Efficient Light Harvesting in a Dark, Hot, Acidic Environment: The Structure and Function of PSI-LHCl from Galdieria sulphuraria

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ABSTRACT Photosystem I-light harvesting complex I (PSI-LHCI) was isolated from the thermoacidophilic red alga Galdieria sulphuraria, and its structure, composition, and light-harvesting function were characterized by electron microscopy, mass spectrometry, and ultrafast optical spectroscopy. The results show that Galdieria PSI is a monomer with core features similar to those of PSI from green algae, but with significant differences in shape and size. A comparison with the crystal structure of higher plant (pea) PSI-LHCI indicates that Galdieria PSI binds seven to nine light-harvesting proteins. Results from ultrafast optical spectroscopy show that the functional coupling of the LHCl proteins to the PSI core is tighter than in other eukaryotic PSI-LHCI systems reported thus far. This tight coupling helps Galdieria perform efficient light harvesting under the low-light conditions present in its natural endolithic habitat.

INTRODUCTION Photosynthesis is the main process on Earth that converts light energy to chemical energy. The development of oxygen evolving (oxic) photosynthesis by the ancestors of cyanobacteria ~2.5 billion years ago led to a dramatic increase in atmospheric O2 levels, which in turn contributed to the oxidizing conditions found today (1,2). Cyanobacteria, algae, and higher plants all perform oxygenic photosynthesis, and the primary photosynthetic steps in these organisms have been well conserved over 1.5 billion years of evolution. In eukaryotes, photosynthesis takes place in the chloroplast, a special organelle that contains a differentiated membrane system known as thylakoids. It has been established that chloroplasts in eukaryotes derived from an ancestor of modern cyanobacteria by endosymbiosis. Photosynthetic reactions are divided into two categories: 1) light reactions, which include the splitting of H2O into O2 + 4H+, electron transfer across the thylakoid membrane, and the production of the high-energy currency metabolites ATP and NADPH; and 2) dark reactions in which ATP and NADPH are used to drive CO2 fixation for the production of carbohydrates (3). In the light reactions, two thylakoid membrane proteins, photosystems I and II (PSI and PSII), catalyze the first and most important step in converting visible light into chemical energy: the light-driven charge separation. The core subunits of both PSI and PSII are well conserved in the cyanobacteria and eukaryotes; differences are found primarily in the oligomeric state, the smaller protein subunits, and the peripheral antenna complexes, which increase the light-harvesting capacity of PSI and PSII.

PSI catalyzes the light-driven electron transfer from plastocyanin or cytochrome c6 at the luminal (interior) side of the thylakoid membrane to ferredoxin at the stromal (exterior) side, and reduced ferredoxin ultimately reduces NADP+ to NADPH via Ferredoxin NADP+ reductase (1). In cyanobacteria, PSI forms a trimer in which each monomer comprises 12 different proteins to which 127 cofactors (mostly chlorophylls and carotenoids) are noncovalently bound (4). In plants, PSI is a monomer consisting of 16 different proteins to which four peripheral light-harvesting complexes (LHCl; Lhca1–Lhca4) are attached (5).

Photosynthetic organisms live in different environmental niches with vast differences in temperature, nutrients, pressure, and light conditions. Many unusual habitats are occupied only by extremophiles—organisms that thrive in extremes of temperature, salinity, or pH. In recent years, extremophiles have received much attention because they contain specialized enzymes that are useful for biotechnological and pharmaceutical applications (6). In addition, the study of extremophiles is helping to broaden our view of how organisms adapt to extreme environments, which has profound implications for our search for life elsewhere in the universe (7). The majority of extremophiles are Bacteria or Archaea; among the few exceptions are the eukaryotic Cyanidiales (6).

Cyanidiales are an order of asexual, unicellular, thermoacidophilic red algae that live at high temperatures (50–55°C) and acidic pH (0.5–3.0) (8). Phylogenetic studies indicate...
that Cyanidiales diverged ~1.3 billion years ago from the other orders in Rhodophyceae, making it the most ancient algal order (8). The photosynthetic apparatus in red algae appears to be in an intermediate state between plants and cyanobacteria. The ultrastructure of red algal chloroplasts is remarkably similar to that of cyano- bacteria due to the presence of phycobilisomes, since their thylakoid membranes do not show the differentiation into stacked and unstacked regions characteristic of plants and green algae (9). PSI and PSII also seem to be transitional: PSI of red algae has a peripheral membrane-intrinsic LHC similar to that of photosynthetic eukaryotes, whereas red algal PSII contains phycobilisomes as the primary light-harvesting antenna protein in place of the chlorophyll (Chl / b or Chl a/e) binding proteins found in green algae and higher plants (10,11).

Currently, Cyanidiales are divided into three genera (Cyanidium, Cyanidioschyzon, and Galdieria) comprising six species (Cyanidium caldararium, Cyanidioschyzon merolae, Galdieria maxima, G. partita, G. daedala, and G. sulphuraria) (12,13). Within Cyanidiales, G. sulphuraria exhibits a variety of unique features: it propagates through endospores, can survive on 50 different carbon sources, represents >90% of the biomass in extreme environments such as hot sulfur springs (pH 0.05–4 and temperatures up to 57°C), is tolerant to toxic metal ions (Al³⁺, Cd²⁺, Hg²⁺, and Hg²⁺), and has the ability to grow photoautotrophically, heterotrophically, and mixotrophically (14). Furthermore, 30% of Galdieria gene sequences are unique and have no paralogs in other Cyanidiales (15). These differences, along with the extreme conditions under which this red alga performs oxygenic photosynthesis, make G. sulphuraria an interesting candidate for biochemical, structural, and functional studies on photosynthetic proteins. Because of its deep-branching position in the photosynthetic family tree, it can also provide insight into the evolutionary relationships among the photosynthetic machineries of cyano- bacteria, algae, and plants.

Our current knowledge of the photosystems of G. sulphuraria is minimal and is mostly inferred from studies of other Cyanidiales. In a recent study, Vanselow et al. (3) analyzed the genes encoding PSI proteins in Galdieria and compared them with the cyanobacterial and eukaryotic PSI complexes. They concluded that Galdieria PSI may be a common ancestor of PSI from cyanobacteria, green algae, and plants. In a study by Marquardt et al. (16), five lhc genes (lhc1–lhc5) were found to be present in the Galdieria genome, and one (lhc4) was confirmed by N-terminal sequencing. Results from that study showed that Lhc4 has a transit peptide of 44 amino acids, with a molecular mass of 20,574 Da for the mature protein (185 amino acids). In this work, our goal was to investigate the structure and composition of PSI-LHCl supercomplexes from Galdieria using electron microscopy (EM) and liquid chromatography with tandem mass spectrometry (LC-MS/MS), and to interrogate their light-harvesting function by ultrafast optical spectroscopy. The resulting picture of Galdieria PSI structure and function yields important new insights into the evolution of this unique organism.

**MATERIALS AND METHODS**

**Cell culture and PSI isolation and characterization**

G. sulphuraria cells were grown at 42°C in 11 L flasks containing a 10× medium at pH 2.0, with a constant supply of air and 2% CO₂ and illumination at 25 μmol photon m⁻² s⁻¹ (model LI-189; LI-COR Biosciences, Allentown, PA) (17). Cells were harvested by centrifugation at 2500 rpm for 2 min at 25°C. Harvested cells were homogenized in MM buffer (20 mM MES pH 6.0, 10 mM CaCl₂, 10 mM MgCl₂, and 500 mM mannitol), and 1 mM phenylmethylsulfonyl fluoride was added before the cells were broken. After cell lysis by means of a bead beater (Biospec Products, Bartlesville, OK) and washing, the thylakoid membranes were solubilized by 1.25% n-dodecyl-β-D-maltoside (β-DDM) at room temperature. Thylakoid membranes were centrifuged at 58,000 rpm for 90 min and the detergent-solubilized PSI-LHCl was further purified from the thylakoid extract by anion exchange chromatography with a step gradient, followed by size exclusion chromatography as described previously (18). A more detailed description of the isolation and biochemical characterization of PSI from G. sulphuraria will be published separately (B. Thangaraj, M. Vaughn, C. Vanselow, I. Sarrou, N. Myers, J. Whitelegge, B. Bruce, and P. Fromme, in preparation). PSI (crystals) of Thermosynechococcus elongatus and His-tagged PSI-LHCl of Chlamydomonas reinhardtii used in this study for comparative purposes were isolated and purified as described by Fromme and Witt (18) and Gulis et al. (19), respectively.

**Low-temperature fluorescence emission spectra**

PSI samples were adjusted to 10 μM Chl with Tris buffer (pH 7.5) containing 60% glycerol and 0.02% β-DDM, and the emission spectra were measured at 77 K with a FLUOROMAX-3 spectrophuorometer between 600 and 800 nm using excitation at 468 nm.

**Differential extinction coefficient of Galdieria P700**

We determined the differential extinction coefficient of P700 by recording flash-induced absorption changes of P700 due to a redox reaction with N,N,N′N′-tetramethyl-p-phenylenediaminedihydrochloride (TMPD) according to a previously published method (20). PSI complexes were diluted to a final chlorophyll concentration of 40 μM in a buffer containing 100 mM Tricine (pH 8.0), 10 mM CaCl₂, 10 mM MgCl₂, 0.02% β-DDM, and 800 μM TMPD. The reaction of PSI with TMPD⁻ was performed in an anaerobic cuvette with a 1 cm path length. Before measurements were obtained, the buffer was degassed and the sample was loaded in an anaerobic chamber. The flash-induced absorbance difference spectra of P700 and TMPD were recorded at room temperature with a JTS-10 LED kinetic spectrometer (Bio-Logic, Grenoble, France). The sample was excited by actinic light (650 nm; intensity: 3000 μE m⁻² s⁻¹; duration: 10 ms). We used interference filters at 703 nm and 573 nm for P700 and TMPD⁻, respectively. Because we measured at 703 nm, to calculate the extinction coefficient at 700 nm, we normalized the absorption based on the dual-beam spectrophotometer readings. As a control, we applied the same method to T. elongatus PSI samples. Previous studies reported extinction coefficient values of 10.6–12 mM⁻¹ cm⁻¹ (20,21) for oxidized TMPD, which we used as an extinction coefficient range for the Galdieria PSI-LHCl complex and T. elongatus PSI. Chlorophyll (Chl a) concentrations were determined after
Galdieria PSI-LHCI supercomplexes by measuring the P700 oxidation. The proteins were suspended in a buffer containing 20 mM MES, pH 6.4, 100 mM MgSO₄, and 0.02% β-DDM. The sample (4 mL) was equally divided between two glass cuvettes (2 mL each). Then 10 μL of ascorbate (0.5 M) were added to one cuvette and 10 μL of potassium ferricyanide (0.1 M) were added to the other. The absorption difference spectrum (reduction – oxidation) was measured between 650 and 850 nm using a Cary dual-beam spectrophotometer. The differential extinction coefficients obtained for Galdieria PSI-LHCI were employed for quantitation of the Chl a/P700 ratio (23).

MS and data analysis

Galdieria PSI samples were precipitated with cold acetone (−20°C) to remove lipids, detergents, chlorophyll, and other cofactors. The precipitated protein was recovered by centrifugation (10,000 × g) and the supernatant was removed. The protein pellet was dissolved in 90% formic acid for immediate injection into a high-performance liquid chromatography system prepared for reversed-phase chromatography. Column preparation, fraction collection, MS instrumentation setup for intact proteins, and data analysis were performed as described elsewhere (24,25). Selected fractions from LC-MS+ were trypsin-digested and analyzed by nano LC-MS/MS, and the obtained MS data were analyzed by Mascot software (Matrix Sciences, London, UK) as described previously (25).

EM and single-particle analysis

Samples of purified PSI-LHCI complex were diluted with buffer (20 mM MES pH 6.4, 100 mM MgSO₄, 10 mM CaCl₂, and 0.03% β-DDM) to a final concentration of ~6 μM and negatively stained with 2% uranyl acetate on glow-discharged, carbon-coated copper grids. EM was performed on a Philips CM120 electron microscope equipped with a LaB6 tip, operated at 120 kV. In total, 925 electron micrographs were recorded with a Gatan 4K slow-scan CCD camera at 130,600 × magnification (after binning the images) of 2.3 Å at the specimen level. GRACE software was used for semiautomated specimen selection and data acquisition (26). The 38,859 single-particle projections (128 × 128 pixel frame) were subjected to multireference alignment and reference-free alignment procedures; multivariate statistical analysis, and hierarchical ascendant classification (all routines within the GRoningen Image Processing software package (27)). The final two-dimensional (2D) projection maps of the presented PSI views were calculated from the best-resolved classes using the correlation coefficient determined in the alignment step as a criterion. The final sums were made by summing ~30% of the class members, with the correlation coefficient in the alignment step as a parameter.

Ultrafast fluorescence spectroscopy

We measured the fluorescence decay kinetics using an ultrafast streak camera as described previously (28–30). Briefly, the sample was illuminated by 130 fs pulses from a mode-locked Ti:S laser (Mira 900; Coherent Laser, Santa Clara, CA) pumped by a frequency-doubled Nd:YVO₄ laser (Verdi; Coherent Laser). The repetition rate was reduced to 4.75 MHz using a pulse picker (Model 9200; Coherent Laser) and the excitement light was frequency-doubled to 400 nm. To avoid singlet-singlet annihilation, the pulse energy was reduced to ~0.1 nJ using a neutral density filter. Fluorescence data were collected at a right angle to the excitation beam and focused on the entrance slit of a Chromex 250IS spectograph coupled to a Hamamatsu C5680 streak camera (Hamamatsu, Tokyo, Japan) with a MS56757 sweep unit. Time-intensity surfaces were recorded at timescales of 0.8, 1.4, and 2 ns on a Hamamatsu C4742 CCD camera. The full width at half-maximum of the overall instrument response function was ~6 ps at the 0.8 ns timescale, ~12 ps at the 1.4 ns timescale, and ~20ps at the 2 ns timescale. Global analysis was carried out with the use of ASUFIT (http://www.public.asu.edu/~laserweb/asufit/asufit.html).

Experiments at room temperature were carried out in a 3 × 3 mm glass cuvette (Starna Cells, Atascadero, CA). Measurements were performed at 77 K with a 1.0 × 0.5 cm plastic cuvette in an N₂-cooled cryostat (Oxford Instruments, Oxfordshire, UK). The samples used at 77 K were diluted in 66% glycerol to ensure freezing as a transparent glass. Fluorescence delay kinetics were also measured using the time-correlated single-photon counting (TCSPC) technique to confirm the results from ultrafast fluorescence spectroscopy (for materials and methods, see Fig. S8 in the Supporting Material).

RESULTS

A biochemical analysis of the isolated Galdieria PSI-LHCI clearly shows that the sample was of high purity with minor contaminations from phycobiliproteins. Fluorescence emission spectra of Galdieria PSI-LHCI supercomplexes measured at 77 k show two shoulder peaks at 685 and 690 nm indicative of fluorescence emission from LHCI complex, and a dominant peak at 723 nm, a typical region of fluorescence emission from PSI (Fig. 1). The spectra show no peaks at 650 nm and 660 nm, the region of fluorescence for phycobiliproteins (phycocyanin and allophycocyanin), indicating that the Galdieria PSI-LHCI sample does not contain significant impurities from phycobilisomes. Emission spectra from Chlamydomonas exhibit a major peak at 715 nm due to fluorescence emission from PSI, and a minor peak at 650 nm from Chl b. For T. elongatus, the single peak at 730 nm corresponds to a typical emission band from PSI. This figure clearly indicates that Galdieria PSI-LHCI has a red chlorophyll pool almost...
similar to that of *T. elongatus*, with significantly more red emission than *Chlamydomonas*.

Depending on the extinction coefficient of TMPD used (see Materials and Methods), a molar extinction coefficient of $115 \pm 5 \text{ mM}^{-1} \text{ cm}^{-1}$ was obtained for *Galdieria* PSI-LHCI complexes at 700 nm (see Fig. S1). For *T. elongatus* PSI, an extinction coefficient of $65 \pm 5 \text{ mM}^{-1} \text{ cm}^{-1}$ was obtained. The chemically oxidized-minus-reduced spectra of P700 for PSI-LHCI complexes of *Galdieria* and PSI trimer for *T. elongatus* are shown in Fig. S2. With the extinction coefficient value range obtained for *Galdieria* PSI-LHCI, we determined a Chl a/P700 ratio range of $237 \pm 7$ for *Galdieria* and a range of $102 \pm 7$ for PSI *T. elongatus*. The Chl a/P700 value obtained for PSI *T. elongatus* agrees well with previous reports and the published x-ray structure (31). The ratio obtained for *Galdieria*, however, is higher than those reported for other red alga (e.g., *Porphyridium purpureum* (136 \pm 20) and *C. caldarium* (103 \pm 10) (32)).

A similar Chl a/P700 ratio was deduced for PSI-LHCI of the green alga *C. reinhardtii* (217 \pm 6).

**Low-resolution intact protein MS analysis**

The presence of all of the PSI subunits except PsaM and PsaN, and all five of the LHCs (Lhcr1–Lhcr5) with different posttranslational modifications (B. Thangaraj, M. Vaughn, C. Vanselow, I. Sarrou, N. Myers, J. Whitelegge, B. Bruce, and P. Fromme, in preparation). Furthermore, MS data analysis of trypsin-digested fractions revealed the presence of at least four different Lhcr peptides from four of the five different LHCs identified in *G. sulphuraria* (Table 1, Fig. S3, Fig. S4, Fig. S5, Fig. S6, Table S1, Table S2, Table S3, and Table S4). The single LHCI peptide missing from Table 1, Lhcr4, was previously identified by N-terminal sequencing (16). Therefore, we assume that all of the five Lhcr proteins were present.

**Electron microscopy**

We performed a single-particle analysis on a set of ~39,000 top-view projections of *G. sulphuraria* PSI particles. The complex appears to have an oval shape in the membrane plane projection, with overall dimensions of $19.6 \times 18.5 \text{ nm}$ (Fig. 2). The most prominent inner feature is an L-shaped ridge in the center, with the corner at the upper-left side; the dotted block arc shape in Fig. 2, A–C, indicates the PsaE, PsaC, and PsaD subunits of the stromal hump. Classification shows, however, that the projection maps have a slight variation in size. The map of frame A represents the largest, most abundant type of projection, and the maps of frames B and C show projections with slightly smaller widths, although they share the same overall shape and inner features. This is clear from the distance of the edge (see white arrowhead) to the ridge between the maps in Fig. 2, A and B; the difference is ~0.1 nm. Nevertheless, because all maps show the same prominent L-shaped inner

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**TABLE 1 Lhcr peptides of Galdieria identified by LC-MS analysis**

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<th>Accession number</th>
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<td>CAB 75583</td>
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<td>CAC 87422</td>
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<tr>
<td>Lhcr3</td>
<td>Q9FEM1</td>
<td>CAC 87419</td>
<td>R.EPGYGGFDPGLAK.D</td>
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<tr>
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<td>Q9FEM0</td>
<td>CAB 87421/CAC 10534</td>
<td>R.LDESMPGYAGFDPGLFSDKFDVK.F</td>
<td>56–78</td>
<td>71</td>
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**FIGURE 2** Image analysis of ~39,000 top-view projections of *G. sulphuraria* PSI particles. (A–D) The most abundant particle classes observed in the projection maps. (E and F) Smaller, less-abundant particle classes. The numbers of averaged particles are (from A to F) 2657, 3771, 3300, 2797, 882, and 948, respectively. The scale bar is 10 nm. The position of a ridge at the core moiety is indicated by an L; the dotted block arc shape indicates the PsaE, PsaC, and PsaD subunits of the stromal hump; and the white arrowhead marks the side where the projection map in A is wider than that in B.
feature and overall oval circumference, the difference in width between the maps in Fig. 2, A–D, is likely not structure-related but caused by a gradual tilt of particles attached to the carbon support film of the EM specimens. Indeed, it is known that the roughness of the carbon support film may cause some particle tilting; a clear example of this is the previously analyzed cyanobacterial PSI (33). Because cyanobacterial PSI forms trimers, a loss of threefold symmetry in the top-view projection directly indicates tilting. In a nonsymmetric particle, such as the G. sulphuraria PSI-LHCI complex, a possible tilt is more difficult to identify. The particles presented in Fig. 2, E and F, have dimensions of ~17 × 14 nm and 16 × 12.4 nm, corresponding to smaller particles with a lower Lhcr content.

The EM images clearly show that Galdieria PSI-LHCI is a monomer with a dense belt of LHC antenna proteins closely surrounding the PSI. This result is experimental proof of the hypothesis, based on a genetic analysis, that Galdieria PSI may contain plant-type monomeric PSI-LHCI (3). Galdieria PSI-LHCI also differs significantly from the complexes in higher plants and green algae: the LHCs in Galdieria are more functionally tightly coupled to the PSI core than in higher plants and green algae. This functional tight coupling is shown by the ultrafast spectroscopy and TCSPC results given below.

Fluorescence decay kinetics

Room-temperature fluorescence decay data were collected at timescales of 800 ps, 1.4 ns, and 2 ns, and all three data sets could be satisfactorily described by three components with lifetimes of 6–13 ps, 48–50 ps, and 400–850 ps. The spectral shapes obtained from global analysis at the three timescales were very similar, and we were able to construct a consensus fluorescence decay-associated spectrum (FDAS) by averaging the fitting results (Fig. 3 A). The 6–13 ps component shows a positive peak centered at ~690 nm, representing fluorescence decay on the blue side of the spectrum at this timescale, whereas a negative peak centered at ~720 nm indicates an increase in fluorescence on the red side. This conservative spectral shape has been observed frequently in spectroscopic studies of PSI and is characteristic of energy transfer from the blue pool of chlorophyll to the red-shifted chlorophylls i.e., the excitation energy transfer between pools of chlorophyll with different transition energies (21–24). The longest timescale (2 ns) showed both a longer lifetime (13 ps, as opposed to 6 ps) and a less conservative spectral shape than the shorter timescales. The data collected at shorter timescales were more accurate due to better temporal resolution.

The second component shows a peak at ~695 nm and a long red tail extending out to at least 770 nm. The lifetimes obtained for this component are timescale-independent and in the 47–50 ps range. Thus, they are longer than those typically seen for PSI cores but shorter than the slow components associated with the trapping of LHCI-originated excitons in eukaryotic PSI-LHCI.

Finally, although a third component was required for an accurate fit, it accounts for only ~2% of the overall decay amplitude. Despite the low amplitude of this component, it consistently fit to lifetimes in the 400–850 ps range, rather than the 3–7 ns range that is typical of uncoupled chlorophyll components. It is possible that this component arises from weakly coupled chlorophyll. It is highly unlikely, however, that this is the same type of LHCI component observed in studies of PSI-LHCI from other eukaryotes. Its extremely low amplitude suggests that it probably does not represent a population of chlorophyll that receives significant initial excitation.

When the sample is cooled to 77 K, the situation becomes significantly more complex. The difficulty of transferring energy out of the red-shifted states at low temperature leads to multietapenential decay behavior that is more kinetically and spectrally heterogeneous than that seen at room temperature. Five exponential components were needed to obtain an adequate fit to the streak camera data, but the lifetimes obtained were not extremely consistent between timescales (Fig. S7). This suggests that PSI from Galdieria contains several pools of red-shifted chlorophyll with potentially complex kinetic relationships.
Because of the fairly broad range of timescales involved in fluorescence decay, there is no obvious reason to consider the lifetimes obtained at one timescale to be universally more reliable than the others. It is probable that the shortest components will be most accurate at the shortest measurement timescale, and the longest components will be most accurate at the longer timescales. Despite this difficulty, however, a comparison of the different FDAS allows us to reach consistent conclusions.

The fastest component measured ranges from 7.7 to 10.1 ps and shows a clear energy transfer character similar to the ~6 ps component observed at room temperature. Like its room-temperature counterpart, it has a roughly conservative shape with a positive peak at ~695 nm and a much broader negative peak centered around 720 nm.

The second and third components also show some energy transfer character, although their lifetimes show a broader variation (15–40 ps for the second, 67–160 ps for the third). As a rule, shorter lifetimes are obtained in the experiments conducted at shorter timescales, indicative of complex multiexponential processes. These components are more consistent in their spectral shapes: the second component indicates energy transfer from a pool centered around 700–705 nm to one centered around 720–730 nm, and the third shows transfer from 710–715 nm to 730–740 nm. Of these three initial components, each successive component is shifted further to the red and shows a less conservative spectral shape, indicating a greater participation of red-shifted chlorophyll sites and an increase in trapping (possibly nonphotochemical) relative to transfer with each successive component. Taken together, these results indicate that the first few hundred picoseconds of fluorescence spectral evolution at 77 K involve a shift from blue to red states, accompanied by increasing levels of trapping by P700 (i.e., quenching by P700") at longer timescales.

The fourth and fifth components also show variation in the lifetimes obtained (320–660 ps and 1.2–3.5 ns, respectively), with the longer measurement timescales yielding longer lifetimes. The 320–660 ps component shows a peak at ~720 nm and comparatively little amplitude to the red of 740 nm, whereas the nanosecond component peaks at ~730 nm and extends further into the red. Both components show exclusively positive amplitude, suggesting that no further energy transfer is taking place on these timescales; these components represent decay processes, either by slow transfer to P700 or effectively uncoupled fluorescence. It is probable that these two components represent excitation decay in two distinct pools of red-shifted chlorophylls: one pool is centered at ~720 nm and is populated on a 15–40 ps timescale, whereas the other is centered at ~730 nm and is populated on a 67–160 ps timescale. As before, the more red-shifted spectral features are associated with longer lifetimes, consistent with a situation in which sites with lower transition energies are more kinetically disadvantaged at low temperatures. A comparison with the steady-state 77 K spectrum (Fig. 1) shows that the small spectral features between ~680 and 700 nm correspond to positive lobes of the rapidly decaying phases, whereas the major peak centered at ~720 nm arises from the slow-decaying components.

We used the fluorescence delay kinetics measured by TCSPC to confirm the results from ultrafast fluorescence spectroscopy. TCSPC was performed from 650 nm to 780 nm with a 10 nm interval. Four exponential components were necessary to fit the decay curves over the entire wavelength region (8 ± 1 ps, 60 ± 10 ps, 140 ± 10 ps, and 3.5 ± 1 ns; see Fig. S8 for more details about the TCSPC results). The TCSPC results further confirm the kinetics for energy equilibration and the overall trapping processes obtained from the streak camera experiments.

**DISCUSSION**

Our analysis using single-particle EM shows that the PSI complex of *G. sulphuraria* is a monomeric, oval-shaped particle. Because several types of PSI complexes have been studied by high-resolution x-ray diffraction or lower-resolution EM, a comparison may provide some clues about structural homologies and differences. PSI from a related red algal species, *C. caldarium*, was found to be somewhat heterogeneous, but the largest particle had overall dimensions with a maximum size of 19.6×18.5 nm (11). In that study, cell cultures grown under high-light conditions (200 μmol photon m⁻² s⁻¹) yielded small and medium-sized PSI complexes, whereas algae grown under low-light conditions (20 μmol photon m⁻² s⁻¹) and some of the PSI projection maps have dimensions that are similar to (Fig. 2, C and D) or even larger than (Fig. 2, A and B) the largest *Cyanidium* PSI structure. However, the projections shown in Fig. 2, E and F, have dimensions that differ from the medium (found both at high- and low-light conditions) and small (found only at low-light conditions) PSI particles from *Cyanidium*. Our *Galdieria* cell cultures are strongly inhibited at high light intensities, and our work focused on low-light-adapted cells because this represents the ecologically relevant growth conditions for this organism.

A 2D map of PSI from the green alga *C. reinhardtii*, shown in Fig. 4 B, permits a more detailed structural comparison (34). *Chlamydomonas* PSI measures 21.3×18.2 nm and comprises a monomeric core flanked by approximately nine light-harvesting complexes (35). A comparison shows that both maps have similar features in the core (asterisks, Fig. 4, A and B) and a similar total surface area. The overall shape, however, is significantly different, with the *Galdieria* PSI-LHCI complex being rounder and showing no visible gap between the PSI core and LHC belt.
A comparison can also be made with the high-resolution x-ray structure of plant PSI (36). Fig. 4 C shows an approximate fit of the map (green overlay). The plant structure fits in the right side of the 2D map of *Galdieria*. We fit the x-ray structure into our 2D map by using the positions of similar densities in the *Chlamydomonas* and *Galdieria* 2D maps (indicated by the dotted block arc shape and three asterisks in Fig. 4, A and B) as fixed points. Details of the fitting are described elsewhere (34). The stromal ridge composed of PsaE, PsaC, and PsaD is clearly visible in Fig. 2, A–C (dotted block arc shape) and was matched with the x-ray structure position of these subunits. This indicates that *Galdieria* PSI binds at least four closely attached LHCs. Although it is difficult to make an exact estimation of the number, a possible estimation would lead to 7–9 LHC/PSI. We propose that the additional LHCs form a second layer around the PSI core, similarly to previous observations (11), but alternate arrangements are also possible. It is clear, however, that most of the ∼7–9 light-harvesting proteins must be at one side of the core complex, in similarity to *C. caldarium* (11).

PSI particles can be broadly separated into two categories based on their fluorescence decay characteristics. Many form a single, strongly coupled energy transfer system—this is the case in all known PSI core complexes, and their major decay lifetimes range from ∼18 ps in the green alga *C. reinhardtii* to >40 ps in the cyanobacterium *S. platensis* (37). Trapping lifetimes in these complexes are determined largely by chlorophyll sites whose transition energies have been lowered due to excitonic coupling; complexes with few of these red chlorophylls (such as *C. reinhardtii* and *Synechocystis*) show faster trapping, whereas those with a larger number (like *T. elongatus* and *S. platensis*) show slower trapping. Exciton transfer from these red-shifted pigments to P700 requires the system to borrow thermal energy from the environment; trapping from these red-shifted states shows a strong temperature dependence and is often rate-limiting. In addition, the iron stress-induced PSI-IsiA complexes formed by many cyanobacteria form strongly coupled light-harvesting systems with trapping lifetimes that can be limited by exciton migration (rather than thermally assisted exciton escape from red sites), but are typically shorter than 50 ps (33,38–40).

In green algae and plants, the PSI core is moderately coupled to extrinsic LHCs such as the PSI-LHCI complexes (28,41,42). In addition to the fast trapping from excitons arising in the PSI core, the PSI-LHCI complex samples of green algae and plants exhibit major fluorescence decay phases with lifetimes of ≥100 ps, which arise from excitations that begin in the extrinsic LHCI proteins and whose transfer to P700 is limited by a pigment gap at the PSI-LHCI interface.

Taken in isolation, the fluorescence decay data from *Galdieria* PSI are somewhat enigmatic. The room-temperature decay kinetics of *Galdieria* PSI are dominated by a single trapping phase, in similarity to the tightly coupled cyanobacterial PSI-IsiA complex and the PSI core complexes described above. This single trapping phase, however, is remarkably slow, and would suggest a large pool of red chlorophylls that substantially retards trapping at room temperature. At the same time, *Galdieria* is a eukaryote and is known to contain LHCI genes, but the >100 ps decay components typically associated with excitations beginning in LHCI appear to be missing (16). Another interpretation is suggested by the EM images: LHCI proteins are clearly visible in these images, but their association with PSI appears to be tighter than in green algae and higher plants (34,43). This raises the possibility that exciton transfer in PSI-LHCI from *Galdieria* could be similar to the PSI-IsiA antenna system in cyanobacteria, where the addition of extrinsic antenna complexes dramatically increases the PSI absorption cross-section without introducing the substantial 100–200 ps trapping components typically associated with excitons originating in LHCI (33,38–40).
fairly slow trapping lifetime of *Galdieria* PSI is probably attributable in part to red-shifted chlorophyll sites (as in the case in *T. elongatus* and *S. platensis*). Our fluorescence emission spectra (Fig. 1) clearly indicate that *Galdieria* has a large red chlorophyll pool as found in *T. elongatus*. Another contribution to the slower trapping could come from the increase in the size of the antenna system and hence the lengthening of random exciton walks that terminate at P700, and a similar effect is probably responsible for the 38-ps trapping time observed in PSI-IsiA supercomplexes from *T. elongatus* (44).

One can consider these spectroscopic and structural features within an ecological context by contrasting *Galdieria* with other photosynthetic eukaryotes. Many of these species live at fairly low cell densities and must cope with variable light conditions, including photoinhibition. Higher plants and green algae have evolved sophisticated cellular machinery for state transitions that regulate the association of LHCII with PSI and PSII based on ambient light conditions, as sensed by the redox state of the plastoquinone pool (45–47).

In contrast, *Galdieria* thrives in hot, acidic crevices, forming a dense cell layer in volcanic rocks and endolithic habitats (14). Besides being an extreme environment in terms of temperature and acidity, this habitat is also very stable and provides consistent low-light conditions. Laboratory cultures of *Galdieria* are strongly inhibited by high-light conditions (>40 μmol photon m⁻² s⁻¹), whereas growth is fairly robust under low-light conditions (15–35 μmol photon m⁻² s⁻¹) and extremely high cell densities (1.7–5 g of dry weight from 1 L of cell culture) can be reached under phototrophic growth (B. Thangaraj, M. Vaughn, C. Vanselow, I. Sarrou, N. Myers, J. Whitelegge, B. Bruce, and P. Fromme. unpublished data). Similar high cell densities of *G. sulphuraria* were reported previously (17), and cell growth inhibition at high illumination has been observed for *Galdieria* and other Cyanidiales (48). In endolithic habitats, <1% of the surface photon flux is available for photosynthesis (14). These factors suggest that *Galdieria*’s photosynthetic systems may have been optimized over 1.3 billion years for low-light conditions. Consistent with this static, low-light environment, *Galdieria* lacks an LHCII gene, suggesting that it may have lost the ability to adapt to changing light conditions via state transitions. Further evidence for an antenna system adapted to static low-light conditions is provided by time-resolved spectroscopy and augmented by electron micrographs, which show a more continuous electron density between PSI and LHCI than is seen in the more loosely coupled complexes from green algae and higher plants. Thus, the PSI-LHCI complex in *Galdieria* forms a functionally tightly coupled complex with faster and more efficient trapping compared to PSI-LHCI complexes in green algae and higher plants.

This is consistent with a scenario in which *Galdieria* PSI diverged from an ancient photosynthetic eukaryote with loosely attached antenna complexes that colonized static, low-light endolithic habitats and adapted to these conditions over billions of years. The selective pressure of growth under low-light conditions led to tighter functional coupling between lhcr proteins and the PSI core in *Galdieria*, as increased photosynthetic efficiency was more critical for survival, possibly at the expense of regulatory flexibility via state transitions or other mechanisms.

This study demonstrates that the oligomeric state of PSI in *Galdieria* is a monomer, with a belt of LHC proteins functionally tightly coupled to the PSI core. To our knowledge, this is the first eukaryotic PSI-LHCI system for which a tight functional coupling has been reported. Our results indicate that despite the broad evolutionary conservation of proteins involved in oxygenic photosynthesis, rather dramatic adaptations are possible under extreme conditions. Because of its ecological niche in a static, low-light environment, *G. sulphuraria* appears to have reorganized the PSI-LHCI interface in a way that improves light-harvesting efficiency. Future studies will need to address whether the close coupling observed in this study is the result of a static, unregulatable arrangement of auxiliary antenna proteins, or *Galdieria* possesses other means (besides traditional state transitions) of adapting its light-harvesting machinery to variable light conditions. As more photosynthetic extremophiles are characterized in detail, we will surely learn more about the adaptive diversity that exists alongside the remarkable universality of oxygenic photosynthesis.

**SUPPORTING MATERIAL**

Eight figures and four tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)01261-0.

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