Effect of Photo-Oxidation on Energy Transfer in Light Harvesting Complex (LH2) from Rhodobacter Sphaeroides 601 *

LIU Kang-Jun(刘康俊)[1,2], LIU Wei-Min(刘伟民)[1], YAN Yong-Li(闫永丽)[1], DONG Zhi-Wei(董志伟)[1], LIU Yuan(刘源)[3], XU Chun-He(徐春和)[3], QIAN Shi-Xiong(钱士雄)[1**]

[1]*Department of Physics, Fudan University, Shanghai 200433*

[2]*National Key Laboratory of Surface Science, Fudan University, Shanghai 200433*

[3]*Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032*

(Received 22 February 2006)

We study the photo-oxidation of bacteriochlorophylls (BChls) in peripheral light harvesting complexes (LH2) from rhodobacter sphaeroides by using the steady absorption and the femtosecond pump–probe measurement, to realize the detailed dynamics of LH2 in the presence of photo-oxidation. The experimental results reveal that BChl-B850 radical cations may act as an additional channel to compete with the unoxidized BChl-B850 molecules for rapidly releasing the excitation energy, while the B800→B850 energy transfer rate is almost unaffected in the oxidation process.

PACS: 82. 50. Nd, 87. 14. −g, 82. 53. Ps

Peripheral light-harvesting complexes (LH2), which serve as pigment antenna to efficiently absorb sunlight and transfer excitation energy to reaction centres (RCs), are an indispensable part of the photosynthetic unit in purple bacteria. The high-resolution x-ray crystal structures of LH2 show that nine monomeric bacteriochlorophylls (BChls) with absorption peak at 800 nm form a ring, labelled as B800, and 18 strongly interacting BChls form the B850 ring, which absorbs light at 850 nm.[1−4]

As crucial species directly participated in the ultrafast photo-induced excitation relaxation process, BChls are treated as the main objects for probing the structural properties and primary events in both the antenna complexes and the RC of the photosynthetic bacteria. The photochemical,[5] chemical,[6,7] and electrochemical[8−10] methods have been widely employed to investigate the redox properties of BChls in different photosynthetic pigment-protein complexes for a long time. The information of structural arrangement and interaction in these pigment-protein complexes was collected from detecting the redox midpoint potential E_m as well as the redox reversibility of the BChls.[6,8,10,11−14] On the other hand, a great deal of research has been focused on the effect of BChl oxidation on both the structural characterization and the energy transfer mechanism by using the steady[5−8] and time-resolved[15−18] absorption, fluorescence emission,[7,15,19] circular dichroic and electron paramagnetic resonance (EPR)[6,11,12,20] measurements. Monshouwer *et al.*[17] found that the dynamics of antenna complexes are different between the low-intensity and the high-intensity excitation at 77 K, high-intensity exciting B800 can make B850 photo-damaged, and this photo-product can effectively trap the excitation energy in the antenna complexes. A consistent result observed in the single molecule spectroscopic experiments performed on LH2 from *Rps. acidophila* displayed the switch-off of fluorescence caused by photo-oxidation of the BChl-B850 ring.[15] In most cases, trapping of excitation in molecular aggregates occurs if an impurity has excited state energy level lower than that of the aggregates.[7,15,21−26] Therefore, in tight connection with the BChl oxidation process, the BChls radical cation, which may have an optical absorption band reported near 925 nm,[27−29] can act as such an impurity for effectively trapping the excitation energy in the bacteria photosynthetic units. Our previous report on the electrochemical oxidation of LH2 complexes revealed that the generation of BChl-B850 radical cation can dramatically influence the energy transfer processes within LH2 complexes.[30]

In this Letter, by using the photo-oxidation treatment, we aim at the crucial dependence of the energy transfer on the BChl radical cation generation in LH2 from *rhodobacter sphaeroides* 601 for a further comprehension of the detailed dynamical evolution of the competitive quenching pathway within the LH2 complexes. The experiments are performed with the simultaneous measurements of the steady absorption and a femtosecond pump–probe process. The experimental results further prove that the BChl-B850 radical cation acts as an additional channel which dramatically and rapidly quenches the excitation energy with almost unchanged B800→B850 energy transfer

* Supported by the National Natural Science Foundation of China under Grant Nos 10274013 and 10374020.

**To whom correspondence should be addressed. Email: sxqian@fudan.ac.cn

©2006 Chinese Physical Society and IOP Publishing Ltd

rate.

Rhodobacter sphaeroides was grown and purified as described elsewhere.[31] LH2 complexes from purple bacteria *rhodobacter sphaeroides* 601 were dissolved in buffer solution (50 mmol/L pH 8 Tris-HCl buffer with 40 mM ubiquione-10 added as the mediator) and the optical density was set to be 0.5 at 850 nm in a 1-mm cuvette in the measurement.

The femtosecond pump–probe measurements were carried out at room temperature by using a Spectra Physics Ti:sapphire laser with the output from the oscillation stage of the laser in pulse duration of 120 fs and repetition rate of 82 MHz. The laser beam wavelength could be tuned from 780 nm to 850 nm, and the average output power was maintained at 400 mW. The photo-oxidation beam from amplifier stage had repetition rate of 1 kHz and wavelength of 800 nm, and the pulse power density was 1×10^{10} W/cm^2.

The sample cell for photo-oxidation experiments was properly placed in the fs pump–probe system. An SM-240 CCD spectrophotometer (CVI Spectral Instruments, Putnam, CT, USA) was also suitably set in the system for achieving the real-time detection of the oxidation process (see Fig. 1). The absorption and the fs pump–probe process were measured with two independent computers, while the photo-oxidation process was intermitted every 3 min.

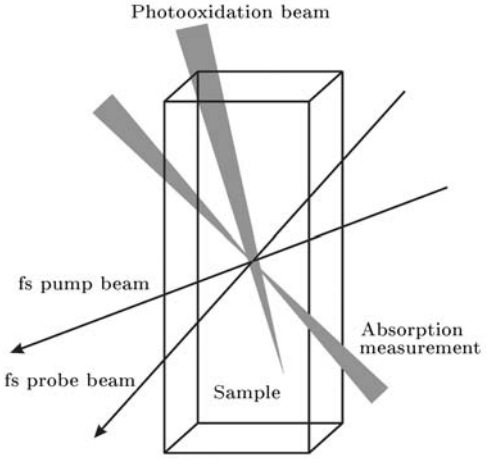

Fig. 1. Schematic view of the experimental setup for the fs pump–probe process and the real-time monitor absorption measurements of the oxidation process in LH2 from *rhodobacter sphaeroides*.

The detailed effect of photo-oxidation on the BChl absorption of LH2 is demonstrated in Fig. 2. The BChl oxidation results in photobleaching (PB) of both the B800 and B850 Q_y absorption bands. Meanwhile, a new absorption peak emerges at 685 nm, which come probably from the formation of the BChl dication (bivalent cation $BChl^{2+}$). By dismutation of $BChl^+$-$BChl^+$ and $BChl^+$-Car^+ cation pairs created by hole transfer in the Car-BChl pigment system resulting in formation of the $BChl^{2+}$ dication, the BChl oxidation is nearly irreversible.[8] Comparing the bleaching of B800 and B850 absorption bands under the same experimental condition (Fig. 2), it can be seen that the absorption peak of B800 bleaches slightly faster than that of B850. It could be understood that the nearest B800-B800 neighbourhood excitonic coupling is weaker than the coupling between B850 pigments.

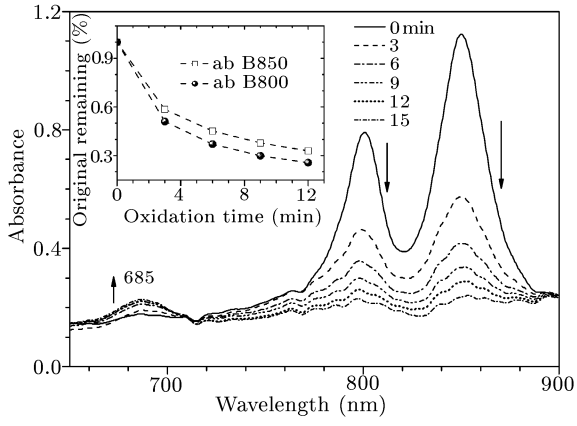

Fig. 2. (a) Evolution of Q_y absorption of LH2 from *rhodobacter sphaeroides* by photo-oxidation. Inset: the intensity of the absorption peak for B800 and B850 bands versus photo-oxidation time.

The fs pump–probe traces of LH2 complexes excited at 800 nm are shown in Figs. 3(a)–3(d). For the unoxidized LH2, the initial dynamics exhibits an ultrafast decay of the photobleaching, and an additional photoabsorption (PA) is attributed to the excited state absorption of the B850 band[32] (Fig. 3(a)). After 6-min photo-oxidation, PA signal nearly disappears (Fig. 3(b)). Then, we can observe a new PB signal with a long lifetime, which gradually increases (see Fig. 3(c)). Eventually, the dynamics evolution is mainly dominated by a long-lasting PB signal as mentioned above (see Fig. 3(d)). The corresponding fitting results reveal that the fast decay time component of 0.7 ps, which mainly reflects the rate of energy transfer from B800 to B850,[33] is nearly independent of the photo-oxidation. It has been suggested that the nearest B800–B800 neighbouring excitonic coupling is very weak, therefore the B800 excitation is considered to be highly localized on the individual B800 molecule.[34] Accordingly, photo-oxidation of BChl-B800 molecules does not greatly affect the excitation energy transfer from the unoxidized BChl-B800 molecules to the B850 ring. Moreover, although the B800-B850 energy transfer rate is not affected by photo-oxidation, a disappeared PA signal accompanied with a long-life PB signal generated is clearly observed. The PA signal is mainly attributed to the excited state absorption in the B850 band. Thus, some other signals may be mixed in this dynamics upon the photo-oxidation: that is, the BChl radical cation in the B850 ring may

act as an additional energy transfer channel to effectively quench the excitation energy. Such pathway may compete with the original energy transfer process to trap the excitation energy and it causes the long-lasting PB signal.[30,35]

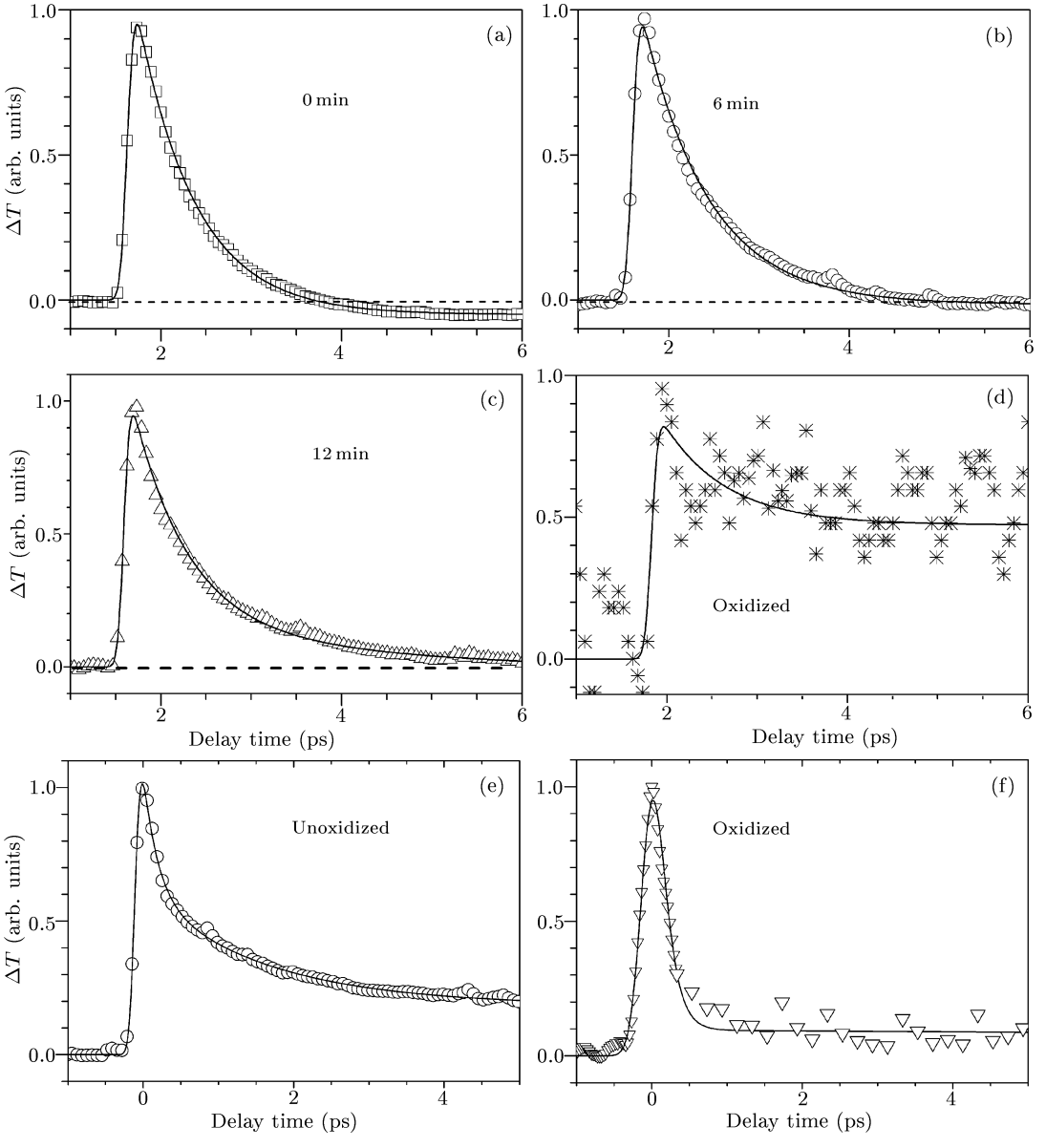

Fig. 3. Effect of photo-oxidation on normalized one-colour fs pump probe dynamics excited at [(a)–(d)] 800 nm and [(e)–(f)] 850 nm for LH2 from *rhodobacter sphaeroides*. The solid lines represent the fitting results.

For the experimental results of LH2 excited at 850 nm (as shown in Figs. 3(e)–3(f)), the characteristic changes in B850 dynamics also indicate that an additional channel generated by oxidation of BChl-B850 molecules acts as the excitation energy trapping pathway. For the unoxidized LH2, the relaxation process of PB signal of the BChl-B850 band initially exhibits an ultrafast decay, followed by a long-lived decay process. Three decay components with lifetimes of about 200 fs, 2 ps, and larger than 100 ps (see Fig. 3(e)) are achieved by data fitting, which reflects the intra/interband energy transfer pathways in the B850 ring.[36] At the oxidation time of 20 min, the dynamics evolution of BChl-B850 shows a bi-exponential decay process with time constants $t_1 < 100$ fs and $t_2 > 100$ ps (shown in Fig. 3(f)). During the photo-oxidation process, as reported in Ref. [17], the photo-product, i.e. the BChl-B850 radical cation with the 925 nm absorption band, may act as an additional trapping channel, and can effectively trap the excitation energy in the antenna complexes. The observed ultrafast process may be interpreted as the quenching process between the excited BChl-B850 and the BChl-B850 radical cation, which is fast to compete with the original intracomplex excited state dynamics and energy transfer within LH2, and the component of about 100 ps could be

probably ascribed to the lifetime of the B850 radical cation.[18]

We propose some schemes of the excitation energy relaxation processes in the photo-oxidized LH2 complex as shown in Fig. 4. Figure 4(a) shows the energy transfer process in unoxidized LH2. When photo-oxidation occurs, a new energy level relating to the BChl-B850 radical cation can be produced to trap the excitation energy (see Fig. 4(b)).

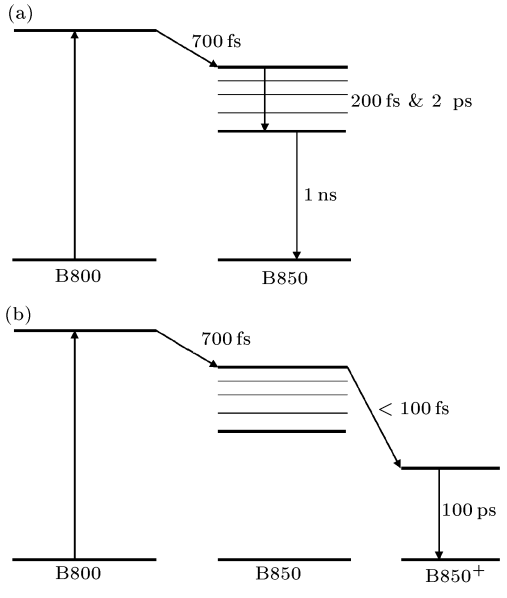

Fig. 4. Proposed energy level diagrams for demonstrating the main transition and relaxation processes in the LH2 complex under the conditions of (a) no oxidation and (b) photo-oxidation.

In this work, we use the photo-oxidation to oxide LH2 and use the fs pump–probe technique to investigate the detailed dynamics of the oxidation process in LH2. By photo-oxidation, the absorption peaks of B800 and B850 bleach with different rates, and a new absorption peak emerges at 685 nm reflecting the generation of $BChl^{2+}$. From the pump–probe result, we conclude that (i) oxidation has little influence on the B800→B850 energy transfer process, (ii) the BChl-B850 radical cation may act as an additional trapping channel, which competes with the unoxidized BChl-B850 molecules for rapidly releasing the excitation energy.

References

[1] Papiz M Z, Prince S M, Howard T, Cogdell R J et al 2003 *J. Mol. Biol.* **326** 1523

[2] Walz T, Jamieson S J, Bowers C M, Bullough P A et al 1998 *Mol. Biol.* **282** 833

[3] Herek J L, Wohlleben W, Cogdell R J, Zeidler D et al 2002 *Nature* **417** 533

[4] Cogdell R J, Howard T D, Isaacs N W, McLuskey K et al 2002 *Photosynth. Res.* **74** 135

[5] Rafferty C N, Bolt J and Sauer K 1979 *Proc. Natl. Acad. Sci. USA* **76** 4429

[6] Picorel R, Lefebvre S and Gingras G 1984 *Eur. J. Biochem.* **142** 305

[7] Law C J and Cogdell R J 1998 *FEBS. Lett.* **432** 27

[8] Kropacheva T N and Hoff A J 2001 *J. Phys. Chem.* B **105** 5536

[9] Lin X, Murchison H A, Nagarajan V et al 1994 *Proc. Nat. Acad. Sci. USA* **91** 10265

[10] Kong J L, Lu Z Q, Lvov Y M, Desamero R Z B et al 1998 *J. Am. Chem. Soc.* **120** 7371

[11] Gomez I, Sieiro C, Ramirez J M, Gomea-Amores S et al 1982 *FEBS. Lett.* **144** 117

[12] Gomez I, Picorel R, Ramirez J M, Perez R et al 1982 *Photochem. Photobiol.* **35** 399

[13] Gomez I, Sanchez R A, Campo F F del 1985 *Physiol. Veg.* **23** 583

[14] Picorel R, Lefebvre S, Gingras G 1984 *Eur. J. Biochem.* **142** 305

[15] Bopp M A, Jia Y, Li L, Cogdell R J et al 1997 *Proc. Natl. Acad. Sci. USA* **94** 10630

[16] Pšenčik J, Polivka T, Nemec P, Dian J et al 1998 *J. Phys. Chem.* A **102** 4392

[17] Monshouwer R, de Zarate I O, van Mourik F, van Grondelle R 1995 *Chem. Phys. Lett.* **246** 341

[18] Bergström H, Sundström V, van Grondelle R et al 1986 *Biochim. Biophys. Acta* **852** 279

[19] Brune D C, King G H, Infosino A, Steiner T et al 1987 *Biochemistry* **26** 8652

[20] Limantara L, Fujii R, Zhang J P, Kakuno T et al 1998 *Biochemistry* **15** 17469

[21] Wang J, Gosztola D, Ruffle S V, Hemann C et al 2002 *Proc. Natl. Acad. Sci. USA* **99** 4091

[22] Barzda V, Vengris M, Valkunas L, van Grondelle R et al 2000 *Biochemistry* **39** 10468

[23] Rajagopal S, Egorova E A, Bukhv N G et al 2003 *Biochim. Biophys. Acta* **1606** 147

[24] Hartwich G, Friese M, Scheer H, Ogrodnik A et al 1995 *Chem. Phys.* **197** 423

[25] Fiedor L, Scheer H, Tschirschwitz F, Ehlert J et al 2000 *Chem. Phys. Lett.* **319** 145

[26] Fiedor L, Leupold D, Teuchner K, Voigt B et al 2001 *Biochemistry* **40** 3737

[27] Chauvet J P, Vlovy R, Santus R and Land E J 1981 *J. Phys. Chem.* **85** 3449

[28] Fajer J, Borg D C, Forman A, Felton R H et al 1974 *Proc. Natl. Acad. Sci. USA* **71** 994

[29] Fuhrhop J H and Mauzerall D 1969 *J. Am. Chem. Soc.* **91** 4174

[30] Liu W M, Lu Y D, Liu Y, Kong J L et al 2006 *BBRC* **340** 505

[31] Boonstra A F, Visschers R W, Calkoen F et al 1993 *Biochim. Biophys. Acta* **1142** 181

[32] Liu W M, Zhu R Y et al 2003 *Chin. Phys. Lett.* **20** 2148

[33] Guo L J, Liu Y, Yang Y, Mi J et al 2002 *FEBS. Lett.* **511** 69

[34] Sundström V, Pullerits T and van Grondelle R J 1999 *J. Phys Chem.* B **103** 2327

[35] Tinnefeld P, Heilemann M and Sauer M 2005 *Chem. Phys. Chem.* **6** 217

[36] Liu D Z, Gao Y L and Kispert L D 2000 *J. Electroanal. Chem.* **488** 140