

The Mechanism of Triplet Energy Transfer from the Special Pair to the Carotenoid in Bacterial Photosynthetic Reaction Centers

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The kinetics of triplet energy transfer from the primary electron donor P, a bacteriochlorophyll dimer, to the carotenoid were measured in the M182HL mutant of *Rhodobacter sphaeroides* photosynthetic reaction centers. In this mutant, the accessory bacteriochlorophyll that is bound in the B_M position in wild-type reaction centers is changed to a bacteriopheophytin. By using phosphorescence data for P in the reaction center and for bacteriochlorophyll *a* and bacteriopheophytin *a* monomers in simple solvents, the activation energy for triplet energy transfer from the special pair to the bacteriopheophytin at B_M in M182HL is predicted to be 910 ± 50 cm⁻¹. Analysis of the decay kinetics of ³P as a function of temperature gives an experimental activation energy of 950 ± 50 cm⁻¹. Thus, the M182HL mutant allows for prediction and quantitative verification of the energetics of triplet energy transfer by a two-step mechanism from the special pair to the carotenoid through the chromophore in the B_M binding site.

Introduction

The triplet state of the special pair primary electron donor, ³P, is formed when electron transfer to the quinone is blocked in bacterial photosynthetic reaction centers (RCs). Once formed, ³P rapidly transfers its energy to a carotenoid molecule.^{1–5} As revealed in the crystal structure of the RC, the carotenoid is about 11 Å from P (see Figure 1). Because triplet energy transfer involves transitions that are dipole forbidden, long-distance energy transfer by the Förster mechanism is not operative, and the process must instead proceed by Dexter-type transfer. The Dexter mechanism requires close contact between donor and acceptor in order to achieve sufficient orbital overlap.⁶ As seen in Figure 1, a monomeric bacteriochlorophyll *a* (Bchl) labeled B_M is found between P and the carotenoid, suggesting a two-step mechanism for triplet energy transfer: ³P → ³B_M → ³Car. Since ³B_M has never been directly observed in carotenoid-containing reaction centers,⁷ it is generally assumed that the second step in this two-step mechanism is much faster than the first, that is, ³P → ³B_M is rate limiting and ³B_M does not accumulate to an appreciable extent.

For this two-step mechanism to be viable, the energy of ³P must be near to or higher than that of ³B_M. The energy difference between these two states can be estimated from spectral data. The energy of ³P can be measured directly in the RC from the phosphorescence spectrum;⁸ the energy of isolated ³Bchl *a* in an organic glass can also be measured by phosphorescence.⁹ Because it is not possible to observe ³B_M in situ, its energy was estimated starting with the absorption maximum of B_M in the RC and then subtracting half of the Stokes shift plus the difference in emission maxima between the Bchl fluorescence and phosphorescence in a glassy matrix. This led to the prediction that the energy of ³B_M is 200 ± 70 cm⁻¹ above that of ³P in wild-type RCs; this analysis is summarized in Table 1. Significantly, the ³P → ³Car process has been found to have an activation energy of approximately this magnitude,^{4,10} thus this two-step mechanism appears consistent with the energetics.⁹

Despite this excellent agreement, several approximations were

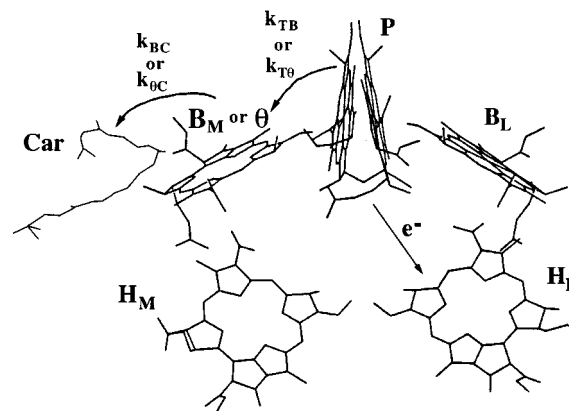


Figure 1. Arrangement of the chromophores involved in the initial electron and triplet energy transfer processes in photosynthetic reaction centers from the X-ray structure of wild type.^{35,36} In the mutant M182HL, the bacteriochlorophyll *a* in the B_M binding site is replaced by a bacteriopheophytin *a* and is designated θ .

TABLE 1: Information Used to Estimate the Activation Energy for ³P → ³B_M (or ³ θ) → ³Car Triplet Energy Transfer in the Reaction Center based on the Measured Phosphorescence Spectra of Bacteriochlorophyll *a* and Bacteriopheophytin *a*⁹ and ³P⁸

| | wild type bacterio- chlorophyll <i>a</i> | M182HL bacterio- pheophytin <i>a</i> |
|---------------------------------------|--|--|
| absorption in B _M site | 12450 ± 60 cm ⁻¹ | 12630 ± 30 cm ⁻¹ |
| – 1/2(Stokes shift) | 50 ± 20 cm ⁻¹ | 60 ± 20 cm ⁻¹ |
| – [ν _{max} (fluorescence) | 4610 ± 30 cm ⁻¹ | 4070 ± 30 cm ⁻¹ |
| – ν _{max} (phosphorescence)] | | |
| – ³ P phosphorescence | 7590 ± 20 cm ⁻¹ | 7590 ± 20 cm ⁻¹ |
| = est activation energy | 200 ± 70 cm ⁻¹ | 910 ± 50 cm ⁻¹ |

made in estimating the absolute energies of ³P and ³B_M. Ordinarily these approximations would be of no consequence; however, in a system where the energy difference is so small, it could be argued that the agreement between the observed activation energy and the estimate from the triplet energies is

fortuitous. For example, the Stokes shifts for both the singlet and triplet states in situ are not known and are assumed to have the same values as in vitro. Furthermore, we assumed that the spectral shift of the singlet state of Bchl in the B_M site to lower energy relative to its energy in solution also shifts the energy of its triplet state by an equal amount, that is, that the singlet-triplet splitting is constant and independent of environment.

In the course of other work, we began investigating a mutant, M182HL, which assembles with a Mg-free bacteriopheophytin *a* (Bpheo) in the B_M binding site in place of the native Bchl.^{11,12} We will designate a Bpheo in this site as θ . Because the phosphorescence from $^3\text{Bpheo}$ isolated in a glassy matrix has been measured,⁹ this mutant offers an independent and *quantitative* test for the two-step triplet energy transfer mechanism. As discussed below, an analysis parallel to that sketched above suggests that the activation energy for the rate-limiting $^3\text{P} \rightarrow ^3\theta$ step should be substantially higher than for $^3\text{P} \rightarrow ^3\text{B}_M$. Precisely this is observed, and the predicted activation energy agrees closely with the observed activation energy. We note that many experiments have demonstrated that the triplet energy transfer process is sensitive to changes in the B_M chromophore and in the environment around B_M ; however, as far as we know, in no case has there been a quantitative analysis of the energetics that permits a definitive test of the mechanism.^{10,13–15}

Experimental Section

The gene coding for the M182HL mutant of *Rb. sphaeroides* RCs was kindly provided by Professor Neal Woodbury, and we inserted this into a strain that produces RCs with a polyhistidine tag for rapid purification.¹⁶ RCs were suspended in 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 1.0 mM EDTA. Q_A was either removed or chemically reduced by the addition of sodium dithionite.¹⁷ For measurement of transient absorption kinetics, the sample was excited with a 532 nm actinic pulse from a Nd:YAG laser at 2 Hz repetition rate. The time dependence of the change in the Q_Y absorption of P was measured with a weak probe beam at 875 nm and detected with a fast photodiode and a digital oscilloscope. The sample temperature was controlled with two cascaded thermoelectric coolers (Melcor Corporation) driven by a variable current power supply. The thermoelectric coolers were themselves cooled with an ethylene glycol/water bath; this allowed for precise temperature control between room temperature and 230 K. For experiments below 230 K, the sample was cooled with a miniature Joule-Thompson refrigerator (MMR Technologies) with precise temperature control from 250 to 117 K. The effects of magnetic fields on the ^3P decay^{18,19} were measured using an electromagnet driven by a 1 kW current-regulated power supply, resulting in a variable magnetic field from 0 to 400 G.

Results

The absorption spectrum of the M182HL mutant is compared with wild-type in Figure 2. The peak assignments refer to the chromophore labels in Figure 1. The shoulder on the low-energy side of the 800 nm feature seen in wild-type is absent in M182HL, and a new peak appears on the high-energy side of the 800 nm feature.³⁷ We ascribe this shift to the formation of RCs in which the Bchl in the B_M binding site is replaced by Bpheo, as has been demonstrated in pigment analyses by Katilius et al.¹² Interestingly, the θ absorption is not blue-shifted as far as the Bpheo molecules in the H_L/H_M binding sites (see Figures 1 and 2) and the integrated intensity of the θ absorption is substantially larger than that of the Bpheo's in those sites. This result parallels that seen in the β mutant, M214LH, which

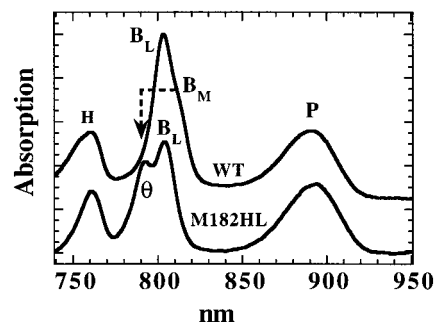


Figure 2. Electronic absorption spectra in the Q_Y region at 77K for *Rb. sphaeroides* wild type and M182HL reaction centers, normalized at 890 nm.

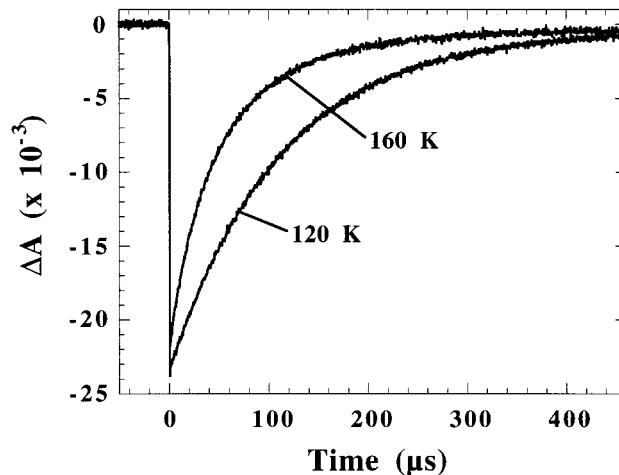


Figure 3. Decay of the bleach of P monitored at 875 nm following a subsaturating actinic flash at 532 nm. Raw data shown are for quinone-depleted M182HL reaction centers at 120 and 160 K. Note that the bleach for 160 K is significantly diminished relative to the bleach at 120 K, because of the temperature dependence of triplet formation. Since the P band undergoes a red-shift at low temperature, the absorbance at the probe wavelength (before arrival of the actinic pulse) is slightly greater (by 0.001) at 120 K than it is at 160 K. This does not measurably affect the bleach amplitudes. For this sample, $A_{532} = 0.12$, $A_{875} = 0.14$ at room temperature.

assembles with a Bchl molecule in the H_L binding site.²⁰ In that case, the peak position and intensity of the Bchl in the H_L binding site are more similar to the Bpheo's in the H_L/H_M binding sites and not to the Bchl's in the B_L/B_M binding sites.²¹ These observations are in contrast to *Chloroflexus aurantiacus* RCs, where the B_M site is occupied by a Bpheo whose absorption overlaps with the Bpheo's in the H site. Interestingly in that case there are additional amino acid changes in the vicinity of the B_M binding site. In both cases in *Rb. sphaeroides*, the properties of the chromophores appear to depend strongly on the site and less on the chemical identity. The origin of this interesting effect is not known.

Two typical ^3P decay curves for the quinone-depleted M182HL mutant are shown in Figure 3. These decays can each be fit well by a single exponential.²² The ^3P decay analysis was performed as a function of temperature, and the decay rates (k_{obs}) are plotted as an Arrhenius plot in Figure 4A. We assume that the temperature dependence of k_{obs} arises solely from a constant energy difference between ^3P and $^3\theta$, and is not complicated by temperature-dependent structural changes or entropy differences between the two states. This analysis also assumes that k_{TG} (see Figure 5) is temperature independent; it has been previously demonstrated in R-26 RCs that this is only weakly dependent on temperature.¹⁹ The small deviation between the

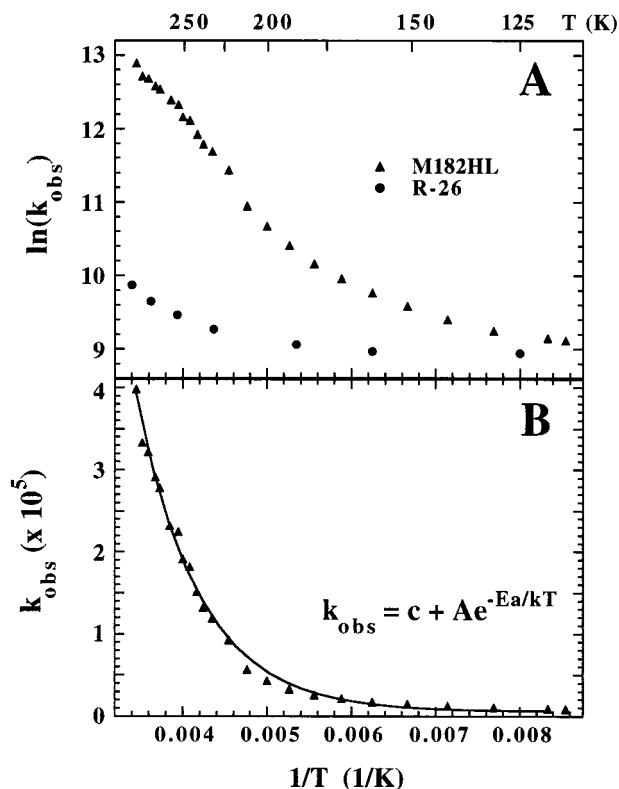


Figure 4. (A) Arrhenius plots of ^3P decay in quinone-depleted M182HL and quinone-depleted R-26 reaction centers. On this plot, $\ln(k_{\text{obs}})$ in wild-type (quinone-reduced, carotenoid-containing and with Bchl in the B_M binding site) RCs is approximately 16.5 and is independent of temperature over the range shown.¹⁴ (b) Quinone-depleted M182HL ^3P decay data along with a quantitative fit to the Arrhenius equation, where c is a nonactivated, low-temperature limit, accounting for k_{TG} (see Figure 5). Fit parameters: $c = 5760 \text{ s}^{-1}$; $A = 4.0 \times 10^7 \text{ s}^{-1}$; $E_a = 936 \text{ cm}^{-1}$.

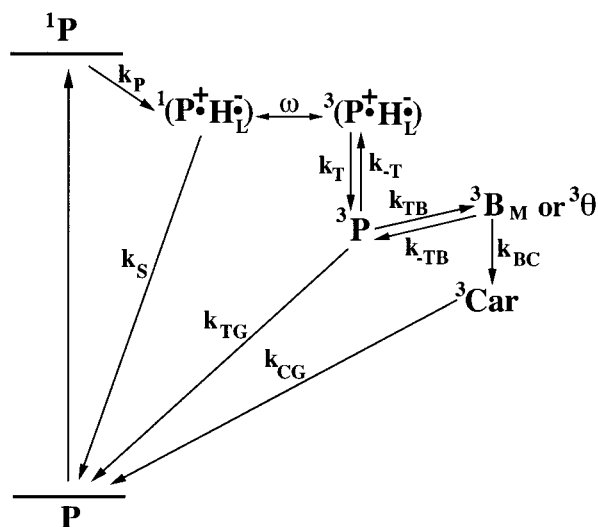


Figure 5. Reaction scheme describing the formation and decay of ^3P .

data and the fit in Figure 4B is possibly due to the limitations of the assumptions used in the data analysis. The quantitative analysis shown in Figure 4B yields an activation energy of 936 cm^{-1} in the M182HL mutant. Repeated data sets for M182HL ^3P decay gave a narrow range of activation energies, resulting in an average value of $950 \pm 50 \text{ cm}^{-1}$. It is evident from the data in Figure 4A that triplet decay is much faster in M182HL than in R-26 RCs, which lack carotenoid and thus cannot decay by triplet energy transfer.²³ The ^3P decay rate for carotenoid-

containing wild-type *Rb. sphaeroides* 2.4.1 RCs, $\ln(k_{\text{obs}}) \approx 16.5$ (not shown in Figure 4A as it is off scale) is much faster than for M182HL and is approximately independent of temperature over the range shown in Figure 4.^{4,14} We note that the preexponential, A, obtained from fitting our M182HL data agrees within a factor of 3 with the preexponential extracted from ^3P decay data from carotenoid containing wild-type reaction centers in a previous study.¹⁴ Considering the complexity of the system and the perturbation introduced by the θ chromophore, agreement within a factor of 3 between the two studies is reasonable.

Discussion

^3P is formed in the RC by $^3(\text{P}^+\text{H}_\text{L}^-)$ radical pair recombination according to the reaction scheme shown in Figure 5. Many of the rate constants have been worked out for this reaction scheme by several labs using the R-26 mutant.^{24–30} Relevant to this discussion are the decay processes available to ^3P , including activated reformation of $\text{P}^+\text{H}_\text{L}^-$, with rate constant $k_{-\text{T}}$,¹⁸ decay to the ground state by nonradiative decay with rate constant k_{TG} , and when carotenoid is present, energy transfer to the carotenoid with rate constant k_{TB} . In wild-type RCs, this latter process is completely dominant at temperatures above about 50 K, given the low activation energy for $^3\text{P} \rightarrow ^3\text{Car}$ energy transfer as described in the Introduction. Clearly the decay of ^3P is much slower in the M182HL mutant, but not as slow as in the carotenoidless R-26 mutant.

We can estimate the energy of the $^3\theta$ state in M182HL following exactly the same method used for the $^3\text{B}_\text{M}$ state in wild-type.⁹ The energy of ^3P is obtained in situ from the RC phosphorescence spectrum.⁸ The energy of monomeric $^3\text{Bp}^{\text{pheo}} a$ in an organic glass has been measured by phosphorescence.⁹ Because it is not possible to observe $^3\theta$ in situ by phosphorescence, its energy was estimated starting with the absorption maximum of θ in M182HL (Figure 2), and then subtracting half of the Bp^{pheo} Stokes shift plus the difference in emission maxima between the Bp^{pheo} fluorescence and phosphorescence in a glassy matrix. This analysis is shown in Table 1 for both wild-type and M182HL. The result is that the predicted activation energy for $^3\text{P} \rightarrow ^3\theta$ is $910 \pm 50 \text{ cm}^{-1}$. This value is in close agreement with the observed activation energy in the M182HL mutant. We stress that the same approximations have been made in estimating the energy of $^3\theta$ and $^3\text{B}_\text{M}$ in situ; however, the phosphorescence, absorption and kinetic data are independent of one another, so this is a rigorous check of the energy transfer mechanism.

In conclusion, the data presented here for M182HL are in quantitative agreement with two-step energy transfer from ^3P to the carotenoid via the triplet state of the occupant of the B_M site. It is interesting to compare this situation with the ongoing debate about the mechanism of primary electron transfer on the functional L-side, where a two-step mechanism, $^1\text{P} \rightarrow \text{P}^+\text{B}_\text{L}\text{H}_\text{L}^- \rightarrow \text{P}^+\text{B}_\text{L}\text{H}_\text{L}^-$, or a one-step superexchange mechanism may operate. This comparison is relevant because triplet energy transfer, operating by the Dexter mechanism, is closely related to conventional electron transfer. For triplet energy transfer, two electrons are, in effect transferred, and the reorganization energy is expected to be small, as there is little charge displacement.^{31,32} What is thus far missing in the debate about the electron-transfer mechanism is a quantitative prediction and observation of an activation energy based on experimental data in situ, analogous to what is described here for triplet energy transfer. Recent experiments that provide quantitative information on the energetics of intermediates in situ^{33,34} may make this possible.

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- (23) 3P decay in R-26 is due to activated reformation of $^3(P^+H_L^-)$ in competition with intersystem crossing and decay to the ground state; triplet energy transfer plays no role as R-26 lacks carotenoid. We note that the activation energies for magnetic field dependent 3P decay in R-26 to reform $^3(P^+H_L^-)$ (rate constant k_{-T} in Figure 5) and for magnetic field independent triplet energy transfer to θ in M182HL are coincidentally similar (Figure 4).
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