PACS numbers: 42.62.Be; 82.53.Ps
DOI: 10.1070/QE2005v035n01ABEH002880

Relaxation dynamics of the LH2 complex from a photosynthetic purple bacterium *Thiorhodospira sibirica* studied by the near-IR femtosecond pump-probe method

A.P. Razjivin, V.O. Kompanets, R.Yu. Pishchal'nikov,
A.A. Moskalenko, Z.K. Makhneva, V.S. Kozlovsky, S.V. Chekalin

Abstract. **Photoinduced changes in the absorption spectrum of the LH2 (B800-830-850) complex from a *Thiorhodospira sibirica* (*Trs. sibirica*) bacterium are studied by the pump-probe method. The complex has the anomalous absorption spectrum exhibiting three bands in the near-IR region at 793, 826.5, and 846.5 nm. At room temperature, the excitation energy transfer from the B800, B830, and B859 bands was detected with the time constants $\tau_1 \sim 0.5$ ps, $\tau_2 \sim 2.5$ ps, and τ_3 of the order of a few hundreds of picoseconds, respectively. A rapid energy transfer from the B830 band compared to energy transfer from the B850 band ($\tau_2 \ll \tau_3$) suggests that all the three bands belong to the same complex (i.e., that the LH2 complex from *Trs. sibirica* is homogeneous). A slower energy transfer (by three – five times) from the B830 band of the LH2 complex from *Trs. sibirica* compared to energy transfer from the B800 band of the LH2 complexes (B800-850 and especially B800-820) from other purple bacteria suggests that the electronic structures of ensembles of bacteriochlorophyll molecules in these complexes are substantially different.**

Keywords: *pump-probe method, femtosecond spectroscopy, excitation transfer, purple bacteria.*

1. Introduction

The structural base of the photosynthetic apparatus of purple bacteria is formed by pigment – protein complexes of the reaction centre and light-harvesting antenna (the central LH1 and peripheral LH2 complexes). The absorption spectra of the LH2 complexes exhibit two bands at 800 and 850 nm. The 850-nm absorption band belongs to bacteriochlorophyll (BChl) molecules forming a ring containing 16 or 18 molecules, while the 800-nm absorption band is related to BChl molecules forming the second ring of 8 or 9 molecules located above the first ring. These rings are referred to as the B850 and B800 rings, respectively. BChl molecules in the B850 ring are closely placed, which results in the exciton interaction and delocalisation of excitation over the ring (the energy of pair interactions between adjacent BChl molecules is a few hundreds of inverse centimetres). The energy of interaction between BChl molecules in the B800 ring does not exceed approximately 30 cm^{-1}, so that these molecules can be treated in the first approximation as monomers (see details in reviews [1, 2]).

Recently, a new photosynthetic purple bacterium *Thiorhodospira sibirica* (*Trs. sibirica*) was described [3]. The LH2 complex from this bacterium has the anomalous spectrum with three maxima (which can be referred as B800-830-850). The optical properties of this complex were explained by using the model of its structure [4], which assumes, first, that the transition dipole of each second BChl molecule in the B850 ring is turned through 90° with respect to its direction in the previous BChl molecule and, second, that the distance between the B850 and B800 rings is approximately only 10 Å rather than 17 Å [1].

The kinetics of energy transfer from the B800 band to the B850 band in the LH2 complex from different bacteria was studied under various conditions in a number of papers (see, for example, review [2]). At room temperature, the time constant of energy transfer from the B800 band to the B850 band lies in the range from 0.65 to 1.2 ps [5 – 8], and that from the B800 band to the B820 band in the range from 0.75 to 0.9 ps [6, 9]. In samples of the extracted LH2 complex, unlike chromatophores, no energy transfer from the B850 (or B820 band) occurs, so that the excited-state lifetime for the B850 (B820) band is no less than 600 ps [5, 6, 10].

In this paper, we studied energy transfer between the absorption bands of the LH2 complex from *Trs. sibirica* excited by 800-nm femtosecond pulses at room temperature.

2. Experimental

The dynamics of the difference spectra of photoinduced changes in optical absorption was studied with the help of a femtosecond laser setup of the Collective Use Centre at the Institute of Spectroscopy, RAS. The setup consists of a Tsunami HP femtosecond laser, a Spitfire HP regenerative amplifier, and a broadband detection system. The femtosecond laser was pumped by a 532-nm, 4.5-W line from a Millenia-V cw solid-state Nd : YVO_4 laser. The amplifier was pumped by 527-nm pulses from an Evolution-X

A.P. Razjivin, R.Yu. Pishchal'nikov, V.S. Kozlovsky A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Vorob'evy gory, 119992 Moscow, Russia; Fax: (095) 939 3181; e-mail: razjivin@belozersky.msu.ru;
V.O. Kompanets, S.V. Chekalin Institute of Spectroscopy, Russian Academy of Sciences, 142190 Troitsk, Moscow region, Russia; Fax: (095) 334 0886; e-mail: chekalin@isan.troitsk.ru;
A.A. Moskalenko, Z.K. Makhneva Institute of Fundamental Problems of Biology, Russian Academy of Sciences, 142290 Pushchino, Moscow region, Russia; e-mail: andrey-mos@issp.serpukhov.su

Received 22 September 2004
Kvantovaya Elektronika **35** (1) 107 – 110 (2005)
Translated by M.N. Sapozhnikov

Nd : YLF laser with a pulse repetition rate of 1 kHz and an average power of 8 W. The energy of 800-nm, 45-fs pulses with a pulse repetition rate of 1 kHz at the amplifier output could achieve 1 mJ. The pulse FWHM was ~ 20 nm. The output radiation of the amplifier was split into two beams with a beamsplitter (Fig. 1). In one of the beams, a computer-controlled optical delay line was placed, whose minimal time step was 1.37 fs, and also a quartz plate and a polariser allowing the rotation of the polarisation plane of radiation in the range from 0 to 90°.

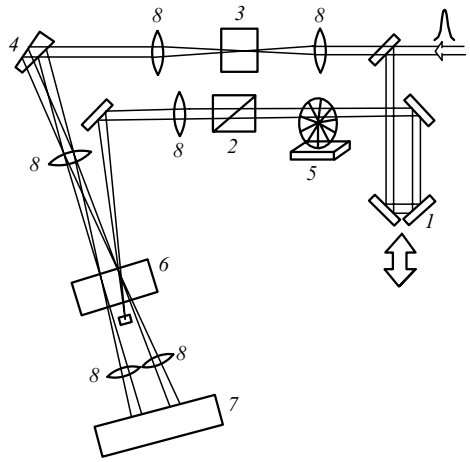

Figure 1. Scheme of the experiment: (*1*) delay line; (*2*) polarisation rotator; (*3*) cell for supercontinuum generation; (*4*) wedge plate; (*5*) optical modulator; (*6*) sample cell; (*7*) spectrometer; (*8*) achromatic lenses.

The attenuated radiation pulses in this channel were focused into a spot of diameter 0.5 mm to pump a sample in a cell. Probing was performed by radiation from another channel transformed to femtosecond-supercontinuum pulses with a broad spectrum between 400 and 1100 nm by focusing the radiation into a 5-mm cell with water. To avoid thermal distortions of the spatial distribution of the beam propagating through the cell, we used a forced laminar water circulation. Then, the probe radiation was split with quartz wedge (*4*) into two equal beams (the signal beam, propagating through the excitation region of the sample, and the reference beam, propagating through the sample outside the excitation region). Both these probe beams were focused with an achromatic lens with the focal distance 80 mm to spots of diameter 0.15 mm on the cell with a sample separated by 8 mm. The angle between the pump and probe beams was 25°. A part of the supercontinuum radiation propagated through the sample was selected with a grating monochromator in the 750 – 1050-nm region and detected with two fast 1024-pixel diode CCD linear arrays. The difference spectra were obtained by modulating the probe beam with modulator (*5*) at the frequency 500 Hz. A change in the optical density at each wavelength was calculated by the expression

$$\Delta A = -\lg\left[\frac{I_1/I_2}{I_1^0/I_2^0}\right],$$

where $I_{1,2}$ and $I_{1,2}^0$ are the intensities of the probe and reference pulses in the presence of excitation and its absence, respectively. A sample was studied in a 5-mm quartz cell of volume 1 cm^3. To avoid the degradation of the sample, the cell was moved up and down at a frequency of 150 Hz. For this purpose, it was attached to the head of a speaker to which a signal from a low-frequency generator was fed.

The number of photons incident on the cell was $(1-2)\times 10^{14}$ for 1 cm^2 per pulse, corresponding to excitation of approximately each third LH2 complex upon absorption of a 800-nm radiation pulse.

3. Results and discussion

Figure 2 shows the absorption spectrum of the LH2 (B800-830-850) complex from *Trs. sibirica*. The theoretical spectrum of this complex and its exciton components were calculated as in [4]. One can see that 800-nm pulses do not provide selective excitation of the shortest-wavelength absorption band at ~ 793 nm. Although the excitation energy is mainly absorbed in the long-wavelength wing of the B800 band, some part of it should be also absorbed in the short-wavelength wing of the B830 band. According to the calculation of exciton components (Fig. 2), direct excitation of the B830 band by 800-nm pulses can be rather efficient, in particular, because the pump pulse FWHM achieves ~ 20 nm.

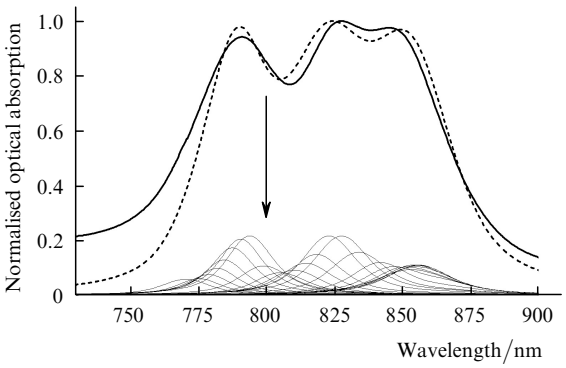

Figure 2. Near-IR absorption spectrum of the LH2 complex from *Trs. sibirica* recorded at room temperature (thick solid curve); theoretical spectrum of this complex (dashed curve), and the exciton components of the theoretical spectrum (thin solid curves). The arrow indicates the wavelength of the ultrashort pump pulse.

Figure 3 shows the spectra of photoinduced changes in the optical absorption for the LH2 complex. One can see that first photobleaching occurs simultaneously in the long-wavelength part of the B800 band and the short-wavelength part of the B830 band (peak near 821 nm). Photobleaching at 821 nm is substantially stronger than, for example, at 800 nm, although, as follows from Fig. 2, absorption in the B800 band at 800 nm is considerably stronger than absorption in the short-wavelength of the B830 band at this wavelength. A weaker photobleaching in the B800 band compared to the B830 band can be explained by fast energy transfer (comparable with the duration of the convolution of the pump and probe pulses) from the B800 band. This energy transfer will also result in additional bleaching of the B830 band during irradiation by the pump pulse.

Then, photobleaching begins to dominate in the long-wavelength wing of the B830 band, resulting in the

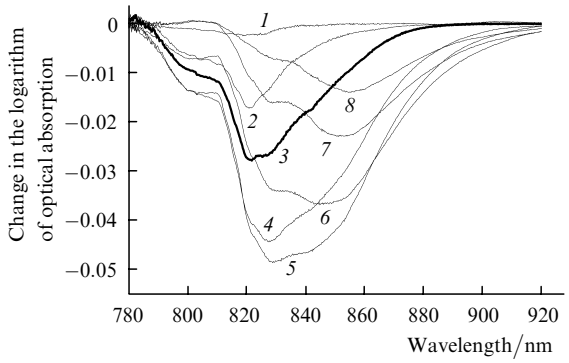

Figure 3. Spectra of photoinduced changes in optical absorption for the LH2 complex from *Trs. sibirica* as functions of the time delay (or advance) of the probe pulse with respect to the pump pulse. Curves (*1*) and (*2*): the advance time is 822 and 137 fs, respectively; curve (*3*): spectrum corresponding to the zero instant (the maxima of the pump and probe pulses coincide); curves (*4*)–(*8*): the delay time is 274, 493, 1178, 4192, and 12 522 fs, respectively.

appearance of a broad maximum at 835 nm. Later, the bleaching maximum shifts to 855 nm and remains here until the disappearance of excitation in the complex. Note that in the case of a large time delay, the 835-nm maximum does not disappear completely but transforms to a shoulder in the short-wavelength wing of the 855-nm band.

A signal at 780 nm is probably caused by the coherent response of the medium [11], and can be considered as a convolution of the pump and probe pulses (the relaxation time is less than 50 fs, otherwise the asymmetry of the convolution curve would be observed). The duration of the 800-nm pump pulse determined with an autocorrelator was ~ 50 fs. However, the duration of the convolution of the pump and probe pulses was substantially longer (~ 550 fs). This was caused by a large thickness of the cell (5 mm), a long duration of the supercontinuum, large diameters of the pump and probe beams (0.5 and 0.15 mm, respectively), and a large angle (25°) between the directions of these beams.

The kinetic curves of changes in optical absorption for different probe wavelengths in the B800, B830, and B850 bands are presented in Fig. 4. The strongest changes in the optical density were detected in the B830 band during the first 2–3 ps after the propagation of the probe pulse. The kinetic curves for all the wavelengths differ from single-

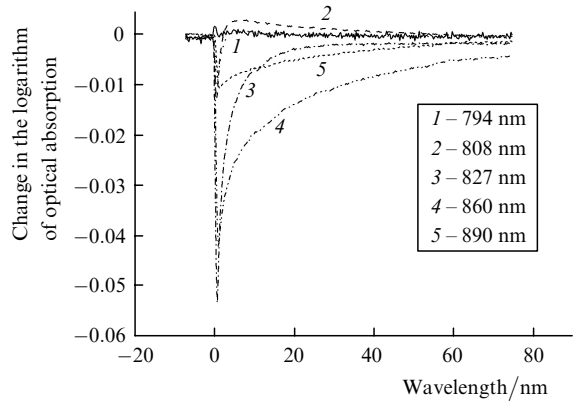

Figure 4. Kinetic curves of photoinduced changes in optical absorption for the LH2 complex from *Trs. sibirica* for different wavelengths of the probe pulse (excitation at 800 nm).

exponential curves. Taking into account the spectral overlap of the B800 and B830 bands and of the B830 and B850 bands, one can expect that the kinetic curves will reflect in each case the time dependence of absorption in both these bands at any probe wavelength. The exception can be only the long-wavelength wing of the B850 band.

In the 810–900-nm range, the delay time of the probe pulse with respect to the 800-nm pump pulse (spectral dispersion) virtually linearly increases with increasing wavelength (5.625 fs nm^{-1}). In the 790–810-nm range (the region of the pump pulse), the wavelength dependence of the delay time deviated substantially from linear. This is explained by the fast relaxation of the photoinduced change in optical absorption (bleaching) in this spectral region. However, in the region $\lambda > 810$ nm, relaxation only weakly affects the kinetic curve describing the rise in photoinduced changes in optical absorption. Therefore, in this case we can compare the fronts of the kinetic curves. For clarity, the kinetic curves in Fig. 5 are plotted so that all of them pass through the same point at the 0.1 level of the maximum. One can see that the fronts of the curves for $\lambda = 810$ and 830 nm virtually coincide, while the delay (at the 0.9 level of the maximum) of the kinetic curve for $\lambda = 900$ nm with respect to curves at $\lambda = 810$ and 830 nm is approximately 180 fs (Fig. 5). This value can be used as an estimate of the minimal time of energy transfer from the B830 (or B800) band to the long-wavelength wing of the B850 band in the LH2 complex from *Trs. sibirica*. A more exact estimate is complicated because of a large width of the convolution of the pump and probe pulses (FWHM ~ 550 fs).

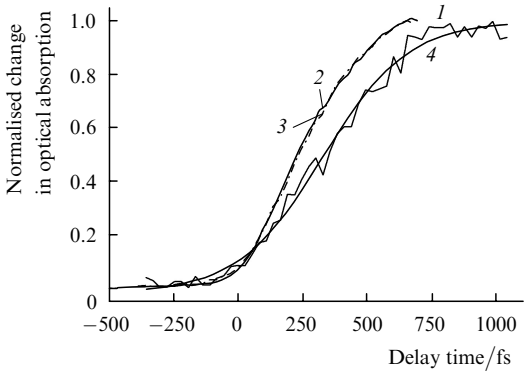

Figure 5. Delay of the rise front of photoinduced changes in optical absorption (at the 0.9 level) in the long-wavelength wing of the B850 band [probing at 900 nm, (*1*)] with respect to the fronts of the B800 [$\lambda = 810$ nm, (*2*)] and B830 [$\lambda = 830$ nm, (*3*)] bands. All the kinetic curves were made coincident at one point at the 0.1 level. S-shaped curve (*4*) approximates the rise curve of changes in absorption for $\lambda = 900$ nm.

It is known from the literature [8, 12] that the time of energy transfer from the B800 band in the LH complexes from different bacteria depends somewhat on the wavelength. Because this effect is not too strong [8, 12], we assumed in the first approximation that the kinetics of energy transfer within each band is the same. However, kinetic curves contain many components due to the overlap of absorption bands, the superposition of excited-state absorption bands, and rapid energy transfer between the bands. The kinetic curves are close to single-exponential (containing only weakly pronounced second exponential)

Table 1. Coefficients at exponentials for different probe wavelengths used in the simulation of the kinetic curves of photoinduced changes in optical absorption for the LH2 complex from *Trs. sibirica* (coefficients are normalised so that $A_1 + A_2 + A_3 = 1$.

Wavelength/nm	A_1	A_2	A_3
810	0.50	0.43	0.07
815	0.40	0.49	0.11
825	0.33	0.49	0.18
835	0.24	0.43	0.33
840	0.24	0.37	0.39
855	0.19	0.26	0.55
870	0.16	0.25	0.59
880	0.10	0.21	0.69
890	0	0.23	0.77
900	0	0.20	0.80

only in the longest-wavelength wing of the B850 band (at 900 nm). At other wavelengths, the curves are fitted by three exponentials.

To simulate the kinetic curves, it is necessary to select appropriately the time constants for excitation deactivation for all the three bands. In the case of a correct (self-consistent) selection of these constants, the simulation of kinetics at any wavelength is reduced to the selection of coefficients at exponentials (without varying the time constants). In our case, all the kinetic curves are well described by exponentials with the time constants $\tau_1 =$ 0.5 ps, $\tau_2 = 2.5$ ps, and τ_3 of the order of a few hundreds of picoseconds. The coefficients A_1, A_2, and A_3 at the exponentials for different wavelengths of the probe pulse are presented in Table 1.

The wavelength dependences of the contributions from all the three components show that the long-lived component (τ_3) is related to the B850 band. Energy transfer from the B830 band ($\tau_2 = 2.5$ ps) occurs for 2–3 ps. The residual long-lived bleaching in the B830 band ('shoulder' in the spectrum at large time delays, Fig. 3) is probably caused by reverse energy transfer from the B850 band (stationary distribution of the excitation energy between these bands at room temperature). It follows from this that the LH2 sample from *Trs. sibirica* does not contain the B800-830 complexes, otherwise the excited-state lifetime in the B830 band would be of the order of a few hundreds of picoseconds. This suggests that the LH2 sample is a homogeneous complex in which energy transfer occurs between all the three B800, B830, and B850 bands.

A short excited-state lifetime in the B800 band of the LH2 complex from *Trs. sibirica* ($\tau_1 = 0.5$ ps) approximately corresponds to the time of energy transfer from the B800 band to the B850 or B820 band in usual LH2 complexes at room temperature (0.6–0.9 ps [5–9]). The excited-state lifetime in the long-wavelength band of this complex (a few hundreds of picoseconds) also agrees with the corresponding lifetime for the B850 and B820 bands of the LH2 complexes from other bacteria [5, 10, 13].

The time constant for the central B830 band of the LH2 complex from *Trs. sibirica*, which is approximately 2.5 ps, poorly agrees with the time constants measured for the LH2 complexes from other purple bacteria. The spectral overlap of the B830 and B850 bands is approximately the same as that for the B800 and B820 bands in the B800-820 complexes from Chromatium vinosum and Phodopseudomonas acidophila. However, energy transfer from the short-wavelength band to the long-wavelength band in these complexes occurred at the subpicosecond scale [6, 9]. The fact that the time constant of energy transfer between the B830 and B850 bands in the LH2 complex from *Trs. sibirica* is approximately 4–5 times greater than that for the B800-820 complexes suggests probably that the electronic structures of ensembles of BChl molecules and (or) mechanisms of energy transfer in these complexes are substantially different. It is not clear now whether the model [4] of the LH2 complex from *Trs. sibirica* can explain this increase in the time constant of energy transfer from the B830 band.

Acknowledgements. This work was partially supported by the Russian Foundation for Basic Research (Grant Nos 03-04-48884 and 03-02-16736).

References

1. Cogdell R.J., Isaacs N.W., Freer A.A., Arrelano J., Howard T.D., Papiz M.Z., Hawthornthwaite-Lawless A.M., Prince S. *Prog. Biophys. Molec. Biol.*, **68**, 1 (1997).
2. Van Grondelle R., Novoderezhkin V. *Biochemistry*, **40**, 15057 (2001).
3. Bryantseva I., Gorlenko V.M., Kompantseva E.I., Imhoff J.F., Suling J., Mityushina L. *Int. J. Syst. Bacteriol.*, **49**, 697 (1999).
4. Pishchal'nikov R.Yu., Moskalenko A.A., Razjivin A.P. *Biol. Membr.*, **20**, 386 (2003).
5. Jimenez R., Dikshit S.N., Bradforth S., Fleming G.R. *J. Phys. Chem.*, **100**, 6825 (1996).
6. Kennis J.T.M., Streltsov A.M., Vulto S.I.E., Aartsma T.J., Nozawa T., Amesz J. *J. Phys. Chem. B*, **101**, 7827 (1997).
7. Herek J.L., Fraser N.J., Pullerits T., Martinsson P., Polivka T., Scheer H., Cogdell R.J., Sundstrom V. *Biophys. J.*, **78**, 2590 (2000).
8. Ihalainen J.A., Linnanto J., van Stokkum I.H.M., Myllyperkio P., Ucker B., Scheer H., Korppi-Tommola J.E.I. *J. Phys. Chem. B*, **105**, 9849 (2001).
9. Ma Y.-Z., Cogdell R.J., Gillbro T. *J. Phys. Chem. B*, **102**, 881 (1998).
10. Bergstrom H., Westerhuis W.H.J., Sundstrom V., van Grondelle R., Niederman R.A., Gillbro T. *FEBS Lett.*, **233**, 12 (2004).
11. Eisentahl K., in *Sverkhkorotkie svetovye impul'sy* (Ultrashor Light Pulses) (Moscow: Mir, 1981) p. 350.
12. Novoderezhkin V., Wendling M., van Grondelle R. *J. Phys. Chem. B*, **107**, 11534 (2003).
13. Sundstrom V., van Grondelle R. *J. Opt. Soc. Am. B*, **7**, 1595 (1990).