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Photoreaction and thermal deactivation of excitation in purple bacteria light harvesting complexes (LH2) with and without reaction centres

M. Hara[a], J. Miyake[a], J. Goc[b], D. Frąckowiak[b,*]

[a]National Institute for Advanced Interdisciplinary Research, AIST/MITI, 1-14 Higashi, Tsukuba, Ibaraki 305, Japan
[b]Institute of Physics, Poznań University of Technology, ul. Piotrowo 3, 60-965 Poznań, Poland

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Abstract

Light harvesting peripheral complexes (LH2) and carotenoids free-reaction centres were isolated from purple photosynthetic bacteria *Rhodospirillum molischianum* and *Rhodobacter sphaeroides*, respectively. The LH2 complexes without and with various amounts of reaction centres were immobilized in polyacrylamide gel. The absorption and photoacoustic spectra of such samples were measured. The changes due to continuous light illumination in absorption and photoacoustic spectra were investigated. The changes in absorption generated by flash were also measured. The conclusions concerning the photoreactions in antenna and reaction centre chromophores, the storage of energy in reaction centres as well as the protection of LH2 chromophores against overexcitation by heat emission and excitation transfer to reaction centres are discussed. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Light harvesting complexes (LH2); Light induced absorption changes; Photoacoustic spectra; Reaction centres; *Rhodobacter sphaeroides*; *Rhodospirillum molischianum*

1. Introduction

The energy absorbed by chromophores of photosynthetic apparatus can be transferred to other chromophores, emitted as luminescence, exchanged into heat or, especially in reaction centres chromophores, used for photoreaction [1]. The absorption bands of various chromophores of organisms are in high degree overlapped. Therefore, it is not easy to distinguish the contributions from different pigment forms to all these processes [1]. The investigations of simpler model systems constructed from parts of photosynthetic apparatus can be useful in interpretation of physiological processes occurring in organisms [1]. In present paper, the interactions between carotenoids-free reaction centres (RC) from *Rhodobacter sphaeroides* (*Rh. sphaeroides*) strain R-26 and LH2 complexes of purple non-sulfur bacteria *Rhodospirillum molischianum* (*Rs. Molischianum*) are investigated. This last bacterium posseses the LH2 complexes and exhibits a carotenoid (Car) band shift enabling to evaluate the change in the membrane potential [2]. The LH2 uncoupled from RC can be easily denatured by overexcitation [3,4]. These antenna complexes have some regulatory mechanisms protecting them against photodamage [5]. One of them is the transfer of chlorophyll excitation to carotenoids where it is converted into heat and the second some aggregation and conformational rearrangement [6,7].

In vivo the LH2 complexes are not located in close surroundings of RC because as is usual in purple bacteria, they are the bulk of peripheral antenna, whereas LH1 are tightly bound to RC. *Rs. molischianum* contains two types of peripheral complexes absorbing in 800–850 nm and in 800–820 nm. In some cases the last type was denoted as LH3 [8] but usually [9] both are named LH2. The LH2 are forming the cylindrical groups with a diameter of 5.3 nm which are located around the core complexes [9,10].

Because of the difference in Q_y energy (LH1 about 870 nm, LH2 800–850 nm) the excitation from LH2 is funnelled predominantly to LH1 and further to RC [10]. In this paper, we want to check how efficient, in our model system, is the direct energy transfer from LH2 to RC and if the RC presence can protect the LH2 complexes against the photo chemical damage.

*Corresponding author. Tel.: +48-61-8782344; fax: +48-61-8782324; e-mail: frackow@phys.put.poznan.pl

Abbreviations: A – absorbance;BChl – bacteriochlorophyll; LH2 – light harvesting peripheral complexes; PAS – photoacoustic spectrum; RC – reaction centres; *Rh. sphaeroides – Rhodobacter sphaeroides*; *Rs. molischianum – Rhodospirillum molischianum*

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Excitation energy transfer seems to be possible because similarities between binding sites of chromophores to protein in LH1 and LH2 complexes of investigated organisms were reported [10]. The RC of both investigated bacteria are in some extent similar, therefore the conclusions obtained on the basis of *Rh. sphaeroides* RC can be extrapolated to interactions between LH2 and RC of *Rs. molischianum*. RC of used strain (R-26) of *Rh. sphaeroides* is carotenoidless [11]. Therefore, the addition of various amount of RC preserve the contents of various carotenoids in all samples. It enables to normalize the absorption of the samples at carotenoids spectral range. Fluorescence emission, heat generation and photochemical reactions are competitive processes. The energy emitted as fluorescence is low in comparison to the energy converted into heat and used for photochemistry [12]. The information concerning the change in thermal deactivation (TD) occurring in photosynthetic organisms as a result of strong illumination by light in 400 nm–700 nm range are scarce [12,13] and all reported data describe the effects observed for higher plants [12–15] or algae [5].

The increase in heat emission followed by the decrease in photochemical activity and fluorescence quenching due to organism illumination was observed for plants [12,13] and was explained as a protecting mechanisms against strong illumination. The molecular mechanism of this protection is not quite clear and the effect was, as far we know, not yet shown for bacteria. Our measurements of photoacoustic signal changes as a result of sample illumination gives a new information about this process.

2. Materials and methods

The non-sulfur bacteria *Rs. molischianum* (ATCC 14031) and *Rh. sphaeroides* strain R-26 were used in our experiments. *Rh. sphaeroides* and *Rs. molischianum* were cultured semi-anaerobically under illumination by tungsten lamp (500 lux) in the medium with 57 mM succinate instead of malate [16].

Bacterial cells were harvested by centrifugation for 10 min at $4000 \times g$, washed with 10 mM Tris HCl (pH 8.2), and then disrupted in a French pressure cell ($1200\ kg/cm^2$). After centrifugation for 20 min at $3000 \times g$ to remove unbroken cells, intracytoplasmic membranes were collected by centrifugation for 60 min at $120{,}000 \times g$, washed with 10 nM Tris HCl (pH 8.2) and then suspended in 10 mM Tris HCl (pH 8.2) as a chromatophore suspension.

LH2 was purified from *Rs. molischianum* chromatophore membranes as follows. Proteins in chromatophore membranes ($A_{862} = 50$) were solubilized for 20 min at room temperature for 20 min in 80 mM Tris HCl (pH 6.8), 5% (w/v) lauryldimethylamine *N*-oxide (LDAO) and 20% (v/v) glycerol. The supernatant liquid, after centrifugation for 1 h at $120{,}000 \times g$, was applied on a 12% (w/v) polyacrylamide gel and electrophoresed [17]. A thick red band in the polyacrylamide gel containing LH2 was excised and homogenized in 10 mM Tris HCl (pH 8.2) containing 0.1% (w/v) LDAO for extraction of the protein. Polyacrylamide gel particles were removed by centrifugation for 20 min at $3000 \times g$ to obtain the purified LH2 suspension.

The content of pigments in LH2 purified from *Rs. Molischianum* and in RC purified from *Rb. sphaeroides* were measured biochemically as was described in [8,18–21]. Bacteriochlorophyll *a* (BChl) was extracted twice from the cell with acetone–methanol mixture (7 : 2). The concentration of BChl in the extract was calculated from the absorbance ($\varepsilon = 75$ mM/cm at $\lambda = 770$ nm). The content of carotenoids was measured and calculated after saponification of acetone–methanol extract for 10 min at 40°C in 12% (w/v) KOH and extracted twice with diethylether ($\varepsilon = 140$ mM/cm, at $\lambda = 454$ nm). The results are shown in Table 1. An experimental value of BChl/Car ratio (2.91) in LH2 is matched well with the value (3.0) calculated from the 3-D structure [8]. Three-dimensional structure shows that *Rs. Molischianum* LH2 consists of eight pairs of α and β subunits. Each pair has two BChl molecules (850 nm), one BChl (800 nm) and one Car. Therefore, one LH2 contains 16 BChl (850 nm), eight BChl (800 nm) and eight Car molecules. Details of the densitogram analyses of SDS-PAGE show that in the LH2 sample some traces RC-LH1 complexes of *Rs. Molischianum* are present.

RC was purified from *Rh. sphaeroides* strain R-26 by method of Clayton et al. [16]. The concentration of RC ($\varepsilon = 288$ mM/cm) can be calculated according to Straley

Table 1
Content of various chromophores in investigated samples (relative values) and concentration of chromophore-protein complexes immobilized in the polyacrylamide gel before drying

Samples	Composition	LH2 (*Rs. molischianum*)				RC (*Rh. sphaeroides*)	
		$BChl_{802}$	$BChl_{852}$	Car	c_{LH2}	BChl	$c_{R}C$
A	LH2	32	64	32	3.2	–	–
B	LH2 + RC	32	64	32	3.2	1	0.2
C	LH2 + 2RC	32	64	32	3.2	2	0.4
D	LH2 + 4RC	32	64	32	3.2	4	0.8
E	LH2 + 8RC	32	64	32	3.2	8	1.6

Note: LH2 — light harvesting complex, RC — reaction centre, BChl — bacteriochlorophyll, Car — carotenoid, *c* — concentration (μmol).

et al. [18]. The samples with different ratios of RC to LH2 complexes (Table 1) were embedded in polyacrylamide gel as was described previously [22]. Table 1 contains the concentration of chromophore–protein complexes immobilized in the polyacrylamide gel before drying, calculated from experimental data. As seen from Table 1, in most samples, the number of RC chromophores is much lower than the number of LH2 chromophores.

Absorbance spectra were measured using UV–Vis spectrometer (Perkin Elmer, Lambda 20, USA). Continuous-light induced spectra in near infrared region were measured by a conventional spectrophotometer (Shimadzu UV-160, Japan) [23] with cross illumination. Illuminating light was supplied through the glass fiber-guided light source (Luminar Ace LA50, Hayashi Watch-works Co., Ltd., Japan). The light was reduced by using a blue glass filter (Corning 4-76, USA) for visible region and sharp cut-off filter (Fuji SC-72, Japan) for near-infrared region. Measurements of the spectra were taken 30 s after the start of illumination. The intensity of the illuminating light through the filter and that of the measuring beam were 950 W/m^2 and 100 mW/m^2, respectively.

Flash induced spectra in near-infrared region (700–950 nm) were measured by a flash-photolysis apparatus (Photal RA-412HS, Otsuka Electronics, Japan) with a xenon flash. In order to separate spectral ranges of the measuring and excitation beams, a pair of filter was used: blue glass filter (Corning 4-76, USA) for visible region and Toshiba ITF-50S-83RT (Japan) filter, for near-infrared region. Data of time course kinetics in xenon flash induced spectral change (in the range of 0.1–800 ms after the flash) were transferred to a personal computer and fitted by a double exponential components plus constant background mode by Igor Pro program (Wave Matrics Inc., USA) for curve fitting to estimate the rate and amplitude.

Besides the difference spectra of absorption, the whole absorption spectra of all samples were measured. These spectra were normalized at carotenoid peak located at 496 nm.

The photoacoustic spectra (PAS) were measured with a single beam spectrometer [24] built in our laboratory and equipped with photoacoustic cell (Model 300, MTEC Photoacoustics, Inc. USA). The steady-state PAS of various samples were also normalized at carotenoid 496 nm peak. Samples diameter for PAS measurements was 9 mm.

The additional illumination for measuring PAS of illuminated sample was provided by fiber light guide from 150 W halogen lamp through the glass filter transferring the 350–600 nm spectral range. The intensity of the illuminating radiant beam at 375 nm was 3.4 μm/m^2 s and was much lower than the intensity of the time modulated Xe 1000 W light beam used in PAS apparatus (at 802 nm it was 72 μm/m^2 s). The intensity of additional illuminating light was only about 5% of the intensity of light used for PAS measurements. Therefore, the observed PAS changes due to the additional illumination were small. The thermal diffusion length, it means thickness of the layer from which the heat is able to reach the boundary between sample and active gas, and therefore can contribute to PAS signal, is given by [24]:

$$\mu_s = \sqrt{\frac{k}{\pi \nu_c \rho c}},$$

where ν_c is frequency of light modulation, k the thermal diffusion coefficient, ρ the material density, and c the specific heat of the sample. The evaluated thermal diffusion length at the frequency of modulation used (40 Hz), was about 2×10^{-5} m, while the sample thickness was $(1.0 \pm 0.2) \times 10^{-4}$ m.

Our samples are embedded in gel, therefore, the eventual oxygen evolution does not overlap on thermal effects. This gives us opportunity to measure the PAS at rather low frequency of light modulation (40 Hz). At low frequency, the PAS apparatus is more sensitive than at high frequencies usually used for photosynthetic samples [25].

The relative changes in PAS signal ($PAS_{il} - PAS_d/PAS_{il}$; where PAS_{il} is the photoacoustic signal with additional illumination, PAS_d the PAS signal without additional illumination) was established from 50 dark–light measurements and were averaged for maxima 802 nm and 852 nm.

3. Results

3.1. Spectra without additional illumination

3.1.1. Absorption spectra

Fig. 1 presents the absorption spectra of investigated samples. Maxima of BChl *a* located in LH2 occur at 802 and 852 nm, respectively. RC maxima (Fig. 1(B)) are located at 802 nm (monomeric BChl form) and at 862 nm (special BChl pair). The maximum at about 800 nm exhibits higher intensity than at 862 nm. Therefore, the ratio of 802 nm to 852 nm maxima is increasing with the increase of RC content (Table 2). At 862 nm special pair of RC is

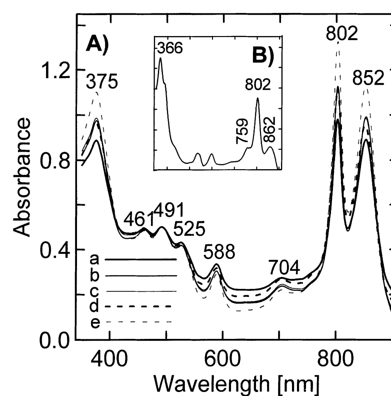

Fig. 1. (A) Absorption of measured samples (described in Table 1); (B) Absorption of reaction centres.

Table 2
The ratios of absorption (A) maxima and photoacoustic (PAS) maxima in various samples

Sample	Composition	A_{802}/A_{852}	PAS_{802}/PAS_{852}
A	LH2	1.10	0.619
B	LH2 + RC	1.13	0.634
C	LH2 + 2RC	1.13	0.665
D	LH2 + 4RC	1.17	0.660
E	LH2 + 8RC	1.18	0.609

absorbing, therefore, one can predict that the long wavelength maximum could be more sensitive to photo-oxidation than the short wavelength maximum at 802 nm. The Q_x maxima of all forms of pigments are located at about 588 nm. This band is rather broad because it contains the contributions from both types of LH2 chromophores as well as from RC chromophores. The carotenoids maxima are located at 461, 491 and 525 nm. The main maximum of Soret band is observed at 375 nm. It is known that exact locations of all maxima depend on the type of strain [26] and additionally, in our sample, can also be changed by complexes immobilized in gel. As seen from Fig. 1 the absorption spectra of all samples are, in first approximation, similar. This is due to the higher number of chromophores (Table 1) in LH2 (24 BChl *a* molecules) than in RC (4 BChl *a* molecules). The exact ratio of the 852 nm to 802 nm maxima in LH2 of investigated bacteria depends on the type of strain [26]. It shows that the ratio of the number of 802 nm chromophores to that absorbing at 850 nm can be different. It can also be changed by the light condition during culturing.

3.1.2. Photoacoustic spectra

The embedding of the sample in the gel preserves the observed eventual effect of oxygen evolution caused by modulated illumination of the sample in photoacoustic apparatus. Therefore, all changes in PAS signal are predominantly due to the change in the yield of the thermal deactivation related to the energy storage in some photochemical reactions or energy transfer to other chromophores.

Fig. 2 shows the PAS spectra. The PAS spectra for different samples are even more similar between themselves than the absorption spectra (Fig. 1). Therefore, only the spectra for sample A (without RC) and sample E (for highest RC content) are shown in Fig. 2. As seen from Table 2 the ratio of the maximum at 802 nm to that at 852 nm is changing in an irregular way, but for all samples the intensity of long wavelength maximum is higher than the intensity at 802 nm. The PAS spectra differ from the absorption spectra predominantly in the following points:

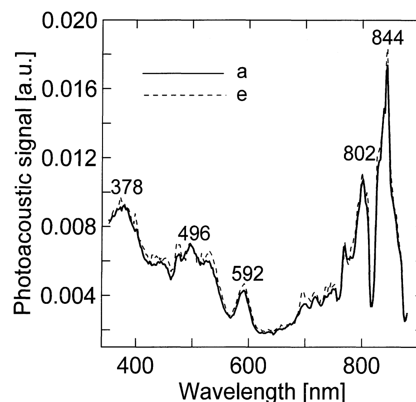

Fig. 2. Photoacoustic spectra of samples A LH2 alone and LH2 with highest content of RC (described in Table 1).

1. The ratio of the Soret band to the red band is much lower than in absorption spectra. It shows that the excitation energy internal conversion from second excited singlet state S_2 to S_1 is strongly competing with TD from S_2 to S_1. Large part of the energy is converted from S_2 to S_1 and after this process it is efficiently converted into heat contributing to the red bands' thermal effects. Of course the energy difference between S_2 and S_1 is also converted into heat.
2. In the PAS spectrum the ratio of signal at 850 nm region to that at about 800 nm is much higher than the ratio of the same absorption maxima (Figs. 1 and 2 and Table 2). It shows the existence of rather efficient excitation energy transfer from 802 nm form to that absorbing at 856 nm. This energy transfer is competing with TD in 802 nm chromophores. For special pair of the RC (absorbing at 862 nm) TD process is competing with photoreaction occurring in this special pair which should cause a decrease in TD in this long wavelength region but the number of RC chromophores is rather low (Table 1). A similar ratio of PAS maxima is observed for LH2 alone (curve a) which shows that the yields of TD of 802 nm and 852 nm LH2 chromophores have to be strongly different. It suggests efficient excitation energy transfer between 802 nm and 852 nm chromophores or/and that these long wavelength chromophores of LH2 have lower yield of fluorescence or higher resistance to photochemical reaction than chromophores absorbing 802 nm.

3.2. Spectra of illuminated samples

3.2.1. Absorption at continuous illumination

The difference absorption spectra of samples located in both spectrophotometer beams are small and not regular (not shown) whereas the differences between samples with additional illumination and 'dark' sample are seen well (Fig. 3). The ratio of changes at 852 nm to that at 802 nm maxima is close to unity for LH2 alone (sample A) and decreases with the increase in RC content till the last sample in which it increases a little once again. It seems that this effect is only partially due to the effect observed previously for the same

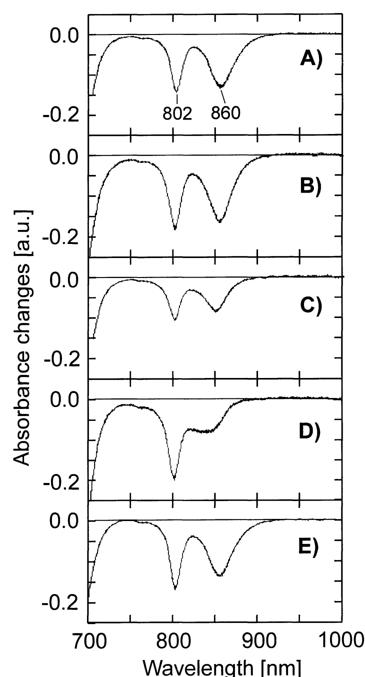

Fig. 3. Absorption changes due to continuous illumination of various samples.

RC [27] and related to special pair photo-oxidation changing the long wavelength maximum. From the results of sample A it follows that LH2 also have contribution to absorption photo-changes which can be related to the RC traces in the sample. The amplitude of absorption changes with RC content are complicated: at low RC content they increase at both maxima, later decrease (sample C) and increase again (samples D and E). Also the ratio of 802 nm to 860 nm peaks changes in a complicated way.

Observed changes are due to following effects: (1) protection of LH2 chromophores by excitation transfer to RC, (2) decrease in RC absorption caused by special pair photo-oxidation and (3) the increase with the increase in RC amount, the contributions to absorption and absorption changes from their chromophores. Also some influence can have the photoreactions occurring in traces of RC connected with LH2. These effects are superposed on absorption changes of LH2.

From Fig. 3 it follows that both maxima of LH2 complexes are undergoing some photochemical changes which are dependent on RC presence. It suggests that excitation can be transfered from LH2 to RC. The changes are shown in near-infrared region (from 700 nm till 1000 nm). Different sensitivities of these two maxima on photoreactions are in agreement with the stronger TD of 860 nm than that at 802 nm (Fig. 2).

The usage of energy in photochemistry on special pair of 850 nm region is shielded by the difference in properties between the two maxima of LH2 complexes. The continuous light illumination is therefore less suitable for the distinction between photoreactions in RC and in antenna than flash

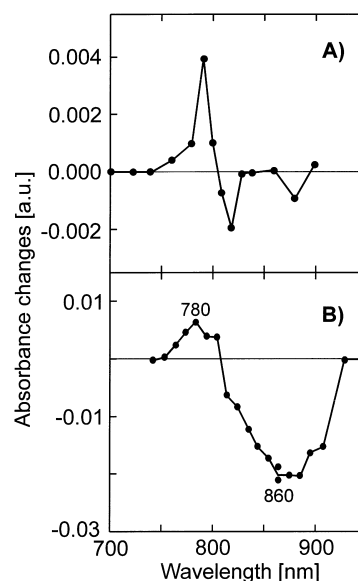

Fig. 4. Absorption changes due to flash illumination: (A) sample A – LH2 only, (B) sample E – LH2 with highest content of RC.

illumination. In flash illumination the observed absorption changes in LH2 and in RC can decay with different kinetics.

3.2.2. Absorption changed by flash illumination

Fig. 4 shows the absorption changes due to flash illumination for the sample with LH2 only (Fig. 4(B)) and for a sample with highest used RC content (Table 1). As we can see, the changes for LH2 complexes alone are much smaller than for the same complexes with RC addition (Fig. 4(B)). From Fig. 4(A) and Fig. 3 it follows that even chromophores of the antenna are undergoing some photochemical changes as a result of illumination. These changes seem to decay very quickly in time because they are much better seen at steady-state measurements (Fig. 3) than as a result of short flash illumination (Fig. 4). These small changes are observed at both IR regions (Fig. 4(A)). Since, in such systems spectral band can undergo also electrochemical shifts as a result of charge generated by the photo-reaction, it is not easy to propose a right interpretation of these small changes. The changes due to flash illumination of the sample with RC can be more easily explained. The negative changes in the 800–900 nm region are due predominantly to RC photooxidation (Fig. 4(B)) because they are much larger than for LH2 alone (Fig. 4(B)). Xenon flash induced spectra in Fig. 4(B)) shows a typical shape of flash induced spectrum of RC, the same as the spectrum in our previous paper [11]. This spectrum is a superposition of bleaching at 867 nm caused by light induced $BChl_2$ oxidation and Stark shift (potential induced band shift) of BChl and that of BPh in shorter wavelength region (760–840 nm). Spectral changes derived from LH2 did not contribute so much to this spectrum. The differences in the changes at continuous and flash illumination can be due to different kinetics of generation and decay of photo-processes in RC and antenna.

Table 3
Relative changes in PAS value due to additional illumination

λ_{PAS} (nm)	Sample	A: LH2	E: LH2 + 8RC	Chl*a*
802	$\Delta PAS/PAS_{il}$	0.15 ± 0.01	0.15 ± 0.01	–
	PAS_{il}	$(5.35 \pm 0.06) \times 10^{-3}$	$(5.72 \pm 0.06) \times 10^{-3}$	–
852	$\Delta PAS/PAS_{il}$	0.20 ± 0.03	0.28 ± 0.02	–
	PAS_{il}	$(5.66 \pm 0.14) \times 10^{-3}$	$(6.18 \pm 0.09) \times 10^{-3}$	–
670	$\Delta PAS/PAS_{il}$	–	–	0.08 ± 0.01
	PAS_{il}	–	–	$(2.76 \pm 0.03) \times 10^{-3}$

$\Delta PAS = PAS_{il}\text{-}PAS_{d}$. il – illuminated, d – dark.

3.2.3. PAS changes for continuous illumination

As the changes are small, they have been measured only for sample with LH2 complexes alone (sample A) and for sample with highest RC content (sample E – Table 1). To have reproducible results the dark and light period measurements were repeated 50 times and averaged. The dark period was 12 s, followed by the light period also 12 s. The results are gathered in Table 3. The energy storage in a sample defined as $(PAS_{il} - PAS_{d})/PAS_{il}$ (where PAS_{il} and PAS_{d} are photoacoustic signal with and without additional illumination, respectively) [12], was calculated for both maxima at 802 and 852 nm. As seen from Table 3 the relative changes in 802 nm band in both samples are similar. It shows that they are predominantly due to LH2 chromophores. But different is the situation at 852 nm. In this region the changes in PAS are stronger in sample with RC than for LH2 alone. But also for LH2 alone the relative PAS changes stronger at 852 nm than at 802 nm. It is in contradiction with the supposition that chromophores at 802 nm are more sensitive for photoreactions than 852 nm chromophores. Therefore, the low steady-state PAS of 802 nm (Fig. 2) has to be due rather to efficient ET between both types of chromophores. Highest changes at additional illumination are observed in 852 nm region for a sample with RC. The difference between these changes and the changes in this region by LH2 chromophores is due to the energy storage in special pair. In order to check if the observed changes in our samples are not given by some photochemistry in chlorophyll separated from complexes, the same experiment was done for Chl *a* solution in nematic liquid crystal. The effect was much lower than for LH2 (Table 3). It is also not due to any apparatus fault because the black body located in the same conditions gives very low effect, practically in the limits of accuracy.

4. Conclusions

The results obtained in our model system suggest following conclusions:

1. The strong changes observed in 852 nm absorption region are dependent on RC presence. The changes for LH2 complexes observed for the same region are much smaller and can be partially due to traces of RC in the sample.
2. The changes in PAS of illuminated samples suggest that not only in plants but also in bacteria the energy storage in RC are changed by strong illumination. This effect is stronger in the case of the RC presence in a sample, but measurable changes in relative PAS values are also occurring in LH2 chromophores.
3. The TD values of 802 nm and 852 nm maxima show efficient excitation energy transfer between the chromophores in LH2.
4. Because of superposition of LH2 and RC absorption maxima and very low intensity of fluorescence of investigated samples it was not possible to draw quantitative conclusions concerning ET between LH2 and RC in our model system. The observed effects in PAS and absorption strongly suggest efficient excitation energy transfer occurring in investigated model system.
5. The energy storage in RC was low, but the intensity of incident light was also low. The changes can be seen because of the method used for averaging the signal from several light–dark periods.

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