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Characterization of Photosystem II Activity and Heterogeneity during the Cell Cycle of the Green Alga *Scenedesmus quadricauda*[1]

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The photosynthetic activity of the green alga *Scenedesmus quadricauda* was investigated during synchronous growth in light/dark cycles. The rate of O_2 evolution increased 2-fold during the first 3 to 4 h of the light period, remained high for the next 3 to 4 h, and then declined during the last half of the light period. During cell division, which occurred at the beginning of the dark period, the ability of the cells to evolve O_2 was at a minimum. To determine if photosystem II (PSII) controls the photosynthetic capacity of the cells during the cell cycle we measured PSII activity and heterogeneity. Measurements of electron-transport activity revealed two populations of PSII, active centers that contribute to carbon reduction and inactive centers that do not. Measurements of PSII antenna sizes also revealed two populations, $PSII_\alpha$ and $PSII_\beta$, which differ from one another by their antenna size. During the early light period the photosynthetic capacity of the cells doubled, the O_2-evolving capacity of PSII was nearly constant, the proportion of $PSII_\beta$ centers decreased to nearly zero, and the proportion of inactive PSII centers remained constant. During the period of minimum photosynthetic activity 30% of the PSII centers were insensitive to the inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea, which may be related to reorganization of the thylakoid membrane. We conclude from these results that PSII does not limit the photosynthetic activity of the cells during the first half of the light period. However, the decline in photosynthetic activity observed during the last half of the light period can be accounted for by limited PSII activity.

The photosynthetic activity of synchronously grown cells of algae is strongly modulated during the cell cycle. In algal cells synchronized by light/dark periods, the rate of photosynthesis can vary more than 2-fold. The photosynthetic activity reaches a maximum during the early phase of the light period, persists for a few hours, and then steadily declines until the end of the light period, which coincides with the onset of cell division (Sorokin, 1957; Sorokin and Mayers, 1957). Respiratory activity exhibits a similar periodic modulation during the cell cycle (Sorokin and Mayers, 1957). Despite decades of research, the factors that control the photosynthetic activity of a cell during its development have not been identified. Some studies indicate that a component of the electron-transport apparatus of the thylakoid membrane may be rate limiting during the cell cycle (Senger and Bishop, 1967, 1969; Schor et al., 1970; Senger, 1970; Frickel-Faulstich and Senger, 1974; Hesse et al., 1976, 1977; Mende et al., 1981), whereas other studies point to a limitation in the C-reduction cycle (Walther and Edmunds, 1973; Myers and Graham, 1975). These observations raise the possibility that the site of control may change during the cell cycle.

Several studies reveal cell-cycle-dependent modifications of the photosynthetic machinery of the thylakoid membrane that could impose limitations on overall photosynthetic activity. For example, Heil and Senger (1986) showed that the increase in photosynthetic activity during the early light phase in *Scenedesmus obliquus* correlates with an increase in phosphorylation of thylakoid membrane proteins, which is involved in controlling the relative antenna sizes of the photosystems (Allen, 1992). Cell-cycle-dependent changes in antenna size were observed in *Euglena gracilis* by Winter and Brandt (1986), who showed that insertion of one of the key PSII light-harvesting proteins into the thylakoid membrane occurs during the middle of the light phase. In addition, changes in the heterogeneity of the PSII antenna size are modulated in synchronously grown cells of *Chlorella fusca* during the cell cycle (Butko and Szalay, 1985; Scheffczyk et al., 1989). Protein phosphorylation also plays a role in the damage/repair process of photoinhibited reaction centers (Prasil et al., 1992; Aro et al., 1993), which can lead to limitations in photosynthetic activity (Sorokin, 1960). In addition to modification of the

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Abbreviations: F_n(100 ms), fluorescence intensity of whole cells measured 100 ms after the *n*th single-turnover saturating flash; F_o, fluorescence intensity of whole cells measured when the primary quinone acceptor Q_A is oxidized; $PSII_A$, PSII reaction centers active in plastoquinone pool reduction; $PSII_X$, PSII reaction centers inactive in plastoquinone pool reduction (non-Q_B-reducing centers).

light-harvesting and electron-transport proteins, there are significant changes in the structural organization of the thylakoid membranes during the cell cycle. Electron micrographs show that in *Scenedesmus quadricauda* (Setlik et al., 1981) and *E. gracilis* (Winter and Brandt, 1986) the minimum photosynthetic activity coincides with the appearance of short stacks of thylakoid membranes known as pseudograna because they resemble the grana stacks of higher plants. Whether these structural changes affect the photosynthetic activity of the cells is not known.

In this study we investigated the green alga *S. quadricauda* during its cell cycle, focusing on the role of PSII in determining the rate of photosynthesis. Specifically, we measured the capacity of PSII reaction centers to harvest light and transfer electrons from water to the plastoquinone pool in synchronously grown cells. Because PSII exists in different forms in cells, we measured two types of heterogeneity, one that determines electron-transport capacity and one that determines the effective size of the antenna system serving individual reaction centers. The electron-transport assay distinguishes between $PSII_A$ and $PSII_X$ reaction centers (Thielen and Van Gorkom, 1981; Lavergne, 1982a, 1982b; Melis, 1985; Graan and Ort, 1986; Chylla et al., 1987; Chylla and Whitmarsh, 1989, 1990; Nedbal et al., 1991). $PSII_A$ centers turn over at rates of a few hundred electrons per second in saturating light, whereas the turnover of $PSII_X$ is 1/10th to 1/1000th that of active centers. The slow turnover of $PSII_X$ centers has been shown to be due to the slow reoxidation rate of Q_A^- (Chylla et al., 1987; Chylla and Whitmarsh, 1989).

Measurements of the effective antenna size serving PSII reaction centers reveal two distinct populations, $PSII_\alpha$ and $PSII_\beta$ (Melis and Homann, 1976). The antenna system serving $PSII_\alpha$ centers is typically twice the size of that serving $PSII_\beta$ centers. Most in vivo studies show that $PSII_\alpha$ centers are active photosynthetically and may overlap or be identical to $PSII_A$. On the other hand, inactive PSII centers are served by an antenna system that is approximately one-half that of active $PSII_A$ centers (Chylla and Whitmarsh, 1990; Nedbal et al., 1991), indicating a strong overlap between $PSII_X$ and $PSII_\beta$ centers. However, the relationship among active $PSII_A$, $PSII_X$, $PSII_\alpha$, and $PSII_\beta$ centers is not straightforward. In vivo studies show that changes in the populations of $PSII_X$ and $PSII_\beta$ centers do not track one another, demonstrating that the two populations are not always identical (Guenther et al., 1988; Oxborough et al., 1996). The different forms of PSII are distributed asymmetrically in the thylakoid membrane. Stroma thylakoid membranes are enriched in both $PSII_X$ and $PSII_\beta$ centers, whereas $PSII_A$ centers are located in the grana membranes (Henrysson and Sundby, 1990). However, the data do not exclude the possibility that some $PSII_X$ and/or $PSII_\beta$ centers are also present in grana membranes (Lavergne and Briantais, 1996).

In this report we describe in vivo measurements of O_2 evolution and chlorophyll fluorescence that reveal significant changes in PSII activity and heterogeneity during the cell cycle of *S. quadricauda*. A comparison of the rate of photosynthetic C reduction with PSII capacity indicates that PSII does not limit the maximum photosynthetic rate during the early and middle phases of the light period during the cell cycle, but the decline in photosynthetic rate late in the light period and during cell division can be accounted for by limited PSII capacity.

MATERIALS AND METHODS

Synchronous Growth of Algal Cultures

Cells of the green alga *Scenedesmus quadricauda* (Turp., Bréb. strain Greifswald from the Culture Collection in Trebon) were grown at 27°C in 0.5 L of liquid medium (Setlik et al., 1981) in a water-jacketed cylinder of 4.5 cm i.d. Cells were mixed by bubbling with air containing 3% CO_2. Photosynthetically active irradiance at 400 to 500 μE m^{-2} s^{-1} was provided by two 500-W tungsten/halogen lamps. Synchronous growth was controlled by light/dark cycles (typically 14–16 h light/8–10 h dark). The cell cycle was monitored by measuring the cell density by optical spectroscopy and by counting the relative numbers of cells undergoing cell division using an optical microscope (Zeiss). The chlorophyll concentration of the suspension was measured at 2-h intervals and adjusted by dilution to maintain 4 to 6 μM. Chlorophyll measurements were done according to the method of Jeffrey and Humphrey (1975) using cells that were broken by vortexing with glass pellets (500 μm in diameter) for 5 min in 0.5 mL of 80% acetone.

Steady-State Rates of O_2 Evolution

Steady-state rates of O_2 evolution were measured using cells in a thermostated cuvette (Gilson Medical Electronics, Middleton, WI) and a Clark-type electrode (model 5331 O_2 probe, Yellow Springs Instruments, Yellow Springs, OH). An OxyMeter (P.S. Instruments, Brno, Czech Republic) controlled the polarizing voltage and timing of the actinic light exposures and digitized the signal. The actinic light was provided by 30 red-light-emitting diodes (model HLMP8103, Hewlett-Packard). Cells were typically exposed to 180 s of actinic light, during which time the O_2 evolution rate reached a stable level under light-limited (50 μE m^{-2} s^{-1}) and light-saturated (500 μE m^{-2} s^{-1}) conditions.

Flash-Induced O_2 Yield

The O_2 yield in a series of single-turnover, saturating flashes was measured for cells using a bare platinum/silver electrode constructed as described by Meunier and Popovic (1988). Samples were prepared by spinning down 1-mL aliquots of cells, which were resuspended in 50 μL of growth medium containing 30 mM KCl. Resuspended cells were placed on the platinum electrode in an agar ring (15% Bacto, Difco, Detroit, MI) that had been soaked in a 300 mM KCl solution. This arrangement provided highly reproducible measurements of O_2 evolution normalized on a chlorophyll basis. Samples were dark adapted for 3 min in an air-saturated medium without the polarizing voltage, which was applied 10 s before the flash-induced yield of O_2 was measured. The cells were exposed to actinic flashes at

2 Hz for 10 s provided by a Xe lamp (model FX1160Q, EG&G, Salem, MA). The electrode-polarizing voltage, flash trigger, and signal processing were provided by a control unit (model FL-100, P.S. Instruments).

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured using a dual-modulation fluorometer (model FL-100, P.S. Instruments). Fluorescence was excited by low-intensity measuring flashes provided by 7 orange-light-emitting diodes (model HLMP-DH08, Hewlett-Packard). The energy, duration (2.5 μs), and timing of the individual flashes were selected to excite less than 0.1% of the PSII reaction centers per flash. An array of 96 red-light-emitting diodes (model HLMP8103, Hewlett-Packard) and a Xe flash lamp (model FX-200, EG&G) provided actinic flashes. The optical compartment of the instrument was essentially as described by Nedbal et al. (1999). The square-wave light-emitting diode actinic flashes (25 μs duration) were saturating. Each sample (1.6 mL) was placed in a 10- $\times$ 10-mm cuvette and dark adapted for at least 10 min before measurement. The experimental protocol consisted of determining F_o, followed by a single-turnover saturating flash, after which the fluorescence decay was measured to determine the kinetics of Q_A^- reoxidation (four data points per decade were recorded starting 32 μs and ending 56 s after the actinic flash). Next, the suspension was dark adapted for 5 min and exposed to a series of 10 saturating, single-turnover flashes given at the frequency of 10 Hz. After each flash the fluorescence was measured to determine the kinetics of Q_A^- reoxidation. Finally, fluorescence induction was measured using a series of subsaturating actinic flashes applied at a rate of 1 kHz.

Electron Microscopy

Electron micrographs were made using synchronously grown cells taken at the time of maximum photosynthetic activity and at the end of cell division. Cells were maintained for 0.5 h in the dark at 10°C and subjected to modified microwave fixation and embedding procedures as described previously (Giammara, 1993; Giberson and Demaree, 1995). The incubation times in all fixatives and rinsing media were doubled to allow sufficient penetration. The Spurr blocks were sectioned with an ultramicrotome (Reichert-Jung, Heidelberg, Germany) and collected on copper hexagonal grids. The sections were shadow coated, stained, and visualized with a transmission electron microscope (model H-600, Hitachi, Tokyo, Japan).

RESULTS

Synchronous Growth

Figure 1 shows that growing cells of *S. quadricauda* in 16-h light/8-h dark cycles synchronized cell division, which starts at the beginning of the dark period. The degree of synchrony was monitored by measuring the density of coenobia (daughter cells from one mother cell of *S. quadricauda* remain linked in the coenobium throughout the cell cycle). All cells divided during each light/dark cycle.

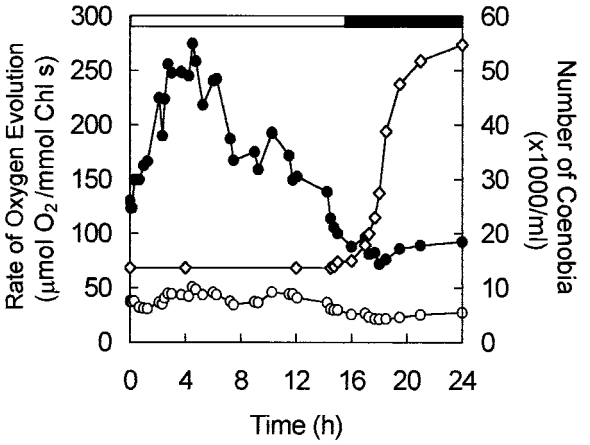

Figure 1. Rate of the CO_2-dependent O_2 evolution was measured for *S. quadricauda* cells in saturating light (500 μE m^{-2} s^{-1}) (●, left axis) and in limiting light (50 μE m^{-2} s^{-1}) (○, left axis) during synchronous growth. Measurements of algal coenobia during synchronous growth showed the release of new coenobia from mother cells starting at the beginning of the dark period and completed by the end of the dark period (◇, right axis). The white and black bars at the top of the figure indicate the light and dark period, respectively. Further details and the experimental conditions are described in the text. Chl, Chlorophyll.

Steady-State Rates of O_2 Evolution

The rate of O_2 evolution was measured for synchronously grown cells using saturating and subsaturating light intensities. Measurements were done under photoautotrophic conditions with CO_2 as the terminal electron acceptor. During the first 3 h of the light period the rate of light-saturated O_2 evolution increased from 125 to 250 μmol O_2 $mmol^{-1}$ chlorophyll s^{-1}, and then remained high for about 4 h (Fig. 1). During the last half of the light period the rate of O_2 evolution steadily declined to about 100 μmol O_2 $mmol^{-1}$ chlorophyll s^{-1}, reaching a minimum just after the onset of the dark period, which coincided with cell division. Figure 1 also shows the rate of O_2 evolution measured in subsaturating light, which showed less variation during the early light period than the light-saturated rate. During the last few hours of the light period the light-limited rate of O_2 evolution decreased nearly 50%.

Flash-Induced O_2 Yield Measurements of $PSII_A$ Centers

To determine the relative number of active PSII centers during the cell cycle we measured the flash-induced O_2 yield in whole cells. Figure 2 shows the typical four-oscillation period of the O_2 yield, with the maximum yield occurring on the third flash (Joliot et al., 1969). During the first 8 to 10 h of the light period the O_2 yield on the third flash exhibited a relatively small variation (±15% of the average yield), indicating that the number of active PSII reaction centers on a chlorophyll basis was fairly constant during this period. During the last 6 to 8 h of the light

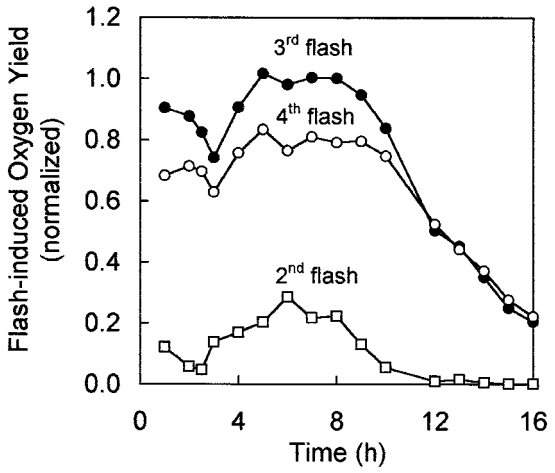

Figure 2. Flash-induced O_2 yield is shown for the second (□), third (●), and fourth (○) flash for synchronously grown cells of *S. quadricauda* during the light period. No O_2 evolution was detected on the first flash. The flash-induced O_2 yield measured for cells during the dark period was substantially lower and is not shown. Cells were loaded onto the electrode on a chlorophyll basis and single-turnover saturating flashes were given at 2 Hz.

period the O_2 yield declined rapidly, to about 20% of the maximum yield. This decline in the flash-induced O_2 evolution shows that the number of $PSII_A$ centers decreased during the last half of the light period. The number of $PSII_A$ centers remained low throughout the dark period (data not shown).

Flash-Induced Fluorescence Measurements of $PSII_A$ and $PSII_X$ Centers

Measurements of the chlorophyll fluorescence intensity subsequent to a single-turnover flash were analyzed to determine the proportion of active and inactive PSII centers in whole cells. The analysis is based on the fact that the fluorescence decay is controlled largely by the reoxidation kinetics of Q_A^-. In $PSII_A$ centers the oxidation of Q_A^- is rapid (a few milliseconds or faster), whereas in $PSII_X$ centers the oxidation of Q_A^- is much slower (Chylla and Whitmarsh, 1989). Figure 3 shows the fluorescence decay induced by a series of four flashes given 100 ms apart for synchronously grown cells harvested during maximum photosynthetic activity and during cell division. The fluorescence decay is shown on a slow time scale to reveal the contribution of $PSII_X$ centers; therefore, rapid decay of fluorescence in active centers due to electron transfer from Q_A^- to Q_B (or Q_B^-) is not resolved. However, $PSII_A$ centers do contribute to the slow fluorescence decay through their S states (Lavergne and Leci, 1993). In contrast, the contribution of $PSII_X$ centers to the slow fluorescence decay is independent of flash number.

To minimize the contribution of $PSII_A$ centers to the slow decay, we analyzed the fluorescence decay after the fourth flash, which is controlled almost entirely by inactive centers (Lavergne and Leci, 1993). As shown by the arrows in Figure 3, the contribution of $PSII_X$ centers was estimated by the difference between the fluorescence level 100 ms after the fourth flash and F_o [i.e. $\Delta F_4 = F_4(100\ ms) - F_o$]. Figure 4 shows that the fluorescence emission originating from $PSII_X$ centers is stable for the first 10 h of the light period and then increases about 2.5 times before cell division. Estimating the proportion of $PSII_X$ from fluorescence emission requires assumptions about the relative antenna size and quantum yields of active and inactive centers (Chylla and Whitmarsh, 1989). If we assume that the antenna size of inactive centers is proportional to that of $PSII_\beta$ (Chylla and Whitmarsh, 1990; Nedbal et al., 1991) and that the quantum yields of $PSII_A$ and $PSII_X$ are the same, then the calculated fraction of inactive $PSII_X$ increases from about 3% in active cells at the middle of the light phase to about 10% in dividing cells at the beginning of the dark period. The increase in $PSII_X$ centers coincides with the decrease observed in steady-state O_2 evolution (Fig. 1) and the decrease in the O_2-evolving capacity of the cells (Fig. 2). However, the concentration of $PSII_X$ is too low to account for the large decrease in photosynthetic activity that occurs at the end of the light period.

Fluorescence-Induction Measurements of $PSII_\alpha$ and $PSII_\beta$ Centers

Measurements of fluorescence induction in the presence of DCMU reveal two distinct antenna sizes serving different reaction centers, $PSII_\alpha$ and $PSII_\beta$ (Melis and Homann, 1976). The antenna system serving $PSII_\alpha$ centers is significantly larger than that serving $PSII_\beta$ centers. To determine the proportion of $PSII_\alpha$ and $PSII_\beta$ centers throughout the cell cycle of *S. quadricauda* we measured fluorescence-induction curves in the presence of DCMU and analyzed the data for antenna heterogeneity (Lavergne and Trissl,

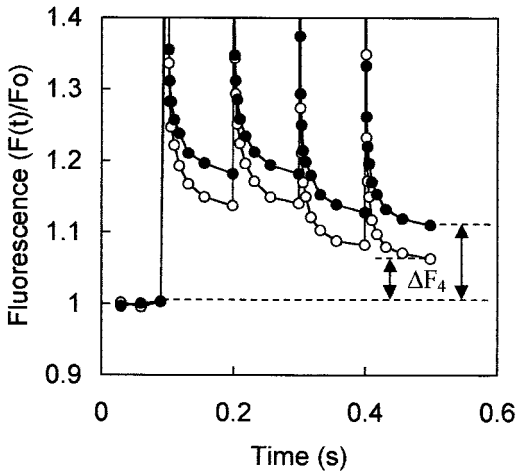

Figure 3. Chlorophyll fluorescence decay induced by a series of single-turnover flashes is shown for *S. quadricauda* cells harvested during maximum photosynthetic activity (○) and during cell division (●). The difference in the fluorescence intensity measured 100 ms after the fourth actinic flash, and F_o [$\Delta F_4 = F_4(100\ ms) - F_o$] is a measure of the relative amount of $PSII_X$ present (see text for further explanation). Single-turnover saturating flashes were given at 10 Hz. $F(t)$, Fluorescence at time t.

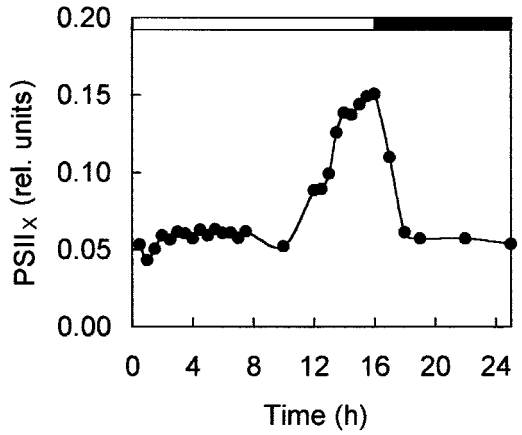

Figure 4. PSII$_X$ assayed by the difference in the fluorescence intensity measured 100 ms after the fourth flash and F_o [$\Delta F_4 = \Delta F_4$(100 ms) − F_o] as shown in Figure 3. Measurements were done using intact cells of *S. quadricauda*. The total variable fluorescence in cells at the peak of photosynthetic activity was approximately 3 times F_o (see Fig. 6B), and the antenna of PSII$_\alpha$ was about 2 times larger than in PSII$_\beta$ (see Fig. 5). Assuming equal fluorescence quantum yield of PSII$_A$ and PSII$_X$, and assuming that PSII$_X$ are β-type antennas, the inactive PSII$_X$ can be estimated to be approximately 3% of total PSII. The fraction of inactive PSII$_X$ can be estimated to represent about 15% of the total PSII in dividing cells using a similar approximation. rel., Relative.

1995). Figure 5 shows that at the beginning of the light phase the effective antenna size of PSII$_\alpha$ was about 2.5 times larger than the effective antenna size of PSII$_\beta$. During the first few hours of the light period, the effective size of PSII$_\alpha$ decreased slightly (about 20%) and then increased to the size measured at the beginning of the light period. The effective antenna size of PSII$_\beta$ was constant throughout the cell cycle (data not shown).

The number of PSII$_\beta$ centers changed drastically during the light cycle. Figure 5 shows that at the beginning of the light period about 35% of the total PSII reaction centers were PSII$_\beta$, but by the 4th h the PSII$_\beta$ centers had nearly disappeared. After the 5th h the proportion of PSII$_\beta$ centers increased steadily, reaching a steady-state level a few hours before the dark period. A comparison of Figures 1 and 5 reveals that the proportion of PSII$_\beta$ centers is inversely proportional to the O_2-evolving capacity of the cells during the light period. During the maximum photosynthetic activity the number of PSII$_\beta$ centers was at a minimum, whereas during the minimum photosynthetic activity the number of PSII$_\beta$ centers was at a maximum. These data (Figs. 4 and 5) support earlier findings that PSII$_\beta$ and PSII$_X$ are not identical sets (Guenther et al., 1988; Oxborough et al., 1996).

Reduction/Oxidation Rates of the Plastoquinone Pool

A comparison of Figures 1 and 2 shows that the greater than 2-fold increase in the rate of O_2 evolution at the beginning of the light period was not due to an increase in the concentration of active PSII centers. This means that the increase in the photosynthetic capacity of the cells must be due to changes occurring subsequent to the reduction of the plastoquinone pool. To test this we measured fluorescence induction of cells collected at various phases of the light/dark cycle. The redox state of the plastoquinone pool is controlled by the rate of electron flow into the pool from PSII versus the rate of electron flow out of the pool through the Cyt *bf* complex (which is driven by PSI). A fully reduced plastoquinone pool indicates that a reaction on the oxidizing side of the plastoquinone pool is rate limiting, whereas a partially oxidized pool indicates that PSII is rate limiting. By comparing fluorescence-induction measurements in whole cells in the absence and presence of DCMU, it is possible to identify whether reactions on the reducing or oxidizing side of the plastoquinone pool are limiting the rate of O_2 evolution (Krause and Weis, 1991).

Figure 6A shows fluorescence-induction curves in the presence and absence of DCMU measured for cells harvested at the beginning of the light period. The maximum fluorescence intensity in the absence of DCMU was nearly the same as the maximum fluorescence intensity measured in the presence of DCMU, indicating that PSII can donate electrons to the plastoquinone pool faster than the Cyt *bf* complex can remove them. Figure 6A shows that PSII was not rate limiting for electron transport in the cells at the beginning of the light period, confirming the conclusion based on a comparison of the data in Figures 1 and 2 discussed above.

Figure 6B shows similar measurements for cells harvested during the 4th h of the light period. In this case the maximum fluorescence measured in the control sample was much lower than that measured in the presence of DCMU, indicating that PSII was becoming a limiting factor in the rate of O_2 evolution when the photosynthetic activity was at a peak (see Fig. 1). The data in Figure 6, A and B, show that the initial increase in the photosynthetic capacity

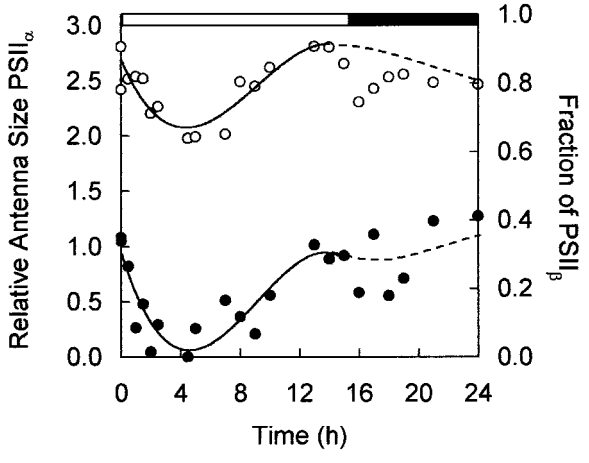

Figure 5. Changes during the cell cycle of the relative antenna size of the PSII$_\alpha$ centers (○) and the fraction of the PSII$_\beta$ centers (●) in intact cells of *S. quadricauda*. The effective antenna size of PSII$_\beta$ centers was stable throughout the cell cycle and was used here as a reference for a relative antenna size of the PSII$_\alpha$ centers (data not shown). The data were obtained by a deconvolution of the fluorescence induction measured in the presence of 10 μM DCMU. The dashed lines interpolate data that were obtained with cells that were not fully inhibited by DCMU (see text for discussion).

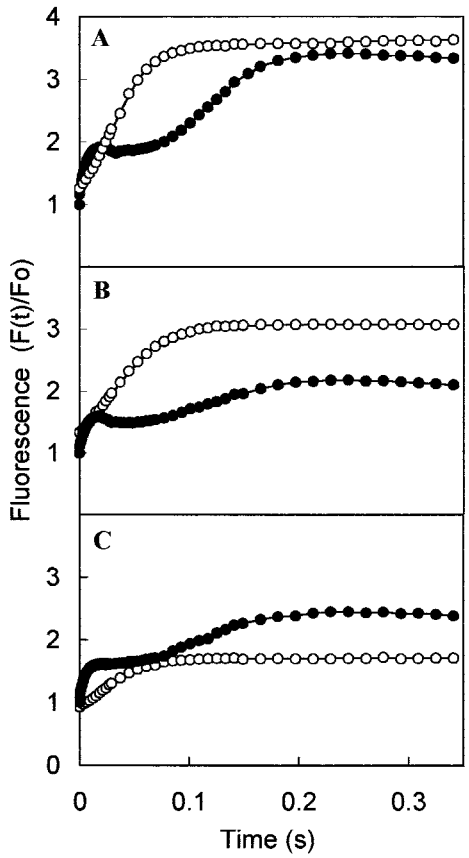

Figure 6. Fluorescence induction measured in cells of *S. quadricauda* at the beginning of the light period (A), in the 4th h of the light period (B), and in the dividing cells (C). The induction measured in the presence of 10 μM DCMU (○) was elicited by actinic light that was 15 times weaker than the light prompting the induction in the absence of DCMU (●).

of the cells in the light period corresponded to an increase in the rate of plastoquinone pool oxidation. Furthermore, the decline in the steady-state O_2 evolution occurring in the second half of the light phase appeared to be controlled by the declining capacity of PSII, which could be observed by declining rates of O_2 evolution in the light-limited regime (Fig. 1) and in single-turnover saturating flashes (Fig. 2).

Figure 6C shows the fluorescence-induction curves for cells harvested during cell division. Surprisingly, the maximum fluorescence in the absence of DCMU was greater than that measured in the presence of DCMU. As shown below, this anomalous behavior was due to PSII centers that were not inhibited by DCMU.

DCMU-Insensitive O_2 Evolution

Analysis of the fluorescence-induction curves for dividing cells indicated that some PSII centers were insensitive to DCMU (Fig. 6C). Measurements of the rate of O_2 evolution as a function of DCMU showed that about 30% of the PSII activity was insensitive to DCMU during cell division (Fig. 7). Because O_2 evolution was measured under light-limiting conditions, the O_2-evolving capacity was directly proportional to the fraction of PSII centers (Whitmarsh and Cramer, 1978). Thus, these data indicate that about 30% of the PSII reaction centers were insensitive to DCMU during cell division. Additional support for this conclusion is provided by flash-induced fluorescence measurements of the reoxidation kinetics of Q_A^- in the presence of 10 μM DCMU, which indicated that about 20% of the PSII centers were not inhibited (data not shown). The O_2-evolution activity measured in saturating light gave titration curves similar to those found under light-limited conditions (Fig. 7, compare solid and open symbols).

Thylakoid Membrane Organization

Figure 8, A and B, shows light micrographs of a four-cell coenobium observed during the peak of photosynthetic capacity (Fig. 8A) and a mother coenobium before release of new coenobia observed at the beginning of the dark period (Fig. 8B), which corresponded to the period of minimum photosynthetic capacity. Corresponding electron micrographs of thylakoid membranes are shown Figure 8, C and D. During the period of maximum O_2-evolving capacity, the thylakoids were strongly stained, yielding sharp contours in the image and showing stacks of appressed membranes (Fig. 8C). The stacked membranes were two to three membranes thick and extended through the entire chloroplast. In contrast, dividing cells were poorly stained, yielding low-quality images. The thylakoids appeared as short stacks and contained many more appressed membranes (Fig. 8D). Because the stacked membranes in dividing cells resemble grana of higher plants, they are sometimes referred to as pseudograna. The difference in staining by osmium tetroxide between the cells in the middle of the light phase and in dividing cells revealed that the thylakoid membranes themselves were quite different. In dividing cells the thylakoids were less accessible to staining, which may be related to the lower accessibility to the DCMU.

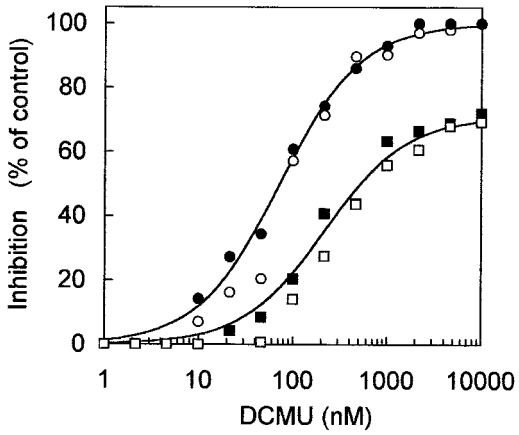

Figure 7. Inhibition of the light-saturated (open symbols) and light-limited (closed symbols) O_2 evolution by various concentrations of the herbicide DCMU in *S. quadricauda* cells harvested in the 5th h of the light period (circles) and during cell division (squares). The cells were incubated with the herbicide for 5 min. Doubling of the incubation time had no effect.

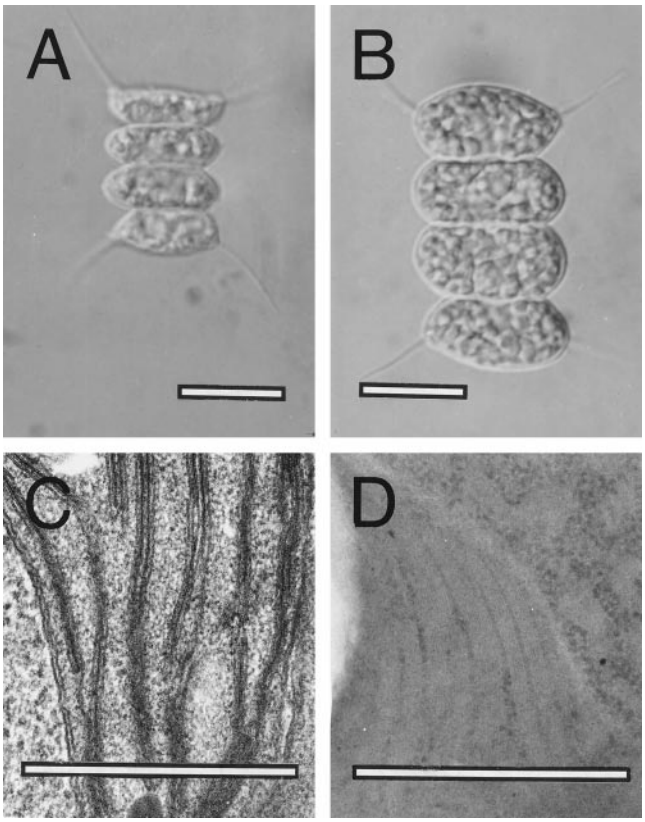

Figure 8. Optical micrographs of *S. quadricauda* cells (A and B) and electron micrographs of thylakoid membranes (C and D) are shown for cells harvested during the 5th h of the light period (A and C) and at cell division (B and D). Bars = 10 μm in A and B and 1 μm in C and D.

DISCUSSION

Measurements of the light-saturated rate of CO_2-dependent O_2 evolution of synchronously grown cells of *S. quadricauda* showed that the maximum rate of photosynthesis changed significantly during the cell cycle (Fig. 1). During the first 3 h of the 14- to 16-h light period, the light-saturated rate of O_2 evolution increased approximately 2-fold. The maximum rate was maintained for a few hours and then steadily decreased during the last 8 h of the light period. The rate of O_2 evolution reached a minimum at the beginning of the dark period (8–10 h), which coincided with the onset of cell division. The PSII capacity remained low throughout the dark period.

The Increase in the Maximum Rate of Photosynthesis in the Initial Phase of the Light Period Is Not Controlled by the Activity or Concentration of PSII Centers

To determine the role of PSII in controlling the overall rate of photosynthesis we measured the flash-induced yield of O_2, which revealed the maximum capacity of PSII centers independently of subsequent electron-transport reactions. This is because the single-turnover flashes were given at a low rate so that the plastoquinone pool into which PSII transfers electrons remained oxidized. The flash-induced O_2 yield at the beginning and at the 4th h of the light period showed no increase in the capacity of PSII on a chlorophyll basis (Fig. 2). During this same period the maximum rate of steady-state O_2 evolution increased more than 100% (Fig. 1). These data reveal that the increase in the rate of steady-state O_2 evolution is not due to an increased PSII capacity but to an increased rate of electron flow subsequent to PSII. This conclusion is supported by measurements of fluorescence induction, which showed an increasing capacity of the electron-transport chain to oxidize the plastoquinone pool during the first few hours of the light period (Fig. 6, A and B).

PSII Limits the Maximum Rate of Photosynthesis at the End of the Light Period, as the Cells Prepare for Division

The steep decline in photosynthetic capacity observed toward the end of the light period (Fig. 1) can be accounted for by limited PSII activity. This conclusion is based on the observation that the capacity of PSII decreased 80% between the 8th h of the light period and the beginning of the dark period (Fig. 2). The pattern of the decrease of PSII activity (Fig. 2) was similar whether the O_2-evolution capacity was measured under light-saturated or light-limited conditions.

Characterization of PSII Heterogeneity during the Cell Cycle

Although it is well known that PSII exists in multiple forms in algae and higher plants, the physiological consequences of this heterogeneity are not understood. To address this problem we investigated PSII heterogeneity throughout the cell cycle of *S. quadricauda* and identified active and inactive centers, $PSII_A$ and $PSII_X$, as well as $PSII_\alpha$ and $PSII_\beta$.

Figure 4 shows that during the first 10 h of the light period the fluorescence emission of $PSII_X$ centers remained fairly constant (Fig. 4). The most significant change during this period was the decline in the number of $PSII_\beta$ centers observed at the onset of the light period. The $PSII_\beta$ centers nearly disappeared by the 4th h of the light period. In parallel with the loss of $PSII_\beta$ centers was a 20% decrease in the effective antenna size of $PSII_\alpha$ centers (Fig. 5).

A comparison of Figures 1 and 2 shows that the decline in photosynthetic capacity during the last half of the light period (Fig. 1) mirrored a decline in the amount of $PSII_A$ (Fig. 2). We estimate that the number of $PSII_X$ centers increased from about 3% to 10% during this same period (Fig. 4). In the dark period the number of $PSII_X$ centers declined rapidly and remained low for the remainder of the dark period and the first half of the light period. These data show that the decline in photosynthetic O_2 evolution during the last half of the light period corresponded to an increase in $PSII_X$ centers, but there was not a one-to-one conversion of active to inactive centers. In the subsequent dark period there was a rapid decline in $PSII_X$ centers, whereas there was no change in the rate of photosynthetic O_2 evolution (Figs. 1 and 4). We conclude that the transient increase in the $PSII_X$ population at the end of the light

phase is likely due to a light-dependent process occurring in dividing cells. One possibility is that $PSII_X$ is related to the PSII repair cycle, as proposed by Neale and Melis (1990).

It is noteworthy that the decline in the activity of PSII reaction centers occurring in the last half of the light phase (Figs. 1 and 2) and the increase in fluorescence emission of $PSII_X$ correlated with changes in the organization of the thylakoid membranes (Fig. 8). To explain these observations we propose as a working hypothesis that the PSII-repair cycle may be modulated because of the dramatic changes occurring in the thylakoid membrane (Fig. 8). The increase in $PSII_X$ centers at the end of the light period is consistent with an impaired repair cycle. In the dark period there was no photodamage, and as a result $PSII_X$ centers gradually returned to the low levels observed at the beginning of the dark period. High photosynthetic activity began to be restored at the beginning of the subsequent light phase, when light-induced PSII activation began (Greer et al., 1986).

A Fraction of PSII Centers Become Insensitive to DCMU during the Cell Cycle

Approximately 30% of PSII centers were insensitive to the inhibitor DCMU during cell division (Fig. 7). This is a surprising result because DCMU is an effective inhibitor of PSII. One possible explanation of these data is that the DCMU-binding site in 30% of the active PSII centers was modified (Shochat et al., 1982; Vermaas and Steinback, 1984). Another possibility is that 30% of PSII centers were physically inaccessible to DCMU. It is noteworthy that electron micrographs revealed significant changes in the organization of the thylakoid membranes during cell division, including an increase in membrane stacking, which could limit the accessibility of some PSII centers to DCMU.

CONCLUSION

As a working hypothesis to account for the data presented here, we propose that during the early light phase of the cell cycle the photosynthetic capacity of the cells increases, whereas the population and activity of the PSII reaction centers remains relatively constant. The maximum rate of photosynthesis is sustained for 3 to 4 h and corresponds to a phase of rapid cell growth. During this time of high photosynthetic activity the cells appear to use PSII at maximum capacity. After the cell accumulates enough reserves for the next cell division, which occurs in the middle of the light period, photosynthetic activity declines and the cells undergo a dramatic reorganization of the thylakoid membranes. During this period energy fixation is not a high priority, and the number of active PSII centers steadily declines, reaching a minimum during cell division. We propose that the altered morphology and composition of the thylakoid membranes observed in dividing cells, as revealed by poor staining and by limited accessibility to DCMU, may inhibit the normal operation of the PSII-repair cycle. Any interruption of the repair cycle would lead to a decline in active PSII centers. This idea is supported by the transient increase in inactive PSII centers during cell division if $PSII_X$ centers are intermediates in the repair cycle. In this model the subsequent decline of $PSII_X$ in the dark is due to partial completion of the repair cycle. The complete restoration of PSII activity, which is a light-dependent process, begins at the start of the next light period.

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