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The Paths of Excitation Energy Deactivation in LH1 Reduced Mutant and Wild-Type Strains of *Rhodobacter sphaeroides*

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The P3 mutant of *Rhodobacter sphaeroides* had an altered ratio of reaction center to core (LH1) and peripheral (LH2) antenna complexes compared to the wild-type strain. Intracytoplasmic membranes from these two strains were purified and then resuspended in buffer or immobilized in isotropic and stretched polymer film. The absorption, photoacoustic, and delayed luminescence spectra were measured. The ratios of infrared absorption and photoacoustic bands (located at about 880 nm for LH1 and at 850 and about 800 nm for LH2) as well as the half-width of these bands are different for the LH2 and LH1 mutants and wild-type strain. The whole yields of thermal deactivation of the two strains were comparable, but in the absorption region of LH2 it was slightly lower in the case of the mutant than for the wild-type strain. The delayed luminescence main maxima were observed at about 860 and 700 nm. The first one could be due to emission of bacteriochlorophyll *a* of LH2 complexes. The emission at about 700 nm is probably due to dihydromesochlorophyll, which is usually, to some extent, produced from bacteriochlorophyll *a* in bacterial complexes. The delayed luminescence emission is competing with excitation energy transfer to the reaction center. The intensity of the delayed luminescence of the mutant strain was higher than that of the wild-type strain when both samples were excited in a region of carotenoid absorption. The mutant contains less carotenoids than the wild-type strain. Carotenoids work as efficient antenna. When they at a lower concentration the excitation can be trapped more easily by some chlorophyll-like pigment isolated from the excitation energy chain. The dependences of delayed luminescence spectra on the light polarization and excitation wavelengths for the wild-type strain and for the mutant were different. The anisotropy of delayed luminescence showed that bacteriochlorophyll *a* molecules of different orientations were contributing to the mutant and the wild-type strain emission. All the results suggest that the excitation energy transfer from the antenna to the reaction center in the mutant and the wild-type strain is similar.

KEY WORDS: Bacteriochlorophyll; delayed luminescence; photoacoustic spectra; reaction centers; light harvesting complexes.

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INTRODUCTION

The light harvesting (LH) system of *Rhodobacter sphaeroides (Rb. sphaeroides)* consists of core (LH1) and peripheral (LH2) antenna complexes. The core light harvesting complexes are present in the photosynthetic membrane in a fixed stoichiometry at approximately 25–29 LH1 bacteriochlorophyll *a* (BChl) molecules per

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reaction center (RC) [1,2], whereas the amount of LH2 complexes is variable and depends on the incident light intensity during culturing [3]. Normally the light energy captured by the peripheral LH2 antenna complexes migrates toward the core LH1 complexes to the RC, initiating the transmembrane electron transport [4]. In previous investigations [5,6] a P3 mutant of *Rb. sphaeroides* with a lower ratio of the contents of LH1 to LH2 complexes calculated by the RC number was described. Despite the obvious rearrangements in the photosynthetic light harvesting system, the mutant exhibits a normal growth rate over a wide range of light intensities. It was suggested that the excitation energy transfer from light harvesting complexes to RC could be similar in both strains [5–8]. This is somewhat in contradiction with earlier observations [9].

It was found [10] that the P3 mutant illuminated with a wavelength of 850 nm (the absorption maximum of LH2) exhibited a 140% higher hydrogen production rate than the wild-type strain at 875-nm light illumination. At 875-nm illumination (absorption of LH1) the P3 mutant shows a hydrogen production rate similar to that of the wild-type strain despite a decreased level of LH1. It is probably due to the alternation of energy transfer pathways in the photosynthetic unit.

The aim of this paper is comparison of the fate of excitation energy in the wild-type strain and P3 mutant. The alterations in LH1 and LH2 contents occurring in the P3 mutant compared to the wild-type strain could result in differences in the paths of energy transfer and deactivation. To establish these differences the thermal deactivation of two samples as well as their time-resolved delayed luminescence spectra were measured. Both these results give information on-paths of deactivation alternative to hydrogen production. These results could be useful in understanding the hydrogen production rates observed for these two strains.

We do not suppose any definite model of our complexes' orientation in polyvinyl alcohol film. It is known [8,11] that the long axis of large elongated objects embedded in an anisotropic matrix can in some cases form an angle with the film axis. Our measurements and discussion of emission anisotropy concern only those parts of the molecules which are efficiently emitting delayed luminescence (DL). This emission could be due to thermal excitation from the triplet to the excited singlet state followed by delayed fluorescence and/or the pigment ionization and delayed recombination to the excited singlet. Both processes can occur with different efficiencies in molecules located in various surroundings.

Results are obtained using polarized light for excitation and for observation of the polarized components of emission [12]. It is known that in the investigated systems fast excitation transfer between pigments occurs, therefore the absorbing and emitting molecules can be different. Usually a larger pool of molecules contributes to absorption than to DL. Efficiently emitting DL molecules are less efficient in excitation transfer to the RC. The strong delayed emission suggests a large pool of molecules is present separated from the chain of excitation energy transfer.

MATERIALS AND METHODS

Preparation of the Wild-Type Strain and P3 Mutant *Rb. sphaeroides Chromatophores*

Both bacterial *Rb. sphaeroides* strains, wild-type and mutant, were grown anaerobically under a light intensity illumination of 60 W/m^2 at 30°C. To obtain the P3 mutant cell suspension of the wild-type strain *Rb. sphaeroides* were spread over agar plates and irradiated with UV light from a transilluminator (Model TM-20; Funakoshi, Japan). Selection of mutants in the photosynthesis apparatus was made according to the color of the colonies from plates in which at least 50% of the colonies survived. Mutants were grown under the same condition as wild-type cultures.

Rb. sphaeroides cells were harvested in the beginning of the stationary growth phase, washed once in 10 mM Tris–HCl, pH 8.0, and then resuspended in 3–5 ml of the same buffer. Cells were broken by sonication at 4°C for 5 min (running at a 50% duty cycle) using a Sonifier Cell Disrupter (Branson Ultrasonics Co.). Unbroken cells and large debris were removed by centrifugation (12,000 rpm, 15 min). Chromatophores were analyzed when the proteins and pigments were isolated by ultracentrifugation at 70,000 rpm [5,6].

Biochemical Analysis of Proteins and Pigments

Harvested bacterial cells were washed once with 10 m*M* Tris–HCl (pH 8.2) and then suspended in the same buffer. The absorbance of the cell suspension was measured at 850 nm. After overnight dialysis against distilled water, cells were lyophilized in *vacuum* for measurements of the dry cell weight. Bacterial cells were digested for 20 min in an aqueous 10% (w/v) KOH solution. Hydrochloric acid was then added to neutralize the solution pH. The protein concentration was measured by the Bradford method using bovine serum albumin as the calibration standard [13].

Bacteriochlorophyll *a* (BChl *a*) was extracted twice from the cell with an acetone–methanol mixture (7:2). The absorption spectrum of the extract in the acetone–methanol mixture was measured by a spectrophotometer (UV-160; Shimadzu, Tokyo). The concentration of BChl in the extract was calculated from the absorbance at 770 nm with a molar extinction coefficient of 75 m*M* cm^{-1}. A portion of the acetone–methanol extract was sonified for 10 min at 40°C in 12% (w/v) KOH, then extracted with diethyl ether twice for carotenoid (Car) measurements. Absorption spectra of the extract in diethyl ether were measured. The concentration of Car in the extract was calculated from the absorbance at 454 nm with a molar extinction coefficient of 140 m*M* cm^{-1}. Proteins in chromatophore membranes were solubilized and electrophoresed on a 10–20% (w/v) gradient polyacrylamide gel [14]. The protein band was stained with Coomassie Brilliant Blue R-250. The amount of LH1 α subunit was established by densitometry using a gel scanner.

Spectroscopic investigations of chromatophores were done in aqueous buffer solutions (0.2 *M* NaCl, 10 m*M* Tris–HCl, pH 8.2)and in polymer films. In the second case chromatophores were introduced into a water solution of polyvinyl alcohol (PVA). The PVA films were prepared and stretched up to 300% of their initial length as described previously [15,16]. The stretched PVA films without chromatophores and unstretched PVA films with and without chromophores were used as the references.

Content of Proteins and Pigments

We measured the content of proteins and pigments in both bacterial strains. Results are shown in Table I. SDS–polyacrylamide gel electrophoresis of chromatophores of the wild-type strain (RV) and of the mutant (P3) was used [10]. The amount of LH1 α subunit measured by densitometry in P3 chromatophores was about half that in RV, although the content of RC was the same in both samples. Meinhard *et al.* [9] proposed a formula to estimate the LH1/LH2 ratio from the absorption spectrum. According to this formula we estimated that P3 contained a reduced level of LH1 (35%) and an enhanced level of LH2 (140%) in comparison with RV chromatophores [6]. Light-harvesting complexes of purple photosynthetic bacteria have been well characterized: the BChl/Car ratio in LH1 is 2 [14], whereas this ratio is 3 in LH2 [17]. Table I shows that the BChl/Car ratio in P3 is higher than that in RV [17]. This is because P3 contains a lower amount of LH1, although the value of absorbance measured at 850 nm for dry cell weight is similar in both samples. From these three pieces of evidence we concluded that the LH1/LH2 ratio in P3 mutant is reduced.

Table I. Biochemical Analysis of Pigments and Protein in Bacterial Cells

Content	Bacterial cell	
	Wild-type strain (RV)	Mutant (P3)
Total protein (% dry cell weight of bacteria)	48.2	36.6
Bacteriochlorophyll *a* (βL[a] mol BChl/g protein)	36.7	57.5
Carotenoids (βL mol Car/g protein)	26.1	21.6
BChl/Car molar ratio	2.26	2.67
Absorbance/dry cell weight (A_{850}/g)	1.48	1.49

[a] βL, subunit.

Spectral Measurements

The absorption spectra were measured using a Specord M40 spectrophotometer (Carl-Zeiss Jena, Germany) equipped with polarizers and with a computer on-line for data acquisition and handling. The polarized spectra of samples in a stretched polymer were measured. For such samples a baseline was established for the vertical and horizontal positions of the polarizers. As the reference similarly stretched unpigmented film was used. The degree of orientation *S* was calculated according to the following formula [18]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} \quad (1)$$

Photoacoustic spectra were taken using a single-beam spectrometer built in the Poznań laboratory, based on the apparatus made in Trois-Rivières, Canada [19]. The xenon lamp used as the light source enables measurements of the spectra in the range from 300 to 900 nm. The photoacoustic spectra were measured with a light modulation frequency of 8 Hz and a phase shift between the modulated light beam and the measured photoacoustic signal of $\Delta\varphi = 130°$. Carbon black was used as a reference sample. On the basis of the photoacoustic and absorption spectra the spectrum of thermal deactivation (TD) was calculated.

Time-resolved DL spectra (in the microsecond range) were measured with an arrangement which has been described in detail previously [20]. The apparatus was equipped with a nitrogen and dye laser (Type LN 120C/LD2C; PRA Laser, Canada). The pulse duration was about 200 ps (full width at half-maximum; FWHM).

The intensity of the exciting light was sufficiently low to avoid nonlinear effects. A Hamamatsu R 928 (Japan) photomultiplier was used as the detector.

The DL spectra, under the experimental conditions used, can be taken in the 600- to 900-nm spectral region. All presented spectra were corrected for the spectral sensitivity of the photomultiplier and monochromator output. The DL spectra were measured in the time range from 0.2 to 100 μs after the laser pulse within a time window of 5 μs.

For the stretched PVA films the following polarized components of delayed luminescence were measured: VVV, VHH, VVH, and VHV (H, horizontal; V, vertical). The first and last letter refer to the direction of the electric vector of the excitation and delayed luminescence emission, respectively, and the middle letter refers to the position of the orientation axis of the PVA film. The same components for unstretched samples were established (the middle character in this case is 0). The polarized components measured for unstretched samples enabled the correction of the results obtained for stretched samples for a different sensitivity of the apparatus for horizontally and vertically polarized light. As usual for a uniaxially oriented sample the four following coefficients of emission anisotropy were calculated [21,22]:

$$r_a = \left(\frac{VVV}{V0V} - \frac{VVH}{V0H}\right) \Big/ \left(\frac{VVV}{V0V} + 2\frac{VVH}{V0H}\right) \quad (2)$$

$$r_b = \left(\frac{VHV}{V0V} - \frac{VHH}{V0V}\right) \Big/ \left(\frac{VHV}{V0V} + 2\frac{VHH}{V0H}\right) \quad (3)$$

$$r_c = (VVV - VHV)/(VVV + 2\,VHV) \quad (4)$$

$$r_d = (VVH - VHH)/(VVH + 2\,VHH) \quad (5)$$

RESULTS AND DISCUSSION

Membranes in Buffer

Figure 1 shows the absorption spectra of the membranes in buffer solution for the sample from the wild-type strain (Fig. 1a) and P3 mutant (Fig. 1b) of *Rb. sphaeroides*. In the wild-type strain the Q_Y absorption band of LH1 is located at 875 nm) [1,2,23]. In Fig. 1a it is shown as a shoulder of the Q_Y band of LH2 core complexes with a maximum at about 852 nm. The Q_Y band of peripheral LH2 complexes is located at 802 nm.

The P3 mutant absorption spectrum exhibits a much narrower 852-nm band because of lower contributions from the LH1 long-wavelength band. Peripheral LH2 complexes exhibit absorption at 798 nm. The lower LH1

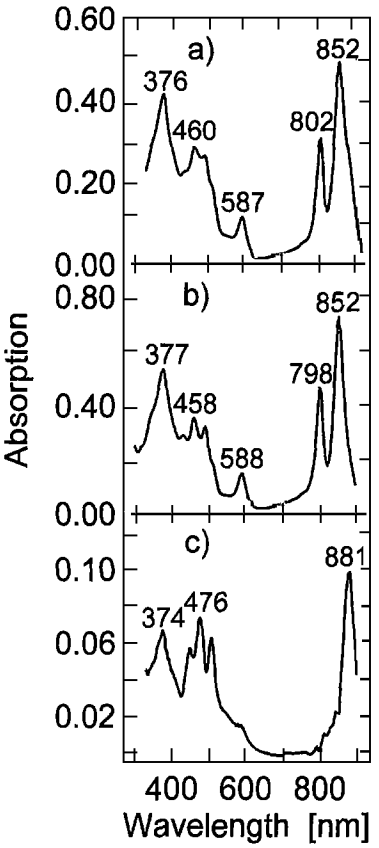

Fig. 1. Absorption spectra of *Rb. sphaeroides* chromatophores in buffer solution: (a) wild-type strain (RV); (b) mutant (P3); (c) difference spectrum (wild-type strain minus mutant absorption normalized at λ = 800 nm).

content in the P3 mutant than in the wild-type strain causes the change in the ratio of absorption at 852 nm (to which LH1 complexes are contributing) to complex absorption at 802 nm (Table II).

In Fig. 1c the difference absorption spectrum (absorption of the wild-type strain minus absorption of the mutant) is shown. Both spectra were, before subtraction, normalized at the maximum of LH2 complexes at 798 nm. From Fig. 1c it follows that there is a much higher LH1 content in the wild-type strain than in the mutant sample. The maximum responsible for LH1 in the difference spectrum is located at 881 nm. This is in agreement with previous results [5,6] that for this mutant the content of LH1 and LH2 complexes compared to that in the wild-type strain was 35 and 140%, respectively, at an unchanged content of RC.

In the 587- to 588-nm absorbance region the Q_X bands of different complexes are superimposed (Figs. 1a and b). In the Soret band the maxima at 376–377 nm belong to BChl and bacteriopheophytin bands. Superpo-

Table II. Spectral Characteristics of *Rb. sphaeroides* Chromatophores in Buffer Solution[a]

Spectral characteristic	Wild-type strain (RV)	Mutant (P3)
ABS		
FWHM (at 852 nm)	45 nm	35 nm
A_L/A_S (λ_L, λ_S)	1.71 (852, 802 nm)	1.60 (852, 798 nm)
	0.94 (460, 802 nm)	0.76 (458, 798 nm)
PAS		
FWHM (at λ_{max})	60 nm (847 nm)	46 nm (849 nm)
PAS_L/PAS_S (λ_L, λ_S)	1.43 (847, 801 nm)	1.43 (849, 801 nm)
TD		
TD = PAS/ABS (a.u.)	0.17 (847 nm)	0.16 (849 nm)
	0.20 (801 nm)	0.18 (801 nm)
TD_L/TD_S (λ_L, λ_S)	0.85 (847, 801 nm)	0.88 (849, 801 nm)
DL		
DL_L (a.u.) (λ_L), λ_{exc} = 430 nm	0.45 (870 nm)	0.65 (860 nm)
DL_L/DL_S (λ_L, λ_S), λ_{exc} = 385 nm	2.29 (860, 710 nm)	2.94 (860, 710 nm)
DL_L/DL_S (λ_L, λ_S), λ_{exc} = 430 nm	0.57 (870, 700 nm)	0.95 (860, 690 nm)

[a] ABS, absorption; PAS, photoacoustic spectra; TD, thermal deactivation; DL, delayed luminescence; FWHM, full width at half-maximum. Ratios of ABS, PAS, TD, and DL maxima are shown at given wavelengths (λ_L, λ_S): λ_L, long-wavelength maximum; λ_S, short-wavelength maximum. Description in text. Accuracy of all amplitudes ratios was about ±0.07.

sition of absorption in the region 458–487 nm is due to carotenoids. The LH1 complex contains a higher ratio of carotenoids to BChl molecules than the LH2 complex; therefore in the mutant, having a global number of BChl molecules similar to that of the wild-type strain (and lower content of LH1), the ratio of carotene to BChl absorption (A_{460}/A_{802}) is lower than in the wild-type strain (Table II). The higher content of carotenoids in the wild-type strain than in the mutant is also shown in Fig. 1c.

The photoacoustic spectra (PAS) of the same samples are presented in Fig. 2. For both types of samples the positions of PAS and absorption maxima are similar but not identical (Table I). This is usually observed in the case of the superposition into PAS of the contributions from pigments exhibiting various yields of thermal deactivation (TD) [24,25]. It is very interesting that the TD values of mutant and wild-type strains are very close one to other (Table II). This shows that in both cases the TD is diminishing to a similar extent the amount of excitation energy transferred to the RC. This suggests that in both cases the efficiency of excitation energy transfer (ET) to the RC is similar, which is in agreement with the supposition that after rearrangement of LH2 and LH1 in mutant complexes an efficient photosynthetically system can be obtained [5,6,8]. The ratio of TD of both PAS maxima (at 847 and 801 nm) is slightly higher for the mutant than for the wild-type strain, but the mutant exhibits a slightly lower TD in both maxima (Table II). The change in TD maxima ratio suggests that the TD spectrum is changed as a result of replacement of the LH1 complexes by LH2 (852 nm) complexes.

Figure 3 shows the DL spectra of membranes from two strains located in buffer and excited at BChl and at Car absorption wavelengths. The DL spectra are located in the spectral region of prompt fluorescence of the same complexes, therefore DL is generated from the first

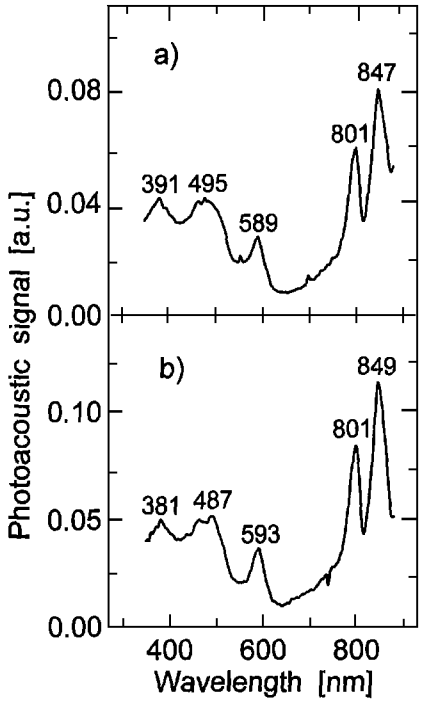

Fig. 2. Photoacoustic spectra of *Rb. sphaeroides* chromatophores in buffer solution: (a) wild-type strain (RV); (b) mutant (P3). Modulation frequency f = 8 Hz, phase shift $\Delta\varphi$ = 130°.

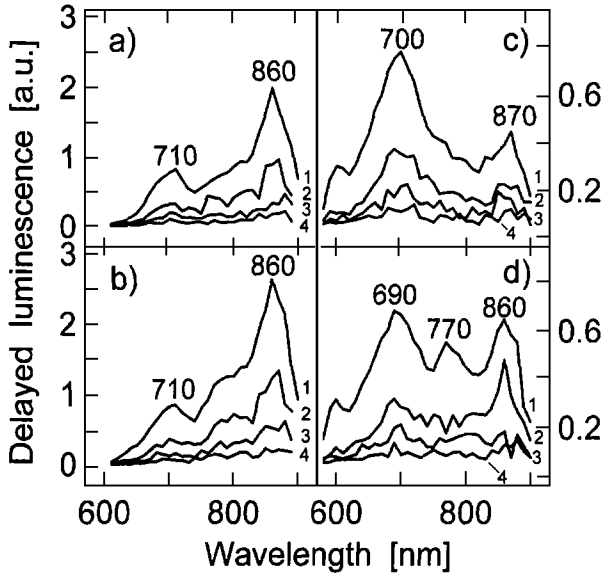

Fig. 3. Delayed luminescence spectra of the *Rb. sphaeroides* chromatophores in buffer solution: (a, c) wild-type strain (RV); (b, d) mutant (P3). (a, b) λ_{exc} = 385 nm; (c, d) λ_{exc} = 430 nm. Spectra taken at time windows of (1) 0.2–5.0 μs, (2) 5–10 μs, (3) 10–15 μs, and (4) 15–20 μs. Curves 2–4 are presented on a 5× expanded scale with respect to curve 1.

excited state [23,24]. It is not possible to decide to which extent the delayed emission is from the delayed recombination of ionized pigment and in which part it is generated from thermal excitation from the triplet to the excited singlet state [23,24].

DL spectra consist of two or three superimposed maxima. The first one, located at 690–710 nm, is due to a small (unobservable in absorption) fraction of dihydromesochlorophyll (DHP). These protonated forms of chromophores usually are not taking an efficient part in the chain of ET processes, therefore they are working as an excitation trap and are emitting efficient DL [24]. For both strains this emission is much higher (Figs. 3a and b) at excitation in the BChl region (at 385 nm) than in the case of excitation in the carotene region (at 430 nm). The lower DL intensity observed for both strains with excitation at 430 nm than at 385 nm is due to lower absorption in the carotenoid than in the BChl spectral region. The DL emission is competing with excitation transfer to the RC. The intensity of the DL of the mutant strain was higher than that of the wild-type strain when both samples were excited in the region of carotenoid absorption. Carotenoids are working as efficient antennae [23]. The mutant contains less carotenoids than the wild-type strain. When carotenoids are at a lower concentration the excitation can be trapped more easily by some chlorophyll-like pigment isolated from the excitation energy chain. At excitation in the carotenoid region the DL of DHP is higher for the wild-type strain than for the mutant, which can be explained by the lower amount of carotenoids present in the mutant. Carotenoids usually preserve BChl degradation. The maximum of the DL spectra located at 860–870 nm is related to the absorption of LH2 complexes at about 850 nm. It is not clear which form of pigment is responsible for the DL shoulder or the small maximum in the region 770–800 nm. Figure 3 shows that the decay time of DL is different for various spectral regions. The decay time values were established from the semilogarithmic graphs (natural logarithm of intensity versus time). For example, for the mutant excited at 430 nm with observation at 690 nm, the DL decay time is 7.1 μs, whereas for the same sample measured at 860 nm it is 3.0 μs. Observed decay times are range from 3.0 to 7.2 μs.

Membranes Immobilized in PVA Film

Absorption spectra of the wild-type strain and mutant samples embedded in PVA films are shown in Fig. 4. From comparison of the spectra in Fig. 4a, obtained for stretched films, with the spectrum of the unstretched

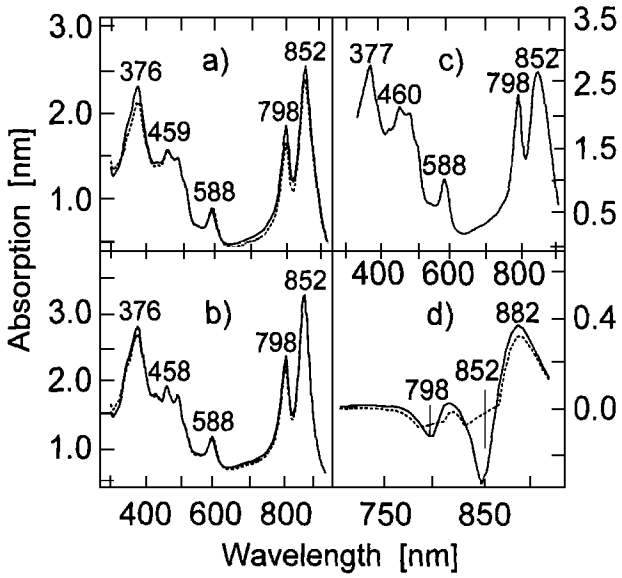

Fig. 4. Absorption spectra of the *Rb. sphaeroides* chromatophores in PVA films: (a, b) polarized spectra of uniaxially stretched (300%) films. Solid lines, parallel polarized components; dashed lines, perpendicular components. (a) Wild-type strain (RV); (b) mutant (P3). (c) Absorption of natural light in unstretched (0%) PVA film with wild-type strain (RV) chromatophores. (d) Difference spectra of uniaxially stretched (300%) films (wild-type strain minus mutant, normalized at λ = 458 nm) calculated from parallel components (solid line) and from perpendicular components (dashed line).

sample (Fig. 4c), it follows that as a result of film stretching the half-width of the 852-nm band diminishes and the ratio of this band intensity to that of the 798-nm band decreases. This effect could only be due to the change in the orientation of the rings of LH1 pigments absorbing in the long-wavelength wing of the 852-nm band (from LH1 at 875 nm). It is possible that the Q_Y transition moment (TM) could be oriented at some angle with respect to the PVA film plane because TM of antenna chromophores are forming mutually different angles [26]. The TM of LH2 absorbing at 852 nm has to be located in a more favorable way for the absorption of such light than TM of 882 nm. The ratio of the LH2 absorption at 852 nm to that at 798 nm is practically the same in both polarized components of absorption of stretched samples (Table III). This shows that these two complexes contribute to a comparable degree to both components.

In both cases, for the wild-type strain and for the mutant, the degree of orientation (S) calculated from the polarized absorption is low, but in the case of the wild-type strain it is slightly higher than for the mutant (Table III).

Table III. Spectral Characteristics of *Rb. sphaeroides* Chromatophores in PVA films[a]

Spectral characteristic	Wild-type strain (RV)	Mutant (P3)
A_L/A_S (λ_L = 852 nm, λ_S = 798 nm)		
Unstretched (0%)	1.2	1.1
Stretched 300%		
(‖)	1.4	1.4
(⊥)	1.5	1.5
$S(\Delta S = \pm 0.01)$		
At λ = 376 nm	0.03	0.02
At λ = 798 nm	0.04	0.02
At λ = 852 nm	0.02	0.00
DL_L/DL_S (λ_L = 790–810 nm, λ_S = 680–700 nm)		
Unstretched (0%)		
V0V	0.94	1.53
V0H	1.03	1.64
Stretched 300%		
VVV	0.69	0.98
VVH	0.80	0.82
VHV	1.01	1.07
VHH	0.87	0.83

[a] A, absorption (nm); S, degree of orientation; DL, delayed luminescence. Ratio of A and DL maxima are shown at given wavelengths (λ_L, λ_S): λ_L, long-wavelength maximum; λ_S, short-wavelength maximum, (‖) Parallel polarized component of absorption; (⊥) perpendicular polarized component of absorption. VVV, VHH, VVH, and VHV—polarized components of delayed luminescence (H, horizontal; V, vertical). Description in text. Accuracy of all amplitudes ratios was about ±0.07.

The structure of antenna complexes of purple bacteria was intensively investigated [8,23,27–31]. Most authors supposed that in LH2 complexes the BChl molecules absorbing in the 850-nm region and at 800 nm have their porphyrin rings oriented differently with respect to the membrane plane.

Figure 4d shows the difference absorption spectra, wild-type minus mutant, normalized to the carotenoid absorption region at 458 nm. They are calculated for parallel (solid line) and perpendicular (dashed line) components. In the carotenoid region anisotropy of absorption is very low. The highest negative difference between the wild-type strain and the mutant is observed in the parallel component in the 852-nm region. The value of the difference between perpendicular components in the same region is close to zero. This shows again that many more LH2 852-nm complexes are present in the mutant than in the wild-type strain and that these complexes exhibit orientation. A similar situation, a negative value of the difference between parallel components and a low difference between perpendicular components, is observed for LH2 complexes absorbing at about 798 nm. In the absorption region of the LH1 complexes (about 882 nm) the difference spectra of both components are positive, showing that there are more such molecules in the wild-type than in the mutant strain. The parallel component is slightly higher than the perpendicular one, showing a low anisotropy of absorption of this form.

The contribution to absorption due to the pigment molecules depends on the membrane location in the film as well as on the direction of the pigment transition moment with respect to the membrane plane [11,31]. In the case of molecules having their Q_Y almost perpendicular to the film plane, the contributions to absorption are low because the electric vector of absorbed light is located in this plane. In the case of a predominantly planar orientation of membranes in a polymer film it is also not possible to conclude definitely, on the basis of the presented absorption data, how the pigment rings of various complexes are oriented. It is sure that there are differences in the TM orientation of LH1 at 882 nm and LH2 at 852 nm.

In both strains located in isotropic film the anisotropy of DL emission is higher in the 700- to 810-nm region than in other regions (data not shown). In whole spectra V0H (the perpendicular component) is higher than V0V (parallel). This shows that the transition moments of absorbing molecules are forming a large angle with the TM of the emitting DL molecules. In PVA films the intensity of DL of the mutant is lower than that of the wild-type strain.

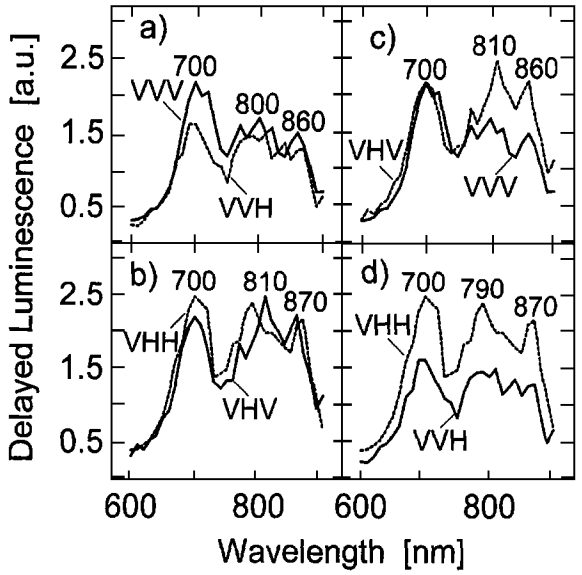

Fig. 5. Polarized delayed luminescence emission spectra of wild-type strain (RV) *Rb. sphaeroides* chromatophores in uniaxially stretched (300%) PVA films. λ_{exc} = 385 nm. Polarizations of components are marked on graphs.

Figure 5 shows the polarized components of DL spectra of uniaxially stretched PVA films with membranes from the wild-type strain, and Fig. 6 the same set for a mutant sample. It follows from comparison of Figs. 5 and 6 with Fig. 3, embedding the sample in PVA causes an increase in DHP emission.

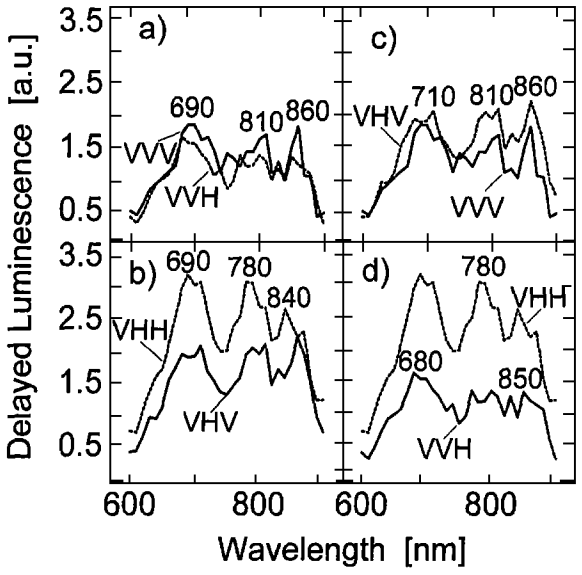

Fig. 6. Polarized delayed luminescence emission spectra of mutant (P3) *Rb. sphaeroides* chromatophores in uniaxially stretched (300%) PVA films. λ_{exc} = 385 nm. Polarizations of components are marked on graphs.

Table IV lists the emission anisotropy coefficient values calculated, as explained under Materials and Methods, on the basis of spectra from Figs. 5 and 6. It follows from the r_a values that the DL emission (at 860 nm) excited parallel to the stretching axis is polarized. For 860 nm emission the mutant exhibits a lower anisotropy of emission than the wild-type strain. The DHP emission (at 700 nm) is polarized to a similar degree for both strains. The observed DL anisotropy is higher than the degree of orientation (S), which shows that not all chromophores engaged in absorption are emitting DL with the same yield (Table III). The anisotropy of emission for molecules with TM located almost perpendicularly to the film axis provides very low and negative values of r_b. Rotation of the sample from a position parallel to the film axis with respect to the exciting electric vector of light to perpendicular one gives a negative anisotropy r_c, well measurable in the long-wavelength band of emission, especially for the wild-type strain. The coefficient r_c vanishes when only photoselection effects are responsible for observed r_a and r_b values. The negative values of r_c show that there are some pools of molecules having TM directed under a large angle with respect to the film axis. In the wild-type strain this pool of molecules emitting at 860 nm is larger than in the mutant, whereas for 700-nm observation the difference between r_c values for both strains is in the limit of accuracy.

A high negative value of r_d is observed when VHH > VVH, i.e., when the TM of absorption forms a large angle with the sample axis and the emission TM is located under a similar angle as the absorption TM. Comparison of r_a with r_d suggests that there are pools of molecules oriented differently with respect to the film axis. In the mutant the pool of "badly" oriented molecules is larger than in the wild-type strain. Comparison of all coefficient of anisotropy values suggests a strong effect of polarized light photoselection on observed emission. It also seems

Table IV. Anisotropy Coefficients Obtained from Polarized Delayed Luminescence Emission Measurements for Uniaxially Stretched PVA films with *Rb. sphaeroides* Chromatophores[a]

Coefficient	Wild-type strain (RV)	Mutant (P3)
r_a (λ_{max})	0.13 (860 nm)	0.08 (860 nm)
	0.13 (700 nm)	0.13 (700 nm)
r_b (λ_{max})	0.00 (860 nm)	−0.05 (860 nm)
	−0.01 (700 nm)	−0.08 (700 nm)
r_c (λ_{max})	−0.12 (860 nm)	−0.06 (860 nm)
	0.00 (700 nm)	−0.01 (700 nm)
r_d (λ_{max})	−0.15 (860 nm)	−0.17 (860 nm)
	−0.13 (700 nm)	−0.19 (700 nm)

[a] Description in text.

that there are pools of differently oriented luminescent molecules obtaining excitation from various donors.

The similar values of TD as well as of the DL intensities for the wild-type strain and mutant show that the yields of the excitation energy transfer from antenna to RC are similar in both strains.

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