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PRIMARY PHOTOCHEMICAL PROCESSES IN ISOLATED REACTION CENTERS OF *RHODOPSEUDOMONAS VIRIDIS*

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Summary

Picosecond and nanosecond spectroscopic techniques have been used to study the primary electron transfer processes in reaction centers isolated from the photosynthetic bacterium *Rhodopseudomonas viridis*. Following flash excitation, the first excited singlet state (P*) of the bacteriochlorophyll complex (P) transfers an electron to an intermediate acceptor (I) in less than 20 ps. The radical pair state (P^+I^-) subsequently transfers an electron to another acceptor (X) in about 230 ps. There is an additional step of unknown significance exhibiting 35 ps kinetics. P^+ subsequently extracts an electron from a cytochrome, with a time constant of about 270 ns. At low redox potential (X reduced before the flash), the state P^+I^- (or P^F) lives approx. 15 ns. It decays, in part, into a longer lived state (P^R), which appears to be a triplet state. State P^R decays with an exponential time of approx. 55 μs. After continuous illumination at low redox potential (I and X both reduced), excitation with an 8-ps flash produces absorption changes reflecting the formation of the first excited singlet state, P*. Most of P* then decays with a time constant of 20 ps. The spectra of the absorbance changes associated with the conversion of P to P* or P^+ support the view that P involves two or more interacting bacteriochlorophylls. The absorbance changes associated with the reduction of I to I^- suggest that I is a bacteriopheophytin interacting strongly with one or more bacteriochlorophylls in the reaction center.

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Abbreviations: ESR, electron spin resonance; ENDOR, electron nuclear double resonance.

Introduction

Recent studies of photosynthetic reaction centers isolated from *Rhodopseudomonas sphaeroides* indicate that the primary photochemical reaction occurs in several steps. The absorption of a photon by a bacteriochlorophyll complex (P) promotes the complex to an excited singlet state (P*) from which an electron transfers to an intermediate acceptor (I). Measurements of optical absorbance changes following the excitation of reaction centers with short flashes have shown that the reduction of I occurs in less than 10 ps [1—4]. The reduced acceptor (I^-) then transfers an electron to another acceptor (X), which appears to be a quinone. This step requires about 200 ps [1,2]. If electron transfer between I^- and X is blocked, either because X is already reduced or because the quinone has been extracted from the reaction centers, the radical pair P^+I^- ("state P^F") survives for about 15 ns before it decays by the return of an electron from I^- to P^+ [5,6]. In most cases, the return of the electron restores P^+ directly to the ground state. However, the 15 ns lifetime of the radical pair provides an opportunity for a rephasing of the spins of the unpaired electrons on P^+ and I^-, so that the return of an electron to P^+ also can place the bacteriochlorophyll complex in a triplet state ("state P^R") [5—7]. In the presence of a magnetic field, state P^R is formed with an unusual spin polarization that probably results from an influence of the field on the spin rephasing in P^+I^- [8—10]. The triplet state has a low quantum yield at room temperature, and it appears to be a side product, rather than an intermediate in the normal pathway leading to P^+X^- [15].

Reaction centers from *Rps. sphaeroides* contain four molecules of bacteriochlorophyll and two of bacteriopheophytin [11]. ESR and ENDOR studies have shown that the spin of the unpaired electron on P^+ is delocalized over two molecules, suggesting that P involves two of the four bacteriochlorophylls [12—15]. Measurements of the absorbance changes that accompany the transient formation of P^+I^- suggest that I is one of the two bacteriopheophytin molecules [1—5,15—17]. In isolated reaction centers and chromatophores from several species of bacteria, it has been possible to trap I in the reduced state for long periods, either by direct reduction or by continuous illumination at low redox potentials [18—29]. In the latter case, P^+ has a chance to extract an electron from a secondary electron donor such as a cytochrome each time the reaction center is excited into the radical pair state P^+I^-. On those occasions when P^+ is reduced by the cytochrome rather than by I^-, the reaction centers are left in the state PI^-. The absorbance changes that occur under these conditions are consistent with the view that I is a bacteriopheophytin. However, the reduction of I also causes major changes in some of the absorption bands that have been attributed to the bacteriochlorophyll of the reaction center, suggesting a close interaction between one or more of the bacteriochlorophylls and the bacteriopheophytin [19—29].

The present paper describes picosecond and nanosecond spectroscopic measurements on reaction centers from *Rhodopseudomonas viridis*. This species differs from *Rps. sphaeroides* in that it contains bacteriochlorophyll *b* and bacteriopheophytin *b* instead of bacteriochlorophyll *a* and bacteriopheophytin *a* [30]. The absorption bands of reaction centers containing bacteriochlorophyll

b and bacteriopheophytin *b* occur at longer wavelengths than do those of reaction centers with bacteriochlorophyll *a* and bacteriopheophytin *a*. Reaction centers from *Rps. viridis* have a major absorption band at 960 nm, and a broad, asymmetrical band at 830 nm [24,26,28,31—33]. At cryogenic temperatures, the 830 nm band splits into four overlapping components, with maxima near 790, 815, 830, and 850 nm [24,33]. The 790 nm component probably is due to the bacteriopheophytin *b* of the complex, and the other bands to bacteriochlorophyll *b* [24,33]. When the bacteriochlorophyll *b* (P) is photooxidized to P^+, the 960 nm band and the 850 nm component of the 830 nm band bleach, the absorbance near 810 nm increases, and the 790 and 830 nm components appear to undergo small shifts in position [24]. When I is reduced to I^-, there is little change in the 960 nm band, but bleaching occurs at 790 and 830 nm, and the strength of the absorption near 810 nm increases again [25,26,28]. Some of these absorbance changes probably result from the disruption of excitonic interactions between different bacteriochlorophyll *b* molecules of the complex, or between bacteriochlorophyll *b* and bacteriopheophytin *b*, when one of the molecules is oxidized or reduced [25,33]. However, little is known about how the six pigments are arranged in the reaction center, or of the interactions that occur among them. Kinetic spectroscopic studies of the isolated *Rps. viridis* reaction centers promised to be particularly informative, because the resolution of the 815, 830 and 850 nm absorption bands is greater than the resolution that occurs in reaction centers from species containing bacteriochlorophyll *a*. Reaction centers from *Rps. sphaeroides* or *Rhodospirillum rubrum* have a broad, asymmetrical band near 800 nm which probably is homologous to the 830 nm band of *Rps. viridis*. The 800 nm band appears to include several different electronic transitions [33—37], but the different components are not well separated. In addition, isolated *Rps. viridis* reaction centers contain bound *c*-type cytochromes which make possible the photochemical trapping of I^-. This has enabled us to study the behavior of P* under conditions that prevent the transfer of an electron to I.

Materials and Methods

Picosecond spectroscopic and kinetic measurements were carried out on the apparatus described by Magde and Windsor [38]. The excitation flash was at 530 nm and had a duration of approx. 8 ps.

Nanosecond spectrophotometric measurements were made as described by Parson et al. [5], with several modifications. The excitation flash had a wavelength of 834 nm and generally had a width of about 20 ns at half-maximum amplitude. For some of the kinetic measurements, the flash width was decreased to about 5 ns by the use of a Pockels cell switched by a laser-triggered spark gap [6]. For most of the measurements, the photomultiplier and preamplifier used previously [5] were replaced by an ITT F4102 photomultiplier, which had a response time of about 1 ns. The anode of the photomultiplier was connected directly to the 50 Ω input of the oscilloscope, via RG-8 cable and General Radio connectors. A 10 mA bias current was introduced via a General Radio insertion unit and a T connector; the photocurrent from the anode nulled this current at the peak of the flash from the Xe measuring

lamp. This equipment increased the signal-to-noise ratio substantially under many conditions. It was less susceptible than the earlier equipment to electrical noise generated by the Pockels cell. However, it was not as satisfactory for measurements at wavelengths shorter than 420 nm or longer than 650 nm, and the original equipment was used for some of these measurements.

Nanosecond measurements employed samples in 1 cm cells which were deoxygenated by bubbling with N_2. Picosecond measurements employed samples in either 1, 2 or 5 mm cells which were usually not deoxygenated. However, for measurements at low redox potential, samples were bubbled with N_2 both before and after the addition of $Na_2S_2O_4$. All of the measurements were made at room temperature.

For some of the experiments described below, reaction center preparations reduced with $Na_2S_2O_4$ were continuously illuminated by white light from either a 150 W or 300 W tungsten-halogen lamp. The beam passed through several cm of water prior to the sample in order to avoid overheating the samples.

Rps. viridis NHTC 133 was grown in 30-l bottles illuminated by two quartz-line lamps on a medium described by Eimhjellen et al. [30]. The harvested cells were stored at $-17^{\circ}C$ until required. Reaction centers were isolated using the detergent lauryldimethylamine oxide, and a slight modification [26] of the procedure described by Pucheu et al. [32]; after chromatography of lauryldimethylamine oxide extracts of the photosynthetic lamellae on DEAE-cellulose, chromatography on hydroxyapatite was used for further purification [26]. The resulting preparation was virtually free of the chlorin impurity that has been a problem in many of the previous studies of reaction centers from this species [15]. The absorption spectrum of the preparation at room temperature has been published elsewhere [26]. At $77^{\circ}K$, the splitting of the 830 nm absorption band into four components occurred essentially as has been described [24] for earlier preparations; the 815 nm component had a well-resolved absorption maximum at 817 nm. For some of the experiments (Fig. 4B), the lauryldimethylamine oxide in the reaction center preparation was exchanged for Triton X-100 by reabsorbing the preparation to DEAE-cellulose equilibrated with 50 mM Tris, pH 8.0, and washing the column (8×1 cm) with 10 column volumes of the Tris buffer. Elution with 1% Triton X-100/50 mM Tris, pH 8.0, desorbed the complex from the column, and the chromatographing, brown fraction was collected. For other experiments (Figs. 2, 6, 7, and 8), Triton X-100 was added to a solution of the lauryldimethylamine oxide preparation, and the lauryldimethylamine oxide was removed by dialysis.

The reaction center concentration was estimated by using assumed extinction coefficients of 300 and 100 $mM^{-1} \cdot cm^{-1}$ at 830 and 960 nm, respectively [24].

Results

Fig. 1 illustrates the absorbance changes that accompany the conversion PX to P^+X^-, on excitation of isolated reaction centers with 20-ns flashes at moderate redox potentials, i.e. with X in its normal unreduced state. The spectrum includes a bleaching in the near ultraviolet (400 nm) and visible (610 nm) absorption bands and an absorbance increase at 450 nm. Absorbance changes in

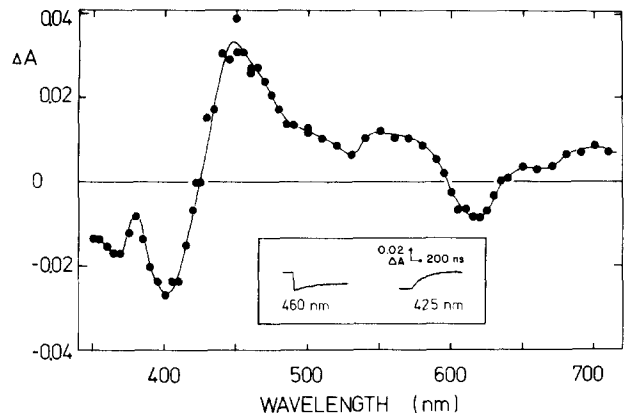

Fig. 1. Optical absorbance changes caused by excitation of *Rps. viridis* reaction centers at moderate redox potential with flashes lasting about 20 ns. The reaction center concentration was approx. 1.3 μM in 50 mM Tris · HCl, pH 8.05, 0.05% Triton X-100; the redox potential was approx. +250 mV; the optical path length was 1 cm. The inset shows typical measurements at 460 and 425 nm; a downward deflection is an absorbance increase. The spectrum represents the initial absorbance changes, immediately after the flash. The flashes were of saturating intensity.

the visible and near infrared regions have been measured previously, using continuous light for excitation [26,28,31—33], but those in the visible region were obscured by absorbance changes due to cytochrome oxidation. The traces in the inset show the kinetics with which the bacteriochlorophyll *b* complex returns to the reduced state by extracting an electron from the high-potential cytochrome *c*-558 [39]. The cytochrome oxidation can be measured separately at 425 nm; it has a time constant of about 270 ns. Under the conditions of these measurements, about 40% of the P that was oxidized by the flash was reduced again rapidly by the cytochrome; the remainder was reduced on a slower time scale.

If the redox potential is lowered so as to reduce X, the 20-ns flash causes a different set of absorbance changes (Fig. 2). Those measured immediately after the flash (filled symbols) are characterized by absorbance decreases at 545 and 380 nm, increases at 450 and 680 nm and a trough at 610 nm; traces A and B in the inset show representative measurements. The absorbance changes are very similar to those associated with the formation of state P^F in reaction centers from *Rps. sphaeroides* [5] or *Rds. rubrum* [6], and we therefore view them as reflecting the creation of the radical pair, P^+I^-. The bleaching at 380 and 545 nm and the formation of the broad band at 680 nm resemble the absorbance changes that accompany the reduction of bacteriopheophytin *b* to an anionic radical in vitro and are consistent with the view that I is bacteriopheophytin *b* [15,16]. The other features of the spectrum, such as the trough at 610 nm, fit the assumption that state P^F also contains the bacteriochlorophyll *b* radical cation, P^+ [15].

The decay kinetics of state P^F ($P^+I^- \rightarrow PI$) are shown in trace C of Fig. 2. The exponential decay time is approx. 15 ns (the decay half-time is approx. 10 ns), the same as it is in reaction centers from *Rps. sphaeroides*. Following the decay of state P^F, there remain absorbance changes associated with a second transient state, which decay in turn with a time constant of about 55 μs (trace

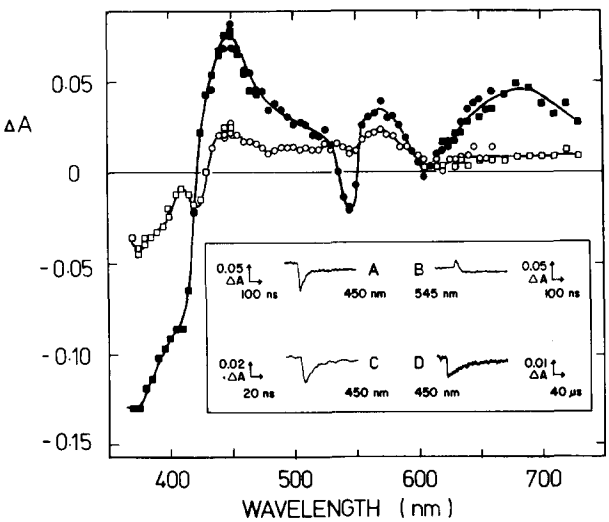

Fig. 2. Absorbance changes caused by excitation with 20-ns flashes, after lowering the redox potential to reduce X by the addition of excess solid $Na_2S_2O_4$ in the dark. The spectra show the absorbance changes measured immediately after the flash (filled symbols) or approx. 150 ns after the flash (open symbols). Traces A and B in the inset show typical measurements at 450 and 545 nm. For these, and for the data shown by circles in the spectra, the reaction center concentration was approx. 4 μM in 50 mM Tris · HCl (pH 8.05)/0.05% Triton X-100, and the path length was 1 cm; the ordinate scale for the spectra applies to these measurements. Data shown by squares in the spectra and trace D in the inset were obtained in a separate series of measurements with approx. 2.4 μM reaction centers; for the spectra, these data were normalized to the others in the region 435—470 nm. For Trace C in the inset, the conditions were as for traces A and B, but the flash width was decreased to about 5 ns; this also decreased the total strength of the flash. The amplitudes of the absorbance changes shown in the spectra probably do not represent complete conversion of the reaction centers into state P^F or P^R, because some of the reaction centers could have had I reduced before the flash, as a result of previous excitations. In addition, the flashes were not sufficiently long to allow extensive recycling of reaction centers that decayed from P^F back to the ground state. Without such recycling, the conversion into state P^R probably cannot be complete [5].

D). The spectrum of the longer-lived absorbance changes is comparatively featureless (Fig. 2, open symbols). It includes very broad absorption bands near 450 and 560 nm and is similar to the spectra associated with state P^R in reaction centers from *Rps. sphaeroides* and *Rds. rubrum* [5,6]. We therefore attribute the longer-lived absorbance changes to the triplet state of the reaction center bacteriochlorophyll *b*. ESR studies at low temperatures have shown that excitation of *Rps. viridis* reaction centers at low redox potentials [26,28,29] causes the formation of a triplet state with a spin polarization that is similar to the polarization observed in *Rps. sphaeroides*. As in *Rps. sphaeroides* reaction centers, the quantum yield of the triplet state appeared to be lower than that of the radical pair state: the longer-lived absorbance changes decreased in size relative to the short-lived ones if the flash intensity or width was decreased. However, we did not investigate this point in detail.

Fig. 3 shows spectra of the absorbance changes resulting from excitation of reaction centers at moderate redox potential with flashes lasting about 8 ps. The open circles represent measurements made at 20 ps after the flash; the filled circles were measured 1 ns after excitation. The measurements at 1 ns show the features characteristic of the formation of P^+X^- (Fig. 1 and refs. 26,

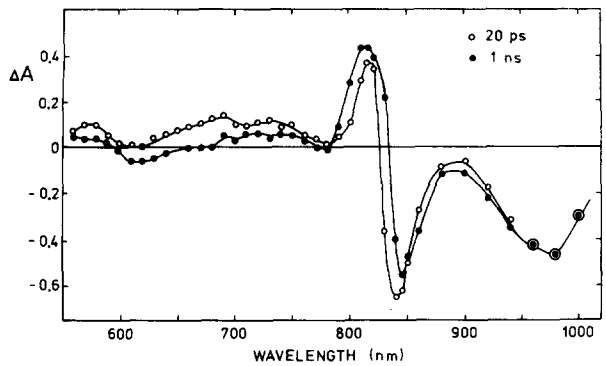

Fig. 3. Difference spectra of the absorbance changes following excitation with an 8 ps flash, measured after time delays of 20 ps (open circles) and 1 ns (closed circles). Measurements employed approx. 52 μM reaction centers in 50 mM Tris · HCl (pH 8.0)/0.1% lauryldimethylamine oxide; the path length was 1 mm. Each point is the average of at least three measurements and typically has a standard deviation of ±0.03.

28,31—33). In addition to the bleaching around 610 nm, there are larger absorbance decreases around 960 and 850 nm, and a major increase near 820 nm.

The spectrum measured at 20 ps (open circles in Fig. 3) matches well with the spectrum measured immediately after the 20-ns flashes at low potentials (filled circles in Fig. 2) in the spectral region that is common to the two figures (550—725 nm). This supports the conclusion that the radical pair P^+I^- is an intermediate in the transfer of an electron from P to X, in agreement with the previous work in *Rps. sphaeroides* [1—4,16]. The 20 ps spectrum includes a trough around 610 nm and a broad (610—780 nm) absorbance increase centered at about 680 nm. The absorption band at 960 nm is bleached to the same extent in the 20 ps and 1 ns spectra, while the band at 830 nm shows additional bleaching in the 20 ps spectrum.

If the reaction centers are in the form P^+I^-X 20 ps after the flash, and P^+IX^- at 1 ns, subtracting the measurements made at 1 ns from those made at 20 ps should give the difference spectrum for $(I^- + X) - (I + X^-)$. Fig. 4A shows a spectrum calculated in this way. One expects the difference spectrum to be dominated by the absorbance changes due to I and I^-, because the reduction of X to X^- probably causes only relatively small absorbance changes at these wavelengths [40,41]. For comparison, Fig. 4B shows spectra of the absorbance changes that accompany the reduction of I when the reaction center preparation is illuminated with continuous light at low redox potentials; these spectra are essentially the same as the ones that have been reported recently elsewhere [23—25]. The spectra in parts A and B of Fig. 4 are very similar. In addition to the features at 545 and 680 nm which are discussed above, they include a major bleaching at 830 nm, a smaller bleaching near 790 nm, where the bacteriopheophytin *b* of the reaction centers probably absorbs (see Introduction), a broad, relatively weak increase in absorbance near 900 nm, and a small trough near 600 nm. The similarities between the spectra support assignment of the 20 ps spectrum to P^+I^-X and the 1 ns spectrum to P^+IX^-. There is, however, a discrepancy in the region around 810 nm: the difference between the 20 ps and 1 ns measurements is negative here, whereas the spectrum obtained by con-

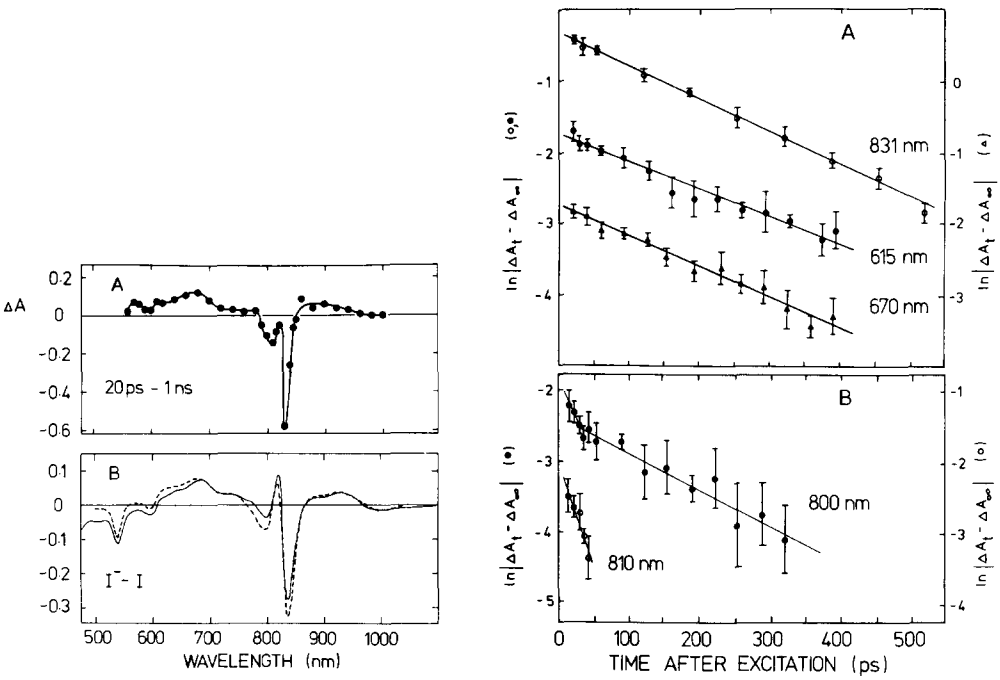

Fig. 4. (A) Difference spectrum calculated from the data of Fig. 3 by subtracting the measurements made at 1 ns from those made at 20 ns. (B) Difference spectrum of absorbance changes caused by continuous illumination of reaction centers at low redox potentials. Solid curve, 3.4 μM reaction centers in 50 mM Tris · HCl (pH 8.0)/0.1% lauryldimethylamine oxide, cuvette thickness 1 cm. Dashed curve, 3.6 μM reaction centers in 50 mM Tris · HCl (pH 8.0)/1.0% Triton X-100. Solid $Na_2S_2O_4$ was added to lower the potential in both cases and the samples were illuminated with white light. (Monochromatic illumination at 760, 836 or 1027 nm caused the same effect.) The spectra were measured with a Cary 14 R spectrophotometer. With the sample in lauryldimethylamine oxide, the difference spectrum was measured with the white light on, because the absorbance changes started to decay when the light was turned off. With the sample in Triton X-100, the absorbance changes lasted for many minutes after the light was turned off, and the spectrum was measured shortly after stopping the illumination.

Fig. 5. Kinetics of the absorbance changes following excitation of reaction centers (approx. 65 μM in 50 mM Tris · HCl (pH 8.0)/0.1% lauryldimethylamine oxide with an 8 ps flash. Path lengths were all 1 mm except for the measurements at 615 nm where a 2 mm cell was used. The ordinates plot the natural logarithm of the difference between the absorbance change measured at a given time after excitation and the absorbance change measured at 1—2 ns, at which time the absorbance changes had reached a constant value. Note the shifts in the ordinate scales for the measurements at different wavelengths. Each point is the average of at least five measurements.

tinuous illumination of reaction centers at low potentials is positive.

Fig. 5 shows measurements of the kinetics of the absorbance changes at five different wavelengths, following excitation of reaction centers with the 8 ps flash at moderate redox potentials. The initial formation of P^+I^-, as measured by the increase in absorbance at 670 nm and the decrease at 831 nm, is complete by the time of the earliest measurements, less than 20 ps after the flash. This agrees with the finding of Netzel et al. [28] that the absorbance increase at 1310 nm, reflecting the formation of P^+, occurs within 10 ps after the excitation.

The transfer of an electron from I^- to X, as measured by the decay of the absorption at 670 nm, the decay of the bleaching at 831 nm, or the appearance

of a net bleaching at 615 nm, occurs with an exponential decay time of about 230 ps (Fig. 5A). The measured decay times are 239 ± 31 ps at 670 nm, 259 ± 31 ps at 615 nm, and 213 ± 23 ps at 831 nm. Within experimental error, these are identical with the decay times that have been measured previously in reaction centers form *Rps. sphaeroides* [1,2]. The absorbance changes at 800 nm also exhibit a step with a time of about 230 ps (200 ± 55 ps), as one would expect if they overlap the absorption band of the bacteriopheophytin *b* at 790 nm (Fig. 5B). However, a small portion of the absorbance changes decays with substantially faster kinetics (43 ± 14 ps). At 810 nm, essentially the entire process occurs with the faster kinetics; the time measured here is 35 ± 8 ps. Apparently, it is this fast component that accounts for the discrepancy between the spectra of Fig. 4 in the region near 810 nm. A similar fast step was detected previously in the 800 nm absorption band of reaction centers from *Rps. sphaeroides*, but was not so clearly resolved spectrally from the other components of the 800 nm band [1]. The fast step represents an increasing absorbance in both species.

We next investigated the response of the reaction centers to excitation with short flashes under conditions that blocked the transfer of an electron from P* to I. When I was converted to its reduced state by continuous illumination at low redox potentials, excitation with the 8 ps flash caused the absorbance changes that are shown in Fig. 6. The predominant feature of the spectrum is a bleaching of the 960 nm absorption band. The bleaching extends to shorter wavelengths, having a distinct shoulder near 850 nm which we take to reflect the 850 nm component of the 830 nm absorption band (cf. ref. 23); there is no absorbance change at 830 nm. At still shorter wavelengths, a very broad absorbance increase extends into the visible region, broken by a trough at 610 nm. Judging from studies on bacteriopheophytin *a* and related compounds in vitro [42,43], the absorbance changes are consistent with those that one expects to accompany the formation of the excited singlet state, P*. Netzel et al. [28] have found that the absorbance increase at 1310 nm which is characteristic of the formation of P* does not occur under these conditions.

The absorbance changes shown in Fig. 6 decay almost completely by 1 ns

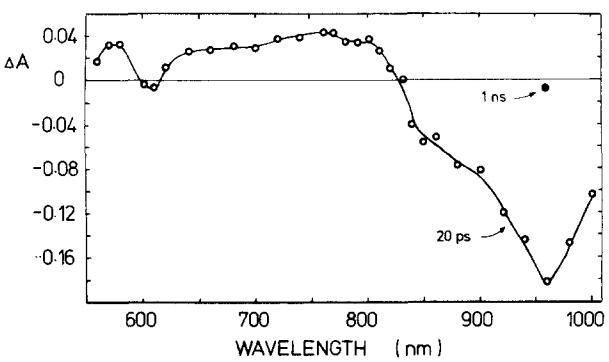

Fig. 6. Difference spectrum for the absorbance changes caused by excitation with 8-ps flashes, after reduction of I by continuous illumination at low redox potential. Reaction centers were approx. 8 μM in 50 mM Tris · HCl (pH 8.0)/0.05% Triton X-100. The path length was 5 mm. Each point reflects the average of at least three measurements.

after the flash (filled circle at 960 nm). Most of the decay occurs with an exponential time of approx. 20 ps, but the decay also includes a slower component (or components) with a time of several hundred ps (Fig. 7).

Fig. 8 shows the results of excitation with 20-ns flashes when I has been reduced previously. Trace A was obtained with isolated reaction centers in which X alone was reduced by lowering the redox potential with $Na_2S_2O_4$ in the dark. The trace shows the formation of states P^F and P^R following the flash, and is similar to A in Fig. 2. When the reaction centers were then illuminated with continuous light, the flash caused the absorbance changes shown in traces B and C. B was measured while the reaction centers were illuminated continuously, and C 2 min after turning the continuous light off. In both cases, the flash caused a small absorbance increase that decayed completely to the baseline. Trace D is similar to B, but with expanded vertical and horizontal scales to illustrate more clearly the completeness of the decay. Trace E was obtained after decreasing the width of the flash to about 5 ns. This affords a more reliable measurement of the decay kinetics than do the measurements with wider flashes, although the smaller amplitude of the signal reduces the signal-to-noise ratio considerably; the time-constant of the decay is approx. 15 ns.

The lower part of Fig. 8 shows the spectrum of the absorbance changes measured as in trace B. The spectrum is featureless except for a trough near 610 nm, resembling the spectra that we have attributed to P* (Fig. 6) and the triplet state P^R (open symbols in Fig. 2). Again, from model studies of bacteriopheophytin *a* and related compounds in vitro, one expects the excited singlet and triplet states to have virtually identical absorption spectra [42,43]. We

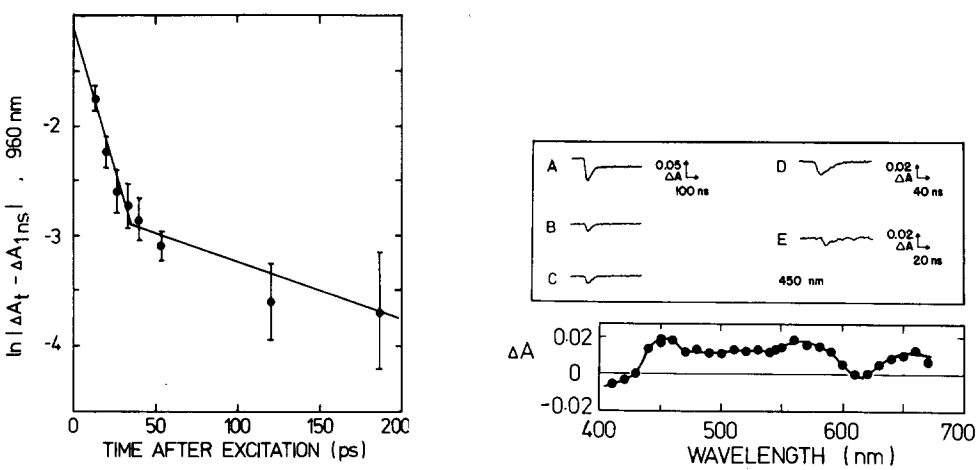

Fig. 7. Kinetics of the decay of the bleaching at 960 nm under conditions as in Fig. 6. Each point is the average of at least five measurements.

Fig. 8. Absorbance changes caused by excitation with 20-ns flashes, after reduction of I by illumination at low redox potential. (A) Approx. 4 μM reaction centers in 50 mM Tris · HCl (pH 8.05)/0.05% Triton X-100; path length, 1 cm; $Na_2S_2O_4$ was added and the sample was allowed to relax in darkness for 4 h. A downward deflection is an absorbance increase. (B) Same as A, after turning on continuous white light. (C) Same as B, 2 min after turning off the light. (D) Same as B, but with expanded scales. (E) Same as B, but with flash width decreased to 5 ns. The spectrum shows the initial absorbance changes, measured as in B.

therefore are unable to identify the transient state shown in Fig. 8 unambiguously. It could be either an unusually short-lived triplet, or a long-lived component of the excited singlet state. Although the absorbance changes associated with P* appeared to decay completely with faster kinetics (Figs. 6 and 7), the absorbance changes measured with the nanosecond apparatus were comparatively small, and they might not have been detectable in the experiments of Figs. 6 and 7. In addition, transient states that are formed with a low quantum yield, such as triplet states, can accumulate as a result of multiple excitation during the 20 ns flash but not during the 8 ps flash.

Discussion

The primary photochemical reaction in *Rps. viridis* is remarkably similar to that in *Rps. sphaeroides*. In reaction centers of both species, the transfer of an electron from P* to I occurs in less than 10 ps, and in both the radical pair P^+I^- transfers an electron to X in about 230 ps. If X is already reduced, so that the removal of an electron from P^+I^- is blocked, the radical pair lives for about 15 ns, and the return of an electron from I^- to P^+ can form a long-lived spin-polarized triplet state. In reaction centers from wild-type strains of *Rps. sphaeroides* and *R. rubrum*, however, the bacteriochlorophyll triplet state P^R decays in 10—20 ns by transfering energy to a carotenoid [6,7]. This seems not to occur in *Rps. viridis*, although reaction centers obtained from this species have been found to contain a carotenoid [31]. The 55 μs life-time of state P^R in the isolated *Rps. viridis* reaction centers is comparable to the life-times that have been measured in carotenoidless strains of *Rps. sphaeroides* and *Rds. rubrum* [5,6].

One of our incentives for studying the preparation from *Rps. viridis* was the potential of resolving the different components in the 830 nm absorption band. As Introduction points out, the spectral separation of these components is greater than that of the homologous components that probably underlie the 800 nm absorption band in the *Rps. sphaeroides* reaction center. The present work has realized this potential to some degree. The difference spectrum in Fig. 6 shows that the 850 nm component of the 830 nm band is bleached along with the 960 nm band when P is converted to P*. Previous work [24,25] has shown that the 850 and 960 nm bands also bleach in concert when P is converted to P^+, and our measurements agree with this. Out results therefore support the conclusion that the 850 and 960 nm bands result from excitonic interactions within the same set of bacteriochlorophyll *b* molecules. It would be these molecules that are most directly involved in the lowest excited singlet state of the reaction center, P*, and in the expulsion of an electron to generate P^+. The interactions that generate the two absorption bands must be disrupted as a result of the removal of an electron from the set. Our observations can be accounted for most simply on the assumption that only two of the four bacteriochlorophyll *b* molecules are responsible for the 850 and 960 nm bands, although they do not exclude additional excitonic interactions with one or both of the other two bacteriochlorophyll *b* molecules. Shuvalov et al. [25] have suggested that such additional interactions occur.

As Introduction mentions, ESR and ENDOR studies of reaction centers from *Rps. sphaeroides* have shown that the unpaired electron in P^+ is deloca-

lized over two molecules of bacteriochlorophyll *a* [12—15]. Similar studies of reaction centers from *Rps. viridis* [15,20,44,45] indicate that the electron is not shared equally by two identical molecules of bacteriochlorophyll *b*. This observation is not inconsistent with the view that P consists of two interacting molecules of bacteriochlorophyll *b*, but it suggests that the environments of the two molecules are significantly different.

The component of the 830 nm band that is centered nearest 830 nm appears not to be altered by the formation of P* (Fig. 6). It is, however, profoundly affected by the conversion of I to I^- (Fig. 4). Although the absorbance changes that occur at 545 and 790 nm provide compelling evidence that I is one of the two bacteriopheophytin *b* molecules of the reaction center, the 830 nm absorption hand seems likely to be due to bacteriochlorophyll *b*. Our results thus support the conclusion [23,25,26] that I involves both bacteriopheophytin *b* and bacteriochlorophyll *b*, with excitonic interactions occurring between the two, and the interactions being disrupted by the reduction of one of the participants. ESR and ENDOR measurements indicate that the unpaired electron on I^- appears to be restricted to a single molecule [44,45], and judging from in vitro studies [15], the bacteriopheophytin *b* molecule would be more likely to be reduced than would the bacteriochlorophyll *b*. Furthermore, the difference spectrum for the reduction of bacteriochlorophyll *b* in vitro shows a definite peak in the region 1000—1100 nm whereas the difference spectrum for the reduction of bacteriopheophytin *b* does not [15]. The lack of such an absorbance increase when I is converted to I^- (Fig. 4B) lends support to the postulate that bacteriopheophytin *b* and not bacteriochlorophyll *b* is the component of I which is reduced.

In the region of 810 nm, one sees an increase in absorbance when P is converted to P^+ (Fig. 3), and also when I is in the I^- state (Fig. 4). As Vermeglio and Clayton [34] have pointed out, disrupting the excitonic interactions between an interacting pair of molecules by oxidizing or reducing one member of the pair should free the other member to act essentially as a monomer. The increased absorbance near 810 nm could be due to the monomeric bacteriochlorophyll *b* that is liberated from exciton interaction when P is oxidized or when I is reduced. In addition, the formation of P^+ or I^- could cause electrochromic shifts of the absorption bands due to the other bacteriochlorophyll *b* molecules of the reaction center. Such shifts could account for part of the increase in absorbance near 810 nm [26,28], but there is no way to evaluate the quantitative importance of the latter effect at present. On either interpretation, one would expect the absorbance increase to occur as rapidly as P is converted to P^+, or I to I^-, and an increase in absorbance at 810 does indeed occur within 20 ps after excitation (Fig. 3). But the initial increase is not as large as one would expect, and the absorbance continues to increase after the flash with a time constant of about 40 ps (Fig. 5B). Similar observations were made previously in reaction centers from *Rps. sphaeroides* [1]. A simple phenomenological interpretation would be that the initial absorbance increase is masked by the bleaching of another absorbance band, and that the 40 ps step is a reversal of this bleaching. In the *Rps. viridis* reaction centers, the 830 nm band includes a component with a maximum near 815 nm, and it could be this component that is bleached. The finding of a similar step in both *Rps. viridis* and *Rps.*

sphaeroides suggests that the absorbance change reflects a significant transformation in the reaction center, such as a structural reorganization of some of the components, but further work will be necessary in order to explore this point. It is possible that bleaching of the 815 nm band reflects the absorption of a second photon by the reaction center, following the oxidation of P.

If I is reduced, so that the removal of an electron from P* is blocked, most of the excited singlet state decays in about 20 ps. Radiationless processes must be responsible for this rapid decay, because the natural radiative life-time of P* calculated from the absorption spectrum is on the order of 10 ns. Fig. 9 outlines some possibilities for these processes: Row A shows the steps that generate P^+I^- and then PI^- during continuous illumination at low redox potentials, and B shows the excitation of PI^- to form the excited singlet state, P*I. In this state, I^- has the possibility of transferring an electron to P*, generating P^-I. Because of the high free energy of P*, this process will almost certainly be thermodynamically favorable, even though the redox potential of P^-/P probably is more negative than that of I^-/I [15,26,29]. Whether it is kinetically favorable is not clear. The great speed of the normal transfer of an electron from P* to I implies that a significant amount of orbital overlap occurs between the two reactants but the back-reaction between P^+ and I^- is comparatively slow, requiring about 15 ns. If P^-I does form from P^*I^-, the reaction center should be able to relax very rapidly from there back to PI^-. This last step is essentially identical to the normal transfer of an electron from P* to I (row A), which occurs in less than 10 ps. If the conversion of P^-I to PI^- is rapid, relative to the rate of formation of P^-I from P*I, our spectrophotometric measurements would not be able to detect the transient formation of P^-I.

In the diagrams of Fig. 9B, the spin of the unpaired electron on I^- is opposed

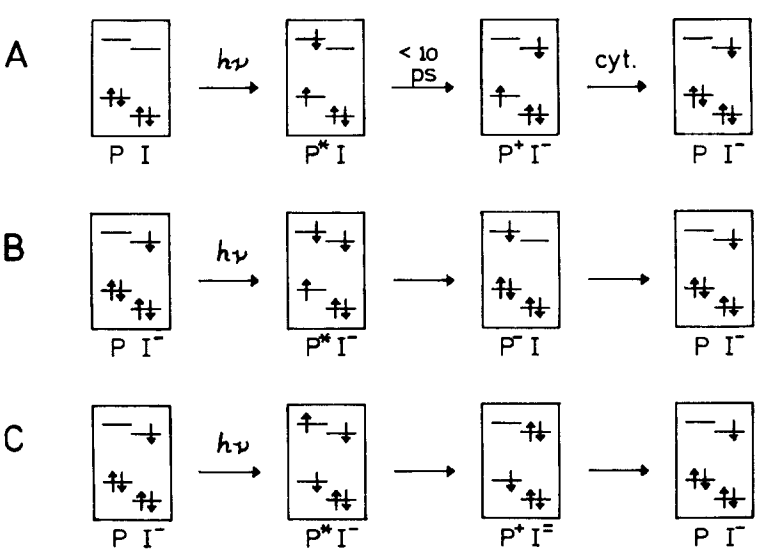

Fig. 9. Scheme illustrating photochemical electron transfer reactions between P and I under various conditions. The horizontal lines represent the lowest unoccupied and highest occupied orbitals for electrons on P and I; arrows represent electrons. See the text for discussion.

to that of the electron in the lower, half-filled orbital of P*. Because the two spins are uncorrelated, this will be the case in approx. 50% of the reaction centers. In the other 50% (Fig. 9C), the electron of I^- will be forbidden to enter the lower orbital on P*, and the decay of P^*I^- must follow a different path. One posibility (not shown) is the transfer of an electron from I^- to the upper half-filled orbital on P*, generating an excited state of P^-. Another, which is shown in Fig. 9C, is the transfer of an electron from P* to I^-, generating P^+I^{2-}, followed by rapid relaxation to PI^-. Again, P^+I^{2-} would not be detected spectrophotometrically if it decayed as rapidly as it formed. These considerations could provide an explanation for the multiphasic decay kinetics of P^*I^- (Fig. 7).

The scheme of Fig. 9C can be developed further to provide a mechanism for generating the triplet state of P, by assuming that the formation of P^+I^{2-} from P^*I^- is reversible. If an electron with spin α moves from P* to I^-, and an electron with spin β returns, the result would be to convert P* from singlet to triplet. This could account for the transient state that was seen in the nanosecond experiments (Fig. 8), although as was pointed out above, we cannot exclude the possibility that the transient state is a very long-lived component of the singlet state. A triplet state that is formed in this way would not be expected to display the marked electron spin polarization that is observed in state P^R. In agreement with this, ESR measurments have shown that the spin-polarized triplet does not form when reaction centers are illuminated after the reduction of I [19,26,28,29].

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