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# Organization of the antenna bacteriochlorophylls around the reaction center of *Rhodopseudomonas viridis* investigated by photoselection techniques

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**The orientation of the long-wavelength ($Q_y$) transition dipoles of the antenna bacteriochlorophylls was examined in isolated quantasomes of *Rhodopseudomonas viridis*. A quantasome consists of a reaction center surrounded by six antenna complexes, each of which probably contains four molecules of bacteriochlorophyll *b*. We measured the photodichroism of the absorbance changes associated with oxidation of the primary electron-donor in the reaction center (P-960), when the long-wavelength absorption band of the antenna was excited selectively with polarized light. The polarization of the fluorescence from the antenna was also measured. Similar measurements were made with isolated reaction centers, in which P-960 could be excited directly, and with intact chromatophores. Reaction centers gave high polarization values for the fluorescence and the photodichroism; chromatophores and quantasomes both gave low values. The results are consistent with the view that the long-wavelength transition dipoles of the antenna molecules lie in a common plane with the corresponding dipole of P-960, but have no preferred orientation in this plane. The antenna thus appears to be circularly degenerated about P-960.**

## Introduction

The organization of the pigments in photosynthetic membranes has been investigated extensively using spectroscopy with plane-polarized light (for reviews, see Refs. 1 and 2). One of the conclusions of these studies is that the chlorophyll or bacteriochlorophyll molecules that serve as the primary electron-donor, and the antenna molecules that absorb at the longest wavelengths, are all oriented so that their long-wavelength ($Q_y$) transition moments lie in, or close to, the plane of the membrane [1,2]. There is, however, little information on the relative orientations of the antenna and reaction center pigments. Although a large number of studies using the photoselection technique have considered the chromophores in isolated reaction centers or in purified antenna complexes, to date only one report gives information on the orientation of the antenna pigments relative to the reaction center. In that study, Junge and Schaffernicht [3] found that the long-wavelength transition dipoles of the antenna surrounding P-700, the primary donor in chloroplast Photosystem I, have a circular degeneracy. The transition dipoles are all in the same plane as the long-wavelength dipole of P-700, but have no identifiable orientation in that plane.

Two main classes of antenna complexes have been recognized in photosynthetic bacteria: the B875 type, which corresponds to the long-wavelength antenna, and the B800–850 type, which

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absorbs at shorter wavelengths. In chromatophores, the B875 antenna probably is organized in pools surrounding and connecting the reaction centers [4], and a specific association of B875 with the reaction centers has been described [5]. In principle, measurements of photodichroism or fluorescence polarization should allow detection of local molecular ordering of the antenna chromophores with respect to the reaction center. To investigate this point, however, one must examine isolated complexes containing only one reaction center and the antenna that is associated with it most directly. In intact membranes containing larger arrays of antenna complexes, extensive energy transfer could lead to depolarization before the excitation reaches the vicinity of the reaction center, obscuring any local order that exists in this region.

Recently, the isolation and biochemical characterization of monomeric photoreceptor units ('quantasomes') from *Rhodopseudomonas viridis* has been described [6]. In the chromatophore membrane, the quantasomes are organized in a hexagonal lattice. Electron microscopy and image-processing techniques [7,8] indicate that a reaction center core is surrounded by a ring of six antenna complexes. The antenna complexes are of the B875 type [9]. Isolated quantasomes appear well suited for an investigation of the relative orientation of the antenna and the primary electron-donor. We present here photodichroism and fluorescence polarization data obtained by selective excitation in the long-wavelength absorption band of the antenna. The results demonstrate that normal energy transfer between the antenna and the trap occurs in isolated quantasomes, and are consistent with a circular degeneracy of the antenna.

## Materials and Methods

Quantasomes were isolated by treating chromatophores of *Rps. viridis* with lauryldimethylamine oxide and deoxycholate, essentially as described by Jay et al. [6]. Reaction centers were isolated according to Clayton and Clayton [10]. For study, reaction centers in 50 mM Tris-HCl buffer (pH 8) containing 0.1% lauryldimethylamine oxide were mixed with two parts of glycerol. 1 mM potassium ferricyanide was added to oxidize the bound cytochromes. The absorbance at 830 nm was approx. 1.0. Quantasomes in 50 mM Tris-HCl (pH 8) containing 1% deoxycholate/0.1% lauryldimethylamine oxide/1 mM dithiothreitol/0.1% $NaN_3$ were mixed similarly with glycerol, as were chromatophores in 20 mM Tris-HCl (pH 7.5) containing 100 mM NaCl; their absorbances at 1010 nm were approx. 1.25.

For measurements at 200 K, the sample was placed in a Dewar cooled with a dry ice-methanol mixture. The apparatus for measuring flash-induced absorption changes included a tungsten-halogen lamp, monochromators before and after the sample, and a Glan-Thompson prism polarizer situated before the sample. Extended-red S-20 (EMI 9558A) or cooled S-1 (RCA 7102) photomultipliers were used for measurements around 830 and 950 nm, respectively. For excitation, a xenon flash of about 20 $\mu$s duration was used at a repetition rate of 0.5 Hz. The excitation beam passed through a long-wavelength-transmitting interference filter (Corion Corp. LL-1000) and a film polarizer (Polaroid type HR), and was perpendicular to the measuring beam. The transmission curve

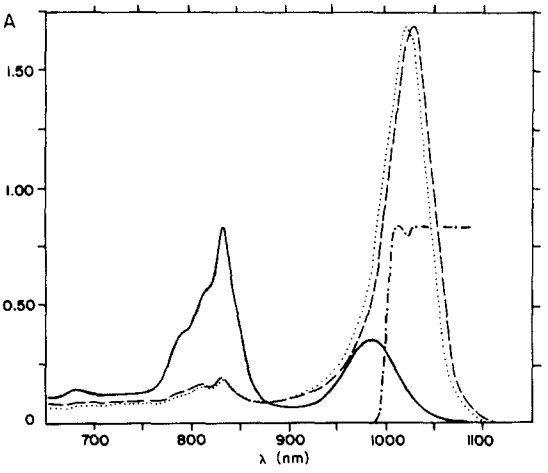


Fig. 1. Near-infrared absorption spectra of isolated reaction centers (———), quantasomes (· · · · · ·) and chromatophores (- - - - - -), at 200 K. The samples were in glycerol-buffer mixtures, as described in the text. Also the fractional transmittance of the excitation filter used for the photodichroism measurements is shown with the same vertical scale (· – · – ·). The excitation light provided by the Xe flash lamp decreases rapidly at wavelengths above 1000 nm.

of the LL-1000 filter is depected in Fig. 1. The output from the phototube was digitized with a Biomation 802 transient digitizer and sent to a computer for signal averaging and analysis. Typically, 100 signals were averaged. The initial amplitudes of the absorbance changes were obtained by nonlinear, least-squares fitting of the data to a single or double exponential decay expression. The polarization $p$ was calculated as $p = (\Delta A_V/\Delta A_H - 1)/(\Delta A_V/\Delta A_H + 1) = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$. Here $\Delta A_V$ and $\Delta A_H$ are the absorbance changes measured with vertically and horizontally polarized measuring light, both with vertically polarized excitation. When the excitation beam was polarized horizontally, $\Delta A_V$ and $\Delta A_H$ were equal within experimental error.

Some of the fluorescence measurements were made by using a Q-switched ruby laser to pump a dye laser (Exciton IR140 dye in dimethylsulfoxide) for excitation at approx. 915 nm. The excitation polarization could be selected by use of a Glan-Thompson prism polarizer and a quarter-wave plate interposed in the laser beam. The fluorescence at 90° passed through a prism polarizer, a monochromator and the LL-1000 filter, and was detected using the cooled S-1 phototube. The signals were digitized with a Tektronix R7912 transient digitizer, and were corrected for variations in the flash intensity. Typically, five signals were averaged.

Fluorescence resulting from excitation at other wavelengths was measured by single-photon counting. Continuous excitation light from a tungsten-halogen lamp passed through either a monochromator and long-wavelength pass filter or a narrow-band interference filter, and a prism polarizer. The emission at 90° passed through the film polarizer, monochromator and long-wavelength pass filter, and was detected with a cooled photomultiplier (Hamamatsu R632) and home-built photon-counting electronics. When necessary, the fluorescence data were corrected for artifacts due to scattered excitation light. These were measured by replacing the photosynthetic sample by a suspension of powdered milk that had been diluted to have the same turbidity as the sample at a wavelength where the absorbance and fluorescence of the latter were negligible.

The fluorescence polarization was calculated as $p = (F_{VV}F_{HH}/F_{VH}F_{HV} - 1)/(F_{VV}F_{HH}/F_{VH}F_{HV} + 1)$, where $F$ stands for the fluorescence intensity and the first subscript (V, H) stands for the excitation polarization and the second for the detection polarization. The factor $F_{HH}/F_{HV}$, which corrects for the polarization dependence of the monochromator between the sample and the photomultiplier, was typically 1.30.

## Results

Absorption spectra of the chromatophores, quantasomes and reaction center preparations of *Rps. viridis* used in this work are shown in Fig. 1. These spectra were measured at 200 K, where the long-wavelength absorption band of P-960, the primary electron-donor, occurs at 985 nm. As previously described for room-temperature spectra [6], the absorption maximum of the quantasomes (1020 nm) is shifted by about 10 nm to shorter wavelengths compared to chromatophores.

Absorbance changes at 950 nm, which reflect the photooxidation of P-960 to the cation radical ($P\text{-}960^+$), were measured with the three different preparations. For the measurements of the absorbance changes in isolated reaction centers, it was found necessary to add 1 mM potassium ferricyanide in order to prevent very fast reduction of $P\text{-}960^+$ by the bound cytochromes [11]. Ferricyanide addition had little effect on the amplitude of the absorbance changes of $P\text{-}960^+$ in chromatophores and quantasomes. The excitation flash was attenuated as necesssary with neutral density filters, so that the absorbance change was always less than 15% of its saturation value, thus insuring adequate photoselection conditions [1]. The amount of attenuation required with quantasomes was similar to that required with chromatophores, and greater than that required with reaction centers, indicating that excitations absorbed by the antenna system in the quantasomes are transferred efficiently to the reaction center. Typical sets of records for the three different preparations are shown in Fig. 2. The traces are the averages of 100 measurements of $\Delta A$ at 950 nm with vertical ($\Delta A_V$) and horizontal ($\Delta A_H$) positions of the analyzing polarizer (with a vertically polarized excitation flash in both cases). The temperature was maintained at 200 K to insure immobilization of

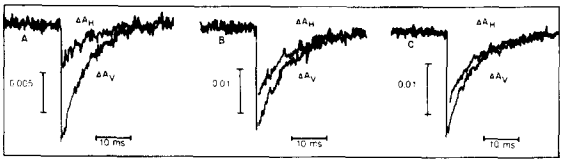


Fig. 2. Absorbance changes at 950 nm in isolated reaction centers (A), quantasomes (B) and chromatophores (C). The samples were in glycerol-buffer mixtures at 200 K, as described in the text. They were excited with vertically polarized light at approx. 1010 nm (see text and Fig. 1). The absorbance changes were measured with the detection polarizer parallel ($\Delta A_V$) and perpendicular ($\Delta A_H$) to the excitation polarizer.

the particles [12]. The decay kinetics of P-960$^+$ are characterized by a single exponential ($\tau \approx 8$ ms) in reaction centers, and by two exponentials ($\tau_1 \approx 2$ ms and $\tau_2 \approx 10$ ms) in quantasomes and chromatophores. The polarization ($p$) values (see Materials and Methods) of the initial absorbance changes at 950 nm are 0.47 for reaction centers, 0.18 for quantasomes and 0.13 for chromatophores (Table I).

Spectra of absorbance changes occurring between 810 and 850 nm are shown in Fig. 3A. The $\Delta A_V/\Delta A_H$ ratios (Fig. 3B) are discontinuous near 835 nm, where the absorbance changes evidently include opposing contributions from several different transitions [12,13]. In the regions where the absorbance changes are largest and can be measured most reliably (810–820 nm and 835–845 nm), the polarization is significantly smaller in chromatophores and quantasomes than it is in isolated reaction centers. When the polarization of the excitation beam was horizontal, $\Delta A_V/\Delta A_H$ was equal to 1.0 within experimental error throughout this region (not shown).

The fluorescence from reaction centers at 293 K was measured at 1030 nm, with excitation at 950 nm. The corrected fluorescence polarization values of 0.43–0.44 (Table I) are close to the value of 0.47 obtained in the photodichroism measurements. The fluorescence from quantasomes was measured at 1060 nm, with excitation at 915, 1000 or 1040 nm.

TABLE I

PHOTODICHROISM AND FLUORESCENCE POLARIZATION IN REACTION CENTERS, QUANTASOMES, AND CHROMATOPHORES OF *RPS. VIRIDIS*

| Preparation | Temp. (K) | Excitation wavelength (nm) | Detection wavelength (nm) | Polarization (p) |
|---|---|---|---|---|
| Photodichroism | | | | |
| Reaction centers | 200 | 1010 [a] | 950 | 0.47 ± 0.03 [e] |
| Quantasomes | 200 | 1010 [a] | 950 | 0.18 ± 0.02 [e] |
| Chromatophores | 200 | 1010 [a] | 950 | 0.13 ± 0.02 [e] |
| Fluorescence polarization | | | | |
| Reaction centers | 293 | 950 [b] | 1030 | 0.43 ± 0.02 [f] |
| | 293 | 950 [b] | 1030 | 0.44 ± 0.02 [f] |
| | 293 | 950 [c] | 1030 | 0.44 ± 0.02 [f] |
| Quantasomes | 293 | 1040 [c] | 1060 | 0.15 ± 0.02 [f] |
| | 293 | 1000 [b] | 1060 | 0.14 ± 0.01 [f] |
| | 293 | 915 [d] | 1060 | 0.16 ± 0.03 [g] |
| | 200 | 915 [d] | 1060 | 0.14 ± 0.03 [g] |
| Chromatophores | 293 | 915 [d] | 1060 | 0.13 ± 0.03 [g] |
| | 200 | 915 [d] | 1060 | 0.12 ± 0.03 [g] |

[a] Xe flash with long-wavelength-pass filter (Fig. 1).
[b] Tungsten lamp with narrow-band interference filter (20 nm bandwidth at 950 nm; 40 nm at 1000 nm).
[c] Tungsten lamp with monochromator (24 nm band width).
[d] Dye laser.
[e] Means of four measurements on chromatophores, nine on quantasomes, and eight on chromatophores.
[f] Single measurements with uncertainties calculated from photon counting statistics by standard error propagation theory.
[g] Single measurements with estimated uncertainties.

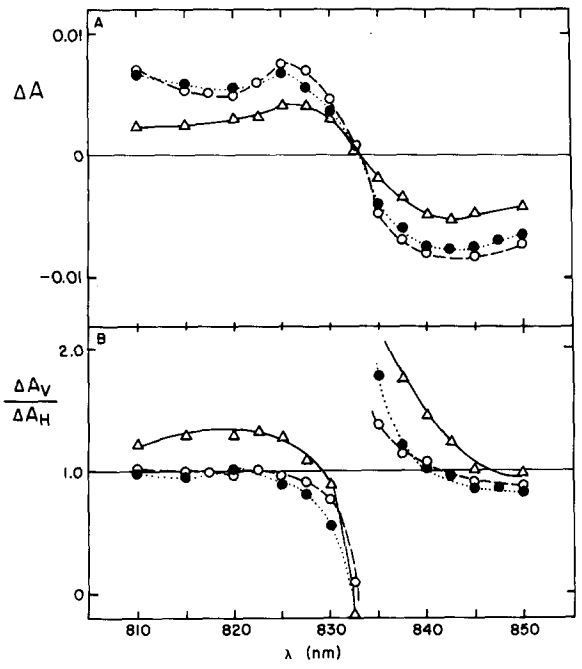


Fig. 3. (A) total flash-induced absorbance changes ($\Delta A_V + 2\Delta A_H$) in isolated reaction centers (△), quantasomes (●) and chromatophores (○) at 200 K. Samples and excitation conditions as described in the text and Figs. 1 and 2. (B) $\Delta A_V/\Delta A_H$, the ratio of the absorbance changes measured with the detection polarizer parallel and perpendicular to the (vertical) excitation polarizer.

The corrected polarization values were 0.14 to 0.16, with no significant dependence on the excitation wavelength or temperature (Table I). Similar values were obtained with chromatophores (Table I).

## Discussion

The photoreceptor unit or quantasome of *Rps. viridis*, first isolated by García et al. [14], and recently further characterized [6–8], is thought to contain one reaction center and six antenna complexes. Taking into account the ratio of the antenna's absorbance at 1020 nm to that of the reaction center at 830 nm (Fig. 1), the antenna can be estimated to consist of approx. 24 bacteriochlorophyll-*b* molecules per reaction center. Each antenna complex thus probably contains four bacteriochlorophyll-*b* molecules. These most likely are bound to an $\alpha_2\beta_2$ complex of two types of polypeptides, as has been suggested for some of the B875-type bacteriochlorophyll-*a*-protein complexes isolated from other species [9].

As visualized by electron microscopy, the quantasome is about 12 nm in diameter, and has an apparent 6-fold rotational symmetry. However, investigations of 2-dimensional [15] and 3-dimensional crystals [16] of isolated reaction centers from *Rps. viridis* have shown [16] that the cross-section of the reaction center in the membrane plane is roughly elliptical ($4.5 \times 6.0$ nm on the average [15], but $3.0 \times 7.0$ nm in the central part). This implies that the molecular contacts between the reaction center and the antenna within a quantasome cannot have rotational symmetry higher than 2-fold. The apparent 6-fold symmetry observed in the density maps derived from Fourier-processed electron micrographs thus probably reflects a rotational average over quantasomes packed with various orientations into the hexagonal lattice [7]. As pointed out above, these observations imply that, in order to investigate whether a local order exists between the $Q_y$ transition moments of the antenna pigments and the primary electron-donor P-960, one must examine isolated quantasomes. In the chromatophore membrane, energy transfer among rotationally averaged, adjacent quantasomes could lead to significant depolarization. The absorption changes and fluorescence measurements reported on here demonstrate that the antenna pigments are functionally coupled to the reaction center in the quantasomes in essentially the same way as they are in the intact chromatophore, thus making such an investigation possible.

The absorbance changes at 950 nm caused by flash excitation are due to the bleaching of the long-wavelength absorption band of the primary electron-donor, P-960. These absorbance changes have a high polarization value of 0.47 when the reaction centers are excited in the same band (Fig. 2 and Table I). The fluorescence of the reaction centers shows a similar polarization (Table I). The measured polarizations are close to the theoretical maximum value of 0.50 that is expected for excitation and detection of the same optical transition in an immobilized chromophore [1]. The long-wavelength absorption band of P-960 evidently consists either of a single transition or of two transitions with closely parallel transition dipole moments

[17–19]. Similar observations have been made with reaction centers isolated from *Rps. sphaeroides* [12,20] and *Rhodospirillum rubrum* [21].

The lower value of the polarization (0.13) observed in the absorbance changes at 950 nm with chromatophores indicates that a significant depolarization occurs as the excitation migrates to the reaction center from the antenna pigment that is initially excited. The polarization of fluorescence from chromatophores is similar (Table I). The value of 0.13 is close to the theoretical value of 0.143 that is expected for excitation and observation within a circularly degenerate system of oscillators [1,22]. Our observations thus are consistent with the notion that many transfer steps occur among antenna dipoles that are oriented essentially parallel to the plane of the membrane [17,23], but have no preferential direction in that plane. Comparable results have been reported for the fluorescence polarization of chromatophores of species that contain bacteriochlorophyll *a* [20,24,25], and of aggregates of antenna complexes isolated from these chromatophores [24–27].

We found that a similar depolarization occurs in isolated quantasomes. The photodichroism polarizations measured at 950 nm and in the 800–860 nm region (Fig. 3) are close to the values obtained with chromatophores, as are the fluorescence polarization values (Table I). This suggests that an approximately circular degeneracy of the antenna is present in these small complexes, as well as in the intact membrane. The observation that the fluorescence polarization is similar to the photodichroism value also indicates that there probably is little contribution to the fluorescence from disconnected pigments. If such contaminants were released from the membrane and remained attached to the quantasomes during the isolation, they presumably would have increased the polarization. In fact, the $p$ value measured for P-960$^+$ photodichroism is slightly higher than the values obtained from the fluorescence polarization (Table I). Although the difference is only marginally significant, it could mean that excitons generated on antenna dipoles oriented parallel to P-960 are trapped slightly more efficiently than are excitons created on antenna dipoles oriented at a large angle with respect to P-960.

The photoselection data for the quantasomes allow exclusion of models in which the $Q_y$ transition moments of the antenna bacteriochlorophyll and P-960 are preferentially either parallel or perpendicular. They do not allow discrimination between two other possible models. In one model, depolarization occurs by extensive energy transfer among the six antenna complexes attached to the reaction center. In the second model, the depolarization occurs within the four bacteriochlorophyll molecules in any individual one of the six antenna complexes. Circular degeneracy could occur even in complexes consisting of only two molecules, if the transition moments of the two molecules have perpendicular transition dipoles. Such an arrangement was originally proposed by Breton et al. [24] to describe the fluorescence polarization of a B850 complex isolated [28] from *Rps. sphaeroides* R26-1, a mutant in which the B800–850 antenna is thought to have lost its B800 component [29]. This model also has been extended to the B800–850 antenna of wild-type *Rps. sphaeroides* [24,26].

The B875-type antenna from species that contain bacteriochlorophyll-*a* has been characterized previously by fluorescence polarization studies of both the isolated complexes and intact chromatophores [25,29]. Although a polarization of 0.13 is observed upon excitation over most of the $Q_y$ absorption band, there is a significant increase in the polarization with excitation on the long-wavelength edge of the band. This is especially clear at low temperatures [25]. The increase in the polarization at long wavelengths suggests that the B875 antenna could contain a small pool of pigments that have a preferential orientation relative to the reaction center. Our measurements of the fluorescence polarization of quantasomes (Table I) give no indication of such a pool in *Rps. viridis*.

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