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Spectroscopic studies of two spectral variants of light-harvesting complex 2 (LH2) from the photosynthetic purple sulfur bacterium *Allochromatium vinosum*

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ABSTRACT

Two spectral forms of the peripheral light-harvesting complex (LH2) from the purple sulfur photosynthetic bacterium *Allochromatium vinosum* were purified and their photophysical properties characterized. The complexes contain bacteriochlorophyll *a* (BChl *a*) and multiple species of carotenoids. The composition of carotenoids depends on the light conditions applied during growth of the cultures. In addition, LH2 grown under high light has a noticeable split of the B800 absorption band. The influence of the change of carotenoid distribution as well as the spectral change of the excitonic absorption of the bacteriochlorophylls on the light-harvesting ability was studied using steady-state absorption, fluorescence and femtosecond time-resolved absorption at 77 K. The results demonstrate that the change of the distribution of the carotenoids when cells were grown at low light adapts the absorptive properties of the complex to the light conditions and maintains maximum photon-capture performance. In addition, an explanation for the origin of the enigmatic split of the B800 absorption band is provided. This spectral splitting is also observed in LH2 complexes from other photosynthetic sulfur purple bacterial species. According to results obtained from transient absorption spectroscopy, the B800 band split originates from two spectral forms of the associated BChl *a* monomeric molecules bound within the same complex.

1. Introduction

Light harvesting complex 2 (LH2) is one of the two types of transmembrane photosynthetic antenna complexes present in the photosynthetic apparatus in purple bacteria. To date, only two high-resolution structures of the LH2 antenna complex have been determined using X-ray crystallography. These were isolated from two non-sulfur purple bacterial species: *Rhodopseudomonas (Rps.) acidophila* strain 10050 and *Phaeospirillum (Phs.) molischianum*. The complexes were determined to ~2.5 Å resolution, though the LH2 from *Rps. acidophila* was later improved to 2.0 Å [1–4]. These structures show that the complexes form circular aggregates consisting of eight (*Phs. molischianum*) or nine (*Rps. acidophila*) identical protein subunits formed by heterodimers of polypeptide chains, denoted α and β with transmembrane orientation. The α/β subunits form a double ring with inner and outer diameters of 36 Å and 68 Å for *Rps. acidophila,* respectively, and 31 Å and 62 Å for *Phs. molischianum*. Each protein subunit binds three bacteriochlorophylls *a* (BChl *a*). The strongly coupled BChl *a* molecules have their bacteriochlorin rings oriented perpendicular to the membrane plane and interact excitonically producing a combined Q_y transition in the vicinity of 850 nm. They form a ring with closest distances between molecules of 3.66 Å for *Rps. acidophila* and 3.54 Å for *Phs. molischianum* in the dimers (a pair of B850 bacteriochlorophylls from α and β subunits) and 3.74 Å and 3.63 Å, respectively, between molecules from neighboring dimers. The monomeric BChl *a* molecules (B800) are oriented so that their tetrapyrole rings lie parallel with the membrane plane and are positioned between β–polypeptides [1–3]. In complexes from both species, each α/β subunit contains also one carotenoid. This is rhodopin glucoside in *Rps. acidophila* and lycopene in *Phs. molischianum*. Due to the same number of conjugated carbon-carbon double bonds in the backbone these carotenoids possess similar spectroscopic properties regardless of the differences in the terminal substituents [5].

In contrast, very little structural information is available for LH2 complexes from purple sulfur bacteria due to partial or incomplete information about primary sequences of their LH2 peptides, as well as difficulties in growing many types of these bacterial cultures. Recently, some progress with structural studies on these complexes was achieved using electron microscopy (EM) [6]. The EM of single LH2 complexes from

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Allochromatium (Alc.) vinosum (formerly *Chromatium vinosum*) revealed that the complex adopts two circular forms, the dominant form built from 13 α/β subunits and a minor form that was shown to consist between 10 and 12 α/β subunits. Based on these statistical projections the outer ring size of the LH2 may vary from 68 to 115 Å [6]. Despite the fact that EM revealed at least two structural forms of the LH2 it was not clear from preparative point of view whether that sample contained more than one type of LH2 complex or not. Nevertheless it is clear that LH2 complexes from sulfur purple bacteria may have different ring sizes compared to their "non-sulfur" counterparts. These structural differences are also reflected in spectroscopic properties. The steady-state electronic absorption spectrum of LH2 complexes from some purple sulfur bacteria grown under normal light conditions show an abnormally broad B800 band, not observed in LH2 complexes from non-sulfur species. Moreover, upon lowering the temperature the B800 band splits into two distinct peaks separated by ~15 nm with almost equal amplitudes [7,8].

Different explanations have been offered for the B800 band splitting. One assumes that the sample is actually a mixture of two spectrally slightly different LH2 complexes that are impossible to separate by the normal biochemical purification procedures [7]. The second hypothesis supported by polarized time-resolved absorption spectroscopy presumes that this LH2 contains two sets of B800 molecules, equivalently distributed within the individual LH2 complex [9]. A possible third hypothesis is that the B800-type BChl *a* molecules are organized as excitonically coupled molecules that give rise to a splitting of the absorption band. The equal intensities of the two absorption bands around 800 nm would indicate, in this model, that the oscillator strength is evenly distributed between two exciton components of the coupled monomers [10].

Bacteria that produce LH2 with a split B800 band like *Alc. vinosum* or *Thermochromatium (Tch.) tepidum* express multiple types of α-and β-apoproteins [11]. Under such circumstances, it is easy to imagine that individual LH2 rings with an inhomogeneous polypeptide composition could result in the second scenario mentioned above. Moreover, if the LH2 could be composed from randomly assembled multiple types of α/β subunits, the spectral properties of the B800-type BChls may differ even within the same LH2 complex.

In order to obtain a definitive answer to this issue we applied femtosecond time-resolved transient absorption spectroscopy at 77 K on these two spectral forms of the LH2 complex from *Alc. vinosum*. One of the forms denoted as Thio-HL (high light) contains the characteristic split B800 band at 77 K, the second form denoted as Sulph-LL (low light) has typical "non-sulfur" character with one sharp B800 peak. In addition, both complexes have different absorption spectra in the region where the carotenoids absorb. We also evaluated how changes in the carotenoid composition affect the path of energy flow in these antenna complexes.

2. Materials and methods

2.1. Bacteria growth and LH2 complex preparation

The cells were grown anaerobically in the light in the presence of either sodium sulfide or sodium thiosulfate as the sources of reduced sulfur [12,13]. The cells grown at high light (220 lux, Osram incandescent 150 watt bulbs) with sodium thiosulphate at 30 °C produced the LH2 with the double B800 band while those grown with sodium sulphide at low light (10 lux, Osram incandescent 60 watt bulbs) and at 30 °C produced the LH2 complex with a single B800 peak. After growth, the cells were harvested by centrifugation, resuspended in 20 mM Tris HCl pH 8.0 and broken by passage through a French Press at 10 tons/sq. inch in the presence of a little DNAse. The chromatophore membranes were then pelleted by centrifugation and resuspended in 20 mM Tris HCl buffer pH 8.0 to an OD at 850 nm of 50. The membranes were then solubilized with the addition of 2% DDM (n-Dodecyl-beta-D-maltopyranoside) for 4 hours while stirring at room temperature. Any unsolubilized material was then removed by a brief centrifugation and the LH complexes fractioned by sucrose density centrifugation for 16 hours at 150,000*g* at 4 °C. The sucrose step gradient was made up of 0.6, 0.8, 1.0 and 1.2 M sucrose steps in 20 mM Tris HCl buffer with 0.02% DDM. The LH2 complexes where removed from the gradients and further purified by a combination of ion exchange and molecular sieve chromatography.

2.2. Carotenoid separation and purification

Carotenoids were extracted by applying a mixture of 1:1 (v/v) acetone:methanol to concentrated LH2 complexes. Carotenoids were separated from the extract by adding small volume of *n*-hexane and partitioning with water. The *n*-hexane layer containing carotenoids was then injected to an Agilent 1100 HPLC system employing a LiChrosorb Silica normal phase column (ES Industries) (250 mm × 4.6 mm) with isocratic flow rate of 1.5 mL/min and *n*-hexane:tetrahydrofuran (90:10, v/v) as a mobile phase. The chromatograms were monitored at wavelengths corresponding to the (0-0) vibronic band of the carotenoids steady-state absorption observed in the applied solvent mixture.

2.3. Steady-state absorption and fluorescence spectroscopy

The samples of the LH2 were diluted in 20 mM Tris-HCl buffer, pH 8 with 0.02% DDM in order to perform room temperature (RT) measurements. For 77 K measurements buffer containing 57% glycerol (v/v) in 20 mM Tris–HCl (pH 8) with 0.02% DDM was used. Low temperature experiments were performed using OptistatDN cryostat (Oxford Instruments, UK). Steady-state absorption and fluorescence spectra were recorded in 1 cm path length cuvettes using a Perkin-Elmer Lambda 950 spectrometer and a Photon Technology International fluorimeter, respectively. Fluorescence was detected at a right angle with respect to the excitation beam and corrected for the instrument response. The sample OD was adjusted to 0.1 at the maximum of the B850 absorption band. Fluorescence excitation spectra were corrected for instrument excitation characteristics using a calibrated reference photodiode. The bandpass for the fluorescence measurements was set to 4 nm.

2.4. Femtosecond time-resolved absorption spectroscopy

Time-resolved pump-probe absorption experiments were carried out using Helios, a femtosecond transient absorption spectrometer from Ultrafast Systems, LCC coupled to a femtosecond laser system. It consists of Solstice, a one box ultrafast amplifier (Spectra-Physics) built of Spitfire Pro XP, a Ti:sapphire regenerative amplifier with a pulse stretcher and compressor, Mai-Tai, a femtosecond oscillator as seeding source and Empower, a diode-pumped solid state pulsed green laser as the pump source. The system produces pulses centered at 800 nm with energy of ~3.5 mJ, ~90 fs duration and 1 kHz repetition rate. Ninety percent of the output beam was used to generate a pump beam in Topas-C, an optical parametric amplifier equipped with Berek extension (Light Conversion Ltd, Lithuania). The remaining 10% was used to produce probe pulses in the Helios spectrometer. A white light continuum probe in the visible (VIS) region was generated by a 3 mm thickness Sapphire plate. A near infra-red (NIR) probe was generated using 10 mm thickness proprietary crystalloid rod. A complementary metal–oxide–semiconductor (CMOS) linear sensor with 1024 pixels was used as a detector in the VIS range and a 256 pixel InGaAs linear diode array in the NIR. To provide an isotropic excitation of the sample and avoid pump-probe polarization effects the pump beam was depolarized. For carotenoids in 2-MTHF the energy of the pump beam was set to 1 μJ with a spot size of 1 mm diameter, corresponding to intensity between 3.4 and 3.5×10^{14} photons/cm^2 depending on the exact excitation wavelength. In order to minimize excited state annihilation processes in the LH2, the energy of the pump was kept between 0.06