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Dynamics of Photosystem II and Its Light Harvesting System in Response to Light Changes in the Halotolerant Alga *Dunaliella salina*[1]

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ABSTRACT

A photosystem two (PSII) core complex consisting of five major polypeptides (47, 40, 32, 30, and 10 kilodaltons) and a light harvesting chlorophyll *a/b* complex (LHC-2) have been isolated from the halotolerant alga *Dunaliella salina*. The chlorophyll and polypeptide composition of both complexes were compared in illuminated and dark-adapted cultures. Dark adaptation is accompanied by a decrease in the chlorophyll *a* to chlorophyll *b* (Chl *a*/Chl *b*) ratio of intact thylakoids without any change in total chlorophyll. These changes occur with a half-time of 3 hours and are reversed upon reillumination. Analyses of PSII enriched membrane fragments suggest that the decrease in the Chl *a*/Chl *b* is due partly to an increase in the Chl *b* content of LHC-2 and partly to changes in the relative levels of the two complexes. Apparently during dark adaptation there is: (a) a net synthesis of chlorophyll *b*, (b) removal of PSII core complexes resulting in a 2-fold drop in the PSII cores to LHC-2 chlorophyll ratio. These changes should dramatically increase the light harvesting capacity of the remaining PSII reaction centers. Presumably this adjustment of antenna size and composition is a physiological mechanism necessary for responding to shade conditions. Also detected, using ^{32}P, are light-induced phosphorylation of the LHC-2 (consistent with the ability to undergo State transitions) and of the 40 and 30 kilodalton subunits of the PSII core complex. These observations indicate that additional mechanisms may also exist to help optimize the interception of quanta during rapid changes in illumination conditions.

Shade plant species, as well as shade-adaptable plants, when grown at low light intensities, are characterized by remarkable alterations in the composition and morphology of their chloroplasts as compared to high-light grown plant (3, 6, 10, 11). Such alterations include an increase in the total Chl and in the relative Chl *b* content, a decrease in the level of proteins involved in electron transport, ATP synthesis, and CO_2 fixation, and an increase in the proportion of stacked thylakoid membranes attributed mainly to the increased size of the photosystem two (PSII) antenna Chl. This latter change in PSII is due to the net synthesis of light harvesting Chl *a/b* complex (LHC-2). All the changes in higher plants are slow and are to a large extent irreversible (3, 6, 10, 11). Similar variations in Chl content and in Chl *a*/Chl *b* ratios, in response to shade adaptation, have also been reported in several algae (14, 18).

The light capturing ability to PSII is also subject of regulation by a redox-activated protein kinase which phosphorylates LHC-2 (1). The phosphorylation of LHC-2 is believed to lead to the diffusion of phosphorylated LHC-2 from stacked region of the grana to the nonappressed stromal lamellae resulting in a redistribution of absorbed quanta in favor of PSI (6).

Presumably this mechanism is advantageous in limiting light conditions when there are fluctuations in the quality and quantity of incident radiation (6). Such conditions occur in the shade environment. In agreement with this, these changes, known as State transitions (5), are rapid and reversible and occur in both higher plants (16) and algae (12).

In addition to LHC-2, several other thylakoid membrane polypeptides become reversibly phosphorylated and recently it has been shown that many of these are associated with the PSII core complex (24). The PSII core complex has been isolated from higher plants (15, 19, 26, 27), from algae (17), and from cyanobacteria (28). It has been found to be highly conserved in composition, containing five polypeptides of 10 kD and above and possibly additional lower mol wt components. Of the five major polypeptides, two are Chl binding; 47 kD and 40 to 43 kD proteins which are products of the chloroplast *psb* A and *psb* C genes, respectively (7). Two other polypeptides of mol wt in the region on 32 kD are encoded by the *psb* B and *psb* D genes (D1 and D2 polypeptides, respectively) also bind Chl and function as the PS2 reaction center (23, 25). The fifth polypeptide of 10 kD is the α-subunit of Cyt *b*-559 and forms a heterodimer with the β-subunit having a mol wt of 4 kD. These two components are the products of the chloroplast *psb* E and *psb* F genes, respectively (21). It has recently been shown that in the case of higher plants a number of these components can become phosphorylated (24).

Recently we have purified PSII core complexes from the halotolerant alga *Dunaliella salina* and demonstrated that the subunit stoichiometry of its five polypeptides is 1:1:1:1:1 and that it does not change upon shade adaptation (20). Here we have further investigated the effect of light-intensity changes on the pigment composition and on phosphorylation of PSII polypeptides in an attempt to understand the dynamics of PSII in *D. salina* upon light-shade adaptation.

MATERIALS AND METHODS

Thylakoid Membrane Preparation. *Dunaliella salina* was grown in a medium containing 1, 2, 3, or 4 M NaCl under

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continuous illumination as previously described (9). For dark adaptation, flasks were covered with aluminum foil. The preparation of isolated thylakoids was achieved with cells collected at 2,500*g* for 7 min. Using the same centrifugation conditions these were then washed repeatedly in 1 M NaCl and subjected to a final wash with 20 mM Na-tricine (pH 8.0) and 15% glycerol. Thylakoids were obtained by an osmotic rupture using a suspension medium containing 20 mM Na-tricine (pH 8.0), 1 mM benzamidine, and 5 mM γ-amino caproic acid. The thylakoids were collected at 12,000*g* for 10 min and washed once in a buffer consisting of 20 mM Na-MES (pH 6.0), 15 mM NaCl, and 5 mM $MgCl_2$ and resuspended in the same buffer.

Preparation of PSII Core Complex, LHC-2, and PSII Enriched Fragments. The isolation of PSII core and LHC-2 from *D. salina* was performed by a modification of the procedure of Gounaris and Barber (19) developed for spinach chloroplasts. Washed *D. salina* thylakoids (2 mg Chl/ml) were incubated for 30 min at 4°C in the above mentioned Mes buffer with 4% (w/v) Triton X-100 present. A pellet, corresponding to PSII enriched fragments, was then collected by centrifugation (30 min at 35,000*g*), washed once in 20 mM Na-Mes buffer (pH 6.0), resuspended at 0.8 mg Chl/ml in the same buffer containing 0.64% Triton X-100 and incubated for 1 h in the dark on ice. Samples containing 1 mg Chl were applied to a 0.1 to 1 M linear source gradient containing 0.1% Triton X-100 and centrifuged at 180,000*g* for 16 h at 4°C. The PSII core complex migrated to about 0.7 M sucrose while LHC-2 was located as a broad band at 0.3 to 0.4 M sucrose. Both PSII and LHC-2 samples were stored in liquid N_2.

Analytical Methods. SDS-PAGE was carried out on a 7 to 17% acrylamide system similar to that given by Laemmli (22). Autoradiography of dried gels was performed at −70°C using Fuji NIH x-ray film and high-speed intensifying screens. Fluorography of gels was carried out according to Bonner and Laskey (13) and Chl levels were determined according to Arnon (4).

Materials. Chemicals were obtained from Sigma Chemical Co., radiochemicals from Amersham International, U.K., and detergents from Boehringer Manneim.

RESULTS

The Chl composition of *Dunaliella salina* cultures is influenced both by the NaCl concentration and by the concentration of cells in the suspension. In all tested cultures there seems to be an inverse correlation between the Chl *a*/Chl *b* ratio and the total Chl concentration (Fig. 1). There was also a pronounced increase in the Chl/protein which correlated with the increase of Chl concentration in the culture. A possible reason for this drop in Chl *a*/Chl *b* is adaptation to low light intensity due to self-shading, similar to previous observations in *Chlorella* (14) and in higher plants (3, 6, 10, 11).

To check this possibility, *D. salina* cultures were dark-adapted for different periods. Table I shows that following 48 h of dark-adaptation there is a significant decrease in the Chl *a*/Chl *b* ratio of the thylakoids and of the PS2 enriched membrane fragments. The half-time for these changes is 3 h (Fig. 2) and the maximal drop is attained within 12 h in the dark. Despite this change in the Chl *a*/Chl *b* ratio the total level of Chl remained essentially constant or slightly increased (see Fig. 2). This maintenance of a constant level of Chl during dark adaptation suggests that a proportion of Chl *a* is converted to Chl *b*. Indeed, as Figure 2 and Table I shows, the isolation and analysis of the PSII core complex and LHC-2 indicates that a decrease in the Chl *a*/Chl *b* ratio during dark adaptation is accompanied mainly by a decrease in the level of Chl *a* in the PSII core fraction. In addition to this change we also observed (Table I) that the level of Chl *a* relative to Chl *b* in isolated LHC-2 decreased after dark treatment indicating that the stoichiometry of these two Chl species within

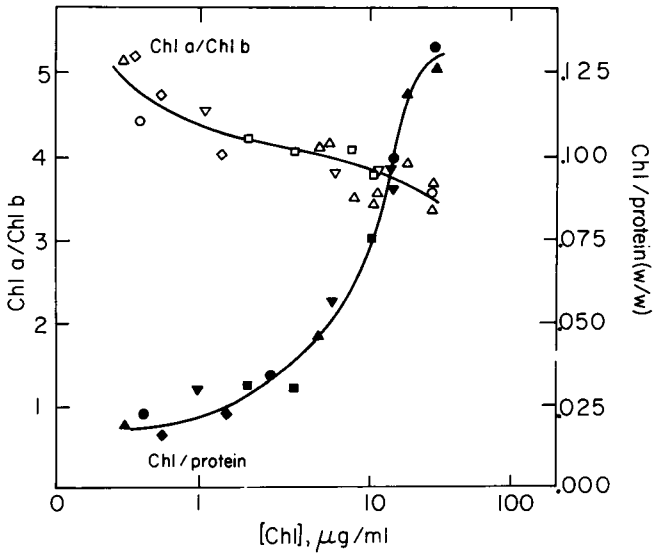

FIG. 1. Correlations between Chl *a*/Chl *b* and Chl/total protein with Chl concentration in *D. salina* cultures. A collection of Chl *a*/Chl *b* data measured for *D. salina* cultures (0.5–4 M NaCl), samples at different growth stages. Cultures were initiated at 0.1 μg Chl/ml and left up to the stationary phase stage. (▲, △), 0.5 M; (●, ○), 1.0 M; (■, □), 2.0 M; (▼, ▽), 3.0 M; and (♦, ◇), 4.0 M NaCl.

Table I. *Effect of Dark Adaptation on Chl a/Chl b and on Chl Recovery in D. salina PSII*

D. salina cultures were grown for 7 d under continuous illumination and the Chl concentration was maintained at 0.3 to 1.0 mg Chl/ml. Half of the culture was dark-adapted for 48 h and both cultures were harvested and used to obtain thylakoids and PSII enriched membrane fragments as described under "Materials and Methods."

Preparation	Chl *a*/Chl *b*		Chl recovery		Dark-induced Decrease in Chl *a*/Chl *b*
	Light	Dark	Light	Dark	
	ratio		%		%
Thylakoids	4.06	3.25	100	100	20.0
PSII enriched membranes	2.62	2.08	42	46	20.6
LHC-2	2.00	1.76	32	40	12.0
PSII cores	>10	>10	5.0	2.8	

LHC-2 can vary. Both changes in Chl *a*/Chl *b* and PSII core/LHC-2 ratios are completely reversed with similar kinetics upon reillumination of dark adapted cells.

Since these changes may reflect either a decrease in PSII core/LHC-2 ratio, or changes in the level of Chl within each PSII core or LHC-2, we determined the Chl level of the PSII core and of LHC-2 complexes in light- and dark-adapted cells. To achieve this, cells were grown 5 to 7 d on [^{14}C]bicarbonate to achieve uniform labeling of PSII and LHC-2. As shown in Table II, dark adaptation does not induce gross changes in the Chl or lipid contents of either PSII or LHC-2. The calculated numbers of Chl molecules per PSII core complex and LHC-2 are 18 to 20 and 8 to 10, respectively. These results therefore suggest that the dark-induced drop in Chl *a*/Chl *b* ratio in *D. salina* is caused partly by an increase in the number of LHC-2 at the expense of a decrease in the number of PS2 core complexes (Table II) and by an increase in the Chl *b* content of LHC-2.

In order to study further the dynamics of PSII and LHC-2 in response to light and dark conditions, we have followed ^{35}S incorporation using [^{35}S]sulphate. In the dark we found essentially no incorporation of ^{35}S into the thylakoid polypeptides

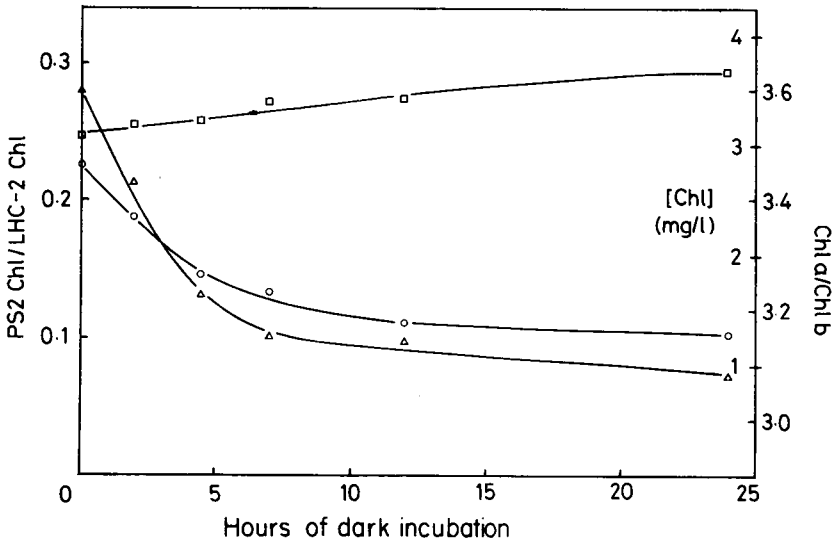

FIG. 2. Effect of dark adaptation on Chl *a*/Chl *b* ratio, total Chl and PSII core Chl/LHC-2 Chl ratio. *D. salina* (1 M cultures) were cultured in the light for 5 d at 3 to 5 mg Chl/L before subjecting them to dark adaptation. Total (Chl) of the cell cultures (□), Chl *a*/Chl *b* of washed thylakoids (Δ), and the Chl level in PSII core complex/LHC-2 fractions (O) obtained by sucrose gradient fractionations were analyzed after different periods of dark adaptation.

Table II. *^{14}C-Distribution in PSII Core Complex and LHC-2 Isolated from Uniformly Labeled D. Salina*

D. salina cultures were continuously illuminated in a sealed container in the presence of [^{14}C]bicarbonate (0.5 mCi/L, 5 mM $NaHCO_3$), for 7 d. Cells were either then kept for 24 h in the light or given a dark treatment for the same time. The Chl/protein was estimated for the PSII core and LHC-2 by assuming mol wt for monomeric complexes of 159 and 28 kD, respectively. It was also assumed that the carbon content is 74% for Chl and 55% for protein.

Constituent	PSII Core		LHC-2	
	Light	Dark	Light	Dark
I. Mol % C in:				
Proteins	80.6	81.9	53.3	53.7
Chl	11.1	10.1	22.2	23.7
Lipids	8.3	8.0	24.5	22.3
II. Chl stoichiometry				
[Chl]/[protein] (mol/monomer)	19.7	18.0	9.0	9.5
III. LHC-2/PSII core	Light		Dark	
[Chl]LHC/[Chl]RC	6.4		14.1	
[Plypeptide monomers]LHC/[monomeric unit]PS2	14.0		26.8	

over an incubation period of 24 h. However, transferring dark-adapted cultures to light results in the incorporation of ^{35}S into the polypeptides of the two complexes. As Figure 3A shows, this incorporation had a lag period of several hours in parallel with the induction of net Chl synthesis. Uniform labeling with ^{35}S, indicated by a constant ^{35}S/Chl ratio was obtained after 24 to 27 h in the light. It was also found that ^{35}S incorporation into the sulpholipids of the thylakoid membrane (extractable by chloroform-methanol) also followed parallel kinetics (data not shown). Analysis of ^{35}S incorporation into the PSII core complex and LHC-2 showed that uniform labeling is obtained in the former faster than the latter complex (Fig. 3B). It has been previously shown (20), using ^{35}S labeling, that of the five major polypeptides of the PSII core complex of *D. salina* only the D1 component rapidly turns over. Therefore, the faster incorporation of ^{35}S-label into the PSII core complex compared to LHC-2 is probably due to the rapid synthesis of this polypeptide. Moreover, it was shown that the stoichiometry of the five polypeptides of the PSII core was 1:1:1:1:1 and that this stoichiometric relationship was not changed during dark-adaptation (20).

Incorporation of ^{32}P into PSII polypeptides of *D. salina* was measured following a prolonged incubation of cell cultures (5 d in continuous illumination with or without additional 24 h in complete darkness) with [^{32}P] inorganic phosphate. The incorporation of ^{32}P into both LHC-2 and PSII core polypeptides was significantly higher in light-grown as compared to dark-adapted cells (Tables III; Fig. 4). Calculation of the ^{32}P content per LHC-2 monomer yields a value close to 1 and the decrease upon dark adaptation is about 30%. ^{32}P content of PSII polypeptides is lower, and is decreased to a greater extent in dark adapted cells (Table III). All LHC-2 polypeptides, and in particular the major 28 kD component, seems to be labeled.

In the PSII core most of the label appears in the 30 kD subunit, less radioactivity appears in the 40 kD subunit. There is also labeling of additional 13 kD polypeptide. This phosphoprotein, however, was found in several different fractions of the sucrose gradients and does not seem to be an obligatory component of the PSII core complex (24).

The rate of dark dephosphorylation of PSII polypeptides is faster than the changes in Chl *a*/Chl *b* and in PSII Chl/LHC-2 Chl ratio in the dark (Fig. 5) indicating that the phosphorylation-dephosphorylation precedes the reorganization in Chl composition and antenna size of PSII in this halotolerant alga.

DISCUSSION

We have shown that when *D. salina* is subjected to a dark period it rapidly adjusts its relative levels of Chl *a* and *b* in a manner similar to that observed with higher plants subjected to low light or shade conditions. This change seems to reflect alterations in the relative levels of PSII core, LHC-2 complexes, and in the Chl *a*/*b* ratio of LHC-2. Of particular importance is that the dark-induced changes in Chl *a*/Chl *b* ratios are not

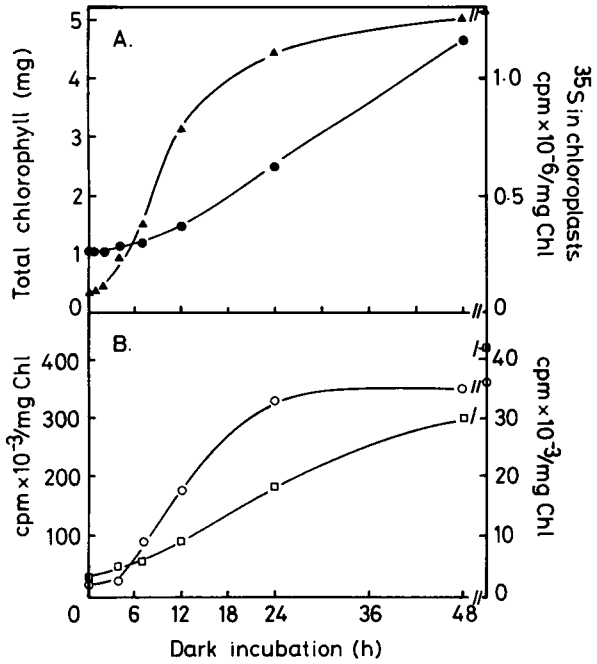

FIG. 3. Time course of ^{35}S incorporated into *D. salina* thylakoid polypeptides in the light. *D. salina* cells were cultured for 48 h in low sulphate medium (0.15 mM instead of 5 mM in the complete growth medium). [^{35}S]Sulphate (0.5 mCi/L) was added and the cells were incubated in complete darkness for 17 h. After different periods of illumination samples were analyzed for total Chl and for ^{35}S content in (A) the chloroplast thylakoid membrane (▲, ^{35}S/mg Chl; ●, Chl) and (B) in PSII core complexes and LHC-2 (○, PS2 core; □, LHC-2). ^{35}S incorporation is expressed in counts/mg Chl.

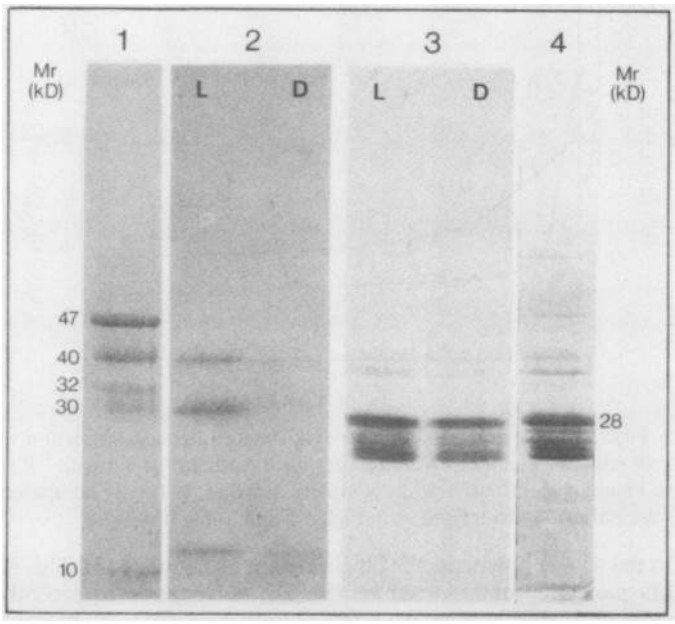

FIG. 4. Phosphorylation of PSII core complex and LHC-2 polypeptides in light or dark adapted *D. salina*. *D. salina* cells were cultured for 5 to 7 d with 100 μM [^{32}P]phosphate (1.0 mCi/L). Dark adapted cultures were incubated for 24 h in complete darkness. Samples of PSII core and LHC-2 complexes containing equal amounts of Chl from light (L) or dark (D) adapted cells were analyzed by SDS-PAGE (7–17% gradient gels) autoradiography. Lanes 1 and 4 are Coomassie blue stained gels of PSII core and LHC-2, respectively, and lanes 2 and 3 their autoradiographs.

accompanied by a net degradation of total Chl. Rather, it seems that the adjustment in this ratio is due mainly to the removal of a portion of PSII core complex coupled with a concomitant increase in the amount of LHC-2. Interestingly, there also seems to be some adjustment of the Chl *a*/Chl *b* within the LHC-2 complex. Overall this adaptation to the dark had the effect of more than doubling the antenna size for the PSII reaction center assuming that LHC-2 can efficiently transfer energy to the primary electron donor P680. The speed at which these changes occur and their reversibility indicates that they are the basis of a physiological mechanism presumably underlying adaptation to changes in light intensity in the natural environment. It is also interesting that the strategy adopted is not to decrease the total level of Chl but to redistribute it so as to optimise the ratio between the PSII reaction center concentration and the level of antenna Chl. The dark-induced increase in the concentration of Chl *b* could be due to a *de novo* synthesis or may result in a direct conversion of Chl *a* to Chl *b*. The latter possibility would be consistent with previous demonstrations of Chl *b* synthesis in the dark from a Chl *a* precursor (8). Prior to the work presented here, Falkowski (18) had shown in another species of *Dunaliella* that rapid changes in Chl *a*/Chl *b* ratios can occur which are reversible and which precede any changes in the total cellular Chl content, consistent with our observation.

Two open questions resulting from these studies are the fate of the PSII reaction centers which disappear, and the origin the extra LHC-2 which appear in the dark. Since we could not detect in *Dunaliella* any turnover of PSII reaction center subunits (20) or incorporation of [^{35}S]methionine into chloroplast polypeptides including LHC-2 (Fig. 3), in the dark it appears that there is neither *de novo* synthesis of LHC-2 nor degradation of PSII reaction center complexes in the dark. These results may imply, therefore, that there is a pool of nonfunctional LHC-2 and of PSII reaction center polypeptides or complexes which may either become assembled or removed from PSII in response to a decrease in light intensity.

Table III. *Summary of In Vivo Incorporation of ^{32}P into RC-2 and LHC-2 in Light and Dark Adapted D. salina*

D. salina cultured for 5 d under continuous illumination in a medium containing 0.1 mM phosphate + 250 μCi/L ^{32}P were harvested either immediately or after 24 h of dark adaptation. Lysis of cells and preparation of PSII cores and LHC-2 was performed in the presence of 10 mM NaF to inhibit phosphatase activity. The stoichiometry of ^{32}P content was calculated by assuming 9 Chl per LHC-2 monomer and 19 Chl per PSII core complex.

Condition	LHC-2		PSII Cores	
	cpm/mg Chl	*^{32}P/monomer*	*cpm/mg Chl*	*^{32}P/unit*
Light	4.5×10^5	0.84	5.8×10^4	0.23
Dark	3.2×10^5	0.60	1.7×10^4	0.07

The above adaptation, in contrast to higher plants, seems to be fast enough to enable the photosynthetic system of *D. salina* to respond to daily changes in light intensity. Nevertheless, the phosphorylation studies indicate that other control mechanisms may also be operating. Using a modulated technique to detect Chl fluorescence we were able to show that this alga, like other algae, has the capability to undergo State 1-State 2 transitions with a time course of a few minutes (data not presented). Therefore, the observation that LHC-2 of *D. salina* is phosphorylated in the light is expected (1) while the dark level of phosphorylation either indicates the existence of some phospho-LHC-2 not involved in State transitions or that the plastoquinone pool remained sufficiently reduced to allow a low level of kinase activity to be maintained. In addition to this, we detected two further phosphoproteins in the PSII core complex. The 40 kD phosphoprotein is almost certainly the *psb c* gene product corresponding

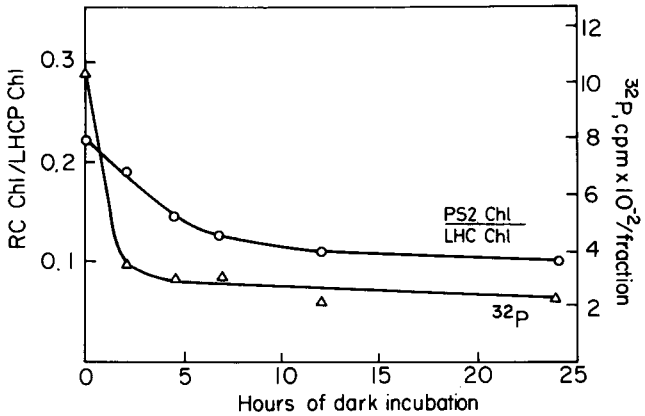

FIG. 5. Time course of *in vivo* dark-induced dephosphorylation of PSII core polypeptides. *D. salina* cells were cultured for 5 d with ^{32}P as in Figure 4 and transferred to complete darkness. PSII core complexes were purified and analyzed as in Figure 2 and Table III.

to the 43 kD polypeptide of higher plant chloroplast (2). The 30 kD phosphoprotein could be the D2 polypeptide as recently suggested from studies with pea chloroplasts (24). At present, there is no clear idea why these polypeptides undergo reversible phosphorylation, and whether it is linked in some way to the disassembly and reassembly of the PSII core complex. The origin of the 13 kD phosphoprotein is unclear and this component only occasionally co-purifies with the PSII core complex (24).

In conclusion, we have demonstrated that in contrast to higher plants, *D. salina* possesses mechanisms for rapidly modifying its relative levels of Chl binding proteins and thus could prove to be an excellent experimental system for studying the dynamics of light adaptation and gene expression.

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