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ARRANGEMENT AND INTERACTION OF PIGMENT MOLECULES IN REACTION CENTERS OF *RHODOPSEUDOMONAS VIRIDIS*

PHOTODICHROISM AND CIRCULAR DICHROISM OF REACTION CENTERS AT 100 K *

V.A. SHUVALOV and A.A. ASADOV

Institute of Photosynthesis, USSR Academy of Sciences, Pushchino, Moscow Region (U.S.S.R.)

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Summary

The linear dichroism of light-induced absorbance changes (photodichroism, *p*) accompanying photooxidation of the primary electron donor (*P*), a bacteriochlorophyll dimer $(\mathrm{BChl})_2$-960, or photoreduction of the intermediary electron acceptor, bacteriopheophytin (BPh), as well as circular dichroism (CD) of the reaction centers of *Rhodopseudomonas viridis* at 100 K in their various redox states: $P \cdot \mathrm{BPh}$, $P^{\dagger} \cdot \mathrm{BPh}$ and $P \cdot \mathrm{BPh}^{\bar{\cdot}}$, have been measured. Photooxidation of *P* is accompanied by bleaching of the absorption and CD bands at 980 nm and a considerable change of the frequency, a decrease of the dipole and rotational strengths of the transition at 850 nm with the preservation of dipole and rotational strengths of the BPh transition at 790 nm. On the other hand, the photoreduction of BPh is accompanied by bleaching the absorption and CD bands of BPh at 790 nm and a considerable change of the frequency, a decrease of dipole and rotational strengths of the transition at 830 nm with the preservation of the dipole and rotational strengths of the transitions at 850 and 980 nm. Consideration of these data in terms of the static quantum treatment of reaction centers as molecular aggregates shows: (i) a direct interaction between BPh and $(\mathrm{BChl})_2$-960 is not observed; (ii) BPh mainly interacts with BChl monomer absorbing at 830 nm (BChl-830); (iii) $(\mathrm{BChl})_2$-960 mainly interacts with BChl monomer absorbing at 850 nm (BChl-850); and (iv) there is also an interaction between BChl-830 and BChl-850 since the dipole strengths of these transitions differ from each other but their sum corresponds to the sum of the

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

dipole strengths of two BChl monomers. The p values of the transitions have been used for the estimations of the angles between the dipole-vectors of the transitions in reaction centers.

Estimations of the distances and interaction energy between the transitions of the pigment molecules in the reaction center are given.

Introduction

Recent studies of the photosynthetic bacteria reaction centers have shown that light induces the charge separation between a special pair of the bacteriochlorophyll (BChl) molecules ((BChl $a)_2$-870 or (BChl $b)_2$-960 abbreviated as P) and the bacteriopheophytin (BPh) molecule during less than 10 ps [1,2] with the formation of a radical ion pair $(\mathrm{BChl})_2^{\dot{+}} \cdot \mathrm{BPh}^{\dot{-}}$ [3—6]. This charge separation is accompanied by an energy loss of 0.1—0.15 eV, as measured from the temperature dependence of the luminescence induced by recombination of the ion pair [3,7] and from the redox titration of (BChl $b)_2$-960 (E_m = +515 mV) and BPh b (E_m = —620 mV) * in *Rhodopseudomonas viridis* reaction centers [7]. Ubiquinone, (or menaquinone in the case of *Rps. viridis* [14]) accepts an electron from this radical ion pair and ferrocytochrome donates an electron to it with the formation of $(\mathrm{BChl})_2^{\dot{+}} \cdot \mathrm{BPh}$ [1,8] or $(\mathrm{BChl})_2 \cdot \mathrm{BPh}^{\dot{-}}$ [3,4,9], respectively. Little is known about the arrangement and interactions among the photoactive BPh [3,10,31] and BChl molecules in the reaction center. This problem may eventually be resolved by X-ray measurements. However some preliminary investigations can be made on the basis of the optical experiments [4,11—13]. For this purpose it is reasonable to measure the optical properties of reaction centers in the various redox states: $(\mathrm{BChl})_2 \cdot \mathrm{BPh}$, $(\mathrm{BChl})_2^{\dot{+}} \cdot \mathrm{BPh}$ and $(\mathrm{BChl})_2 \cdot \mathrm{BPh}^{\dot{-}}$. Oxidation of $(\mathrm{BChl})_2$ or reduction of BPh results in the changes of frequencies, dipole and rotational strengths of the transitions of the certain pigment molecules of the reaction center. This information can be used for the estimation of the interaction energy between the transitions of pigment molecules and for the estimation of the arrangement of chromophores in the reaction center. With this aim we have studied the absorption spectra, photodichroism and circular dichroism spectra of *Rps. viridis* reaction centers in various redox states at 100 K.

Materials and Methods

Rps. viridis reaction centers were obtained by the method described by Pucheu et al. [14] using dodecyl dimethyl aminooxide for solubilization. Concentrated solution of reaction centers was diluted with 80% glycerol and frozen at ≈100 K, with the E_h of the medium either at ≈+250 mV or <—300 mV. To

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* Previous redox titration of BPh/$\mathrm{BPh}^{\dot{-}}$ in *Rps. viridis* reaction centers was carried out at pH 11.5, using neutral red as the mediator and dithionite at high concentration as the titrant; under those conditions a potential ≅ —600 mV could be reached (see Fig. 3 of ref. 7). Prince et al. [5] reported a more positive redox potential (—400 mV) for the *Rps. viridis* reaction center. The resolution of this discrepancy requires further examination.

obtain the state $P \cdot \mathrm{BPh}$, the sample was frozen in the dark at $E_h \approx +250$ mV. The states $P^+ \cdot \mathrm{BPh}$ and $P \cdot \mathrm{BPh}^-$ were obtained by illuminating the samples (during freezing) poised at $E_h \approx +250$ mV and <-300 mV, respectively (see also refs. 4 and 7). Difference (light minus dark) absorption spectra in polarized light were measured as described earlier [13]. Linear dichroism of the light-induced absorbance changes (photodichroism) was measured as $(\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + \Delta A_{\perp}) = p$ where $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are the absorbance changes measured in the light polarized parallel and perpendicularly to the exciting light, respectively. Circular dichroism spectra were measured by means of a set-up, similar to that described by Breeze and Ke [15], using an elasto-optic modulator for circular polarization of the linearly polarized measuring light.

Results

Fig. 1 shows the absorption and circular dichroism spectra of *Rps. viridis* reaction centers at ≈100 K: at $E_h \approx +250$ mV (Fig. 1A), after photooxidation of $(\mathrm{BChl})_2$-960 at 20°C and $E_h \approx +250$ mV (Fig. 1B) and after photoreduction of BPh at 20°C and $E_h < -300$ mV (Fig. 1C). In the CD spectrum of the unmodified reaction centers four transitions at 787, 831, 849 and 980 nm are

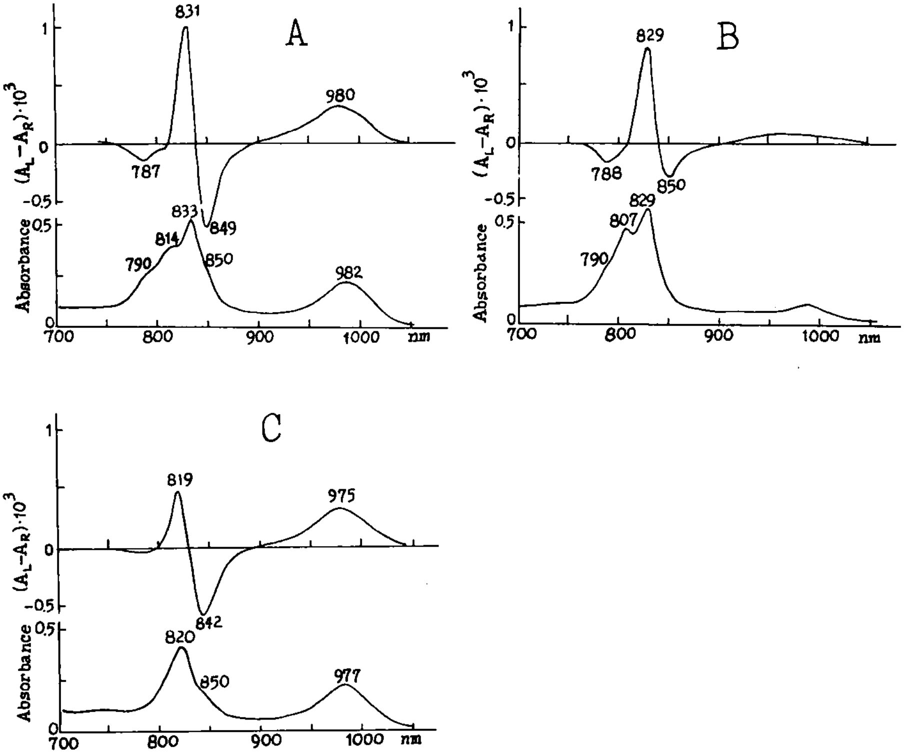

Fig. 1. Absorption and circular-dichroism spectra of the various redox states of *Rhodopseudomonas viridis* reaction centers at ~100 K. (A) Reaction centers at $E_h \sim +250$ mV (state $P \cdot \mathrm{BPh}$). (B) Reaction centers at $E_h \sim 250$ mV frozen under illumination (state $P^+ \cdot \mathrm{BPh}$). (C) Reaction centers at $E_h < -300$ mV frozen under illumination (state $P \cdot \mathrm{BPh}^-$).

observed. The same transitions and also the transition at 814 nm are observed in the absorption spectrum of the unmodified reaction centers. $(BChl)_2$-960 photooxidation induces a bleaching of the absorption band at 982 nm, a decrease and the shift of the band at 833 to 829 nm, the decrease and the blue shift of the band at 850 nm (see below), and a slight red shift of the band at 790 nm. This photoreaction is accompanied by a decrease of CD bands at 980 and 849 nm, by a slight decrease of the CD band near 830 nm without changes of the CD band intensity at 787 nm. BPh photoreduction results in a decrease of absorbance at 790 nm, in a decrease and a shift of the band at 833 to 820 nm and in a shift of the band at 982 nm to 977 nm. In this case a decrease of the CD band at 787 nm, the decrease and a shift of the CD band at 831 to 819 nm are observed. The absorption and CD bands at 850 and 980 nm are slightly shifted to the blue without changes of their intensity. The frequencies, the dipole and rotational strengths of the four transitions for the three redox states ($P \cdot$ BPh, $P^{\ddagger} \cdot$ BPh and $P \cdot$ BPh$^{\bar{}}$) of *Rps. viridis* reaction centers are given in Table I. The transition at 814 nm of reaction centers in state $P \cdot$ BPh and the transition at 807 nm, which appears after $(BChl)_2$-960 photooxidation, do not contribute to CD spectra of reaction centers and are not considered.

Upon illumination at 980 nm of reaction centers at $E_h \simeq +250$ mV and ≈ 100 K, the p value of the 980 nm band as measured for the rapid decaying component of absorbance changes after the light is turned off, is near 0.46 (Fig. 2A). The p value for the slow component is smaller. This agrees with the results reported by Vermeglio et al. for *Rhodopseudomonas spheroides* reaction centers [16] and with our results reported for *Rhodospirillum rubrum* reaction centers (see Fig. 2 in ref. 13). However, since we measure the p values in a wide spectral range (500—1150 nm) where the absorbance changes are not always intense enough for the separation of the rapid- and slow-decaying components, the p values are given for the sum of the two components. For example the p value for the transition at 980 nm as measured in such a way is 0.25 which agrees with the earlier data for *Rhs. rubrum* reaction centers [13,17]. The p values for the difference-spectrum bands bleached at 850, 837, and 775 nm are ≈ 0, +0.2 and —0.12, respectively (Fig. 2B and C). Near the isosbestic point of the difference absorption spectrum at 830 nm, the absorbance decrease has the p value of +0.5 and the absorbance increase, —0.4. The difference-spectrum bands developed at 825 and 805 nm have p values of +0.01 and +0.15, respectively.

Sharp changes of LD values in the region of 820—870 nm indicate the presence of two bands (α and β) with different polarization (p_α and p_β). In this case the measured value of p (p_{meas}) is given by:

$$p_{\mathrm{meas}} = \frac{(\Delta A_{\parallel}^{\alpha} + \Delta A_{\parallel}^{\beta}) - (\Delta A_{\perp}^{\alpha} + \Delta A_{\perp}^{\beta})}{\Delta A_{\alpha} + \Delta A_{\beta}}$$

where $\Delta A_\alpha = \Delta A_{\parallel}^{\alpha} + \Delta A_{\perp}^{\alpha}$ and $\Delta A_\beta = \Delta A_{\parallel}^{\beta} + \Delta A_{\perp}^{\beta}$, then:

$$\frac{\Delta A_\alpha}{\Delta A_{\mathrm{meas}}} = \frac{p_{\mathrm{meas}} - p_\beta}{p_\alpha - p_\beta} \qquad (1)$$

where $\Delta A_{\mathrm{meas}} = \Delta A_\alpha + \Delta A_\beta$. The spectrum of $(\Delta A_{\parallel} + \Delta A_{\perp})$ (Fig. 2B) can

TABLE I

DIPOLE AND ROTATIONAL STRENGTHS OF THE TRANSITIONS FOR VARIOUS STATES OF *RHODOPSEUDOMANAS VIRIDIS* REACTION CENTERS AT ≈100 K

Reaction center transitions for state ($P \cdot$ BPh, nm)	Dipole strengths (D) [a] (debye^2)			Rotational strengths (R) [b] (debye magneton)		
	$P \cdot$ BPh [f]	$P^{+\cdot} \cdot$ BPh	$P \cdot$ BPh$^{-\cdot}$	$P \cdot$ BPh	$P^{+\cdot} \cdot$ BPh	$P \cdot$ BPh$^{-\cdot}$
982	67 ± 5 [c]	—	67 (977 nm)	+3.2 ± 0.2	—	+3.0
850	17 [c] ± 2	17-4 [e]= 13 ± 2 (825 nm)	17 (850 nm)	—3.0 ± 0.2	—1.4	—3.1
833	60 [c] ± 5	60-6 [e]= 54 ± 5 (830 nm)	60-9 [e]= 51 ± 5 (820 nm)	+2.8 ± 0.18	+2.5	+1.9
790	10 [d] ± 2	10 ± 2 [g] (790 nm)	—	—0.7 ± 0.05	—0.7	—

[a,b] The values of dipole (D) and rotational (R) strengths were estimated using the following equations:

$$D = \frac{3hc\ 10^3 \ln 10 \cdot 10^{36}}{8\pi^3 N} \int \frac{\epsilon(\nu)}{\nu}\, d\nu \ (\text{Debye}^2)$$

$$R = \frac{3hc\ 10^3 \ln 10 \cdot 10^{38}}{8\pi^3 N \cdot 0.93} \int \frac{\Delta\epsilon(\nu)}{\nu}\, d\nu \ (\text{Debye magneton})$$

where h is Planck's constant (erg. $\cdot$ s^{-1}), c is the speed of light (cm/s), N is Avogadro's number, ϵ is molar extinction coefficient (M$^{-1} \cdot$ cm^{-1}), and $\Delta\epsilon = (A_L—A_R)$/cl. The ϵ value of $1.07 \cdot 10^5$ M^{-1}: cm^{-1} for the difference absorption spectrum band at 980 nm has been found from a comparison of the bleaching the 960 nm band on photooxidation of (BChl)$_2$-960 and the development of the 680 nm band ($\epsilon = 2.64 \cdot 10^4$ M$^{-1} \cdot$ cm^{-1} [19]) on photoreduction of BPh in the same sample (see Fig. 2 in ref. 7).

[c] The dipole strength has been estimated by resolving the absorption spectrum into the gaussian components in the region of 750—1500 nm. The obtained band widths correspond to those of CD bands.

[d] The dipole strength of the photobleached part of the 790 nm transition (during BPh photoreduction) has been found using $\epsilon = 1.07 \cdot 10^5$ M$^{-1} \cdot$ cm^{-1} for ΔA_{980} (see [a] and [b]) and the ratio $\Delta A_{545}/\Delta A_{980}$ = 0.225 in the reaction center (see fig. 2 in ref. 7).

[e] The changes of dipole strengths of the transitions which are shifted during the photochemical reaction have been estimated by substracting the negative part of light-induced absorbance changes from the positive one and by comparison of the obtained area with the area of 980 nm band bleaching which has a dipole strength of 67 debye^2.

[f] The sum of oscillator strengths which are proportional to $D \cdot \nu_A$, is $1.73 \cdot 10^6$ debye$^2 \cdot$ cm^{-1} for these four transitions in the reaction center. The sum of oscillator strengths of Q_y transitions of the five isolated chromophores, four BChl molecules and one BPh molecule ($D_{\text{BChl}\,b} \cong 30$ debye^2 and $D_{\text{BPh}\,b} \cong 19$ debye^2 as measured from absorption spectra, presented in ref. 19) is $1.74 \cdot 10^6$ debye^2 $\cdot$ cm^{-1}.

[g] A red shift of the 790 nm band is only observed for rapid component of ΔA and does not occur for ΔA with $\tau_{1/2} \cong 30$—60 s at 100 K (Shuvalov, V.A. and Asadov, A.A. (1978), in preparation). These data show that the red shift of the 790 nm band is caused by the influence of the electric field of the reduced quinone-iron complex, rather than of P^+ (see also ref. 3).

differ from the spectrum of ΔA observed with unpolarized light for both measuring and exciting beams (plot ($\Delta A_{\parallel} + 2\Delta A_{\perp}$) should be used for this case). However, since the observed maximal value of positive polarization is 0.25 and that of negative polarization is —0.1, the difference between the two

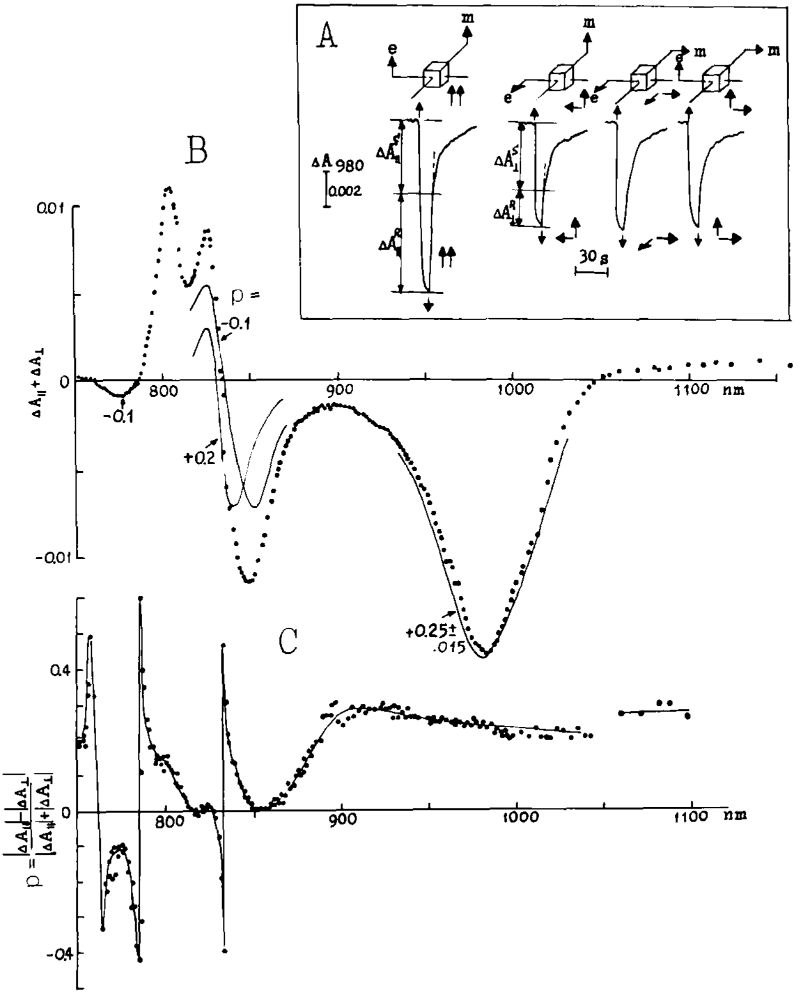

Fig. 2. Linear dichroism of absorbance changes induced by illumination at 980 nm of *Rps. viridis* reaction centers at ~100 K and E_h ~ +250 mV. (A) Kinetics of absorbance changes (ΔA_{980}) at various mutual orientations of E-vectors of the measuring (m) and exciting (e) lights. Dark kinetics shows the rapid (ΔA^R) and slow (ΔA^S) components. (B) Spectrum of absorbance changes ($\Delta A_{\parallel} + \Delta A_{\perp}$) induced by the exciting light at 980 nm, which was polarized in parallel ($\Delta A_{\parallel}$) or perpendicularly ($\Delta A_{\perp}$) to the measuring light. The exciting light induced the oxidation of about 6% of reaction centers in the sample. Solid curves indicate the resolved components of the difference absorption spectrum (see text). (C) Spectrum of $p = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + \Delta A_{\perp})$.

spectra is only ±5% of ΔA. On the other hand, the plot ($\Delta A_{\parallel} + \Delta A_{\perp}$) is convenient for resolving the difference absorption spectrum into components using Eqn. 1.

The difference absorption spectrum of *Rps. viridis* reaction centers at 77 K [18] shows that two bands at 837 and 848 nm have equal intensities. If the p value of the absorption spectrum band at 830 nm is 0.2 (see below) one can resolve the difference absorption spectrum in the region of 820—870 nm into two components: a blue shift and a decrease of the band at 830 nm ($p = +0.2$) and a shift to 825 nm and a decrease of the band at 850 nm ($p = -0.1$)

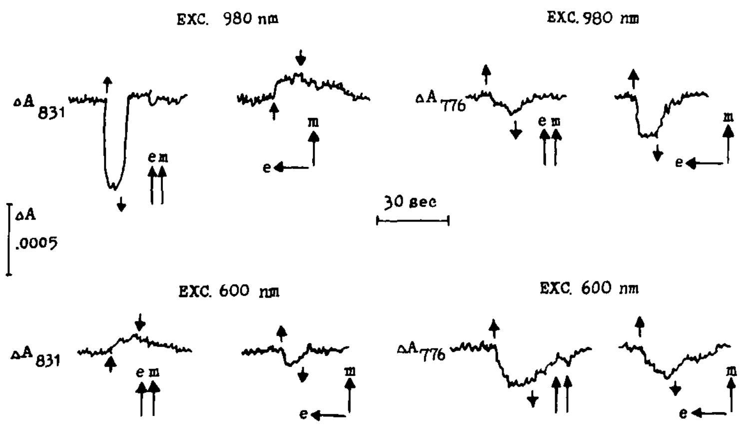

Fig. 3. Kinetics of absorbance changes at the parallel and mutually perpendicular orientation of the E-vectors of the measuring (m) and exciting (e) lights. Other conditions are the same as in Fig. 2.

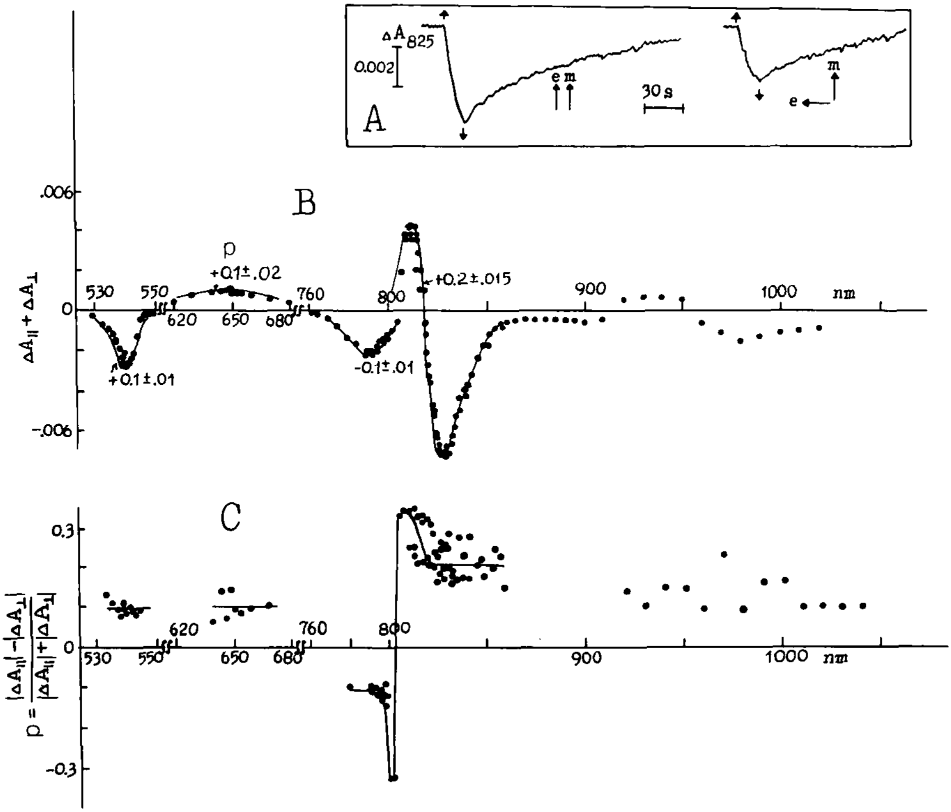

Fig. 4. Linear dichroism of absorbance changes induced by illumination at 980 nm of *Rps. viridis* reaction centers at ~100 K and $E_h < -300$ mV. (A) Kinetics of absorbance changes (ΔA_{825}) induced by the exciting (e) light, which was polarized in parallel or perpendicularly to the measuring (m) light. (B) Spectrum of absorbance changes ($\Delta A_{\parallel} + \Delta A_{\perp}$) induced by the exciting light at 980 nm, which was polarized in parallel ($\Delta A_{\parallel}$) or perpendicularly ($\Delta A_{\perp}$) to the measuring light. The exciting light induced the reduction of about 4% of reaction centers in the sample. Solid curves indicate the resolved components of the difference absorption spectrum (see text). (C) Spectrum of $p = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + \Delta A_{\perp})$.

(Fig. 2B). The 850 nm CD band of the oxidized reaction centers (Fig. 1B) rather indicates that a small fraction of the centers was not bleached (a somewhat residual absorption at 980 nm can also be seen).

Fig. 3 shows that in contrast to illumination at 980 nm, the bleaching of the band at 776 nm upon illumination at 600 nm is observed with the positive p value of +0.07, and near the isosbestic point at 831 nm the absorbance decrease has a negative p value and the absorbance increase a positive p value.

Fig. 4 shows the p spectrum for the absorbance changes induced by the photoreduction of BPh at $E_h < -300$ mV and ≈100 K upon illumination at 980 nm. The photoreduction at 100 K is a small part of the reaction observed at 20°C and is characterized by bleaching of the BPh bands at 790 and 546 nm (p values of −0.1 and +0.1, respectively), by a decrease and shift of the band at 833 to 820 nm, by a shift of the band at 982 to 977 nm and by the formation of the band of BPh radical anion at 650 nm (p values of +0.1). The p spectrum in the region of 812—860 nm has a constant p value of +0.2 including the isosbestic point near 820 nm. Thus the BPh photoreduction results in the shift of the band at 833 nm which has a p value of +0.2, and no change of the 850 nm band.

Discussion

The transition at 980 nm has a dipole strength of ≈67 debye^2 which approximately corresponds to the sum of the dipole strengths of the Q_y transitions of the two BChl *b* monomers (≈30 debye^2 for isolated chromophores as measured from absorption spectrum data in ref. 19). The transition at 980 nm has a constant high value of polarization (+0.46) in the region of 925—1050 nm which agrees with the high polarization value of the corresponding band at 870 nm of BChl *a*-containing reaction centers [16,20]. This implies that at 980 nm there is either one exciton transition or two nonexciton transitions with parallel dipole vectors. The second possibility can be ruled out since the large red shift (≈2300 cm^{-1}) of the band at 980 nm with respect to the band of BChl *b* monomer (798 nm) indicates the strong exciton coupling of the two BChl *b* monomers rather than an environmental shift. Therefore we consider the dimer $(BChl)_2$-960 as one molecule with the allowed transition at 980 nm, the dipole strength of which is ≈67 debye^2.

This dimer can have a forbidden transition (see ref. 32), the dipole vector of which must be perpendicular to that of the transition at 980 nm. Holten et al. [6] following the assumption of Vermeglio and Clayton [12] suggest that the forbidden transition of the dimer is the band at 850 nm. The oxidation of $(BChl)_2$-960 can be accompanied by the bleaching of the allowed and forbidden transitions and the development of the monomer transition, the dipole vector of which is approximately parallel to that of the allowed transition at 980 nm. However, the transition at 850 nm is only shifted but does not disappear and keeps the negative polarization when $(BChl)_2$-960 is photooxidized. The half band-width of the transition at 850 nm rather indicates that this transition belongs to the BChl monomer (BChl-850).

There are reasons to consider that the four transitions in the near infrared spectrum of *Rps. viridis* reaction centers belong to four nonidentical chromophores: the 790 nm transition to BPh *b* molecule, 980 nm transition to the BChl *b* dimer, which is the primary electron donor, and the 830 and 850 nm transitions to two BChl *b* molecules which are in different environments since the changes of frequencies, dipole and rotational strengths of the transitions at 830 and 850 nm are not similar when (BChl)$_2$-960 is oxidized or when BPh is reduced. The 814 nm transition in reaction centers in the state $P^{\cdot} \cdot$ BPh probably belongs to a contaminate BChl molecule which does not interact with the reaction center pigments, since there is no corresponding CD band. This pigment seems to be reduced irreversibly upon illumination at low redox potentials. The intensity of the transition at 814—817 nm is also dependent on the method used in isolating the reaction centers [31]. The transition at 807 nm which appears seems to belong to the (BChl)$_2^{+}$-960 [31]. The positive polarization of this band is consistent with this assumption, but the *p* value of this band cannot be measured correctly because of the presence of BPh band shift with negative polarization in this region (corrected *p* value for rapid component of ΔA_{807} can reach 0.44). The assignments of the transitions to certain molecules in the reaction centers are similar to that described earlier [6,31], except the band at 850 nm, which can probably be attributed to (BChl)$_2$-960. The main difficulty of this attribution is related to the explanation of the spectrum of *p* values (see above).

The experimental data show that the optical properties of the reaction centers are consistent with the first-order expressions for average oscillator strengths ($\mu_{\mathrm{OA}}^2 \nu_{\mathrm{A}}$) and average rotational strengths (R_{A}) of aggregates with non-degenerate transitions. These expressions have been obtained for such aggregates by the static quantum method based on the first-order time-independent pertubation theory [21] and they are in close agreement with the corresponding expressions obtained by other independent methods (see ref. 22) *.

$$\nu_{\mathrm{A}}\mu_{\mathrm{OA}}^2 = \boldsymbol{\mu}_{\mathrm{ioa}} \cdot \boldsymbol{\mu}_{\mathrm{job}} - 4 \sum_{j \neq \mathrm{i}} \sum_{\mathrm{b} \neq \mathrm{a}} \frac{V_{\mathrm{ioa;job}} \boldsymbol{\mu}_{\mathrm{ioa}} \boldsymbol{\mu}_{\mathrm{job}} \nu_{\mathrm{b}} \nu_{\mathrm{a}}}{h(\nu_{\mathrm{b}}^2 - \nu_{\mathrm{a}}^2)} \tag*{(2) **}$$

$$R_{\mathrm{A}} = -\frac{2\pi}{C} \sum_{\mathrm{j} \neq \mathrm{i}} \sum_{\mathrm{b} \neq \mathrm{a}} \frac{V_{\mathrm{ioa;job}} \nu_{\mathrm{a}} \nu_{\mathrm{b}} (\mathbf{R}_{\mathrm{j}} - \mathbf{R}_{\mathrm{i}}) \cdot (\boldsymbol{\mu}_{\mathrm{job}} \cdot \boldsymbol{\mu}_{\mathrm{ioa}})}{h(\nu_{\mathrm{b}}^2 - \nu_{\mathrm{a}}^2)} \tag*{(3) **}$$

$$\sum_{\mathrm{A}} \nu_{\mathrm{A}} \boldsymbol{\mu}_{\mathrm{OA}}^2 = \sum_{\mathrm{i}} \nu_{\mathrm{a}} \boldsymbol{\mu}_{\mathrm{ioa}}^2 \tag{4}$$

$$\nu_{\mathrm{A}} = \nu_{\mathrm{a}} \tag{5}$$

Where ν_{A}, $\boldsymbol{\mu}_{\mathrm{OA}}$ and R_{A} are the frequency, electric dipole transition moment and rotational strength of the transition O → A in aggregate, respectively, ν_{a} and

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* The validity of the application of all these methods to our case is based on the following data: EPR and ENDOR studies [3,23,24,31] show that the unpaired electron of the reaction center state (BChl)$_2^{+}$ · BPh or state (BChl)$_2$ · BPh^{-} is located in the dimer (BChl)$_2$ or in the single molecule of BPh, respectively. This can indicate the absence of electron exchange between (BChl)$_2^{+}$ and other BChl and BPh molecules as well as between BPh^{-} and the four BChl molecules.

** These formulae are derived for non-degenerate electrically allowed ($\boldsymbol{\mu}_{\mathrm{ioa}} \neq 0$), magnetically forbidden ($m_{\mathrm{ioa}} = 0$) transitions of chromophores in aggregate. However the transition at 830 nm has an intrinsic CD, which shows that $m_{\mathrm{ioa}} \neq 0$ for this transition.

μ_{ioa} refer to the frequency and electric dipole moment of the transition o → a in chromophore i which is in the time-average or static field of the rest of the aggregate * (the self-consistent field approximation). In the case of non-degenerate transitions, the aggregate frequency ν_A will be essentially the same as the monomer frequency ν_a (Eqn. 5) (see ref. 21); ν_b and μ_{job} are the same for the transition o → b in chromophore j; $|R_j - R_i| = R_{ij}$ is the distance between the centers of chromophores i and j; $V_{ioa;job}$ is a transition-transition interaction energy which is approximated by the dipole transition-dipole transition interaction energy between chromophores i and j as follows:

$$V_{ioa;job} = \frac{1}{R_{ij}^3}\left[\mu_{ioa}\mu_{job} - 3\,\frac{(\mu_{ioa}\cdot R_{ij})\cdot(\mu_{job}\cdot R_{ij})}{R_{ij}^2}\right] \qquad (6)$$

The sum of the oscillator strengths of the transitions at 790 nm (only the photobleached part of the band at 790 nm), 833, 850 and 980 nm corresponds to the sum of the oscillator strengths of Q_y transitions of five isolated chromophores: four BChl *b* molecules and one BPh *b* molecule (Table I), in agreement with Eqn. 4. At the same time the oscillator strengths of the transitions at 850 and 790 nm are smaller, but the oscillator strengths of the transitions at 833 and 980 nm are larger than that of isolated chromophores (Table I). These data are consistent with Eqns. 2 and 4, which show that the interaction between the transitions of chromophores leads to a redistribution of oscillator strengths between the transitions with the preservation of the sum of the oscillator strengths. For example, the reduction of BPh, the dipole strength of which is smaller by ≈ 9 debye^2 than that of the isolated chromophore, induces a decrease of the dipole strength of the transition at 833 nm by ≈ 9 debye^2 without the change of the dipole strengths of other transitions. Probably the interaction between the transitions of BPh and BChl-830 molecules is accompanied by an increase of the dipole strength of the 833 nm transition and by a decrease of that of the 790 nm transition by 9 debye^2.

The decrease in dipole strengths of the transitions at 830 and 850 nm when $(BChl)_2$-960 is photooxidized (see Table I) can indicate the interaction between the transitions of BChl-850 and $(BChl)_2$-960 and between BChl-830 and $(BChl)_2$-960. The sum of the dipole strengths of the two transitions at 830 and 850 nm corresponds to that of the Q_y transitions of two BChl *b* monomers but there is a strong difference between the dipole strengths of the transitions at 850 and 830 nm. In agreement with Eqn. 2, these data show that there is an interaction between the transitions of BChl-830 and BChl-850 molecules.

The near infrared CD spectrum of the reaction centers consists of four bands at 790, 830, 850 and 890 nm of both positive and negative sign with large rota-

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* The protein matrix participates in the formation of the static field of the reaction center. However, its contribution to $\nu_A \cdot \mu_{OA}^2$ and R_A from the transitions o → b in near infrared region, weighted by the factor $(\nu_b^2 - \nu_a^2)^{-1}$, is negligible. The oxidation (or reduction) of one of the chromophores changes the static field of aggregate especially for neighbouring chromophores for which the frequency ν_a can be markedly changed in this case [25]. However, the theory of the electrochromism [26] and experimental data [27] show that the change of the static field induces only a slight change of the dipole strength of the chromophore transition. This implies that the large change of the dipole strength of the transition O → A, induced by the oxidation (or reduction) of *P* (or BPh) in reaction centers in mainly related to the second term of Eqn. 2, which comes from coupling between the electronic transitions of chromophores i and j.

tional strengths in contrast to the small CD value of a single positive component of isolated chromophores [28]. Eqn. 3 shows that the interaction between the transitions of, for example, two non-coplanar and non-parallel chromophores can result in the appearance of two CD bands with opposite signs and with the frequencies corresponding to those of the transitions of two non-identical chromophores, since $\nu_A = \nu_a$ (Eqn. 5). The reduction (or oxidation) of one of the two chromophores must be accompanied by a decrease of two CD bands with opposite signs. This is the case for the oxidation of $(BChl)_2$-960 which is accompanied by a decrease of two bands with opposite signs at 980 and 850 nm and for the BPh reduction which induces a decrease of two CD bands with opposite signs at 790 and 830 nm. These data show that there is an interaction between the transitions of BPh and BChl-830 and those of BChl-850 and $(BChl)_2$-960, in agreement with the data on the changes of transition dipole strengths (see above).

Fig. 5 shows the mutual interaction of four chromophores: BPh, BChl-830, BChl-850 and $(BChl)_2$-960. The absence of a direct interaction between BPh and $(BChl)_2$-960 chromophores is supported by the experimental data which show that the photooxidation of $(BChl)_2$-960 does not change the dipole and rotational strengths of the transition at 790 nm, while the photoreduction of BPh does not change the dipole and rotational strengths of the transition at 980 nm. The maximal interactions between the transitions are observed for the two pairs of chromophores: between the transitions of $(BChl)_2$-960 and BChl-850 and between those of BPh and BChl-830, since the oxidation of $(BChl)_2$-960 induces considerable changes in the frequency, dipole and rotational strengths of the transition at 850 nm, while the reduction of BPh induces con-

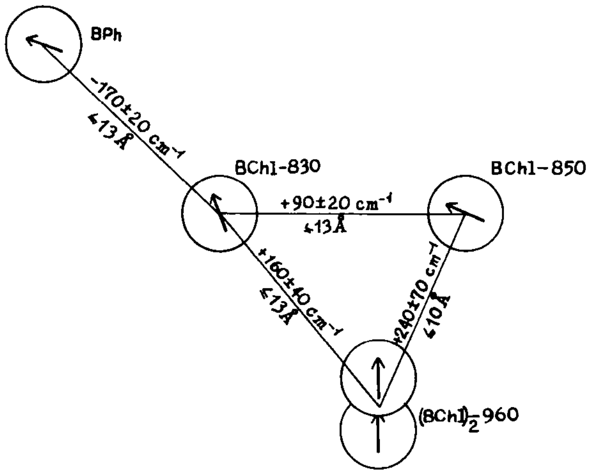

Fig. 5. Arrangement of the pigment molecules in *Rps. viridis* reaction centers. The arrows show the mutual orientation of the Q_y dipole transitions in each pigment molecule of the reaction center. The figures indicate the pairwise interaction energy (in cm^{-1}) between the transitions of the chromophores and the distances (in Å) between the centers of the chromophores. If the macrocycle planes of the chromophores are approximately parallel, the perpendicular distance between the macrocycle planes of BPh and BChl-830 is ~4Å, as estimated by Eqn. 3. The perpendicular distance between the plane of the BChl-850 macrocycle and the plane which passes through the center of $(BChl)_2$-960 dimer and parallel to the macrocycle planes of its component molecules (see model for special pair in ref. 30), is ~8Å.

siderable changes of those of the transition at 830 nm. There seems to be an interaction between BChl-830 and BChl-850 (see above), which probably are intercalated between BPh and $(BChl)_2$-960 in agreement with previous data [4], and which can participate in the electron transfer from $(BChl)_2$-960 to BPh.

The photodichroism measurements upon illumination at 980 nm can be used for the estimation of the angles (α) between the dipole vector of the transition at 980 nm and those of the other transitions, since the p value is given by:

$$p = \frac{3\cos_\alpha^2 - 1}{\cos_\alpha^2 + 3} \tag{7}$$

We assume that low ΔA polarization of the slow-decaying components for both photooxidation of P and photoreduction of BPh is related to depolarization of absorbance changes during long periods of observation (30—90 s). Therefore, to obtain the proper angles, p values should be corrected in agreement with p value for rapid component of ΔA induced by photooxidation of P. The estimated values of α between the dipole vector of the transition at 980 nm and dipole vectors of the transitions at 850, 833, 790 and 545 nm (Q_y transition of photoactive BPh molecule) are 70 ± 10, 23 ± 3, 71 ± 10 and $42 \pm 6^\circ$, respectively.

The obtained angles and the magnitudes of the changes of dipole and rotational strengths of the transitions of reaction centers induced by the oxidation of $(BChl)_2$-960 and the reduction of BPh (Table I) allow to estimate the interaction energies between the transitions of the chromophores in reaction centers and the distances between the centers of the chromophores using Eqns. 2, 3 and 6 (see Fig. 5).

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