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Energy transfer in an LH4-like light harvesting complex from the aerobic purple photosynthetic bacterium *Roseobacter denitrificans*

Dariusz M. Niedzwiedzki [a,*], Marcel Fuciman [d], Harry A. Frank [d], Robert E. Blankenship [a,b,c]

[a] Photosynthetic Antenna Research Center, Campus Box 1138, Washington University in St. Louis, St. Louis, MO 63130, USA
[b] Department of Biology, Washington University in St. Louis, MO 63130, USA
[c] Department of Chemistry, Washington University in St. Louis, MO 63130, USA
[d] Department of Chemistry, University of Connecticut, U-3060, 55 North Eagleville Road, Storrs, CT 06269–3060, USA

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ABSTRACT

A peripheral light-harvesting complex from the aerobic purple bacterium *Roseobacter (R.) denitrificans* was purified and its photophysical properties characterized. The complex contains two types of pigments, bacteriochlorophyll (BChl) *a* and the carotenoid (Car) spheroidenone and possesses unique spectroscopic properties. It appears to lack the B850 bacteriochlorophyll *a* Q_y band that is typical for similar light-harvesting complex 2 antennas. Circular dichroism and low temperature steady-state absorption spectroscopy revealed that the B850 band is present but is shifted significantly to shorter wavelengths and overlaps with the B800 band at room temperature. Such a spectral signature classifies this protein as a member of the light-harvesting complex 4 class of peripheral light-harvesting complexes, along with the previously known light-harvesting complex 4 from *Rhodopseudomonas palustris*. The influence of the spectral change on the light-harvesting ability was studied using steady-state absorption, fluorescence, circular dichroism, femtosecond and microsecond time-resolved absorption and time-resolved fluorescence spectroscopies. The results were compared to the properties of the similar (in pigment composition) light-harvesting complex 2 from aerobically grown *Rhodobacter sphaeroides* and are understood within the context of shared similarities and differences and the putative influence of the pigments on the protein structure and its properties.

1. Introduction

Light-harvesting complexes (LHC) are essential elements of the photosynthetic apparatus in all photosynthetic organisms. LHCs absorb incident light and transfer the excitation energy to the reaction center where it is converted into chemical energy in a series of electron-transfer steps. Despite having the same functionality among many different photosynthetic organisms, LHCs are not structurally unified and diverge into many subgroups having different structural properties and pigment composition [1].

Purple bacteria produce two general types of LHCs, the light harvesting complex 1 (LH1), closely associated with the reaction center (RC) and light-harvesting complex 2 (LH2), peripherally arranged around LH1-RC. The structure of the LH2 antenna complex has been determined for *Rhodopseudomonas (Rps.) acidophila* strain 10050 and *Phaeospirillum (Phs.) molischianum* bacterial species using X-ray crystallography. Both complexes were determined to ~2.5 Å resolution; however the LH2 from *Rps. acidophila* was later improved to 2.0 Å [2–5]. The LH2 complex from *Rps. acidophila* forms a circular structure consisting of nine identical protein subunits formed by heterodimers of polypeptide chains denoted α and β oriented across the photosynthetic membrane. The protein subunits form a double ring with inner and outer diameters of 36 Å and 68 Å. Each of the subunits accommodates three bacteriochlorophylls *a* (BChl *a*) molecules and one Car, rhodopin glucoside. Rhodopin glucoside spans the space between the α and β polypeptide pairs and is in close contact with a pair of the strongly coupled BChl *a* molecules (B850). The B850 BChls are oriented perpendicular to the membrane plane and form a ring of 9 pairs of BChl molecules. The minimum distances between B850 BChls are 3.66 Å within dimers and 3.74 Å between molecules from neighboring dimers. The monomeric BChl *a* molecules (B800) are oriented parallel with the membrane plane and positioned between β–polypeptides [2–4]. The LH2 complex from *Phs. molischianum* has a similar circular structure with eight α/β subunits forming inner and outer rings with dimensions of 31 Å and 62 Å with 8 dimers of B850 BChl pigments and 8 monomeric B800 BChls. Different structural packing in both complexes affects the distance of closest approach between the B850 BChls which are 3.54 Å (within dimer) and 3.63 Å (between pairs). The LH2 complex from *Phs. molischianum* contains also 8 lycopene molecules. Lycopene is a carotenoid that adopts in the LH2 complex of *Phs. molischianum* a similar orientation as rhodopin glucoside in the *Rps. acidophila* LH2 complex despite its different structure [5].

* Corresponding author. Tel.: +1 314 935 8483; fax +1 314 935 4925.
E-mail address: niedzwiedzki@wustl.edu (D.M. Niedzwiedzki).

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The spectroscopic properties of the LH2 antenna complexes are determined by their structure. The Q_y absorption band of BChls absorbing at 800 nm originates from the monomeric, widely-spaced (21 Å between Mg atoms) B800 BChls, while the band appearing at 850 nm is associated with the closely-spaced ring (9 Å between Mg atoms) of the strongly coupled dimeric B850 molecules. Specific spacing, contiguity of the molecules in the rings, and alignment of the transition dipole moments lead to excitonic coupling that shifts absorption of both molecular arrangements to longer wavelength to different degrees compared to the absorption of individual BChl *a* molecules.

Several purple bacteria species express "anomalous" peripheral light harvesting antenna complexes under stressed conditions such as low light or low temperature. Complexes discovered in *Rps. acidophila* strain 7050 and in *Rps. cryptolactis* have the B850 band blue shifted to 820 nm. Due to its spectroscopic character, this complex is referred to as B800–B820 or LH3. Its crystal structure has been determined with a resolution of 3.0 Å for *Rps. acidophila* strain 7050 and revealed only very small differences in comparison to the "standard" LH2 structure from strain 10050 [6]. It was concluded that the observed blue-shift of the BChl bands is caused by minor differences in the primary structure of the apoprotein which affects the hydrogen bonds (H-bonds) between BChls and the protein [6]. A spectrally similar complex B800–B830 was also found in *Chromatium (Chr.) purpuratum* [7]. In response to low light conditions, another purple bacterium, *Chr. vinosum* produces a complex with a suppressed B850 band in its absorption spectrum and is also called the B800 complex [8]. Similarly, *Rhodopseudomonas palustris* expresses a complex with a greatly reduced B850 band under low light condition. This complex has been called both B800 and LH4. A high-resolution crystal structure of this LH4 is still not available; however, a 7.5 Å resolution density map was published by Hartigan et al. [9]. Such resolution is too low to draw definitive conclusions about pigment organization in the structure. Nevertheless, using a combination of spectroscopic and biochemical methods, the authors concluded that LH4 is built from eight α/β peptide pairs and each subunit contains four BChls *a* (32 in total) in contrast to three in the "standard" LH2 [9].

In this work, we present studies on the low-light peripheral light-harvesting complex from the purple aerobic anoxygenic phototrophic bacterium *Roseobacter (R.) denitrificans* (known also as *Erythrobacter sp.* strain OCh114), as a candidate for another LH4 complex. This complex was initially described by Shimada et al. in the 1990s as a B806 complex due to the BChl Q_y band absorbance at that wavelength. Since then, only a very basic spectroscopic characterization of this protein has been performed [10,11]. We used several different optical spectroscopic techniques including steady state absorption, fluorescence, circular dichroism, femtosecond and microsecond time-resolved transient absorption, and nanosecond time-resolved fluorescence at both room and cryogenic temperatures to reveal how these spectroscopic properties influence light harvesting and excitation transfer in this unusual complex.

2. Materials and methods

2.1. Cell growth

R. denitrificans strain OCh114 was grown under aerobic conditions on Difco Marine Broth 2216 media. A small volume of liquid culture was used to inoculate 1 L media, split into 4 equal flasks, which were closed with foam stoppers. The cultures were placed in the dark, in an incubator at 30 °C and continuously shaken. The cells were harvested after 7 days of growth when they were assumed to be in a stationary stage.

2.2. Preparation of the peripheral LH antenna complex

The cells were resuspended in 20 mM Tris buffer (pH = 8.0) and the membranes were released by ultrasonication process and then pelleted by centrifugation at 250,000*g* for 2 h. Subsequently, the pellet was resuspended in 20 mM Tris buffer (pH = 8.0) ($OD_{800nm} \approx 20$) and mixed with lauryldimethylamine-oxide (LDAO) to a final concentration of 1% for 20 minutes at 4 °C. The mixture was then centrifuged at 250,000*g* for 1 h to separate insoluble material. Further purification of the complexes was carried out using an anion exchange chromatographic column (Q Sepharose High Performance, GE Healthcare) equilibrated with 20 mM Tris–HCl buffer (pH 8) with 0.1% LDAO by applying a linear gradient of NaCl between 0 and 500 mM. The protein-containing fraction eluted with 250–300 mM NaCl. For low temperature measurements, the sample was resuspended in a 50:50 (vol./vol.) glycerol:buffer solution.

2.3. Steady-state absorption, fluorescence and CD spectroscopy

Room temperature steady-state absorption spectra were recorded using a Perkin Elmer Lambda 950 spectrophotometer. Room temperature fluorescence emission and fluorescence excitation spectra were recorded using a Photon Technology International fluorometer. The 10 K absorption spectrum was recorded using Varian Cary-50 spectrophotometer equipped with a liquid helium cryostat Janis-100. The 10 K fluorescence emission and fluorescence excitation spectra were recorded using a Fluorolog-3 fluorometer from Jobin-Yvon Horiba equipped with the same cryostat. In all cases, fluorescence was monitored at a right-angle relative to the excitation. Excitation and emission monochromator slits were set to a bandpass of 4 nm. Fluorescence excitation spectra were corrected using a calibrated reference diode. Circular dichroism was measured using a Jasco 815 CD spectrometer at room temperature with 1 nm spectral resolution.

2.4. Time-correlated single-photon-counting spectroscopy

Fluorescence lifetime measurements were done using a time-correlated single-photon-counting (TCSPC) system of a Jobin-Yvon Horiba Fluorolog 3 equipped with a Single Photon Counting Controller FluoroHub 2.0 (J-Y Horiba) and a pulsed NanoLed-370 diode (as excitation light source), with excitation at 370 nm and a pulse duration less than 1.3 ns. The sample emission was monitored at the maximum of the emission peak.

2.5. Femtosecond time-resolved transient absorption spectroscopy

Transient pump–probe absorption experiments were carried out using Helios, a femtosecond transient absorption spectrometer (Ultrafast Systems, LCC), coupled to a femtosecond laser system previously described in detail [12,13]. The system is based on a Spitfire-50 fs, Ti:sapphire amplifier with pulse stretcher and compressor (Spectra-Physics) pumped at 1 kHz repetition rate by Evolution 15, Q-switched Nd:YLF laser (Coherent) and seeded by pulses from Tsunami, mode-locked Ti:sapphire oscillator (Spectra-Physics), that is pumped by Millenia VsJ, diode-pumped Nd:YVO_4 CW visible laser (Spectra Physics). Output pulses, with center wavelength of 800 nm, energy of 600 μJ/pulse, ~50 fs duration, and 1 kHz repetition were split into two beams by a beam splitter. Ninety percent of the signal was sent to OPA-800C optical parametric amplifier (Spectra-Physics) to generate a pump beam. The remaining 10% was used to derive probe pulses. A white light continuum probe 450–800 nm in the visible region (VIS), and 850–1450 nm in the near infrared (NIR) was generated by a 3 mm Sapphire plate. A charge-coupled detector S2000 with a 2048 pixel array from Ocean Optics was used as a detector in the VIS range. In the NIR, a 512 pixel array SU-LDV high resolution InGaAs Digital Line Camera from Sensors Unlimited was used. The pump and probe beams were overlapped at the sample at the magic-angle (54.7°) polarization. The signals were averaged over 5 seconds. The samples were pumped into the 0–0 vibronic band of the $S_0(1^1A_g^-) \rightarrow S_2(1^1B_u^+)$ steady-state absorption