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Dark Heterotrophic Growth Conditions Result in an Increase in the Content of Photosystem II Units in the Filamentous Cyanobacterium *Anabaena variabilis* ATCC 29413[1]

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The filamentous nitrogen-fixing cyanobacterium *Anabaena variabilis* ATCC 29413 is capable of heterotrophic growth in complete darkness. After 6 months of continuous dark growth, both the autotrophic and heterotrophic cultures were found to have the same doubling time of 14 h. On a cellular basis, the chlorophyll content remained the same and the phycobilin content showed an increase in the dark-grown cultures. Fluorescence emission spectra at 77 K of dark-grown cells indicated that the phycobilisomes are functionally associated with photosystem II (PSII). Moreover, upon transfer to light, the dark-grown cells readily evolved oxygen. Although photosystem I (PSI) and whole chain-mediated electron transfer rates were comparable in both types of cultures, the rate of PSII-mediated electron transfer was found to be 20% higher in dark-grown cells. The PSI to PSII ratio changed from 6:1 in autotrophic cultures to 4:1 in the dark-grown cells. These changes in the rate of PSII electron transfer and in the stoichiometry between the two photosystems under dark, heterotrophic growth conditions were brought about by a preferential increase in the number of PSII units while the number of PSI units remained unchanged. The advantages of using this organism in the selection of PSI-deficient mutants are discussed.

Although cyanobacteria are generally considered to be photoautotrophic organisms, a number of species are able to grow either photoheterotrophically or heterotrophically in dark (Rippka, 1972). The ability of the unicellular, easily transformable cyanobacterium *Synechocystis* sp. PCC 6803 to grow photoheterotrophically in the absence of a functional PSII complex has been made use of in the selection and maintenance of a large number of PSII mutants (Pakrasi and Vermaas, 1992). On the other hand, cyanobacterial mutants lacking a functional PSI complex are found to be extremely light-sensitive, and the selection of such mutants has been possible only under heterotrophic growth conditions (Rochaix, 1992).

In cyanobacteria two different types of heterotrophic growth conditions have been recognized. Some cyanobacterial species, such as *Synechocystis* 6803 (Anderson and McIntosh, 1991) and *Anabaena variabilis* (from the culture collection of the Institute of Microbiology, University of Tokyo; Ohki and Katoh, 1975), show growth under light intensities too dim to support any photoautotrophic growth, but not under complete darkness. This phenomenon, now generally known as LAHG, has been well characterized in *Synechocystis* 6803 (Anderson and McIntosh, 1991; Smart et al., 1991). This organism grows in Glc-containing media in dark when illuminated for 5 to 10 min every day with a photon-flux density of 40 μmol m^{-2} s^{-1}. The selection of PSI reaction center-deficient mutants of *Synechocystis* 6803 has been made possible under LAHG conditions (Smart et al., 1991; Smart and McIntosh, 1993).

On the other hand, a number of cyanobacterial species, such as *A. variabilis* ATCC 29413 (Wolk and Shaffer, 1976), *Synechocystis* sp. PCC 6714 (Vernotte et al., 1992), and *Plectonema boryanum* (Raboy et al., 1976), are able to grow heterotrophically in complete darkness without the need for any periodic light exposure. Among these cyanobacterial species, *A. variabilis* American Type Culture Collection No. 29413 (henceforth called *Anabaena* 29413) is amenable to reverse genetic analysis (Maldener et al., 1991), and mutants insertionally inactivated in the *psaA*, *psaB* (Toelge et al., 1991; Nyhus et al., 1993), and *psaC* (Mannan et al., 1991) genes, encoding three cofactor-binding proteins of PSI in this organism, have been isolated under heterotrophic growth conditions.

Heterotrophic growth in complete darkness as well as under LAHG conditions is known to bring about a number of significant changes in both the structural and functional organization of the photosynthetic apparatus of various cyanobacterial species. In *Synechocystis* 6803 cells grown under LAHG conditions, there is a 75% decrease in the content of Chl on a cellular basis. This decrease is brought about mainly through a decrease in the number of PSI units, leading to a decrease in the ratio of PSI to PSII (Smart et al., 1991). Vernotte et al. (1992) have observed a 90% decrease in the Chl content in *Synechocystis* 6714 cells grown heterotrophically. Moreover, in this latter organism, heterotrophic growth results in a block in energy transfer to PSII from its major antenna complex, the phycobilisomes. In *P. boryanum*, Raboy et al. (1976) observed a 50% decrease in the content of Chl and a similar decrease in the rate of in vivo photosynthetic CO_2 assimilation in heterotrophically grown cells. In *Chlorgloea fritschii*, Evans and Carr (1975) observed an 80% decrease in the capacity for light-induced oxygen evolution in the dark-grown cells. However, a desirable trait for a cyanobacterial strain to be used for molecular genetic analysis

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Abbreviation: LAHG, light-activated heterotrophic growth.

of the light reactions of photosynthesis is that it undergoes minimal changes in the structural and functional organization of its photosynthetic apparatus under heterotrophic growth conditions. In particular, biochemical analysis of directed PSI mutants will benefit from the use of an organism that maintains the same number of PSI units under both photoautotrophic and dark heterotrophic conditions.

Although *Anabaena* 29413 has become an important system for molecular genetic analysis of the PSI complex (Mannan et al., 1991; Toelge et al., 1991; Nyhus et al., 1993), there is little information available about the status of the photochemical apparatus in the dark-grown cells. In this investigation we have performed a detailed analysis of the changes in the photosynthetic apparatus of *Anabaena* 29413 cultures during dark growth. Our results indicate that in this strain, the only significant change during heterotrophic growth was a small but reproducible increase in the number of PSII units while the content of PSI units remained essentially unaltered. A preliminary report of some of these findings was made at the IXth International Congress on Photosynthesis (Mannan and Pakrasi, 1992).

MATERIALS AND METHODS

Cyanobacterial Strains and Culture Conditions

The wild-type strain of *Anabaena variabilis* American Type Culture Collection 29413 and the mutant strain IM141 were grown in BG11 medium supplemented with 10 mM Tes-KOH, pH 8.2, at 30°C on a rotary shaker (150 rpm). Illumination was provided at a photon flux density of 60 μE m^{-2} s^{-1}. For dark heterotrophic growth, the media were supplemented with 5 mM filter-sterilized Fru. The mutant strain IM147 (a kind gift of Prof. C.P. Wolk) was grown in BG11 medium supplemented with 50 μg/mL of neomycin sulfate. In this strain, the *prcA* gene encoding a calcium-dependent protease has been inactivated by the insertion of a neomycin resistance cartridge (Maldener et al., 1991). Growth in neomycin-containing media helped to minimize bacterial contamination during repeated transfers under continuous dark heterotrophic growth conditions. Under autotrophic growth conditions, the IM141 strain grows on N_2 as the sole source of nitrogen with the same doubling time as the wild-type strain and has no apparent change in its photosynthetic properties (Maldener et al., 1991; Nyhus et al., 1993).

Optical Spectroscopy

Absorption spectra and P700 chemical difference spectra were recorded at room temperature on a DW2000 spectrophotometer (SLM Aminco Instruments, Urbana, IL) in the split-beam mode with a 2-nm slit width. P700 concentration was estimated from ascorbate-reduced minus ferricyanide-oxidized chemical difference spectra of thylakoid samples as described elsewhere (Mannan et al., 1991).

Fluorescence emission spectra at 77 K were obtained on a home-built spectrofluorometer consisting of an INSTASPEC 1 diode array (Oriel Corp.) with a 1-nm slit width. The spectra are presented with the correction for the instrument response. We used the KALEIDAGRAPH program (Abelbeck Software) on a Macintosh computer to normalize the spectral data.

Estimation of Pigment Concentrations

The molar ratio of phycocyanin to Chls was determined from absorption spectra of whole cells according to Arnon and co-workers (1974). Molar extinction coefficients of 890 cm^{-1} and 556.7 cm^{-1} were used to determine the concentrations of Chl and phycocyanin, respectively. In the measurement of photosynthetic electron transfer rates and in the determination of Chl:P700 and Chl:PSII ratios, the concentration of Chl in intact cells as well as in thylakoid membranes was determined after methanolic extraction (Lichtenthaler, 1987).

Measurement of Room Temperature Chl *a* Fluorescence Transients

Anabaena filaments were pelleted down and resuspended in fresh BG11 medium containing 40 μM DCMU. The samples were incubated in the dark for 30 min at room temperature before recording the transients on a PAM Chl fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Illumination was provided with broad-band white light filtered through a Corning CS 4-96 filter at a photon flux density of 400 μE m^{-2} s^{-1}. The data were recorded on a Tektronix 2230 oscilloscope and subsequently processed on a Macintosh computer using the KALEIDAGRAPH program (Abelbeck Software).

Determination of Chl:PSII Ratio

To estimate the concentration of PSII in whole cells, the binding of [^{14}C]DCMU was measured according to the procedure described elsewhere (Nyhus et al., 1993).

Immunoblotting and Protein Analysis

Thylakoid membranes were isolated essentially as described elsewhere (Nyhus et al., 1992). Thylakoid membranes equivalent to 5 μg of Chl were fractionated on urea-containing 16% SDS-polyacrylamide gels. The fractionated proteins were transferred to 0.2-μm nitrocellulose filters (Schleicher & Schuell) using a semidry blotting apparatus (Bio-Rad) for 2 h at 10 V. Immunodetection was carried out according to a procedure described elsewhere (Pakrasi et al., 1991). The secondary antibodies were alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma Immunochemicals). Rabbit antibodies raised against the PsaD protein from *Nostoc* sp. PCC 8009 were from Dr. D.A. Bryant, those raised against the PsaA and PsaB proteins of *Synechococcus elongatus* were from Dr. I. Enami, and those raised against the D1 and D2 proteins of spinach PSII were from Drs. M. Ikeuchi and Y. Inoue.

For the estimation of protein content, thylakoid membranes were extracted twice with 80% acetone and twice with cold diethyl ether, and the final pellet was dissolved in 0.1 N NaOH (Lowry et al., 1951). BSA was used as the standard.

Measurement of Rates of Electron Transfer Reactions

A Clark-type oxygen electrode was used to measure the rates of photosynthetic electron transfer reactions at 30°C

Table I. *Growth rate, pigment content, and photosynthetic properties of autotrophic and heterotrophic cultures of Anabaena 29413*

Asc, Sodium ascorbate; DAD, diaminodurene; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; FeCN, potassium ferricyanide; MV, methyl viologen.

	Autotrophic	Heterotrophic
Doubling time (h)	14.0	14.4
Pigment ratios (mol/mol)		
Phycocyanin:Chl[a]	38.1	43.4
Chl:PSII[b]	785 ± 24	503 ± 42
Chl:P700[b]	133 ± 10	131 ± 12
PSI:PSII	5.9	3.8
Protein:Chl (w/w)[b]	14.1 ± 1.5	14.7 ± 2.0
Rates of electron transfer reactions[b] (μmol O_2 mg^{-1} Chl h^{-1})		
H_2O to CO_2	245 ± 30	250 ± 35
H_2O to DCBQ:FeCN	450 ± 40	545 ± 39
DAD/Asc to MV	440 ± 42	480 ± 50
DCIP/Asc to MV	713 ± 116	725 ± 69

[a] Based on data for Figure 1. [b] Average of six different experiments; see "Materials and Methods" for further details.

with saturating (3500 μE m^{-2} s^{-1}) red light (CS 2–63, Corning). Filaments from exponentially growing cultures were pelleted by centrifugation at 3000*g* for 5 min at room temperature and the pellets were resuspended in 20 mM Tris-Cl (pH 7.0). Whole-chain electron transport was measured in the presence of 10 mM $NaHCO_3$. PSII-mediated electron transport was measured in the presence of 0.1 mM 2,6-dichloro-*p*-benzoquinone and 1 mM $K_3Fe(CN)_6$. PSI electron transport was measured in medium containing 20 μM DCMU, 1 mM sodium ascorbate, 2 mM methyl viologen, and 1 mM 3,6-diaminodurene (Fluka). The samples were adjusted to a final Chl concentration of 10 μg/mL. The rates of PSI electron transport in thylakoid membranes were measured in a reaction mixture containing 50 mM Hepes, pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM KCN, 20 μM DCMU, 10 μg/mL superoxide dismutase (Sigma), 0.5 mM sodium ascorbate, 0.1 mM 2,6-dichlorophenolindophenol, 1 mM methyl viologen, and thylakoid membranes equivalent to 6.6 μg/mL Chl.

RESULTS

Growth of Autotrophic and Heterotrophic Cultures

After 1 year of maintenance under our laboratory conditions, the photoautotrophic culture of *Anabaena* 29413 grew with a 14-h (Table I) doubling time, which is similar to the growth rate reported earlier for an autotrophic culture by Maldener et al. (1991). When such an autotrophic culture was transferred to the dark (heterotrophic growth conditions), the doubling time during the initial period was found to be 38 h (Mannan et al., 1991). However, after 6 months of growth in complete darkness, the doubling time of this heterotrophic culture reached approximately 14 h, which is similar to that of the autotrophic culture.

Optical Spectroscopic Analysis

Under dark growth conditions, the photochemical apparatus of the cells remain unused. It is possible that during prolonged periods of dark heterotrophic growth, there may be a significant reduction in the photochemical apparatus to prevent unnecessary use of cellular metabolic resources. Such decreases have been observed in photosynthetic bacteria (Kiley and Kaplan, 1988) and the green alga *Chlorella protothecoides* (Aoki and Hase, 1964). To determine whether the photochemical apparatus of *Anabaena* 29413 cells had undergone any change during heterotrophic growth, we analyzed the content and composition of the Chl and phycobilin pigments in both the autotrophic and heterotrophic cultures. In vivo, Chl absorb maximally at 680 nm in the red region of the spectrum. Thus, the *A* at this wavelength can be taken as a direct measure of the content of Chl. Using the scattering at 730 nm as a measure of cell density, we observed that the A_{680} was nearly identical in autotrophic and heterotrophic cells (see Fig. 1), indicating that there is no appreciable change in the cellular Chl content during dark growth. The major difference between these two spectra was in the region of absorption by phycobilins. The heterotrophic culture showed an increase in the absorption around 620 nm, indicative of an increase in the content of phycobilins relative to that of Chls. As shown in Table I, there was a 14% increase in the phycobilin to Chl ratio in the dark-grown cells.

In cyanobacteria, the light energy absorbed by the phycobilin pigments is ultimately transferred to the two photosystems through the process of excitation energy transfer (Fork and Mohanty, 1986). In *Synechocystis* sp. PCC 6714 grown under dark, heterotrophic growth conditions, the assembly of the phycobilisomes occurs normally. However, the transfer of excitation energy from phycobilisomes to the two photosystems is blocked in these cells (Vernotte et al., 1992). Figure 2 shows the 77 K fluorescence emission spectra from autotrophic and heterotrophic cultures of *Anabaena* 29413, illuminated with 560-nm light, which preferentially excites the

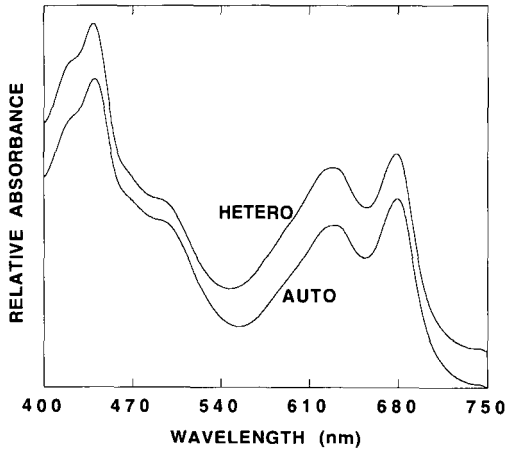

Figure 1. Room-temperature absorption spectra of cells of autotrophic (AUTO) and heterotrophic (HETERO) cultures. The cell suspensions were diluted with the culture medium to A_{730} = 0.1. The spectra were offset for ease of viewing.

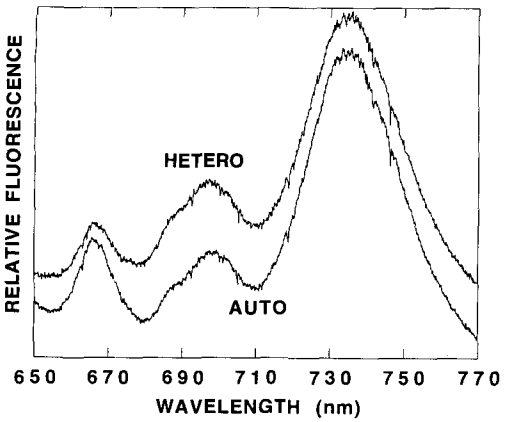

Figure 2. Fluorescence emission spectra at 77 K of intact cells of autotrophic (AUTO) and heterotrophic (HETERO) cultures adjusted to 5 mg Chl/mL. The spectra were offset for ease of viewing. Fluorescence was excited with 560-nm light (half band width = 20 nm).

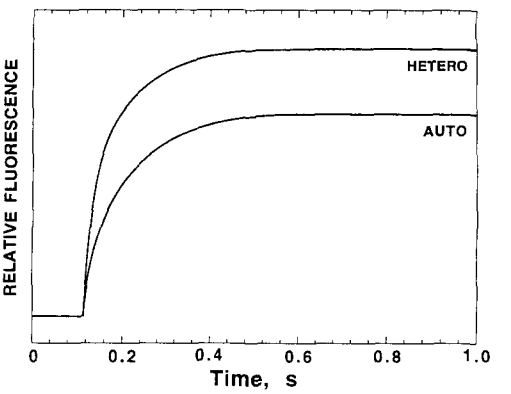

Figure 3. Room temperature fluorescence transients of autotrophic (AUTO) and heterotrophic (HETERO) cultures of *Anabaena* 29413. The cells were incubated in the presence of 5 μM DCMU in the dark for 30 min before the transients were recorded for a period of 1 s. Only the variable fluorescence traces have been shown. The horizontal line at the beginning of each trace indicates the F_0 level.

phycobilin pigments. The spectra of the autotrophic cells had major emission bands at 666 nm (F666), 685 nm (F685), 695 nm (F695), and 735 nm (F735). Among these, F666 is emitted from phycobilisomes, F685 and F695 originate from PSII, and F735 is emitted from PSI (Fork and Mohanty, 1986). These data indicate that the light energy absorbed by phycobilins was transferred to both PSII and PSI in these cells. In contrast to the findings with *Synechocystis* 6714, we observed that F685, F695, and F735 bands were present at significant levels in heterotrophically grown *Anabaena* 29413 cells. The F666 band was considerably lower, indicating that phycobilins in these cells are energetically more tightly coupled to the Chl in PSII and PSI. Another difference between these two spectra was that in the dark-grown cells, there was a relative increase in fluorescence emission from PSII, resulting in increases in the F685:F735 and F695:F735 ratios.

Rates of Photosynthetic Electron Transfer Reactions

Table I shows the rates of whole-chain electron transfer as well as PSI- and PSII-mediated electron transfer reactions. Although the rates of whole-chain and PSII-mediated electron transfer were measured only in intact cells, the rate of PSI-mediated electron transfer was measured both in intact cells and in thylakoid membranes. As indicated by the data shown in Table I, the dark-grown cells had a fully functional photosynthetic electron transfer chain. The rates of whole-chain and PSI-mediated electron transfer reactions were not significantly different between the autotrophic and heterotrophic cultures. However, the rate of PSII-mediated electron transfer was significantly (20%) higher in the dark-grown cultures. Moreover, the dark-grown cells evolved oxygen immediately after transfer to light (without any lag period), indicating that these cells had functional PSII complexes and did not require any photoactivation to gain competence for water oxidation.

Analysis of Room Temperature Chl Fluorescence Transients

Figure 3 shows the room-temperature fluorescence transients of DCMU-treated cells of autotrophic and heterotrophic cultures of *Anabaena* 29413. With samples containing equal amounts of Chl, the variable fluorescence levels were found to be 25 to 30% higher in the dark-grown cells compared with that in the light-grown cells. At room temperature, the fluorescence emission occurs mainly from the PSII complex. Thus, an increase in the yield of fluorescence at room temperature suggested that there had been an increase in the PSII content of the dark-grown cells.

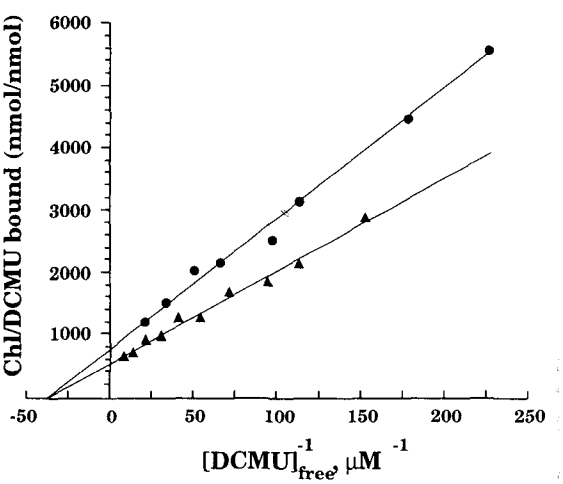

Figure 4. Double-reciprocal plot of DCMU binding to whole cells of autotrophic (●) and heterotrophic (▲) cultures of *Anabaena* 29413. [^{14}C]DCMU was added at various concentrations to 1-mL samples in 25 mM Hepes-NaOH, pH 7.5. The concentration of Chl in each sample was 25 μg/mL.

Determination of Chl:PSII and Chl:P700 Ratios

Assuming that each PSII unit has one DCMU binding site, the number of PSII units in intact cells can be quantitated on a Chl basis using [^{14}C]DCMU, which binds specifically to PSII. Figure 4 shows the double-reciprocal plots for the binding of [^{14}C]DCMU in the autotrophic and heterotrophic cells. Although the affinity for [^{14}C]DCMU binding remained approximately the same (approximately 25 nM) in both types of cells, there was a significant decrease in the ratio of Chl:PSII in the heterotrophic cells (Table I). These data implied that in the dark-grown cells, there was an approximately 50% increase in the number of PSII units.

The Chl:P700 ratio was determined for the thylakoid membranes from both the autotrophic and heterotrophic cultures by chemical difference spectroscopy. As shown in Table I, this ratio remained unaltered between the two types of cells, indicating that the number of PSI units was essentially unchanged during heterotrophic growth.

Taken together, these data indicate that the PSI:PSII ratio was approximately 6:1 in autotrophic cells and approximately 4:1 in heterotrophic cultures.

Analysis of Thylakoid Membrane Proteins

The protein:Chl ratio of the thylakoid membranes from the heterotrophic cultures was very similar to that from the autotrophic cultures (Table I). Western analysis of these membranes with antibodies specific to PSII and PSI polypeptides provided additional evidence for the relative increase in the number of the PSII units. As shown in Figure 5, the relative amounts of PsaA/PsaB, the reaction center proteins, as well as PsaD, a membrane-associated stromal protein, of PSI were similar in autotrophic and heterotrophic cells. However, the levels of both D1 and D2, the reaction center proteins of PSII, were significantly increased in the dark-grown cells.

DISCUSSION

The ability of a number of unicellular and filamentous cyanobacteria to grow chemoheterotrophically in the dark has been known for a long time (Rippka, 1972; Raboy et al., 1976). However, it has been generally believed that the heterotrophic metabolism in cyanobacteria is less efficient than their autotrophic metabolism (Pelroy et al., 1972; Raboy et al., 1976). In this communication, we have demonstrated that the filamentous cyanobacterium *Anabaena* 29413 can grow equally well under dark, heterotrophic and photoautotrophic growth conditions. As described earlier, wild-type *Anabaena* 29413 cells maintained in darkness for >6 months had a growth rate comparable with that of the autotrophic cultures. Similar adaptation to faster growth rates has been observed with a *psaC*-less mutant strain (Mannan et al., 1991) that is extremely light sensitive and has to be maintained in darkness (data not shown). This type of acclimation to heterotrophic growth conditions has been shown earlier for *Plectonema boryanum*. These cells have a doubling time of 24 h under autotrophic growth conditions. Upon transfer to the heterotrophic conditions, they have a generation time of 200 h, which decreases to 40 h after 4 years of continuous heterotrophic growth (Raboy et al., 1976). Our data clearly indicate that the heterotrophic capacity of *Anabaena* 29413 cells can be equal to its autotrophic capacity.

In our opinion, the most interesting aspect of a dark-grown culture of *Anabaena* 29413 is its ability to maintain the functional integrity of its photosynthetic apparatus during long periods of dark growth. Upon transfer to light, the dark-grown cultures of *Anabaena* 29413 immediately evolved oxygen at rates comparable with that from the autotrophic cultures. These data are in contrast to those found with a number of other cyanobacterial species grown heterotrophically. For example, when dark-grown cultures of *Synechocystis* 6714 and *Chlorogloea fritschii* are transferred to light, the rate of oxygen evolution was 20% of the rates observed from the autotrophic cultures. In addition, the recovery to the 100% level occurs in a biphasic manner in 2 to 4 h. The second phase of this recovery process was inhibited by chloramphenicol, suggesting that the recovery process involved a restructuring of the PSII complex as well as new protein and pigment synthesis (Evans and Carr, 1975; Vernotte et al., 1992).

Synechocystis 6803 cultures grown under LAHG conditions are able to evolve oxygen at a rate that is 4-fold higher than that from autotrophic cultures (Smart et al., 1991). This increase in the rate of PSII electron transfer can be explained in terms of extensive changes in the structural organization of the photosynthetic apparatus of cells in the LAHG cultures. In these cells, there was a 75% decrease in the Chl content, brought about mainly by a decrease in the number of PSI units. As a result of this preferential decrease in the number of PSI units, the number of PSII units on a Chl basis went up 4-fold, and, consequently, there was a corresponding increase in the rate of PSII-dependent oxygen evolution when expressed on a Chl basis.

Similar decreases in the content of photosynthetic pigments have also been observed in two other species of cyanobacteria grown under heterotrophic conditions. In heterotrophic cells of *Synechocystis* 6714, the levels of both Chl and phycocyanins decrease. Moreover, the decrease in the Chl content is more than that of the phycocyanin (Vernotte et al., 1992). In the case of dark-grown *P. boryanum* cells, the content of

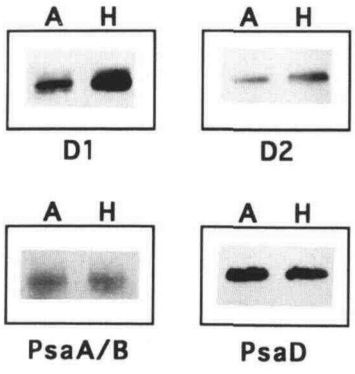

Figure 5. Immunoblot analysis of membrane samples. Replicate samples of the thylakoid proteins from autotrophic (A) and heterotrophic (H) cultures were fractionated on denaturing SDS-PAGE, transferred to nitrocellulose filter, and immunostained with rabbit antibodies against D1, D2, PsaA/PsaB, and PsaD proteins, respectively.

Chl decreased by 50% while the phycocyanin content remained unchanged. In contrast, the content and the composition of these pigments in the heterotrophically grown *Anabaena* 29413 cells showed only minor differences. There was no decrease in the content of the Chl and a small increase in the phycocyanin content that can be correlated with the observed increase in the number of PSII units under heterotrophic conditions (see Table I).

From the comparison of the Chl:P700 and Chl:PSII ratios of autotrophic and heterotrophic cultures, we can conclude the following: (a) The PSI:PSII ratio in the heterotrophic cultures reached a value of 4:1 from a value of 6:1 found in autotrophic cultures. This decrease in the ratio of PSI:PSII would amount to a 50% increase in the number of PSII units. (b) This change in the stoichiometry is brought about by a preferential increase in the number of PSII units whereas the number of PSI units remains the same. Support for this conclusion also comes from the western analysis of the relative amounts of component polypeptides of PSII and PSI. Under heterotrophic growth conditions we observed an increase in the levels of PSII polypeptides, but the amounts of PSI polypeptides remained essentially unchanged (Fig. 5).

In cyanobacterial thylakoid membranes, the ratio of PSI:PSII is usually found to be 7:1 to 8:1 (Burnap et al., 1989). The number of Chls in a cyanobacterial PSII complex has been estimated to be approximately 40 (Rögner et al., 1990). We have previously determined that isolated PSI particles from *Anabaena* 29413 have approximately 100 Chls in a PSI complex (Nyhus et al., 1992). As a result, nearly 95% of the Chls present in cyanobacterial thylakoid membranes are associated with PSI. Consequently, a 50% increase in the number of PSII units in the dark-grown cells did not result in a significant difference in the Chl:P700 ratio (Table I).

Upon transfer of *Anabaena* 29413 cultures from autotrophic to heterotrophic growth conditions, the changes in the ratio of the two photosystems could be observed within 3 weeks, whereas the adaptation to dark growth (in terms of a decrease in the doubling time) occurred over a period of approximately 6 months. Thus, the changes in the stoichiometry of the photosystems occurred as an immediate physiological response, whereas adaptation to the heterotrophic mode of metabolism involved longer-term changes.

The contrasting responses of *Synechocystis* 6803 and *Anabaena* 29413 cells to heterotrophic growth conditions deserve a special mention. During dark growth, the ratio of PSI:PSII decreases in both of these cyanobacterial species. However, *Synechocystis* 6803 responded to the LAHG conditions through a preferential decrease in its content of PSI units, whereas heterotrophic *Anabaena* 29413 cells showed an increase in their PSII content while the number PSI units remained unchanged. These differences may be explained in terms of the different growth conditions, namely LAHG and continuous dark growth. However, *Anabaena* 29413 cells grown under LAHG conditions had characteristics similar to those of cells grown under continuous dark conditions (data not shown). Therefore, the difference in the response of *Synechocystis* 6803 and *Anabaena* 29413 cells to dark growth conditions must be a result of inherent metabolic differences between these species.

The ability of *Anabaena* 29413 cells to maintain the structural and functional integrity of their photosynthetic apparatus in general, and the number of the PSI units in particular, makes this organism an attractive candidate for molecular genetic analysis of the PSI complex. These features of this organism are highly advantageous in the isolation of PSI particles from mutants that assemble their PSI reaction centers and are still light sensitive so that the cells need to be grown under dark, heterotrophic conditions. For example, we have been able to purify large amounts of photoactive PSI complex from a mutant strain of *Anabaena* 29413 (Mannan et al., 1991) in which the PSI reaction center assembles even in the absence of the PsaC protein (R.M. Mannan, K. Sonoike, and H.B. Pakrasi, unpublished observations).

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