantitatively be transferred to form hydrogen in a direct biophotolysis process [Weaver et al. 1980, Greenbaum 1984, 1988, Ghirardi et al. 1997, Benemann 1997]. Table 2 summarizes the parameters involved in these calculations. It is shown that fully pigmented cells will generate at most 25% of the hydrogen (mass or volume) generated by the Chl antenna deficient cells.

Table 2. Solar conversion efficiencies and hydrogen production estimates. The optical properties and pigment contents of fully pigmented and Chl antenna deficient cells are given in Table 1 and Fig. 1.

	Fully pigmented cells	**Chl antenna deficient cells**
Daily incident PAR, mol photons m^{-2}	60	60
Minimum number of mol photons required to produce 1 mol H_2	4	4
Integrated daily photosynthetic solar conversion efficiency	17%	65%
Upper limit of H_2 mass produced (mol H_2 m^{-2} d^{-1})	2.55	9.75
Upper limit of H_2 volume produced (L H_2 m^{-2} d^{-1})	~57	~218

In all certainty, however, hydrogen yields will be lower from those shown in Table 2. Reasons for such attenuation include:

- competition for reduced ferredoxin (electrons) between the hydrogen producing pathway (via the bi-directional hydrogenase) and other metabolic pathways in the chloroplast.
- photoinhibition of green algal photosynthesis under bright sunlight [Baroli and Melis 1996]. In general, this adverse phenomenon lowers photosynthetic productivity [Powles 1984]. Photoinhibition will be significantly more pronounced in the fully pigmented than in the Chl antenna deficient cells [Baroli and Melis 1998].
- mutual cell shading in a mass algal culture, a phenomenon that is significantly more pronounced in the fully pigmented than in the Chl antenna deficient cells (Fig. 1).

The combined effect of the above mentioned attenuations in the production of hydrogen will depend on the case-by-case environmental and physiological conditions prevailing. A precise assessment of the effect of these parameters is, however, beyond the scope of this feasibility study.

Conclusions

Results in this feasibility study demonstrated a novel method for maximizing solar conversion efficiencies and photosynthetic productivity in microalgae by minimizing the number of the light-harvesting antenna pigments of the photosystems. Direct experimental evidence showed that a highly truncated light-harvesting Chl antenna size in the green alga *Dunaliella salina* could result in a

- >6-fold greater photosynthetic productivity (on a per Chl basis), compared to that of normally pigmented cells (Fig. 5)
- >4-fold greater yield of hydrogen production under mass culture, compared to that of normally pigmented cells (Table 2)

In summary, microalgae with a truncated Chl antenna size will be ideal for a variety of commercial applications including CO_2 mitigation, and rare biochemical, biomass or hydrogen production. Cultivation of green algae under continuous illumination of high irradiance resulted in the formation of a highly truncated Chl antenna size. However, this condition readily reverts to that of the fully pigmented cells upon lowering of the light intensity (Fig. 4B) or upon the fall of darkness [Polle and Melis, unpublished]. On the contrary, fully pigmented cells do not readily downscale their Chl antenna size whenever they encounter a HL condition, suggesting that once assembled, the Chl antenna is stable and that minimizing it could occur only over time during further growth and development of the

organism [Kim et al. 1993, Melis 1998]. Thus, HL-acclimated cells, although instrumental in this feasibility study, cannot be used outside the laboratory., For purposes of industrial application and hydrogen production, it would be desirable to develop microalgal mutants with a permanently truncated light-harvesting Chl antenna size, i.e., a cell-type with a photosynthetic unit size that is similar to that of the HL-acclimated cells under all growth irradiances.

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