Pressure and low temperature effects on the fluorescence emission spectra and lifetimes of the photosynthetic components of cyanobacteria

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ABSTRACT The effects of hydrostatic pressure on the excited state reactions of the photosynthetic system of cyanobacteria were studied with the use of stationary and dynamic fluorescence spectroscopy. When the cells were excited with blue light (442 nm), hydrostatic pressure promoted a large increase in the fluorescence emission of the phycobilisomes (PBS). When PBS were excited at 565 nm, the shoulder originating from photosystem II (PSII) emission (F685) disappeared under 2.4 kbar compression, suggesting suppression of the energy transfer from PBS to PSII. At atmospheric pressure, the excited state decay was complex due to energy transfer processes, and the best fit to the data consisted of a broad Lorentzian distribution of short lifetimes. At 2.4 kbar, the decay data changed to a narrower distribution of longer lifetimes, confirming the pressure-induced suppression of the energy transfer between the PBS and PSII. When the cells were excited with blue light, the decay at atmospheric pressure was even more complex and the best fit to the data consisted of a two-component Lorentzian distribution of short lifetimes. Under compression, the broad distribution of lifetimes spanning the region 100–1,000 ps disappeared and gave rise to the appearance of a narrow distribution characteristic of the PBS centered at 1.2 ns. The emission of photosystem I underwent 2.2-fold increase at 2.4 kbar and room temperature. A decrease in temperature from 20 to −10°C at 2.4 kbar promoted a further increase in the fluorescence emission from photosystem I to a level comparable with that obtained at temperatures below 120°K and atmospheric pressure. On the other hand, when the temperature was decreased under pressure, the PBS emission diminished to very low values at blue or green excitation, suggesting the disassembly into the phycobiliprotein subunits.

INTRODUCTION

Cyanobacteria and red algae have three antennae systems: the chlorophyll antennae complexes associated with photosystem I (PSI)[1] and photosystem II (PSII) located in the thylakoid membrane and an extramembraneous antennae complex called phycobilisomes (PBS) (Glazer, 1985). The PBS are formed by pigmented proteins, the biliproteins. The strong absorption bands of the major biliproteins lie in the region of 470–650 nm, whereas those of the chlorophyll-a complexes are located at 430–440 and 670–680 nm. This separation in the major absorption bands permits analysis of the relative contributions of the biliproteins and of chlorophyll-a to the action spectra of PSI and PSII (Wang et al., 1977; Butler, 1978; Diner, 1979). The PBS are in close association with PSII to where the absorbed energy is funneled (Meyers et al., 1980; Manodori et al., 1984; Ohki et al., 1987). Since PBS provide most of the light-harvesting capacity of cyanobacterial and red algal cells, photosynthesis yield greatly depends on the efficiency of this energy transfer process.

Intrinsic fluorescence has been used to probe the energy transfer in photosynthetic units (Grondelle and Amesz, 1986). More recently, the application of time-resolved fluorescence has enlarged the understanding of the energy transfer routes (Yamazaki et al., 1984; Hodges and Moya, 1988; Mimuro et al., 1989; Mullineaux and Holzwarth, 1991). Under blue light excitation at room temperature, the main emission in the fluorescence spectra of cyanobacteria is attributed predominantly to the PSII chlorophyll antennae (Goedheer, 1965; Murata et al., 1966; Krause and Weis, 1991). At liquid nitrogen or liquid helium temperatures, the fluorescence spectra measured in vivo show a dramatic increase in the emission bands when compared with ones obtained at room temperature principally at long wavelength range (Brody, 1958; Cho and Govindjee, 1970*a, b;* Rijgersberg et al., 1979; Rijgersberg and Amesz, 1980). This long wavelength band emission is attributed to low-energy antenna chlorophylls in PSI and varies from 710 to 740 nm depending on the cyanobacteria species (Rijgersberg and Amesz, 1980; Wittmershaus et al., 1992).

Hydrostatic pressure has been used to study protein–protein interactions with minimal direct effects on the tertiary structure of the polypeptides (Heremans, 1982; Weber and Drickamer, 1983). At room temperature, pressures of up to 3.0 kbar appear to have minimal effects on the properties of single-chain proteins (Heremans, 1982; Weber and Drickamer, 1983) and can thus be trusted to provide reliable information on the thermodynamics and kinetics of multisubunit protein assembly (Weber, 1987; Silva and Weber, 1988; Ruan and Weber, 1988; Silva et al., 1989, 1992; Bonafe et al., 1991; Erijman and Weber, 1991). The effect of medium pressure

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[1] *Abbreviations used in this paper:* APC, allophycocyanin; Chl a, chlorophyll a; DCMU, 3-(3'4'-dichlorophenyl)-1',1-dimethyl urea; PBS, phycobilisomes; PSI, photosystem I; PSII, photosystem II; Tris, tris(hydroxymethyl)-aminomethane.

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(1–1,200 bar) on the steady-state fluorescence emission of red algae has been studied by Schreiber and Vidaver (1973*a, b*). Their data suggested pressure perturbation of the energy transfer between accessory pigments and chlorophyll. More recently, Hoganson et al. (1987) have studied the effects of high pressure (up to 3,000 bar) on the photochemistry of photosynthetic reaction centers of bacteria. Whereas pressure promoted acceleration of electron transfer from quinones to the primary electron donor bacteriochlorophyll in *Rhodobacter sphaeroides*, it slowed the charge recombination in *Rhodopseudomonas viridis*.

The present article describes the combined use of high pressure (1–2,500 bar) and temperature (20 to −10°C) to study the fluorescence properties of the cyanobacteria *Anabaena sp.* Steady-state fluorescence spectra and lifetimes were measured at atmospheric and high pressure. The lifetimes measured with blue or green light excitation give a clear-cut evidence of the pressure suppression of energy transfer. The titration of the pressure-induced changes allowed a calculation to be made of the volume change related to the attachment of the PBS to PSII. The increase of PSI fluorescence promoted by high pressure at temperatures below the water freezing point introduces a novel tool to study the excited state reactions that precede the photochemistry.

MATERIALS AND METHODS

Algal culture

Anabaena sp. (29151; American Type Culture Collection, Rockville, MD) was grown photoautotrophycally at 30°C in ASM-1 medium (Gorhan et al., 1964) supplied with Na/K PO_4 5 mM and was flushed by air. Cells were collected in the early stationary growth phase, washed with distilled water and resuspended in a solution containing 0.2 M sucrose and 50 mM Tris-HCl (pH 7.5) at 5 μg chlorophyll/ml just before the fluorimetric measurements. Sucrose was present to avoid cell deposition during the experiments. Chlorophyll-a was estimated spectrophotometrically in methanolic extracts (MacKinney, 1941).

Steady-state fluorescence studies under pressure

The high pressure bomb has been described by Paladini and Weber (1981), and fluorescence spectra were recorded on a spectrofluorimeter (model 200; ISS Inc., Champaign, IL). The bomb was kept at different temperatures with the aid of a water circulator, whereas a dry nitrogen gas flush prevented water condensation on the optical surfaces.

Phase fluorometry lifetime measurements

Lifetime measurements were performed in a multifrequency cross-correlation phase and modulation fluorometer. A detailed description of phase fluorometry lifetime measurements and data analysis has been fully described previously (Gratton et al., 1984; Lakowicz et al., 1984; Alcala et al., 1987; Beechem et al., 1991). The quality of fits was assessed by χ^2 values and by plots of weighted residuals. For the experiments with green-excitation light (565 nm), we used the harmonic content of a high repetition rate, mode-locked Nd-YAG laser. This laser is used to synchronously pump a dye laser (Alcala et al., 1985).

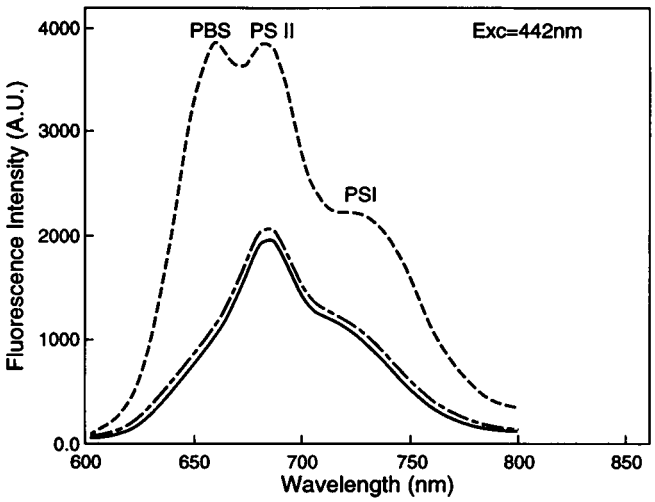

FIGURE 1 Fluorescence emission spectra measured at room temperature from cells excited with blue light (442 nm) at atmospheric pressure (——), under 2.4 kbar (---), and after return to atmospheric pressure (- - -). The cells were suspended in 200 mM sucrose and 50 mM Tris-HCl (pH 7.5).

Excitation wavelength was 565 nm, and the emission was observed through a long-wave pass filter (R62) with a cutoff wavelength at 620 nm. For the lifetime studies at atmospheric and high pressure, light scattering of the cells at 565 nm (interference filter from Corion Corp., Holliston, MA) was utilized as reference. The same results were obtained when the measurements were performed at atmospheric pressure inside the pressure bomb or in a regular cuvette and by utilizing Ficoll as the light-scattering reference.

The lifetime measurements with blue excitation were performed by a multifrequency cross-correlation phase and modulation fluorometer using the 458 nm line of an argon ion laser (model 2025; Spectra-Physics, Mountain View CA) to excite the sample. The emission was observed through the R62 filter.

Chemicals

All reagents were of analytical grade. Tris-HCl buffer was selected because the dependence of its pKa on pressure is small. At 3 kbar, the value of pKa increases by only 0.1 unit (Neuman et al., 1973).

RESULTS

Pressure effects on the fluorescence emission spectra

Fig. 1 shows the fluorescence emission spectra measured at room temperature when the cells were excited with blue light (442 nm), which is predominantly absorbed by Chl-a. At atmospheric pressure, a main emission that originates from Chl-a associated with PSII is observed at 685 nm. The spectrum at atmospheric pressure also presented a shoulder in the blue region related to PBS emission (645–655 nm), as well as a red shoulder (710–740 nm) emerging from PSI Chl-a fluorescence. Dramatic changes occurred in the fluorescence emission spectra when the cells were subjected to high pressures. At 2.4 kbar, the small shoulder of PBS emission became the

major peak (Fig. 1). This large increase in the PBS fluorescence yield suggests that hydrostatic pressure produces inhibition of energy transfer from accessory pigments to the chlorophyll antennae of PSII (Chl-aII). Under compression, the PSII Chl-a emission peak (685 nm) showed a twofold increase that originates from the contribution of the tail of PBS emission. When deconvolution is performed, there is practically no change in the emission of PSII. On the other hand, it is noteworthy that PSI fluorescence (710–740 nm) increased under pressure at room temperature. After gaussian deconvolution of the contributions of PSII, the emission at 735 nm increased by a factor of 2.2.

The changes promoted by pressure at room temperature were completely reversible in a time of 30 min after decompression as seen by the recovery of the original spectrum (Fig. 1). After pressure incubation, the shape and filamentous aspect of the cells in the optical microscopy were similar to the nonpressurized cells.

To further verify the pressure effects on the energy transfer between the accessory pigments and the Chl-a antennae complex associated to PSII, the cells were excited with green light (565 nm), which is in the largest measure absorbed by the PBS. This set of experiments was performed in the presence of 3-(3′4′-dichlorophenyl)-1′,1-dimethyl urea (DCMU) with the purpose to maintain the PSII reaction center in a closed state. At atmospheric pressure, the fluorescence emission spectrum from DCMU-treated cells presented a major band at 655 nm due to PBS emission. The fluorescence intensity at this wavelength increased several times as a function of pressure (Fig. 2 *A*), which can be explained by the decrease in energy transfer reactions between the PBS and the PSII chlorophyll antennae. The spectrum measured at atmospheric pressure also contained a shoulder at 686 nm related to the PSII Chl-a emission, which can be observed in the normalized spectrum (Fig. 2 *B*). It is important to note that at this wavelength excitation, the PSII emission arises from the PBS absorption and subsequent energy transfer, since the chlorophyll molecules do not absorb in this spectral range. The mentioned shoulder at 686 nm (Fig. 2 *B*) disappeared completely under pressure due to the pressure-induced suppression of energy transfer between the accessory pigments and the PSII.

It also can be noticed in Fig. 2 that upon compression the main emission peak attributed to PBS emission presented a red shift (655–662 nm) that became more pronounced as higher pressure was utilized. These data suggest dissociation of the PBS from the membrane surface that brings about emission of allophycocyanin (APC) present in the PBS core. The emission spectrum obtained at high pressure and room temperature is characteristic of isolated PBS in which the fluorescence emission is mostly due to APC. It should be pointed out that essentially the same results were obtained when DCMU

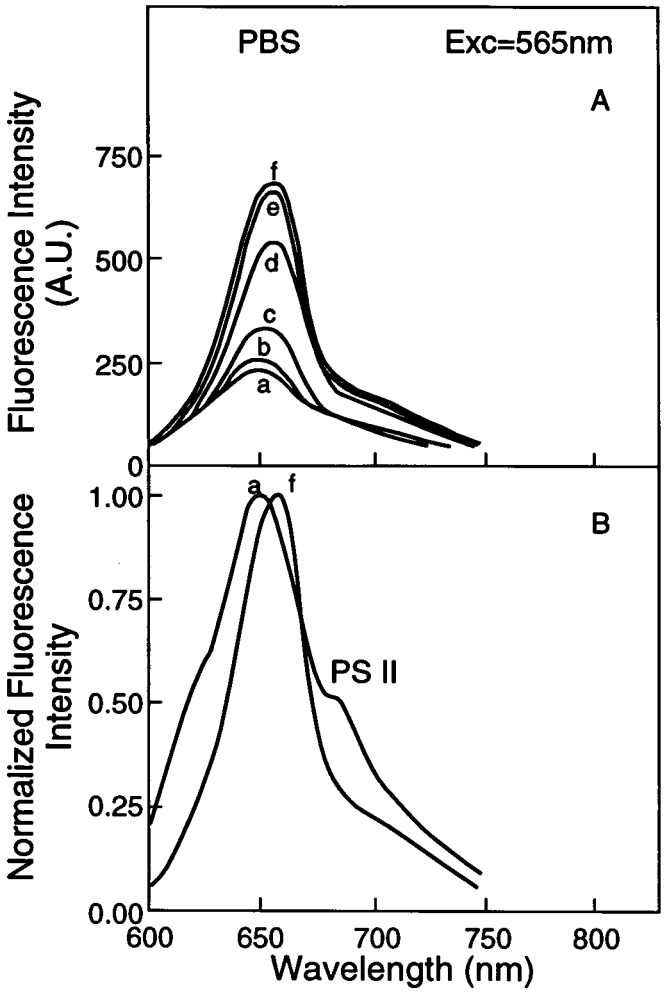

FIGURE 2 (*A*) Fluorescence emission spectra at room temperature from cells excited with green light (565 nm) at (*a*) atmospheric pressure, (*b*) 1.0 kbar, (*c*) 1.4 kbar, (*d*) 1.7 kbar, (*e*) 2.0 kbar, and (*f*) 2.4 kbar. (*B*) Normalized spectra at 1.0 bar (*a*) and 2.4 kbar (*f*). All spectra were performed in the presence of 10 μM DCMU in the suspension medium described in Fig. 1.

was absent from the suspension medium, except that the shoulder at 686 nm was less pronounced.

Fig. 3 illustrates the titration of the changes in the energy transfer as a function of hydrostatic pressure on exciting the cells with green light (565 nm). The inhibition of energy transfer promoted by pressure is unimodal and levels off at ~2.0 kbar. Considering that the transition is due to a second-order association reaction:

$$\text{PBS.PSII} \leftrightarrow \text{PBS} + \text{PSII}.$$

The equilibrium constant for this reaction will be given by (Weber, 1992):

$$K_{do} = \alpha^2 C/(1 - \alpha), \qquad (1)$$

where α is the degree of dissociation and C is the molar concentration of the complex PBS.PSII. From pressure-dissociation data, it is possible to calculate both the stan-

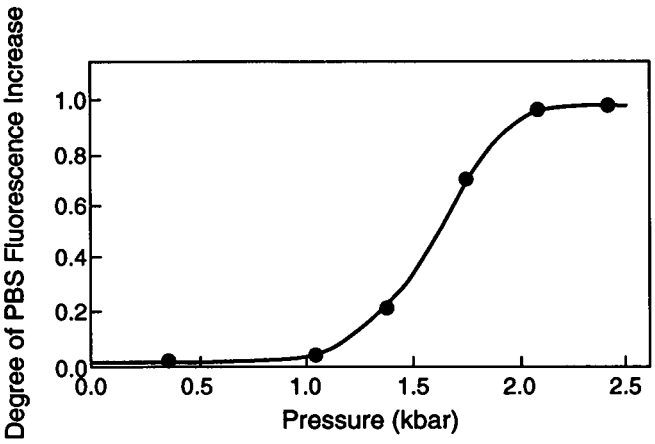

FIGURE 3 Degree of PBS fluorescence increase as a function of pressure. The degree of PBS fluorescence increment is expressed using the ratio $(F_p - F_{atm})/(F_{2.4kbar} - F_{atm})$, where F_p represents the intensity of the PBS emission in each pressure applied. Other conditions as Fig. 2.

dard volume change of association (ΔV) and the dissociation constant at atmospheric pressure (K_{do}), based on the thermodynamic relation

$$K_d(p) = K_{do} \exp(p\Delta V/RT), \quad (2)$$

where $K_d(p)$ is the dissociation constant at pressure p. If we introduce the degree of dissociation at pressure p, α_P, we can write Eq. 2 for the case of a dissociation reaction between a complex (PBS.PSII) and its dissociated products:

$$\ln(\alpha_P^2/(1 - \alpha_P)) = p(\Delta V_P/RT) + \ln(K_{do}/C). \quad (3)$$

The values of α_P correspond to the degrees of PBS fluorescence increase in Fig. 2. The volume change of the reaction (ΔV) is determined from the slope of the plot $\ln(\alpha_P^2/(1 - \alpha_P))$ versus pressure (Eq. 3). The volume change (ΔV) of association derived from the data was 276 ml/mol, a value that may be considered typical for a protein complex. The extrapolation to the ordinate axis (Eq. 3) provides the ratio between the dissociation constant at atmospheric pressure and the concentration of the complex (C). Since intracellular concentration of PBS.PSII complexes is difficult to estimate, we could not determine K_{do}.

Fluorescence lifetime studies

If hydrostatic pressure decreases the singlet-singlet energy transfer between PBS and Chl-a antennae complex associated to PSII, it is expected that the lifetime of the excited state under pressure would become longer than the one observed at atmospheric pressure. To pursue this hypothesis, we performed lifetime measurements at 1.0 and 2,400 bar in a multifrequency cross-correlation phase and modulation fluorometry using the harmonic content of a mode-locked laser (Alcala et al., 1985; Silva et al., 1992).

Fig. 4 *A* shows both the phase shift and relative modulation as a function of frequency obtained with green excitation (565 nm) at atmospheric pressure and at 2.4 kbar. High pressure drastically changed both relative modulation and phase shift. The decay data were analyzed using continuous distribution of lifetimes (Alcala et al., 1987; Govindjee et al., 1990). The best fit to the data was obtained using a Lorentzian distribution rather than a sum of two or three exponentials. Mullineaux and Holzwarth (1991) recently have assigned five exponential lifetime components to PSII, PSI, and PBS in cyanobacteria by using decay data recorded at different excitation and emission wavelengths. In our experiments, we use only two different excitations, and the emission was detected at wavelengths above 620 nm. In the absence of a time-resolved excitation-emission matrix, the use of a distribution seems to provide a more realistic representation of the data (Govindjee et al., 1990).

Lorentzian lifetime distribution analysis of the data (Fig. 4) using just one component for the fitting provided the lower χ^2 values. The average lifetime increased

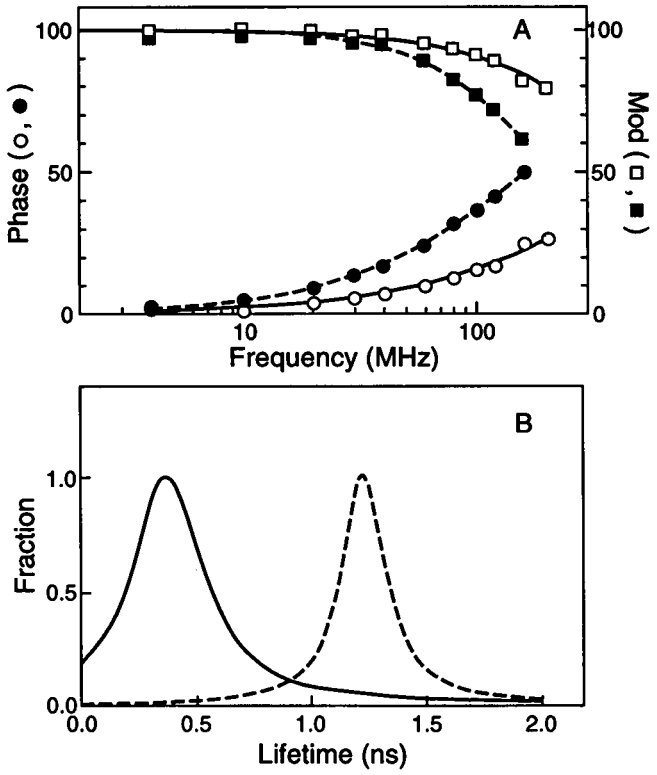

FIGURE 4 (*A*) Plot of phase angle (○, ●) and modulation (□, ■) versus frequency for excitation of *Anabaena sp* cells with green light (565 nm) at room temperature and atmospheric pressure (○, □) and at 2.4 kbar (●, ■). The lines correspond to the best fit for each set of data, using a single-component Lorentzian distribution analysis. (*B*) Lorentzian distribution analysis of the lifetime data obtained in *A* at atmospheric pressure (——) and 2.4 kbar (- - -). The best fit for atmospheric pressure consisted in a single-component distribution: center = 0.385 ns; halfwidth = 0.363 ns; χ^2 = 5.57. The values obtained for 2.4 kbar are center = 1.230 ns; halfwidth = 0.223 ns; χ^2 = 2.71.

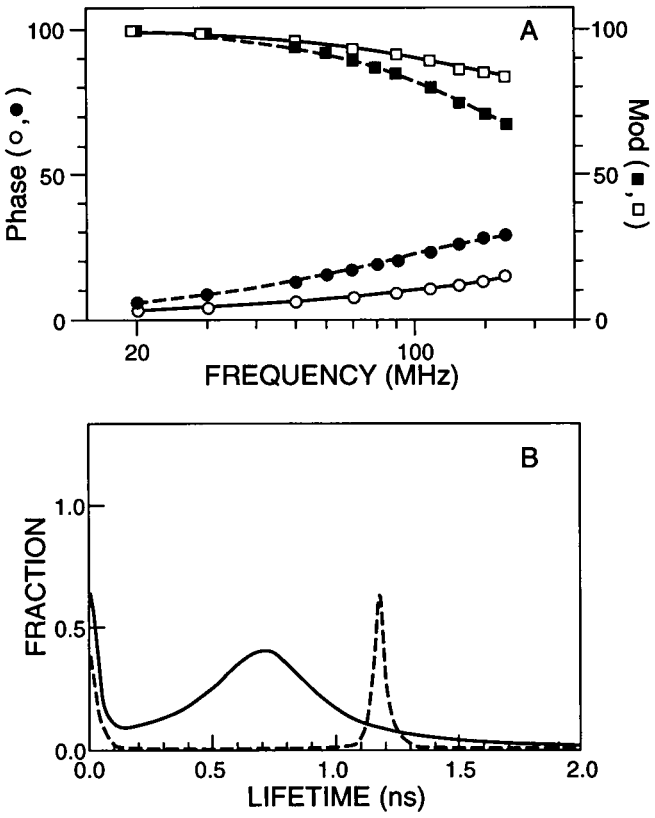

FIGURE 5 Phase and modulation as a function of frequency of cyanobacteria excited with blue light (458 nm) at atmospheric pressure (○, □) and at 2.4 kbar (●, ■). Lorentzian distribution analysis of the lifetime data obtained at blue light excitation at atmospheric pressure (——) and 2.4 kbar (- - -). The lines correspond to the best fit for each set of data, using a two-component Lorentzian distribution analysis. Calculated values for atmospheric pressure: center [1] = 0.703 ns; halfwidth [1] = 0.516 ns; fraction [1] = 0.406; center [2] = 0.004 ns; halfwidth [2] = 0.050 ns; fraction [2] = 0.594; χ^2 = 7.57. Calculated values for 2.4 kbar: center [1] = 1.179 ns; halfwidth [1] = 0.050 ns; fraction [1] = 0.650; center [2] = 0.009 ns; halfwidth [2] = 0.050 ns; fraction [2] = 0.350; χ^2 = 4.38.

threefold on compression (Fig. 4 *B*). At atmospheric pressure, there is a broad population with short lifetime values between 0 and 700 ps (center = 390 ps). At 2.4 kbar the distribution shifted to longer lifetime values centered at 1.23 ns (Fig. 4 *B*). The halfwidth of the distribution at high pressure was much narrower in comparison to the broad distribution obtained at 1.0 bar. These findings provide a clear-cut evidence that hydrostatic pressure is hindering the energy transfer from PBS to PSII antennae.

In Fig. 5, the lifetime values were obtained at blue light excitation (458 nm). The decay at atmospheric pressure was more complex, and the best fit to the data consisted of a two-component Lorentzian distribution of lifetimes (Fig. 5). The first component of very short lifetimes (in the range 0–50 ps) is probably due to the emission of Chl-a associated to PSI with a minor contribution of PSII (Holzwarth et al., 1985; Moya et al., 1986; Mullineaux and Holzwarth, 1991). The second component, similar to the excitation with green light, is very broad spanning the region between 100 and 1,300 ps (center = 703 ps). This distribution of lifetimes are clustered in the region where Mullineaux and Holzwarth (1991) found four exponential lifetime components in whole cyanobacteria cells. These components were assigned to energy transfer between PBS rods to PBS core (90–120 ps); PBS terminal emitters (200 ps), PSII core (400–650 ps), and closed PSII (1,100–1,400 ps). This latter component is not predominant in our data because the experiments were performed in the absence of DCMU and there was no preillumination.

Under compression (2.4 kbar), the broad distribution of lifetimes from the second component disappeared and gave rise to the appearance of a narrow distribution with lifetime centered at 1.18 ns (Fig. 5). The steady-state emission spectra under pressure at similar conditions (blue excitation) showed the predominance of PBS emission (Fig. 1) that permits the assignment of the most part of the 1.18-ns component to uncoupled PBS. This component might also contain the contribution of PSII emission resulted from direct excitation of Chl-aII. However, this contribution would be smaller since, under pressure, fluorescence resulting from PSII is <30% of the total emission (Fig. 1). The relative fraction of the shortest component (center = 9 ps) decreased under pressure, probably because of the predominance of PBS emission. The lifetime data with blue-light excitation also confirm the suppression of energy transfer between PBS and PSII.

Temperature and pressure effects on the fluorescence emission spectra

The PSI fluorescence emission underwent a 2.2-fold increase when the cells were subjected to high hydrostatic pressure (Fig. 1). At atmospheric pressure, this emission only increases when temperature is diminished to values below 77°K (Cho and Govindjee, 1970*a*, *b*; Rijgersberg et al., 1979; Rijgersberg and Amesz, 1980; Wittmershaus et al., 1992). To study the PSI fluorescence, pressure and low temperatures were combined. It is noteworthy that under 2.4 kbar the freezing point of water is around −20°C (Bridgman, 1964). Therefore, it was not necessary to add any kind of antifreezing or protective solvent resulting in a very suitable environment for spectral observations. In the following experiments, the cells were initially subjected to 2.4 kbar and thereafter the temperature was progressively decreased to −10°C. On blue light excitation (Fig. 6), a striking effect could be observed: whereas PSI emission rose and became the major peak, concomitantly the PBS fluorescence yield presented a dramatic decrease becoming insignificant at −10°C. After subtraction of the contribution of the PSII emission, the PSI fluorescence increased 3.4 times by decreasing temperature from 20 to −10°C at 2.4 kbar. When compared with the emission at room temperature and

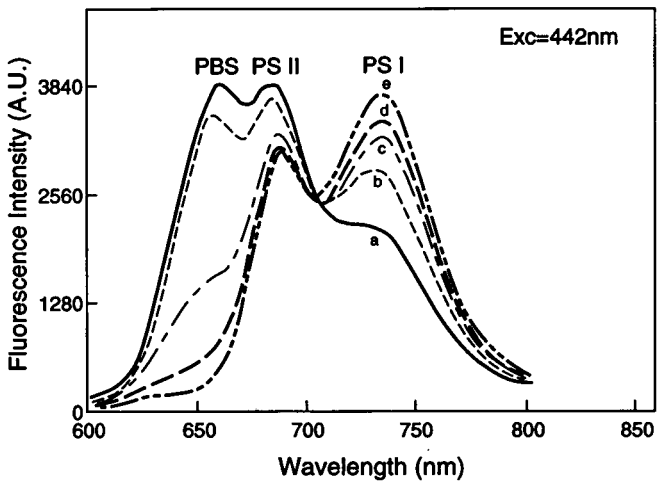

FIGURE 6 Effects of temperature on the fluorescence emission spectra of cells excited with blue light (442 nm) at 2.4 kbar. The temperature was progressively decreased: (*a*) 20°C; (*b*) 5°C; (*c*) 0°C; (*d*) −5°C to (*e*) −10°C. Other conditions as Fig. 1.

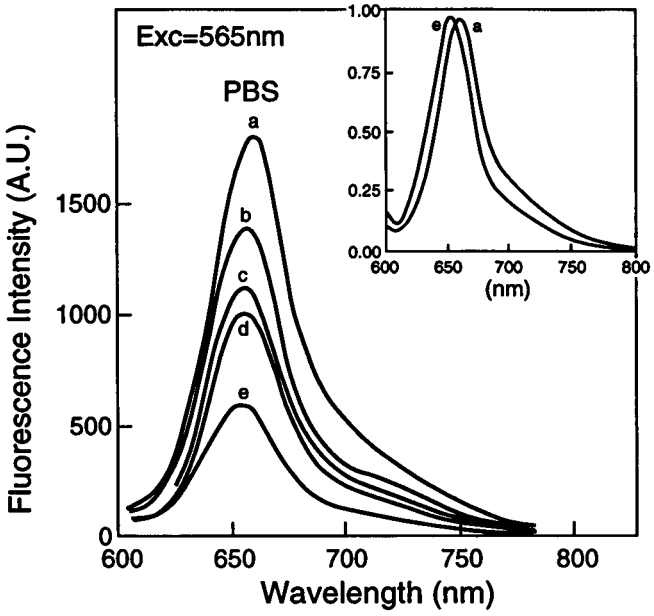

FIGURE 8 Fluorescence emission spectra of cells excited with green light (565 nm) and subjected to 2.4 kbar, in which the temperature was decreased in steps: (*a*) 25°C; (*b*) 15°C; (*c*) 8°C; (*d*) 3°C to (*e*) −2°C. *Inset,* Normalized fluorescence spectra (λ_{exc} = 565 nm) under 2.4 kbar at the temperatures 25°C (*a*) and −2°C (*e*).

atmospheric pressure, the quantum yield increased by a factor of 7.5. We also observed that PSII fluorescence underwent a small decrease when the temperature was lowered (Fig. 6).

The decrease in the emission of PBS under pressure and low temperatures (Fig. 6) can be explained by cold-induced disassembly of its structure. In Fig. 7, this effect was better quantified by ploting the ratio (F655/F685) as a function of temperature at fixed pressure (2.4 kbar). The same transition curve was observed when the fluorescence intensities at 655 nm were plotted against pressure (not shown). It is remarkable that the curve obtained by cooling has larger ratios (F655/F685) than the one obtained by warming, indicating the lack of reversibility of the cooling effects under pressure. On the other hand, the effects of temperature under pressure on the PSI as measured by the ratio F735/F685 were completely reversible without hysteresis (data not shown). In addition, the value of F735/F685 obtained before compression and cooling was equal to that found after warming and decompression.

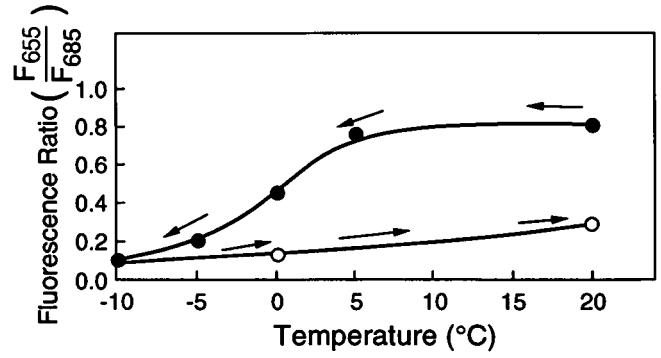

FIGURE 7 Hysteretic behavior of temperature on the ratio F_{PBS}/F_{685}. Symbols (●) are for decreasing temperature and (○) for increasing temperature at 2.4 kbar. Other conditions as Fig. 6.

Fig. 8 shows the decline in quantum yield of the PBS emission promoted by the decrease in temperature at high pressure when the cells were excited with green light (565 nm), giving additional support to the cold and pressure-induced dissociation of PBS. The inset of Fig. 8 shows the normalized spectra at room temperature and at −2°C under 2.4 kbar. The maximum emission of the spectra shifted from 662 to 655 nm caused by the disassembly of the PBS structure. The emission at 655 nm can be attributed to phycocyanin, present in the PBS rods, that were separated from the core.

DISCUSSION AND CONCLUSIONS

This article shows that hydrostatic pressure reversibly inhibits the singlet-singlet energy transfer from accessory pigments (PBS) to Chl-a antennae associated to PSII in a blue-green algae. Lifetime studies at different excitation wavelengths unambiguously demonstrated the pressure-induced suppression of energy transfer. The appearance at high pressures of a characteristic population of emitting species centered at 1.2 ns is in line with the large increase in the emission intensity of PBS. Under 2.4 kbar and blue excitation, the accessory pigments emission increased about 10-fold. At this wavelength, only the peripheric components of PBS (phycoerythrocyanin) absorb, but because of its high quantum yield and the

highly efficient energy transfer between the PBS components, emission of allophycocyanin appears. It is noteworthy that this emission under pressure had the same amplitude or even higher than the emission of Chl-a associated to PSII (Fig. 1), even though the blue light is predominantly absorbed by Chl-a.

The spectra and lifetime data suggest that pressure promotes uncoupling between the PBS and the PSII chlorophyll antennae complex hindering the normal energy transfer pathway. Up to date, it is well established that hydrostatic pressure is able to efficiently promote dissociation of dimers and tetramers (Silva et al., 1986, 1992; Weber, 1987; Ruan and Weber, 1988; Erijman and Weber, 1991), larger aggregates (Silva et al., 1989; Bonafe et al., 1991), and viral structures (Silva and Weber, 1988). In the case of the PBS-PSII complex, dissociation may occur since the PBS is an extramembraneous complex attached to the thylakoid membrane by the high molecular weight linker peptide. When the cells were excited with green light, pressure promoted not only a large increase in the quantum yield but also a red shift in the emission maximum from 655 to 662 nm (Fig. 2). This latter emission is attributed to APC contained in the PBS core complex (Glazer, 1985; Mimuro et al., 1989). The maximum emission at 662 nm also suggests that the detached PBS lack the high molecular weight linker and APC-B that emit around 680 nm (Glazer, 1985). If they were present in the detached PBS, then the emission should be even more shifted to red wavelengths. The pressure inhibition of the energy transfer between PBS and PSII is further supported by the complete disappearance of the prominent shoulder at 685 nm. This fluorescence originates from the emission of chl-aII excited by dipole–dipole interaction with PBS components when the cells were exposed with green light. The volume change (ΔV) of association of the complex PBS-PSII (276 ml/mol) is slightly large in comparison with other dissociation systems (Weber, 1987; Silva et al., 1992). It can be explained by an extensive surface of contact between the PBS and the chlorophyll-protein complexes of PSII.

The short lifetime values obtained at 1.0 bar at blue or green excitation are generated by the energy transfer and photochemical reactions that occur during the lifetime of the excited state. Under pressure, there was a large increase in the center of lifetime value due to the suppression of energy transfer from PBS to PSII. The lifetime values obtained under compression (1.2 ns) were essentially the same in both regimes of light utilized and might be attributed to the terminal energy acceptors in the PBS (Glazer, 1985). When excited with green light, isolated PBS from these cells have an emission decay similar to that of the cells under pressure (Foguel et al., unpublished results). At blue excitation, there was an additional very short lifetime component (<50 ps) that might correspond to the 40-ps component found by Mullineaux and Holzwarth (1991). In their assignment, they suggest that there are actually two components with lifetimes of ~40 ps: one originating from PSII and the other component coming from PSI. The PSI component is predominant especially at excitation wavelengths strongly absorbed by Chl-a, such as the case of excitation with blue light. This fast component was present at atmospheric pressure and under 2.4 kbar.

At room temperature, pressure also promoted a 2.2-fold increase in the fluorescence quantum yield of PSI. On decreasing temperature in the range between 20 and −10°C under pressure, there was a further increase in the fluorescence of Chl-a associated with PSI. The total increase in fluorescence emission of PSI on subjecting to pressure (2.4 kbar) and lowering the temperature to 263°K was 7.5. Increases in the emission of PSI of similar magnitude at atmospheric pressure only occurs at temperatures below 150°K (Cho and Govindjee, 1970*a*, *b*; Rijgersberg and Amesz, 1980; Wittmershaus et al., 1992). At 20°K, the PSI emission at 725 nm is ~40 times greater than at room temperature (Wittmershaus et al., 1992). In contrast to the experiments performed at ultralow temperatures (120–20°K), the cells were not frozen in our study. The temperature dependence of PSI emission at wavelengths > 700 nm in plants (Butler et al., 1979; Rijgersberg et al., 1979) and cyanobacteria (Rijgersberg and Amesz, 1980; Wittmershaus et al., 1992) suggest the presence of low energy chlorophylls. However, there is no consensus to explain the temperature-induced effects on low energy Chls. In one of the models (Butler et al., 1979; Wittmershaus et al., 1992), it is proposed that the low energy Chls (F725) may function as a trap transferring their excited-state to the reaction center (P700) aided by thermal energy (kT). The fluorescence emission prevails as the temperature is diminished. Our results demonstrate that pressure facilitates the effect of low temperature in increasing the fluorescence quantum yield of the low energy Chls. The decrease in lipid fluidity of biological membranes by hydrostatic pressure (Chong and Weber, 1983) might explain the observed effects of pressure on PSI. By reducing the membrane motions, pressure would decrease the vibrational and rotational degrees of freedom of the low energy Chls, disabling the transfer of their excited state to the reaction center (P700).

Lowering the temperature under pressure also promoted a substantial decrease in the fluorescence emission of the PBS (Figs. 6 and 8). Probably, in this condition, the low temperature is promoting disassembly of the PBS supramolecular structure, which was previously uncoupled from the thylakoid membrane by the compression work at room temperature. The disassembly is clearly shown by the blue shift (662–655 nm) observed when the green excited cells were subjected to 2.4 kbar at low temperatures (Fig. 8). The fluorescence emission at 655 nm is characteristic of phycocyanin present in the

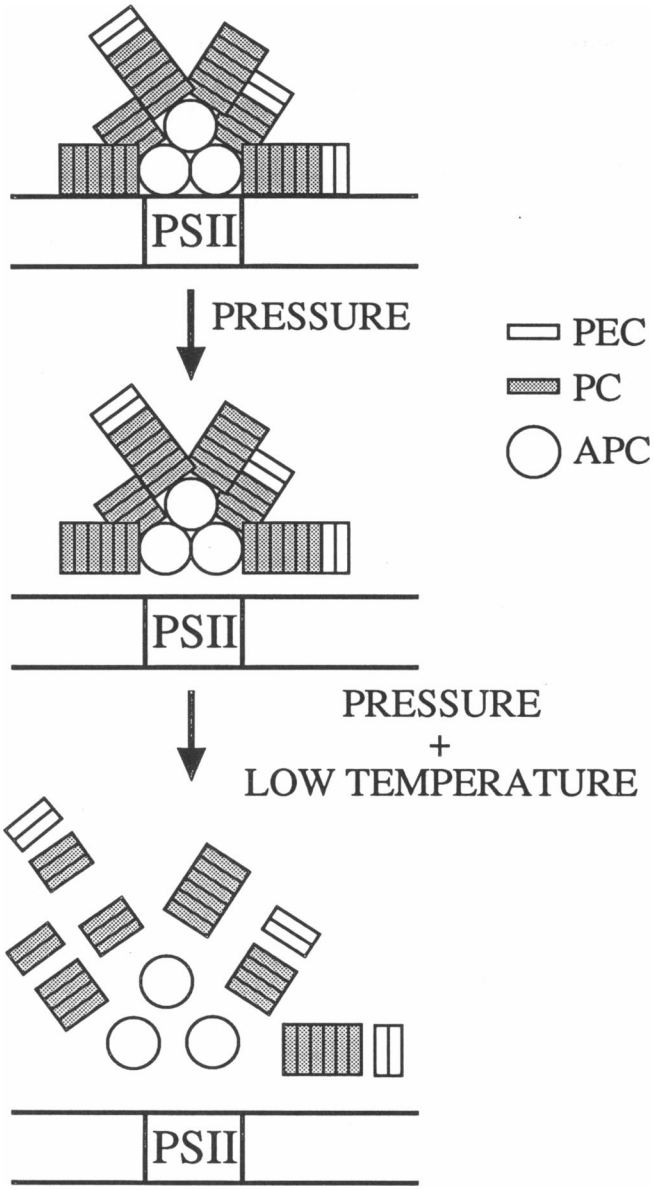

FIGURE 9 Schematic representation of the pressure-induced dissociation of the PBS.PSII complex at room temperature and of the disassembly of PBS by the combined effect of pressure and low temperature. PC, phycocyanin; PEC, phycoerythrocyanin.

PBS rods that were excited by the green light (565 nm). The application of high pressure to isolated PBS at room temperature did not show any significant spectral alteration (unpublished results). The spectra from the isolated PBS were only shifted to the blue when the temperature was lowered under pressure, suggesting disassembly of the PBS supramolecular structure (unpublished results). Fig. 9 shows a model for the effects of pressure and temperature on the PBS.PSII complex as well as on the PBS structure. The model stresses that high pressure at room temperature promotes only dissociation of the PBS from PSII. High pressure and cold temperatures elicit the disassembly of the PBS into smaller units. Our data do not permit to ascertain the size distribution of the dissociated PBS components inside the cell.

The complete disappearance of the PBS emission at blue excitation promoted by 2.4 kbar and −10°C raises additional questions. Cold denaturation under pressure is a reasonable explanation for these observations. Denaturation would affect the ground states of the PBS, decreasing their absorption. At room temperature, hydrostatic pressure in the range 1–3 kbar does not provoke denaturation of single-polypeptide proteins (Heremans, 1982; Weber and Drickamer, 1983; Weber, 1992). Nevertheless, denaturation induced by cold and pressure in those cases were not investigated. Cold denaturation has been predicted to occur (Privalov, 1990) and has been observed for few proteins as a transient in supercooled solutions at atmospheric pressure. Recently, denaturation of isolated APC monomers by pressure and low temperature (−15°C) has been observed (Foguel, D., and G. Weber, manuscript in preparation). These results reinforce the possibility of cold denaturation under pressure of some of the components of PBS inside the cell. Cold denaturation would also explain the irreversibility. The effects of low temperature and high pressure on isolated PBS and isolated phycobiliproteins should provide further understanding of the assembly and folding stability of these structures.

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