Biochimica et Biophysica Acta, 975 (1989) 59–65
Elsevier

BBA 43009

Chlorophyll fluorescence lifetime studies of greening in yellow mutants of *Chlamydomonas reinhardtii*: assembly of the Photosystem I core complex

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(Received 19 August 1988)
(Revised manuscript received 13 February 1989)

Key words: Photosynthesis; Etiolated cell; Chloroplast development; Photosystem I; Fluorescence decay; Photon counting; (*C. reinhardtii*)

Changes in the functional organization of chlorophyll occur during light-dependent chlorophyll accumulation in yellow mutants of *Chlamydomonas reinhardtii*. These changes were studied using single-photon counting techniques to determine the chlorophyll fluorescence decay kinetics at various times after transfer of de-greened cultures to the light. Several different yellow mutants were analyzed: y-1, a photoautotrophic strain; LM18-a12b, a Photosystem-II (PS II) -deficient derivative of y-1; and C2, a strain deficient in both Photosystem I (PS I) and PS II. The results demonstrate that the residual chlorophyll present in de-greened cultures has a similar organization, independent of the chlorophyll-protein composition of the fully greened strains. Relative to the composition in light-grown cultures, the chlorophyll present in de-greened cells is enriched in chlorophyll *b* and has fluorescence decay kinetics similar to monomeric chlorophyll in solution. The reaction-center core complexes accumulate preferentially during the first 2 h of greening, and the light-harvesting complexes begin to accumulate more rapidly thereafter. After 5–6 h, during the stage of maximal chlorophyll accumulation rate, reaction centers and antenna complexes accumulate in constant proportions. The PS I reaction-center core complex has a constant apparent size of about 130 Chl / P-700 at all stages of greening under the conditions we examined.

Introduction

In higher plants, the accumulation of the core chlorophyll-protein complexes of the photosynthetic reaction centers (CCI and CCII) and of the antenna chlorophyll-protein complexes, LHC I and LHC II, is strictly dependent upon chlorophyll synthesis, which requires light in most species [1–5]. In the absence of light, the apoproteins do not accumulate because they are either not translated [6,7] or are degraded either during or soon after synthesis [2,4,6,7]. The same tight link between chlorophyll and apoprotein accumulation is also observable in the yellow mutants of the green alga *Chlamydomonas reinhardtii* which, like higher plants, require light for chlorophyll synthesis [8–10]. As in higher plants, the messages for the core complex apoproteins accumulate in these mutants in the absence of light, but the proteins themselves are either not translated or are degraded before they reach mature size (Ref. 11; see also Herrin, D., unpublished data). Newly synthesized LHC apoproteins are also degraded in the absence of chlorophyll [11,12], although in this case, light also affects the level of mRNA accumulation [11,13,14].

Numerous investigators have studied the light-driven etioplast to chloroplast transition in an effort to understand how the relative accumulation of the various chlorophyll-protein complexes is regulated. In the early stages of greening, the accumulation of the Chl *a*-containing reaction-center core complexes predominates with the subsequent addition of LHC complexes [15–17].

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Abbreviations: PS I (II), Photosystem I (II); Chl, chlorophyll; CCI (CCII), core chlorophyll-protein complex of the photosynthetic reaction centers; LHC I (LHC II), antenna chlorophyll-protein complex; Mes, 4-morpholineethanesulphonic acid.

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Mutations that alter this sequence have been found [18], suggesting that it is under active genetic control. However, the mechanisms that determine the sequence are still obscure.

We are interested in exploiting the early appearance of reaction centers during greening to learn more about how the core complexes themselves are assembled. Ohad and his colleagues [19] have reported that PS I activity can be detected in the *C. reinhardtii* yellow mutant, y-1, under some conditions in which the core complex is not detectable. This raises the possibility that the apoproteins may be stabilized against degradation after binding only a subset of the total chlorophyll molecules and that there may be selectivity in the early binding of chlorophyll to particular binding sites on the proteins. To examine the assembly of PS I during the early stages of greening without interference from PS II, we have crossed a PS II-deficient mutant [20] with y-1 and studied its greening.

Analysis of the time-dependence of excitation decay in photosynthetic membranes has proven to be a valuable tool for probing the underlying energy transfer and trapping processes (for reviews, see Refs. 21 and 22). Using single-photon counting techniques, fluorescence decays from chloroplasts and green algae generally can be fit to a sum of three exponential decay components with lifetimes of 60–130 ps (fast component), 300–800 ps (middle component) and 1–3 ns (slow component). With photochemically active reaction centers, the fast component has been attributed to excitation trapping in both PS I and PS II core antennae [23–25]. The middle component has been assigned to the fluorescence from PS II antennae, including core antenna and LHC complexes [24,25]. However, PS I-associated fluorescence decays with lifetimes of 300–800 ps have also been reported in PS II-deficient mutants of both *Chlamydomonas reinhardtii* [20,26,27] and maize [23]. Similar middle component decays have also been observed in detergent-isolated PS I particles [28,29], and have been related to the presence of Chl *b* containing peripheral antennae coupled to the PS I core antenna complex [20,27]. The slow component has been attributed to excitation migration time from closed to open reaction centers or to fluorescence from chlorophylls decoupled from antennae ('free' chlorophylls) [24,25]. It has also been proposed that this slow component arises from charge recombination in the reaction center [30,31].

Although the assignments of the middle and slow components of the chlorophyll fluorescence decay curves have not reached general consensus, the overall shape of the curves is clearly sensitive to both reaction center dynamics and core antenna composition and organization [20,23,26–28,32–37]. In particular, isolated PS I core complexes show a linear relationship between the short fluorescence lifetime and the size of the core antenna complex [20]. Thus, the fluorescence lifetime associated with PS I in vivo should be indicative of changes in Chl P-700 ratios in individual core complexes during greening of yellow *Chlamydomonas* mutants.

Materials and Methods

Three yellow mutants of *C. reinhardtii*, y-1, LM18-a12b and C2, were used in these studies. The greening of mutant y-1, an otherwise wild-type strain, has been studied extensively [11,15,38]. LM18-a12b is a double mutant generated by crossing y-1 with mutant B1 [20], which lacks PS II because of a symmetrical deletion of the chloroplast *psb*A gene. The C2 strain is deficient in both PS I and PS II because of deletions of the chloroplast genes *psb*A and *tsc*A (Roitgrund, C. and Mets, L.J., unpublished data). C2 has no detectable P-700 activity and no variable fluorescence [39]. In addition, C2 requires light for chlorophyll synthesis like y-1, but in this case the mutation is located in the chloroplast rather than in the nucleus (Roitgrund, C. and Mets, L.J., unpublished data).

Culture inocula were grown in the light in Tris-acetate-phosphate (TAP) liquid medium (pH = 7.0) [40] at 25 °C and then 'de-greened' [8] by growth in complete darkness for either 5 days (y-1) or 6 days (LM18-a12b and C2). For C2 cells, 9 mM of 4-morpholine-ethanesulfonic acid (Mes) was added to the TAP medium used for all growth stages. At the end of de-greening (cell density of $(3–5) \cdot 10^6$ cells/ml), cells were harvested by centrifugation, resuspended in fresh TAP medium at the same cell density and then illuminated with 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ using cool white fluorescent lamps at 25 °C.

For fluorescence decay measurements, 200 ml of cell culture were collected after 0 h (dark) and 100 ml each after about 1, 2, 4, 6, 9 and 12 h of illumination. Cells were harvested by centrifugation at $2000 \times g$ for 5 min and resuspended in fresh growth medium at a concentration of 2–5 μg chlorophyll per ml. Chlorophyll was extracted from cells with 80% (vol/vol) acetone and the Chl *a* and *b* concentrations were measured by the Arnon method [41]. Fully greened samples of y-1, LM18-a12b, C2 and also wild-type strain DES-15 were also analyzed in the same way. To measure the fluorescence decay with closed PS II reaction centers in y-1, 5 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added to the resuspended cells 20 min before measurement and the sample was illuminated with low intensity light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$ incandescent light) during measurement.

Fluorescence decay measurements were carried out with the time-correlated single-photon counting apparatus as described in detail by Chang et al. [42]. In brief, excitation pulses of 8–10 ps at full width half maximum (FWHM) are generated by a cavity-dumped

DCM dye laser synchronously pumped by an argon ion laser (Coherent) mode-locked at 514.5 nm. The dye laser wavelength was selected by an intra-cavity three-plate birefringence filter. The pulse repetition rate was controlled by the cavity dumper driver. For LM18-a12b and C2 samples, the repetition rate was 3.8 MHz while 0.95 MHz was used for y-1 mutant. The maximum single-pulse intensity was approx. $2 \cdot 10^{11}$ photons per cm^2. The reverse counting configuration was used in which the start input to the time-to-amplitude converter (TAC) is produced by the fluorescence signal while a portion of the excitation beam is split to a fast photodiode (Telefunken BPW28) to provide the stop signal. Samples were flowed through a 2 mm × 2 mm fluorescence cuvette by a peristaltic pump. Fluorescence photons were collected at right angle to the excitation beam, selected at the magic angle (54.7°) polarization and detected by a microchannel plate PMT (Hamamatsu R2809U). Emission wavelength was selected by a grating monochromator (JY 7/667 IR) with 4 nm bandpass. Interference filters were used in front of the monochromator to block actinic light scattered from the sample. The instrument response function was obtained by scattering excitation light using non-dairy creamer suspended in water as a scattering agent and had a FWHM of 55 ps and a full width at tenth maximum of 120 ps. The sample flow rate is about 75 ml per min and the cuvette cross section is 2 mm × 2 mm. This corresponds to a linear sample flow rate of 300 mm per s through the cuvette. Under our measuring conditions (950 kHz pulse repetition rate, 0.2 mm beam width), the cells experience about 600 pulses during passage through the beam. Although the intensity of the beam is low enough to prevent annihilation ($2 \cdot 10^{11}$ photons per cm^2 per pulse), there is still a definite possibility that a portion of the PS II reaction centers may be closed at the time of excitation by a subsequent laser pulse.

The measured fluorescence decays were fitted to a weighted sum of exponentials through iterative convolution with the instrument response function. A nonlinear least-squares fitting program was used [42]. The fluorescence decay impulse response was described by

$$F(t) = \Sigma A_i \, e^{-t/\tau_i}, \quad \text{with } \Sigma A_i = 1.0$$

where τ_i is the lifetime of each individual decay component and A_i is the corresponding preexponential amplitude. The quality of the fits was judged by a reduced χ^2 criterion, a runs test and a plot of weighted residuals. The fluorescence decay of oxazine 725 (Exciton) was used as a standard. The lifetimes of oxazine 725 were measured to be 750 ± 20 ps in methanol and 490 ± 20 ps in water (24°C; the lifetime of oxazine 725 is temperature sensitive); both are in agreement with the previously reported values [26,43].

Results

Chlorophyll accumulation

The time-courses of chlorophyll accumulation during greening of etiolated cultures of the three strains we studied are qualitatively similar. As shown in Fig. 1, all three strains show a reproducible lag of several hours after transfer to light before the rate of chlorophyll accumulation becomes maximal. In comparison with y-1, the lag is shorter in LM18-a12b and less pronounced in C2. The maximal rates of chlorophyll accumulation are also quite different as are the steady-state levels of total chlorophyll in cells grown in the light (1000–2000, 500–600, and 300–500 μg per 10^9 cells in y-1, LM18-a12b, and C2, respectively). By 24 h in the light, both y-1 and LM18-a12b reach chlorophyll levels similar to those of light-grown cells without undergoing any cell division. Etiolated C2 cultures accumulate chlo-

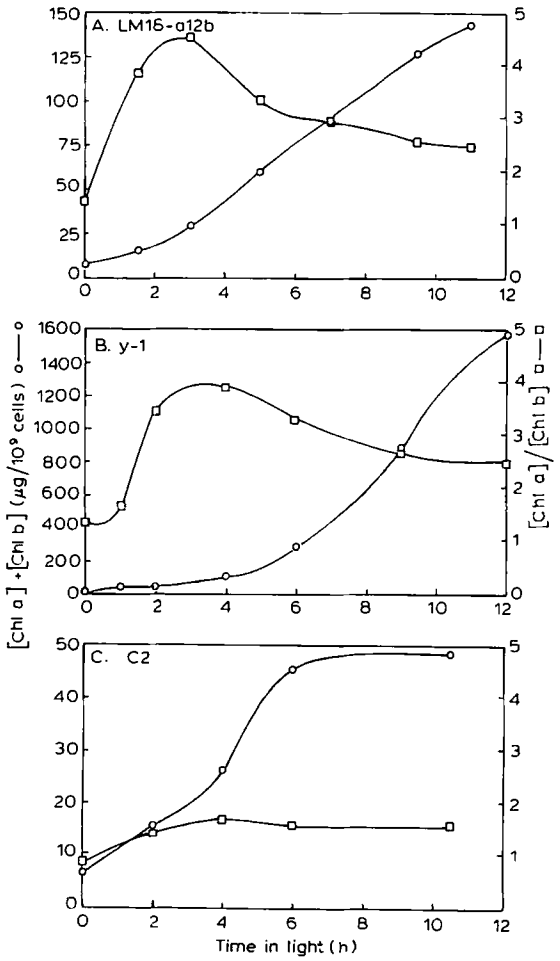

Fig. 1. Chlorophyll accumulation during greening of yellow mutants of *Chlamydomonas*. At time zero, de-greened cultures were transferred to light (see Materials and Methods). At each time point, both the total chlorophyll and the Chl *a*/Chl *b* ratio were determined from the same sample of cells. The data are from the same cultures analyzed for Tables I–III.

rophyll more slowly and do not reach the levels present in light-grown cultures until they begin to divide.

Although Chl *a* and Chl *b* both accumulate, they do so at different rates. The ratio of Chl *a* to Chl *b* in de-greened cells (time 0 in Fig. 1) is always less than that for the greened cells in each case. During the first few hours of greening, Chl *a* rises faster than Chl *b* in y-1 and LM18-a12b (Fig. 1). Then, the rate of Chl *b* accumulation increases preferentially, with the ratio gradually declining to the value characteristic of fully green cultures by 12 h. Strain C2 does not show a comparable peak in the Chl *a*/Chl *b* ratio, accumulating Chl *a* and Chl *b* at the same rates throughout.

Fluorescence lifetime measurements

The time-courses of changes in the chlorophyll fluorescence lifetime characteristics of these strains were determined using 665 nm pulse excitation and analyzing 690 nm emission (680 nm in the case of C2, since its emission at 690 nm is very low). For the most part, these decay curves require a sum of three exponential components for an adequate fit. For all of the strains we analyzed in this study, the fast, middle and slow components are defined by lifetimes of about 85 ps, 500–900 ps, and 1700–3800 ps, respectively, with relative amplitudes that vary during the greening process. Typical results for the three strains (the same cultures analyzed for Fig. 1) are listed in Tables I–III.

The principle features of these time-courses are most easily seen in the analysis of LM18-a12b. The dark-grown culture completely lacks a fast component and the data are adequately fit by a sum of two exponential decays (time 0 in Table I). The first timepoint in the light-exposed culture (1.5 h in this experiment) always shows a significant amplitude of fast 85 ps decay. The amplitude of this component increases steadily during the greening process, reaching the steady-state value in the range of 60% within 4–6 h. It is important to note that during these changes in amplitude, the lifetime remains constant at about 85 ps. In contrast, the lifetimes of the middle and slow components decrease during the greening process, as do their relative pre-exponential amplitudes. It is striking that the fluorescence decay kinetics are relatively stable from about 6 h on, during the period of maximal chlorophyll accumulation rate.

Results from the analysis of the greening process in y-1, although complicated by the influence of PS II reaction centers present in this strain, provide general confirmation of the PS I-related development seen in LM18-a12b. In the de-greened cultures (time 0 in Table II), the fluorescence decay is dominated by the middle

TABLE I

Summary of fluorescence decay kinetics of LM18-a12b as a function of greening time

Samples were excited with 665 nm light and fluorescence was detected at 690 nm. The χ^2 value is the reduced square deviation between the experimental decays and the fit derived by the parameters listed.

Time (h)	$\%A_1$	τ_1 (ps)	$\%A_2$	τ_2 (ps)	$\%A_3$	τ_3 (ps)	χ^2
0	–	–	41	1423	59	3780	1.14
1.5	28	85	36	922	36	2867	1.21
3	39	91	38	969	23	2889	0.95
5	57	84	23	685	20	2138	1.08
7	58	91	25	624	17	1905	1.07
9.5	59	81	24	531	17	1707	1.26
11	58	80	26	544	16	1738	1.21
4 Days [a]	59	94	26	537	15	1653	1.17

[a] Fluorescence measurement was preformed with cells grown in constant light for 4 days.

TABLE II

Summary of fluorescence decay kinetics of y1 as a function of greening time

Samples were excited with 665 nm light and fluorescence was detected at 690 nm. The χ^2 value is the reduced square deviation between the experimental decays and the fit derived by the parameters listed.

Time (h)	$\%A_1$	τ_1 (ps)	$\%A_2$	τ_2 (ps)	$\%A_3$	τ_3 (ps)	χ^2
0	17	85	39	1417	44	3723	1.10
1	44	76	24	508	32	1976	1.17
2	46	79	28	501	26	1743	1.35
4	54	80	26	510	20	1577	1.20
6	57	83	24	485	19	1304	1.06
9	55	78	23	424	22	1167	1.22
12	54	83	25	416	21	1176	1.14
4 Days [a]	56	79	25	388	19	1090	1.07
DES-15 [b]	54	78	27	417	19	1217	1.26

[a] Fluorescence was measured from y-1 cells grown in constant light for 4 days.

[b] DES-15 is a wild-type strain. Fluorescence was measured from DES-15 cells grown in constant light for 4 days.

TABLE III

Summary of fluorescence decay kinetics of C2 as a function of greening time

amples were excited with 665 nm light and fluorescence was detected at 680 nm. The χ^2 value is the reduced square deviation between the experimental decays and the fit derived by the parameters listed.

Time (h)	$\%A_1$	τ_1 (ps)	$\%A_2$	τ_2 (ps)	χ^2
0	60	1430	40	3806	1.11
2	38	786	62	2311	1.01
4	49	976	51	2346	1.14
6	50	947	50	2243	1.06
10.5	47	934	53	2115	1.02
4 Days [a]	47	1221	53	2581	1.24

[a] Fluorescence decay from C2 cells grown in constant light for 4 days.

and slow components (1417 ps and 3723 ps). The appearance of an 85 ps component at this point may reflect the incomplete loss of reaction center traps (either PS I or PS II) during de-greening or their formation during the fluorescence decay measurement itself. Results from greening of etiolated plants [5,44] indicate that both active PS I and PS II reaction centers start to accumulate very soon after the onset of illumination. In our studies, obtaining fluorescence decay curves from etiolated cells required 30–40 min of data collection, possibly allowing a small amount of PS I or PS II to be accumulated. As in LM18-a12b, the amplitude of the fast component in y-1 increases rapidly in the first 4 h of greening, without a significant change in lifetime. Again, the lifetimes of the middle and slow components are markedly shorter in the first timepoint following the onset of illumination and continue to decrease slow during the rest of the greening period. After the first timepoint following illumination, the increase in relative pre-exponential amplitude of the fast component is almost exclusively at the expense of the slow component, with the middle component remaining relatively constant.

The fluorescence decay of de-greened C2 cells (0 h greening) can be fitted to a double exponential function with lifetimes of 1430 ps and 3806 ps (Table III). The decay curve is very similar to those of dark-grown y-1 and LM18-a12b cells. The fluorescence decay kinetics of light-exposed cells, although differing from those of etiolated cells, can still be described by a sum of two exponentials, but with lifetimes of about 900 ps and 2200 ps and relative amplitudes of about 1 : 1 (Table III). No further changes in fluorescence decay kinetics occur through the 11 h greening time-course we followed. Fluorescence measurements with excitation wavelength of 652 nm and with emission wavelength of 690 nm (where the count rate is much lower) gave indistinguishable results (data not shown).

Discussion

The pattern of chlorophyll-protein complex accumulation during greening of y-1 and LM18-a12b can be roughly divided into three phases. The first phase involves an immediate response to illumination that occurs within the first hour. The two major changes in this phase are the appearance of a PS I-associated fast fluorescence decay component and a rapid decrease in overall fluorescence yield related primarily to the shortening of the slow and middle component lifetimes. During the second phase (from 1 to 5 h in the time-course), adjustments are made in the relative rates of accumulation of individual chlorophyll-protein complexes. First, there is a selective accumulation of Chl *a* and active reaction center core complexes. This is followed by a selective increase in the Chl *b* and LHC accumulation rates. Eytan and Ohad [45] have shown that the effective antenna sizes coupled to the reaction-center complexes increase during this phase in y-1. The final, accumulation phase begins at about hour 6 in the timecourse, when the chlorophyll accumulation rate is maximal. The relative constancy of the chlorophyll fluorescence decay curves during this period, in which the bulk of membrane synthesis occurs, suggests that the various chlorophyll-protein complexes are added in constant ratios.

Organization of residual chlorophyll in de-greened cultures

The residual chlorophyll present in the de-greened cultures appears to have similar organization in all three strains we studied, even though light-grown cells of each differ considerably from one another. Light-grown y-1 cells are indistinguishable from wild-type cells in the content and organization of photosynthetic reaction centers and light harvesting protein complexes [9,19,45]. In comparison, LM18-a12b is similar to other *psb*A deletion strains in that it lacks the entire PS II core complex [46], and C2 is missing the PS I reaction-center core complex as well. Nevertheless, the de-greened cultures of all of the strains show virtually identical lifetimes of middle ($\tau \approx 1420$ ps) and slow ($\tau \approx 3800$ ps), decay components reflecting an organization of chlorophyll that is unique to and consistent among de-greened yellow strains.

It is interesting that all of the strains show lower Chl *a*/Chl *b* ratios when de-greened than when fully greened. Bednarik and Hoober [47] have obtained evidence that chlorophyllide *b* can be synthesized directly from protochlorophyllide in the absence of light in *Chlamydomonas* y-1 strains. Thus, it may be that some Chl *b* synthesis can continue during de-greening, even though Chl *a* synthesis is blocked.

The lifetime of the slow component in the fully de-greened cells is longer than that of any known chlorophyll protein complex, including the LHC II protein that lacks photochemical traps [39,48]. It is more similar to the fluorescence lifetime of chlorophyll dissolved in organic solvents, and we think it likely that this component arises from free chlorophyll dissolved in the lipid membrane.

The changes that occur during the first hour of light exposure, including the formation of active PS I core complexes, must reflect the formation of new protein associations of at least a portion of the chlorophyll that existed in the de-greened cells. Persistence of a $\tau = 3800$ ps component from the original chlorophyll pool would be readily evident in these experiments, since net chlorophyll content only doubles during this period. Thus, the formation of normal chlorophyll-protein complexes must require light, even when both the chlorophyll and proteins [11] are available.

Biogenesis of PS I reaction-center core complexes

Whenever a PS I-associated fast decay component is present in these cultures, its lifetime is always around 85 ps, even in the face of large changes in all other aspects of the decay curve. In biochemical preparations, the fluorescence lifetime of PS I core complexes shows a linear dependence on the ratio of core antenna chlorophyll to P-700, as expected from modeling studies [20]. Holzwarth and co-workers [49] also suggest a linear dependence of fluorescence lifetime on array size in PS II. Assuming that the same relationship holds in vivo, the constancy of the fast component suggests that the core antenna size is constant throughout the time-course of PS I core complex accumulation. Hence, the only active complexes that accumulate appear to have full-size core antennae. By applying the relationship observed in isolated PS I particles between fluorescence lifetime and the size of the PS I core antenna [20], the PS I core antenna size of LM18-a12b cells is estimated to be about 130 Chl *a*/P-700.

Karukstis and Sauer [50] also reported a constant lifetime (100 ps) of the fast decay component during greening of etiolated and intermittent light-grown pea leaves. Again, this result would argue for a constant core complex size at all stages in the greening. Their findings differ from ours for *Chlamydomonas,* however, in that the relative amplitude of the fast-decay component is large (45%) at the first timepoint, even in plants that have not been exposed to any light prior to the beginning of the experiment (see Fig. 10 in Ref. 50). The reason for this difference is not known.

Slow component

Time-resolved studies of the slow decay component (1.3–1.8 ns) by Owens et al. (1989, Biophys. J., in press) have shown that the spectral properties of this component are very similar to the steady-state emission of C2 [29] and isolated LHC II complexes [48]. They assigned this component to peripheral LHC I and LHC II complexes which are uncoupled to the core antennae. In this case, any variation in the fluorescence lifetime during the greening process reflects a change in the structure of and the coordination between subunits within these energetically 'isolated' pigments. The continuous decrease of the lifetime of the slow component may result from increased connectivity of the pigment aggregates so that an excitation may now encounter some kind of trap, such as a pair of chlorophyll molecules less than 12 Å apart [33].

An alternative is that the decrease of the slow component lifetime below 2 ns might be due to improved connectivity between LHC II protein complexes and the PS I and PS II core antennae. This suggestion is supported by the result of greening C2 cells. The C2 mutant strain only consists of LHC II protein complexes, it has neither PS I nor PS II. In C2 greening, the only change in the slow-decay component is the decrease of lifetime from 3800 ps in etiolated cells to 2200 ps after 2 h of illumination. From these data, we suggest that the change of fluorescence lifetime from 3800 ps to 2200 ps after transferring etiolated cells to the light may be due to the association of chlorophyll with the chlorophyll *a*/*b*-binding protein. In the absence of PS II, as in the case of LM18-a12b, the shortening of the long fluorescence lifetime must reflect increased connectivity between LHC II protein complexes and the PS I core antenna/reaction center complexes. In y-1 cells, the long lifetime is shorter than that of LM18-a12b. It is believed that the LHC II protein complexes are more closely associated with PS II than with PS I. This can be seen by the results obtained by Gulotty et al. [26] in which the amplitude of the slow component (2.2 ns) is 31% in a mutant lacking PS II reaction center/core antenna. Thus, it is not surprising that the presence of PS II in y-1 cells causes a greater shortening of the slow-component lifetime than occurs in the absence of PS II.

Summary

(1) The residual chlorophyll present in de-greened cultures of several yellow mutants of *Chlamyodomonas reinhardtti* has a similar organization, in spite of major differences in chlorophyll-protein composition of the fully greened strains. Relative to the composition in light-grown cultures, the chlorophyll present in de-greened cells is enriched in Chl *b* and an appreciable portion has fluorescence decay kinetics similar to monomeric chlorophyll in solution.

(2) The reaction-center core complexes accumulate preferentially during the first 2 h of greening, and the light-harvesting complexes begin to accumulate more rapidly thereafter. After 5–6 h, during the stage of maximal chlorophyll accumulation rate, reaction centers and antenna complexes accumulate in constant proportions.

(3) The PS I reaction-center core complex has a constant apparent size of about 130 Chl/P-700 at all stages of greening under the conditions we examined.

Acknowledgements

The authors would like to thank Steve P. Webb for his initial work on this investigation and Aida Pascual for her help in growing samples. G.L.G. is supported by the Alexander Hollaender Distinguished Postdoctoral Fellowship Program of the Department of Energy. L.M. is the Ben Horwich Scholar at the University of Chicago. This research was supported by a grant from the National Science Foundation.

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