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Research paper

Orientation assignment of LH2 and LH1-RC complexes from *Thermochromatium tepidum* reconstituted in PC liposome and their ultrafast excitation dynamics comparison between in artificial and in natural chromatophores

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ABSTRACT

In this study, the artificial chromatophore was constructed by reconstitution of light-harvesting complex 2 (LH2) and core-complex (LH1-RC) from *Thermochromatium tepidum* in egg phosphatidylcholine (PC) liposome. By calcium ion dependent absorption changes, the orientations of protein complexes in liposomes solely were assigned. Ultrafast EET dynamics were studied by *fs* time-resolved absorption spectroscopy on both the artificial and natural chromatophores at their RC 'open' and 'close' states, respectively. The results indicated that these two membrane systems have similar EET dynamic profiles, and the orientations of LH2 and LH1-RC complexes in artificial chromatophores were opposite to those in natural chromatophores.

1. Introduction

The light-absorbing pigments in purple photosynthetic bacteria are organized into two transmembrane pigment-protein complexes, i.e. the peripheral LH2 complexes and the core LH1-RC complexes. In most species, the light energy is initially captured by LH2 and is then transferred *via* LH1 to the RC.

X-ray crystallography has revealed the high-resolution structures of RC [1,2], LH2 [3,4], and LH1-RC [5,6] from various species, while the organization and architecture of supramolecules in native and reconstituted photosynthetic membranes have been studied extensively by atomic force microscopy (AFM) [7–22] and cryo-transmission electron microscopy (cryo-TEM) [23–27], respectively. Usually, the LH2 and LH1-RC complexes form clusters in lipids bilayers. The orientation of pigment-protein complexes in membrane mainly detected by AFM, e.g. Simon Scheuring et al. found reconstituted LH2 from *Rubrivivax gelatinosus* incorporated in egg phosphatidylcholine (PC) bilayers in both orientations [8]. By droplet transfer method, Emiliano Altamura et al. prepared highly oriented photosynthetic Reaction Center (RC) reconstituted in membranes and the orientation assay was performed by biochemical method [28].

Thermochromatium tepidum is a species grown in hot springs, where a high concentration of calcium ions has been reported [29,30]. The calcium ions have been demonstrated to enhance the thermostability of its LH1-RC complex and determine the long wavelength absorption maximum (Qy) at 915 nm in LH1, and this Qy band could be mutually transformed between 915 nm and 880 nm by removal or addition of calcium ions [31–34]. Its structural mechanism has been confirmed by the recent 3.0 Å crystal structure [6]. The LH2 complex of this species was also proved to own the Ca^{2+}-dependent absorption response not only in its isolated state [35] but also in reconstituted liposome [36]. The ultrafast EET dynamics of isolated LH1-RC and LH2 [37–39] have been studied in detail.

The ultrafast excitation energy transfer dynamics in isolated complexes have been extensively studied and reviewed [38,40–43]. In contrast, there have been relatively fewer detailed studies on the energy transfer dynamics occurring between LH2 and LH1 complexes in native photosynthetic membranes [44–46]. The inter-complexes energy transfer efficiency in reconstituted membrane systems has been studied by fluorescence spectroscopy [21,47]. No ultrafast EET dynamics study on artificial photosynthetic membrane was reported yet.

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To address the orientation assignment of protein complexes and the ultrafast EET dynamics in reconstituted photosynthetic membrane of purple bacteria, the reconstitution of isolated LH2 and LH1-RC from *Tch. tepdium* in PC liposome were performed individually and together in this study. By Ca^{2+} sensitive Qy absorption responses and ultrafast EET dynamics comparison between reconstituted LH2-LH1-RC-liposome and natural chromatophore, the orientations of LH2 and LH1-RC complexes were assigned and discussed.

2. Experimental

2.1. Sample preparation

Tris(hydroxymethyl)aminomethane (>99%) was purchased from NOVON Scientific, hydrochloric acid, $CaCl_2$, and EDTA (both A.R.) were purchased from Beijing Chemical Co., and lauryldimethylamine oxide (LDAO, 30%) from Kao Corp. *n*-Dodecyl-β-D-maltoside (DDM), *n*-Octyl-β-D-glucopyranoside (β-OG) and dialysis bag (MWCO: 3500) were from Sigma-Aldrich, and egg phosphatidylcholine, PC, from Wako, Japan.

2.1.1. Preparation of chromatophore (natural), LH2 and LH1-RC from Tch. Tepidum

The preparation was the same as previously reported [37,48]. Briefly describes as follows. *Tch. tepidum* was cultured anaerobically at 48 °C for 7 d. The cells were disrupted at 4 °C by ultrasonication, and the obtained chromatophores were suspended in 20 mM Tris-HCl buffer (pH 8.5) at a concentration of $OD_{850\ nm} \sim 50\ cm^{-1}$ (OD: optical density). After being solubilized with 0.35% (v/v, volume fraction) LDAO for 60 min in the dark, the suspension was centrifuged (145,400*g*, 4 °C, 100 min), and the supernatant was collected as crude LH2. The crude LH2 was further purified by using DEAE-cellulose (Whatman DE52) column chromatography in the presence of 0.05% DDM. The LH1-RC complex with LH1-Qy absorption maximum at 915 nm, named as 'intact LH1-RC', was prepared following previously reported procedures [48]. The 'Ca^{2+}-depleted LH1-RC' with Qy band at 884 nm was prepared by adding 200 mM EDTA into the 'intact LH1-RC'.

2.1.2. Preparation of reconstituted LH2 in liposome (LH2-liposome)

Phospholipids PC was dissolved in chloroform to make a 1 $mg{\cdot}mL^{-1}$ stock solution. 3 mL PC stock solution were put into Eppendorf tube, and the solvent was removed by N_2. 2.4 mL of Tris-HCl buffer (20 mM, pH 8.0), denoted as **buffer A** (without Ca^{2+}) or **buffer B** (with 200 mM Ca^{2+}) respectively, and 0.1 mL of 30% LDAO were added in the tube successively. Sonication was performed for PC solubilization, and then 500 μL concentrated LH2 from *Tch. tepidum* ($OD_{798\ nm} = 22.8\ cm^{-1}$) were added. The resulted suspension with 1 $mg{\cdot}mL^{-1}$ PC and 1% LDAO was vortexed for 30 s and then dialyzed against **buffer A** (or **B**) in the dark at 4 °C for 3 times (2 h, 2 h, and 20 h successively). The resulted sample were homogenized by ultrafiltration with filter pore size of 0.22 μm for 10 times to get the final 'LH2-liposome'. The 'LH2-liposome' with various Ca^{2+} conditions were prepared as follows. For the preparation of both inside (*i*) and outside (*o*) of liposomes with Ca^{2+} (denoted as **C1**), all the preparation was performed in **buffer B**. For the preparation of both *i* and *o* of liposomes without Ca^{2+} (**C2**), 200 mM EDTA was added into the LH2 mother solution firstly to remove the bounded Ca^{2+} and then the preparation was performed all in **buffer A**. For the preparation of only *i* of liposome with Ca^{2+} (**C3**), the sample preparation was performed firstly as **C1**, subsequently 200 mM EDTA were added to remove the Ca^{2+} outside of the liposome, and then dialysis against **buffer A** were performed. For the preparation of only *o* with Ca^{2+} (**C4**), 200 mM EDTA was added into the LH2 mother solution firstly to remove the bounded Ca^{2+} and the preparation was performed in buffer A. Additional dialysis against buffer B was performed twice (12 h for each time).

2.1.3. Preparation of reconstituted LH1-RC in liposome (LH1-RC-liposome)

The same amount of PC was prepared as described in Section 2.1.2. 2.5 mL of 20 mM Tris-HCl buffer (pH 8.0) with 0.8% β-OG to solubilize the PC firstly. 500 μL concentrated LH1-RC ($OD_{918\ nm} = 20.6\ cm^{-1}$) from *Tch. tepidum* was added in the tube. After vortexed for 30 s the mixed suspension was dialyzed against various buffers for 3 times at 4 °C in the dark (2 h, 2 h, and 20 h successively). The LH1-RC-liposome with various Ca^{2+} conditions were prepared as follows. For the preparation of **C1**, 'intact LH1-RC' was used, and dialysis was performed against **buffer C** (20 mM Tris-HCl, pH 8.0, 20 mM $CaCl_2$). For the preparation of **C2**, 'Ca^{2+}-depleted LH1-RC' was used and dialysis was performed against buffer A. For the preparation of **C3**, excessive EDTA was added to the preparation of **C1** and the mixture was incubate for 1 h at 4 °C, then dialysis was performed against buffer A to remove the free EDTA. For the preparation of **C4**, 20 mM $CaCl_2$ was added to the preparation of **C2** during the third dialysis.

2.1.4. Preparation of reconstituted LH2-LH1-RC in liposome (artificial chromatophore)

The same amount of PC was prepared as described in Section 2.1.2. 2.5 mL of Tris-HCl buffer (20 mM, pH 8.0, 0.8% β-OG) was added. Sonication was performed for solubilization of PC thoroughly, 500 μL concentrated LH2 and LH1-RC mixture (LH2 to LH1-RC ratio is 3:1) were added, and the resulted mixture was incubated at 4 °C for 30 min. The resulted suspension was dialyzed against **buffer A** for 3 times (2 h, 2 h, and 20 h successively). To prepare the Reaction Center (RC) 'open' state, 20 mM sodium ascorbate and 2 μM phenazine methosulfate were added.

2.2. Spectroscopic measurements

Steady state absorption spectra in the UV-to-near infrared regions were measured with a Cary-50 absorption spectrometer (Varian). Each spectrum was an average of a few independent measurements, which were all performed at room temperature.

Dynamic light scattering (DLS) was measured on Zetasizer Nano ZS90 (Malvern, United Kingdom). The data was an average of 3 times accumulation.

For *fs* time-resolved absorption measurement, a white light continuum probe (400–1350 nm) was generated from a 3 mm thick sapphire plate and was detected by an InGaAs detector (OMA-V, Princeton Instruments) for the NIR region attached to individual imaging spectrographs (SpectraPro 2300i, USA). An optical parametric amplifier (OPA-800 CF-1, Spectra Physics) provided the actinic laser pulses at desired wavelengths (~120 fs, full width at half-maximum, FWHM). The laser system was run at a repetition rate of 333 Hz for NIR region measurement. To improve the signal-to-noise ratio, each transient spectrum was obtained by averaging 500 individual measurements, and the typical detection sensitivity of the differences absorption (ΔOD) was better than 10^{-4}. All measurements were performed at room temperature (298 K).