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# PICOSECOND PHOTODICHROISM (PHOTOSELECTION) MEASUREMENTS ON TRANSIENT STATES IN REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES, RHODOSPIRILLUM RUBRUM* AND *RHODOPSEUDOMONAS VIRIDIS*

CHRISTINE KIRMAIER [a], DEWEY HOLTEN [a] and WILLIAM W. PARSON [b]

[a] *Department of Chemistry, Washington University, St. Louis, MO 63130, and* [b] *Department of Biochemistry, University of Washington, Seattle, WA 98195 (U.S.A.)*

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**The primary photochemical processes in reaction centers isolated from *Rhodopseudomonas sphaeroides*, *Rhodospirillum rubrum* and *Rps. viridis* have been investigated with weak excitation flashes at 867 or 962 nm lasting approx. 30 ps. Photodichroism (photoselection) measurements of the absorption changes accompanying the formation of the transient states $P^+I^-$ and $P^+Q^-$ have been obtained with unoriented samples at room temperature. (Here P is a bacteriochlorophyll dimer; I, an initial electron acceptor complex that involves a bacteriopheophytin and possibly also bacteriochlorophyll; and Q, a quinone.) For state $P^+Q^-$, calculated spectra of the absorbance changes parallel and perpendicular to the 870-nm (*Rps. sphaeroides* and *R. rubrum*) or 960-nm (*Rps. viridis*) transition dipole of P are in excellent agreement with those from previous measurements on much slower time scales. This comparison indicates that the components of the reaction center probably do not move significantly with respect to each other in the time interval between 2 ns and 10 ms after excitation; faster movements cannot be excluded. The photodichroism spectra for $P^+Q^-$ and $P^+I^-$ both indicate that the bacteriopheophytin transition at 760 or 790 nm is perpendicular to the 870- or 960-nm transition, which also agrees with previous work. The spectra for $P^+I^-$ suggest that the reduction of I, in addition to bleaching the absorption bands of a bacteriopheophytin, alters the absorption spectra of two bacteriochlorophylls that have different orientations relative to the 870- or 960-nm transition.**

## Introduction

The primary photochemical reactions in photosynthetic organisms occur in specialized pigment-protein complexes called reaction centers. Light energy reaching the reaction center generates a series of transient charge-transfer or radical-pair states, each consisting of an oxidized donor molecule and a reduced electron acceptor [1,2]. Picosecond transient absorption studies of reaction centers isolated from purple nonsulfur photosynthetic bacteria have revealed that the first few steps in the charge separation process are complete on a time scale much less than a nanosecond [3–19]. Photon absorption first prepares an excited singlet state (P*) of the primary electron donor (P), a complex involving two of the four bacteriochlorophyll (BChl) molecules present in the reaction center. P* releases an electron, which appears to arrive on one of the two bacteriopheophytins (BPh) with a time constant of 4–10 ps [4–12,15,17]. The electron subsequently is transferred from $BPh^-$ to a quinone (Q) with a time constant of about 200

Abbreviations: P, a complex of two bacteriochlorophylls (BChl); I, a complex consisting of interacting bacteriopheophytin (BPh) and BChl; B, BChl absorbing near 800 or 830 nm; Q, the reaction center quinone.

ps at room temperature [4,5,7,8,11–19].

Transient reduction of the BPh has been detected by the bleaching of the BPh's $Q_x$ absorption band at 545 nm, and by the formation of a broad, new band near 650 nm [4,5,7,8,13–18]. There is also a bleaching of the $Q_y$ band, which is near 760 nm in reaction centers of *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum* and near 790 nm in *Rps. viridis*. Reaction centers from the first two species contain BChl *a* and BPh *a*; those from *Rps. viridis* contain BChl *b* and BPh *b*.

It has been suggested, but not proven, that one of the other two BChls (B) acts as an intermediary electron carrier between P* and BPh [13–16]. Transient absorption changes that probably are due to one or both of the other BChls can be seen at early delay times following excitation, along with the absorbance changes due to the oxidation of P and those associated with the BPh [4,5,8,13–18]. In *Rps. sphaeroides* and *R. rubrum*, the additional absorption changes include a partial bleaching and hypsochromic shift of a band near 800 nm; in *Rps. viridis,* there are similar absorption changes near 830 nm. It is not clear, however, whether these absorption changes reflect the actual reduction of B, or simply a change in its interaction with a BPh or with P. Under some conditions, the absorption changes attributed to B have appeared to precede those due to BPh [13–16].

If electron transfer from $BPh^-$ to Q is prevented by chemical reduction or removal of Q, the transient state generated by excitation ($P^F$) decays with a time constant of about 10 ns at room temperature [20–22]. It has been suggested [22] that $P^F$ is an equilibrium mixture of the radical pair states ($P^+BPh^-$) and ($P^+B^-$). The mixture of reduced electron acceptors has been referred to as '$I^-$', and the corresponding set of unreduced acceptors as 'I'. Continuous illumination in the presence of a reductant can convert reaction centers into the long-lived reduced state $PI^-Q^-$ [8,18,23–29]. Again, this causes absorption changes in the 800- or 830-nm bands due to BChl, as well as in the 545- and 760- or 790-nm bands of BPh.

Attempts to understand the role of B in the electron-transfer sequence have been complicated by the fact that the 800- or 830-nm absorption bands of the reaction center contain contributions from both of the BChls that are not components of P, and possibly also from P itself. The main long-wavelength band of P is at 870 nm in *Rps. sphaeroides* and *R. rubrum* and at 960 nm in *Rps. viridis,* but the dimer may also have a weak band at shorter wavelengths [27–29,30–35]. In addition, the oxidation of P causes changes in the absorption bands of the other BChls and BPhs. One approach to sorting out the contributions from the six different pigments is to study the linear dichroism of the light-induced absorption changes (photodichroism) in oriented samples, or the induced dichroism obtained by excitation of unoriented samples with polarized light (photoselection). These techniques have been particularly helpful in analysis of the absorption changes that accompany the photooxidation of P [27–29,31–37].

In the measurements of dichroism that have been made to date, the reaction centers have been illuminated with either continuous light or flashes lasting tens to hundreds of milliseconds. The experiments thus have probed relatively relaxed states that could be significantly different from the transient states that are initially created by the excitation. In most cases, the long time scales of the measurements also have required that the samples be immobilized by cooling or dehydration. These conditions prevent rotation of the reaction center particules, but do not necessarily inhibit small movements of the pigments with respect to one another. In the present paper, we describe photoselection measurements with a time resolution of about 10 ps. The measurements were made at room temperature on reaction centers in solution, using weak excitation flashes that pumped the long-wavelength absorption band of P. The characteristics of the excitation flashes deserve emphasis, because many of the previous studies of picosecond transients have used flashes that were excessively strong or that excited components other than P. In addition, we present the results of measurements on reaction centers from three species of purple bacteria: *Rps. sphaeroides, R. rubrum* and *Rps. viridis*. Except for the wavelength of excitation, measurements on the three reaction center preparations were carried out under conditions as identical as possible in order to facilitate direct comparison of the results.

## Materials and Methods

The picosecond transient absorption spectrometer is based on a passively mode-locked Nd : YAG laser system that delivers single, 35-ps, 1064-nm, 10-mJ flashes at 10 Hz. Half of the fundamental is used to generate 25–35-ps excitation pulses at a variety of wavelengths by harmonic generation and stimulated Raman scattering. Of particular importance are the excitation flashes at 532 nm (frequency-doubled 1064-nm fundamental), 600 nm (first Stokes Raman line from 532 nm in $C_6{}^2H_{12}$), 867 nm (first anti-Stokes Raman line from 1064 nm in $C_6{}^2H_{12}$) and 962 nm (first anti-Stokes Raman line from 1064 nm in $C_6H_6$). Flashes at 532 nm (2 mJ) and 600 nm (greater than or equal to 400 $\mu$J) are relatively strong and permit intensity dependence studies, but must be reduced in intensity to avoid nonlinear effects in the sample. They are normally polarized at 45° with respect to the vertical. The 867- or 962-nm excitation pulses are weak (less than 200 $\mu$J per flash) and cause photooxidation of about 50% of the reaction centers on each flash. The direction of linear polarization of the 867- or 962-nm pump pulses is controlled (vertical or horizontal) by a half-wave plate in the fundamental used to generate them. Any small residual radiation of the unwanted polarization is removed with film polarizers (Polaroid HR for the near-infrared). Pulses at the desired excitation wavelength are isolated with dichroic beam splitters, colored glass and interference filters.

The remaining 5-mJ pulses at 1064 nm are used to generate weak, 35-ps broad-band (450–1000 nm) white-light probe pulses. This radiation is expanded vertically with cylindrical lenses, passed through both excited and unexcited regions of the sample, and dispersed by a 0.25-m polychromator onto two tracks of a vidicon detector (PAR 1205B) coupled to a Cromemco Z-80 microcomputer-controlled optical multichannel anlayzer (PAR 1205A). The data analysis procedures and precautions necessary to avoid artifacts with this type of two-dimensional detection system are discussed elsewhere [38]. The polarization of the probe pulses arriving at the sample is chosen with linear film polarizers (Polaroid HR for the near-infrared). The directions of propagation of pump and probe pulses intersect at the sample at an angle of approx. 6°.

Reaction centers were prepared from *Rps. sphaeroides* (R-26) and *Rps. viridis* essentially as described previously [21,25]. The preparation from *R. rubrum* (G-9) was obtained by treating chromatophores with octylglucoside, followed by chromatography on DEAE-Sepharose in the presence of the same detergent. Details of this procedure will be described elsewhere (Fenderson, F. and Herriott, J.R., unpublished observations). The absorption spectrum of freshly prepared *R. rubrum* reaction centers was similar to those of preparations described previously, but the BPh absorption band at 760 nm tended to increase in height as the reaction centers were stored. The measurements shown in Figs. 5 and 8 were made with a sample whose absorbance at 760 nm was about 80% of that at 800 nm.

All the experiments were carried out at room temperature. Reaction centers suspended in 10 mM Tris-HCl, pH 8.0, 10 $\mu$M EDTA were at moderate redox potential (P, BPh, Q all in their normal states prior to excitation). Samples of *Rps. sphaeroides* and *R. rubrum* contained 0.05% Triton X-100 and had an absorbance of 0.8 at 800 nm (2 mm path length), except for the measurements of Fig. 2 for which $A_{800} = 2.0$; those of *Rps. viridis* contained 0.01% lauryldimethylamine *N*-oxide and had an absorbance of 0.9 at 830 nm. The samples were flowed to minimize sample degradation and to avoid reexcitation of unrelaxed sample by successive laser shots. A transient difference spectrum (corrected for vidicon dark current and inhomogeneities in the probe light between excited and unexcited regions of the sample [38]) encompassing a 170-nm wavelength interval representing the average of approx. 600 spectra takes about 15 min to acquire, plot, and store on floppy disk. Standard deviations in $\Delta A$ vary depending on the wavelength region and sample ground-state absorption, but are less than or equal to 0.005 when 2400 spectra are averaged.

## Results

*Near-infrared transients with 600-nm pump*

Fig. 1B shows near-infrared transient absorption spectra for *Rps. sphaeroides* reaction centers at two delay times following excitation with sub-

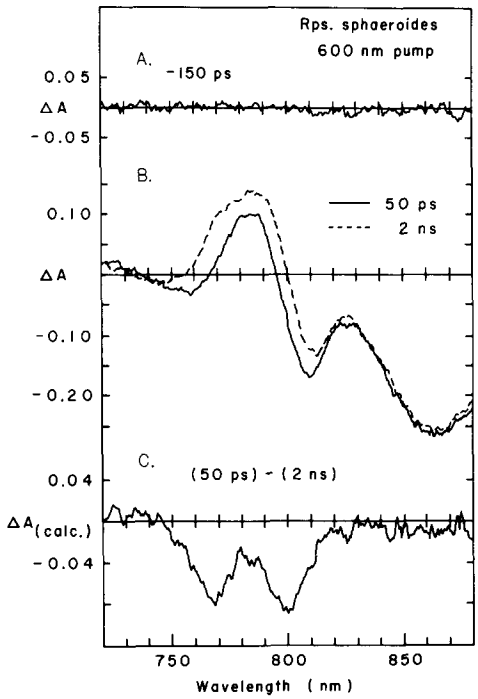


Fig. 1. Near-infrared absorption changes resulting from excitation of *Rps. sphaeroides* reaction centers with approx. half-saturating 35-ps 600-nm flashes. In panel A, the probe pulses arrived at the sample 150 ps before the pump pulses. The baseline indicates that no unrelaxed reaction centers remained in the excited volume between pump pulses, which occur every 100 ms (10 Hz repetition rate). Panel C shows the difference between the 50-ps and 2-ns spectra of panel B. Each spectrum represents the average of 600 spectra.

saturating, 35-ps flashes at 600 nm. These difference spectra, acquired with the two-dimensional data acquisition techniques described above, show in detail the spectral features observed previously with the point-by-point method [4,5,8,13,15,17,18]. Similar spectra in the previous studies have been attributed to the formation of $P^+I^-$ (50-ps spectrum) and $P^+Q^-$ (2-ns spectrum). The spectra at both delays show bleaching in the 870-nm band, due to the oxidation of P to $P^+$. There are complex absorption changes in the 800-nm region, including an absorption increase with a maximum near 785 nm and an absorption decrease near 810 nm. The spectra also reveal the absorption changes accompanying electron transfer from $I^-$ to Q. Subtraction of the absorption changes at a long delay, due to $P^+IQ^-$, from those at the shorter delay, due to $P^+I^-Q$, gives rise to the difference-difference spectrum shown in Fig. 1C. On the assumption that absorption changes due to Q or $Q^-$ are negligible in the near-infrared, the calculated spectrum $[(P^+I^-Q - PIQ) - (P^+IQ^- - PIQ)]$ represents the difference spectrum for the formation of the reduced intermediary electron carrier $I^-$. Similar absorption changes have been observed previously by various methods [8,17,23–26,36]. The absorption decrease near 765 nm has been attributed to the reduction of BPh, and the absorption changes near 800 nm to an electrochromic shift and/or bleaching of an absorption band of B. Polarization effects are not important in these particular measurements for two major reasons. First, the inherent polarization of the near-infrared bands is small with 600-nm pump light [33]. Second, the 600 nm excitation light was polarized at 45° with respect to the probe radiation (see Materials and Methods).

*Transients in the 500–600 nm region with 867-nm pump*

Fig. 2 shows a series of difference spectra for

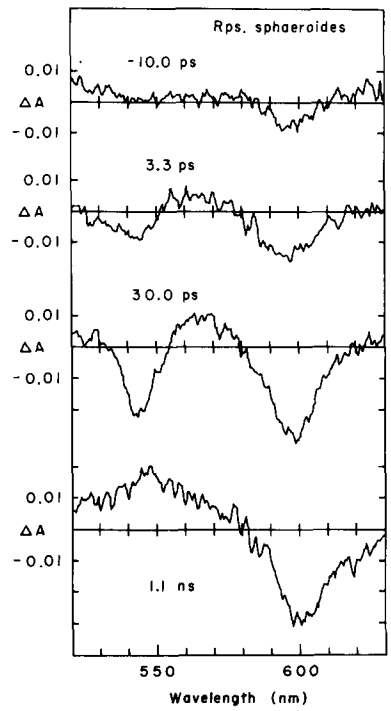


Fig. 2. Absorption changes in the $Q_x$-band region for *Rps. sphaeroides* reaction centers excited with weak 867-nm flashes lasting approx. 30 ps. Each spectrum is the average of 600 spectra.

*Rps. sphaeroides* in the 520–630 nm region as a function of delay time. The well resolved spectra show the bleachings of the 600-nm band due to P (and possibly B as well) and the growth and decay of the bleaching in the 545-nm band of the photoactive BPh. Recovery of the BPh bleaching reflects the step $P^+I^-Q \rightarrow P^+IQ^-$. Decay kinetics measured at 545 nm are in agreement with the measurements in the near-infrared (not shown) and with previous results [4–8,11–19], placing a time constant of approx. 200 ps on this step. The rise time of the absorption changes in both the visible and near-infrared, along with the question of the possible transient reduction of B prior to BPh, will be discussed in a subsequent article. These measurements suggest that the time constant for the reduction of BPh in *Rps. sphaeroides* is approx. 6 ps.

*Transients in the near-infrared with 867- or 962-nm pump*

Fig. 3 shows difference spectra in the near-infrared measured at 45 ps and 2 ns following excitation of *Rps. sphaeroides* reaction centers with weak 867-nm flashes. At each delay time, difference spectra are shown with both horizontal (solid) and vertical (dashed) polarizations of the probe light, for horizontally polarized pump pulses. Complementary results were obtained with vertically polarized pump pulses (V/H was essentially identical to H/V, and V/V to H/H). This is what one would expect for photoselection of unoriented preparations with pump and probe pulses arriving nearly colinearly at the sample. (Note that 90° geometry was not used; see Materials and Methods.)

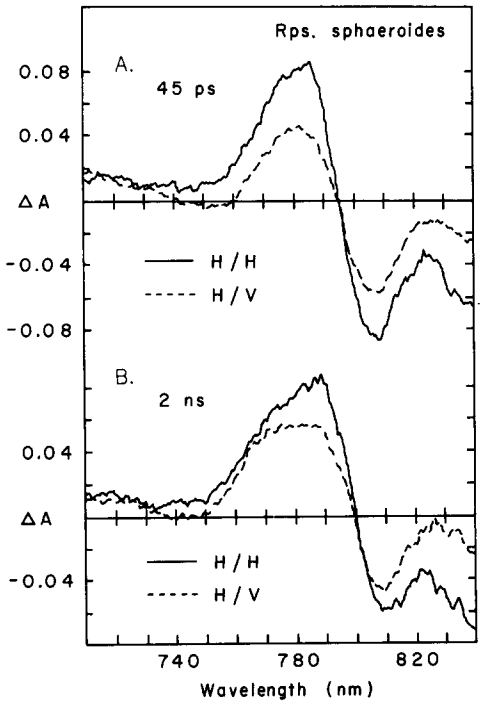


Fig. 3. Near-infrared absorption changes for *Rps. sphaeroides* reaction centers at 45 ps (A) and 2 ns (B) after excitation with weak 867-nm flashes. Spectra for parallel (———) and perpendicular (— — —) pump/probe polarization are given. Each spectrum is the average of 2400 spectra.

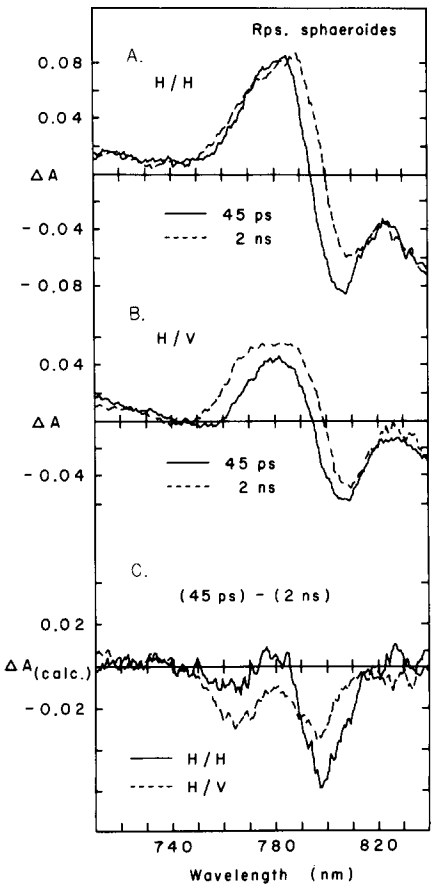


Fig. 4. Panels A and B have the data for *Rps. sphaeroides* of Fig. 3 replotted to show the time dependence for the two pump/probe polarizations. Panel C gives the difference between the 45-ps and 2-ns difference spectra for the parallel (———) and perpendicular (— — —) polarizations of pump and probe pulses.

In Fig. 4, data of Fig. 3 are replotted so as to show more clearly the time dependence of the polarization effects. Fig. 4A shows that for the parallel pump/probe polarization electron transfer from $I^-$ to Q results in an apparent red shift of the absorption spectrum near 800 nm. The isosbestic point shifts from 795 to 800 nm between approx. 45 ps and several nanoseconds. On the other hand, the difference spectra with the perpendicular polarization of pump and probe (Fig. 4B) indicate that electron transfer from $I^-$ to Q also results in a general increase in absorption (and/or reduction in bleaching) between 750 and 790 nm in addition to a shifting of the absorption near 800 nm. These observations are more clearly seen in the difference-difference spectra (45 ps spectra minus 2 ns spectra) shown in Fig. 4C for both relative polarizations of pump and probe pulses. Comparison of Fig. 4 (obtained with 867-nm excitation flashes) with Fig. 1 (obtained with 600-nm excitation flashes) indicates the importance of defining the pump/probe polarization when exciting in the long-wavelength band of P.

Similar photoselection measurements were made on *R. rubrum* reaction centers with weak 867-nm excitation flashes. The transient spectra for this species at short ($P^+I^-$) and long ($P^+Q^-$) delays for parallel (Fig. 5A) and perpendicular (Fig. 5B) polarizations of pump and probe pulses are in general very similar to those observed for *Rps. sphaeroides* (Fig. 4). The main difference is the stronger increase in absorbance between 750 and 770 nm for *R. rubrum* as compared to *Rps.*

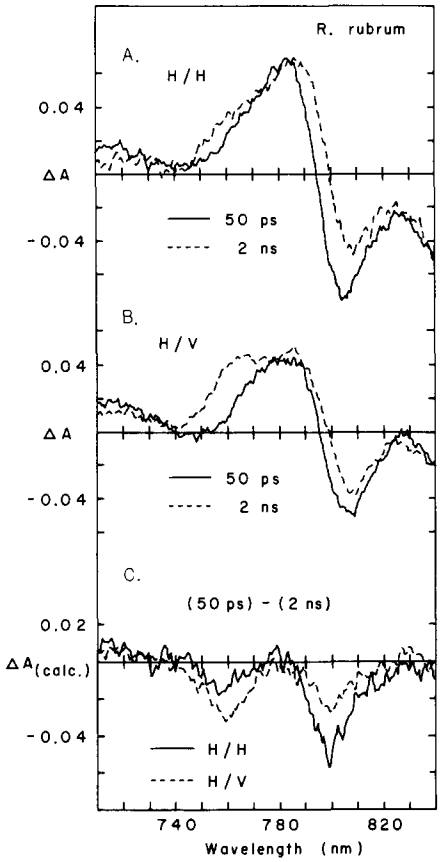


Fig. 5. Near-infrared transient difference spectra for *R. rubrum* reaction centers at 50 ps (———) and 2 ns (— — —) following excitation with weak 867 nm flashes with parallel (A) and perpendicular (B) pump/probe polarizations. Panel C shows the difference between the 50-ps and 2-ns spectra for the two polarizations. Each spectrum is the average of 1200 spectra.

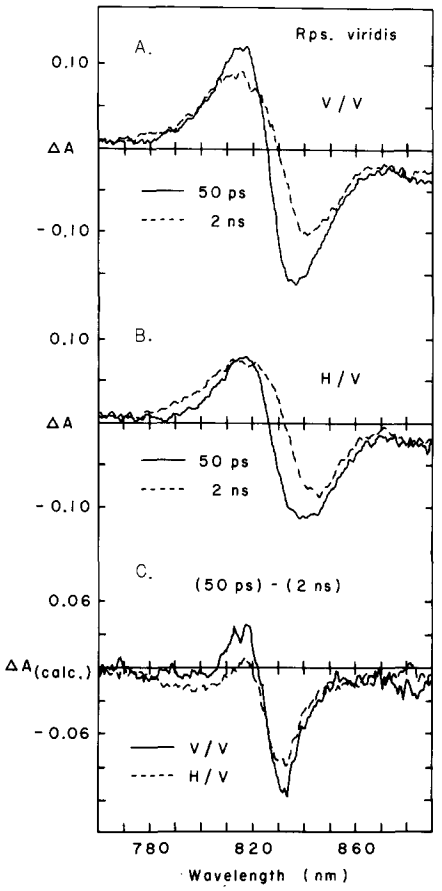


Fig. 6. Near-infrared absorption changes for *Rps. viridis* reaction centers excited with weak 962-nm flashes lasting approx. 30 ps. Each spectrum is the average of 1200 spectra.

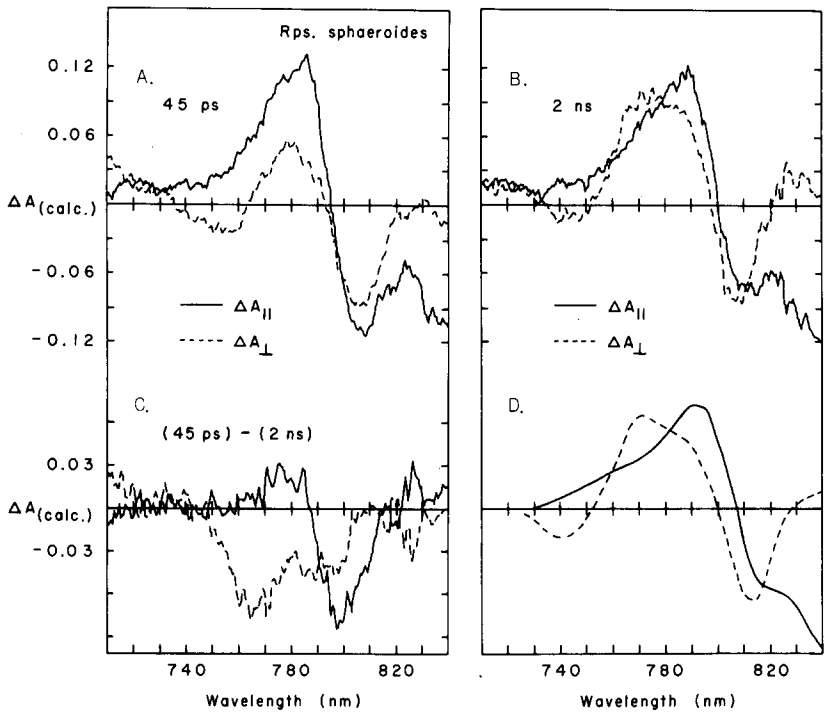


Fig. 7. Calculated absorption changes parallel ($\Delta A_{\parallel}$) and perpendicular ($\Delta A_{\perp}$) to the 870 nm transition for *Rps. sphaeroides* reaction centers. Panels A–C give spectra calculated from the spectra in Fig. 4 according to the formulae given in the text. These formulae and the calculated spectrum shown in panel D for state $P^+Q^-$ are taken from Ref. 33.

*sphaeroides.* This can be seen more clearly with perpendicular pump and probe (Fig. 5B vs. Fig. 4B), where it is also seen that the absorption near 785 nm does not increase in going from 50 ps to 2 ns as it does for *Rps. sphaeroides.* It is unclear at present whether these two small differences are due to the extra BPh ground-state absorbance at 760 nm in the *R. rubrum* sample (see Materials and Methods). All other spectral features for these two species are the same within experimental error. The calculated spectra ($P^+I^-$ minus $P^+Q^-$) are essentially identical for the two types of reaction centers (Figs. 4C and 5C).

The dichroism of the absorption changes in states $P^+I^-$ and $P^+Q^-$ in *Rps. viridis* reaction centers is much different from that in the BChl *a*-containing reaction centers. The near-infrared absorption changes at long and short delays for vertical probe polarization relative to vertically or horizontally polarized 962-nm pump pulses are shown in Fig. 6. (As in the cases of *Rps. sphaeroides* and *R. rubrum,* spectra taken with V/V are the same as those with H/H, and spectra with V/H compare to those with H/V.) With parallel pump/probe polarization in *Rps. viridis* (Fig. 6A), the decay of $P^+I^-$ (50 ps spectrum) to $P^+Q^-$ (2 ns spectrum) results in a clear decrease in absorbance near 815 nm and an increase in absorbance (or decay of an absorbance decrease) between 830 and 850 nm. This behavior is more complex than that observed in *Rps. sphaeroides* (Fig. 4A) and *R. rubrum* (Fig. 5A) where there is no significant change in absorbance near 785 nm as $P^+I^-$ decays to $P^+Q^-$.

The differences between the transient spectra at short and long delays with perpendicular polarizations for *Rps. viridis* (Fig. 6B) also are in contrast to those for *Rps. sphaeroides* (Fig. 4B) and *R. rubrum* (Fig. 5B). These differences show up clearly in the calculated spectra shown in Fig. 6C for *Rps. viridis.* The BPh bleaching near 790 nm is not as strong as that near 760 nm in the BPh *a*-containing reaction centers (Figs. 4C and 5C). In addition, the negative feature at approx. 830 nm in

*Rps. viridis* resulting from the reduction of I has almost the same amplitude with H/V polarization as it does with V/V polarization (Fig. 6C); in *Rps. sphaeroides* and *R. rubrum* (Figs. 4C and 5C), the negative feature at 800 nm is more pronounced when the polarizations are parallel.

*Calculated photodichroism spectra*

Figs. 7–9 show spectra calculated from the observed transient spectra for the three species (Figs. 3–6) according to the formulae derived by Vermeglio et al. [33]:

$$\Delta A_{\parallel} = 2\Delta A_{HH} - \Delta A_{HV}$$

$$\Delta A_{\perp} = 3\Delta A_{HV} - \Delta A_{HH}$$

Again, data for V/V could be substituted for H/H, and V/H for H/V polarizations. These calculated spectra represent the difference spectra for absorption parallel ($\Delta A_{\parallel}$) and perpendicular ($\Delta A_{\perp}$) to the 870- or 960-nm band that was pumped. For comparison with previous work, Fig. 7D shows calculated spectra for *Rps. sphaeroides* obtained by Vermeglio et al. [33] from photoselection studies on $P^+Q^-$ at room temperature. These were measured with millisecond-duration excitation flashes at 900 nm, using reaction centers dried on a glass slide. Except for the wavelength of the isosbestic point near 800 nm, the agreement between our data for $P^+Q^-$ (Fig. 7B) and the slower time scale data on $P^+Q^-$ (Fig. 7D) is excellent, especially considering the small magnitudes ($\Delta A \approx 0.1$) of the absorption changes (Figs. 3 and 4) that gave rise to our calculated spectra. This comparison gives us confidence in the calculated spec-

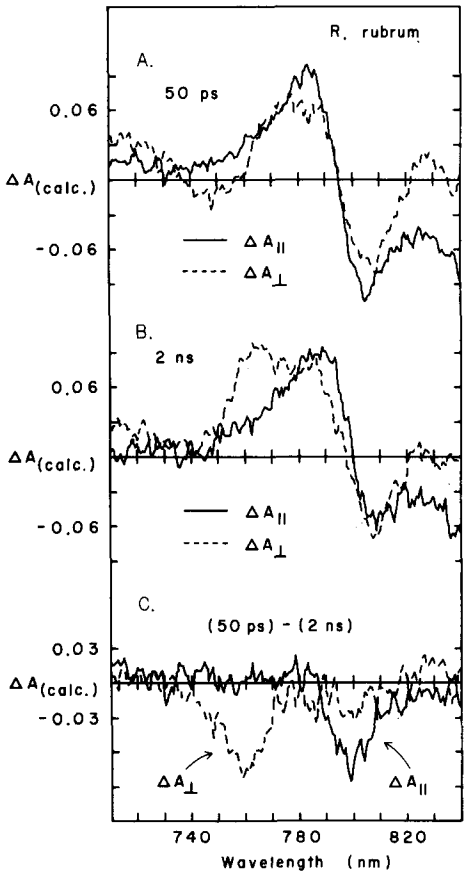


Fig. 8. Photodichroism spectra calculated from the data of Fig. 5 for *R. rubrum* reaction centers.

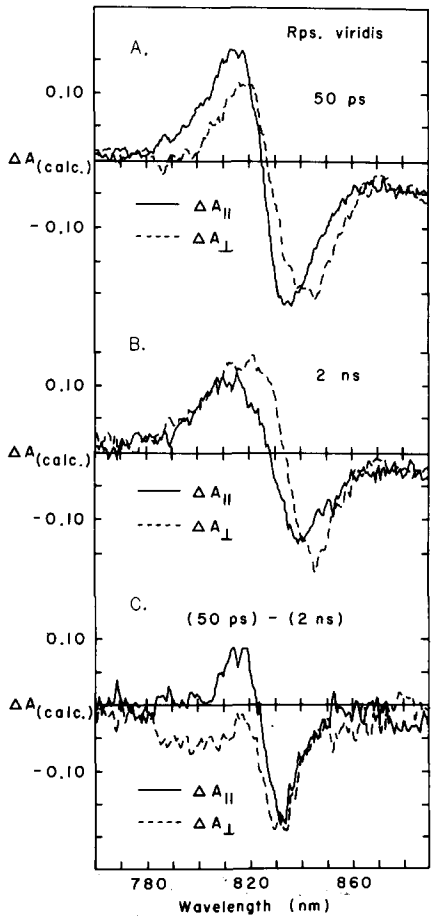


Fig. 9. Photodichroism spectra calculated from the data of Fig. 6 for *Rps. viridis* reaction centers.

tra at shorter delay times (Fig. 7A).

The calculated spectra for $P^+I^-$ measured at a 45 ps delay (Fig. 7A) are dramatically different from those for $P^+Q^-$ measured at long delays (Fig. 7B). The differences between the spectra obtained for states $P^+Q^-$ and $P^+I^-$ are emphasized in the calculated $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra shown in Fig. 7C.

The calculated $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra for *R. rubrum* (Fig. 8) are very similar to those for *Rps. sphaeroides* (Fig. 7) except for the extra increase in absorption near 765 nm in the former, as mentioned above. This difference appears to affect the spectra at both delays nearly equally, since the calculated photodichroism spectra for $P^+I^-$ minus $P^+Q^-$ are very similar for reaction centers of the two species (Figs. 7C and 8C).

The calculated absorption changes parallel and perpendicular to the 960-nm absorption band of P in *Rps. viridis* reaction centers are shown in Fig. 9. The calculated spectra for state $P^+Q^-$ (Fig. 9B), are in general similar to those obtained by Vermeglio and Paillotin [29] for *Rps. viridis* reaction centers immobilized at 160 K, except that the absorption bands were sharper at the lower temperature and splitting was observed in the absorption increase near 810 nm. As noted by Vermeglio and Paillotin [29], both the positive and negative features in the $\Delta A_{\perp}$ spectrum occur at longer wavelengths than the corresponding features in the $\Delta A_{\parallel}$ spectrum. Similar differences can be seen in the spectra for $P^+I^-$ (Fig. 9A).

## Discussion

*Absorbance changes associated with oxidation of P*

The photodichroism measured in the transient states of the reaction center contains much information on the relative orientations and interactions of the electron carriers. However, interpretations of the measurements are complicated by three factors. First, the absorption changes in some regions of the spectrum are made up of contributions from several overlapping absorption bands. Second, each of the underlying absorption bands could contain excitonic contributions from two or more of the six pigments. And third, each exciton band could contain contributions from at least four major optical transitions that occur in the isolated pigments.

For analyzing regions of the spectrum where several absorption bands overlap, Vermeglio et al. [33] have shown that it is informative to consider the quantities $\Delta A_{\parallel}$ and $\Delta A_{\perp}$, rather than the more customary polarization ratio, $p$. Figs. 7B, 8B and 9B show spectra of $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ for the formation of $P^+Q^-$. The spectra for *Rps. sphaeroides* (Fig. 7B) are in excellent agreement with spectra that Vermeglio et al. [33] have obtained with unoriented reaction centers from this species at room temperature (Fig. 7D). The spectra for *R. rubrum* (Fig. 8B) are similar to these. We used flowed samples and measured the absorbance changes 2 ns after the excitation flashes; Vermeglio et al. used reaction centers that were dried on a glass plate, and measured the absorbance changes on a time scale of 10–100 ms.

The calculated absorption changes for *Rps. viridis* (Fig. 9B) are similar to those obtained previously by continuous illumination of reaction centers at temperatures of 160 K or lower [29] or of whole cells oriented in a magnetic field at room temperature [28].

Depolarization due to rotation of the reaction centers is not expected to be significant on the time scale of our measurements, because the rotational correlation time is probably at least 50 ns. (The molecular weights of *Rps. sphaeroides* and *R. rubrum* reaction centers are of the order of 150 000, including bound detergent [39,40]; reaction centers of *Rps. viridis* are probably even larger [31].) The close agreement between our spectra for $P^+Q^-$ and those obtained previously [28,29,33] thus implies that the orientations of the $Q_y$ transition dipoles do not change detectably in the time interval between 2 ns and 10 ms after the formation of $P^+Q^-$. This in turn indicates that the relative orientations of the pigments and the interactions between the pigments do not change significantly in this interval. As we discuss below, Vermeglio and Paillotin [29] have suggested that a change in the relative orientations of the BChls occurs upon or after the formation of $P^+Q^-$ in *Rps. viridis*.

In *Rps. sphaeroides* and *R. rubrum*, the formation of $P^+Q^-$ causes an absorbance decrease on the long-wavelength side of the 800 nm absorption band, and an increase on the short-wavelength side. Similar absorbance changes occur on either side of 830 nm in *Rps. viridis*. The absorption

changes in the 800- or 830-nm region probably result, at least in part, from an electrochromic (Stark) effect of $P^+$ on the absorption bands of the other two BChls in the reaction center, or from a change in the excitonic interactions of P with the other BChls. As previous workers [27–29,31–37] have pointed out, however, the absorbance changes cannot be attributed simply to a hypsochromic shift of a single absorption band, because the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra are not proportional to each other. In *Rps. sphaeroides* and *R. rubrum,* the $\Delta A_{\perp}$ spectrum is displaced to shorter wavelengths relative to the $\Delta A_{\parallel}$ spectrum (Figs. 7B and 8B). In *Rps. viridis,* $\Delta A_{\perp}$ is displaced to longer wavelengths relative to $\Delta A_{\parallel}$ (Fig. 9B). Several different explanations have been offered for these observations. First, the 800- or 830-nm absorption band must contain contributions from both of the BChls that are not part of P; these two molecules could have different orientations and could lie at different distances from P. The oxidation of P could therefore affect their spectra differently. It has been suggested that the oxidation of P causes the spectrum of one of the BChls to shift to the blue, and the other to shift to the red [28,29,37]. Second, the oxidation could cause the other BChls to move, so that their dipoles take on new orientations [29,34]. Finally, the absorbance changes in the 800- or 830-nm region could be partly due to absorption bands that belong to P itself [27–35].

Vermeglio et al. [33] have concluded that the $\Delta A_{\perp}$ spectrum for $P^+Q^-$ in *Rps. sphaeroides* reflects the bleaching of a weak band centered near 805 nm, in addition to a shift of a band centered near 800 nm. In *Rps. viridis,* the $\Delta A_{\perp}$ spectrum appears to include the bleaching of a band near 850 nm, in addition to a shift that is centered near 830 nm [28–30]. The $\Delta A_{\parallel}$ spectra appear to contain contributions from the shifts of the 800- or 830-nm bands, but not from the bleaching of the 805- or 850-nm components. It has been proposed that the perpendicularly polarized absorption band at 805 or 850 nm is the higher-energy band formed by $Q_y$ exciton interactions of the two BChls that make up P [28,29,31,33]. When P is oxidized, the higher-energy band should bleach along with the lower-energy band at 870 or 960 nm. The $\Delta A_{\perp}$ and $\Delta A_{\parallel}$ spectra both may also reflect the formation of a new absorption band near 790 nm in *Rps. sphaeroides,* or near 810 nm in *Rps. viridis* [27–29,31–33]. This new band has been attributed to the molecule of BChl that remains unoxidized when one electron is removed from the dimer. There are, however, other possible interpretations of the spectra. For example, the 805- or 850-nm band could be an exciton band that arises primarily from the two BChls that are not part of P, but whose dipole strength is enhanced by interactions with P. Recent studies of BChl and BPh aggregates in vitro have shown that the $Q_y$ absorption bands can experience hyperchromism, resulting from a mixing with the Soret and $Q_x$ bands (Scherz, A. and Parson, W.W., unpublished results). Depending on the orientations of the four BChls, the oxidation of P thus could cause an increase or decrease in the dipole strengths (and rotational strengths) of absorption bands that are due largely to the other BChls. Numerous other suggestions concerning the absorption spectrum of P have been offered [2,27,34–37,41–43].

Our view of the absorption changes due to states $P^+Q^-$ and $P^+I^-$ (the latter to be discussed below) in all three bacteria is that the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra in the 800- or 830-nm region probably contain differently weighted contributions from the two BChls that are not part of P. The formation of $P^+$ could affect the two molecules differently, depending on how they are placed in the reaction centers, as suggested by Paillotin et al. [28,29] and Shuvalov et al. [37]. On the other hand, our measurements do not rule out the possibility that the BChls undergo significant movements in the time interval between 0 and 2 ns. Some of the differences between the spectra measured for $P^+I^-$ and $P^+Q^-$ could result from such movements.

The absorbance changes between 740 and 770 nm in the $\Delta A_{\perp}$ spectra for $P^+Q^-$ in *Rps. sphaeroides* and *R. rubrum,* (Figs. 7B and 8B) appear to reflect a bathochromic shift superimposed on a net absorbance increase; the $\Delta A_{\parallel}$ spectra show a relatively featureless absorbance increase. The $\Delta A_{\perp}$ spectra in this region probably emphasize absorbance changes due to the BPhs, which evidently are oriented so that their $Q_y$ transition dipoles are perpendicular to the 870-nm dipole [31,33]. The absorbance changes probably result at least partly from interactions of the BPhs with $Q^-$, because similar absorption changes occur

when reaction centers are converted to the state $PQ^-$ [44,45]. By photoselective excitation in the $Q_x$ bands at low temperature, Vermeglio et al. [33] have shown that the absorption changes between 740 and 770 nm for *Rps. sphaeroides* include slightly different contributions from each of the two BPhs. The effect of the reduction of Q on the BPh absorbing near 790 nm in *Rps. viridis* is not apparent in spectra obtained at room temperature (Fig. 9B), in agreement with previous results [28]. Measurements at lower temperature, where the near-infrared bands are better resolved, have led to the conclusion that the 790-nm transition of BPh is perpendicular to the 960-nm transition of P [29].

*Absorbance changes associated with reduction of I*

Figs. 7C, 8C and 9C show the difference spectra calculated by subtracting the measurements of $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ made at 2 ns from those made at 45 or 50 ps (or alternatively by calculating these spectra from the calculated absorption changes of Figs. 4C, 5C and 6C). These spectra give the parallel and perpendicular components of the absorption changes resulting from the initial reduction of I, minus any changes due to the reduction of Q at the later time and any changes due to movements of the pigments in the time interval between 45 ps and 2 ns. Q and $Q^-$ themselves do not absorb significantly in the near-infrared, but as mentioned above, $Q^-$ does appear to shift the absorption bands of the two BPhs to longer wavelengths. This effect evidently does not make a major contribution to the difference spectra of Figs. 7C and 8C for *Rps. sphaeroides* and *R. rubrum,* because the $\Delta A_{\perp}$ spectra show only a bleaching between 740 and 770 nm. The $\Delta A_{\perp}$ spectrum obtained with *Rps. viridis* shows a similar, but less pronounced, bleaching at 790 nm (Fig. 9C). The $\Delta A_{\parallel}$ spectra for *Rps. sphaeroides* and *R. rubrum* are essentially featureless in the 760-nm region (Figs. 7C and 8C), as is that for *Rps. viridis* near 790 nm (Fig. 9C), in agreement with the previous evidence that the $Q_y$ transition dipoles of the BPhs are oriented perpendicular to the 870- or 960-nm dipole of P [27–29,33,36,37].

The $\Delta A_{\perp}$ spectrum for *Rps. sphaeroides* (Fig. 7C) shows an absorbance decrease centered near 790 nm. The $\Delta A_{\parallel}$ spectrum includes a significantly stronger absorbance decrease near 800 nm, and a small absorbance increase near 780 nm. Similar features are evident in the spectra for *R. rubrum* (Fig. 8C), although the absorption decrease in the $\Delta A_{\perp}$ spectrum is not well defined. The $\Delta A_{\perp}$ and $\Delta A_{\parallel}$ spectra for *Rps. viridis* (Fig. 9C) show similar features in the 810–840-nm region, but the absorption decrease at 830 nm is nearly the same with the two polarizations. In all three species, the $\Delta A_{\parallel}$ spectrum could be interpreted as a combination of a bleaching and a hypsochromic shift.

In principle, the absorbance decreases at 790- or 830-nm regions in the $\Delta A_{\perp}$ spectra could result from a change in the interactions between a BChl and BPh upon the reduction of the BPh. If the unreduced BPh and BChl were suitably oriented, excitonic interactions in the unreduced complex could strengthen the $Q_y$ absorption band of the BChl at the expense of the higher-energy $Q_y$ band (and possibly also the $Q_x$ and Soret bands) of the BPh. This transfer of dipole strength would be lost when the BPh was reduced, resulting in an absorbance decrease in the longer-wavelength band. The exciton interactions between the unreduced molecules also could lower the energy of the longer-wavelength band, so that the reduction of the BPh would result in a shift to shorter wavelengths in addition to the decrease in dipole strength. Similar interpretations have been suggested previously [8,27]. It is not clear, however, that a change in exciton interactions involving the $Q_y$ band of a BPh could explain the bleaching and shift seen in the 800- or 830-nm region of the $\Delta A_{\parallel}$ spectrum. The 760- or 790-nm absorption bands of the BPhs would not interact strongly with a BChl transition dipole that was parallel to the 870- or 960-nm dipole, because they are oriented perpendicular to this dipole. It might be possible to explain the parallel-polarized absorption decrease if hyperchromism involving the $Q_x$ and $B_x$ ($x$-polarized Soret) bands of the BPh contributes to the strengths of the 800- or 830-nm absorption in the unreduced reaction centers. But the $B_x$ and $Q_x$ transitions are too far removed in energy to have a significant effect on the energy of the long-wavelength absorption band. Interactions involving these transitions thus probably could not account for the hypsochromic shift that is seen near 800 or 830 nm in the $\Delta A_{\parallel}$ spectrum.

A possible explanation for the dichroism of the absorption changes in the 800- or 830-nm region is that $I^-$ consists partly of a BChl radical, $B^-$. The bleaching component of the absorption changes then could be explained simply by the loss of absorption due to B. The shift could be explained by a change in the interaction between B and the fourth (unreduced) BChl, or by an electrochromic effect of $B^-$ or $BPh^-$ on the fourth BChl. The shift could be more pronounced in the $\Delta A_{\parallel}$ spectrum than in the $\Delta A_{\perp}$ spectrum if the $Q_y$ transition dipole of the fourth BChl is oriented more or less parallel to the 870- or 960-nm dipole and the $Q_y$ transition dipole of B is oriented at an appreciably larger angle with respect to this dipole. The idea that the two BChl dipoles have different orientations would be consistent with the dichroism of the $P^+Q^-$ spectra, as discussed above. The idea that $I^-$ consists of a mixture of $BPh^-$ and $B^-$ would be in accord with the observation that the absorption spectrum of *Rps. sphaeroides* reaction centers in state $P^F$ varies with temperature [2,22]. It was proposed that the added electron density of $I^-$ resides largely (approx. 80%) on the BPh at room temperature, and increasingly so at lower temperature. Similar conclusions have been drawn from ESR and ENDOR measurements on *Rps. viridis* [25,46]. One interpretation of the larger contribution of the perpendicular component to the absorption decrease at 830 nm in *Rps. viridis* (Fig. 9C), as compared to the absorption decrease at 800 nm in *Rps. sphaeroides* and *R. rubrum* (Figs. 7C and 8C), is that the energy gap between $P^+B^-$ and $P^+BPh^-$ could be smaller in *Rps. viridis,* so that the electron spends a greater percentage of the time on B in this species. Comparative studies of the temperature dependence of the photodichroism in the three species could test this possibility.

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