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The polarized photoacoustic spectra of *Rhodobacter sphaeroides* cells embedded in polymer and strongly irradiated by polarized radiation

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Abstract

The cells of purple photosynthetic bacterium *Rhodobacter sphaeroides* embedded in stretched polymer films were irradiated by strong polarized "white light" with an electric vector parallel to the direction of film stretching. The polarized absorption and photoacoustic spectra before and after strong irradiation were measured. Measurements of absorbance showed no confident anisotropy before and after strong irradiation. In contradiction, the photoacoustic method showed after strong irradiation some changes in anisotropy of thermal deactivation due to the perturbation of the fate of excitations. The increase in yield of thermal deactivation, higher in a region of light-harvesting complex 2, can be explained by the irreversible changes in the conformation of the complexes due to strong irradiance reported up to now predominantly for thylakoid antenna complexes.

Additional key words: absorption; bacteriochlorophyll; carotenoids; chromophore; light-harvesting complex; purple photosynthetic bacteria; thermal deactivation.

Introduction

The structure and related to it excitation energy transfer (ET) of photosynthetic purple bacteria were already established (McDermott *et al.* 1995, Sundström and Grondelle 1995). Strong reversible changes in the conformation of antenna complexes were up to now observed predominantly by circular dichroism spectra of irradiated thylakoid samples (Garab and Mustárdy 1999). We applied a different technique: polarized absorption and photoacoustic spectra (PAS) of oriented, strongly irradiated purple bacteria cells. Havaux *et al.* (1991) and Barzda *et al.* (1995a) showed that some conformational changes of pigments are associated with a change in excitation electron transfer (ET) and increase in thermal dissipation of radiant energy. This could be a mechanism preventing the destruction of the antenna system occurring as a result of too strong irradiance (Gruszecki *et al.* 1990, Barzda *et al.* 1995a,b).

On the basis of polarized absorption and photoacoustic (PAS) spectra it is possible to find the orientation and role of various chromophores in the photosynthetic apparatus of organisms (Frąckowiak *et al.* 1986, 1995, Cegielski *et al.* 1992). The strong polarized irradiation can perturb natural function of dye molecules with transition moments (TMs) parallel to the direction of electric vector of acting radiation. The comparison of polarized spectra taken before and after strong irradiation can give information about the role of various chromophores in the photosynthetic apparatus. In many cases anisotropy of PAS is larger than that of absorption which gives the opportunity to evaluate the orientation of various forms of chromophores. The change in efficiency of ET in a chain of pigments that transport excitation to reaction centres (RC) strongly influences the yields of pigment thermal deactivation (TD) (Cegielski *et al.* 1992, Enomoto *et al.* 2000). In this paper we investigated the influence of strong polarized irradiation on spectral properties of oriented cells of the purple photosynthetic bacterium *Rh. sphaeroides* and wanted to find whether the treatment causes irreversible changes in the spectral properties of dyes.

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Abbreviations: BChl *a* – bacteriochlorophyll *a*; Car – carotenoid; ET – energy transfer; LHC – light-harvesting complex; PAS – photoacoustic spectra; PVA – polyvinyl alcohol; RC – reaction centre; TD – thermal deactivation; TM – transition moment.
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Materials and methods

Rb. sphaeroides was cultured anaerobically in a culture medium at "white light" of 20 mW m^{-2} at 28 °C (Drews and Giesbrecht 1966). Whole bacteria were sonicated for 15 min at 4 °C and centrifuged at 36 000 × *g* for 30 min. Bacterial suspension was introduced into an aqueous solution of polyvinyl alcohol (PVA, *Sigma*, USA) mixed with resin *AG1-XB* (*Bio-Rad Lab.*, USA) (Martyński *et al.* 1998), poured onto glass substrate, and dried for 24 h. Then the PVA films were stretched uniaxially in 100 % humidity up to 300 % of its initial length (Fiksiński and Frąckowiak 1980). Such procedure enabled to obtain stretched PVA films with whole bacteria dispersed homogeneously in volume of polymer foil. Both absorption spectra and polarized PAS were taken for the sample before and after additional strong polarized irradiation.

Strong irradiation was carried out by polarized (through *ADP Glan-Thompson* polarizer, *Carl Zeiss*, Jena, Germany) xenon radiation source (*XBO 101*, 100 W, *Carl Zeiss*, Jena, Germany) during one hour. Irradiance of 300 W m^{-2} was selected after many experiments. Irradiance should to be strong enough to perturb ET in the bacterial organism, but should not, in a measurable degree, denature the pigments. The direction of electric vector of acting polarized radiation was parallel to stretching axis of the sample. During irradiation, the sample was cooled down to room temperature. The samples before and after additional strong irradiation were called irradiated and non-irradiated.

Absorption spectra of the samples were obtained using an *M40* spectrophotometer (*Carl Zeiss*, Jena, Germany) equipped with polarizers (*M*: 350-660 nm and *IR*: 660-920 nm; *Carl Zeiss*, Jena, Germany). Polarized absorption measurements were done for the electric vector of measuring beam (E) forming 0°, 30°, or 90° angle with the direction of film stretching axis.

Polarized PAS were measured using a single beam spectrometer built in our laboratory, similar to that described by Ducharme *et al.* (1979), equipped with polarizers (*M*: 350-660 nm and *IR*: 660-920 nm, *Carl Zeiss*, Jena, Germany). Spectra were measured at a frequency of modulation of f = 10 Hz and were corrected for the spectral distribution of the lamp using carbon black as a reference. Measurements were done with electric vector of exciting beam that formed angles 0°, 30°, and 90° with the direction of film stretching axis.

Polarized absorption spectra as well as polarized PAS of whole cells of *Rb. sphaeroides* embedded in stretched PVA films were measured for non-irradiated and irradiated samples. PAS were corrected for the apparatus function for both visible (350-660 nm) and IR (660-920 nm) range of wavelengths. All spectroscopic measurements were carried on at room temperature (295±1 K).

TD was calculated by dividing the PAS signal by absorbance:

$$TD = \frac{PAS}{ABS} \quad (1)$$

where ABS and PAS are absorbance and PAS, respectively. This parameter in approximation has a meaning of thermal deactivation because applied values of absorbance are proportional to energy absorbed by the sample. All spectral measurements with polarized radiation were done in the same conditions therefore TD was always proportional to the part of energy exchanged into heat. Thermal deactivation defined by Eq. (1) is very useful to compare the fate of radiant energy conversion of each chromophore group.

The anisotropy of polarized absorption S(A) and photoacoustic S(P) spectra was calculated according to the formulae:

$$S(A) = \frac{S(A)_{II} - S(A)_{\perp}}{S(A)_{II} + 2S(A)_{\perp}}, \quad (2)$$

$$S(P) = \frac{S(P)_{II} - S(P)_{\perp}}{S(P)_{II} + 2S(P)_{\perp}}, \quad (3)$$

where $S(A,P)_{II}$ and $S(A,P)_{\perp}$ are absorbance or PAS signals measured when electric vector of measuring beam was parallel (0°) and perpendicular (90°) to the film stretching axis.

Preparation, irradiation, and all measurements were done with two reproducibly series of samples. Both series gave similar results, but the presented values correspond to one of them.

Results and discussion

The shape of PAS of whole cells of *Rb. sphaeroides* embedded in stretched PVA films before and after additional strong irradiation (Fig. 1) was typical for whole bacteria. They showed well-resolved LHC2 maxima at 805 and 854 nm as well as a carotenoid (Car) maximum at 499 nm (Clayton and Sistrom 1978). The Soret band transitions B_Y and B_X of various forms of BChl *a* complexes were strongly mutually overlapped. The antenna complex LHC1 875 nm was seen as a shoulder of the complex LHC2 854 nm wide band. The ratio of absorbance maxima of all chromophore forms after and before irradiation did not vary markedly (less than 10 %; Table 1). Thus additional strong irradiation did not induce dramatic denaturation of pigments and photosynthetic units still

efficiently absorbed energy. According to Martyński *et al.* (1998) the bacteria cells and their fragments embedded in stretched PVA films were oriented and as a result the TMs of the chromophores were also usually oriented. The degree of cell orientation depended on the conditions applied during film preparation. In our case when whole bacteria cells were introduced to PVA, the anisotropy of absorbance S(A) of all chromophore forms was relatively low (Table 2). Additional strong polarized irradiation did not change markedly the S(A). For all chromophore forms, S(A) was lower than 0.03, except of region of the Car band at 499 nm where S(A) = –0.04. The comparison of absorbance spectra measured at three configurations of the electric vector of measuring polarized beam (0°, 30°, 90° with respect to the PVA stretching axis), taken before and after additional strong irradiation, did not show large differences (Fig. 1, Table 1). Thus almost the same part of energy was absorbed by various parts of bacterial system independently on applied strong irradiation.

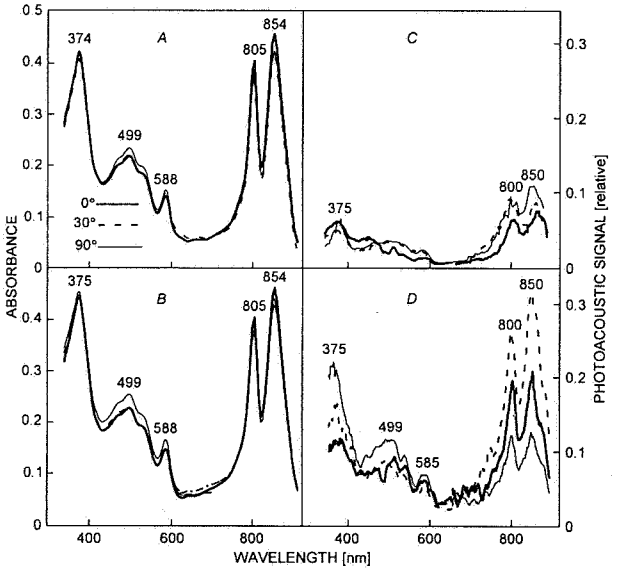

Fig. 1. Polarized absorption (*A, B*) and photoacoustic (*C, D*) spectra of whole cells of *Rb. sphaeroides* embedded in stretched PVA films before (*A, C*) and after (*B, D*) additional strong polarized irradiation. Curves correspond to the measurements with electric vector of measuring beam forming angles of 0°, 30°, and 90° with the direction of film stretching axis.

Fig. 1*C,D* shows PAS of *Rb. sphaeroides* cells embedded in stretched PVA for three directions of exciting polarized radiation, before and after strong irradiation. Strong irradiation caused multiple increase of photoacoustic signal. The ratio of PAS signal after and before additional irradiation depended on the chromophore form (PAS spectral region) and radiation polarization, and varied from 1.02 to 4.40 (Table 1). As shown earlier for another bacteria and their fragments in a model system (Frąckowiak *et al.* 1986, Frąckowiak and Planner 2000), polarized PAS are more sensitive to pigment orientation in polymer matrix than the absorbance spectra. For whole cells of *Rb. sphaeroides* in PVA films the polarized PAS of various pigment forms were much better resolved than absorption spectra (Fig. 1). There were large differences in PAS signal measured at three components of polarized radiation. The anisotropy of photoacoustic signal S(P) was much higher than that of absorbance S(A) (Fig. 1, Table 2). The highest value of S(P) ≈ 0.2 was observed for both LHC2 forms, but additional strong polarized irradiation changed sign of anisotropy to opposite. The ratio of photoacoustic maxima after and before irradiation was almost one for both LHC2 regions at the perpendicular component of polarized radiation while it was over 3 at the parallel one. To the perpendicular PAS component contribute chromophores with TMs not parallel to the electric vector of acting radiation. Chromophores oriented in parallel were perturbed and therefore ET between them was strongly changed. The observed strong increase of parallel component of PAS after irradiation indicated that strong irradiation destroyed some native function of bacterial chromophores in photosynthetic system with TMs parallel to the electric vector of acting radiation. This implicated a more efficient conversion of absorbed radiant energy into heat. But similar to the absorbance spectra, it did not perturb the pigment absorbance functions.

In contradiction to the small changes in a shape of absorbance spectrum due to irradiation, the ratios of various PAS maxima for different BChl forms were changed strongly. For example, the PAS maxima ratio of LHC2 805 to Soret 375 band increased as a result of strong irradiation for radiation component parallel to the stretching axis from 1.04 before irradiation to 1.66 after irradiation. But for the perpendicular component the ratio of LHC2 805 to Car 499 decreased after irradiation from 45.1 to 4.7. Hence Car molecules were less sensitive to perturbation by strong irradiation. Analysis of the Soret 375 as well as Q_x 588 bands did not give definite information because of the strong overlap of various forms. Therefore they were not analysed.

From absorbance and PAS measurements TD was calculated. As could be expected from low changes in absorbance and strong changes in PAS, the characters of TD and PAS changes were similar. Polarized irradiation of samples caused a very high increase of TD of most of the chromophores (Table 3). The highest TD value was observed after irradiation with the 30° component for both LHC2. The ratio of TD before and after irradiation depended on polarization and varied from 1.0 for perpendicular component of LH2 854 to 4.3 for parallel component of Q_X 588. Strong polarized irradiation with the E vector parallel to the PVA stretching direction gave strong increase in TD value of the parallel component whereas it almost did not change TD of the perpendicular

Table 1. The ratio of absorbance $R(A) = ABS_{ir}/ABS_0$ and photoacoustic $R(P) = PAS_{ir}/PAS_0$ maxima measured after and before sample irradiation of whole cells of *Rb. sphaeroides* embedded in stretched PVA films before (0) and after (ir) additional strong polarized irradiation. R(A) correspond to the ABS measurements with electric vector of measuring beam forming angles 0°, 30°, and 90° with the direction of film stretching axis. R(P) correspond to the PAS measurements with electric vector of exciting beam forming angles 0°, 30°, and 90° with the direction of film stretching axis.

Spectra	Angle (form)	B_Y, 375 nm	Car, 499 nm	Q_X, 588 nm	LHC2, 805 nm	LHC2, 854 nm
				R(A)		
ABS	0° (II)	1.06±0.02	1.04±0.04	1.03±0.04	0.99±0.02	1.02±0.02
	30°	1.10±0.02	1.05±0.04	1.00±0.04	1.03±0.02	1.05±0.02
	90° (⊥)	1.09±0.02	1.08±0.04	1.08±0.04	1.00±0.02	1.01±0.02
				R(P)		
PAS	0° (II)	1.9±0.3	3.5±0.6	4.4±0.7	3.0±0.3	3.3±0.3
	30°	3.6±0.4	2.9±0.5	2.2±0.4	2.9±0.3	3.6±0.3
	90° (⊥)	3.4±0.4	3.4±0.6	3.3±0.5	1.2±0.2	1.0±0.2

component. This shows that perturbation by radiation gave the decrease in yield of excitation ET what causes the increase in TD. The values in Table 3 show that perpendicular TMs were almost not disturbed due to the strong irradiation for almost all chromophore groups. The only exception was in the Car region (about 500 nm) where TD after irradiation was also increased about three times, but no evident difference in TD anisotropy was observed. It means that Cars are distributed forming various angles with film stretching axis.

Table 2. Anisotropy of absorbance $S(A) = (ABS_{II} - ABS_{\perp})/(ABS_{II} + 2\ ABS_{\perp})$ and photoacoustic $S(P) = (PAS_{II} - PAS_{\perp})/(PAS_{II} + 2\ PAS_{\perp})$ of chromophore forms of whole cells of *Rb. sphaeroides* embedded in stretched PVA films before (0) and after (ir) additional strong polarized irradiation. ABS – absorbance, PAS – photoacoustic signal.

Chromophore form	ABS $S(A)_0$	$S(A)_{ir}$	PAS $S(P)_0$	$S(P)_{ir}$
B_Y 375	0.00±0.01	–0.01±0.01	–0.01±0.01	–0.18±0.06
Car 499	–0.03±0.01	–0.04±0.01	–0.08±0.03	–0.07±0.03
Q_X 588	–0.03±0.01	–0.04±0.01	–0.14±0.06	–0.05±0.03
LHC2 805	0.03±0.01	0.02±0.01	–0.14±0.04	0.16±0.04
LHC2 854	0.02±0.01	0.03±0.01	–0.20±0.06	0.17±0.06

The strong increase of TD of Cars observed as a result of sample irradiation showed that Cars are important in converting the excess radiant energy into heat and in such a way they protect other pigment chromophores against photodestruction. Such observation is in agreement with the findings of Gruszecki *et al.* (1990), Havaux *et al.* (1991), and Barzda *et al.* (1995a,b). For non-irradiated LHC2 the TD at polarized excitation with electric vector forming 0°, 30°, or 90° in respect to the film-stretching axis were similar. After strong irradiation the 30° component was higher than those at 0° and 90°.

All our results support the literature data (Barzda *et al.* 1995a,b, Garab and Mustárdy *et al.* 1999) that irradiation changes the conformation of the complexes and that as a result of irradiation the thermal dissipation in LH and in Car increases. We applied strong polarized irradiation to the bacteria cells embedded in stretched films, with irradiance that did not denature pigments but, as we

Table 3. Thermal deactivation TD = PAS/ABS [relative] of whole cells of *Rb. sphaeroides* embedded in stretched PVA films before and after additional strong polarized irradiation; max. accuracy ΔTD/TD = 7 %.

	Angle (form)	B_Y 375	Car 499	Q_X 588	LH2 805	LH2 854
Before irradiation	0° (II)	0.15	0.13	0.10	0.16	0.14
	30°	0.12	0.15	0.17	0.25	0.21
	90° (⊥)	0.16	0.15	0.14	0.27	0.29
After irradiation	0° (II)	0.27	0.42	0.42	0.49	0.44
	30°	0.39	0.41	0.37	0.69	0.70
	90° (⊥)	0.49	0.47	0.43	0.33	0.30

showed, changed their ET function. The changes obtained by strong irradiance were irreversible as a result of embedding cells into a rigid matrix. Measurements of absorbance spectra and polarized PAS showed that such irradiation did not change absorption function of pigments but affected multiple increase of thermal deactivation of LHC pigments with TMs parallel to the direction of electric vector of acting strong irradiance. Irradiation perturbed the excitation ET to RC and a result it prevented the destruction of photosynthetic apparatus. We showed previously (Cegielski *et al.* 1992) that when ET in a chain of donor and acceptor molecules is blocked, the other paths of deactivation, *e.g.*, TD, increase.

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