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Formation and decay of radical-pair state P^+I^- in *Chloroflexus aurantiacus* reaction centers

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We have examined the room temperature kinetics of the absorption changes associated with the formation of state P^+I^- (P^+BPh^-) and its subsequent decay to state $P^+Q_A^-$ in reaction centers from *Chloroflexus aurantiacus*. Our data, acquired using 30-ps excitation flashes, strongly suggest that formation of P^+I^- (P^+BPh^-) takes longer in *Chloroflexus* than in reaction centers from *Rhodopseudomonas sphaeroides*. The reduction of the photoactive bacteriopheophytin (BPh) could take as long as 13 ps. Absorption changes different from those due to P^+I^- are observed early in the excitation flash, but the detailed identity of the transient remains unclear. We also find that the kinetics observed subsequent to P^+I^- formation differ with detection wavelength. The time constant measured in the anion band (I^-) at 655 nm is 324 ± 20 ps and probably reflects the rate of electron transfer from I^- (BPh^-) to Q_A. However, the kinetics measured in the BPh ground-state absorption bands are slightly longer: 365 ± 19 and 367 ± 21 ps at 538 and 760 nm, respectively. At 810 nm, a wavelength normally associated with the monomeric bacteriochlorophyll (BChl) in the *Chloroflexus* reaction center, a slightly faster (281 ± 19 ps) time constant is observed. This detection-wavelength dependence of the kinetics is similar to that observed recently in *Rps. sphaeroides* reaction centers. Comparison of these results suggests that the kinetics observed in the ground-state absorption bands of the BPhs and BChls in *Chloroflexus* may contain contributions from readjustments of the pigments and/or protein in response to the charge separation process.

Introduction

The current view of the primary charge separation process in reaction centers isolated from the purple photosynthetic bacteria *Rps. sphaeroides* and *Rps. viridis* and from the green bacterium *Chloroflexus aurantiacus* can be summarized as follows:

$$PIQ_A \xrightarrow{h\nu} P^*IQ_A \xrightarrow{<10\ ps} P^+I^-Q_A \xrightarrow{<500\ ps} P^+IQ_A^-$$

Excitation produces the excited singlet state (P^*) of the primary electron donor (P), a complex involving two bacteriochlorophyll (BChl) molecules [1–4]. The view that P is a dimer in *Rps. sphaeroides* and *Chloroflexus* has received strong support from the recently published crystal structure of the *Rps. viridis* reaction center [5]. An electron from P^* appears very rapidly (approx. 4

Abbreviations: BPh, bacteriopheophytin; BChl, bacteriochlorophyll; P, primary electron donor; P^*, excited singlet state of the primary electron donor; I, electron acceptor; Q_A, primary quinone.

ps in *Rps. sphaeroides* [6–8]) on an electron acceptor, I. Recent hole-buring [9–11] and accumulated photon echo [11] experiments indicate that the lifetime of P* may be less than 200 fs. This suggests that another state or relaxation contributes to the initial photochemistry. However, the earlier suggestion [12,13] that P^+BChl^- (where BChl represents one of the two monomeric BChls not part of P) is a resolved intermediate between P* and P^+I^- is not supported by the most recent work [7,8,14].

Early measurements on reaction centers from the purple bacteria indicated that I involves at the minimum a bacteriopheophytin (BPh) molecule [15–24]. However, it has been suggested that the state P^+I^- could be an equilibirum (thermal) mixture of $[P^+BChl^-]$ and $[P^+BPh^-]$ [25,26] or that it could be $P^+[BChl\text{-}BPh]^-$ (see Ref. 1). Recent picosecond measurements, however, have led to the view that P^+I^- is simply P^+BPh^- in *Rps. sphaeroides* [27,28]. State P^+I^- (P^+BPh^-) transfers an electron to yield state $P^+Q_A^-$ (where Q_A is the primary quinone) with an approx. 200 ps time constant at room temperature [6,16,17,24,27].

The overall charge separation process in *Chloroflexus* has been shown to be similar to that in *Rps. sphaeroides*. A BPh has been implicated as an initial electron acceptor in the state P^+I^-, which forms well within a 30-ps excitation flash and subsequently transfers an electron to a quinone in approx. 310 ps [29,30]. Notable differences between the reaction centers of *Chloroflexus* and *Rps. sphaeroides* are that the former contains three BChl, three BPh, a manganese atom and menaquinone as Q_A [31–35], whereas the latter has four BChl, two BPh, an iron atom and ubiquinone [36].

A recent study has revealed an unexpected detection-wavelength dependence of the kinetics normally associated with electron transfer from BPh^- to Q_A in *Rps. sphaeroides* reaction centers [27]. At 285 K, a time constant of approx. 205 ps was measured in the 665 nm absorption band of BPh^-. However, a slightly longer time constant (approx. 250 ps) was measured by decay of bleachings in the visible (545 nm) and near-infrared (765 nm) ground-state absorption bands of the BPhs. At 795 nm, a wavelength associated with BChl, slightly faster (approx. 150 ps) kinetics were determined. These results indicate that the primary photochemistry is not quite as simple as outlined above. It was proposed [27,28] that the absorption changes measured at some wavelengths (795 nm, for example) might reflect nuclear relaxations rather than electron transfer. These relaxations could involve readjustments of the pigments and/or protein in response to changing charge distributions on the pigments resulting from electron transfer.

Because of these recent results in *Rps. sphaeroides*, we re-examined the $P^+I^-Q_A \rightarrow P^+IQ_A^-$ reaction in *Chloroflexus*, since in our previous study we measured the kinetics at only one wavelength (765 nm) [29]. Here we report the results of kinetic measurements for the process $P^+I^-Q_A \rightarrow P^+IQ_A^-$ in four different regions of the visible and near-infrared spectra. We confirm the earlier measurement that the rate of electron transfer from I^- to Q_A is somewhat slower in *Chloroflexus* than in *Rps. sphaeroides*, but as for *Rps. sphaeroides* we find that the observed kinetics differ with detection wavelength. In our earlier study [29] we also observed that the absorption changes early in the 30-ps excitation flash had characteristics of those due to both $P^+Q_A^-$ and P^+I^-, and based on this we suggested that the initial formation of P^+I^- (P^+BPh^-) also is somewhat slower in *Chloroflexus* than in *Rps. sphaeroides*. Here we present additional spectral and kinetic data which further support this idea and indicate that the initial reduction of the photoactive BPh may require as long as 13 ps.

Materials and Methods

Chloroflexus reaction centers were prepared as described previously [29], and suspended in 20 mM Tris-HCl (pH 8), 0.05% LDAO. Immediately before the start of picosecond measurements, samples were made 1 mM in freshly prepared sodium ascorbate. Samples were flowed through a 2 mm pathlength cell and maintained at approx. 10°C under an argon atmosphere during experiments. For measurements in the visible (500–750 nm) the samples had a ground-state $A_{865} \approx 1.0$ (in a 2 mm path), and for the near-infrared (710–920 nm) the samples were 50% as concentrated. The reaction centers showed no decomposition over the course of a typical 5-h kinetic measurement as monitored

by ground-state absorption spectra taken before and after experiments.

The dual-beam picosecond transient absorption spectrometer used in these studies has been described elsehwere [37]. Excitation flashes at 600 nm, 30-ps in duration and containing 200 μJ were generated by stimulated Raman scattering from 532-nm pulses in $C_6{}^2H_{12}$, and focused to a diameter of 1.5–2 mm at the sample. The excitation flashes were attenuated so that 50–60% of P's ground-state absorption at 865 nm was bleached; typically, 50–100 μJ were used depending on the focusing. The excitation flashes were polarized at 45° with respect to the probe pulses so that dichroism of the absorption changes is not expected; the dichroism is small with visible excitation in any case [38].

Results

Fig. 1 shows near-infrared difference spectra taken 47 ps (solid) and 2.5 ns (dashed) after the center of a 30-ps 600-nm excitation flash. The 47-ps spectrum can be ascribed to state P^+I^-, and the 2.5-ns spectrum to state $P^+Q_A^-$. These spectra are essentially identical to those reported previously for *Chloroflexus* [29]. Fig. 2 shows spectra for the two states taken in the visible region using a sample twice as concentrated as that used to acquire the near-infrared spectra. The bleaching at 538 nm in the P^+I^- (47 ps) spectrum is shown here with better resolution that that reported previously for *Chloroflexus* [29]. Not reported previously is the transient absorption band centered at 655 nm in the 47-ps spectrum.

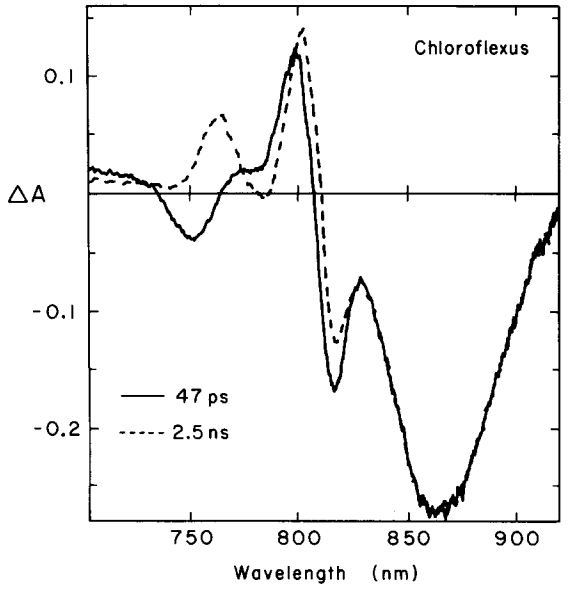

Fig. 1. Near-infrared transient-state difference spectra for *Chloroflexus* reaction centers taken 47 ps (solid) and 2.5 ns (dashed) after the center of a 30-ps excitation flash at 600 nm. Both spectra were acquired in two 150-nm intervals that agree within experimental error in the regions of overlap. These spectra represent the average of data acquired using approx. 300 excitation flashes and have an error in ΔA of approx. ± 0.005 over the wavelength region shown.

Many of the features in the P^+I^- and $P^+Q_A^-$ spectra for *Chloroflexus* have qualitatively similar counterparts in the corresponding difference spectra of reaction centers from *Rps. sphaeroides* and *Rps. viridis* [1,6–8,12–30,37]. The bleaching at 538 nm in the P^+I^- spectrum of Fig. 2 reflects bleaching of a BPh Q_X band. A similar Q_X bleaching near 540 nm has been described for both *Rps. sphaeroides* and *Rps. viridis* reaction centers. The small trough near 500 nm probably represents bleaching of the BPh $Q_X(1,0)$ band, as has been observed in *Rps. sphaeroides* [28]. The absorption band centered at 655 nm in the P^+I^- spectrum is analogous to similar absorption bands found at 665 and 680 nm in the P^+I^- spectra of *Rps. sphaeroides* and *Rps. viridis*, respectively. This broad absorption band is consistent with the presence of either BPh^- or $BChl^-$, based on in vitro

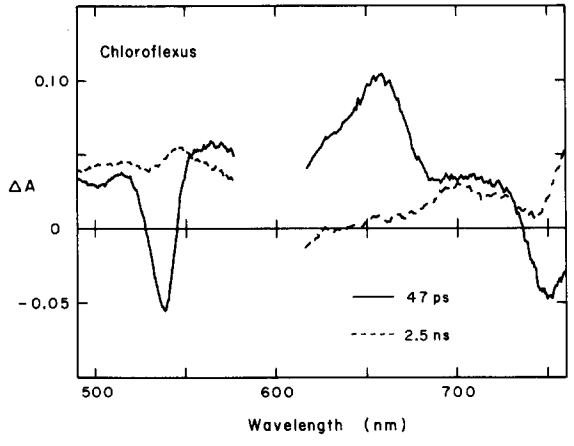

Fig. 2. Transient-state difference spectra acquired in the visible region for *Chloroflexus* reaction centers having twice the concentration as for Fig. 1. Other conditions as in Fig. 1.

spectra of these anion radicals [19,39,40]. The P^+I^- and $P^+Q_A^-$ difference spectra between 740 and 830 nm (Fig. 1) are quite complex. Many of the features in these spectra are, again, similar to those observed for both *Rps. sphaeroides* and *Rps. viridis* reaction centers, but for neither these species nor for *Chloroflexus* are the origins of the absorption changes fully understood [16,17,27–29,37,38,41]. However, for *Chloroflexus* it appears likely that the absorption changes between 740 and 780 nm are due to the BPhs, and those observed between 780 and 830 nm are due predominantly to the monomeric BChl and/or P (or P^+). Bleaching of P's band at 865 nm is unchanged between 47 ps and 2.5 ns.

Spectra calculated by subtracting a $P^+Q_A^-$ (2.5 ns) spectrum from a P^+I^- (47 ps) spectrum are shown in Fig. 3. These absorption changes are normally associated with the reduction of I. These spectra show more clearly, particularly in the near-infrared (Fig. 3b), the wavelength regions where kinetics can be monitored during the transformation $P^+I^-Q_A \rightarrow P^+IQ_A^-$.

We have measured kinetics in each of the four wavelength regions depicted in Fig. 3. Within each region the absorption changes were averaged over 5–10 nm intervals and fit to the function $\Delta A = \Delta A_\infty + \Delta A_0 \exp(-t/\tau)$, where ΔA_∞ is the absorption change at the asymptote (long time), $\Delta A_0 + \Delta A_\infty$ is the absorption change at $t = 0$, and τ is the time constant. Only data at delay times of 47 ps and longer were fit. The observed decay kinetics at all wavelengths are fit well by this single exponential function and a typical set of kinetic data and a computer fit are shown in Fig.

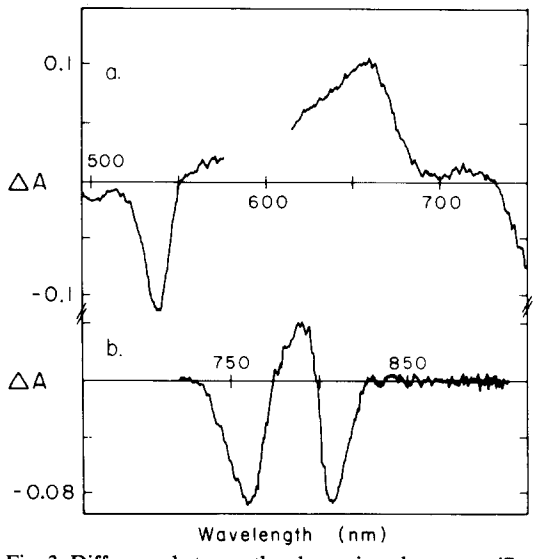

Fig. 3. Difference between the absorption changes at 47 ps and 2.5 ns (i.e., 47 ps-2.5 ns) calculated from the spectra in Figs. 1 and 2. (a) Visible region; (b) near-infrared region.

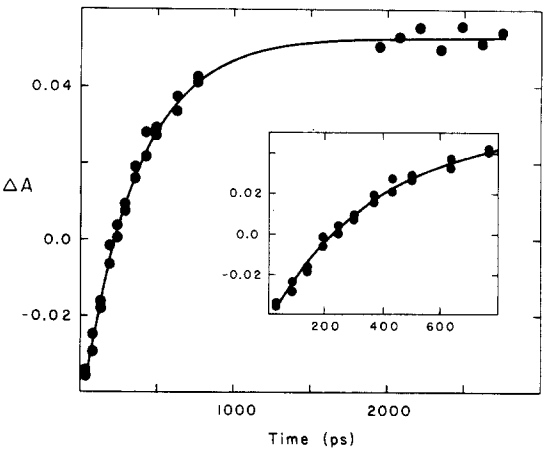

Fig. 4. Growth of the 755–760-nm absorption increase (see Fig. 1) with respect to excitation with a 30-ps 600-nm flash. The initial portion of the kinetics is shown on an expanded time (picoseconds) scale in the inset. The solid curve in the main figure and in the inset is a non-linear least-squares fit to a single-exponential function (see text), giving a time constant of 381 ± 22 ps. Data in two adjacent intervals gave lifetimes of 398 ± 25 ps (750–755) nm and 375 ± 21 ps (760–765 nm). The average value of 385 ± 23 ps is reported in Table I.

TABLE I

SUMMARY OF DECAY KINETICS IN *CHLOROFLEXUS AURANTIACUS* REACTION CENTERS

Data are lifetimes in picoseconds obtained using 30-ps 600-nm excitation flashes. All measurements were performed on samples at approx. 10°C. The wavelengths listed give the boundaries of the regions within which the kinetics were determined, as discussed in Results. The error limits represent one standard deviation. The weighted averages and standard deviations are listed in the bottom row.

530–545 nm	640–670 nm	750–765 nm	805–815 nm
356 ± 17	323 ± 23	370 ± 21	289 ± 20
374 ± 21	324 ± 18	346 ± 20	266 ± 23
365 ± 20	326 ± 20	385 ± 23	287 ± 15
365 ± 11	324 ± 12	365 ± 13	283 ± 11

4. Table I summarizes the results of individual measurements. The wavelengths listed at the top of Table I give the boundaries of the regions within which the data were fit. Within each region adjacent 5–10 nm intervals gave time constants within experimental error of one another, and the values in Table I represent the averages of the time constants determined for these smaller intervals. For example, (see also legend of Fig. 4) one measurement gave time constants of 331 ± 25, 317 ± 20 and 319 ± 23 ps over 640–650, 650–660 and 660–670 nm, respectively, and the average value of 323 ± 23 ps is reported in Table I (first entry in the 640–670 nm column).

In the 538-nm Q_X and 760-nm Q_Y bands of BPh the measured time constants are the same within experimental error: 365 ± 19 ps and 367 ± 21 ps, respectively. A slightly shorter time constant of 324 ± 20 ps is measured in the anion (I^-) absorption band centered at 655 nm. Shorter still is the 281 ± 19 ps lifetime measured in the 810-nm band of BChl. This detection-wavelength dependence of the kinetics observed in *Chloroflexus* parallels that reported recently in *Rps. sphaeroides* reaction centers [27]. There the measured time constants were 253 ± 10 ps and 249 ± 12 ps in the BPh Q_X and Q_Y ground-state bands at 545 nm and 765 nm, respectively, 207 ± 12 ps in the BPh^- transient absorption band at 665 nm, and 150 ± 17 ps near 795 nm, a wavelength normally associated with BChl.

We also examined the evolution of the absorption changes during the 30-ps excitation flash. The early spectra show some characteristics that are different from those observed for P^+I^-, as we reported previously [29]. This is demonstrated in Fig. 5, which compares the 2.5-ns $P^+Q_A^-$ (dashed) and 47-ps P^+I^- (dotted) spectra with one acquired at 0 ps (solid), the time at which the 30-ps pump and probe pulses maximally overlap. The 0-ps spectrum has been multiplied by 2 to normalize the bleaching of P's band to that in the two later spectra. At wavelengths other than in P's band, the 0-ps spectrum does not normalize to the P^+I^- (nor to the $P^+Q_A^-$) spectrum. The ratio of the 812-nm trough to the 800-nm absorption increase is slightly larger in the 0-ps spectrum (1.9) than it is in the 47-ps P^+I^- spectrum (1.5). However, notably absent in the 0-ps spectrum, and particularly in the earlier spectra, is any large 'extra' bleaching in the 800–820 nm region which one might expect for a substantial population of P^+BChl^-. (The earlier spectra we acquired correspond to the delay times shown in Fig. 6; the earliest one was at −33 ps.) The absorption changes in this region are all smaller in the early spectra than they are for the P^+I^- spectrum when normalized at 865 nm as in Fig. 5. Turning to the BPh region, the 0-ps spectrum shows a small absorption decrease near 750 nm also present in the 47-ps spectrum, and contains an absorption increase near 770 nm and a valley near 785 nm, which give it some character of the $P^+Q_A^-$ spectrum. Again, the absorption changes in the 740–780-nm region in all the early (−33 to +33 ps) spectra are significantly smaller than in the 47-ps P^+I^- spectrum when normalized to the bleaching in P's band.

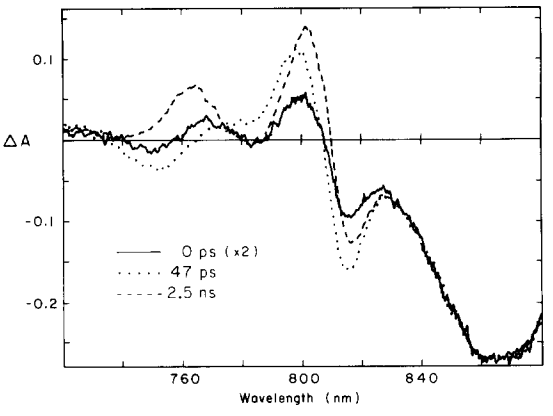

Fig. 5. Near-infrared transient-state difference spectra at 47 ps (·····) and 2.5 ns (------) replotted from Fig. 1. The spectrum taken at 0 ps (———) has been multiplied by 2 to normalize it to the other two spectra over the 830–900-nm region of P's bleaching. The spectra are truncated at 880 nm (see Fig. 1) in order to emphasize the differences observed at wavelengths shorter than 830 nm, where the 0-ps spectrum does not normalize to either spectrum taken at the later times.

The rise-time of the near-infrared absorption changes at several wavelengths in *Chloroflexus* are shown in Fig. 6. The closed circles show the growth of the bleaching of P's band (averaged over the interval 840–870 nm), and the open circles show the growth of the absorption increase at 797–801 nm (see Fig. 1). The data were normalized at delay times of 100 ps and longer. Note that

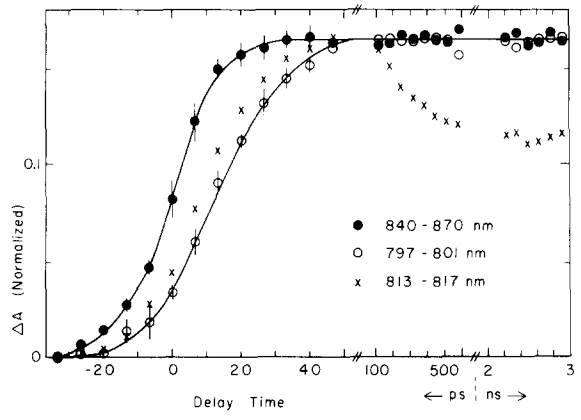

Fig. 6. Growth of bleaching in P's band in *Chloroflexus* reaction centers averaged over the interval 840–870 nm (●), of the absorption increase over 797–801 nm (○), and of the absorption decrease over 813–817 nm (×). These data were acquired with 30-ps 600-nm excitation flashes. Representative error bars are shown only for a few points for purposes of clarity; the typical standard deviation in ΔA for these data is ± 0.005. The 797–801-nm data were normalized to P's bleaching at delay times of 100 ps and longer, while the 813–817-nm data was normalized based on the points at 40–47 ps only.

799 nm is an isosbestic wavelength between the P^+I^+ and $P^+Q_A^-$ difference spectra (see Figs. 1 and 3). This means that the rise-time of the absorption change at this wavelength is not perturbed by a convolution with the subsequent $P^+I^-Q_A \rightarrow P^+IQ_A^-$ kinetics. It is very clear from Fig. 6 that the absorption increase at 799 nm does not develop as quickly as does the bleaching of P's 865-nm ground-state absorption band. In order to estimate the rise-time of the 799-nm absorption change in Fig. 6, the 840–870 nm bleaching data were first differentiated to obtain an instrument response function. This was then convoluted with an exponential plus a constant and the resultant function was used to fit the 799-nm data. This procedure yielded a value of 13 ps for the apparent time constant associated with the formation of the absorption increase at 799 nm. (This lag cannot be ascribed simply to an excited state of the monomeric BChl, since studies on *Rps. sphaeroides* using 0.8-ps 600-ns flashes indicate that energy transfer from BChl to P takes less than 1 ps [7].)

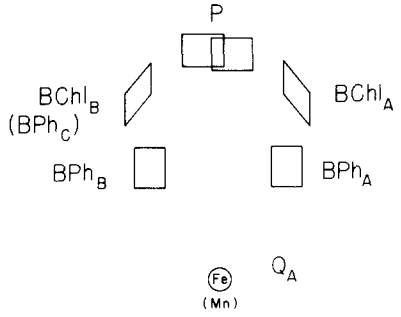

Fig. 7. Hypothetical arrangement of the pigments in *Rps. sphaeroides* and *Chloroflexus* reaction centers as might be predicted based on the known *Rps. viridis* crystal structure [5]. *Rps. sphaeroides* contains the Fe atom and BChl$_B$, while *Chloroflexus* has a Mn atom and BPh$_C$. The other chromophores are common to both reaction centers. A C_2 axis of symmetry runs from P to the Fe atom in the *Rps. viridis* crystal structure. The Q_B binding site is hence presumably symmetrically displaced from Q_A. (Q_B was lost from the *Rps. viridis* reaction center during crystallization [5].)

A lag is seen at all wavelengths between 740 and 830 nm, but the apparent rise-times may be different. When normalized at 40–50 ps the absorption decrease at 815 nm (crosses in Fig. 6) appears to develop more quickly than the absorption increase at 799 nm. The absorption decrease near 755 nm (see Fig. 5) appears to develop slightly more slowly (kinetic data not shown); however, the maximum ΔA at this wavelength is only -0.04 and when scaled ($\times 4$) to the data of Fig. 6 the error bars on the 755-nm points significantly overlap the 799-nm data. It is important to note that subsequent kinetics make rise-times appear different than they really are. The 815- and 755-nm rise-times will be affected in this manner, and further will be affected differently, since the subsequent decays are different at the two wavelengths (280 and 365 ps, respectively; Table I). The 799-nm rise-time is not affected, since there are no other kinetics at this wavelength. Thus, obtaining rise-times at 815 and 755 nm requires deconvolution of the 30-ps pump and probe flashes as well as the subsequent kinetics. Therefore, we are reluctant to cite different values for the rise-times of the absorption changes in the ground state regions of the BPh (755 nm) and the BChl (815 nm). However, clearly the 799- and 855-nm kinetic data of Fig. 6 strongly suggest that the formation of P^+I^- is slower in *Chloroflexus* than in *Rps. sphaeroides*.

Discussion

Formation of state P^+I^- (P^+BPh^-)

We first compare *Chloroflexus* to *Rps. sphaeroides* in regard to the observed rise-times of the absorption changes in the near-infrared, and address the accuracy of the 13-ps time constant derived from the 799- and 855-nm data of Fig. 6. Under identical experimental conditions as used in the present study, i.e., 30-ps 600-nm flashes, we have estimated an approx. 7-ps lag in the appearance of the near-infrared absorption changes compared to bleaching at 865-nm in *Rps. sphaeroides* [14]. Similar results (approx. 7-ps time constants) have been estimated in the visible and near-infrared from studies using 20–30-ps flashes in the long-wavelength band of P [12–14,37,42]. There have been three more direct measurements of this time constant employing flashes of 1 ps or less. Holten et al. [6] using 700-fs 610-nm flashes measured approx. 4 ps, Martin et al. [8] using 150-fs 860-nm flashes have reported 2.8 ± 0.2 ps, and Woodbury et al. [7] using 800-fs 610-nm flashes measured 4.1 ± 0.2 ps. These time constants were measured at a variety of wavelengths in the visible and near-infrared, whereas bleaching at 865 nm was always faster and instrument-limited. All these measurements give a time constant somewhat shorter than estimated from experiments employing 20–30-ps flashes. Thus, in regards to the *Chloroflexus* data presented here, the 13-ps time constant probably represents an upper limit. However, we feel our spectral and kinetic data suggest that the actual value, when measured more directly with shorter duration flashes, will be longer than the approx. 4 ps time constant found in *Rps. sphaeroides*.

Recent hole-burning experiments on *Rps. sphaeroides* and *Rps. viridis* [9–11] indicate that the P* lifetime may be less than 200 fs, and possibly as short as 20 fs. Such a short-lived state could not contribute to the initial spectra and kinetics in our experiments. So what process is responsible for the apparent 13-ps early kinetics in *Chloroflexus* and the early spectra observed prior to P^+I^- formation? One possibility is that there may be very rapid (less than 200 fs?) charge separation between the two BChls of P [7–11,23]. Such a charge-transfer state might be expected to exhibit some of the features of a P^+ (or $P^+Q_A^-$) spectrum, particularly if in P^+ the charge is localized on one of the two BChls of the dimer on the optical time scale (as has been suggested in *Rps. sphaeroides* [51]). The chemically induced P^+ difference spectrum in *Chloroflexus* [30] is essentially the same as the 2.5-ns $P^+Q_A^-$ spectrum shown in Figs. 1 and 5; both spectra show a distinct absorption increase centered near 765 nm. In the 47-ps P^+I^- spectrum this peak apparently is masked by bleaching in the BPh Q_Y band. Since the 0-ps spectrum contains features of both the P^+ ($P^+Q_A^-$) and P^+I^- spectra, it is possible that the apparent 13-ps kinetics reflects electron transfer from a charge-separated state within P to the BPh. This process conceivably could involve the transient reduction of the monomeric BChl. However, when compared to the 47-ps spectrum as in Fig. 5, there is no large or extra bleaching (expected for P^+BChl^-) between 790–820 nm in the 0-ps spectrum or in any of the four other earlier spectra that we acquired during the 30-ps flash (corresponding in time to the points indicated in Fig. 6). Indeed, the opposite is true. There is less bleaching in the BChl region in all of these early spectra than there is at 47 ps (e.g., see the 0-ps spectrum in Fig. 6). These observations lead us to conclude that if P^+BChl^- forms, it is not present in an amount sufficient to contribute substantially to the early absorption changes. Similar conclusions have been reached, also in studies using 30-ps flashes, from the early spectra of *Rps. sphaeroides* [14]. More directly, the most recent studies on *Rps. sphaeroides* using subpicosecond flashes have led to the conclusion that P^+BChl^- is not a resolved intermediate prior to P^+BPh^-, which apparently forms in approx. 4 ps, as discussed above [7,8].

Thus, even when subpicosecond flashes are employed, possibilities such as initial charge separation within the dimer and disruption of interactions between P, the BChls, the BPhs and the protein upon excitation or electron transfer complicate interpretation of the initial absorption changes and kinetics [6–11]. In addition, the recent observation of a detection-wavelength dependence of the kinetics normally associated with electron transfer from I^- to Q_A (see [27] and below) suggests the possibility that kinetics mea-

sured for the earliest events also may not simply reflect the rate of electron transfer; they may be skewed by additional relaxations involving motions of the pigments and/or the protein caused by excitation or electron transfer. Such relaxations or the transient formation of one of the charge-transfer states mentioned above could result in different rise-times for the absorption changes in the near-infrared, e.g., 815-nm the data of Fig. 6. Because of these numerous possibilities, uncertainties in the expected difference spectra, and our time resolution, it is difficult to assign the initial absorption changes to a particular state, and the apparent 13-ps kinetics to a single transient event. However, in view of the recent hole-burning experiments [9–11], it is unlikely that the initial photochemistry that we have observed in *Chloroflexus* can be assigned to the simple single-step process $P^*BPh \rightarrow P^+BPh^-$. The data presented here suggest that it may be especially informative to investigate further the earliest events in photosynthesis in reaction centers of *Chloroflexus*, which exhibit spectra with more well separated near-infrared features, and slower photochemistry.

Detection-wavelength dependence of the kinetics normally associated with electron transfer from I^- to Q_A

The absorption changes that accompany the conversion of state P^+I^- to state $P^+Q_A^-$ in *Chloroflexus* are very similar to those observed previously in *Rps. sphaeroides*. The calculated (P^+I^- minus $P^+Q_A^-$) difference spectra (Fig. 3), which are normally taken to reflect the absorption changes associated with the reduction of I, are also very similar for the two species. For *Chloroflexus* the four major features of the calculated spectra of Fig. 3, occur at 538, 655, 760 and 810 nm. In *Rps. sphaeroides* analogous features are found at 542, 665, 765 and 795 nm [27]. Kinetics measured in these respective regions at 285 K are, for *Chloroflexus*, 365, 324, 367 and 281 ps (Table I), and for *Rps. sphaeroides*, 253, 207, 249 and 150 ps. The detection-wavelength dependence of the kinetics is strikingly similar in the two reaction centers; the time constants are about 115 ps longer in each of the corresponding regions in *Chloroflexus*. These similarities must be contrasted with the fact that reaction centers from the two species have (amongst other differences) different pigment contents [31–33,36,43].

The most consistent interpretation of the detection-wavelength dependence of the room temperature kinetics observed here for *Chloroflexus* is the same as that expressed recently for *Rps. sphaeroides* [27]. In short, it appears that the kinetics measured at some wavelengths may contain contributions from molecular readjustments involving the pigments (and/or the protein), and do not reflect directly the rate of electron transfer. At room temperature, we interpret the kinetics at 655 nm as being due to the disappearance of BPh^- and, thus, to the rate of electron transfer from BPh^- to Q_A. The kinetics measured in the BPh Q_X (538 nm) and Q_Y (760 nm) ground-state absorption bands (which one would expect to be the same as those measured at 655 nm) are somewhat longer, apparently due to additional relaxations involving the other BPh(s); at room temperature the ground-state absorption bands of the three BPhs overlap. Consistent with this explanation is the previous observation in *Rps. sphaeroides* that the time constants measured at 5 and 76 K in the 545, 665 and 765 nm regions were all the same (approx. 100 ps), while a slower (approx. 135 ps) time constant was measured at 755 nm, a wavelength associated with the second BPh.

It is useful to have a picture with which to further discuss our results on *Chloroflexus* and compare them with those obtained recently on *Rps. sphaeroides*. Fig. 7, derived from the crystal structure of the *Rps. viridis* reaction center [5], provides such a picture. Although we make use of the *Rps. viridis* crystal structure to discuss the results on *Chloroflexus* and *Rps. sphaeroides* mainly for illustrative purposes, this approach seems reasonable in view of the overall similarities in the spectroscopy of the three reaction centers. Studies using polarized light, for example, suggest that *Rps. sphaeroides* and *Rps. viridis* [37,38,41, 44–48], as well as *Chloroflexus* [30,49,50] have similar overall orientations of the six bacteriochlorin pigments, and that the third BPh in *Chloroflexus* (BPh_C in Fig. 7) may be oriented similarly to one of the monomeric BChls in reaction centers from the purple bacteria [50]. Our replacement of $BChl_B$, rather than of $BChl_A$, with BPh_C in *Chloroflexus* is the least disruptive to the apparent

active electron-transfer chain, as discussed below.

In this model the photoactive BPh is BPh_A, which receives an electron and then transfers it to Q_A with an approx. 325 ps time constant in *Chloroflexus* (approx. 205 ps in *Rps. sphaeroides*). *Rps. sphaeroides* has only one other BPh (BPh_B in Fig. 7) to which the slower relaxation kinetics observed previously were ascribed [27]. *Chloroflexus* has two other BPhs (BPh_B and BPh_C). The slower time constant observed here in the 538- and 760-nm regions (compared to 655 nm) of *Chloroflexus* could contain a similar contribution from some type of relaxation involving either BPh_B or BPh_C or both.

It seems reasonable that $BChl_A$, rather than $BChl_B$, in *Rps. sphaeroides* would be the one more greatly affected by formation of $P^+BPh_A^-$ and thus could contribute to the 795 nm kinetics (approx. 150 ps) observed previously [27]. *Chloroflexus* reaction centers contain only one monomeric BChl, which appears to be responsible for most of the ground-state absorption near 810 nm. Consequently, we have replaced $BChl_B$ (in *Rps. sphaeroides*) by the third BPh (BPh_C) in *Chloroflexus* in Fig. 7. In this scheme, the 810-nm kinetics in *Chloroflexus* (approx. 280 ps) also could involve $BChl_A$. These kinetics, in both reaction centers, are faster than those measured at wavelengths due to the BPhs, and have been tentatively ascribed in *Rps. sphaeroides* [27] to a molecular relaxation (readjustment) involving this BChl. By analogy, again, this same explanation seems a likely possibility for *Chloroflexus*. The absorption spectrum of $BChl_A$ might be perturbed by a change in any interaction with P and/or BPh_A and also by the Stark (electrochromic) effect of the nearby charges on P^+ and BPh_A^-. The kinetics of the absorption changes might result from a movement of the BChl itself, a possibility suggested previously for *Chloroflexus* [30] and *Rps. sphaeroides* [28,37] to explain the complex dichroism of the near-infrared absorption changes, or by movements of nearby charged or polar protein groups in response to the formation of $P^+BPh_A^-$. Such movements also could affect P^+ as well. If P^+ has a (neutral) monomer-like BChl absorption near 800 nm in the transient-state spectra (Fig. 1), as originally proposed for *Rps. sphaeroides* [51], then molecular readjustments involving P^+ could give rise at least in part to the 810-nm kinetics in *Chloroflexus* (and the 795-nm kinetics in *Rps. sphaeroides* [27,28]). It should be noted again that small structural changes resulting from excitation or electron transfer may also contribute to the earliest absorption changes and kinetics as well. Such a possibility was raised [9] to explain the apparent discrepancy between the hole-burning and subpicosecond absorption results. A photoinduced conformation change has been proposed from slower time-scale measurements as well [52].

One might attempt to argue for *Chloroflexus* that the fast (approx. 280 ps) time constant measured near 810 nm and the slower (approx. 370 ps) time constant measured in the 538- and 760-nm bands could represent electron transfer from $BChl_A^-$ and BPh_A^-, respectively, to Q_A. (The approx. 325 ps time constant measured in the anion band then would be an average value.) A similar argument was considered for *Rps. sphaeroides*, but thought unlikely for two reasons [27]. First, considering only the distances involved (assuming Fig. 7 is appropriate), electron transfer from $BChl_A^-$ to Q_A would be expected to be slower, not faster, than from BPh_A^- to Q_A. More directly, the kinetics and low-temperature photodichroism spectra indicate that in *Rps. sphaeroides* P^+I^- is essentially exclusively P^+BPh^- [27,28]. A similar conclusion has been drawn from measurements on *Rps. viridis* reaction centers containing trapped I^- (Tiede, D.M. and Breton, J., personal communication). These results tend to suggest that $P^+BChl_A^-$ is farther in energy above $P^+BPh_A^-$ than estimated for reaction centers having Q_A reduced [25,26], so that the properties of P^+I^- in native (Q_A oxidized) reaction centers appear to be dominated by $P^+BPh_A^-$. Additional measurements on *Chloroflexus* are planned to further examine this question in this species.

A second alternate origin for the detection-wavelength dependence of the kinetics that was considered previously for *Rps. sphaeroides* [27] appears even more unlikely now in view of the present measurements on *Chloroflexus*. The kinetics might be accounted for if, in some fraction of the reaction centers, electron transfer occurs down the secondary chain, with electron transfer from BPh_B^- to Q_B being slower than between BPh_A and Q_A. (Q_B presumably occupies the site near BPh_B

symmetrically disposed with respect to Q_A and BPh_A in Fig. 7.) However, the *Chloroflexus* reaction center contains only Q_A, since Q_B is always completely lost during the reaction center preparation [31,32]. Thus, electron transfer down the secondary chain could give $P^+Q_A^-$ only if electron transfer occurs from BPh_B^- to Q_A. Considering the distances involved (assuming Fig. 7 is appropriate) this process would be expected to be too much slower than the rate of electron transfer from BPh_A^- to Q_A to account for the observed kinetics.

This leaves the question of possible effects of electron transfer on the third BPh in *Chloroflexus* (i.e., BPh_C). Suppose that this pigment is in the same site as the second monomeric BChl ($BChl_B$) in *Rps. sphaeroides* and *Rps. viridis*, as discussed above (Fig. 7). Recently, it was found that the absorption changes due to the two monomeric BChls in *Rps. sphaeroides* can be resolved, at least partially, at 800 and 812 nm in low-temperature polyvinyl alcohol films [27,28]; $BChl_A$ appears to be largely responsible for the 800-nm absorption and may contribute to the 795-nm absorption changes and kinetics. On the other hand, the 812-nm BChl ($BChl_B$) appears to simply blueshift upon the oxidation of P and remain unchanged as the electron passes from BPh_A^- to Q_A. If this result can be extended to BPh_C in *Chloroflexus*, then a similar blue shift of the Q_Y band of this pigment might overlap the absorption changes due to the other two BPhs in the 730–780-nm region (Fig. 1) [30]. Low temperature spectral and kinetic measurements are planned to help analyze this possibility. These studies should also help to clarify whether the kinetics of absorption changes that can be associated with one of the BPhs (i.e., BPh_A) correlate with the decay of the 655-nm anion band, as has been found for *Rps. sphaeroides*.

Conclusions

We have observed a detection-wavelength dependence of the kinetics on the time scale (several hundred picoseconds) normally associated with electron transfer from $I^-(BPh^-)$ to Q_A in *Chloroflexus* reaction centers. Our tentative interpretation of these results, in analogy to recent work on *Rps. sphaeroides*, is that the kinetics of the absorption changes measured at some wavelengths contain contributions from readjustments of the pigments and/or the protein in response to electron transfer. Similar molecular readjustments could conceivably contribute to the early (< 15 ps) absorption changes and kinetics. Such pigment/protein dynamics, in addition to the rapid formation and decay of charge-transfer or radical-pair states prior to P^+BPh^-, would make it difficult to assign (or resolve) distinct conventionally defined electronic states; electron transfer may involve non-thermally equilibrated states of the pigments and the protein. The results presented here suggest that it may be especially informative to test these possibilities in *Chloroflexus* reaction centers, which have a different pigment content, more well-resolved near-infrared absorption changes, and slower photochemistry compared to the more commonly studied *Rps. sphaeroides*.

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