Kinetics of Photoacclimation in Response to a Shift to High Light of the Red Alga *Rhodella violacea* Adapted to Low Irradiance

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The unicellular rhodophyte *Rhodella violacea* can adapt to a wide range of irradiances. To create a light stress, cells acclimated to low light were transferred to higher irradiance and the kinetics of various changes produced by the light shift were analyzed. The proton gradient generated by excess light led to a non-photochemical quenching of the chlorophyll fluorescence and some photoinhibition of photosystem II centers was also produced by the light stress. After the shift to higher irradiance, the mRNA levels of three chloroplast genes that encode phycoerythrin and phycocyanin apoproteins and heme oxygenase (the first enzyme specific to the bilin synthesis) were negatively regulated. A change in the amount of thylakoids and in the total pigment content of the cells occurred during light acclimation after a light stress. The change in the size of the phycobilisome was limited to dissapearance of the terminal phycoerythrin hexamers in some of the rods. The ability of *R. violacea* to photoacclimate depends both on large changes in thylakoid number and pigment content and on smaller changes in the antenna size of photosystem II.

Different groups of algae are able to grow under a broad range of light irradiances (Levy and Gantt, 1988; Andreasson and Melis, 1995; Shapira et al., 1997). At low intensities light can be limiting. When algae are exposed to irradiances in excess of that required to saturate photosynthesis, the excess light becomes a stress. Several protective mechanisms can operate (Asada et al., 1998; Asada, 1999; Niyogi, 1999). One occurs at the level of the light-harvesting antenna of photosystem (PS) II: Excess photons absorbed by the antenna can be dissipated through a down-regulation of PSII associated with the proton gradient (ΔpH) generated across the thylakoid membrane (Demming-Adams and Adams, 1992). However, if these relaxation mechanisms are unable to dissipate all of the excess energy, the remaining flux of excess photons leads to the formation of harmful radicals. These reactive molecules then attack target molecules in the thylakoids resulting in photoinhibition. The first target of photoinhibition is the PSII reaction center which is associated with a loss of photochemical activity and variable fluorescence (Baker and Horton, 1987; Kirilovsky et al., 1988, 1990; Prasil et al., 1992; Vass et al., 1992).

Studies on photoprotection and photoinhibition have often been done on a time scale of less than a day. They mostly concern higher plants and algae where the distal PSII antenna is a light-harvesting complex (LHC) called LHCII and where a xanthophyll cycle is operating (Olaizola et al., 1994; Olaizola and Yamamoto, 1994; Ting and Owens, 1994; Casper-Lindley and Björkman, 1998). In rhodophytes the peripheral antenna of PSII consists of a large extramembrane complex, the phycobilisome (PBS) rather than LHCII, and there is no xanthophyll cycle (Hager, 1980; Gantt, 1981). However a ΔpH-dependent chlorophyll (Chl) *a* fluorescence quenching can also be formed under strong illumination (Delphin et al., 1996, 1998) and its photoprotective role in the unicellular red alga *Rhodella violacea* has recently been studied(Ritz et al., 1999).

As a longer term response to light stress, photoacclimation takes place. This requires photoregulation of gene expression and several changes at the cellular level. The information about the change in light intensity is transferred through signal transduction pathways to the regulatory elements that control gene expression. Studies on photoacclimation have compared steady-state exposures to high light (HL) with low light (LL) exposures. These investigations have focused on various aspects of the differences between cells acclimated to different intensities at the level of their ultrastructure, pigmentation, light-harvesting antenna size and PSII to PSI ratios (Cunningham et al., 1989; Andreasson and Melis, 1995; Baroli and Melis, 1996). There are some reports on the kinetics of photoacclimation processes. Andreasson and Melis (1995) have shown a declining amount of LHCII when LL-grown cells of the green alga *Dunaliella salina* were transferred to HL. Neidhardt et al. (1998) examined the relationship between chronic photoinhibition and antenna size in *D. salina*. In the unicellular green alga *Chlamydomonas reinhardtii*, Sha-

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pira et al. (1997) showed that LL-grown cells were able to photoacclimate to HL by down-sizing their PSII antennae and increasing the rate of D1 synthesis 10-fold. The differential regulation of chloroplast gene expression during a light stress was characterized and *psbA* and *rbcL* transcripts levels were found to be much less regulated than the corresponding proteins D1 and Rubisco.

In rhodophytes, emphasis has been placed on the regulation of the size and pigment content of the PBS (Levy and Gantt, 1988; de Lorimier et al., 1992; Algarra and Rüdiger, 1993; Grossman et al., 1993; Bernard et al., 1996). Cunningham et al. (1989) showed that in *Porphyridium cruentum* the number of thylakoids per cell varied with light irradiance. Our aim was to investigate the major changes occurring during photoacclimation and the possible occurrence of regulatory events specifically triggered by a light stress, which would lead to formation of transient changes in the levels of specific proteins. In the present work we undertook a comparative study of the kinetics of changes triggered by a shift of LL-acclimated cells to higher irradiances, analyzing the ultrastructure of the chloroplast as well as PBS composition in the unicellular red alga *R. violacea*. We first studied comparative properties (growth rate, pigment content, ultrastructure, and PBS composition) of algae grown under three different irradiances, LL, medium light (ML), and HL. Then, to create a light stress, LL-grown cells were shifted to ML or HL. The consequences at the level of the electron transfer chain, the occurrence of photoprotection and partial photoinhibition were followed over a period of 3 d after the shift to higher irradiances. In parallel, ultrastructure modifications were monitored and changes in pigment content were determined. The regulation at the mRNA level of some of the genes involved in the biosynthesis of PBS components as well as the de novo synthesis of PBS were also studied.

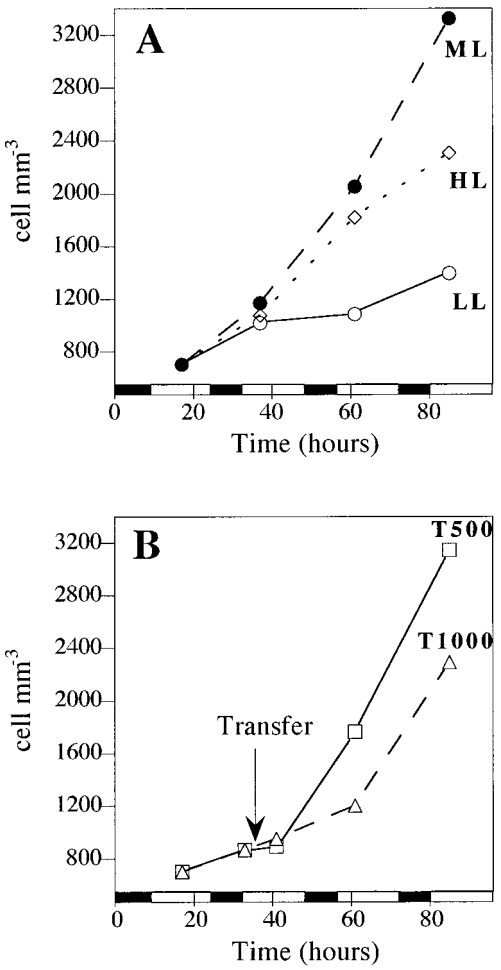

Figure 1. Growth curves during 3 d after the dilution to 700 cell mm^{-3}. The 16-h photoperiod and 8-h dark period are indicated as white and black segments respectively. A, Control cultures, LL cells (○), ML cells (●), and HL cells (◇). B, Growth curves of the LL-adapted cells transferred either to ML (T500 culture) or HL (T1000 culture). The 1st d after the dilution, LL-adapted cultures were transferred to ML (□) or HL (△) conditions, 1 h after the beginning of the light period. The data shown are from one representative experiment. Similar data were obtained from five independent experiments.

RESULTS AND DISCUSSION

Characterization of Cells Acclimated to Steady-State Illumination at 40, 500, and 1,000 μmol m^{-2} s^{-1}

Algae fully acclimated to the three different light intensities had very different characteristics. They grew at different rates as shown in Figure 1A. The maximum rate was found for the ML cells, the LL cells were obviously light-limited. Presumably, the HL cells utilized energy to resist light stress and therefore had less ability to divide. The cell generation times were 70 h (LL cultures), 30 h (ML cultures), and 40 h (HL cultures). The highest light intensity used in these experiments (1,000 μmol m^{-2} s^{-1}) was close to the upper limit of tolerance for *R. violacea* cells. Beyond this value, cells remain viable, but were unable to divide owing to diversion of energy resources to the processes needed to survive light stress. Chloroplast thylakoid content also depended strongly on light intensity. The ultrastructure of cells and of thylakoid membranes in the three cultures is shown in Figure 2. In LL-acclimated cells, the thylakoid membranes were densely packed and arranged in parallel arrays (Fig. 2, A and H) whereas in HL-grown cells, the volume occupied by the chloroplast was much smaller, the thylakoids were further apart and their arrangement was less organized (Fig. 2, C and J). In ML culture, when cells were growing faster than in HL, the thylakoids were less packed than in LL cells but they were still organized in parallel arrays (Fig. 2, B and I).

In agreement with the observations just described, pigment contents of cells were very different in the three growth conditions. The amount of pigment per

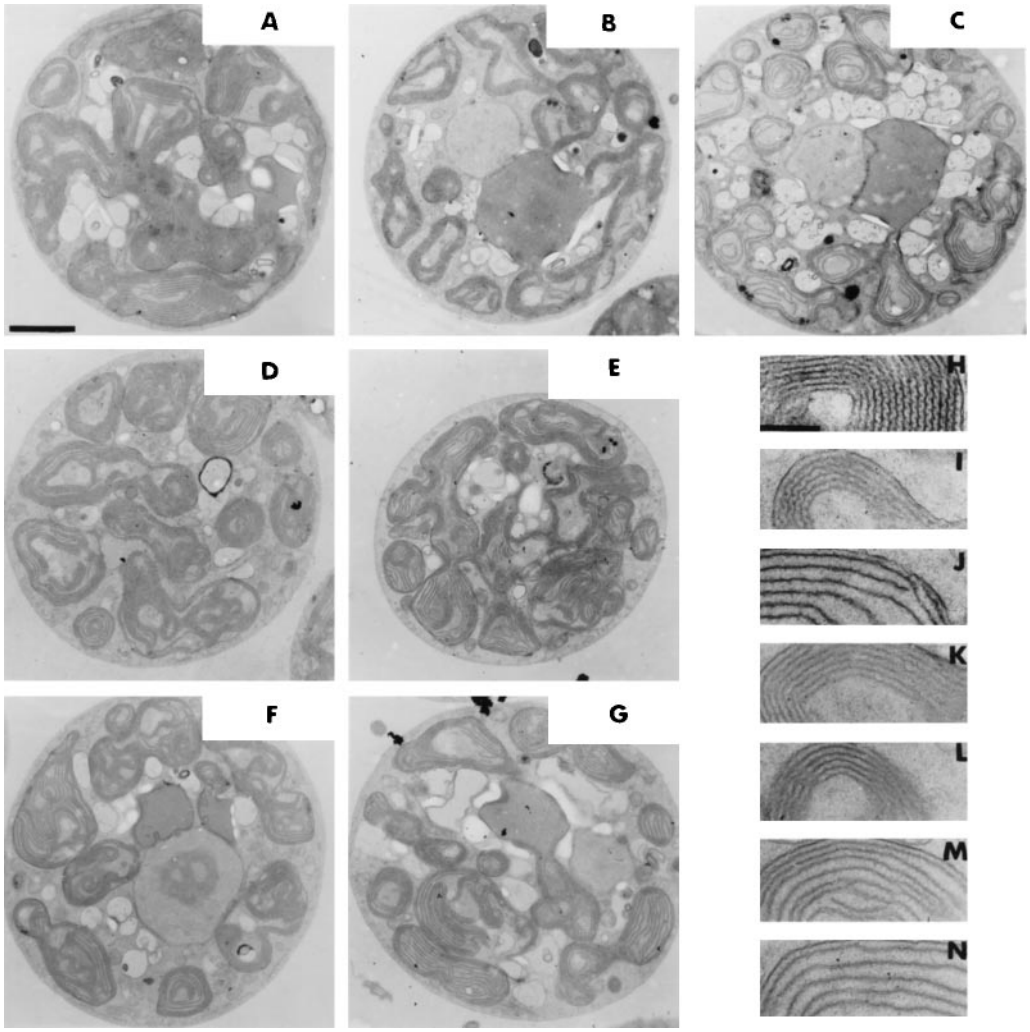

Figure 2. Ultrastructure of cells and thylakoids of *R. violacea*. A to G, Cell sections with the same magnification (scale bar on A is 2 μm). H to N, Thylakoid membranes: higher magnification of the same chloroplast surface (scale bar on H is 0.5 μm). Cells from the three fully acclimated cultures were examined: LL-adapted culture (A and H), ML-adapted culture (B and I), and HL-adapted culture (C and J). The 1st d after the dilution, LL-adapted cultures were transferred to ML (T500 culture) or HL (T1000 culture) conditions, 1 h after the beginning of the light period. Samples were collected 24 h (T500, D and K; T1000, F and M) and 48 h (T500, E and L; T1000, G and N) after the transfer.

cell was inversely correlated with the photon flux density. Pigment contents are shown in Table I. In rhodophytes, Chl *a* is the only Chl present, and it is mainly associated with the LHCI component of PSI (Gantt, 1996), with a small fraction of the pigment found in the CP47 and CP43 and in the PSII reaction center. The Chl *a* content was much smaller in ML- or HL-grown cells than in LL-grown cells (Table I). Phycobiliproteins (PBPs) and their associated linkers are located in the peripheral antennae of PSII; the PBS is bound to the external surface of the thylakoid membranes (Gantt, 1986). In *R. violacea*, under LL conditions, the PBS rods contain one proximal hexamer of phycocyanin (PC) and two distal hexamers of phycoerythrin (PE), whereas under HL conditions, some of the distal hexamers are missing (Koller et al., 1977; Mörschel et al., 1977; Klotz and Glazer, 1985; Bernard et al., 1996). According to the results in Table I, the PC to allophycocyanin (AP) ratio was approximately constant in all light conditions, whereas the ratio of PE to AP was lower under HL than under LL (2.6 for HL cells and 3.4 for LL cells). Comparing HL to LL grown cells, the deficit of the PE content per PBS in HL grown cells is around 25%. This corresponds to a significant loss in chromophores since PE hexamers contain almost twice as much chromophores as PC hexamers. Cultures grown at 500 and 1,000 μmol m^{-2} s^{-1} had a smaller PBP content per cell compared to the culture grown at 40 μmol m^{-2} s^{-1} (Table I). It was the pigment content per cell that changed the most when comparing the properties of the cells grown under various irradiances. The HL-acclimated cells contained four to five times less pigments than LL-acclimated cells. The ability of *R.*

Table I. *Steady-state pigment contents per cell for the three adapted cultures of R. violacea*

Values represent four independent measurements over the 3 d after the dilution and SDS are given.

Culture	Pigment			
	PE	PC	AP	Chl *a*
	10^6 μg cell^{-1}			
LL	48.3 ± 3.2	13.8 ± 1.1	14.1 ± 0.9	7.9 ± 0.2
ML	10.3 ± 1.6	3.7 ± 0.6	3.4 ± 0.8	2.1 ± 0.2
HL	9.5 ± 2.3	4.2 ± 1.1	3.6 ± 0.9	1.5 ± 0.5

violacea to acclimate to various light intensities appear to depend more on very large changes in thylakoid number and pigment content than it does on changes in the antenna size of PSII.

Consequences of a Shift to Higher Irradiances on LL-Acclimated Cells: Short- and Long-Term Responses

In addition to comparing cells acclimated to three light regimes, our aim was to follow the kinetics of photoacclimation by shifting LL-grown cells to higher irradiances. Cells transferred from LL to ML conditions are termed T500, and cells transferred from LL to HL, T1000. Growth curves of T500 and T1000 cultures are shown in the Figure 1B. At both intensities, after an initial lag, growth resumed. The growth rate of the T500 cells transiently exceeded that of the ML-acclimated cells. The doubling time of T500 cells (25 h) was shorter than that of the ML control cells (30 h) and T1000 cells had a doubling time equal to that of the HL-acclimated cells (40 h). The lag in the growth that immediately followed the transfer of the cells may be a result of the stress induced by the increase of light intensity which transiently inhibited cell division.

The fluorescence and oxygen yield changes were measured during the first 3 d after the irradiance shift (Fig. 3). Samples were removed from the culture vessel three times a day during the light period, and the values of F_m' were measured under two different conditions: first, to assay gross non-photochemical quenching (NPQ) due to both photoinhibition and ΔpH, and then in the presence of nigericin to determine the effect of photoinhibition alone. The results were averaged for each day. F_m was determined using dark-adapted LL-grown cells before their transfer to higher irradiance. The average oxygen yield (Ym) during the light period was also determined. Figure 3A reveals that gross NPQ after the transfer from LL to ML was mainly due to a persistent ΔpH with some contribution from photoinhibited PSII centers. Figure 3B shows that photoinhibition after the transfer from LL to HL and the gross NPQ are larger than after the transfer to ML and that at the end of the 3rd d, Ym reaches a value close to that measured in HL-grown cells (0.54), which was lower than Ym of LL- or ML-grown cells, 1 and 0.75, respectively.

The changes in the ultrastructure of the cells were examined during the first 2 d. Twenty-four hours after the shift, either in ML or in HL, modifications were observed (Fig. 2, D, E, K, and L): The volume of the chloroplast decreased and the number of thylakoids per cell diminished. The changes were more marked after 48 h (Fig. 2, F, G, M, and N).

Regulation of gene expression must occur to produce the observed changes in pigment content and thylakoid number required for light acclimation. This was monitored by characterization of the biosynthesis of the PBP. PBPs, the final products of the biosynthetic pathway, were examined for 3 d after the trans-

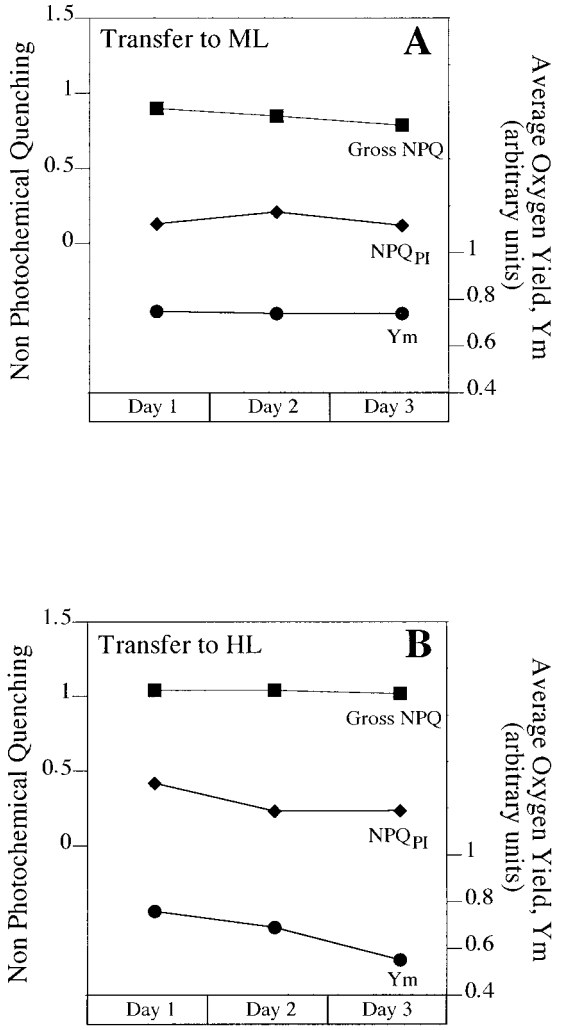

Figure 3. Changes in the gross NPQ, and in the NPQ due to photoinhibition NPQ_{PI} calculated from fluorescence data during 3 d after the transfer from LL to ML (A) or to HL (B). The 1st, 2nd, and 3rd d after the transfer are indicated. The average of NPQ and NPQ_{PI} of different measurements made at different times during the light period is plotted for each day. The same is done for the oxygen yield per flash (Ym) normalized to Ym of LL-grown cells. Similar data were obtained in three independent experiments.

fer from LL to higher irradiances and these results were compared to changes of the Chl *a* content. During the first 3 d after the light shift the pigment content per milliliter of culture varied in a way similar to the pigment content in LL-acclimated cells (data not shown). The variations in the PE, PC, AP, and Chl *a* content per cell were very similar after the light shift; a small increase during the first hours after the transfer was followed by a decrease toward values observed with ML- or HL-acclimated cells (from LL to ML, Fig. 4A or to HL, Fig. 4B). For a better comparison of the kinetics, the first values (LL values) were normalized (Fig. 4, A and B, insets). It

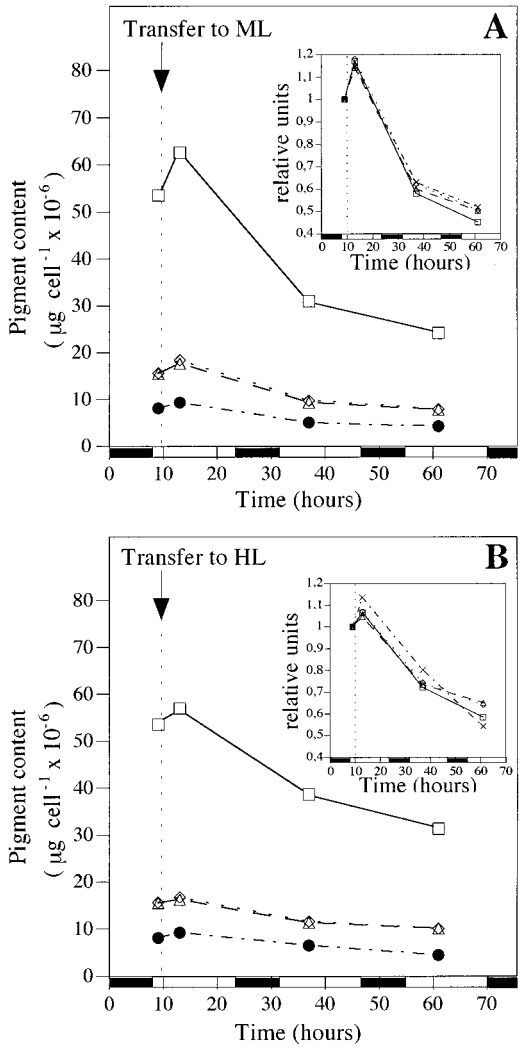

Figure 4. Changes in the pigment contents per cell in cultures transferred from LL to ML (A) or HL (B). The 1st d after the dilution, LL-adapted cultures were transferred to higher light, 1 h after the beginning of the light period. The first point of the curves was taken from LL-adapted culture at the end of the dark period and before the transfer. These measurements were performed on the same samples used for Figure 1. The 16-h photoperiod and 8-h dark period are indicated as white and black segments, respectively. PE, □; PC, △; AP, ◇; Chl *a*, ●. Insets present the data with the pigment contents measured 1 h before the transfer normalized to 1.

can be seen that the PE decrease was faster than the decreases of PC or AP, and that following the transfer to HL the Chl *a* content per cell decreased faster than the PBP. The small increase at the beginning of the transfer can be explained by cell division being blocked but PBP synthesis continuing at the rate determined by the pre-existing conditions (see below, de novo synthesis experiments). Thus, the amount of PBP per cell increased until cell division resumed and down-regulation of gene expression began. The kinetics of down-regulation were somewhat slower in T1000 cells than in T500 cells probably because T500 grows faster than T1000 (Fig. 1B). Because PBP and PBS are very stable and not degraded (Schwarz and Grossman, 1998), decreases in the amount of PBS per cell (and of the PBS size) can only result from cell division and changes in de novo synthesis, that is to say by progressive substitution of the pre-existing large structures by the newly synthesized smaller structures. After transfer, cultures were kept in ML or HL conditions for more than 20 d, with a dilution every 3 d. The pigment content of fully acclimated cells was reached only after 8 to 10 d of growth (data not shown).

Regulation of the gene expression at the transcriptional level was examined for three of the chloroplast-encoded genes involved in the biosynthesis of the PBP: the genes encoding PE and PC apoproteins, and the heme oxygenase (HO). The mRNA level of the chloroplast-encoded *psaA* gene (encoding the P700 apoprotein A1) was used as a control. Figure 5A shows examples of autoradiograms of RNA blots, corresponding to samples from cells acclimated to LL, ML, and HL conditions, hybridized with the four probes for the three cultures. The histograms (Fig. 5B) illustrate the relative transcript abundance and show a clear inverse relationship between transcript abundance for PE, PC, and HO, and light intensity. Changes as a function of time after the shift of LL cultures to ML or HL are shown in Figures 6, A and B. Decreases in PE, PC, and HO mRNA levels were detected 8 h after the transfer (except for HO in T1000) and leveled off at the end of the experiment (except for PC in T1000) at values close to those found in ML or HL fully acclimated cells. Genes coding for PE, PC and HO are negatively regulated by light intensity at the mRNA level. This photoregulation is not valid for all the chloroplastic genes, as shown by the increase in the *psaA* mRNA level. To look for a possible regulation at the translational level, de novo synthesis of the PBS polypeptides was studied. To determine the time needed after the transfer before the regulation becomes effective at the protein level, in vivo labeling of protein with radioactive sodium bicarbonate was performed. The resulting autoradiogram (Fig. 7) shows newly synthesized PBS polypeptides in the cells acclimated to LL and ML conditions (first and last lanes in Fig. 7) and in cells transferred from LL to ML (2, 8, and 27 h after

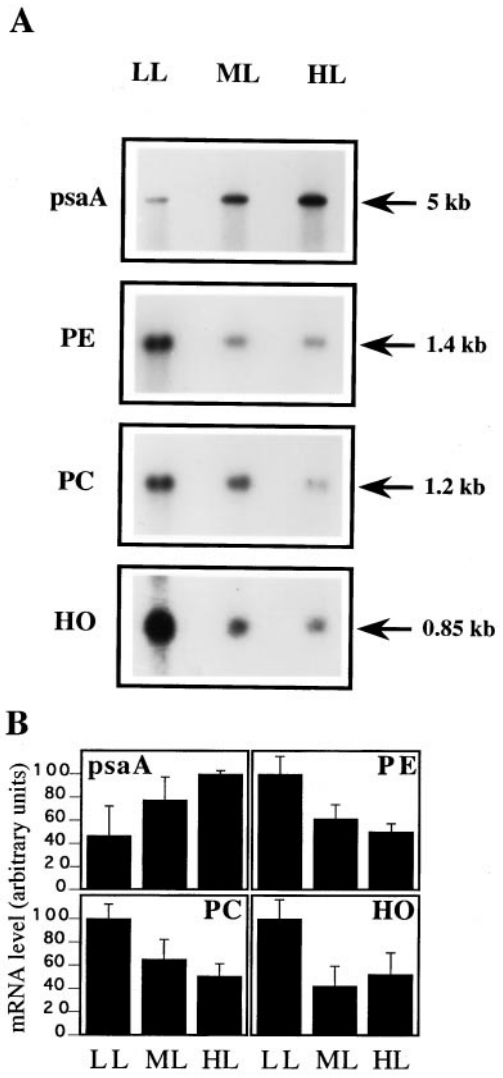

Figure 5. RNA blot hybridizations. A, Autoradiograms of RNA blots hybridized with the *pbsA* (HO), *rpeB* (PE), *cpcAB* (PC), and *psaA* probes, for LL-, ML-, and HL-adapted cultures. The same RNA samples (10 μg per lane) were used for four different blots. The film for the autoradiography of the membrane hybridized with the HO probe was exposed 48 h; 1 h for the PE and PC probes and 27 h for the *psaA* probe. On the top of each lane, the light intensities used for the adapted cultures are indicated. The probes used and the molecular masses of the mRNA are indicated on the left and right of the figure, respectively. B, Histograms show the chloroplast transcript abundance for *pbsA* (HO), *rpeB* (PE), *cpcAB* (PC), and *psaA* genes in cells acclimated to LL, ML, and HL conditions. Values represent the means of three to seven independent sets of samples and one to four replicates of gels for RNA blots with the same set of samples hybridized with the probes for HO, PE, PC, and *psaA* genes; SDs are shown. The values of the spots were determined with a phosphor imager system. For each probe, the level of the strongest signal was taken as 100% and all other levels were expressed with reference to this value. The averages were calculated with samples collected during the 3 d after the dilution. Transcript levels are stable in control cultures as shown by low SD (from 2.5–25.1).

the transfer, lanes marked T2, T8, and T27, respectively). As reported by Bernard et al. (1996), in the ML profile, the L_R^{32} band (linker associated with the distal hexamer of PE) was much less intense than that corresponding to the L_R^{33} band (linker associated with the proximal hexamer of PE). The ratio of L_R^{32} to L_R^{33} started to decrease 2 h after the transfer and by 27 h it was close to that of fully acclimated ML cells. These results agree with the measurements of pigment contents. After the shift, pigment synthesis occurs and LL-PBS were synthesized. A progressive modification of the pigment composition up to 27 h produces a situation approximating that observed in ML-acclimated cells.

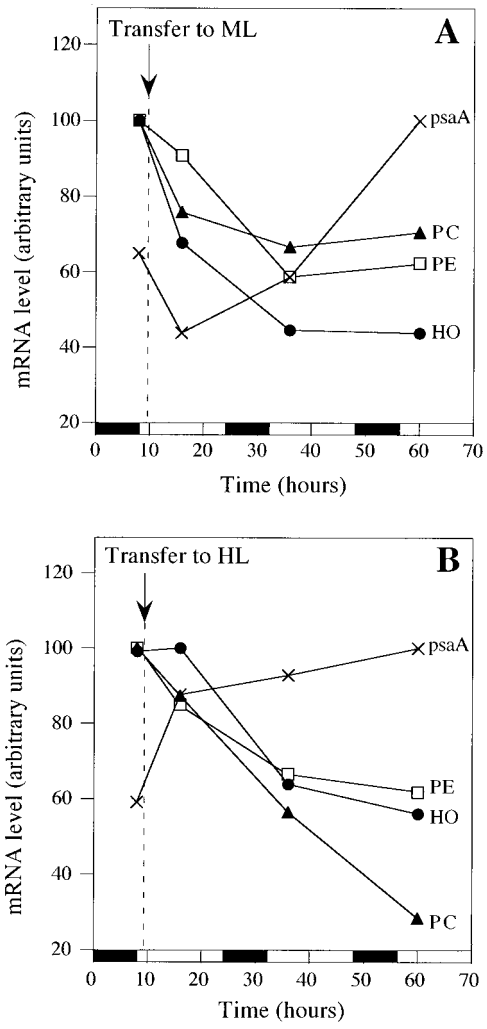

Figure 6. Relative transcript abundance of *pbsA* (HO), *rpeB* (PE), *cpcAB* (PC), and *psaA* genes plotted versus time of culture for T500 (A) and T1000 (B) cultures. The 16-h photoperiod and 8-h dark period are indicated as white and black segments respectively. The 1st d after the dilution, two LL-adapted cultures were transferred to ML (T500) or HL (T1000) conditions, 1 h after the beginning of the light period. Values represent at least three to seven independent sets of samples; and one to four replicates of gels for RNA blots with the same set of samples were hybridized with the probes for HO (●), PE (□), PC (▲), and *psaA* (X) genes. For each probe, the level of the strongest signal detected by the phosphoimager was taken as 100% and all other levels were expressed with reference to this value. The SDs are not shown to maintain clarity; they vary from 2.2 to 44.5.

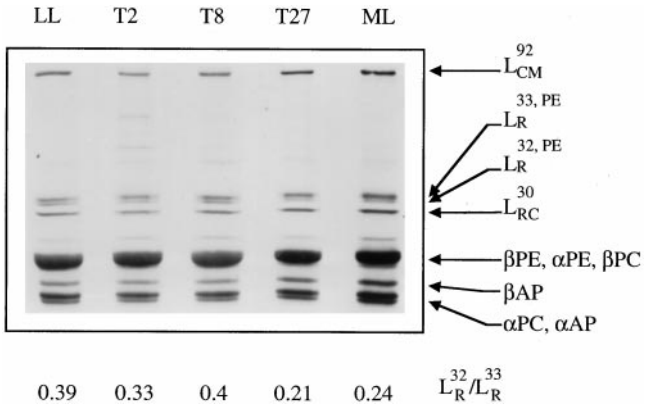

Figure 7. De novo synthesis of PBS polypeptides under different light conditions. Autoradiogram of the de novo synthesized PBS polypeptides separated by lithium dodecyl sulfate-PAGE. The in vivo protein labeling was performed under the different culture conditions: LL- and ML-acclimated cells and 2 h (T2), 8 h (T8), and 27 h (T27) after the transfer from LL to ML. The positions of PBP subunits and linkers are indicated on the right of the figure. At the bottom of each lane, the ratio of L_R^{32} to L_R^{33} is indicated. The quantification of the two bands corresponding to the polypeptides was performed as described in "Materials and Methods."

CONCLUSION

When LL-acclimated cells were transferred to higher irradiance, they experienced a light stress, which was severe enough to block cell division for some time. When growth resumed, the growth rate was lower after the transfer to HL than after the transfer to ML. Chronic photoinhibition persisted after photoacclimation (as was previously described by Neidhardt et al. [1998] for a green alga) and was larger after transfer to HL than after the transfer to ML. After the shift to ML, the decrease of the mRNA levels of the three chloroplast-encoded genes PE, PC apoproteins, and HO enzyme began soon after the transfer and reached levels close to those found in fully acclimated cells within 3 d. Other authors have also found chloroplast gene regulation at the mRNA abundance level in a rhodophyte (Apt and Grossman, 1993). After the transfer of LL-grown cells to higher irradiance, a large change in the total pigment content of the cells occurs during photoacclimation as a result of cell division and down regulation of gene expression. The modification in the antenna size, which requires a specific regulation of L_R^{32} and PE, is limited to disappearance of the distal PE hexamers in some of the rods.

Photosynthetic activity is often believed to be the photon-sensing receptor that transfers information to the effector that controls gene expression machinery. The cytochrome f/b_6 complex, the plastoquinone pool, thioredoxin, and the proton gradient have been proposed as possible redox sensors (Pearson et al., 1993; Danon and Mayfield, 1994; Escoubas et al., 1995; Mühlbauer and Eichacker, 1998; Pfannschmidt et al., 1999). In *R. violacea*, the persistent ΔpH under high irradiance or the redox state of one of the electron transfer components could be part of the system that transduces the light intensity signal to the effector of the gene expression.

Shapira et al. (1997) have introduced the concept of a stress-induced gene regulation. In the work reported here the only stress-specific regulations are the transitory inhibition of cell division, the build-up of a proton gradient, the generation of a ΔpH nonphotochemical fluorescence quenching and some photoinhibition that remains despite the change of the PSII antenna size. We do not rule out the possibility of more rapid regulation for genes encoding specific stress-induced proteins (Zheng et al., 1998).

Previous work has ascribed photoacclimation in different algae to a reduction of the PSII antenna size and a decrease of the number of thylakoids per cell. *R. violacea* have a similar behavior. After the shift of LL-grown cells to higher irradiances, it would be interesting to compare the extent of photoprotection due to the ΔpH-dependent NPQ in algae with LHCII and a xanthophyll cycle with algae with PBS and small Chl antenna.

MATERIALS AND METHODS

Plant Material, Culture Conditions, and Shift to Higher Light Conditions

Rhodella violacea (strain 115-79 from Göttingen University, Germany) was grown photoautotrophically in sterile artificial seawater (Jones et al., 1963) with the addition of vitamin B12 at 25 μg L^{-1}. Cultures of 300 to 700 mL were incubated at 20°C in glass culture flasks continuously flushed with sterile air, and illuminated with fluorescent tubes with a 16-h light/8-h dark photoperiod. Three different light intensities were used, 40, 500, and 1,000 μmol m^{-2} s^{-1}, defined as LL, ML, and HL conditions. To standardize the culture conditions and minimize self-shading, cells were regularly diluted every 3 d to 700 cell mm^{-3} with fresh medium.

Transfer from LL to ML or HL was performed as follows: On d 0, the cultures were diluted to 700 cell mm^{-3}. On d 1, 1 h after the beginning of the light period, the LL culture was transferred to ML (T500 culture) or HL (T1000 culture) conditions. From d 1 to 3, samples were collected at different times, from the LL, ML, and HL adapted cultures and from the transferred culture (T500 and T1000 cultures). On the 3rd d, in the middle of the light period, the cultures were diluted to 700 cell mm^{-3}.

Growth Rate

Cell densities were determined daily with a Thoma (Societe Precis Briare, France) hemocytometer, using the public domain NIH Image program (National Institutes of Health, Bethesda, MD). The generation time was calculated for cells in exponential growth.

Electron Microscopy

R. violacea cells were fixed at 4°C for 1 h with 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer, pH 6.8, plus 0.25 M Suc, and post-fixed in 1% (w/v) OsO4 for 2 h. Fixed cells were embedded after dehydration in Spurr's epoxy resin (Spurr, 1969). Sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (CX2, JEOL, Tokyo).

Pigment Extraction and Quantification of Pigment Contents

Pigments were extracted in 0.15 M sodium-phosphate buffer (pH 7.2) after cell breakage by sonication. One part of the resulting suspension was centrifuged (182,000*g* in a SW55 rotor [Beckman Instruments, Fullerton, CA] for 1 h at 4°C) to remove cell debris. PBP concentrations in the supernatant were estimated spectrophotometrically using extinction coefficients and equations according to Koller et al. (1977). The other part of the suspension was used to extract Chl *a* in 90% (v/v) methanol. After centrifugation (15,000*g* in a 2K15 Nr 12141 rotor [Sigma, St. Louis] for 10 min at 4°C) the Chl *a* concentration was determined from the supernatant using extinction coefficients according to Mackinney (1941). Optical densities were measured with a spectrophotometer (Varian, Sunnyvale, CA).

Fluorescence Measurements

Fluorescence induction kinetics of the PSII Chl at 20°C were measured in a laboratory-built continuous fluorimeter using green-light-emitting diodes with an interference filter (550 ± 20 nm, Corion, Franklin, MA) to provide continuous illumination of adjustable intensity and duration that served both as actinic and as detecting beam. The fluorescence was detected at 680 nm by a photomultiplier (H 5700–50, Hamamatsu Photonics, Hamamatsu City, Japan) protected by an interference filter (680 ± 10 nm, Corion). Data were collected in a computer with a PCL 818 (Advantech, Taipei, Taiwan) data acquisition card, with a 33-μs time resolution. The system used software developed in this laboratory. Data were collected with different time resolutions and averaging was done to obtain the maximum information with a minimal file size. The results were displayed on logarithmic time scale to display all of the kinetics together. The software controlled both the light-emitting diodes and data acquisition.

At room temperature, fluorescence emanates mainly from the Chl *a* antenna of PSII (Dau, 1994). Fluorescence yield is dependent on photochemical and NPQ (Buschmann, 1999). Photochemical quenching is dependent on the redox state of the primary acceptor of PSII, the plastoquinone Q_A (Duysens and Sweers, 1963; van Gorkom, 1974). When all PSII centers are open (Q_A oxidized), they are efficient exciton traps and the fluorescence yield of PSII is low. When Q_A is reduced, the centers are unable to trap excitons, the photochemical quenching is suppressed. Chl fluorescence can also be decreased by NPQ ascribed to two main processes occurring under strong light: ΔpH-dependent quenching and photoinhibition (Satoh and Katoh, 1981; Delphin et al., 1996, 1998; Sauer and Debreczeny, 1996; Ritz et al., 1999). In the absence of photochemical quenching and NPQ, fluorescence reaches a maximum level F_m. F_m was measured as the maximum of a fluorescence induction during a strong green illumination of 100 μmol m^{-2} s^{-1} dark-adapted samples. NPQ was determined by the maximum level F_m' of a fluorescence induction of pre-illuminated samples. The ΔpH-dependent quenching can be suppressed by nigericin (an uncoupler acting as a proton-transporting ionophore) (Delphin et al., 1998; Ritz et al., 1999). In the presence of nigericin the remaining NPQ is attributable to photoinhibited PSII centers. Algal cells were used at a constant concentration of Chl *a* during an experiment (6 μg Chl *a* mL^{-1} for the transfer to ML, 4 μg Chl *a* mL^{-1} for the transfer to HL). F_m was measured on dark-adapted samples of the LL-grown cells. F_m' was measured at different times after the transfer to higher irradiances in the absence or in the presence of 100 μM nigericin. NPQ was then computed from: $NPQ = (F_m/F_m') - 1$ (Buschmann, 1999). Gross NPQ, the sum of NPQ due to ΔpH and NPQ due to photoinhibition (NPQ_{PI}) was computed with the values of F_m' measured in the absence of nigericin. NPQ_{PI} was computed with the values of F_m' measured in the presence of nigericin.

Oxygen Yield Measurements

The oxygen yield per flash was measured with a rate electrode already described (Joliot and Joliot, 1968; Kirilovsky et al., 1990). The short (5-μs) saturating flashes were produced by a Strobotac (General Radio, Concord, MA). The spacing between flashes was 0.5 s. Cells (100 μg Chl *a* mL^{-1}) were dark adapted prior to each flash sequence. The average oxygen yield (Ym) is indicative of the number of active PSII centers and therefore the extent of photoinhibited PSII centers can be evaluated by this technique. Ym was measured for the fully acclimated cultures and also at different times after the transfer of LL-grown cells to higher irradiances. The comparison between different samples was difficult. The amount of cells deposited on the electrode cannot be calibrated and for *R. violacea*, the amplitude of the signal for a given sample increases with time. Averaging on several samples and sequences enabled us to indicate a clear trend.

RNA Isolation, Blot Hybridization, and mRNA Quantification

Total RNA from *R. violacea* culture samples (10^8 cells rinsed in fresh medium and frozen at −80°C) were isolated as described for cyanobacteria (Mohamed and Jansson, 1989) using hot phenol for extraction and LiCl as the precipitating agent, except that higher volumes (1–2 mL) of phenol heated up to 65°C were used. Total RNA levels were quantified by A_{260}. Electrophoresis of RNA (10 μg per lane) in agarose gels containing formaldehyde, transfer onto nitrocellulose filters, and hybridization were performed as previously described (Damerval et al., 1989). For

the study of PBP biosynthesis, four probes were used. The PBP (PE, PC, and AP) are composed of chromophores (bilin) covalently bound to apoproteins, while the HO is the first enzyme specific of bilin biosynthesis pathway. The probe for the *pbsA* gene coding for HO was a PCR product corresponding to exon 1 (174 nucleotides) (Richaud and Zabulon, 1997). The *rpeB*, *cpcA*, and *cpcB* genes code respectively for the apoprotein of the PE β-subunit and those of the PC α- and β-subunits. An internal *Hin*dIII fragment of 330 bp in the *rpeB* gene (Bernard et al., 1992) was used to probe for PE mRNA, and a 0.6 kb *Pst*I-*Hin*dIII fragment containing the 3′ end of the *cpcB* gene and the *cpcA* gene of *R. violacea* (Garnier et al., 1995) was used to probe for PC mRNA. The *psaA* gene codes for the PSI P700 apoprotein A1. The probe for *psaA* mRNA is an 840-bp PCR product (corresponding to the sequence from amino acids 311–602) obtained in our laboratory on *R. violacea* plastidial genome (C. Richaud and G. Zabulon, unpublished data). All the genes described above are chloroplast encoded in *R. violacea*. The quantification of the spots was performed with a phosphor imager system (Fujix BAS 1000, Fuji, Tokyo) associated with a program for digital image analysis. The level of the strongest signal was taken as 100% and all other levels were expressed with reference to this value.

In Vivo ^{14}C-Protein Labeling and Separation of the PBS Polypeptides

For in vivo ^{14}C-protein labeling, the incorporation of 1.1×10^7 MBq (300 μCi) [^{14}C]sodium bicarbonate by whole cells was analyzed. The protein labeling was performed for the different culture conditions (LL, ML, and cells transferred from LL to ML). The ^{14}C-substrate was added to samples (10^8 cells) and the incubation was performed for 90 min under each culture conditions. PBSs were isolated according to the method of Bernard et al. (1996). Then PBS polypeptides (same quantity of radioactivity loaded in each lane) were separated by lithium dodecyl sulfate-PAGE (denaturating 14% [w/v] polyacrylamide slab gel, 1.5 mm thick, in the Tris[tris(hydroxymethyl)aminomethane]-Tricine (*N*-tris [hydroxymethyl]glycine) buffer system of Schagger and von Jagow [1987]). For autoradiography the gel was soaked after electrophoresis for 30 min in an enhancer (Enlightning, DuPont, Wilmington, DE), dried, and exposed to x-ray film. The film for the autoradiography of the gel was exposed for 4 d. Autoradiograms were digitalized with a scanner (Studio ScanIIsi Agfa, Agfa-Gevaert N.V., Mortsel, Belgium) calibrated in optical density with a Scanner Transmission Tablet (gray scale, Eastman Kodak, Rochester, NY). The quantification of the bands was performed with the public domain NIH Image program. The resulting values were divided by their respective apparent M_r, and then divided by the value for the L_{CM} (one of the linker polypeptide), which is assumed to be representative of the number of PBS since it links the PBS to the PSII.

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