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# Thermal deactivation of excitation in bacterial reaction centres embedded in gel

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**Abstract**

Bacterial photosynthetic reaction centres from *Rhodobacter sphaeroides* strain (R-26) were immobilized in polyacrylamide gel. The samples of RC in gel were incubated with reducing–oxidizing agents in order to reach four various redox potentials of samples and then they were dried in vacuum to fix the obtained redox states of chromophores of reaction centres. For such samples the absorption and photoacoustic spectra were measured with and without additional illumination. The illumination caused the changes in absorption and in photoacoustic spectra. The changes depend on redox potential of the sample and spectral region. The yield of thermal deactivation for various bacteriochlorophyll and bacteriopheophitin bands was different what suggests that the contributions to various bands from the pigment forms characterized by different yield of photochemical reaction are different. The electron transfer process caused by illumination and thermal dissipation of excitation are mutually competitive processes. The chromophores engaged in electron transfer are less effective in thermal deactivation of excitation. Electron back transfer from quinone to photo oxidized special pair of reaction centre embedded in gel occurs in about 0.1 s, for all investigated redox states. 

*Keywords:* Light-induced absorption changes; Photoacoustic spectra; Reaction centres; *Rhodobacter sphaeroides*

## 1. Introduction

It was shown [1] that it is possible to control the redox state of reaction centres (RC) immobilized in dried polyacrylamide gel previously incubated with reducing or oxidizing agents.

Using the set of samples consisting of RC from *Rhodobacter sphaeroides* (*Rh. sphaeroides*) embedded in gels of different redox potentials we compare the effect of additional illumination on absorption and photoacoustic spectra. Occurring as a result of illumination the electron transfer reaction, shown as the change in absorption of giving chromophore, is competing with thermal deactivation of exciting light, therefore, the photothermal data provide additional information about the fate of the excitation of chromophore in various redox states.

The effect of charge separation and transfer occurring in photosynthetic RC is not only the crucial part for the life process of photosynthesis but also, as it was lately shown [2,3], there are several attempts to use this process in photoelectrical devices and in biological sensors. For such applications the proper arrangement of RC is important, what can be reached using Langmuir–Blodgett monolayer deposition method [4].

The RC separated from *Rhodobacter sphaeroids* strain R-26 (carotenoid less) contains four bacteriochlorophyll *a* (BChl) molecules, two bacteriopheophitin *a* (BPh) molecules and quinons ($Q_A$ and $Q_B$) [5].

The optical changes due to the reduction of pigments were widely reported in literature [1,6,7].

## 2. Material and methods

The RC from *Rhodobacter sphaeroides* strain R-26 were separated and purified according [8].

10 μM RC in 10 mM Tris–HCl (pH = 8.2) and 0.06% (W/V) lauryldimethylamine-N-oxide (LDAO) buffer was immobilized in a flat 5% (W/V) polyacrylamide gel. A

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*Abbreviations:* BChl, bacteriochlorophyll; BPh, bacteriopheophitin *a*; Eh, redox potential; PAS, photoacoustic spectrum; Q, quinon; RC, reaction centres; *Rh. sphaeroides*, *Rhodobacter sphaeroides*

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Table 1
The values of redox potentials $E_h$ of *Rhodobacter sphaeroides* reaction centres immobilized in polyacrylamide gel

| No. | Redox agent | Ambient $E_h$ (mV) |
|---|---|---|
| 1 | 1 mM K-ferricyanide ($Fe^{3+}$) | 508 |
| 2 | 1 mM K-ferri/ferrocyanide ($Fe^{3+} : Fe^{2+} = 3 : 1$) | 439 |
| 3 | 1 mM K-ferri/ferrocyanide ($Fe^{3+} : Fe^{2+} = 1 : 3$) | 386 |
| 4 | 0.5 mM Na-ascorbate | 142 |

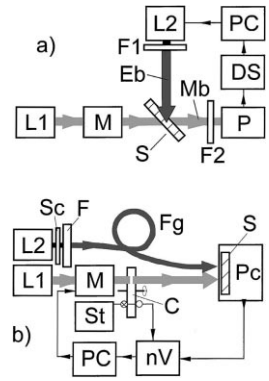


Fig. 1. (a) Scheme of flash photolysis apparatus. Mb – measuring beam, L1 – lamp, M – monochromator, S – sample, F1, F2 – filters, DS – digital storage scope, P – photomultiplier, L2 – flash lamp, PC – personal computer, Eb – excitation beam. (b) Scheme of single beam photoacoustic spectrophotometer. L1 – lamp, M. – monochromator, C – chopper, St – stabilizer of chopper, PC – personal computer, nV – nanovoltmeter, Pc – photoacoustic cell, S – sample L2 – lamp (additional illumination, Sc – shutter, F – filter.

piece of gel was incubated for 1 h at room temperature in a sealed test tube with the incubation solution under nitrogen atmosphere. As incubation solutions the 10 mM Tris–HCl with 1 mM redoxy agent (Table 1) were used. The redox potential of the solution ($E_h$) was measured before and after incubation by platinum electrode [9]. The values of redox potentials $E_h$, obtained as it was described in [1], are summarized in Table 1. The incubated gel was sandwiched between two cellophane sheets and dried in vacuum using a conventional gel drier. The thickness of the samples after gel drying was $(1.0 \pm 0.2) \times 10^{-4}$ m, and RC concentration was about 100 μM.

Spectra of continuous light-induced absorbance changes were measured by a specially designed spectrophotometer with a cross illumination (JASCO, Tokyo, Japan) [1]. Continuous light-induced absorption changes were recorded in visible (400–625 nm) and near infra-red (700–1100 nm) region, while continuous illumination was applied at red and Soret band region, respectively. Intensities of excitation light were: 950 W $m^{-2}$ (for 400–625 nm) and 140 W $m^{-2}$ (for 700–1100 nm), while intensity of measuring beam was 0.1 W $m^{-2}$ (at 550 nm).

Flash-induced absorption spectra were measured by Photal RA-412HS (Otsuka Electronics, Tokyo, Japan). Intensity of measuring beam was 0.14 W $m^{-2}$. Xenon flash lamp intensity was applied to obtain half saturation intensity (50% of RC was bleached). The arrangement used for flash photolysis is shown in Fig. 1(a). Time course kinetics data of flash-induced spectral changes (in the range of miliseconds after the flash) were fitted by a double exponential plus constant background mode by Igor Pro computer program (Wave Matrics, USA) to estimate the rate and amplitude.

In both type of light-induced experiments, measuring and excitation (flash) beams were applied at 45° to the plane of sample. In order to avoid the artifacts due to superposition of acting and measuring beams the filters Corning 4-76 and Toshiba (ITF-50N-76IR) were used.

Besides the difference spectra of absorption also the whole absorption of all samples were measured.

The photoacoustic (PAS) spectra were measured with a single beam spectrometer build in our laboratory and equipped with photoacoustic cell (Model 300, MTEC Photoacoustics, USA). The intensity of modulated xenon 1000 W light beam was 72 μmol $m^{-2}$ $s^{-1}$ at 802 nm. The scheme of photoacoustic spectrometer is shown in Fig. 1(b). 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 (Fig. 1(b)). The acting radiant beam intensity at 375 nm was 3.4 μmol $m^{-2}$ $s^{-1}$. The intensity of additional acting 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 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 [10]:

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

where $\nu_c$ is the frequency of light modulation, $k$ is the thermal diffusion coefficient, $\rho$ is material density, and $c$ represents special heat of sample. Evaluated thermal diffusion length, at used frequency of modulation 40 Hz, is about $2 \times 10^{-5}$ m, while sample thickness is about $1.0 \times 10^{-4}$ m. The relative intensities (intensity ratio of BChl *a* $B_y$ at 386 nm to intensity at other maxima) for PAS and absorption spectra were calculated. Such calculations enable to evaluate the contributions from various chromophore transition moments to the absorption and to the thermal deactivation processes. The direct calculation of the yield of thermal deactivation for the samples changing absorption as a result of illumination, especially at different conditions of additional illumination in photoacoustic and absorption apparatus is not possible.

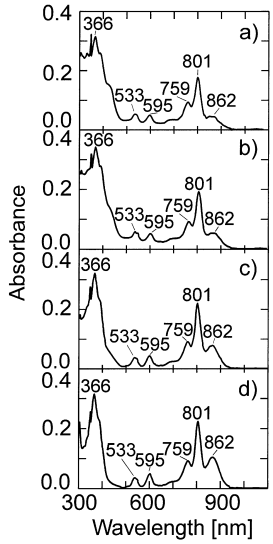


Fig. 2. Absorption spectra of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel (without additional illumination). Samples with various redox potentials ($E_h$): (a) 508 mV, (b) 439 mV, (c) 386 mV, (d) 142 mV.

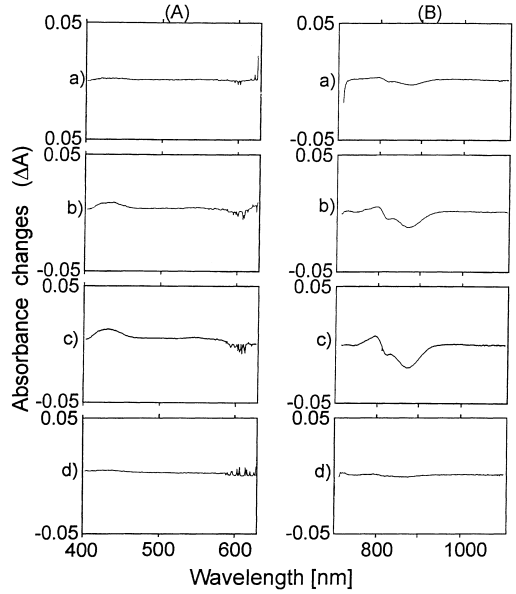


Fig. 3. The changes in absorption spectra of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel, occurring at additional continuous light illumination. The changes were recorded in: (A) visible (400–625 nm) and (B) near infra-red region (700–1100 nm). Samples with various redox potentials ($E_h$): (a) 508 mV, (b) 439 mV, (c) 386 mV, (d) 142 mV.

## 3. Results and discussion

Fig. 2 presents the absorption spectra of all investigated samples measured without additional illumination. The characters of absorption spectra of samples with various redox potentials are similar. Only the $(BChl)_2$ peak at about 860 nm decreases with the increase in $E_h$. A small shoulder peak at 420 nm were shown in Fig. 1(a) and (b) but not in (c) and (d). This is thought to be derived from ferricyanide remained in the sample gel. Other peak intensities are not changing strongly.

Table 2 gathers the band positions in absorption and in photoacoustic spectra for various BChl and BPhe chromophore transitions. PAS maxima are shown with and without additional illumination. As it follows from Table 2 the illumination causes only slight shifts of PAS band with exception of the band belonging to 'special pair' $(BChl)_2$ which at $E_h = 508$ and 439 mV exhibits 6 nm long wavelength shift and of the $Q_x$ band of BPh at 386 mV.

Fig. 3 shows the changes in absorption spectra occurring at continuous illumination. The changes were recorded in visible (400–650 nm) and in near infra-red region (700–1100 nm) for samples with various $E_h$. Light-induced absorption changes are rather low, probably special pair is already partially oxidized before additional illumination. Negative peak at 866 nm belongs to the light-induced oxidation of $(BChl)_2$. A negative shoulder at 815 nm and positive asymmetrical peak at 792 nm can be due to some electrochromic shift of BChl and BPh bands from 803 to 815 nm and from 759 to 792 nm, respectively. Several mechanisms can be responsible for the creation of local

Table 2
The absorption and photoacoustic band positions for various BChl and BPhe chromophore transitions, of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel, with various redox potentials ($E_h$)

| $E_h$ (mV) | $B_Y$ (BChl) | $Q_X$ (BPh) | $Q_X$ (BChl) | $Q_Y$ (BPh) | $Q_Y$ (BChl) | $Q_Y$ $(BChl)_2$ |
|---|---|---|---|---|---|---|
| *Absorbance* | | | | | | |
| 508 | 366 | 533 | 595 | 759 | 801 | 862 |
| 439 | 366 | 533 | 597 | 759 | 802 | 862 |
| 386 | 366 | 534 | 598 | 759 | 803 | 863 |
| 142 | 367 | 534 | 598 | 760 | 804 | 862 |
| *PAS* | | | | | | |
| 508 | 368 (368) | 536 (536) | 596 (594) | 754 (756) | 798 (798) | 854 (860) |
| 439 | 368 (368) | 536 (536) | 596 (594) | 754 (756) | 798 (798) | 854 (860) |
| 386 | 368 (368) | 534 (540) | 596 (594) | 756 (754) | 798 (800) | 856 (856) |
| 142 | 368 (366) | 536 (540) | 598 (594) | 756 (754) | 798 (796) | 856 (862) |

Data in brackets – PAS maxima with additional illumination.

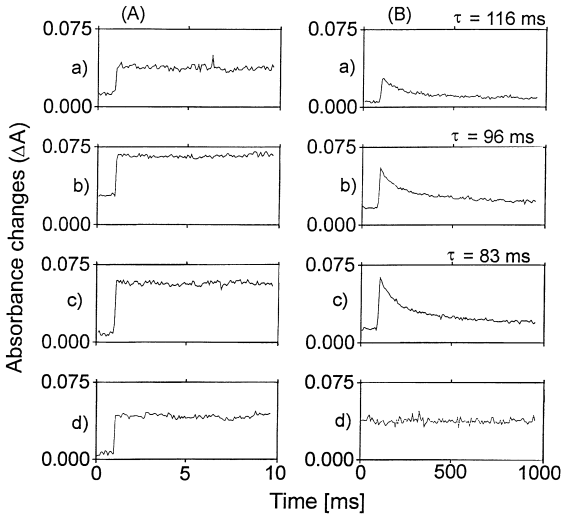


Fig. 4. The absorption changes of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel at wavelength 450 nm due to flash illumination. The changes were recorded in: (A) 10 ms time scale and (B) 1 s time scale. Samples with various redox potentials ($E_h$): (a) 508 mV, (b) 439 mV, (c) 386 mV, (d) 142 mV. All data were averaged from five sets of results. The decay time ($\tau$) are given in the figure.

electric field in such complicated systems as pigment–protein complexes with ions addition [11–13]. Very small changes at 142 mV are caused by quick reduction of oxidized $(BChl)_2$ by ascorbate present in this sample.

Figs. 4 and 5 show the absorbance changes due to flash illumination. As it follows from Fig. 2 the absorbance of all samples are similar. Therefore, the data were not normalized on total absorption values. The data were represented in full time scale 10 ms (Fig. 4(A) and Fig. 5(A)) and 1 s

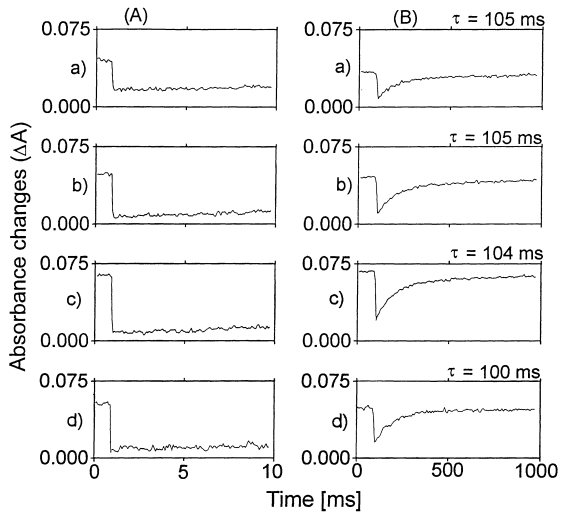


Fig. 5. The absorption changes of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel, at wavelength 867 nm due to flash illumination. The changes were recorded in: (A) 10 ms time scale and (B) in 1 s time scale. Samples with various redox potentials ($E_h$): (a) 508 mV, (b) 439 mV, (c) 386 mV, (d) 142 mV. All data were averaged from five sets of results. The decay time ($\tau$) is given in the figure.

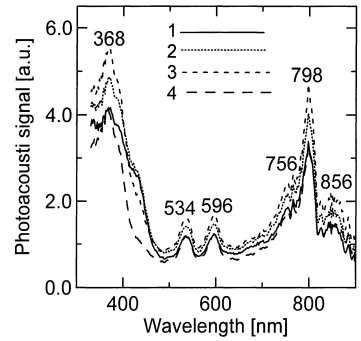


Fig. 6. The photoacoustic spectra of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel without additional illumination. Samples with various redox potentials ($E_h$): (a) 508 mV, (b) 439 mV, (c) 386 mV, (d) 142 mV.

(Fig. 4(B) and Fig. 5(B)). The changes were measured at 450 nm (Fig. 4) and at 867 nm (Fig. 5), it means in the region of $(BChl)_2$ absorption changes due to photooxidation. All data were averaged from five sets of results. The absorption changes are negative at 867 nm and positive at 450 nm. The decrease in time in such changes is due to back transfer of electron from quinone $Q_A$ and $Q_B$ to light-oxidized $(BChl)_2$. The decay of changes can be represented by the formula:

$$A = A_0 + k_1 \exp(-k_2) \quad (1)$$

where $A_0$ is a background and $k_2$ is monoexponential decay. The rate $k_2$ is in both spectral region at 450 nm and at 867 nm about 10 $s^{-1}$. This value agreed to the rate (8.7 $s^{-1}$) of electron back transfer from $Q_A^-$ to $(BChl)_2^+$ as reported for RC in solution [14]. It seems that, for sample immobilized in a gel, the time of electron back transfer to oxidized special pair is about 0.1 s.

Fig. 6 presents the PAS of samples without additional illumination whereas Fig. 7 presents PAS of the same samples taken during additional illumination.

As it follows from Table 3 the ratios of PAS maxima measured with and without additional illumination are

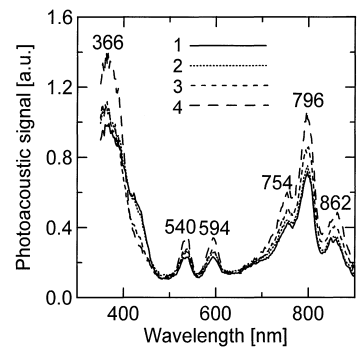


Fig. 7. The photoacoustic spectra of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel with additional illumination. Samples with various redox potentials ($E_h$): 1 – 508 mV, 2 – 439 mV, 3 – 386 mV, 4 – 142 mV.

Table 3
The ratios of Soret $B_Y$(BChl) band to various BChl and BPhe chromophore transitions maxima for absorption and PAS, of *Rhodobacter sphaeroides* strain (R-26) reaction centres immobilized in polyacrylamide gel, with various redox potentials ($E_h$)

| $E_h$ (mV) | $B_Y$ (BChl)/$Q_X$ (BPh) | $B_Y$ (BChl)/$Q_X$ (BChl) | $B_Y$ (BChl)/$Q_Y$ (BPh) | $B_Y$ (BChl)/$Q_Y$ (BChl) | $B_Y$ (BChl)/$Q_Y$ (BChl)$_2$ |
|---|---|---|---|---|---|
| *Absorbance* | | | | | |
| 508 | 5.98 | 6.60 | 3.41 | 1.82 | 7.20 |
| 439 | 5.38 | 5.93 | 3.54 | 1.77 | 5.56 |
| 386 | 7.75 | 6.74 | 3.33 | 1.45 | 3.83 |
| 142 | 9.48 | 6.66 | 3.48 | 1.43 | 3.04 |
| *PAS* | | | | | |
| 508 | 3.49 (4.28) | 3.36 (4.25) | 2.24 (2.31) | 1.23 (1.38) | 2.89 (2.99) |
| 439 | 3.46 (4.05) | 3.31 (4.18) | 2.24 (2.41) | 1.21 (1.43) | 2.74 (3.09) |
| 386 | 3.48 (3.83) | 3.29 (3.95) | 2.31 (2.11) | 1.18 (1.17) | 2.62 (2.64) |
| 142 | 3.50 (4.03) | 3.38 (4.09) | 2.39 (2.32) | 1.25 (1.30) | 2.79 (2.69) |

Data in brackets – PAS ratios maxima with additional illumination.

different and they are also different for the various redox potentials of the samples. These changes are caused by two reasons: (1) the change in thermal dissipation efficiency of chromophore and (2) by the change in absorption values due to sample illumination.

## 4. Conclusions

On the basis of the results presented it is possible to the draw following conclusions:

1. The positions and intensities of the absorption maxima of the samples with different redox potentials are similar, only the $(BChl)_2$ peak decreases with $E_h$ increase (Fig. 2).
2. The changes in absorption, observed predominantly in a region of special pair absorption, are due to sample illumination causing photo oxidation of ($BChl_2$). This effect depends on redox potential of the sample (Figs. 3–5).
3. Small shifts between PAS and absorption maxima positions, occurring for samples without additional illumination (Table 2), show that most of bands contribute slightly different chromophores (located in various surroundings) characterized by different thermal deactivation efficiency but similar absorption. The superposition of PAS bands of such pools of chromophores can be responsible for a shift between absorption and PAS maxima.
4. The relative intensities of absorption bands and PAS bands are strongly different. It shows that various chromophores dissipate the excitation energy with different efficiencies (Table 3).
5. The dependence of the maxima ratios on redox potential are stronger in absorption than in PAS. As it was possible to predict the strongest absorption changes with $E_h$ are observed in $(BChl)_2$ spectral region (Table 3).
6. The ratios of PAS maxima of the samples with and without additional illumination are different. This observation supports the supposition that there are different pools of chromophores located in different surroundings undergoing different light reactions (Table 3).

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