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Far-red light-regulated efficient energy transfer from phycobilisomes to photosystem I in the red microalga *Galdieria sulphuraria* and photosystems-related heterogeneity of phycobilisome population

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ABSTRACT

Phycobilisomes (PBS) are the major photosynthetic antenna complexes in cyanobacteria and red algae. In the red microalga *Galdieria sulphuraria*, action spectra measured separately for photosynthetic activities of photosystem I (PSI) and photosystem II (PSII) demonstrate that PBS fraction attributed to PSI is more sensitive to stress conditions and upon nitrogen starvation disappears from the cell earlier than the fraction of PBS coupled to PSII. Preillumination of the cells by actinic far-red light primarily absorbed by PSI caused an increase in the amplitude of the PBS low-temperature fluorescence emission that was accompanied by the decrease in PBS region of the PSI 77 K fluorescence excitation spectrum. Under the same conditions, fluorescence excitation spectrum of PSII remained unchanged. The amplitude of P700 photooxidation in PBS-absorbed light at physiological temperature was found to match the fluorescence changes observed at 77 K. The far-red light adaptations were reversible within 2–5 min. It is suggested that the short-term fluorescence alterations observed in far-red light are triggered by the redox state of P700 and correspond to the temporal detachment of the PBS antenna from the core complexes of PSI. Furthermore, the absence of any change in the 77 K fluorescence excitation cross-section of PSII suggests that light energy transfer from PBS to PSI in *G. sulphuraria* is direct and does not occur through PSII. Finally, a novel photoprotective role of PBS in red algae is discussed.

"I see no principle saying that the two photosystems had to have different action spectra though it was a great help that they do". Jack Myers, Photosynth. Res. 2002. 73. 21–28.

1. Introduction

Phycobilisomes (PBS) are highly ordered external antenna complexes of cyanobacteria and red algal chloroplasts [1,2]. These water-soluble, giant protein assemblies associated with the cytoplasmic surface of the thylakoid membrane are composed of phycobiliproteins and several colorless linker peptides [1–4]. Hemidiscoidal PBS present in most cyanobacteria and some red microalgae consist of a tricylindrical allophycocyanin core and a fan of six lateral cylinders containing phycocyanin or, in some species, a combination of phycocyanin with phycoerythrin or phycoerythrocyanin. PBS carry up to 20% of cell proteins [5], and during nitrogen starvation, the degradation of PBS plays a critical role to meet cellular nitrogen demand [6].

Cyanobacteria and red algae are characterized by relatively low PSII/PSI ratios [7]. In addition, the core complex of PSII [8] incorporates 2.7 fewer chlorophylls than the core of PSI [9]. Therefore, PBS are generally assumed to be employed mainly as an external antenna compensating for both the low levels and the shortage of chromophores in PSII [10,11]. Nevertheless, it was later found that a proportion of the energy absorbed by PBS was transferred to PSI [12–15], particularly under unbalanced light conditions, when PSI receives too little excitation energy relative to PSII [16]. The mechanism by

Abbreviations: ApcD, one of the two terminal phycobilisome emitters; DCMU, 3-(3,4-dichlorphenyl)-1,1-dimethylurea; L_{CM}, phycobilisome core-membrane linker protein and terminal emitter; PBS, phycobilisome(s); P700, primary electron donor of the PSI reaction center; $P700^+$, cation radical of P700; PSI (PSII), photosystem I (photosystem II)

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which light energy is redistributed between the two photosystems in this way, to rebalance electron transport, is known as the state transitions [17]. It has been suggested that the contacts of PBS with reaction centers of both photosystems are unstable and transient. Indeed, PBS mobility has been shown to correlate with state transitions in cyanobacteria [18]. Nonetheless, in spite of intensive study on the role of PBS in state transitions and contrary to the progress made in understanding the phenomenon in green plants [19], the cause of state transitions in cyanobacteria is still under debate [20] and there is little progress on the mechanisms of state transitions in red algae [21–23].

All the existing data on the interaction of PBS with PSI were obtained with different species of cyanobacteria, and their numerous mutants, and did not extend to red algae [12–15]. Both PSI and PSII are distributed throughout the photosynthetic membrane in red algae [24]. The PSII complex in the thylakoid membrane identically forms dimers in both cyanobacteria and red algae [25] (although see data of Takahashi et al. [26] about possible forming of PSII dimers during the isolation procedures). However, PSI is organized differently in the two clades. In cyanobacteria, PSI mainly forms trimers, while in photosynthetic membranes of red algae, PSI exists in monomeric form and is surrounded by additional light-harvesting chlorophyll *a* binding proteins [25,27,28].

The goal of this study was to investigate the interaction between PBS and PSI and to look for changes in PSI cross-section induced by far-red light in cells of the red microalga *Galdieria sulphuraria* (hereinafter *Galdieria*). Three types of independent measurements provided evidence of energy transfer from PBS to the PSI in *Galdieria.* Firstly, the appearance of PBS characteristic bands in the PSI action spectra; secondly, the sensitization of PSI chlorophyll emission by PBS observed in low-temperature fluorescence excitation spectra; and thirdly, the enhanced photooxidation of P700 brought about by PBS-absorbed light. Using all three methods as well as the study of PBS degradation under nitrogen starvation, we provide evidence on the existence of a PBS fraction directly attached to PSI in chloroplasts of *Galdieria*. A far-red light-regulated alteration in energy transfer between PBS and PSI is observed in *Galdieria* cells, which occurs without affecting the interaction between PBS and PSII. The far-red light is found to reduce the quantum efficiency of light absorbed by PBS by about 60% for P700 photooxidation—a significant regulatory alteration in the cells of red alga *Galdieria*.

2. Materials and methods

2.1. Strain and growth conditions

Pure culture of the unicellular thermoacidophilic red alga *G. sulphuraria* (*Galdieri*) Merola, strain IPPAS P-513, was obtained from the Microalgae Collection of Timiryazev Institute of Plant Physiology RAS. Cells were grown autotrophically at permanent shaking in 150 ml flasks at 37 °C in a mineral Allen medium [29] under 40 μE m^{-2} s^{-1} light provided by cool-white Philips fluorescent tubes. Cells at the log growth phase from 7- to 10-day-old cultures were harvested by centrifugation at 2500×*g* for 5 min and resuspended with the growth medium to an appropriate chlorophyll *a* concentration. In case of nitrogen starvation study, the sedimented cells were washed with nitrogen-free Allen medium and divided into two equal portions. The cells of the first portion, used as a control, were resuspended in complete Allen medium and the cells of the second one were resuspended in the same medium free of nitrogen and additionally grew upon the same light and temperature for 1–4 days.

2.2. PBS isolation

PBS were obtained according to the method of Glazer [30]. The isolation procedure was performed at room temperature. The cells of *Galdieria* were harvested by gentle centrifugation and washed twice in 0.85 M sodium phosphate buffer, pH 7.0, with 1 mM PMSF. The cells were resuspended in the same buffer and disrupted in French Press Thermo Cell Disrupter at 500 psi. Triton X-100 was added to the broken cells to a final concentration of 2% (v/v) to relieve the dissociation of the PBS complex from the thylakoid membrane. After incubation for 30 min with occasional shaking, unbroken cells and debris were removed by centrifugation at 15,000×*g* for 20 min. The supernatant was loaded onto 0.25–0.80 M sucrose density gradient in 0.85 M sodium phosphate buffer, pH 7.0, for ultracentrifugation. The fraction of intact PBS that formed the lower colored band in the gradient was gathered with a syringe and repeatedly ultracentrifuged under the same conditions. The final fraction was gathered with dilution in buffer containing 0.9 M KCl to avoid dissociation of PBS to the free phycobiliproteins.

2.3. Photosynthetic action spectra of PSI and PSII

Polarographic measurements of the flash-induced gas exchange reactions related to both photochemical activity of PSII (O_2 evolution) and PSI (photoinhibition of aerobic respiratory O_2 uptake in the presence of 20 μM DCMU to inhibit PSII function) were performed as described in detail previously [31]. A bare platinum electrode at a potential of –0.7 V vs. Ag/AgCl reference electrode was used to measure photoinduced O_2 exchange rate. A sample of cell suspension in 0.02 ml of growth medium, with optical density of 0.1 at 678 nm and chlorophyll equivalent layer density of 1.5 μg cm^{-2}, was placed on a 6-mm platinum disc in a groove of 0.65-mm depth, covered by a cellophane membrane forming the assay microchamber. The action spectra measurements of O_2 exchange were carried out using cycles of intermittent monochromatic 1-s illumination followed by 20- to 60-s dark period between the successive wavelengths at 5- to 10-nm intervals. The spectral half-width of the beam obtained from the monochromator and fiber optics was 1–6 nm. Light intensity at the surface of the sample in the setup varied in the ranges 0.1–0.25 μE cm^{-2} s^{-1} in the spectral range of 550–720 nm. The spectra were corrected with the use of a calibrated detector RTN-20 S (Russia) for nonlinearity of the light energy-dependent curves and for the spectral coefficient of light reflection by platinum surface through the settled cells. The cell density of the samples and room-temperature absorption spectra were recorded using a Varian 2300 spectrophotometer (Aminco) with a slit width of 2 nm in 0.2-cm cuvette to diminish light scattering. The chlorophyll concentration of the samples was determined by the method of Lichtenthaler [32].

2.4. Low-temperature fluorescence emission and fluorescence excitation spectra

The fluorescence emission and fluorescence excitation spectra of the *Galdieria* cells and PBS samples were recorded at 77 K using a SPEX Fluorolog FL3-22 spectrofluorimeter (SPEX Industries Inc.) with the spectral resolution of 1 nm. The spectra were automatically corrected for the distribution of the exciting light and for response of the photomultiplier during data acquisition. In fluorescence emission measurements, excitation of PBS was achieved at 570 nm with a 5-nm spectral bandwidth. Fluorescence excitation spectra were obtained for emission band at 695 nm (F695, predominantly arising from chlorophyll *a* of PSII) and for emission at 740 nm of PSI (F740), with a 3-nm spectral bandwidths. Four fluorescence spectra were routinely collected and averaged. The samples in a glass tube with a 1-mm optical path had an optical density of ~0.1 in the red absorption peak of chlorophyll *a*, and reabsorption of the emitted fluorescence was negligible. Cells preliminarily plunged in the glass tube holder were adapted to darkness for 5 min or illuminated by far-red light for 3 min; immediately after all, in both cases, dipped in liquid nitrogen and fluorescence emission and fluorescence excitation spectra were run. For actinic illumination, far-red light of 15 μE m^{-2} s^{-1} was

provided from KL-1500 illuminator (Schott, Mainz, Germany) equipped with interference filter (transmission at 700 nm, 20-nm bandwidth). Cells were frozen without glycerol to prevent uncoupling of the PBS from thylakoid components, as glycerol increases hydration at the particle surface and induces artificial fluorescence of PBS [33].

2.5. Photooxidation of P700 by PBS-absorbed actinic light

Redox transients of P700, the primary PSI electron donor, were measured from changes in the absorbance difference at 810 and 870 nm at room temperature with a Walz modulated detection system (Effeltrich, Germany) consisting of a PAM-101 control unit and a dual-wavelength emitter–detector unit ED-P700 DW (Walz, Germany). A multi-branch fiber optic cable was used to guide modulated measuring beams, actinic light and preilluminating far-red light toward the sample and to direct transmitted infrared light to the detector. Measurements were performed in the presence of 20 μM DCMU using dark-adapted cell samples and the same samples preilluminated with far-red light from a 70-W halogen lamp fitted with a 717-nm interference filter. The photon flux density of far-red light was 100 μE m^{-2} s^{-1}; the 2-min period of preillumination was terminated by 5-s dark interval preceding the exposure to actinic light pulse. Actinic light pulses of 10-s duration and intensities varying from 10 to 90 μE m^{-2} s^{-1} were provided from a Luxeon LXK2-PWN2-S00 light-emitting diode (4100 K; Lumileds, United States) equipped with BPF620 (620-nm wavelength, 35-nm bandwidth) glass band-pass filter (Photooptic, Russia) for specific excitation of PBS. The plots of P700 absorbance difference at 810 and 870 nm (ΔA) were fitted with exponential function to determine the amplitude of saturating light, ΔA_{max}. The gradient of the plot calculated by linear regression on a semi-logarithmic scale as a function of ln $[(\Delta A_{max} - \Delta A)/\Delta A_{max}]$ vs. light intensity provides a measure of the relative absorption cross-section of PSI [16]. The sample chlorophyll concentration was 15 μg ml^{-1}.

3. Results

3.1. Absorption spectra and action spectra of PSI and PSII under physiological and nitrogen starvation conditions

In a complete mineral medium, the *Galdieria* cell culture is very similar to many cyanobacterial species having blue –green color since, contrary to the majority of red algae, the hemidiscoidal PBS of *Galdieria* [34] do not contain phycoerythrin. The nitrogen removal from nutrient medium causes various changes in cell morphology and ultrastructure that, for *Galdieria*, have been in detail described earlier [35]. They include suppression of cell division, some diminishing of cell size, cell wall becoming thinner, accumulation of nitrogen-free storage compounds, mainly the storage granules [36], reduction of chloroplasts, and reduction of photosynthetic pigment apparatus including PSI, PSII, and PBS that have much in common with the starved cyanobacteria [37].

In medium lacking nitrogen, *Galdieria* cells show increased bleaching. This change in color is confirmed by the change in the absorption spectra of the cell culture. In a complete medium or in medium depleted of nitrogen, the *Galdieria* cells are characterized by the red chlorophyll absorption peak at 678 nm (Fig. 1A). In control sample, the PBS peak at 620 nm has virtually equal amplitude to the chlorophyll band (Fig. 1A, spectrum 1). Under our experimental conditions, changes in the absorption spectrum occurred after 1 day of nitrogen starvation when the decrease of PBS peak was already evident (Fig. 1A, spectrum 2). After the fourth day of starvation, when PBS were no longer clearly visible in the thin sections of chloroplast thylakoids [29], the absorption in the region of 620 nm was greatly reduced relative to that at 678 nm and was slightly red shifted (Fig. 1A, spectrum 3). The 620-nm absorption band is now dominated by the short-wavelength vibrational satellite of the 678-nm chlorophyll peak rather than PBS absorption (Fig. 1A). The recorded absorption changes correspond well to those described previously for nitrogen starved cyanobacteria [37].

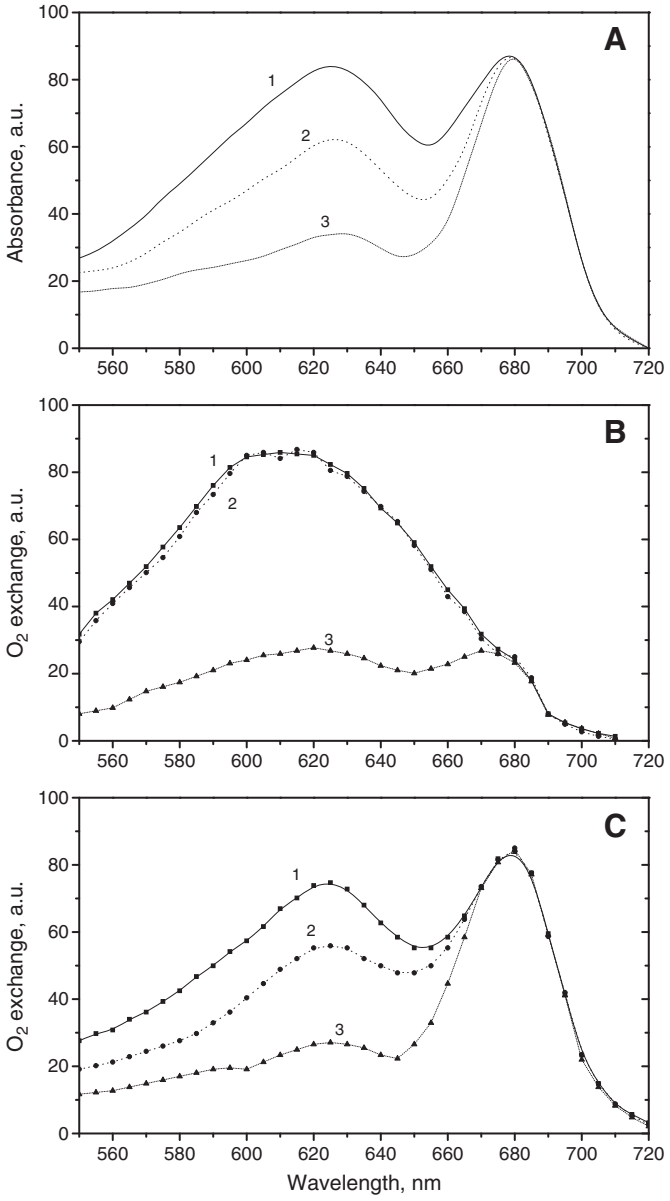

Fig. 1. Absorption spectra and action spectra of PSI and PSII of *Galdieria* cells under physiological and nitrogen starvation conditions: (1) control samples, (2) cells after 24 h of starvation, and (3) cell after 84 h of starvation. (A) Absorption spectra; (B) action spectra of PSII, and (C) action spectra of PSI. All the spectra were normalized in chlorophyll band position at 678 nm.

In *Galdieria*, the PSII action spectrum for O_2 evolution (Fig. 1B) is significantly different from the absorption spectrum. In the control sample, the 620-nm peak of PBS dominates over the 678-nm chlorophyll band, indicating a very high PBS to chlorophyll ratio in PSII. The intensity differences of the PBS and chlorophyll bands are so large that a 620-nm peak overlaps with chlorophyll band, which is observed as just a small shoulder on the long-wavelength slope of the former (Fig 1B, spectrum 1). The shape of the photosynthetic action spectrum for O_2 evolution (PSII) is typical for PBS-containing cyanobacteria [14,38]. After the first day of starvation, surprisingly and contrary to the absorption spectrum, the action spectrum of PSII remained unchanged (Fig. 1B, spectrum 2). The loss of PBS was observed in spectrum following the fourth day of starvation; however,

the PBS peak remained much more evident in the PSII action spectrum than in the cell absorption spectrum (Fig. 1B, spectrum 3).

In the control sample, the action spectrum of PSI, measured separately from PSII inactivated by DCMU, exhibits the PBS band as well, but with the PBS/chlorophyll peak ratio of about 0.8:1.0, which is more similar to the absorption spectrum (Fig. 1C, spectrum 1). Therefore, the data suggest that PBS take part in functions of both photosystems. Contrary to PSII action spectra but similar to absorption spectra, the partial and then almost complete disappearance of 620-nm peak from PSI action spectra was observed after the first and the fourth days of nitrogen starvation, correspondingly (Fig. 1C, spectra 2 and 3). The finding that the PBS peak started to disappear from the PSI action spectrum on the first day of starvation while in PSII action spectrum it stayed invariable suggests that *Galdieria* cells have two PBS fractions. One of them is related to PSII, and the second one is an antenna part of PSI.

It is useful to note that according to erythrosine dye method, during the first 4 days of nitrogen starvation, the fraction of the perished *Galdieria* cells remained constant and did not exceed 2.5% as in control population [35]. These data mean that changes of action spectra revealed upon nitrogen starvation did not correspond to the *Galdieria* cell mortality and have to be explained by stress conditions.

3.2. Low-temperature fluorescence emission and fluorescence excitation spectra of the red-light and dark-adapted samples

To explore further the observed heterogeneity of PBS, we induced state transitions in *Galdieria* cells using far-red light illumination and analyzed them using low-temperature fluorescence and excitation fluorescence spectroscopy. Fig. 2A shows the 77 K fluorescence emission spectra of *Galdieria* cells excited in the region of PBS absorption (570 nm). Spectra for both, dark-adapted and far-red light treated cells show the groups of bands with only small overlap between them: the PBS bands in the region of 640–670 nm, the unresolved common peak of terminal PBS emitters and PSII at 685 nm, narrow peak at 695 nm belonging to the core antenna of PSII, and an intense broad band at 728 nm belonging to the chlorophyll antenna of PSI (Fig. 2A). The shoulder at 645 nm and the peak at 662 nm arise from C-phycocyanin and allophycocyanin emission from PBS, respectively. A 3-min far-red light treatment of the cells caused a relative increase in the PBS fluorescence emission in comparison to the dark-adapted sample (Fig. 2A). When the two emission spectra are normalized to the 728-nm peak, the PBS emission in the far-red light treated cells is approximately twice larger than in the dark-adapted cells. The far-red light treated-*minus*-dark adapted difference spectrum (Fig. 2A) demonstrates that the main changes correspond to the PBS-related bands at 645 and 662 nm and an additional difference peak is formed at 681–683 nm. The long-wavelength spectral changes in emission can be mainly ascribed to the vibronic satellites of the main PBS bands.

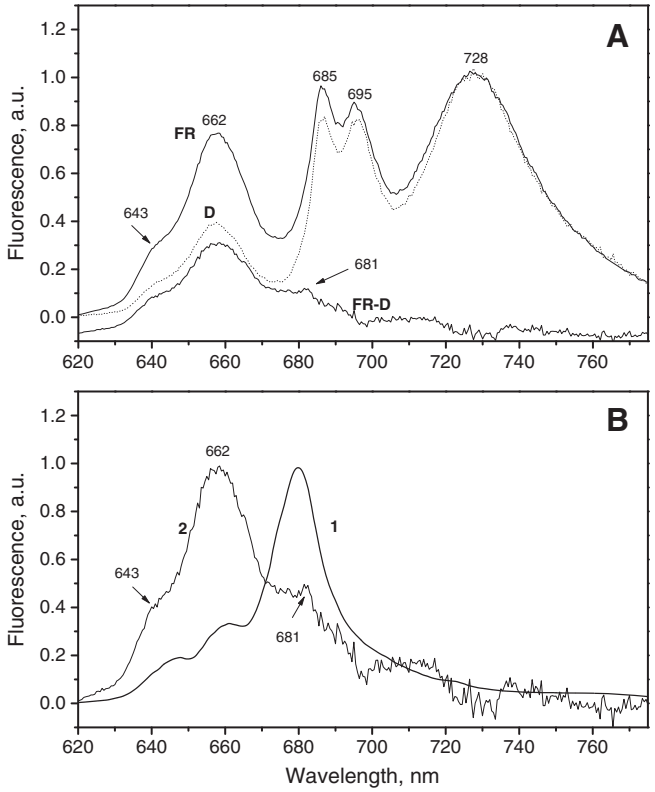

Fig. 2. 77 K fluorescence emission spectra of the whole *Galdieria* cells (A) and isolated PBS (B) after excitation performed at 570 nm. (A) Dark (D) and far-red (FR) light-adapted samples. Spectra were normalized at 728 nm. (FR-D) is a difference spectrum between the two adaptive states. (B) PBS spectrum (1) compared with the enlarged (FR-D) difference spectrum (2) and normalized at the main peak positions.

The conclusion about changes in PBS emission yield was tested by comparing the obtained difference spectrum with the fluorescence emission spectrum of isolated PBS. It is known that low-temperature fluorescence emission spectroscopy is used to provide a measure of the functional integrity of a PBS preparation [5,30]. At 77 K, isolated PBS routinely have a dominant fluorescence emission peak at ~ 681–682 nm, independent of excitation wavelength, and belonged to the terminal PBS emitters [30] (Fig. 2B). It also consists of the low-intensity bands at 645 nm belonging to C-phycocyanin and the band of allophycocyanin at 662 nm (Fig. 2B). When compared with the far-red light-treated-*minus*-dark difference spectrum, it is seen that the spectral positions of the main bands in one spectrum coincide well with the band positions in the other. The similarities in peak positions between the two spectra indicate that the observed differences in fluorescence emission between the far-red light-treated and dark-adapted *Galdieria* cells are explained by an increase in emission of PBS, or the PBS core. However, clearly the spectrum of isolated PBS is characterized by much higher relative intensity of the peak at 681 nm.

The observed increase in the PBS fluorescence emission of far-red light-treated cells could be the result of a reduction in the energy transfer from the PBS to chlorophyll *a*. 77 K excitation spectra provide information on the spectral cross-section of PSI and PSII emissions by utilizing the large spectral differences between them. The F740 fluorescence excitation spectra of PSI measured in the whole visible region for both far-red light-treated and dark-adapted *Galdieria* cells are characterized by three bands in the Soret region at 440 nm (chlorophyll *a*), 470 and 505 nm (carotenoids) (Fig. 3A). In the red region, the main band is observed at 678 nm with a long-wavelength shoulder at ~710 nm, both belonging to chlorophyll *a*. The spectral region between 530 and 660 nm is characterized by the PBS bands with the main peak at 632 nm (Fig. 3A). It was clear from heights ratio of the 632 nm band to chlorophyll *a* band at 678 nm that the excitation cross-section of PSI emission was decreased in far-red light-treated compared to dark-adapted *Galdieria* cells. The dark-adapted-*minus*-far-red light excitation difference spectrum indicates that the loss in cross-section of PSI observed in far-red light coincides with PBS absorption, while the contribution from the Soret region belonging to chlorophyll *a* and carotenoids is unchanged (Fig. 3A). In other words, the excitation spectra for PSI chlorophyll *a* emission indicate reduced energy transfer from the PBS to PSI in samples treated with far-red light. This conclusion is supported by the strong similarity between the difference spectrum and the fluorescence excitation spectrum of isolated PBS (Fig. 3B). Although, slight differences are observed between the two spectra in the long-wavelength PBS absorption area, where the difference spectrum has higher peak intensities than spectrum of isolated PBS does. Thus, the increase in PBS emission in far-red light treated *Galdieria* cells (Fig. 2A) is accompanied by a

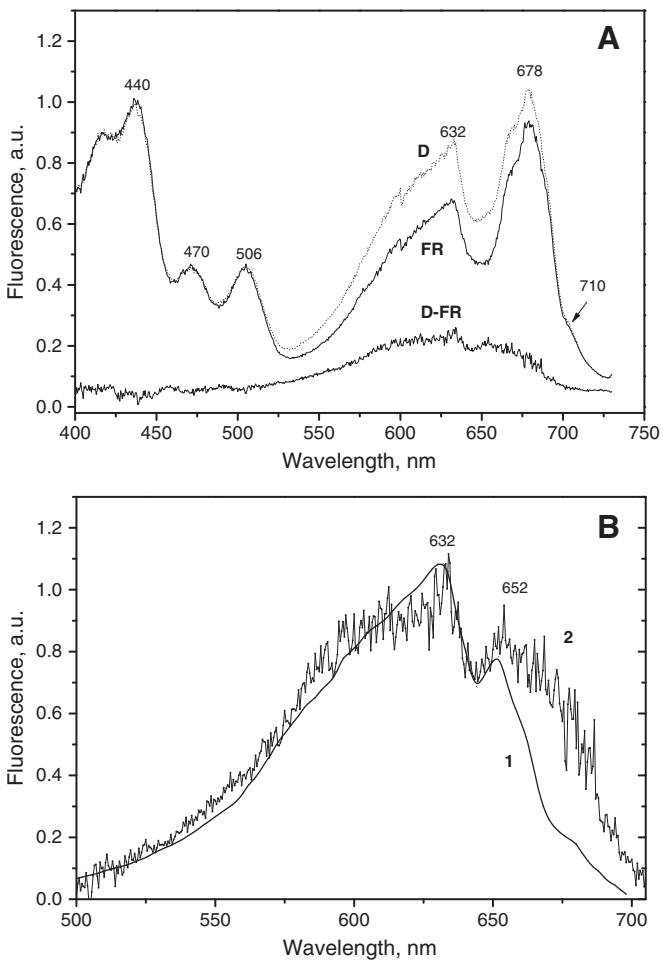

Fig. 3. 77 K fluorescence excitation spectra of the whole *Galdieria* cells (A) and isolated PBS (B) registered for emission of PSI at 740 nm. (A) Dark (D) and far-red (FR) light-adapted samples. Spectra were normalized at Soret peak of chlorophyll *a* at 440 nm. D-FR is a difference spectrum between the two adaptive states. PBS spectrum (1) compared with the enlarged (FR-D) difference spectrum (2) and normalized at the main peak positions.

concomitant decrease in the excitation cross-section of PSI (Fig. 3A). Actually, the cross-section decrease of PSI expressed as a ratio between the areas of the difference spectrum and the excitation spectrum of the far-red light-adapted cells is more than 30%.

The F695 fluorescence excitation spectra of PSII (Fig. 4) measured under the same conditions differ strikingly from the spectra of PSI by the very intense PBS bands at 632 and 652 nm in relation to chlorophyll *a* band at 678 nm. This difference corresponds to the high PBS/chlorophyll *a* ratio found in the pigment apparatus of PSII compared to PSI [14,38]. The bands at 632 and 652 nm belong to C-phycocyanin and allophycocyanin, the main pigment parts of PBS. The relative height of the Soret band at 440 nm is very low, and the spectral band of chlorophyll *a* at 678 nm is seen only as a shoulder (Fig. 4). The overall shape of the spectra repeats that previously observed in cyanobacterial species [38]. Contrary to PSI, the PSII excitation cross-section (Fig. 4) is largely unchanged when comparing far-red light treated and dark adapted *Galdieria* cells.

3.3. P700 photooxidation by excitation energy delivered from PBS

A direct means of assessing whether the energy obtained by PBS can be used in electron transport reactions related to PSI is to demonstrate the effectiveness of monochromatic light absorbed by PBS in bringing about photooxidation of P700. The level of $P700^+$ that accumulates at certain photon flux density depends strongly on light-

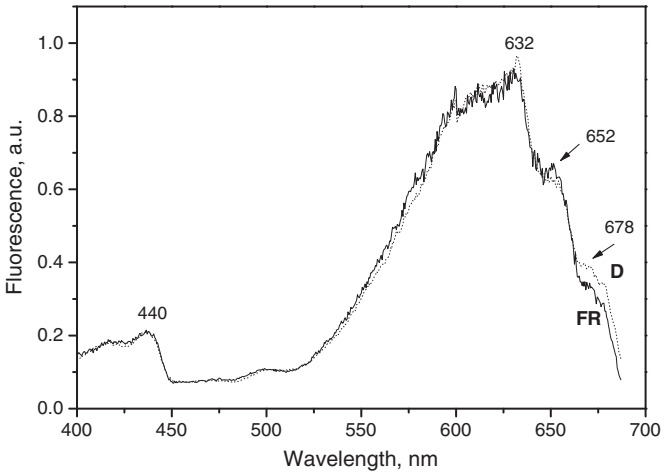

Fig. 4. 77 K fluorescence excitation spectra of the whole *Galdieria* cells registered for emission of PSII at 695 nm. Spectra of the dark (D) and far-red light (FR) adapted samples were normalized at Soret peak of chlorophyll *a* at 440 nm.

harvesting capacity and on the turnover rate of the PSI reaction centers. The result of these effects is a saturation curve: $P700^+$ will accumulate with increasing light intensity. P700 oxidation was measured for DCMU-treated *Galdieria* cells incubated in darkness or treated with far-red light. To investigate the transfer of light energy absorbed by PBS to PSI reaction centers, the actinic light was 620 nm, primarily absorbed by PBS, to minimize direct chlorophyll *a* excitation. In dark-adapted samples, the kinetics of ΔA increase

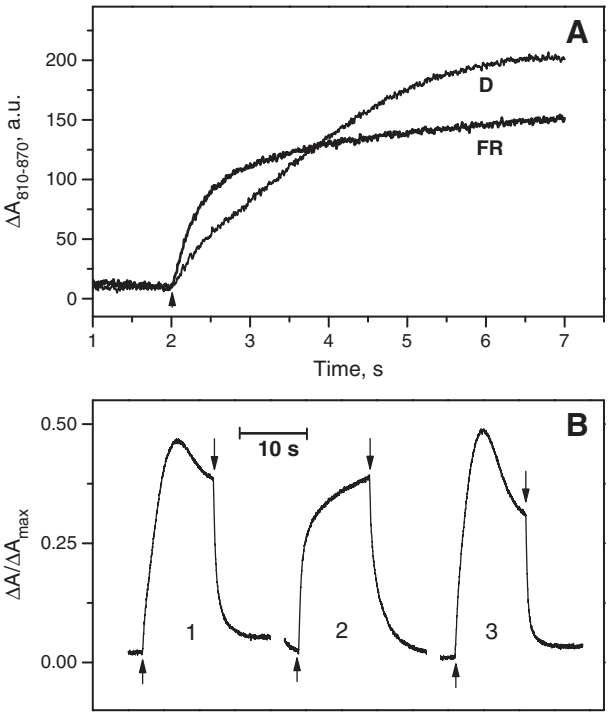

Fig. 5. Kinetics of P700 photooxidation of dark-adapted and preilluminated by far-red light *Galdieria* cells. Arrows indicate light switched on and off. (A) Enlarged scale for the front of the signal for P700 redox state changes induced with 620 nm actinic light in dark adapted (D) and far-red light preilluminated (FR) samples. (B) Reversible effect of far-red light on absorbance changes of P700 induced by excitation of PBS for the cells pre-incubated in the dark for 3 min (curve 1), then preilluminated with far-red light for 3 min (curve 2) and repeatedly incubated in the dark for 3 min (curve 3). Redox state changes of P700 were induced with 620 nm actinic illumination of light intensity that caused 50% of fully oxidation of primary electron donor P700 in the dark-adapted cells.

were nonmonotonic, indicating the involvement of cyclic electron transfer that decelerates P700 photooxidation (Fig. 5A). Dark adaptation is known to promote cyclic electron transport in higher plants [39]. In contrast, the increase of ΔA after far-red light treatment, known to suppress cyclic electron transport [39], proceeds monotonically (Fig. 5A). In order to avoid complications associated with cyclic electron flow, we based our analysis on comparison of the peak amplitudes for the ΔA signals rather than the kinetics. In dark-adapted sample, the P700 oxidation reached its peak after about 5 s after the actinic light was switched on; in the same sample preilluminated with far-red light, the extent of P700 photooxidation was substantially smaller (Fig. 5A). The diminishing of PBS participation in P700 photooxidation is reversible. Under 620-nm light flash, the level of the relative P700 absorbance change of the dark-adapted *Galdieria* cells dropped when the same sample was exposed to far-red light actinic illumination and was restored again after 3–5 min of repeated dark incubation (Fig. 5B, curves 1 to 3). The observed involvement of PBS in P700 photooxidation indicates that different ΔA signals in dark-adapted and far-red light preilluminated cells are manifestation of a kind of short-term light adaptations.

To better quantify the difference in quantum yield of energy migration between PBS and P700, photooxidation of PSI reaction centers was determined at different intensities of 620-nm illumination (Fig. 6). The 620-nm light absorbed by PBS caused a significantly decreased rate of P700 oxidation in cells treated with far-red light vs. in those pre-incubated in the dark. The light response curves shown in Fig. 6A were fitted with exponential function to determine by extrapolation ΔA_{max} values, which as expected were nearly identical and equal to 440 μE^{-1} m^2 s^1, irrespective of light/dark pretreatment. For illustrative quantitative analysis, we compared the plots defined by the equation $\Delta A = \Delta A_{max} \times [1 - \exp(-k \times \text{relative flash intensity})]$ for dark-adapted and far-red light preilluminated samples (Fig. 6B).

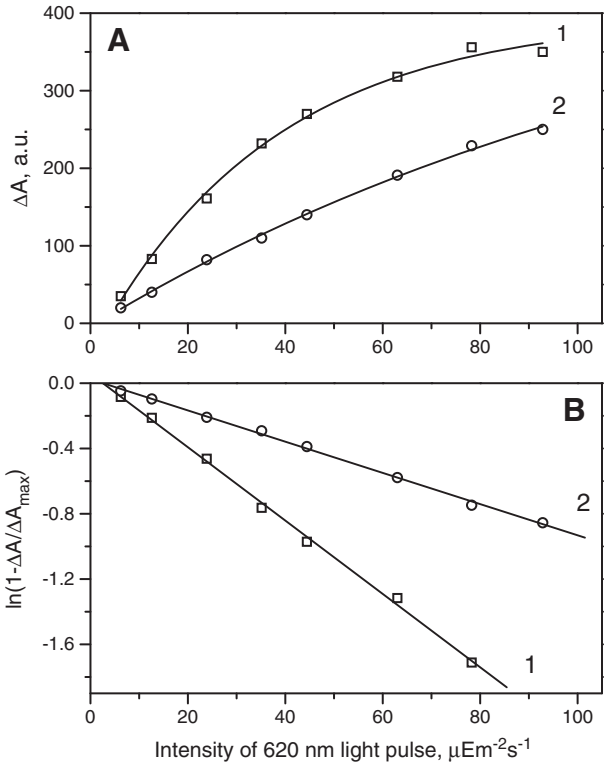

Fig. 6. Plots of P700 photooxidation as a function of light pulse intensity at 620 nm. P700 redox state changes were induced by 10-s pulses of actinic light absorbed predominantly by PBS. Cells of *Galdieria* were incubated for 3 min in the dark (1) or preilluminated for 2 min with far-red light (2). (A) Experimental data fitted with exponential curves. (B) The same as panel (A) in a semi-logarithmic scale.

The steepness of such plots is a measure of the efficiency of photon capture [16,40]. The calculated efficiency of PBS absorbed quanta in P700 photooxidation in the far-red light-treated sample was 0.43 of that in the dark-adapted *Galdieria* cells (Fig. 6B). The difference in rates of P700 oxidation reflects a decrease in energy flow from PBS to P700 after exposure to far-red light. Therefore, in *Galdieria* cells, the dark incubation induced an increase in P700 oxidation by light absorbed by PBS in agreement with the results obtained from 77 K fluorescence measurements.

4. Discussion

4.1. Participation of PBS in function of PSI

PBS-related peaks were observed in this study in the low-temperature excitation spectra of *Galdieria* cells, indicating that a significant amount of light energy absorbed by PBS is delivered to PSI. While the migration of excitation energy at 77 K does not necessarily reflect the pathways that are operative at room temperature [41], the two additional techniques presented here strongly suggest that PBS has a light-harvesting function in PSI. In *Galdieria*, photosynthetic action spectra of PSI, measured after inactivation of PSII with DCMU as photoinhibition rate of O_2 uptake, and the data for photobleaching of P700 by PBS-absorbed light strongly support the 77 K fluorescence measurements. The lower PBS/chlorophyll peak ratio in PSI fluorescence excitation and action spectra comparing to PSII of *Galdieria* likely reflects the about three times bigger chlorophyll core and additional antenna chlorophylls of the PSI when compared with PSII [8,9,27,28].

4.2. Existence of PBS fraction coupled to PSI

In *Galdieria* chloroplasts, the delivery of energy from PBS to PSI could be attributed to the reversible migration of PBS from PSII to PSI on the surface of thylakoid membrane as it was proposed for the fluorescence changes accompanying the state transitions in cyanobacteria [16]. The surface migration in red algae is limited at least in species containing hemispherical (hemiellipsoidal) PBS due to their quite densely packing on thylakoid membrane [42]. It has been also generally assumed to be due to spillover of excitation from PBS-coupled PSII centers to PSI core chlorophylls as is stated in the theory by Shimada et al. [43], who argue for energy transfer from PBS to PSI via PSII. This theory rests on interpretation of fluorescence emission spectra at 77 K without analysis of PSI activity at physiological temperature. The invariability of PSII fluorescence excitation spectra in *Galdieria* cells pre-incubated in the dark or in far-red light in parallel with the marked changes of excitation spectra of PSI suggests that spillover and/or the reversible diffusion of PBS between PSII and PSI cannot explain our data.

Under conditions of heterotrophic and mixotrophic growth, pigment content of *Galdieria* cells decreases as well; however, the mechanism of this process has been substantially different. In this case, there is no direct phycobiliprotein degradation, and the pigment content drops mainly due to inhibition of phycobilin chromophores biosynthesis during active cell division [44,45].

In *Galdieria* cells, during the course of nitrogen starvation, the earlier disappearance of PBS transferring the energy to PSI compared with PBS related to the photosynthetic activity of PSII also indicates the presence of the two PBS cell fractions. The PBS belonging to PSI are more sensitive to stress conditions and, upon nitrogen starvation, disappear from the cell earlier than does the more resistant fraction of PBS coupled with PSII. The alternative is that PSI does not have its own fraction of PBS. Then PBS being docked to PSII complexes could get closer in the plane of the thylakoid membrane, via their lower lateral C-phycocyanin cylinders or via outside part of allophycocyanin core, to PSI complexes neighboring PSII and therefore deliver PSI some part

of absorbed energy. However, if this were the case, the action spectrum of PSII would also be expected to change, because PBS size decreases upon nitrogen starvation starting from the lateral cylinders [37]. Nevertheless, such changes are inconsistent with the unchanged shape of PSII action spectrum on the first day of starvation. Besides, the presence of allophycocyanin-caused peak at 652 nm in fluorescence excitation spectrum also indicates the energy transfer to PSI by means of PBS core.

To date, it remains unclear how PBS substructures can be loosened during nutrient deprivation that causes a massive degradation of the external antenna [4,6]. The mechanisms controlling biliprotein degradation are generally unknown, although one of the suggested models is the binding of chaperone proteins that would activate a degradation pathway [46]. It is suggested that the binding of chaperones to PBS can mark proteins for their further recognition by proteases or can soften the PBS structure by increasing the distances between single PBS segments so that proteases will get increased access to their substrates [47]. Because, upon nitrogen starvation, the proteases degrade the PSI fraction of PBS more rapidly, it suggests that in PSI of *Galdieria* chloroplasts PBS are bound more loosely and could be more accessible to the PBS degrading enzymes than in PSII.

The major linker polypeptide in the PBS core, the core-membrane L_{CM} linker is present in two copies and provides an anchoring the PBS to the photosynthetic membrane [3]. The L_{CM} linker is fragmented into several domains with different functions. Particularly, the peptide domain of L_{CM} called arm2 and known to be composed principally of basic amino acids has been proposed to play the key role in docking of the PBS to the thylakoid membrane [48]. Most probably, the unspecific electrostatic interaction between this L_{CM} arm of PBS core and the photosystems occurs since the presence of phospho- and sulpholipids gives a partial negative charge to the membrane surface [49]. Negatively charged lipids are found exclusively at the cytoplasmic side of the PSII and the overall symmetry and dimension of the PBS core match those of the PSII dimer [50]. Furthermore, the PSII cytoplasmic surface is flat and could accommodate a PBS on its top, which would provide favorable conditions for PBS attachment. The PSI complex contains less negatively charged lipids [9]; moreover, some peripheral subunits of PSI protrude from the cytoplasmic surface of the thylakoid membrane potentially weakening PBS attachment. Thus, electrostatic interaction between PSI and PBS might be less favorable compared to PSII and thus PSI-associated PBS is lost more rapidly upon nitrogen starvation being more accessible to a degradation pathway.

In *Galdieria*, the presence of LHC belt around the monomeric PSI core [25] does not appear to hinder its interaction with PBS. The docking of PBS to monomeric PSI has previously been shown in the cyanobacterium *Spirulina platensis*, where PSI trimers and PSI monomers are spectrally distinguishable *in situ*. It has been demonstrated that PBS act as a peripheral antenna system for PSI trimer as well as for PSI monomer thylakoid fractions [14]. Our data suggest independent pathway of energy migration from PBS to PSI in *Galdieria* chloroplasts and are in line with most observations of PBS–PSI interactions obtained in the last years for cyanobacterial species [14,15,49,51].

Recently, Bush et al. [52] provide structural data of PSI association with PBS-subparticles in another thermoacidophilic alga, *Cyanidioschyzon merolae*. This unicellular species is very closely related to *Galdieria* being also a member of the one the same red algal class Cyanidiophyceae. The results of Bush et al. nicely correlate with our findings. Nevertheless, the case of *Galdieria* differs from specific association of PSI with the CpcG1-like phycocyanin subunit in *C. merolae* [52] and the CpcG2 complexes of phycocyanin described for *Synechocystis* sp. 6803 [51]. In the last two cases, allophycocyanin is excluded from the CpcG2 and the CpcG1-like types of reduced PBS while presence of allophycocyanin in PBS associated with PSI in *Galdieria* is clearly evident from low-temperature fluorescence excitation spectra.

4.3. Association of PBS with PSI is far-red light-dependent

In *Galdieria*, the ability to affect light energy transfer from PBS to PSI under conditions of over-excitation of PSI by far-red light is shown in this study. The rate of P700 photooxidation demonstrates a ~60% decrease in 620-nm light excitation energy transfer to the PSI reaction centers after far-red light pretreatment of the samples. In addition, fluorescence excitation spectra measurements suggests ~30–40% of the energy absorbed by the fraction of PBS docked to PSI has not reached the chlorophyll core in far-red light. The difference between the two measurements can be explained by the higher intensity of far-red light used in study of P700 light oxidation compared to the treatment prior to the fluorescence excitation study. The results indicate the adaptive decrease of PSI cross-section in the region of PBS absorption and the diminishing of the PSI activity.

The energy-migration efficiency from the terminal PBS emitters to chlorophyll *a* depends on the orientation and the distance between neighboring chromophore molecules. Thus, a small change of a clearance between PBS and PSI core would greatly affect the energy transfer and PBS quantum yield of emission. Under far-red light conditions, a local electrochemical trans-membrane gradient generated by PSI-dependent cyclic electron transfer, most probably, results in a small change in the thylakoid conformation, sufficient to change the electrostatic attraction of PBS to PSI into repulsion force [53]. The forming in red light cation radical $P700^+$ transmits to the core of PSI an additional positive charge that weakens PSI interaction with also positively charged arm2 of L_{CM} linker and therefore with the ApcD long-wavelength terminal emitter that, in cyanobacteria, is known to be a main channel of energy transfer from PBS core to PSI embedded in the lipid bilayer [15,54]. In *Galdieria*, the simultaneous and opposite changes of the PBS peak heights in fluorescence emission and fluorescence excitation spectra of PSI suggest a detachment of PBS from PSI under far-red light conditions and its reverse attachment in the dark. This proposition corresponds well to the known diffusive mobility of hemidiscoidal PBS. On the other hand, the observed changes of fluorescence emission at 662 nm (Fig. 2) and of fluorescence excitation at 652 nm (Fig. 3) belong to allophycocyanin but not terminal emitters of intact PBS. One could also speculate that changes under far-red light conditions are caused by rearrangement within the PBS that leads to disconnection of allophycocyanin-composed core and L_{CM} polypeptide rather than by detachment of complete PBS from the membrane surface. Both speculations have to be taken into consideration with caution because the supramolecular organization of the red algal and cyanobacterial membranes is not understood in detail and we do not know how the PBS are precisely anchored with PSI and PSII.

The band positions of PBS revealed in 77 K fluorescence emission and fluorescence excitation difference spectra of PSI and of isolated PBS of *Galdieria* coincide or are very similar while the relative intensity of 681-nm fluorescence peak of terminal emitters is different. The higher amplitude of 681-nm peak in the spectrum of isolated PBS would possibly mean that the terminal emitters are very effectively quenched in situ due to delivery of excitation energy to chlorophyll *a*.

4.4. Far-red light regulation of PSI activity in Galdieria would be a variety of state transitions

State transitions are a short-term regulatory mechanism, which maintains an efficient light-energy conversion by ensuring balance between the PSI and PSII. In higher plants and green algae, the mechanism is well documented and has been shown to involve the redox status of the plastoquinone pool, the activation of kinase, and the phosphorylation/dephosphorylation of chlorophyll *a*/*b*-containing light-harvesting complex allowing its reversible displacement from one photosystem to the other [19,55]. In cyanobacteria and red algae,

the analogous mechanism of this adaptation is not quite clear, concerning both the triggering events and the associated structural changes [20]. Except the abovementioned reversible migration of PBS between the PSII and PSI and "spillover", the main models of light-state transitions that had been proposed include the partial dissociation/association of PBS and PSII without participation in the process of PSI and some hybrid mechanisms [54,56,57].

Our experimental approach was designed to measure, in *Galdieria*, the distribution of PBS-absorbed energy to PSI under far-red light compared with dark conditions. The obtained data allow to distinguish between the selective detachment of PBS from PSI in order to compensate for under-excitation of PSII in far-red light, and the other possible models of PBS-dependent light acclimation. Significantly the PBS detached from PSI do not become energetically coupled to PSII, rather far-red light simply diminishes energy transfer from PBS to PSI. To our knowledge, this kind of short-term far-red light adaptation related exclusively to PSI has not previously been demonstrated for cyanobacteria or red algae. Indeed, earlier described short-term fluorescence emission changes at 77 K in other red algal species were expressed mainly as changes in the PSII bands peaking at 685 and 695 nm [7,20,21,58]. The demonstrated action of far-red light on PBS–PSI interaction could be considered as an earlier unnoticed part of state transitions in the red algae or as an independent adaptive phenomenon. The selective reversible detachment of PBS from PSI is perhaps the most rapid way of short-term light adaptation compared to diffusion of PBS between PSI and PSII in the plane of the membrane or to the spillover movement of the PBS-docked PSII dimers to PSI in the lipid bilayer. It has already been demonstrated here that, under certain conditions, hemidiscoidal PBS could be detached from PSI core complexes and thereby diminish the PSI activity and balance PSI and PSII turnovers. (It is important to note that the uncoupling of energy transfer from the PBS to the reaction centers by addition of glycerol or by any other chemicals was excluded in our study.) Based on obtained results, we speculate that the quantum efficiency of energy transfer from PBS to PSI could be an important factor that influences the photosynthetic activity of *Galdieria* and, possibly, of other unicellular red algae. Further studies will be required to understand the detailed pathway of energy transfer from PBS to PSI and its regulation in red algae.

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