Biochimica et Biophysica Acta, 408 (1975) 189–199

BBA 47006

CAROTENOID TRIPLET STATES IN REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES* AND *RHODOSPIRILLUM RUBRUM*

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(Received June 10th, 1975)

SUMMARY

Purified photochemical reaction centers from three strains of *Rhodopseudomonas sphaeroides* and two of *Rhodospirillum rubrum* were reduced with $Na_2S_2O_4$ so as to block their photochemical electron transfer reactions. They then were excited with flashes lasting 5–30 ns. In all cases, absorbance measurements showed that the flash caused the immediate formation of a transient state (P^F) which had been detected previously in reaction centers from *Rps. sphaeroides* strain R26. Previous work has shown that state P^F is an intermediate in the photochemical electron transfer reaction in the reaction centers of that particular strain, and the present work generalizes that conclusion.

In the reaction centers from two strains that lack carotenoids (*Rps. sphaeroides* R26 and *R. rubrum* G9), the decay of P^F yields a longer-lived state (P^R) which is probably a triplet state of the bacteriochlorophyll of the reaction center. In the R26 preparation, the decay of P^F was found to have a half-time of 10 ± 2 ns. The decay kinetics rule out the identification of P^F as the fluorescent excited singlet state of the reaction center.

In the reaction centers from three strains that contain carotenoids (*Rps sphaeroides* 2.4.1 and Ga, and *R. rubrum* S1), state P^R was not detected, and the decay of P^F generated triplet states of carotenoids. The efficiency of the coupling between the decay of P^F and the formation of the carotenoid triplet appeared to be close to 100 % at room temperature, but somewhat lower at 77 °K. Taken with previous results, this suggests that the coupling is direct and does not require the intermediate formation of state P^R. This conclusion would be consistent with the view that P^F is a biradical which can be triplet in character.

INTRODUCTION

The primary photochemical reaction in bacterial photosynthesis is the transfer of an electron from a bacteriochlorophyll complex, P, to an unidentified acceptor. The reaction occurs within pigment-protein complexes called "reaction centers", which contain four molecules of bacteriochlorophyll and two molecules of bacteriopheophytin [1–3]. Recent studies of reaction centers isolated from *Rhodopseudomonas sphaeroides* strain R-26 have demonstrated that the photooxidation of P involves the

formation of an intermediate state of the reaction center, called state P^F [4, 5]. Following picosecond flash excitation, P^F forms in < 20 ps. It then decays with a half time of approx. 165 ps, giving rise to the oxidized bacteriochlorophyll complex, P^+.

The photochemical electron transfer reaction is prevented if the primary electron acceptor is chemically reduced before the flash. Under these conditions, flash excitation of reaction centers still generates state P^F with a high quantum yield, but as P^F decays another transient state, P^R, appears instead of P^+ [6]. The yield of P^R is high at cryogenic temperatures, but is much lower at room temperature, when the major path for the decay of P^F appears to return the reaction center directly to the ground state.

The identification of states P^F and P^R is unclear. When the primary acceptor is reduced, the lifetime of P^F appears to be too long to be consistent with an identification of P^F as the lowest excited singlet state of the reaction center [6], but measurements of the lifetime have not been sufficiently accurate to rule out this possibility decisively. As an alternative, Rockley et al. [4] have suggested that P^F could be a biradical [7] in which an electron has moved from one bacteriochlorophyll molecule to another, or possibly from bacteriochlorophyll to one of the bacteriopheophytin molecules of the reaction center. Parson et al. [6] have suggested that state P^R is the lowest triplet state of the bacteriochlorophyll complex.

The foregoing studies have been confined to reaction centers prepared from the carotenoidless strain of *Rps. sphaeroides*, R26. In this paper we describe similar experiments with reaction center preparations from two carotenoid-containing strains, 2.4.1 and Ga. We also report on experiments with reaction center preparations from strains Sl (wild type) and G9 (carotenoidless) of *Rhodospirillum rubrum*. Reaction centers from *Rps. sphaeroides* strain 2.4.1 contain spheroidene [8], those from strain Ga contain chloroxanthin (Cogdell, R. J., Kerr, M.A. and Parson, W. W., manuscript in preparation) and those from *R. rubrum* Sl contain spirilloxanthin [9], all in approximately 1 : 1 mol ratios with the bacteriochlorophyll complex. An extension of the studies to these strains seemed important in order to investigate whether the formation of state P^F is peculiar to strain R-26, or whether it is a general feature of bacterial photosynthesis. It also seemed likely to aid in the characterization of states P^F and P^R, because carotenoids are avid quenchers of triplet states of chlorophylls in solution [10–12].

MATERIALS AND METHODS

Cultures of all of the photosynthetic bacteria were obtained from Dr. R. K. Clayton. The bacteria were grown in batch cultures with succinate as the sole carbon source. The cells were harvested, disrupted by passage through a French Press and chromatophores were prepared and purified as described by Clayton and Clayton [13]. Reaction centers *Rps. sphaeroides* strain R26 were prepared by treatment of chromatophores with 1 % *N,N*-dimethyllaurylamine oxide as described by Clayton and Wang [14]; those from strains 2.4.1 and Ga were prepared by treatment of chromatophores with 0.25 % *N,N*-dimethyllaurylamine oxide at 26 °C, using a modification of the procedure of Jolchine and Reiss-Husson [15] for strain 2.4.1. In our experience, a single detergent treatment failed to release the reaction centers from the 2.4.1 or Ga chromatophores. However, if the pellet from the high speed centrifugation was

collected, resuspended in buffer, and treated with detergent for a second time, a good yield of reaction centers was obtained. Reaction centers from *R. rubrum* were prepared from strain G9 by treatment of chromatophores at pH 7.0 with 0.04 % *N,N*-dimethyllaurylamine oxide after the method of Okamura et al. [16], and from strain S1 by the method of Noel et al. [17]. The purified reaction centers usually were dialyzed for 2–3 days against 0.05 % Triton X-100, 50 mM Tris · HCl, pH 7.5, to remove the *N,N*-dimethyllaurylamine oxide. In some cases the *N,N*-dimethyllaurylamine oxide was removed by treatment with dithionite followed by passage of the reaction centers over a DEAE-cellulose column [18].

In a typical experiment, the medium (50 mM Tris · HCl, pH 7.5, with 0.05 % Triton X-100) was bubbled with N_2 to remove oxygen. Then the sample was added, along with excess sodium dithionite, to reduce the primary electron acceptor. The 1-cm cuvette was stoppered and bubbled with N_2 again, and then the contents were mixed.

Absorbance changes following laser flash excitation were measured essentially as described by Parson et al. [6]. For most of the experiments, the actinic light source was a dye laser ($2 \cdot 10^{-5}$ M 3,3'-diethylthiatricarbocyanine iodide in dimethylsulfoxide) which was pumped axially by a Q-switched ruby laser. The maximum emission of the dye laser was at 834 nm and the width of the pulse at half height was usually about 20 ns. For some experiments, the width of the laser flash was reduced to about 5 ns by the use of a Pockels cell (Lasermetrics, Inc., Model 1073) which was pulsed with a laser-triggered spark gap (Lasermetrics, Inc.). The Pockels cell was positioned between the ruby and dye lasers. Flash energies were measured with a ballistic thermopile (TRG, Inc., Model 100), and were varied by calibrated neutral density filters. Flash profiles were measured with a fast vacuum photodiode (ITT, Inc., Type F4000). The detection circuitry of the spectrophotometer had a response half-time of 6.5 ± 1 ns, as measured from the apparent rate of formation of the oxidized reaction center (P^+), when reaction centers at moderate redox potentials were excited with a short flash. The low temperature measurements were performed with a simple liquid N_2 Dewar and used a 1-mm cuvette.

RESULTS

Figs 1A and 1B show typical kinetic traces of the absorbance changes that result from flash excitation of reaction centers of the carotenoidless *R. rubrum* strain G9. For both measurements, the redox potential was sufficiently negative to reduce the primary electron acceptor, preventing the photochemical electron transfer reaction. Under these conditions, the flash causes an immediate absorbance increase at 420 nm, followed by a relaxation which has a half-time of approx. 20 ns at room temperature (Fig. 1A). The spectrum of the initial absorbance change is shown in Fig. 2. It is very similar to the difference spectrum that has been described [4, 6] for the formation of state P^F in reaction centers from *Rps. sphaeroides* R 26, except that the negative peak near 600 nm is somewhat more pronounced in the *R. rubrum* spectrum. The relaxation kinetics also are very similar to those of state P^F in *Rps. sphaeroides* R 26. We therefore interpret the initial absorbance change in the *R. rubrum* reaction centers as reflecting the transient formation of state P^F.

Flash excitation of reaction centers from any of the three carotenoid-con-

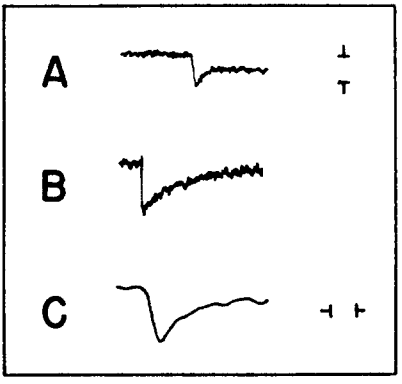

Fig. 1. Kinetics of flash-induced absorbance changes at 420 nm in reaction centers from *R. rubrum* G9 and *Rps. sphaeroides* R26. (A) 2.9 μM *R. rubrum* G9 reaction centers suspended in 50 mM Tris · HCl, pH 7.5, 0.05 % Triton X-100; reduced with excess $Na_2S_2O_4$ prior to the flash. A downward deflection of the trace represents an absorbance increase; the vertical scale marker (ΔA) indicates an absorbance change of 0.043. The horizontal scale marker (ΔT) indicates 100 ns. Lightpath, 1 cm. (B) Same as (A), except that the ΔA scale marker indicates an absorbance change of 0.011, and the ΔT marker indicates 40 μs. (C) 33.7 μM *Rps. sphaeroides* R 26 reaction centers. Conditions as in (A), except $\Delta T = 20$ ns. The excitation flash had a width of approx. 5 ns at half-maximum amplitude. It was not of saturating intensity.

taining strains also results in the formation of a short-lived state which appears to be identical with state P^F. A typical kinetic trace showing the formation and decay of P^F in *Rps. sphaeroides* 2.4.1 reaction centers is shown in Fig. 3A. The spectra of the initial absorbance changes appear to be essentially the same as those for the formation of P^F in the carotenoidless reaction centers, although we could not make accurate measurements at all wavelengths in the carotenoid-containing preparations.

Parson et al. [6] estimated that the decay of P^F in *Rps. sphaeroides* R 26 reac-

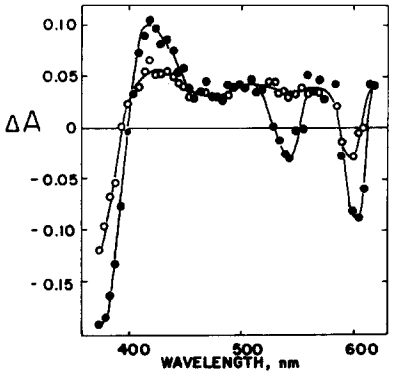

Fig. 2. Spectra of flash-induced absorbance changes in reaction centers from *R. rubrum* G9. Conditions as in Fig. 1A. ●, initial absorbance change ($P \rightarrow P^F$). ○, absorbance change remaining approximately 200 ns after the flash ($P \rightarrow P^R$). At wavelengths where the two absorbance changes had the same value, the open symbols have been omitted for clarity.

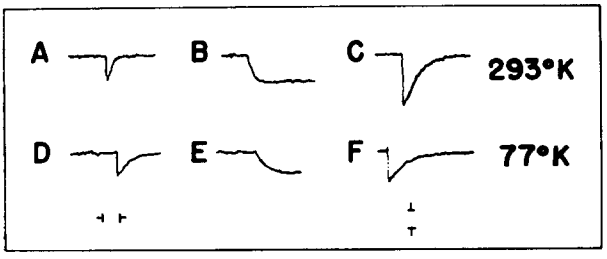

Fig. 3. Kinetics of flash-induced absorbance changes in reaction centers from *Rps. sphaeroides* 2.4.1, following reduction with excess $Na_2S_2O_4$. (A) 4.4 μM reaction centers in 100 mM K phosphate, pH 7.5, 0.05 % Triton X-100. Wavelength, 420 nm. ΔA scale marker = 0.044; ΔT = 100 ns; light path = 1 cm.(B) Same as (A), except wavelength, 520 nm. (C) same as (A), except 2.9 μM reaction centers; wavelength, 530 nm; ΔA scale = 0.035; ΔT = 10 μs. (D) 8.8 μM reaction centers, 90 mM potassium phosphate, pH 7.5, 0.05 % Triton X-100, in 70 % glycerol; temperature 77 °K; ΔA scale = 0.011; ΔT = 100 ns; light path = 1 mm; 420 nm. (E) same as (D), except wavelength 535 nm; ΔA scale = 0.044. (F) same as (D), except wavelength 545 nm; ΔA scale = 0.44; ΔT = 10 μs.

tion centers had a half-time of approx. 6 ns at room temperature. They were not able to measure the decay kinetics accurately, because the excitation flash that they used was not sufficiently short. We have obtained an improved measurement by the use of a shorter flash (Fig. 1C). The decay of the absorbance change reflecting P^F fits an exponential curve with a half-time of 12 ± 1 ns (mean$\pm$S.D. of four measurements). Allowing for the response time of the measuring apparatus, we calculate the actual half-time for the decay of P^F to be 10 ± 2 ns.

As in the *Rps. sphaeroides* R 26 reaction centers, state P^F in the *R. rubrum* G9 reaction centers decays to a longer lived state (Figs 1A and 1B). The difference spectrum for the formation of this state is included in Fig. 2. It is essentially identical to the spectrum that has been described [6] for the formation of state P^R in *Rps. sphaeroides* R 26 reaction centers. In the *R. rubrum* G9 reaction centers, however, the decay of P^R has a half-time of 50 μs at room temperature (Fig. 1B). This is ten times slower than the decay of P^R in the *Rps. sphaeroides* reaction centers at the same temperature. As in the reaction centers from *Rps. sphaeroides* R 26 [6], the quantum yield of state P^R at room temperature was significantly lower than that of P^F. With flashes that were weaker than those that were used for Figs 1 and 2, the absorbance changes due to P^R were smaller, relative to those due to P^F.

In the reaction centers from the strains that contain carotenoids, P^F decays rapidly to reveal a new longer-lived state which is decidedly different from state P^R. Difference spectra for these new states are shown in Fig. 4. The spectrum obtained from *Rps. sphaeroides* 2.4.1 reaction centers includes a broad trough in the 400–490 nm region and a sharp peak around 545 nm (Fig. 4A). Those for the reaction centers from *Rps. sphaeroides* Ga or *R. rubrum* Sl are similar, but are shifted to shorter wavelengths in one case and longer wavelengths in the other (Figs 4B and 4C).

For the following reasons, we identify the new longer-lived states as carotenoid triplet states, and we shall refer to them collectively as car^T. First, these states are seen only in the reaction centers which contain carotenoids. Second, the spectra of the new states are very similar to the spectrum of the triplet state of β-carotene seen in chloroplasts [19, 20] and the spectra of triplet states of other carotenoids in organic solution [21–23]. For carotenoids and various polyenes in solution, Truscott et al.

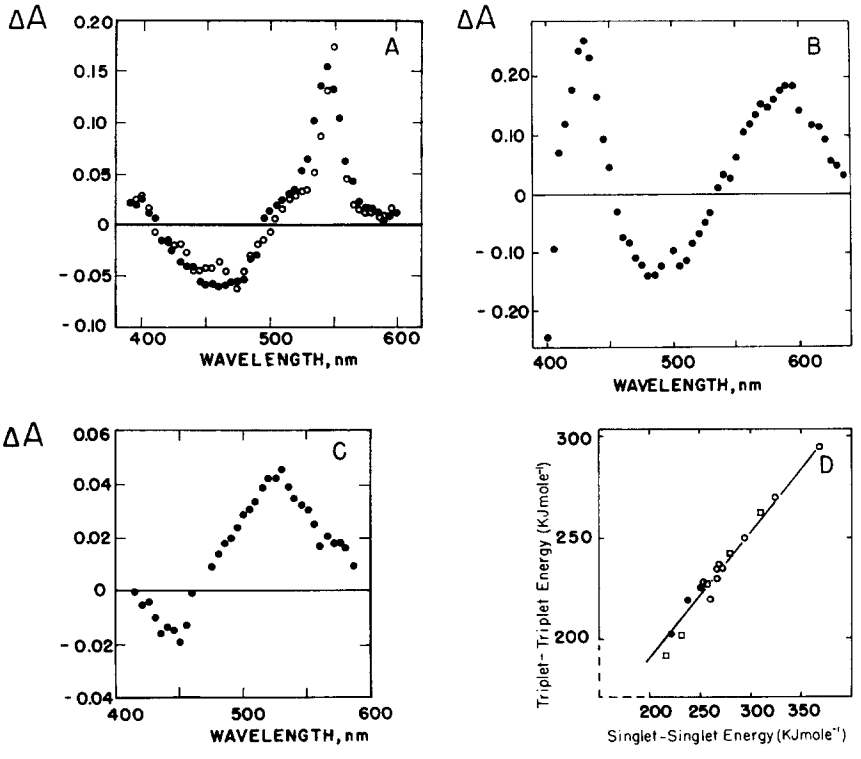

Fig. 4. Flash-induced difference spectra for the formation of the car^T states in reaction centers from three carotenoid-containing strains. (A) ●, Reaction centers of *Rps. sphaeroides* 2.4.1 at room temperature; conditions as in Fig. 3C. ○, Reaction centers of *Rps. sphaeroides* 2.4.1. at 77 °K; conditions as in Fig. 3D. (B) Reaction centers of *R. rubrum* S1 at room temperature. 7.9 μM reaction centers in 50 mM Tris · HCl, pH 7.5, 0.05 % Triton X-100, excess $Na_2S_2O_4$; light path = 1 cm. (C) Reaction centers of *Rps. sphaeroides* Ga (1.13 μM in 50 mM Tris · HCl pH 7.5, 0.05 % Triton X-100; excess $Na_2S_2O_4$; light path = 1 cm; room temperature). (D) Energy of the long wavelength triplet-triplet absorption band vs the energy of the long wavelength singlet-singlet absorption band. ○, and □ data for carotenoids and various polyenes in solution, taken from refs 22 and 23. ●, data for carotenoids in reaction centers; triplet-triplet energies were calculated from the flash-induced difference spectra of Figs 4A–4C and singlet-singlet energies were calculated from the absorption spectra of the corresponding reaction center preparations.

[22] and Mathis and Kleo [23] have found a linear relationship between the energy of the first singlet-singlet transition and the first triplet-triplet transition. We sought a similar relationship in our data, taking the lowest singlet-singlet energy levels of the carotenoids from the absorption spectra of the reaction centers and the triplet-triplet energy levels from the long wavelength maxima in the light-induced difference spectra (Figs 4A–4 C). Our three experimental data points fit the relationship found by Truscott et al. [22] and Mathis and Kleo [23] reasonably well (Fig. 4D). Finally, the decay of the new reaction center states occurs in the microsecond time range (Fig. 3C), as does the decay of the triplet states of carotenoids in solution [21–23].

In all of the preparations that contain carotenoids, the car^T states appear to form in parallel with the decay of state P^F. The half-time for the formation of car^T in *Rps. sphaeroides* 2.4.1 reaction centers is approx. 10 ns at room temperature (Fig. 3B)

TABLE I

KINETICS OF FLASH-INDUCED ABSORBANCE CHANGES IN VARIOUS REACTION CENTER PREPARATIONS AT LOW REDOX POTENTIALS

N.D., not determined.

Reaction center preparation	P^F Decay half-time		P^R Decay half-time		car^T			
					Rise half-time		Decay half-time	
	293 °K (ns)	77 °K (ns)	293 °K (μs)	77 °K (μs)	293 °K (ns)	77 °K (ns)	293 °K (μs)	77 °K (μs)
Rps. sphaeroides								
R 26	10±2	20–30*	6	120	–	–	–	–
2.4.1	~10	~50	–	–	~10	~50	4–5	4–5
Ga	10–20	N.D.	–	–	10–20	N.D.	5–6	5–6
R. rubrum								
G9	~20	N.D.	50	N.D.	–	–	–	–
S1	N.D.	N.D.	–	–	~50	~200	2–3	2–3

* 15 °K (ref. 6).

and this is the decay half-time of P^F (Fig. 3A). The same kinetic correlation can be seen more clearly at 77 °K (Figs 3D and 3E). At this temperature P^F decays with a half-time of approx. 50 ns and this is also the rise time of car^T. The spectrum of car^T in 2.4.1 reaction centers at 77 °K (Fig. 4A) is similar to the room temperature spectrum, but it reveals additional structure in the trough near 460 nm and the peak near 545 nm is sharper and slightly shifted to the red. The half-time for the decay of car^T in the 2.4.1 reaction centers is 4–5 μs and is independent of temperature between 77 °K and room temperature (Figs 3C and 3F). The rise and decay kinetics of car^T in *Rps. sphaeroides* Ga reaction centers are very similar to those in the 2.4.1 reaction centers (Table I).

Fig. 5 shows kinetic traces of car^T in *R. rubrum* S1 reaction centers. In Fig. 5A, the initial fast absorbance increase is indicative of state P^F and the slower rise represents the formation of car^T. The half-time for the formation of car^T is approx. 50 ns at room temperature (Fig. 5B) and 200 ns at 77 °K (Fig. 5C). The decay of car^T (Fig. 5D) has a half-time of 2–3 μs, and is independent of temperature between 77 °K and room temperature. It is perhaps worth noting at this point that the rise and decay kinetics of state car^T in the *R. rubrum* S1 reaction centers are very similar to those of the flash-induced absorbance changes which earlier workers [24, 25] have observed in chromatophores of *R. rubrum* and *Chromatium vinosum*. The difference spectrum for the formation of car^T (Fig. 4C) is also similar to that which Seibert and DeVault [25] reported for *C. vinosum* chromatophores, although their measurements included only a small portion of the spectrum around 425 nm. Because they overlooked the absorbance changes that occur at longer wavelengths, Seibert and DeVault [25] attributed the absorbance changes near 425 nm to the bacteriochlorophyll of the reaction center, rather than to carotenoids. *Chromatium vinosum* resembles *R. rubrum* in containing spirilloxanthin as its major carotenoid [26].

Kinetic data for the transient states of all of the reaction center preparations

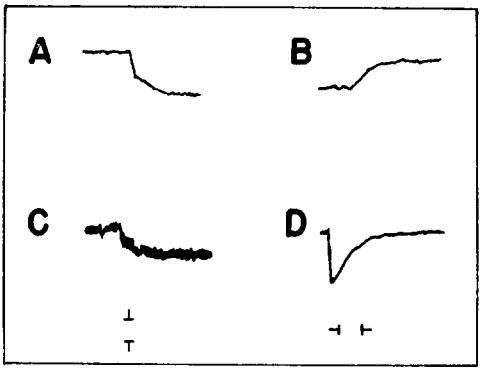

Fig. 5. Kinetics of flash-induced absorbance changes in reaction centers from *R. rubrum* S1. (A) Conditions as in Fig. 4B; wavelength, 420 nm; ΔA scale marker = 0.087; ΔT = 100 ns. (B) Same as (A), except wavelength 510 nm. (C) 13.4 μM reaction centers, 12.5 mM Tris · HCl, pH 7.5, 0.013 Triton X-100, 67 % glycerol; excess $Na_2S_2O_4$; temperature 77 °K; wavelength, 425 nm; ΔA scale = 0.017; ΔT = 400 ns; light path = 1 mm. (D) Same as (A), except wavelength 435 nm; ΔA scale = 0.087; ΔT = 4 μs.

are summarized in Table I.

In principle, car^T might be formed from either of the two states P^F or P^R. Although the rise time of car^T parallels the decay of P^F, and there is no indication of state P^R in any of the reaction centers which contain carotenoids, it is possible that P^R does form, but that it decays immediately to state car^T. One way to test this possibility is the following. State P^F has a quantum yield of formation near unity from 15 °K to room temperature, whereas P^R has a low quantum yield (near 0.1) at room temperature and (at least in *Rps. sphaeroides* R26 reaction centers) a high yield at 15 °K [6]. If car^T forms via P^R, its quantum yield of formation should be significantly lower than that of P^F at room temperature, but the yield might rise as one lowered the temperature. Conversely, a high quantum yield of formation of car^T at room temperature would support the view that car^T forms directly from P^F. This reasoning obviously rests on the assumption that the quantum yield of P^R in reaction centers from *Rps. sphaeroides* strain 2.4.1 would behave like that in the preparation from R 26. We shall examine this assumption more critically in the Discussion.

Fig. 6 shows the amounts of P^F and car^T that are formed in *Rps. sphaeroides* 2.4.1 reaction centers, as a function of the intensity of the excitation flash. The flashes that were used for this experiment were 20–30 ns wide at half-maximum amplitude. This was long enough so that any reaction centers that might decay from state P^F to the ground state without forming car^T would have an opportunity to be re-excited, returning to P^F and perhaps yielding car^T. Thus, if the quantum yield of car^T were less than that of P^F, flashes that were saturating with respect to the formation of P^F would still be subsaturating with respect to car^T. Fig. 6A shows, however, that at room temperature the formation of P^F and car^T have essentially the same dependance on the flash intensity. Apparently, the quantum yields of the two states are identical. We obtained the same results with the reaction centers from *R. rubrum* Sl. Fig. 6B shows the results of similar measurements with the *Rps. sphaeroides* reaction centers at 77 °K. At this temperature, the quantum yield of car^T appears to be slightly lower than that of P^F. These results support the conclusion that car^T forms directly from state P^F.

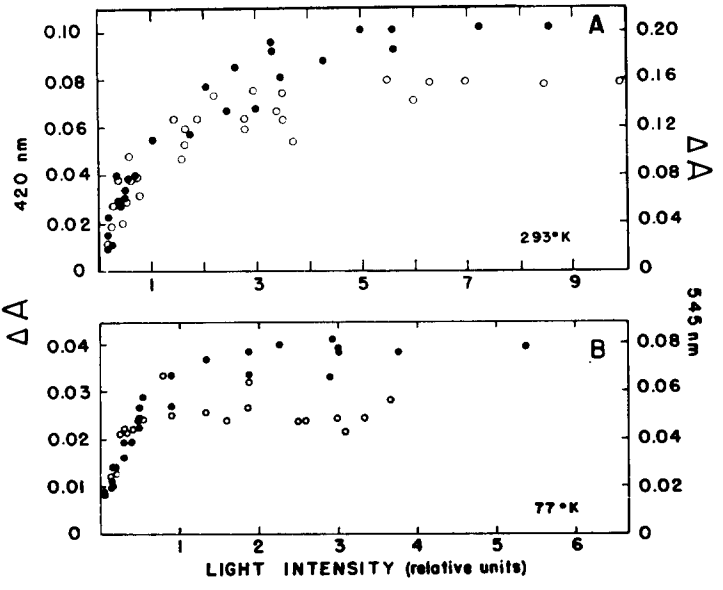

Fig. 6. Formation of states P^F and car^T in reaction centers from *Rps. sphaeroides* 2.4.1, as a function of the strength of the excitation flash. The abscissae give the incident flash intensity in relative units. The strongest flashes had an energy of approx. 46 mJ. (A) Room temperature; conditions as in Fig. 3A. ●, car^T, as measured from absorbance changes at 545 nm. ○, P^F, measured at 420 nm. (B) 77 °K; conditions as in Fig. 3D, except 5.8 μM reaction centers. ●, car^T measured at 545 nm. ○, P^F measured at 420 nm.

When the reaction centers from any of the types used were excited with supersaturating flashes at redox potentials that were positive enough to hold the primary electron acceptor in the oxidized form prior to the flash, only absorbance changes due to the formation of P^+ were seen. There was no evidence for the production of car^T under these conditions.

DISCUSSION

One of the questions arising from the recent demonstration that P^F is an intermediate in the photochemical reaction in reaction centers from *Rps. sphaeroides* R26 is whether P^F is part of a general mechanism for the primary electron transfer reaction in bacterial photosynthesis. The finding that P^F also can be seen in reaction centers from *R. rubrum* and carotenoid-containing strains of *Rps. sphaeroides* supports the view that the same reaction mechanism does operate throughout bacterial photosynthesis.

In the reaction centers which contain carotenoids, the decay kinetics of state P^F appear to be identical with the rise kinetics of car^T. Since the quantum yields of formation of the two states are indistinguishable at room temperature, it seems clear that P^F is converted quantitatively into state car^T. Because we have no evidence for the formation of state P^R in any of the carotenoid-containing reaction centers, these findings are most simply explained by the assumption that car^T forms directly from P^F, without the intermediate formation of state P^R.

This conclusion raises one difficulty. In the reaction centers which lack carotenoids, P^F appears to decay by two paths, one yielding P^R and the other returning the reaction center directly to the ground state [6]. If the presence of a carotenoid opens up a third decay route in addition to these paths, one might expect that the total rate of decay of P^F would be greater in the carotenoid-containing reaction centers than it is in the reaction centers that lack carotenoids. This is not the case. The decay is no faster in the reaction centers from *Rps. sphaeroides* 2.4.1 than it is in those from strain R 26. In the *R. rubrum* Sl reaction centers, the decay of P^F even appears to be slightly slower than it is in the G9 preparation. This suggests that the presence of a carotenoid is associated with a decrease in the rate constant for one or both of the other decay routes. If this is correct, our finding of a high quantum yield for car^T does not rigorously exclude the possibility that state P^R is an intermediate in the formation of car^T from state P^F.

If the decay of P^F is coupled directly with the promotion of a carotenoid to a triplet state, it would appear likely that P^F itself is a triplet state, or is in rapid equilibration with one. This would be consistent with the identification of P^F as a biradical (charge-transfer) state [4], because a biradical could be either singlet or triplet.

The half-time of 10 ns for the decay of P^F in the *Rps. sphaeroides* R 26 reaction centers (Fig. 1C) definitely rules out the identification of state P^F as the fluorescent excited singlet state which is populated initially by the absorption of light. This measurement was made under conditions that held the primary electron acceptor in the reduced form. If the electron acceptor is in the oxidized form, P^F decays with a half-time of approx. 165 ps, giving rise to P^+ [4]. This means that, if P^F were fluorescent, the yield of fluorescence would be approx. 60 times greater under the former conditions than it is under the latter. In fact, the fluorescence yields differ only 3- to 5-fold [27, 28].

The finding that the rate of conversion of P^F to P^+ is much greater than the rates of the side reactions that yield P^R or the ground state is consistent with the high quantum yield with which the electron transfer reaction normally occurs. A 60-fold difference in the rates would allow the electron transfer to occur with a yield of greater than 98 %, in good agreement with actual measurements of the yield [29]. One should bear in mind, however, that the rates of the alternative reactions of P^F are necessarily measured under rather different conditions. The reduction of the electron acceptor could cause changes in the rates of the side reactions, in addition to blocking the electron transfer.

In reaction centers from *Rps. sphaeroides* R-26, excitation at low redox potentials causes the appearance of a triplet EPR signal [30] which probably reflects state P^R [6]. Leigh and Dutton [30] and Uphaus et al. [31] have observed the same EPR signal in chromatophores and cells from carotenoid containing strains. This observation appears to conflict with our finding that reaction centers from carotenoid-containing strains exhibit carotenoid triplets and state P^F, but not state P^R. There are several possible explanations for this discrepancy. First, our measurements show that at 77 °K the formation of car^T is slower than it is at room temperature (Table I), and the quantum yield is somewhat lower (Fig. 6). The EPR measurements were made at

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Note added in proof (Received October 16th, 1975): Subsequent studies of the 2.4.1 reaction have shown that the formation of P^R does replace that of car^T at temperatures below 77 °K.

an even lower temperature (10 °K), and it is possible that the transfer of energy to the carotenoid fails altogether under these conditions. A second possibility is that a triplet state of the reaction center bacteriochlorophyll can be formed if the reaction center absorbs a second quantum of light during the lifetime of car^T. Such a double excitation may have occurred in the EPR experiments, because of the use of continuous exciting light. Additional work at very low temperatures will be necessary to distinguish between these two possibilities.

ACKNOWLEDGEMENTS

We acknowledge with thanks Dr. R. K. Clayton's gift of the bacterial cultures. National Science Foundation Grant No. BMS 74-19857 supported this work.

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