Biochimica et Biophysica Acta, 765 (1984) 138–146
Elsevier

BBA 41469

PICOSECOND PHOTODICHROISM STUDIES ON REACTION CENTERS FROM THE GREEN PHOTOSYNTHETIC BACTERIUM *CHLOROFLEXUS AURANTIACUS*

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(Received September 26th, 1983)
(Revised manuscript received January 13th, 1984)

Key words: Reaction center; Photodichroism; Photoselection; Bacterial photosynthesis; (C. aurantiacus)

Picosecond photodichroism (photoselection) measurements have been carried out on reaction centers from the facultative green photosynthetic bacterium *Chloroflexus aurantiacus* using weak 30 ps flashes in the long-wavelength band of the primary electron donor, P. Absorption changes due to the chemical and photochemical oxidation of P and the reduction of quinone also have been examined. Our results on *Chloroflexus* suggest that the Q_y transition-dipoles of the bacteriopheophytin molecules participating in, or affected by, the primary reactions are oriented essentially perpendicular to the 865 nm transition dipole of P. This is in agreement with previous work on reaction centers from purple bacteria, such as *Rhodopseudomonas sphaeroides*. The data also suggest that the 812 nm ground-state transition is oriented at an angle of 45–65° with respect to the 865 nm transition. The new band that appears near 800 nm upon oxidation of P is polarized mainly parallel to the 865 nm band. These relative polarizations of the absorption bands are in very good agreement with the results of recent linear dichroism studies (Vasmel, H., Meiburg, R.F., Kramer, H.J.M., De Vos, L.J. and Amesz, J. (1983) Biochim. Biophys. Acta 724, 333–339). Possible origins for the absorption changes and the photodichroism spectra are discussed. The data are consistent with either a monomeric or dimeric structure of P-865.

Introduction

Reaction centers in photosynthetic organisms are highly organized pigment-protein complexes that convert the light energy gathered by the antenna system into chemical potential. This is accomplished via a charge separation process initiated in the lowest excited singlet state, P*, of the primary electron donor, usually called P. In reaction centers from photosynthetic bacteria, a considerable body of data has been interpreted to mean that P is a complex involving two bacteriochlorophyll (BChl) molecules [1]. Recently, however, this view has been questioned and the nature of P and its absorption spectrum remains a point of some controversy [2,3]. An electron from P* ultimately arrives on an acceptor that has ready access to the secondary electron transfer components. This acceptor is a quinone (Q) in most bacterial reaction centers and in Photosystem II of plants [1]. The near unity quantum yield of photochemistry appears to be achieved by employing a series of rapid electron-transfer steps that spatially separate the oxidized and reduced photoproducts and thereby prevent recombination. A number of molecular and electronic factors combine to

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; LDAO, lauryldimethylamine *N*-oxide; V, vertical; H, horizontal; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine.

achieve this efficient charge separation, including the distances and relative orientations of the pigments participating in the primary reactions, the interactions that occur among these pigments and the free energy changes that accompany the reactions [4].

In bacterial reaction centers a radical pair state P^+I^- forms within 4–10 ps of excitation and decays to P^+Q^- in several hundred picoseconds at room temperature [1,5–9]. I appears to involve a bacteriopheophytin (BPh) interacting with a BChl (B) not intimately involved in P [10–12].

Experimental techniques employing polarized light have been very helpful in attempts to understand the relative orientations of pigments in reaction centers of photosynthetic organisms [13]. Linear dichroism studies have been carried out on oriented samples of bacterial reaction centers and chromatophores in the ground state, oxidized chemically (P^+), under continuous illumination (P^+Q^-), or trapped under special conditions in state PI^-Q^- [14–18]. Photodichroism studies, which measure the linear dichroism of the absorption changes produced by a flash of light, have been performed on bacterial systems with samples oriented in some way, such as in a magnetic field [19]. Alternatively, unoriented samples have been investigated by employing polarized actinic light (photoselection) [20–24].

Picosecond photodichroism measurements at room temperature have been reported recently on flowed, unoriented reaction centers of the purple bacteria *Rhodopseudomonas sphaeroides, Rhodospirillum rubrum,* and *Rhodopseudomonas viridis* excited with weak 30 ps flashes in the long-wavelength band of P [24]. The dichroism of the absorption changes was investigated in the early transient state P^+I^-, as well as in the longer-lived state P^+Q^- shortly after its formation. Unoriented samples at room temperature can be studied in picosecond photoselection measurements because the rotational correlation time for a particle the size of the reaction center appears to be 25 ns or longer [25].

In this paper we present the results of photoselection photodichroism studies at room temperature on reaction centers from the facultative green photosynthetic bacterium *Chloroflexus aurantiacus,* excited with weak 867 nm flashes lasting approx. 30 ps. The results of picosecond measurements on the early transient states in reaction centers from *Chloroflexus* and *Rps. sphaeroides* pumped with subsaturating 600 nm flashes were recently compared [9]. Some of the contrasting observations between the two reaction centers were attributed to possible differences in pigment content and/or pigment-pigment interactions. It was previously suggested on the basis of the ground-state absorption spectrum that *Chloroflexus* reaction centers contain three BChl *a* and three BPh *a* [26] instead of the four BChl and two BPh found in reaction centers of all the purple bacteria that have been analyzed [27–29]. Pigment extraction studies on *Chloroflexus* reaction centers have now shown that the BChl/BPh ratio is 1 : 1 [30], as compared to 2 : 1 for the purple bacteria discussed above. All available evidence indicates that the reaction centers of *Chloroflexus* are more similar to those of the purple bacteria than they are to the anaerobic green bacteria [9,26,30,31]. Picosecond photodichroism studies on the transient states in *Chloroflexus* are therefore helpful not only in understanding further the primary events, pigment orientations, and possible molecular interactions in reaction centers of this green bacterium, but also in those from other species of photosynthetic bacteria.

Materials and Methods

Chloroflexus reaction centers were prepared by a modification (L. Mancino and R. Blankenship, to be published) of the procedure described by Pierson and Thornber [26] and suspended in 20 mM Tris-HCl (pH 8), 0.05% LDAO (a gift from Onyx Chemical, Jersey City, NJ), plus 10 mM sodium ascorbate. For the picosecond measurements, samples were flowed through a 4 mm pathlength cell ($A_{812} = 0.8$) and maintained at approx. 25°C. Reaction centers showed no decomposition over the course of the experiments.

The dual-beam picosecond transient absorption spectrometer and the configuration for photodichroism measurements has been described elsewhere [24,32]. Weak excitation flashes of approx. 30 ps duration at 867 nm were about half-saturating with respect to bleaching in the 865 nm band of P. Since the pump and probe pulses intersect at the sample at an angle of approx. 6°, the results of measurements using V/V and V/H pump/probe polarizations were essentially the same as those employing H/H and H/V polarizations, respectively. The frequency of the system was 10 Hz.

Ground state absorption spectra and difference spectra for reaction centers in various states were taken on a Cary 219 spectrometer coupled to an Apple computer. Light-minus-dark difference spectra (P^+Q^--PQ) and those for chemical oxidation of P with potassium ferricyanide (P^+Q-PQ) were obtained by computer subtraction of spectra obtained under various conditions. Difference spectra for the reduction of Q (PQ^--PQ) were acquired by illuminating reaction centers with blue light in the presence of 130 μM DAD and 10 mM ascorbate. Electron transfer from DAD to P^+ is fast compared to the recovery of P^+Q^-, so that in steady-state reaction centers accumulate in the state PQ^- [33]. Blocking filters were used to shield the photomultiplier tube from the actinic light. Several difference spectra of each type were averaged.

Results

Transient difference spectra

Fig. 1A shows transient difference spectra at a 60 ps time delay obtained with vertical (solid) and horizontal (dashed) polarizations of the broadband probe pulses with respect to vertically polarized 867 nm pump pulses. On the basis of a previous picosecond study of *Chloroflexus,* we attribute the absorption changes at the 60 ps time delay to the formation of state P^+I^- [9]. Transient difference spectra attributable [9,26,31] to the formation of state P^+Q^- are shown for both pump/probe polarizations at a 2.3 ns time delay in Fig. 1B. The time constant for electron transfer from P^+I^- to P^+Q^- was measured previously to be 310 ± 30 ps [9].

The spectra for both polarizations at both delay times are similar between 790 and 820 nm in that they show an absorption increase at 800 nm and an absorption decrease near 815 nm. However, for both P^+I^- (Fig. 1A) and P^+Q^- (Fig. 1B), the magnitudes of these features are greater for parallel than for perpendicular polarization. The bleaching at longer wavelengths proceeding toward the 865 nm ground-state maximum of P also is clearly larger, as expected, for probe flashes polarized parallel to the 867 nm excitation flashes. The difference spectrum for P^-I^- at the 60 ps time delay obtained with perpendicular pump/probe polarization (dashed spectrum in Fig. 1A)

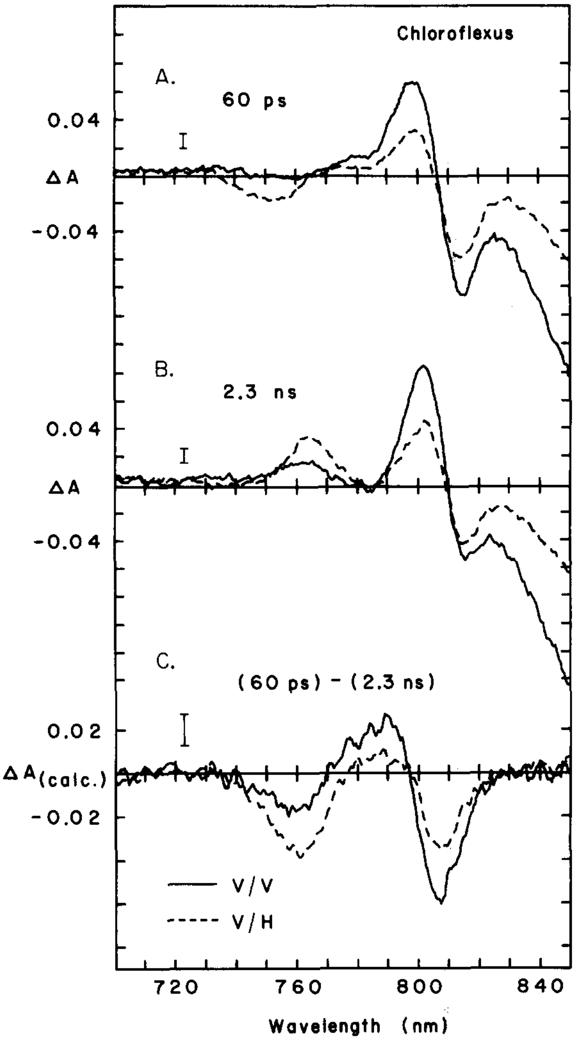

Fig. 1. Near-infrared absorption changes for *Chloroflexus* reaction centers at 60 ps (A) and 2.3 ns (B) after excitation with weak 30 ps 867 nm flashes. Spectra for parallel (V/V; ———) and perpendicular (V/H; — — —) pump/probe polarizations are given. Each spectrum is the average of approx. 2400 spectra. Part C shows the difference between the 60 ps and 2.3 ns spectra for parallel (V/V; ———) and perpendicular (V/H; — — —) polarizations of pump and probe pulses. The maximum standard deviation in ΔA over the spectral region covered is shown on the left of each panel.

shows an absorption decrease in the region of BPh ground state absorption near 750 nm; the absorption changes for parallel polarization are essentially nil in this region (solid spectrum). The difference spectra in the region of BPh ground state absorption at the 2.3 ns time delay (Fig. 1B) exhibit a distinct absorption increase at 765 nm characteristic of the formation of P^+Q^- [9,26,31]. This feature is much larger for perpendicular than for parallel polarizations of the pump and probe flashes.

Differences between the spectra at short and long delay times are seen in the calculated spectra of Fig. 1C, in which the absorption changes at 60 ps minus those at 2.3 ns are plotted for both polarizations. The calculated spectra for both parallel and perpendicular pump/probe polarizations contain absorption decreases in the region of BChl ground state absorption near 810 nm and in the region of BPh ground state absorption near 760 nm, and a positive region in between. However, the magnitudes of each feature depend on the relative polarizations of pump and probe flashes.

Calculated photodichroism spectra

Fig. 2 shows difference spectra for *Chloroflexus* calculated from the data of Fig. 1 according to the formulae derived by Vermeglio et al. [21]:

$$\Delta A_{\parallel} = 2\Delta A_{V/V} - \Delta A_{V/H}$$

$$\Delta A_{\perp} = 3\Delta A_{V/H} - \Delta A_{V/V}$$

These calculated spectra represent the absorption changes polarized parallel (solid) or perpendicular (dashed) to the 865 nm transition dipole being pumped. The calculated difference-difference photodichroism spectra (60 ps minus 2.3 ns spectra) are shown in Fig. 2C. Fig. 3 presents the data of Figs. 2A and 2B replotted, so as to show more clearly the time-dependence of the absorption changes for each pump/probe polarization. Difference spectra corresponding to excitation with unpolarized light can be calculated as $\Delta A = 2\Delta A_{V/H} + \Delta A_{V/V}$ [21].

The gross features of the calculated $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra of Figs. 2 and 3 are similar to those seen in the measured V/V at V/H spectra of Fig. 1. Subtle differences between the two sets of spectra can be seen mainly near the isosbestic wavelengths, between 795 and 800 nm, and in the region of strong BPh ground state absorption between 750 and 770. Comparison of Figs. 3A and 3B indicates that the absorption changes that probably can be associated mainly with the BPhs, between 740 and 770 nm, are polarized largely perpendicular to the 865 nm band. The absorption

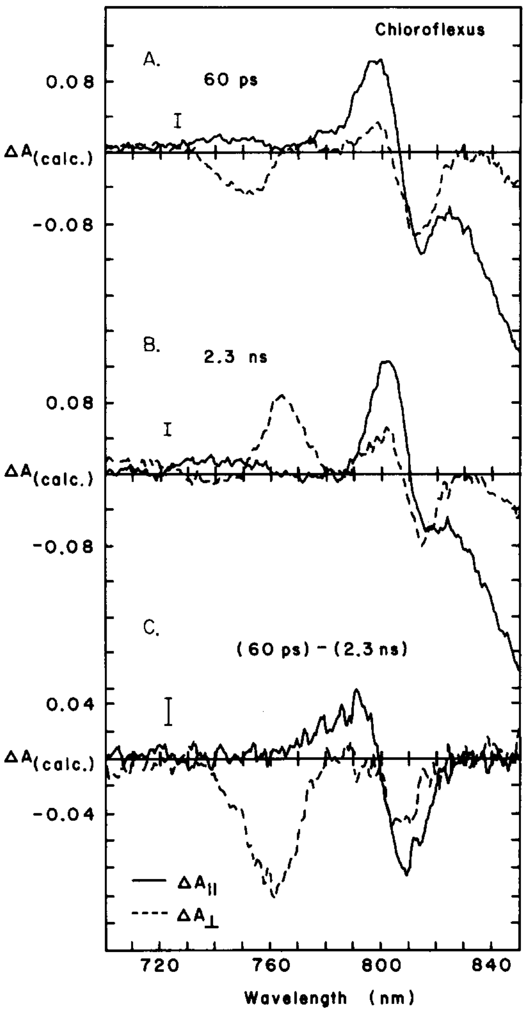

Fig. 2. Calculated absorption changes parallel ($\Delta A_{\parallel}$; ———) and perpendicular ($\Delta A_{\perp}$; — — —) to the 865 nm transition for *Chloroflexus* reaction centers. These spectra were calculated from the spectra in Fig. 1 using the formulae given in the text. (A), 60 ps; (B), 2.3 ns; (C), 60 ps − 2.3 ns.

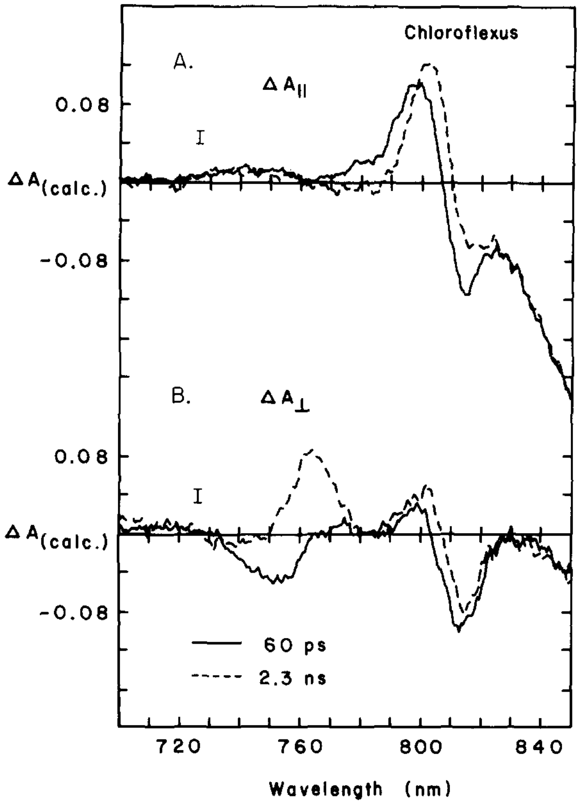

Fig. 3. Photodichroism spectra from Figs. 2A and 2B replotted to show more clearly the time dependence (60 ps, (———); 2.3 ns (— — —)) of the absorption changes for the two pump/probe polarizations. (A), $\Delta A_{\parallel}$; (B) $\Delta A_{\perp}$.

changes between 790 and 820 nm, which can be associated mainly with BChl(s), contain both polarizations, but generally are of larger magnitude in the $\Delta A_{\parallel}$ spectra as compared to the $\Delta A_{\perp}$ spectra.

Steady-state spectra

Absorption changes associated with the chemical oxidation of P are shown in Fig. 4A, and those due to the photochemical reduction of Q in the presence of DAD are presented in Fig. 4B. Note the expanded vertical scale in Fig. 4B, indicating the much smaller amplitude of the absorption changes due to the formation of Q^- compared to those due to the formation of P^+. To first order, the sum of these two difference spectra should reproduce the P^+Q^--PQ, light-minus-dark difference spectrum (not shown). This is found to be the case, except that the sum of Figs. 4A and 4B gives a positive feature near 760 nm that is about 20% larger than found in the measured P^+Q^- difference spectrum. It is unclear at present whether this difference is due to an effect on the BPh ground state absorption caused by the simultaneous oxidation of P and reduction of Q, or to imperfections in the normalization procedure at 865 nm. The ground state absorption spectrum of *Chloroflexus* reaction centers is shown in Fig. 4C.

Oxidation of P results in bleaching of the 865 nm band of P, the apparent blue-shift of the BChl

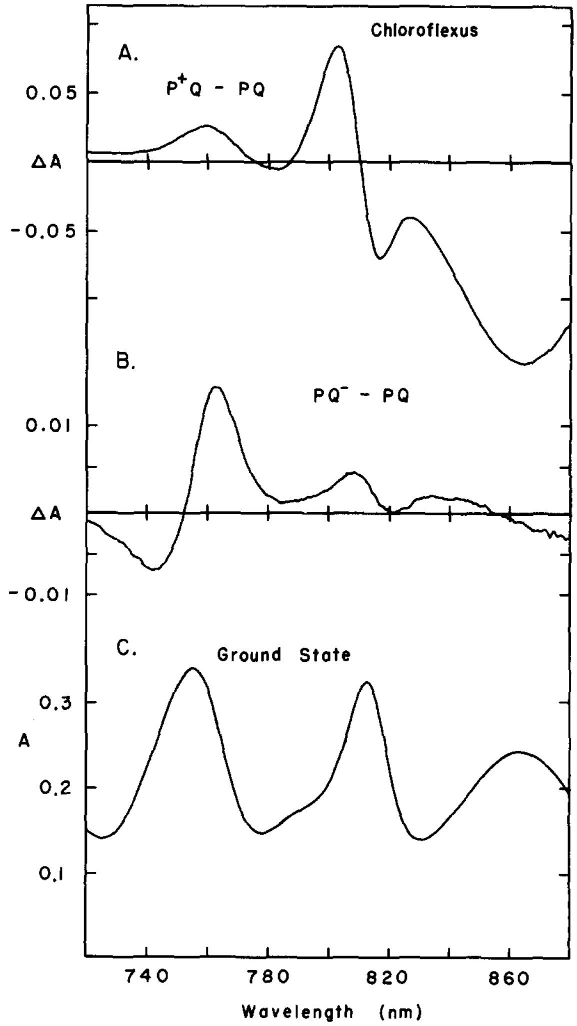

Fig. 4. Difference spectra for the formation of states P^+Q (A) and PQ^- (B) from ground state PQ in *Chloroflexus* reaction centers. These spectra were obtained by the procedures detailed in Materials and Methods. The ground state absorption spectrum is shown in (C).

absorption at 810 nm, and the appearance of a net positive absorption near 760 nm (Fig. 4A). Reduction of Q results in a red-shift of the BPh ground state absorption near 760 nm and a slight blue-shift of the BChl absorption near 810 nm (Fig. 4B). The latter absorption changes are similar to those observed previously in *Rps. sphaeroides* reaction centers upon the reduction of Q [33] and are essentially the same as those reported recently for *Chloroflexus* [34]. Comparison of Figs. 4A and 4B with the picosecond data for the formation of P^+Q^- (Figs. 1B, 2B and 3) indicates that the absorption changes attributable to BChl, at wavelengths longer than 780 nm, are due mainly to the oxidation of P, while the absorption changes in the region of BPh ground state absorption near 760 nm contain contributions from both the oxidation of P (60–70%) and the reduction of Q (30–40%).

Discussion

Absorption changes associated with the oxidation of P

Bleaching between 830 and 850 nm, on the short-wavelength side of the 865 nm band of P, is seen mainly in the $\Delta A_{\parallel}$ difference spectra for the formation of both P^+I^- (Fig. 2A) and P^+Q^- (Fig. 2B). This is as expected for pumping and probing in the same optical transition.

Comparison of the bleachings near 815 nm in Figs. 2A and 2B, indicates that the net ground state transition at 812 nm (Fig. 4C) contains nearly equal contributions of components parallel and perpendicular to the 865 nm band. This observation is consistent with the 77 K linear dichroism spectra reported by Vasmel et al. [18]. They found that the 813 nm transition is close to the magic angle of approx. 55° with respect to the press axis of the gel (i.e. $A_{\parallel} = A_{\perp}$), and that the strong absorption increase at 806 nm that occurs upon oxidation of P is polarized largely parallel to the press axis of the gel. (The near-infrared ground-state peaks are shifted to longer wavelengths at low temperature relative to the positions at room temperature.) These results also are in accord with our P^+Q^- and P^+I^- spectra, in which the positive feature near 800 nm is much larger in the $\Delta A_{\parallel}$ than in the $\Delta A_{\perp}$ spectra (compare Figs. 3A and 3B).

Interpretation of these absorption changes is complex. The absorption increase near 800 nm and the absorption decrease near 815 nm due to the formation of P^+Q^- (Figs. 2B and 3) cannot be interpreted entirely as an electrochromic blue-shift of a single absorption band (such as due to the BChl, B). This is so because the relative magnitudes of the positive and negative features are not equal, and the absorption changes in the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra between 780 and 830 nm are not proportional to one another.

The asymmetry of the $\Delta A_{\parallel}$ spectrum at 2.3 ns could be derived from an electrochromic shift plus, as Pierson and Thornber have suggested [26], an additional absorption at 800 nm due to monomer-like absorption of netural BChl which might appear when one component of P is oxidized. Vermeglio and Clayton [14] previously made a similar proposal for the absorption increase near 790 nm observed upon oxidation of P in *Rps. sphaeroides* reaction centers. Similarly, the asymmetry of the $\Delta A_{\perp}$ spectrum at 2.3 ns could be derived from the same electrochromic shift plus, now, an additional bleaching near 815 nm due to the bleaching of P's higher-energy excitonic component. A previous suggestion by Pierson and Thornber [26] that the ground state shoulder near 790 nm is due to the higher-energy excitonic component of P is not compatible with recent 77 K linear dichroism measurements [18] which indicate that the 790 nm absorption is polarized largely parallel to the 870 nm band. The idea that the higher-energy excitonic component of P is at 815 nm and the monomer-like absorption of P^+ is at 800 nm in *Chloroflexus* is questionable in view of the fact that features near 805 and 790 nm have been interpreted similarly in *Rps. sphaeroides*, even though the 865 nm band of P is at the same wavelength in both species. Similar reasoning also applies to the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra in this region for P^+I^- (Fig. 2A), with the added complication that in this state the absorption changes due to the oxidation of P are superimposed on an absorption decrease centered near 805 nm caused by the reduction of I (see Fig. 2C and below).

Thus, other possibilities for the origins of the absorption changes between 780 and 830 nm in *Chloroflexus*, in addition to being partially due to an electrochromic blue-shift, should be considered.

It has been suggested for *Rps. sphaeroides* (and *Rps. viridis*) reaction centers that the primary reactions may affect differently two BChls absorbing in the 800 nm (or 830 nm) region [17,19,20,23,24]. The two BChls in these species could be oriented differently with respect to P, could interact differently with other components, or they could interact with each other. Disruption of such interactions and the formation of new ones caused by the oxidation of P could account for the complex dichroism of the absorption changes in the 800 or 830 nm regions in *Rps. sphaeroides* and *Rps. viridis*, if the pigments are suitably oriented [24].

Similar reasoning could be applied to *Chloroflexus*. However, pigment extraction studies on this species give a BChl/BPh ratio of 1 : 1, indicating a likely pigment content of three BChl and three BPh [30]. A similar pigment content has been proposed on the basis of the reaction-center ground-state absorption spectrum [26]. Thus, one must consider fewer BChl molecules in *Chloroflexus* when accounting for the 812 nm ground-state absorption band, and the 790 nm shoulder (Fig. 4C), and for the observation that the 812 nm transition appears to be oriented differently with respect to P's at 865 nm than does the 800 nm transition in reaction centers in which P is oxidized (Figs. 2 and 3 and Ref. 18).

One possibility is that P is not an excitonic dimer [2,3]; the 865 nm band would be assigned to a monomeric primary electron donor, the 812 nm band mainly to B, and the 790 nm absorption mainly to the third BChl. Interaction between this third BChl and B, or between either molecule and P or a BPh, could give rise to a stronger ground-state absorption band at 812 than at 790 nm (Fig. 4C). Oxidation of P could disrupt these interactions and cause a redistribution of dipole strength. Another possibility, consistent with P being a dimer, is that one BChl (B) gives rise to the 812 nm ground-state band, and that B moves and/or has an interaction with a BPh that changes when P is oxidized. This argument requires that the 790 nm ground-state shoulder be due to a BPh; the approx. 30 nm red-shift from the normal BPh absorption wavelength is large but cannot be ruled out.

Although the formation of P^+Q^- causes absorption changes that can be associated mainly with BChl(s) in the 800 nm region in both the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra, those in the region of the BPh Q_y ground-state absorption near 755 nm are largely polarized perpendicular to the 865 nm transition. This latter observation is similar to that made previously for *Rps. sphaeroides* (at 760 nm) and *Rps. viridis* (at 790 nm) [19–24] and in low temperature linear dichroism measurements at 755 nm on *Chloroflexus* [18].

The $\Delta A_{\perp}$ spectrum of Fig. 2B exhibits a prominant absorption increase near 760 nm; the $\Delta A_{\parallel}$ spectrum is relatively featureless in this region. The difference spectra of Figs. 4A and 4B indicate that about 60% of the absorption increase near 760 nm in the P^+Q^- spectrum (Fig. 2B) is due to the oxidation of P. The positive feature at 760 nm without any substantial corresponding decrease elsewhere in the BPh absorption band suggests that at least part of the absorption changes in this region could be due to a gaining of intensity at 760 nm upon oxidation of P, possibly at the expense of the 812 nm band, i.e. a hyperchromic effect (A. Sherz and W.W. Parson, personal communication). On the other hand, reduction of Q causes a red shift of the BPh absorption centered near 755 nm (Fig. 4B), an effect similar to that observed in *Rps. sphaeroides* reaction centers [33]. The magnitude of the absorption changes are smaller than those due to the oxidation of P in this region (Fig. 4A vs. 4B). Thus, the net result of these effects is that the $\Delta A_{\perp}$ difference spectrum for the formation of P^+Q^- in the region of BPh ground state absorption (dashed curve in Fig. 2B) is dominated by an absorption increase near 760 nm due to P^+ formation, with the small trough centered at 740 nm arising from reduction of Q.

Absorption changes associated with the reduction of I

The $\Delta A_{\perp}$ spectrum for the formation of P^+I^- shows an absorption decrease at 750 nm, in the BPh ground state band (Fig. 2A); again the $\Delta A_{\parallel}$ spectrum is relatively featureless in the region. A previous picosecond study of *Chloroflexus* reaction centers employing 600 nm flashes [9] has shown that the 535 nm band of BPh also bleaches upon formation of P^+I^-; a similar effect was found here with 867 nm flashes (not shown). These features suggest that the formation of I^- consists, at least in part, of the reduction of one of the three BPhs. The apparent shift of the BPh bleaching from 750 nm in Fig. 2A to 760 in the difference-difference spectrum in Fig. 2C may result from a partial cancellation in Fig. 2A of a strong bleaching due to BPh reduction and the absorption increase in this region due to P^+ formation (see Fig. 4A). If so, then the hyperchromic effect on the BPh absorption induced by oxidation of P is localized on a BPh molecule other than the one that is reduced in I^-. An electrochromic red-shift of the BPh ground state absorption due to the reduction of Q also contributes to the absorption increase near 760 nm in Fig. 2B and, thus, to the large negative feature in this region in Fig. 2C.

In addition to bleachings in the BPh ground state absorption bands, the reduction of I also causes absorption changes in spectral regions where the BChl absorb, as is found to be the case in reaction centers from purple bacteria [10–12,35]. The absorption changes in the 780–820 nm region due to the reduction of I consist mainly, but not completely, of a net absorption decrease centered near 805 nm. One effect of this decrease is to make the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra for P^+I^- at the 60 ps delay (Fig. 2A) appear blue-shifted and the negative feature more pronounced than in the P^+Q^- spectra at the 2.3 ns delay (Fig. 2B). This can be seen more clearly when the spectra of Fig. 2A and B are replotted in the manner shown in Fig. 3, and in the difference-difference spectra of Fig. 2C. The negative features at 805 nm in both the $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ spectra of Fig. 2C could be due to an absorption decrease in the Q_y absorption band of B, upon the reduction of I to I^-. It is unclear at present whether this bleaching is due to the unpaired electron of I^- spending part of the time on both BPh and B, or to the loss of intensity in B's ground state absorption band upon disruption of interactions between B and other components.

Conclusions

Dichroism spectra of the absorption changes due to the formation of states P^+I^- and P^+Q^- in *Chloroflexus* reaction centers have been reported. The Q_y transition(s) at 755 nm of the BPh molecule(s) participating in or affected by the primary electron transfer steps are oriented essentially perpendicular to the 865 nm, long-wavelength transition of the primary electron donor P. The ground state absorption band near 812 nm is oriented at 45–65° (i.e. near the magic angle of approx. 55°) with respect to the 865 nm band, while the new band near 800 nm in reaction centers having P oxidized is oriented mainly parallel to the long-wavelength band. These relative orientations of the near-infrared transitions are in excellent agreement with results of recent low temperature linear dichroism studies on *Chloroflexus* [18].

We have discussed a number of possible origins for the ground state and photodichroism spectra between 780 and 830 nm; the absorption changes in this region cannot be due solely to an electrochromic blue-shift of a single absorption band. The results have been interpreted in terms of pigment content of three BChl and three BPh molecules. One interpretation assumes that P is not a dimer, and of the three BChl molecules one absorbing at 865 nm acts as the primary electron donor, while the remaining two give rise to the strong band at 812 nm and the weaker one at 790 nm. The relative intensities of the latter two transitions could be determined mainly by interaction between the two BChl molecules. These interactions or those with BPh or P would be disrupted and new ones formed upon oxidation of P and/or reduction of I. Another possibility, consistent with a dimeric structure for P, is that B moves and/or has an interaction with a BPh that changes in response to the initial electron transfer steps. Besides causing a bleaching in the BPh ground state bands at 535 and 755 nm, the reduction of I also causes a net absorption decrease near 805 nm. This suggests that I involves interacting BPh and B, a finding similar to that proposed in reaction centers of purple bacteria.

Our data, along with the linear dichroism data of Vasmel et al. [18], indicate that neither the 812 nm band nor the 790 nm band are the higher-energy excitonic component of P. The data are, however, consistent with either a monomeric or dimeric structure for P, although the monomeric structure seems to require fewer assumptions. Den Blanken et al. [36] have interpreted the triplet-singlet spectrum of *Chloroflexus* as evidence in favor of dimeric model. Further work will be required before the nature of P can be fully settled.

Acknowledgements

This work was supported by grants from the Competitive Research Grants Office of the U.S. Department of Agriculture to R.E.B. (80-CRCR-1-0473) and to D.H. (59-2294-1-1-678-0). We thank W.W. Parson and R.M. Pearlstein for helpful discussion and H. Vasmel, J. Amesz and coworkers for preprints of their work.

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