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Development of Photosystem II Complex during Greening of *Chlamydomonas reinhardi* y-1

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ABSTRACT

The relative content of organized pigment, active centers, and acceptor pools of photosystem II and their interconnection during the development of the photosynthetic membranes of *Chlamydomonas reinhardi* y-1 have been measured using the fluorescence induction technique. The degree of connectivity and efficiency of the developing system has been assessed also from measurements of maximal rates, quantum yield, and flash yield of 2,6-dichlorophenolindophenol photoreduction using H_2O as the electron donor. The results obtained indicate that the process of membrane development in this organism consists of two phases: an initial phase of reorganization and connection between pre-existing components, and a second phase of actual accumulation of newly formed, complete, and active units. The ratio of active centers to Chl remains practically constant throughout the process while the degree of connectivity between the active center and the plastoquinone pool was doubled during the early phase of the greening. In addition the degree of connectivity between the plastoquinone pool and the rest of the electron transport chain increases as demonstrated by a 10- to 20-fold rise in the quantum yield and a 10-fold rise in the maximal rate and the flash yield. The ratio of light harvesting Chl to active centers remains apparently constant during the second phase of the greening as indicated by light saturation experiments and by the constancy of the apparent photosynthetic unit size. Electron donation from H_2O seems to develop slower than the activity of the rest of the complex as demonstrated by measurements of 2,6-dichlorophenolindophenol photoreduction using 1,5-diphenylcarbazide as the electron donor. The value of all the above parameters which remain constant during the second phase of the greening are comparable to those obtained with membranes of light-grown cells.

The process of chloroplast membrane biogenesis has been the subject of extensive work carried out in many laboratories. The results obtained so far have been reviewed recently (5, 18, 28). The process of assembly of components and membrane development can be summarized as follows. The chloroplast membranes grow by progressive insertion of newly formed components within pre-existing membranes. The addition of the new components can occur simultaneously or stepwise, and in the second case can result in continuous rearrangements and organizational changes within the membrane, detected at the ultrastructural level (17) and accompanied by drastic changes in photosynthetic activity (12, 27, 37). The stepwise addition of membrane components in *Chlamydomonas reinhardi* was so far demonstrated for two classes of polypeptides (11, 16) associated with the binding of Chl and the formation of the active centers of both photosystems (2, 11, 16). In addition, a developmental sequence of events related to the formation of P_{700} and light harvesting Chl has been described (10).

The development of O_2 evolution activity during greening of a dark-grown *Chlorella* mutant has been described by Herron and Mauzerall (15) and measurements of both activation of O_2 evolution and variable fluorescence during greening of another *Chlorella* mutant have been reported by Dubertret and Joliot (7). The data obtained by both groups indicate that in these mutants active centers connected to the electron transport chain are present in the membrane at the very beginning of the greening and additional units are made during the process. It appears that in both mutants the photosynthetic unit size increases during greening due to addition of Chl *b* (7) or light harvesting Chl (15). However, the above data give only partial information on the process of photosystem II development.

Herron and Mauzerall (15) measured O_2 evolution connected to CO_2 fixation, that is overall photosynthetic activity. On the other hand, Dubertret and Joliot (7) measured only the primary photoact of photosynthesis isolated out of the total activity of PSII. The data reported by the above-mentioned authors have been obtained using whole cells. More information on the development of PSII activity should be expected if isolated membranes are used in which different partial reactions can be measured.

Extensive information is now available on the development of the photosynthetic membranes of *C. reinhardi* using either wild type synchronized cultures (4, 33) or the greening process of the y-1 mutant (28). This information includes development of the membrane-bound and soluble proteins (11, 30), phosphorylation (3, 37), synthesis of Chl, carotenes, and membrane lipids (14), electron carriers such as Cyt *f* (30, 34), plastocyanin (10), ferredoxin, CO_2 fixation cycle enzymes (30), and the underlying process of control by light of the chloroplast differentiation (28).

A detailed analysis of the development of PSII activity in the y-1 mutant would be useful because of the ability to integrate the data within the framework of already existing information.

The PSII complex of the membrane can be considered to consist of the water-splitting system, the active center including the primary acceptor Q, the light harvesting Chl, and the connection via the plastoquinone pool to the rest of the electron transfer chain (20).

Since one deals with a developing system possessing initially membrane remnants (29, 30), one should consider that certain components of the PSII complex might be present, while others can be made *de novo*; some might be unconnected or misassembled and become properly organized in the early phase, and

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others might be added only at a later phase of the process. Measurements of the functionality of the whole chain will disclose only the presence of complete units, while measurements of partial reactions in combination with measurements of overall activity will permit identification of the components which are not yet part of a complete unit. By use of assay systems which measure overlapping partial reactions, it should be possible to gain information on both the presence and synthesis of components and the process of their integration into a complete active unit. Measurements of overall electron transfer activity from natural or artificial donors, intrinsic fluorescence, variable fluorescence, relative induction time, quantum yield, and flash yield could enable us, when combined simultaneously, to obtain such information (see below).

In the present work, the sequence of events leading to the formation of the active center and the light harvesting system of PSII and their connection with the electron transfer chain during the greening process of *Chlamydomonas reinhardi* y-1 has been investigated using this approach. The results show that during the early phase of the greening (0 to 4 hr) a drastic reorganization of the membranes occurs, expressed as a sharp decrease in the apparent size of the photosynthetic unit and an increase in the efficiency of the system as measured by the quantum yield and maximal rate of DCIP[3] reduction. This indicates an extensive connection of existing active centers to electron transfer chains which occurs during this phase. In addition a marked decrease in the relative amount of nonvariable fluoresence is observed implying organization of Chl into the photosystems. During the remainder of the greening process, complete and active PSII units are added to the developing membrane.

MATERIALS AND METHODS

Preparation of Cells and Greening Experiments. *Chlamydomonas reinhardi* y-1 cells were grown either in the dark or in the light on a mineral medium containing acetate as a carbon source as previously described (29). Cells were harvested in the logarithmic phase of growth (1.5 to 2.5 $\times$ 10^6 cells/ml). For greening experiments cells grown in the dark for five to six generations were used (29). The cells were harvested by centrifugation at 3000*g* for 5 min, washed in fresh growth medium, and resuspended in the same medium to a final concentration of 0.8 to 1.2 $\times$ 10^7 cells/ml. The cell suspension was then exposed to white fluorescent light (about 10^4 ergs cm^{-2} sec^{-1}) as described (29). Samples of cells were taken after different periods of exposure to the light and a photosynthetic membrane enriched fraction was prepared as described below. During greening the cell concentration remained constant, *i.e.*, no division occurred before 14 to 18 hr of illumination (29).

Preparation of Membranes. The cells were washed by centrifugation and then resuspended in a medium containing 30 mM tris-HCl buffer, pH 7.5, 10 mM KCl, and 1 mM $MgCl_2$ (TKM buffer), to a final concentration of 0.5 to 1 $\times$ 10^9 cells/ml. The cell suspension was then passed twice through an ice-cooled Yeda Press operated at 1,400 p.s.i. using argon as the gas phase. In some cases the cells were broken using a French press operated at 3,000 p.s.i. (29). The broken cells were centrifuged at 3,000*g* for 2 min and the supernatant centrifuged again at 10,000*g* for 10 min in a Sorvall refrigerated centrifuge at 0 C. The pellet obtained was resuspended in the same buffer, stored in ice, and used within 1 to 1.5 hr. The concentration of cells and final volume of membrane suspension were chosen in such a way as to obtain when possible about 200 to 500 μg Chl/ml. The minimum concentration used at the early phase of greening was 50 μg/ml. The dark-grown cells contain a relatively high concentration of starch (29) and thus the membranes prepared from such cells, as well as those obtained at the beginning of the greening process, were usually contaminated slightly with starch particles. In order to avoid an unnecessary increase in the turbidity of the sample, and thus render spectrophotometrical measurements difficult, it was essential to remove the membrane pellet without disturbing the starch particles, which sedimented as a very tight pellet below the membranes.

Tris Treatment of Membranes. For measurements of electron transfer activity using DPC as an electron donor (36), the membrane pellet was resuspended in 0.8 M tris-HCl buffer, pH 8.3, and kept in ice for 3 min. The membranes were then centrifuged for 10 min at 10,000*g*, and resuspended in TKM buffer as described above. The concentration of tris required to abolish electron transfer from H_2O while preserving maximal activity of electron transfer from DPC was found to be 0.6 M to 0.8 M.

Measurements of Photosynthetic Activity. Hill reaction using DCIP as an electron acceptor was carried out in spectrophotometric cells. The reaction mixture consisted of 2 ml TKM buffer containing 5 μM DCIP and chloroplast membranes to give a final concentration of 5 to 10 μg Chl/ml. In some experiments the Hill reaction was measured in tris-HCl buffer without magnesium (TK) and using 0.2 mM DCIP. The activities measured in this way were similar to those obtained using the above-mentioned system. The reduction of DCIP was followed spectrophotometrically using an Amino-Chance dual wavelength spectrophotometer provided with side illumination and employed in the double-beam mode. The difference in absorbance was measured at 580 to 520 nm ($\Delta\epsilon$ = 1.05 $\times$ 10^4 liter$\cdot$$mol^{-1}$) or 590 $-$ 520 nm ($\Delta\epsilon$ = 1.2 $\times$ 10^4 liter$\cdot$$mol^{-1}$) depending on the turbidity of the membrane preparation used. In flash yield experiments the wavelengths used were 625 and 695 nm ($\Delta\epsilon$ = 1.45 $\times$ 10^4 liter$\cdot$$mol^{-1}$).

Excitation light was obtained from a Braun slide projector equipped with a 500-w tungsten lamp connected to a stabilized DC power supply. The exciting beam was filtered through a 685 nm interference filter (5 nm half-width; Baird Atomic Inc). For maximal rate measurements excitation was passed through a broad band K-6 Balzer's filter (650 to 690 nm) (Balzer's Aktion Gesellschaft, Lichtenstein). The light intensity was varied, using neutral density filters, from 10^{-11} to 10^{-8} einsteins $cm^{-2}\cdot sec^{-1}$. The photomultiplier was protected with two broad band filters (DT green, Balzer's) and one Corning 4-96 filter transmitting between 500 and 600 nm. For flash experiments an ILC 268 lamp (ILC, Sunnyvale, Calif.) was used with a flash duration of ~5 μsec and at a repetition rate in the range of 5 to 20 flashes/sec.

For calculating quantum yield of the DCIP reduction, the fraction of light absorbed in the reaction mixture was measured with an integrating sphere built in this laboratory. Light intensity of the exciting beam was measured with a silicon cell photometer assembled in our laboratory calibrated against a Yellow Spring radiometer or directly measured by the second instrument.

Fluorescence induction measurements were done as described previously (24). The apparatus consisted of a photomultiplier at 90° angle to the exciting light beam. Exciting light (4 to 6 $\times$ 10^{-9} einsteins $cm^{-2}\cdot$ sec^{-1}) was filtered through a 520 to 640 nm broad band Balzer's filter and a Schott OG-590 cut-off filter. The photomultiplier received light through a 685-nm Baird Atomic interference filter of a very low intrinsic fluoresence. The signal was displayed on a Tektronics 564 oscilloscope equipped with a memory screen. Far red light was given between measurements on the same sample using a 726 nm Baird Atomic interference filter. When DCMU was used it was added after the far red illumination. The opening time of the shutter was 2 to 3 msec. For measurements of the fast and slow phases of fluorescence induction a time scale of 10 to 20 msec/division and of 0.1 to 1 sec/division were used, respectively. All measurements were done at room temperature.

[3] Abbreviations: DCIP: 2,6-dichlorophenolindophenol; DPC: 1,5-diphenylcarbazide.

Chl was quantitated as described by Vernon (35) using a Cary model 1608 spectrophotometer. Cell concentration was measured with a hemocytometer.

ANALYSIS OF DATA

Active and Inactive Pigments. For the interpretation of the data we shall distinguish between two kinds of pigment populations: *(a)* disconnected, photochemically inactive pigments, which are not yet assembled into active reaction centers, and *(b)* connected, active pigments. Since we consider only the data relevant to PSII, any pigment which belongs specifically to PSI is classified as (*a*). Such a distinction assigns for each Chl of population (*a*) a zero probability of excitation transfer to a PSII reaction center. Similarly, each Chl of population (*b*) has a high probability of excitation transfer. Alternatively, to some extent at least, the results may be interpreted in terms of a probability distribution for excitation transfer. For example, if $f = [Chl_a]/[Chl_t]$ represents the fraction of active Chl with respect to the total Chl, according to the concept of two populations of Chl, it can also be defined as

$$f = \sum_{i=1}^{N} p(i)/N$$

where $p_{(i)}$ is the probability of energy transfer from a Chl molecule i to the reaction center and N is the total number of Chl molecules.

Fluorescence Induction. We adopt the point of view that the fluorescence rise during illumination, of initially dark adapted membranes, follows directly the reduction of the primary acceptor (8, 13). The fluorescence parameters are: F_0, the initial level after dark adaptation, F_m, the maximal level, and A, the normalized area above the fluorescence curve, which expresses the induction time (24). The scheme in Figure 1 illustrates a typical induction curve. In the presence of DCMU, only a fast phase of rise exists, reflecting the reduction of Q only. Without DCMU, there is in general a combination of fast and slow rise phases, which may be interpreted as reflecting, respectively, units in which Q is disconnected or connected to the plastoquinone pool (25).

The parameter F_v can be expressed by the constants of the photosynthetic unit (24):

$$\frac{F_v}{Chl} = \alpha \cdot \frac{I}{Chl} \cdot \frac{[Chl_a]}{[Chl_t]} \left(\frac{k_F}{k_F + k_H} - \frac{k_F}{k_F + k_H + k_P} \right) \quad (1)$$

where α is a geometrical factor of the measuring apparatus (including detector sensitivity), I is the intensity of absorbed light, $[Chl_a]/[Chl_t]$ is the fraction of excitation channeled to the photosynthetic primary reaction; k_P, k_H, and k_F are rate constants for excitation decay by photochemistry, radiationless transition, and fluorescence, respectively. These constants define the behavior of the pigment system which is connected to an active reaction center, *i.e.* active pigments; $k_F/k_F + k_H$ is the fluorescence quantum yield for a "closed" reaction center (corresponding to F_m, Fig. 1) and $k_F/k_F + k_H + k_P$ is the fluorescence quantum yield for an "open" reaction center (corresponding to F_0, Fig. 1). However, we cannot equate the experimental F_0 with $k_F/k_F + k_H + k_P$ since there is an undefined background fluorescence, which may come from inactive pigments (26). We believe, and this is further supported by the consistency of the data, that the "true" F_0 is small compared to the background and the change of the apparent F_0 during development reflects the conversion of the inactive to the active form of the pigments (see also Clayton [6]).

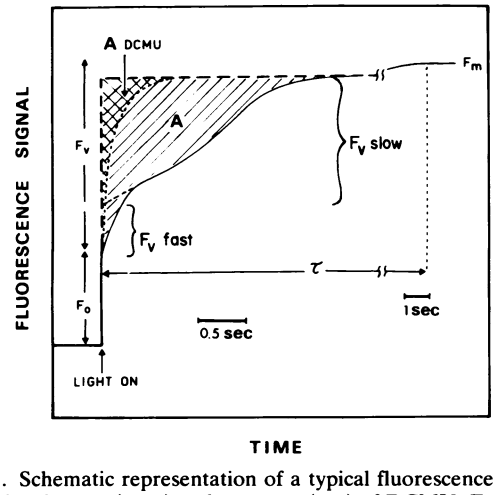

FIG. 1. Schematic representation of a typical fluorescence induction curve in the absence (——) and presence (· · ·) of DCMU. For explanation see "Analysis of Data."

F_v is an extensive parameter, *i.e.* it depends on the quantity of material in the measured sample. Therefore, it is normalized to a given amount of Chl. At the very low Chl concentrations, which were used in our experiments, the fraction of light absorbed is proportional to the Chl concentration. For samples obtained at different times during greening it is not evident that the fraction of light absorbed at 520 to 640 nm is the same for the same total Chl concentration, taking into account the changes in the ratio of Chl *a* to Chl *b*. We have found that such changes do occur; the absorption decreases during greening by a factor of 1.5 to 1.8. Since the incident light intensity was kept constant in all experiments, it is I/Chl which is affected by this change.

The normalized induction area in presence of DCMU (A_{DCMU}) (Fig. 1) reflects the concentration of PSII active reaction centers (C_{II}). Since C_{II} is also an extensive parameter, one has to normalize it for a given amount of Chl. Using the same reasoning as above we may write:

$$\frac{C_{II}}{Chl} = \frac{I}{Chl} \cdot \frac{a}{[Chl_t]} \cdot \frac{k_P}{k_F + k_H + k_P} \cdot A_{DCMU} \quad (2)$$

(In this formula $[Chl_a]/[Chl_t]$ takes the place of α_{II} and $k_P/k_F + k_H + k_P$ replaces ϕ_{II} in reference 24.)

Normal fluorescence induction (without DCMU) reflects the reduction of both the primary acceptor and the plastoquinone pool, and usually consists of a fast and a slow phase (Fig. 1). The fast phase reflects the units which are not connected to the pool. They are active with respect to the primary reaction, but inactive with respect to the electron transfer. The ratio of connected units to total units is given by the ratio of extent of the slow phase to extent of the total variable fluorescence (25).

The normalized area above the induction curve, A, is proportional to the concentration of the total plastoquinone pool [PQ] connected to and including the electron acceptor Q. The normalized [PQ] to Chl ratio ([PQ]/Chl) will be given by:

$$\frac{[PQ]}{Chl} = \frac{I}{Chl} \cdot \frac{[Chl_a]}{[Chl_t]} \cdot \frac{k_P}{k_F + k_H + k_P} \cdot A \quad (3)$$

The plastoquinone pool can be detected as soon as there is a measurable fraction of units connected to the pool. Since several PQ molecules can be reduced by the same PSII center (25), all of the available pool is reduced during induction, even though a considerable fraction of reaction centers might not yet be connected to the pool.

Actual experimental fluorescence induction curves showed additional complexity which has not been observed before in other organisms: *i.e.* DCMU did not abolish completely the slow phase. Even at a very high DCMU concentration a certain additional slow increase in the fluorescence (10% of the total extent) always remained. This can be interpreted in two alternative ways: *(a)* a slow adjustment of the parameters k_F, k_H, k_P to

the new conditions formed by the illumination occurs, which in turn changes the parameters F_0 and F_m but does not reflect any change in the reduction state of Q. Such an adjustment might happen if for instance the closed reaction centers which are electrically polarized may influence secondary structures, which modify in turn the pigment arrangements. *(b)* part of the reaction centers may be inaccessible to DCMU.

In our analysis of A_{DCMU} we simply disregarded this slow phase, and normalized the extent of the fast phase only. This could introduce an error of about 10 to 15% in the relative estimation of A_{DCMU}.

A very slow phase of additional increase in fluorescence occurs also in absence of DCMU (10% of the total extent) and may be explained in a similar way as in *a* above. This slow phase introduces an additional parameter τ shown in Figure 1. For the sake of completeness, changes in the value of τ will also be reported.

Flash Yield (FY) of DCIP Reduction. This parameter is obtained by a variation of the Emerson and Arnold (9) technique for the determination of the photosynthetic unit size. It is reasonable to assume that only reaction centers connected to an intact electron transport chain (to the point of DCIP entry) can contribute to the flash yield. Hence:

$$\frac{FY}{\text{Chl}} = \frac{C_{II} \cdot \beta}{\text{Chl}} \tag{4}$$

where β is the fraction of total reaction centers which can transfer electrons to DCIP.

Quantum Yield (γ) of DCIP Reduction. The steady state quantum yield of DCIP reduction is a product of three factors: (*a*) the fraction of the light channeled to active centers; (*b*) inherent quantum yield of the active centers; and (*c*) the fraction of units connected to an intact electron transport chain. Hence:

$$\gamma = \frac{[\text{Chl}_a]}{[\text{Chl}_t]} \cdot \frac{k_P}{k_F + k_H + k_P} \cdot \beta \tag{5}$$

Maximal Rate. The maximal rate (MR) is a measure of the rate-limiting step in the reduction of DCIP. If one assumes a unimolecular reaction, which includes an electron transport chain component, ETC, as a rate-limiting step, then the maximal rate is given by: $k[\text{ETC}]$ where k is the rate constant of the rate-limiting step and presumably does not depend on the greening process (38).

The implication that $[\text{ETC}] = C_{II} \cdot \beta$ is not self-evident since convergence or divergence of electron transfer chains to one or more PSII centers is an open possibility (22, 24).

RESULTS

Changes in Photoreduction of DCIP during Greening. The photoreduction of DCIP is an overall measure of electron transfer activity related to photosystem II (21, 31). Measurements of this activity close to or at saturating light intensity during the greening process show that the DCIP photoreduction/cell is rather low at the early phase of the greening and increases more than 10-fold as Chl synthesis proceeds (Fig. 2A). The same activity was observed also in tris-treated membrane preparations using DPC as an electron donor. In this case the activity also increased during greening in a way similar to the normal activity, except at the initial stage of the greening, when the DPC-dependent activity was higher (Fig. 2). The same results with DPC were reproduced at this initial stage even with membrane preparations which were not treated with tris.

Since the rise in activity/cell parallels the rise in Chl content, one could assume that the overall increase in photosynthetic electron transfer/cell might be due to accumulation of newly formed photosynthetic membranes having equal and constant specific activity/Chl. However, when the results are calculated on a Chl basis we find a drastic increase in the activity of electron transfer from H_2O or DPC per Chl (Fig. 2B) indicating that the specific photosynthetic activity of the membranes increases as membranes are formed.

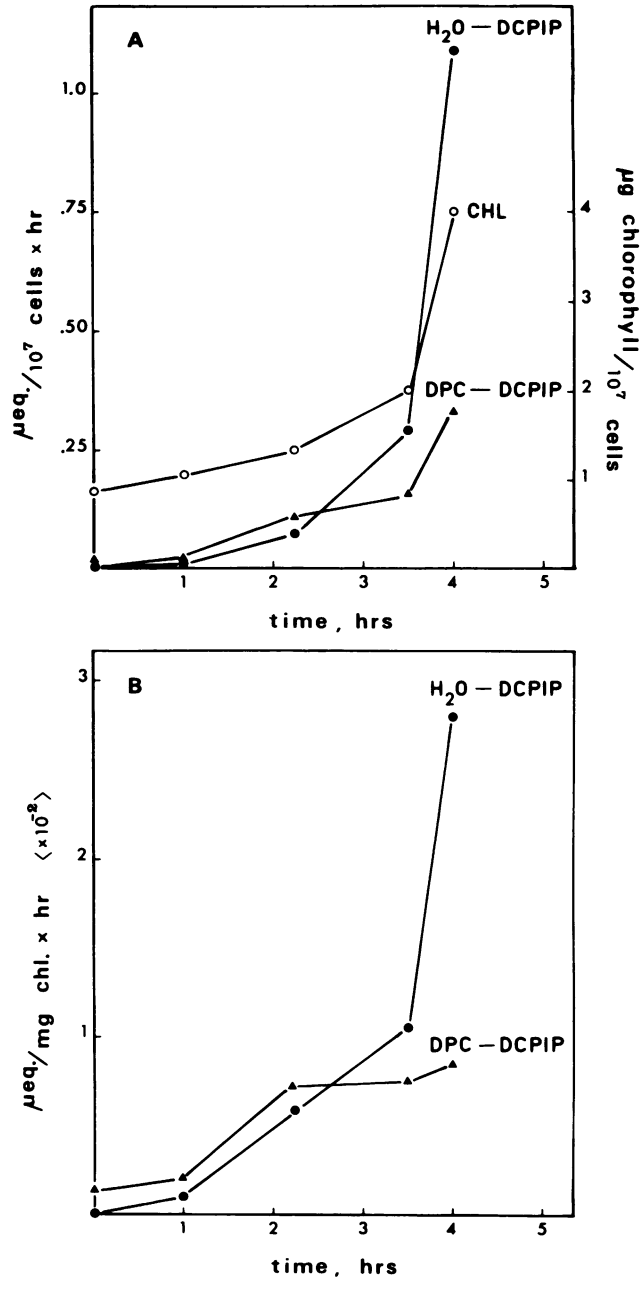

FIG. 2. Changes in the maximal photoreduction rate of DCIP (DCPIP) during greening using H_2O or DPC as electron donors. The activity is expressed on a cell basis in A and on a Chl basis in B. The rate of DCIP reduction, using DPC as an electron donor, was the same at time zero when measured with membranes which have been treated with tris, as well as in the absence of tris treatment. The reaction was carried out in TK buffer; the DCIP concentration was 2×10^{-4} M. For more experimental details see "Materials and Methods."

Changes in Quantum Yield, Maximal Rate, and Flash Yield of DCIP Reduction during Greening Process. For accurate calculation of the quantum yield it is essential to ensure that the measurements are carried out in a range of light intensities that gives a linear response with changes in the exciting photon flux. For measurements of maximal rates one should be sure that light saturation is achieved at each time point of the greening process.

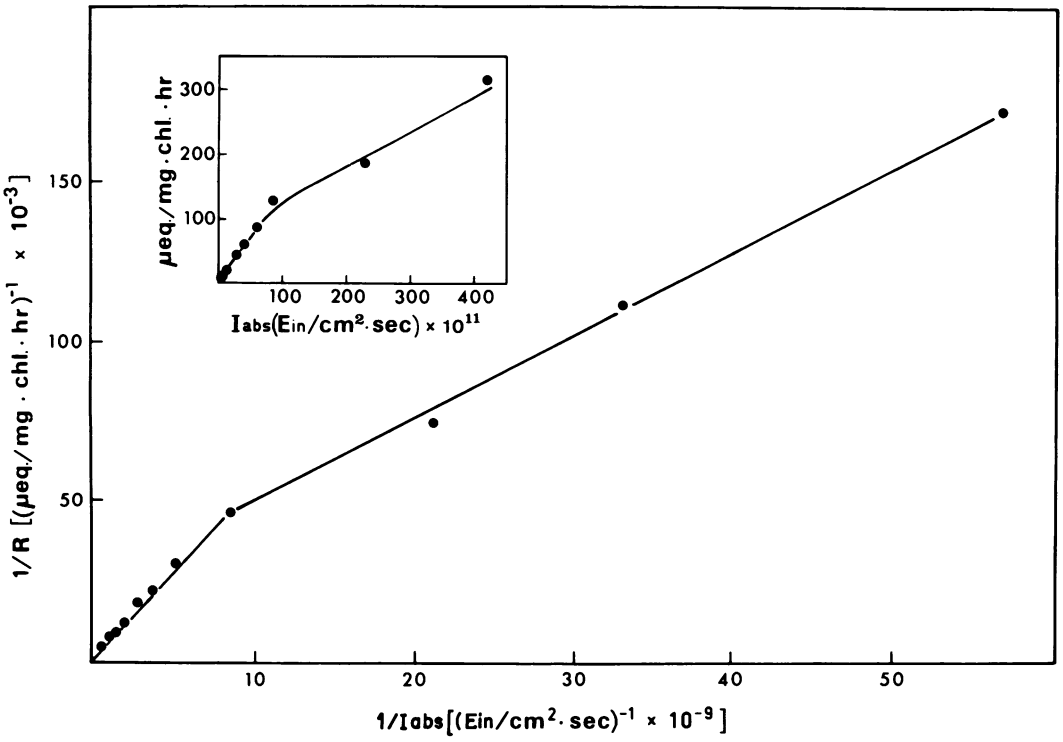

FIG. 3. Double reciprocal plot of the rate of DCIP reduction (R) *versus* light intensity (I_{abs}). The plot is expected to be linear according to the formula $R = \gamma \cdot MR \cdot I/(\gamma I + MR)$. The parameters γ and I are the same as under "Analysis of Data"; MR, maximal rate. The inset represents the raw data (rate *versus* light absorbed). In this particular experiment ([Chl] = 6 μg/10^7 cells) the high light intensity value of γ was 40%. Typically, this value ranged from 20 to 40%.

Measurements of reaction rates *versus* light intensity have been carried out and unexpectedly it was found that the resulting plot shows two linear parts with a break at absorbed light intensities of about 10^{-10} einsteins $cm^{-2} \cdot sec^{-1}$ throughout the greening process. A typical experimental result is shown in Figure 3. In this particular case one can calculate from the slope that the photoreduction of DCIP has a higher quantum yield (0.97 $e^-/h\nu$) at light intensities up to 10^{-10} einsteins $cm^{-2} \cdot sec^{-1}$ and decreases to 0.43 $e^-/h\nu$ at light intensities above this level. Saturation rates were obtained at values of 10^{-7} to 10^{-8} einsteins $cm^{-2} \cdot sec^{-1}$ depending on the time of greening. In all experiments in which quantum yield was measured the results were calculated from the slope of the first part of the plot[4] (Fig. 3) which is obtained at light intensities above 10^{-10} einsteins $cm^{-2} \cdot sec^{-1}$. The quantum yield was not measured in the low range of light intensity, since the activity especially at the early phase of greening was too low to be measured accurately. Membranes from green cells required lower light intensity to reach saturation of DCIP reduction. In the later stages of the greening process the saturating intensity remained rather constant while the saturated reduction rate increased approximately 20-fold.

Since, technically, it is extremely difficult to perform all the types of measurements used in this work on a single culture, one is compelled to combine the results obtained from different experiments performed on different cultures.

Different cultures green at slightly different rates, due to their previous history of growth in the dark. Especially the length of the lag period during which the rate of Chl synthesis increases progressively, varies between 2 and 4 hr (30). Thus, the Chl content/cell at a given time of illumination might vary in different experiments by a value of ± 1 μg/10^7 cells. The behavior of the cells in different experiments could be, generally, correlated with their Chl content. Therefore results of several experiments in which the maximal rate, quantum yield, and flash yield for the photoreduction of DCIP were measured as a function of the Chl content of the cells are shown in Figure 4. The data show a low value for these parameters at Chl levels of 0.5 to 2.0 μg/10^7 cells, a sharp rise of up to about 10-fold for the flash yield and maximal rate and up to 20-fold for the quantum yield at levels of 2.5 to 4 μg/10^7 cells, with no additional changes when the Chl content rises further to levels of up to 7 to 8 μg/10^7 cells or more (Fig. 4). The data in Figure 4 can be compared with those obtained in the light-grown wild type cells which show a maximal rate of about 400 to 700 μeq/mg Chl · hr and a corresponding quantum yield of 20 to 40% indicating that after greening the y-1 mutant indeed regains the wild type behavior.

The flash yield/Chl is the reciprocal of the apparent photosynthetic unit size. Measurements of the number of Chl molecules absorbing light which participate in the process of one electron transfer using the flash yield technique are difficult to perform at low Chl levels because of the low rate of electron transfer. Therefore the spread of data is rather large at a Chl level below about 3 μg Chl/10^7 cells. The typical value is about 5,000 Chl/unit[5] (Fig. 5). In several experiments (not shown here) values as large as 20,000 Chl/unit or more were calculated at Chl concentrations of 1 to 1.5 μg/10^7 cells. A fast decrease is obtained in the apparent size of the photosynthetic unit at levels between 3 and 5 μg/10^7 cells and a leveling off afterwards, when the value

[4] One should notice that a break point was obtained qualitatively at all times during the greening and was present also in light-grown cells as well as in isolated lettuce chloroplasts. On the other hand, such a behavior was not found by Sauer and Park (32) using the same range of light intensities. At the present time we cannot offer an explanation for this phenomenon. A nonlinearity at low light intensity of a different nature was observed in *Chlorella* by Herron and Mauzerall (15).

[5] The calculation of the photosynthetic unit size $(FY/Chl)^{-1}$ given here represents the apparent value of the unit without taking into consideration the fraction of reaction centers which are not connected.

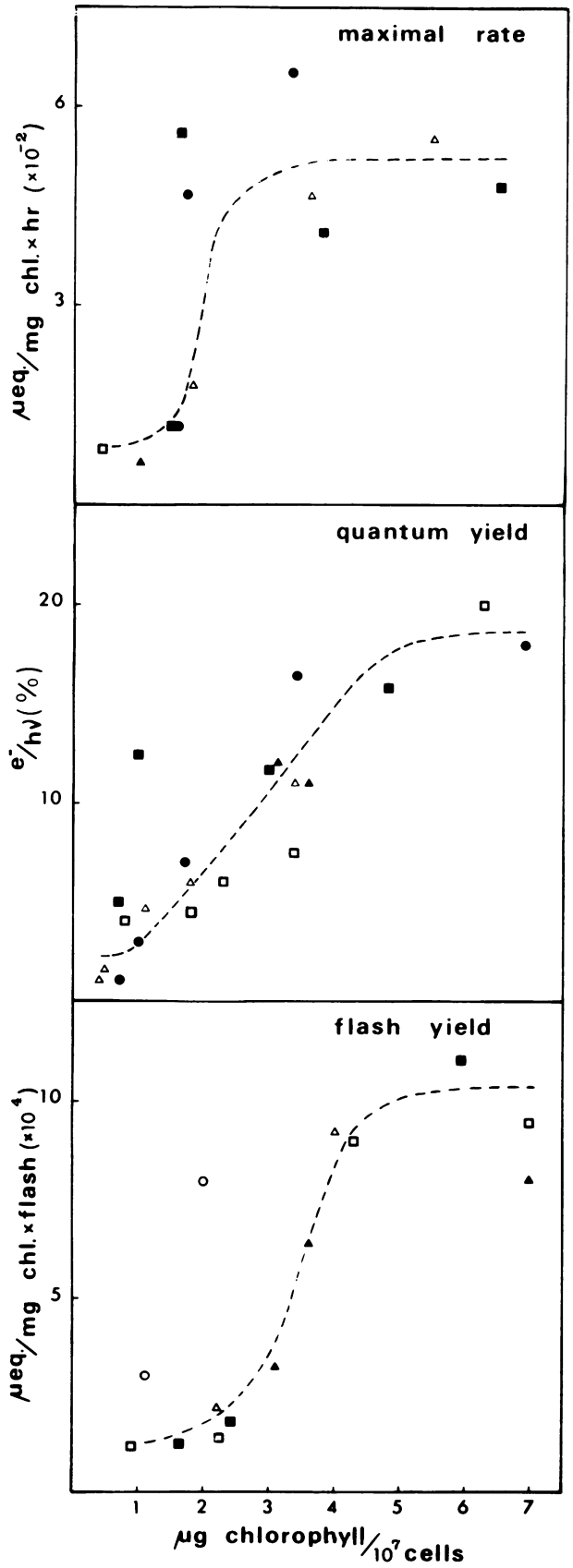

FIG. 4. Changes in maximal rate, quantum yield, and flash yield as a function of greening. Each curve represents data obtained from several experiments. For each measurable the points obtained from the same experiment are indicated by the same symbol. For additional experimental details see "Materials and Methods."

measured is about 1,000 Chl/unit and remains constant even at a Chl level of 14 μg/10^7 cells (Fig. 5). From these data it is evident that the number of units active in electron transport to DCIP relative to the Chl content of the membranes is very small at the onset of the greening process. However, during the part of the process in which most of the new membranes are formed, and the Chl content rises from about 3 to 14 μg/10^7 cells, the apparent size of the photosynthetic unit tends to become constant and similar to that found in light-grown cells, having a Chl content of up to 30 μg/10^7 cells. According to the analysis of data the apparent photosynthetic unit size contains the factor β and must be corrected by multiplying with the quantum yield γ in order to obtain the effective photosynthetic unit size. This value (about 200 Chl/unit) seems to change relatively little during most of the greening period except for the initial phase when it may reach values of up to 1,500 Chl/unit.

Changes in Fluorescence Induction during Greening. Fluorescence induction develops during greening as shown in Figure 6. It is evident that the slow phase of the induction is practically absent at the onset of the greening process; it develops during the first 2 to 3 hr of illumination and becomes characteristic of that obtained in normal light-grown cells after 4 to 5 hr of greening. Typical traces of the fluorescence kinetics obtained at different times are shown in Figure 7. From such traces the values of the parameter F_v, F_0, and A (see "Analysis of Data") were obtained. The results of such measurements are shown in Figures 8 and 9.

The value of F_v/Chl remains relatively constant throughout the greening process, indicating that the active PSII Chl/total Chl does not change significantly at all Chl concentrations/cell. However, the value of F_0/Chl decreases sharply (about 7-fold) when the Chl content/cell rises (from 1 to about 4 μg/10^7 cells) and remains constant afterward (Fig. 8). As expected F_v and F_0 are not affected by the presence of DCMU. Additional information can be obtained from the values of F_v (slow phase)/F_v (total extent), A and A_{DCMU}. The value of A_{DCMU} is very low and thus difficult to measure accurately. However, it appears that A_{DCMU} is rather constant during the greening process, whereas the value of A rises 2-fold during the initial greening period and becomes constant at Chl levels equal to or higher than 3 μg/10^7 cells (Fig. 9).

An attempt was also made to measure the parameter τ (see "Analysis of Data"). Its effect on the values of F_v is small ($\leq$5%). Accurate measurements of τ were difficult to make. However, a definite trend was observed toward an increase in its value at the early phase of greening followed by a decrease and leveling off at around 8 sec toward the end of the process, when Chl content rises to about 4 to 5 μg/10^7 cells.

DISCUSSION

The process of PSII formation in *C. reinhardi* y-1 appears to consist of two major phases: (*a*) an initial phase in which components of the system present in a disorganized state become properly connected and active. In this phase there is relatively little synthesis of new Chl compared to the change in the other parameters. In some experiments the extreme situation was encountered where changes in the observables were obtained without any accompanying increase of the Chl content/cell. (*b*) A phase of synthesis and constant accumulation of complete and active units which are added to the developing membranes.

Obviously the distinction between these two phases is somewhat artificial as they do overlap partially in time for any given batch of cells, and the degree of overlapping is variable for different cultures. This is evident from the wide spread of data in different experiments around the transition point which is defined as that of the drastic rise in the maximal rate, quantum yield, flash yield, and relative fluorescence rise time and the onset of rapid Chl synthesis (Figs. 4 and 9).

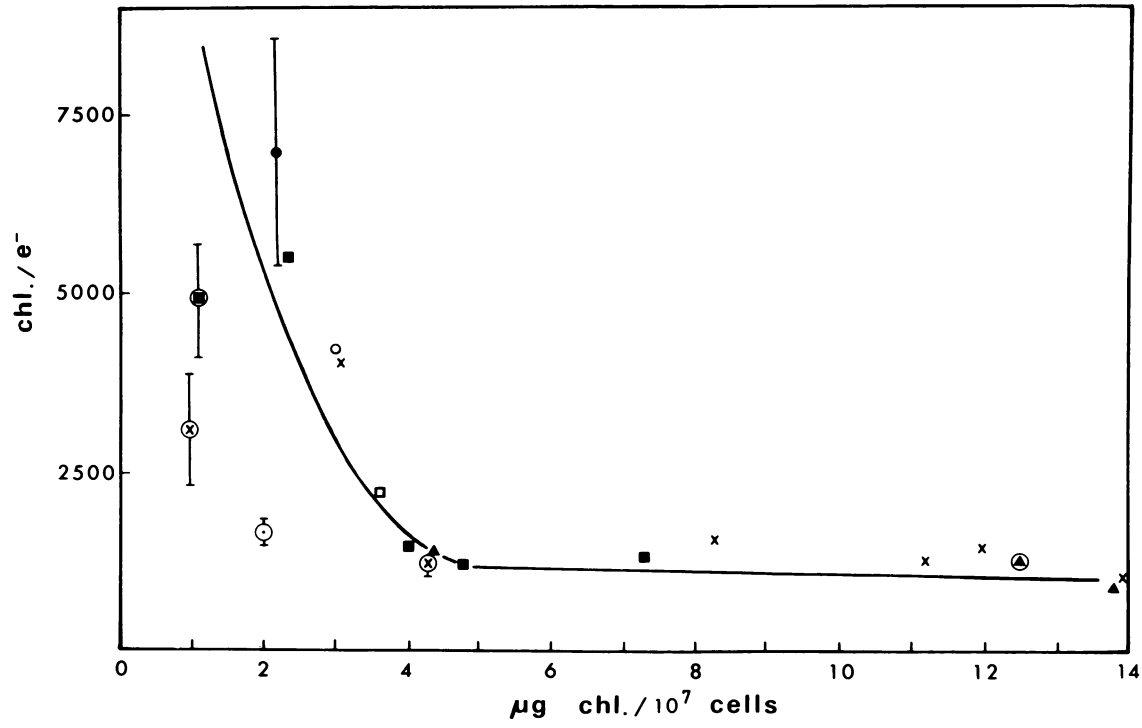

FIG. 5. Changes in the apparent size of the photosynthetic unit as a function of the Chl content/cell during the greening process. The reduction of DCIP using H_2O as an electron donor was measured using the flash yield technique.

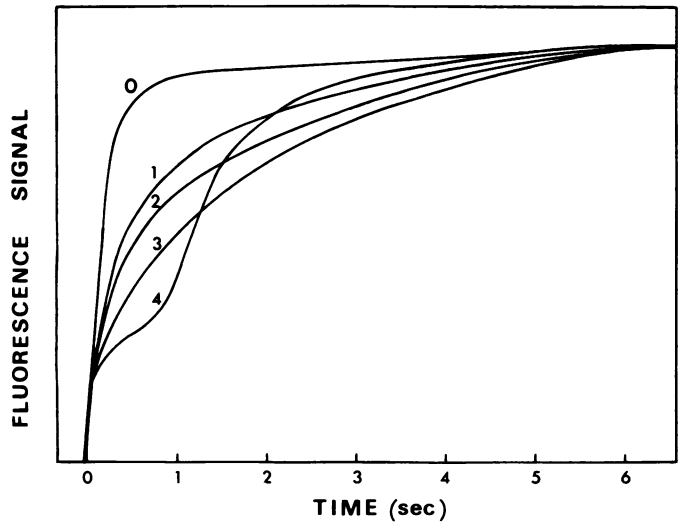

FIG. 6. Schematic representation of the changes in fluorescence induction curves obtained at different times during the greening process. The curves were taken from actual oscilloscope traces obtained at equal light intensities and were normalized to equal F_v/Chl levels; 0,1, 2, 3, and 4 represent different times of the greening process at which the Chl content was 0.8, 1.8, 2.3, 3.5, and 6.4 μg/10^7 cells, respectively (0, 1.8, 2.4, 3.4, and 5 hr, respectively).

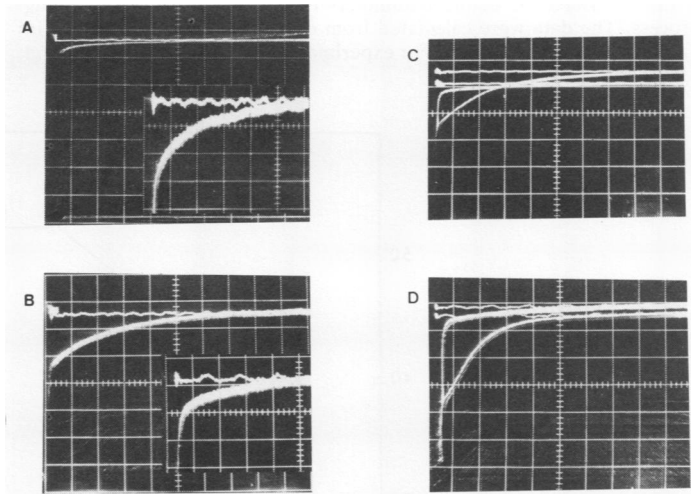

FIG. 7. Typical fluorescence induction traces obtained during the greening process of dark-grown y-1 cells. A, time zero (no greening), Chl content, 0.8 μg/10^7 cells; 100 mv and 0.1 sec/division. Inset: same sample, but with base line suppressed, 10 mv and 0.5 sec/division. At time zero no significant change is observed in the curves upon addition of DCMU. B, 2 hr greening; Chl content: 2 μg/10^7 cells; 1 sec and 20 mv/division. Inset: same sample with DCMU added to a final concentration of 10^{-5} M; 1 sec and 10 mv/division, base line suppressed. C, 5 hr greening, Chl content: 5.4 μg/10^7 cells; upper trace: without addition of DCMU, 0.5 sec and 50 mv/division; lower trace: with addition of 10^{-5} M DCMU, 0.5 sec and 50 mv/division. D, 8.3 hr greening, Chl content: 6.9 μg/10^7 cells; lower trace: without addition of DCMU, 1 sec and 20 mv/division; upper trace with addition of 10^{-5} M DCMU, 1 sec and 20 mv/division. Vertical scale: mv; horizontal scale: time.

The validity of the interpretation for the experimental results presented in this work is based on the fact that data obtained by different techniques and measuring independent different parameters concur in supporting the conclusions. Table I summarizes the relevant parameters extracted from the experimental observables based on the formulae derived under "Analysis of Data." The extensive parameters and observables (*i.e.* those proportional to the quantity of material) are expressed on a Chl basis. These are F_v, F_0, A_{DCMU}, A, flash yield, quantum yield, maximal rate, [PQ], [ETC], and C_{II}. Parameters such as β or $[Chl_a]/[Chl_t]$ are intensive parameters and do not depend on the quantity of the material. A specific parameter may appear in the formulae for several observables. A typical example is $[Chl_a]/[Chl_t]$ which appears in the expressions for F_v, QY, A, and A_{DCMU}. Because there are more parameters than equations, this overlap will help in arriving at some reasonable and consistent conclusions.

EARLY PHASE OF GREENING

Organization of Existing Components. This phase is characterized by a drastic reduction in the value of F_0 without a concomitant change in the variable fluorescence, F_v. The reduction in the value of F_0 is probably due to the conversion of nonorganized Chl having a relative high yield of fluorescence to a form which is intimately organized into the photosystem and having a low fluorescence yield.

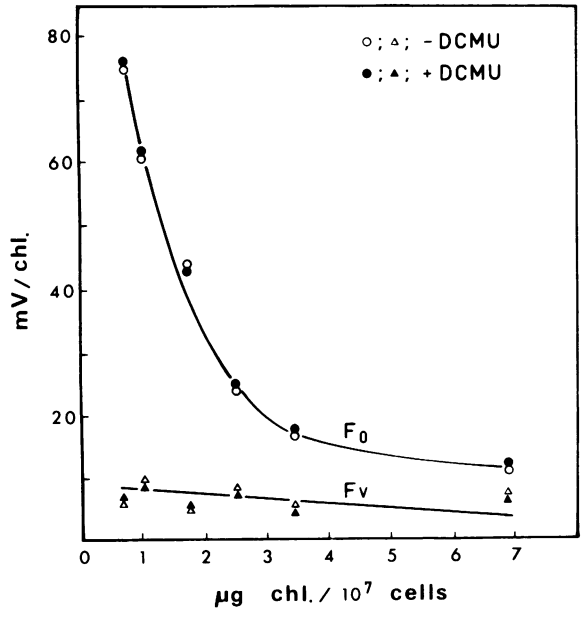

FIG. 8. Behavior of the parameters F_v and F_0 during the greening process. The data were calculated from oscilloscope traces as shown in "Analysis of Data" (Fig. 1). For experimental details see "Materials and Methods."

It is probable that the unorganized Chl present in the membranes of dark-grown cells becomes part of the PSI light harvesting system. This is suggested by the following data. (*a*) It has been shown that during this phase of the greening, the light harvesting Chl of PSI rises without a parallel rise in the total Chl content of the membrane (10). (*b*) As we shall show by analyzing the data of F_v and $A_{(DCMU)}$, the ratio $[Chl_a]/[Chl_t]$ (active Chl of PSII/total Chl) remains probably constant. Hence no additional organized Chl is incorporated in PSII. Further support for the hypothesis that part of the Chl present in the membrane of dark-grown cells is not properly integrated into the thylakoid membranes and thus causes a high F_0 value can be found in (*a*) the absorption spectrum of dark-grown cells showing a maximum at 671 nm as compared to 687 nm in light-grown, fully greened cells (10), and (*b*) the absence of the PSII protein-Chl complex in membranes from dark-grown cells. The Chl present in the membranes in the early phase of the greening migrates in SDS acrylamide electrophoresis as free Chl as compared to that found in the membranes of the later stages, which comigrates with a specific group of membrane peptides (Bar-Nun, Schantz, and Ohad; unpublished results). Some of these peptides (peptide V, a, b [2]) are absent in the dark-grown cells and are synthesized following exposure of the cells to the light. These peptides apparently form a complex with the Chl which in its absence might become soluble in the lipid phase of the membrane.

The organization of Chl, as shown by the F_0 decrease, could be due to a dilution of existing unorganized Chl by newly synthesized Chl which is properly organized. Although in some cases this explanation is plausible (when comparing the decrease in F_0 to the extent of dilution), it is not general. In several experiments we observed a decrease of F_0 (as well as changes in other

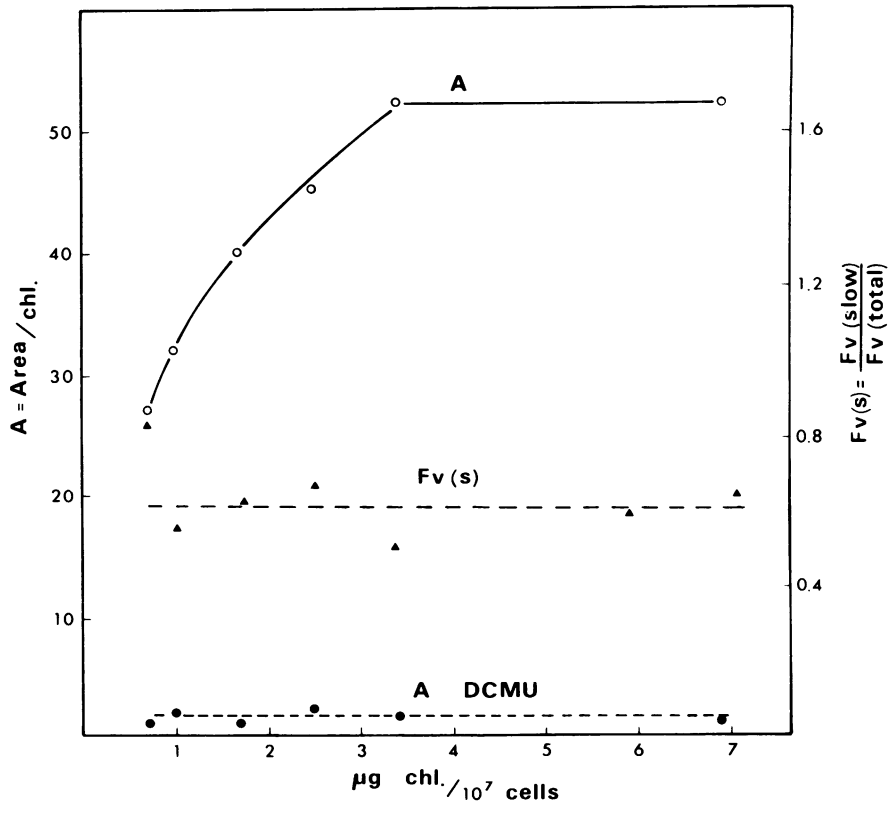

FIG. 9. Measurements of the value of A, A_{DCMU}, and F_v (slow)/F_v (total) during the greening process. The data were calculated from fluorescence induction oscilloscope traces (see "Analysis of Data", Fig. 1) and are expressed as a function of Chl content/cell.

Table I. *Behavior of Different Parameters Derived from Experimental Measurables during Greening Process*

The parameters and measurables are the same as described under "Analysis of Data." The early phase and the late phase of the greening are defined under "Discussion."

Parameter	Derived from F_v	Early phase (connection)	Late phase (synthesis and accumulation)
$[chl_a]/chl_t]$	QY	constant	constant
(Fraction of chl active in PSII relative to total chl)	A A_{DCMU}		
β (Fraction of reaction centers which can transfer electrons to DCIP)	QY FY A_{DCMU}	increases x 10-20	constant
C_{II} (Concentration of PSII reaction centers)	FY	constant	constant
[PQ] (Concentration of plastoquinone connected to PSII reaction centers)	A	increases x 2	constant
[ETC] (Concentration of electron transport chains connected to PSII reaction centers)	MR	increases x 10	constant
"Unorganized chlorophyll"	F_o	decreases x 7	constant

observables) with very little change in the Chl content, according to the concept of the initial phase of development (*cf.* above). According to equation 1, F_v (per Chl) is proportional to the product of three independent factors: I/Chl, $[Chl_a]/[Chl_t]$, and a certain expression of k_F, k_H, and k_P. There is a parallel decrease of similar magnitude of both F_v/Chl and I/Chl during greening. This implies the constancy of the product of the two remaining factors, indicating either that these factors change exactly in a reciprocal way, which seems a very remote possibility indeed, or that both remain constant. Accepting this last possibility, one infers from equation 2 that C_{II}/Chl will vary with the product I/Chl × A_{DCMU}. The results show that A_{DCMU} fluctuates randomly around a mean value by ±40%, while I/Chl decreases by a factor of 1.5 to 1.8. The rise in the value of A_{DCMU} causes an uncertainty in the estimation of C_{II}, but certainly one can consider it to be constant within the limits of the change of I/Chl.

Changes in Quantum Yield and Flash Yield. The quantum yield and the flash yield (per Chl) increase on the average by the same factor (Fig. 4). From the constancy of C_{II} (per Chl) comparing equations 4 and 5, and the assumptions of the constancy of $[Chl_a]/[Chl_t]$ and $k_P/k_P + k_H + k_F$ one arrives at the conclusion that β is the common factor which changes drastically during the early phase of greening and affects the flash yield and the quantum yield in the same way. Thus it appears that one of the main factors which changes during this phase is β, the degree of connectivity of reaction centers to the rest of the electron transfer chain. However, the ratio of F_v (slow)/F_v (total) which represents the degree of connectivity of reaction centers to the plastoquinone pool remains approximately constant. Hence, very probably β is related to a connection between the pool to a further point in the electron transport chain and not to the connection of active centers to the pool. This point is further supported by the fact that the plastoquinone pool (per Chl) measured by fluorescence induction, increases during this phase of greening, by a factor of about 1.5 to 2 (Fig. 9 and equation 3) and that a typical inflection (24) appears after a certain period of greening. This inflection very probably reflects the addition of an undefined second component to the pool (19, 24), which fills a gap between plastoquinone and the point of entry of DCIP.

At this stage we cannot decide whether a pre-existing quinone pool or a newly formed one is involved. Measurements of the relative contents of Cyt *f* in greening *C. reinhardi* y-1 cells have shown that it is present in the membrane found in dark-grown cells in rather large amounts (29, 30). However, this Cyt is not photoactive unless the cells are exposed to the light from 1 to 3 hr (14). Thus, at this phase of the greening, all the Cyt *f* becomes photoactive indicating its connection to the newly activated PSII and PSI centers. Also, it has been reported that plastocyanin is found in excess in the membranes of dark-grown cells (10). Thus, it is attractive to consider that the quinone pool is also present and becomes connected to the active centers and the electron transport chain during the early phase of the greening.

In addition, the maximal rate increases during greening by the same factor on the average as the flash yield and the quantum yield, indicating that β is probably the main factor influencing these observables. More accurate measurements are needed in order to determine if any changes in the convergence of electron transport chains and reaction centers occur.

Development of Water-splitting System. The measurements of electron donation from H_2O as compared with DPC show that the development of the water-splitting activity lags behind the development of DPC oxidation. Addition of DPC to membranes that were not tris-treated obtained from dark-grown cells increases the extent of the DCIP reduction indicating that a rate-limiting step exists on the donor site of PSII at this point. However, during the second phase of greening the water-splitting activity increases severalfold, while that of DPC levels off at values of about 30% the maximal rate obtained with H_2O (Fig. 2B). These results indicate that as the membranes become better organized and more active the ability of DPC to donate electrons becomes rate-limiting. In this respect the *Chlamydomonas* membranes appear to differ from some higher plants in which the

activity measured with DPC is equal to, or higher than that obtained with H_2O (1). However, data basically similar to those obtained in this work have been reported for barley (N. K. Boardman, personal communication).

Measurements of the apparent photosynthetic unit size by the flash yield technique have been performed in several other systems. In most of these the unit size was found to be constant during development, while in others it has been reported to increase (15). Indeed, during the second phase of the greening of *C. reinhardi*, that of actual accumulation of membranes, the size of the unit remains constant also in this system. The changes observed during the initial phase apparently are correlated with the change in β. It is not clear whether in the other systems reported so far β changes or remains constant.

PHASE OF SYNTHESIS AND ACCUMULATION OF NEW UNITS

This phase is characterized by a tendency toward a constant value for all the parameters measured. Such constancy indicates that the relative proportion of active centers to light harvesting Chl is constant throughout this phase of the greening. That this indeed might be the case, is also shown by the small variation found in the requirement for light saturation during this phase. On the other hand, data have been reported showing an increase in the light harvesting Chl/reaction center of PSI in these cells (10). The constancy of the PSII unit during the accumulation phase is also in agreement with the constancy of the quantum yield found at Chl contents above 3 to 4 $\mu g/10^7$ cells.

The concept that PSII, when present, and active, behaves as a complete constant entity finds support also in the data reported by Regitz and Ohad (31), who have measured the sensitivity to trypsin of *Chlamydomonas* thylakoids at different stages of the greening process. While rearrangements occur in the membrane as demonstrated by the exposure or protection of peptides toward trypsin digestion, the PSII units present, measured by DCIP reduction, behave in the same way at all times during the greening.

GENERAL CONSIDERATIONS

The ratio F_v (slow) (connected active centers) to F_v (total) (total active centers) is smaller than unity and remains constant. This can be interpreted as evidence for the existence of a constant small proportion of newly formed centers which remain unconnected during the whole greening process. During the greening of *Chlorella* mutants a change was reported in the fluorescence induction curve in the presence of DCMU from an exponential to a sigmoidal shape. This change was considered as an indication for the development of energy transfer between different units (7). In all our experiments such a change was not observed. However, indications for development of energy transfer between units were found in results of experiments in which the flash yield was measured as a function of flash intensity (23). Preliminary results show a change from a state of lack of energy transfer toward a state of partial energy transfer during the greening process. A more detailed report of these data will be presented elsewhere. Data obtained from other types of experiments corroborate the interpretation of the results presented here. Thus, it has been shown that membrane polypeptides which can be identified as being part of the PSII active centers are synthesized during the greening by the chloroplast ribosomes (2). Greening of *Chlamydomonas* y-1 cells in the presence of chloramphenicol, which inhibits synthesis of chloroplast-made proteins, results in the formation of nonphotoactive membranes. Activity can be regained by reactivation of the chloroplast ribosomes (11, 16). Preliminary results show that in this case F_v (total) increases in absence of Chl synthesis as expected from our interpretation, if new active centers were to be formed.

The understanding of the mechanism of interaction and the association and dissociation between PSI and PSII units related to electron and energy transfer between the units is important for the understanding of the mechanism of photosynthesis. The developing system presents obvious advantages for the investigation of these parameters.

LITERATURE CITED

1. Akoyunoglou, G. and M. Michelinaki-Maneta. 1974. Development of photosynthetic activity in flashed bean leaves. *In*: M. Avron, ed., Proceedings of the Third International Congress on Photosynthesis. Elsevier Publishing Co., Amsterdam. pp. 1885-1896.
2. Bar-Nun, S. and I. Ohad. 1974. Cytoplasmic and chloroplastic origin of chloroplast membrane proteins associated with PSII and PSI active centers in *Chlamydomonas reinhardi* y-1. *In*: M. Avron, ed., Proceedings of the Third International Congress on Photosynthesis. Elsevier Publishing Co., Amsterdam. pp. 1627-1637.
3. Bar-Nun, S., D. Wallach, and I. Ohad. 1972. Changes in the photosynthetic specific activity and the relationship between the light harvesting system and photosynthetic electron transfer chain during greening of *Chlamydomonas reinhardi* y-1. Biochim. Biophys. Acta 267: 138-148.
4. Beck, D. P. and R. P. Levine. 1974. Synthesis of chloroplast membrane polypeptides during synchronous growth of *Chlamydomonas reinhardi*. J. Cell Biol. 63: 759-772.
5. Boardman, N. K., J. M. Anderson, A. Kahn, S. W. Thorne, and E. T. Treffry. 1971. Formation of photosynthetic membranes during chloroplast development. *In:* N. K. Boardman, W. A. Linnane, and R. M. Smillie, eds., Autonomy and Biogenesis of Mitochondria and Chloroplasts. North Holland, Amsterdam. pp. 70-84.
6. Clayton, R. K. 1969. Characteristics of prompt and delayed fluorescence from spinach chloroplasts. Biophys. J. 9: 60-76.
7. Dubertret, G. and P. Joliot. 1974. Structure and organization of System II photosynthetic units during the greening of a dark-grown *Chlorella* mutant. Biochim. Biophys. Acta 357: 399-411.
8. Duysens, L. N. M. and H. E. Sweers. 1963. Mechanism of two photochemical reactions in algae as studied by means of fluorescence. *In:* Studies in Microalgae and Photosynthetic Bacteria. Japan Society of Plant Physiology. University of Tokyo Press, Tokyo. pp. 353-372.
9. Emerson, R. and W. Arnold. 1932. The photochemical reaction in photosynthesis. J. Gen. Physiol. 16: 191-205.
10. Eytan, G., R. C. Jennings, G. Forti, and I. Ohad. 1974. Changes in photosystem I activity and membrane organization during degreening and greening of a *Chlamydomonas reinhardi* mutant y-1. J. Biol. Chem. 249: 738-744.
11. Eytan, G. and I. Ohad. 1970. Cooperation between cytoplasmic and chloroplast ribosomes in the synthesis of photosynthetic lamellar proteins during the greening process in a mutant of *Chlamydomonas reinhardi* y-1. J. Biol. Chem. 245: 4297-4307.
12. Eytan, G. and I. Ohad. 1972. Modulation of chloroplast lamellae composition and function induced by discontinuous illumination and inhibition of ribonucleic acid and protein synthesis during greening of *Chlamydomonas reinhardi* y-1 mutant cells. J. Biol. Chem. 247: 122-129.
13. Forbush, B. and B. Kok. 1968. Reaction between primary and secondary electron acceptors of photosystem II of photosynthesis. Biochim. Biophys. Acta 162: 243-253.
14. Goldberg, I. and I. Ohad. 1970. Lipid and pigment changes during synthesis of chloroplast membranes in a mutant of *Chlamydomonas reinhardi* y-1. J. Cell Biol. 44: 563-571.
15. Herron, A. H. and D. Mauzerall. 1972. The development of photosynthesis in a greening mutant of *Chlorella* and an analysis of the light saturation curve. Plant Physiol. 50: 141-148.
16. Hoober, J. K. 1970. Site of synthesis of chloroplast membranes polypeptides in *Chlamydomonas reinhardi* y-1. J. Biol. Chem. 245: 4327-4334.
17. Hoober, J. K., P. Siekevitz, and G. E. Palade. 1969. Formation of chloroplast membranes in *Chlamydomonas reinhardi* y-1. Effects of inhibitors of protein synthesis. J. Biol. Chem. 244: 2621-2631.
18. Kirk, O. T. J. 1970. Biochemical aspects of chloroplast development. Annu. Rev. Plant Physiol. 21: 11-42.
19. Kok, B. and G. M. Cheniae. 1966. Kinetics and intermediates of the oxygen evolution step in photosynthesis. *In*: R. Sanadi, ed., Current Topics in Bioenergetics, Vol. 1. Academic Press, New York. pp. 1-47.
20. Kok, B., S. Malkin, O. Owens, and B. Forbush. 1966. Observations on the reducing side of the O_2-evolving photoact. Brookhaven Symp. Biol. 19: 446-459.
21. Lumry, R. and J. D. Spikes. 1957. Chemical-kinetic studies of the Hill reaction. *In*: Gaffron, G. H., ed., Research in Photosynthesis. Interscience, New York. pp. 373-391.
22. Malkin, S. 1969. The topological structure of the photosynthetic unit. Kinetic experiments and considerations. *In*: H. Metzner, ed., Progression in Photosynthetic Research, Vol. II. H. Laupp, Jr., Tubingen, Germany. pp. 845-856.
23. Malkin, S. 1974. Energy transfer in the photosynthetic unit. I. The concept of independent units for photosystem II analyzed by flash yields for dichlorophenolindophenol reduction. Biophys. Chem. 2: 327-337.
24. Malkins, S. and B. Kok. 1966. Fluorescence induction studies in isolated chloroplasts. Number of components involved in the reaction and quantum yields. Biochim. Biophys. Acta 126:413-432.
25. Malkin, S. and G. Michaeli. 1971. Fluorescence induction studies in isolated chloroplasts. IV. The inhibition of electron transfer from primary to secondary electron carriers of PSII at low temperature and by DCMU. *In*: G. Forti, M. Avron, and A. Melandri, eds., Proceedings of the 2nd International Congress on Photosynthesis, Vol. I, Stresa. pp. 149-167.
26. Malkin, S. and Y. Siderer. 1974. The effect of salt concentration on the fluorescence parameters of isolated chloroplasts. Biochim. Biophys. Acta 368: 422-431.

27. OHAD, I. 1972. Biogenesis and modulation of chloroplast membranes. *In*: L. Bolis, R. D. Keynes, and W. Wilbrand, eds. Role of Membranes in Secretory Processes. North Holland Publishing Co., Amsterdam. pp. 24–51.
28. OHAD, I. 1975. Biogenesis of chloroplast membranes. *In:* A. Tzagoloff, ed. Membrane Biogenesis. Plenum Press, New York. pp. 279–350.
29. OHAD, I., P. SIEKEVITZ, AND G. E. PALADE. 1967. I. Plastid differentiation in a dark-grown algal mutant (*Chlamydomonas reinhardi*). J. Cell Biol. 35: 521–552.
30. OHAD, I., P. SIEKEVITZ, AND G. E. PALADE. 1967. II. Plastid differentiation during greening of a dark-grown algal mutant (*Chlamydomonas reinhardi*). J. Cell Biol. 35: 553–584.
31. REGITZ, G. AND I. OHAD. 1974. Changes in the protein organization in developing thylakoids of *Chlamydomonas reinhardi*. y-1 as shown by sensitivity to trypsin. *In*: M. Avron, ed., Proceedings of the Third International Congress on Photosynthesis. Elsevier Publishing Co., Amsterdam. pp. 1615–1626.
32. SAUER, K. AND R. B. PARK. 1965. The Hill reaction of chloroplasts. Action spectra and quantum requirements. Biochemistry 4: 2791–2798.
33. SCHOR, S., P. SIEKEVITZ, AND G. E. PALADE. 1970. Cyclic changes in thylakoid membranes of synchronized *Chlamydomonas reinhardi*. Proc. Nat. Acad. Sci. U. S. A. 66: 174–180.
34. SCHULDINER, S. AND I. OHAD. 1969. Light-dependent induction of proton pump activity in whole cells and its correlation to cytochrome *f* photooxidation during greening of a *Chlamydomonas reinhardi mutant* (y-1). Biochim. Biophys. Acta 180: 165–177.
35. VERNON, L. P. 1960. Spectrophotometric determination of chlorophyll and pheophytins in plant extracts. Anal. Chem. 32: 1144–1150.
36. VERNON, L. P. AND E. R. SHAW. 1969. Photoreduction of 2,6-dichlorophenolindophenol by diphenylcarbazide: a photosystem II reaction catalyzed by tris-washed chloroplasts and subchloroplast fragments. Plant Physiol. 44: 1645–1649.
37. WALLACH, D., S. BAR-NUN, AND OHAD. I. 1972. Development of photophosphorylation and proton pump activities in greening *Chlamydomonas reinhardi* y-1 as measured with an open-cell preparation. Biochim. Biophys. Acta 267: 125–137.
38. WILD, A. 1969. The size of the photosynthetic unit and its variability in yellow mutants. *In*: H. Metzner, ed., Progress in Photosynthesis Research, Vol. II. H. Laupp, Jr., Tubingen, Germany. pp. 871–876.