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Study on the Excited Energy Transfer in Light-harvesting Complex (LH2) of *Rhodobacter sphaeroides*

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A green mutant of *Rhodobacter sphaeroides* 601 was acquired by chemical induction. The blue-shifted of the carotenoid absorption was found in the Light-harvesting complex II (LH2) of the mutant. With the excitation at different wavelength, we observed that the evolution of excited-state dynamics in LH2 of *Rhodobacter sphaeroides* 601. The dynamical traces demonstrate a dominant absorption followed concomitantly by an ultrafast transmission increase and decay with 818nm excitation.

Key words: Photosynthesis, Light-harvesting complex, Cartenoid, Excited-state dynamics

INTRODUCTION

In photosynthesis, photons are first absorbed by light-harvest antenna complex and then transported to reaction center (RC). Purple bacteria contain two types of antenna complex. Core antenna is compactly associated with the RC. Peripheral antenna is arranged at periphery. In both, the light absorbing pigments, bacterochlorophyll a ($Bchl_a$) and carotenoids are noncovalently attached to apoproteins, α and β. In LH2 of *Rhodopseudomonas acidophila*, eighteen $Bchl_a$ are coordinated to conserved His on either α or β apoprotein, forming a continuous ring. $Bchl_a$-850 molecules are named for its absorb at 850nm. Other nine molecules of $Bchl_a$ lying toward the cytoplasmic side constitute a loose ring. These $Bchl_a$ molecules' whose absorption is at 800 nm are named as $Bchl_a$-800. Having an all-trans configuration, the carotenoids are observed to locate among $Bchl_a$ molecules. All-*trans*-carotenoids in LH2 of purple bacterial play an important function of light-harvesting. [1]. Energy absorbed by carotenoids is quickly transferred to both $Bchl_a$-800 and $Bchl_a$-850. From $Bchl_a$-800, energy migrates to $Bchl_a$-850 with a time constant of 0.7ps at room temperature [2]. The exciton concept was applied to the strongly coupled BChl-B850 ring in LH2, in which the excitation is delocalized over a number of pigment molecules [3,4].

In this study, we separated a green mutant with different carotenoid absorb in LH2. LH2 of *R. sphaeroides 601* or green mutant are favorable to be used in comparatively study of energy transfera.

Furthermore, We use the conventional pump-probe technique to observe the excited-state dynamics of LH2 of *R. sphaeroides 601.* Different traces were found with excitation at different wavelength.

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MATERIALS AND METHODS

Bacterial growth and LH2 isolation:

R. sphaeroides mutants were grown anaerobically at 28 ℃ for 3 days with a light intensity of 3000 lux in RCVBN medium[5]. The cells were harvested and suspended with Tris-HCl buffer(5 mmol/L, pH8.0). After cells were disrupted at 0℃ by using a ultrasonifier, the fraction of 30000-48000rpm was collected as chromatophores. Chromatophores were suspended in Tris-HCl buffer(10 mmol/L, pH8.0) with 1%(v/v) LDAO and NaCl(0.1mol/L) for 2 hour in the dark. After solubilization, the suspension was added $(NH_4)_2SO_4$ to a concentration of 0.2-0.4g/ml, Afterwards fractions was collected by using centrifugation of 10000rpm, 30min. The deposition was solubilized of TL buffer(10 mmol/L Tris-HCl, pH8.0, 0.1%LDAO). Salt ion was then removed by dialysis for 20 hr. The final purification was done by the centrifugation in a sucrose density gradient following by chromatography on a DEAE-cellulose(DE52, Whatman) column.

Transient spectroscopy:

LH2 was suspended in Tris-HCl buffer (50 mmol/L pH 8.0) and the optical density was set to be 0.5 at 850 nm in a 1 mm cuvette. The femtosecond pump-probe experiments were performed on a home-built system. A Ti:sapphire laser (Tsunami, Spectral Physics), was pumped by a diode laser. The output pulses had a 80-100 fs duration with a repetition rate of 82 MHz. The wavelength of the output pulses can be tuned from 750 to 870 nm with a 10 nm bandwidth (fwhm) by changing the position of the mode-locked slit across the beam profile. The pump power used in this measurement was less than 0.2 nJ/pulse with a beam diameter of about 50 μm in the beam overla mmi n t he he measurements in this work were carried out at room temperature.

RESULTS AND DISCUSSION

A strain of *R.sphaeroides 601* was grown in RCVBN liquid culture medium. At exponential phase of grown, Tris-HCl (0.1 mol/L, PH7.5 with 1% EMS) was added to the bacterium. After 1 hour, dilution of bacterium was spreaded in plates of LB cultures medium. After the aerobically culture of 3 days in the dark, green color cells were selected to repeat culture. At last a strain whose absorption was changed in the range of 400-550nm was separated as the green mutant.

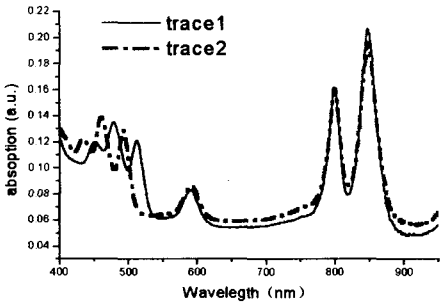

Fig.1.Room-temperature absorption spectra of LH2 of *R. sphaeroides 601* (trace 1) and the green mutant (trace 2).

Fig.1. shows absorption spectra of LH2 of *R. sphaeroides 601* (trace 1) and the green mutant (trace2). They have the same transitions bands of Qx and Qy located at 590, 800 and 850nm. The absorption peaks at 512, 479 and 451nm are attributed to carotenoids in LH2 of *R. sphaeroides 601*. For LH2 of the green mutant, the carotenoid transition peaks are 493, 460 and 434nm.

For long-chain all-*trans* polyenes with ideal C_{2h} symmetry, two excited states of carotenoids are named as $1B_u^+$ and $2A_g^-$ states. Energy states of $1B_u^+$ and $2A_g^-$ states have been confirmed in LH2 of *R.sphaeroides*2.4.1. The state designated as $2A_g^-$ had not been observed in absorption due to the fact that $1A_g^-(S_0) \rightarrow 2A_g^-$ transition is dipole forbidden. While $1A_g^-(S_0) \rightarrow 1B_u^+$ transition is allowed and it should be accounted for the intense absorption band of carotenoids[6,7]. The blue-shifted of the carotenoid absorption bands shows that energy of excited state has been changed. Mechanism of energy transfer in this new green mutant of purple bacteria deserves further study.

The transient dynamics of LH2 of *R. sphaeroides 601* at 800 nm excitation (Fig.2-800nm) demonstrated a ground-

state bleaching. The decay of bleaching of 800 nm excitation has a single component with a 0.7 ps time constant. The component of 0.7 ps represents the energy transfer from B800 to B850 occurring in this time region at room temperature. With the excitation wavelength shifting to the BChl-B850 absorption band, a ground-state bleaching was also showed (Fig.2-848nm).

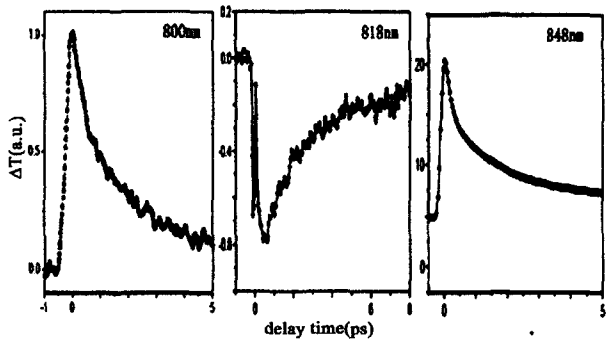

Fig 2. Transient dynamic traces of LH2 of *R. sphaeroides 601* with excitations at 800 nm, 818 nm and 848 nm respectively with parallel pump probe polarization.

Fig.2-818nm shows a different transient dynamics trace of LH2 *R. sphaeroides 601* with a 818 nm excitation. A rapid absorption and an ultrafast transmission increase can be clearly observed at the near-zero time point. This trace can be decomposed into two processes, a decay of absorption with about 4 ps time constant plus a "bleaching" with pulse-width limited time scale. In the BChl-B850 ring, excitation energy probably experiences equilibration for the delocalized exciton coupling. The special trace demonstrates that there probably exists a distribution mechanism to control energy transfer in the BChl-B850 ring before energy is transferred to LH1. The origin of the trace is complex that it is needed further study in the future.

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