Biochimica et Biophysica Acta 1857 (2016) 1514–1523

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Excitation dynamics and structural implication of the stress-related complex LHCSR3 from the green alga *Chlamydomonas reinhardtii*

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ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 28 April 2016
Accepted 30 April 2016
Available online 2 May 2016

Keywords:
LHCSR3
Chlamydomonas reinhardtii
Light-Harvesting Complex
Redfield model
transient absorption

ABSTRACT

LHCSR3 is a member of the Light-Harvesting Complexes (LHC) family, which is mainly composed of pigment-protein complexes responsible for collecting photons during the first steps of photosynthesis. Unlike related LHCs, LHCSR3 is expressed in stress conditions and has been shown to be essential for the fast component of photoprotection, non-photochemical quenching (NPQ), in the green alga *Chlamydomonas reinhardtii*. In plants, which do not possess LHCSR homologs, NPQ is triggered by the PSBS protein. Both PSBS and LHCSR3 possess the ability to sense pH changes but, unlike PSBS, LHCSR3 binds multiple pigments. In this work we have analyzed the properties of the pigments bound to LHCSR3 and their excited state dynamics. The data show efficient excitation energy transfer between pigments with rates similar to those observed for the other LHCs. Application of an exciton model based on a template of LHCII, the most abundant LHC, satisfactorily explains the collected steady state and time-resolved spectroscopic data, indicating that LHCSR3 has a LHC-like molecular architecture, although it probably binds less pigments. The model suggests that most of the chlorophylls have similar energy and interactions as in LHCII. The most striking difference is the localization of the lowest energy state, which is not on the Chlorophyll a (Chl a) 610-611-612 triplet as in all the LHCB antennas, but on Chl a613, which is located close to the lumen and to the pH-sensing region of the protein.

1. Introduction

Rapid changes in sunlight quality and quantity are everyday occurrences for plants and algae [1]. The success of photosynthesis relies on the ability of these organisms to quickly and reversibly adapt to the changing conditions [2]. Accordingly, all photosynthetic organisms of the green lineage have evolved strategies to deal with the rapid light changes occurring in their environment.

During the first steps of photosynthesis, plants and green algae employ various Light Harvesting complexes (LHCs) to capture solar photons and transfer the excitation energy to the reaction centers of Photosystems I and II (PSI and PSII) where charge separation takes place (for reviews see [3,4]). LHC antenna complexes consist of three transmembrane helices, and bind each up to 15 chlorophylls a/b (Chls a/b) and 4 carotenoids (lutein (lut), violaxanthin (vio) and neoxanthin (neo)) [5].

A sub-group of LHCs are expressed in stress related conditions. These include early light-induced proteins, ELIPs (plants, mosses and algae) [6–8], and LI818 proteins, also known as LHCSR (mosses and algae) [9, 10], which are related to the LHCX complexes found in diatoms [11]. LHCSR3 protein expressed in the green alga *C. reinhardtii* [10] has been shown to trigger a complex process known as non-photochemical quenching (NPQ) [12], which dissipates a large part of the absorbed energy as heat. In plants, NPQ activation depends on PSBS [13], which is constitutively expressed, while in mosses, intermediates in the evolution between green algae and vascular plants, both PSBS and a homolog of LHCSR are employed [9]. These proteins are responsible for the fastest component of NPQ, called qE, during which the fluorescence quantum yield of the LHC antennae is significantly reduced within seconds, alleviating the potential oxidative stress caused by high light exposure [2]. While the exact molecular quenching mechanism is still a matter of debate [14] it has been proposed that both PSBS and LHCSR3 "sense" pH changes in the thylakoid lumen which correlate with photosynthetic activity, and switch conformation, activating (at low pH) or de-activating (at high pH) the quenching processes [15–17].

However, PSBS and LHCSR3 have several different properties. PSBS does not bind pigments [18,19] while LHCSR3 does [16,20], and while the first is constitutively expressed in plants, the latter is only expressed upon exposure of *C. reinhardtii* to a variety of stress conditions [10,21]. Additionally, a very short fluorescence lifetime component (below 100 ps) probed in vitro in reconstituted LHCSR3 complexes supported the hypothesis that LHCSR3 is in a default quenched conformation and it becomes even more quenched at low pH [20]. These observations

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http://dx.doi.org/10.1016/j.bbabio.2016.04.285
0005-2728/

have led to the suggestion that LHCSR3 is not involved in light harvesting and its bound pigments are only instrumental for the energy quenching [10,20,21].

However, in our previous work [16] we have shown that LHCSR3 has an excited state lifetime of 2 ns at neutral pH, compatible with the fluorescence lifetime of an antenna, and it becomes shorter only after acidification, in contrast to the earlier reports [20]. Similar conclusions were reached by measuring the fluorescence lifetime of PSII-LHCSR3 supercomplexes at different pHs [17].

Structural information on LHCSR3 is currently missing, preventing a direct comparison between the architecture of LHCSR3 to other antenna complexes such as LHCII [22], CP29 [23], and the unpigmented NPQ trigger PSBS in plants [19].

In this work, by combining ultrafast spectroscopy and modeling, we propose a model for the functional organization and the excited state dynamics in LHCSR3. Excitation energy transfer between the individual pigments is very sensitive to their spatial organization and, together with the excitonic dynamics, gives information about the structural organization of the complex. The model provides information on binding sites and pigment organization and can guide future mutagenesis studies aimed at understanding the role of this complex in photosynthesis and photoprotection in the model organism *Chlamydomonas reinhardtii*.

2. Materials and methods

2.1. Sample preparation

Recombinant LHCSR3 from *C. reinhardtii* (with a His6 tail) was overexpressed in *Escherichia Coli*. The resulting inclusion bodies were then reconstituted with pigments following the protocol described in [16,24]. The pigment composition was determined by fitting with the individual pigment forms the absorption spectra recorded after 80% acetone-pigment extraction and via HPLC analysis (see Table 1) [25]. Results of the pigment analysis are listed below in Table 1. For all of the measurements the sample was diluted to the required OD in a buffer consisting of HEPES (pH 7.6), 0.5 M sucrose and 0.06% beta-DM.

2.2. Sequence homology modeling

Primary sequence alignment of LHCII, CP29 (*Arabidopsis thaliana*) and LHCSR3 (*C. reinhardtii*) was done with ClustalW2 [26]. Sequence homology modeling aligning LHCSR3 against the LHCII structure-template [22] was run on SWISS-MODEL [27].

2.3. Steady-state spectra

Absorption spectra were acquired on a Varian Cary 4000 UV–Vis-spectrophotometer. Fluorescence emission and excitation spectra were acquired at OD << 0.1 cm^{-1} on a HORIBA Jobin-Yvon FluoroLog-3 spectrofluorometer. CD spectra were recorded on a Chirascan CD Spectrophotometer (Applied Photophysics) at a temperature of 10 °C. All the other steady-state spectra were recorded at room temperature.

2.4. Femtosecond transient absorption and global analysis

Transient absorption spectroscopy experiments were run on a Coherent Ti:Sa MIRA seed (mode-locked oscillator) and Rega 9050 (regenerative amplifier) system described elsewhere [28], adapted to a repetition rate of 20 kHz. Briefly, mode-locked 800 nm-seed pulses (Coherent-MIRA seed) were first stretched to be amplified (Coherent-Rega 9050) and then compressed to a pulse width of ~80 fs (autocorrelated). The beam was then split with a 60%/40% ratio of the initial power respectively to the pump and probe beam-pathways. Depending on the experiment, pump was tuned to 489, 652, 662 and 672 nm by means of optical parametric amplification (Coherent OPA 9400) and the bandwidth of the tuned pump-pulse was further reduced to a FWHM of 10 nm via interference filters (THORLABS) centered at the above mentioned wavelengths. White-light continuum for the probe was generated after focusing the 800 nm-probe beam into a sapphire crystal. Probe light was then dispersed and collected on a 76-channels photodiode array, overall covering a ~ 130 nm-spectral window. Pump beam-pathway was deviated to a delay line (up to 3.5 ns) before hitting the sample.

Energy per pulse was adjusted to 5 nJ/pulse (489, 652 and 662 nm) and 3 nJ/pulse (672 nm). A power study at 662 nm excluded the possibility of annihilation effects at the power used for the measurements (See Fig. S5). OD of the sample was adjusted to ~0.6 mm^{-1} in a 1 mm-Quartz cuvette. Oxygen scavenging system was used for all the measurements to prevent sample degradation [29]. The sample-cuvette was shaken throughout the measurement.

Per each different excitation wavelength, raw 2 dimensional (time and wavelength) TA data were then subjected to global analysis with an unbranched sequential scheme [30]. A total of 4 (chl excitations) or 6 (cars excitation) compartments exponentially evolve into the next one (e.g. 1 → 2 → 3 → 4). From this analysis we retrieved the spectrum corresponding to each compartment, Evolutionary Associated Difference Spectrum (EADS) and the time constant associated to the decay [30]. Two scans per excitation wavelength were acquired and averaged before global analysis. Analysis were run on Glotaran 1.5.1 software [31]. Global analysis of the TA traces acquired upon cars excitation (489 nm) was made by fitting simultaneously spectrally-dispersed traces belonging to two distinct and partially overlapping data-acquisition windows. One window was centered in the Soret absorption region (window range ~ 470–599 nm) and the other one in the chlorophyll Q_X/Q_Y region (window range ~584–713 nm). The instrument response function (IRF) for the experiment was estimated via global analysis, and ranged between 126 (662 nm excitation) and 145 fs (652 nm excitation).

2.5. Exciton model

Modeling of the linear and TA spectra of LHCSR3 was done following a protocol developed previously [32]. Modeling of the linear spectra used the modified Redfield theory in a pure exciton basis. To model the TA kinetics, we used the combined Förster–Redfield theory, where relaxation within stromal-side (Chls a602-a603, a610-a611-a612, b609) and luminal-side (Chls a604, a613) compartments was calculated with modified Redfield, while the stromal-to-lumenal transfers were modeled with generalized Förster theory. Note that the luminal-side (Chls a604-a613) transfer rates are the same in the Redfield and Förster pictures (due to big energy gap between the a604 and a613 pigments).

In the modified Redfield, the pulse duration is supposed to be less than the time constants of the modeled kinetics. In other words we neglect the dynamics during the pump and probe pulses. But the calculated TA response is still dependent on overlap of the pulse spectrum and the spectra of the exciton bands of the LHC. In the calculation we suppose a pulse duration of 120 fs, but this is not critical, i.e. variation within 80–140 fs will not change significantly the results (because we model the kinetic components of 150–200 fs and longer).

The spectral density of exciton-phonon coupling is constricted as a sum of overdamped Brownian oscillator (with dumping constant $\gamma = 30\ cm^{-1}$ and coupling strength $\lambda = 40\ cm^{-1}$) and 48 high-frequency underdumped vibrational modes with the total Huang-Rhys factor of $S = 0.84$ for Chl a [32]. The frequencies and relative amplitudes of the

Table 1
Pigment binding properties of recombinant LHCSR3 as from 80% acetone extraction (1) or HPLC analysis (2).

	Chl a/b	Chls/Cars	Chl a + Chl b	Chl a	Chl b	lut	vio	neo
LHCSR3	5.5[1]	3.8[1]	8	6.8	1.2	39%[1] 35%[2]	61%[1] 55%[2]	0%[1] 10%[2]