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Energetics of initial charge separation in bacterial photosynthesis: the triplet decay rate in very high magnetic fields

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The triplet state of the primary electron donor is formed in bacterial reaction centers when electron transfer to the quinone is blocked. The relative quantum yield of formation and the rate of decay of the triplet state were measured in quinone-depleted reaction centers from *Rhodobacter sphaeroides* as a function of temperature and magnetic field up to 135 kG. By analyzing these dependences we obtain a standard free-energy difference at room temperature between the radical pair intermediate and the triplet state of 1370 ± 30 cm^{-1} (0.170 ± 0.004 eV), and a standard enthalpy difference of 1450 ± 70 cm^{-1} (0.180 ± 0.009 eV). These results differ substantially from those obtained previously using lower fields. This difference is ascribed primarily to effects of nuclear spin polarization. In combination with the known energies of the excited singlet and triplet states of the primary electron donor, we calculate that the standard free-energy change for the initial charge separation reaction is 2120 cm^{-1} (0.263 eV) and that the entropy change is small.

Introduction

The initial electron transfer reaction in bacterial photosynthesis occurs between the photoexcited singlet state of the electron donor (denoted ^{1}P) and the electron acceptor (denoted I) within about 3 ps at room temperature [1,2], and about twice as fast at cryogenic temperatures [1,3]. Extensive spectroscopic studies [4] and analyses of the X-ray structures of reaction centers (RCs) from the photosynthetic bacteria *Rhodopseudomonas viridis* [5] and *Rhodobacter sphaeroides* [6-9] have identified the electron donor as a bacteriochlorophyll dimer, often called the special pair. The precise identity of the initial electron acceptor is less certain, but it is believed to be a bacteriopheophytin monomer [10]. The resulting radical pair state, $P^{\dot{+}}I^{\dot{-}}$, decays in about 200 ps as the electron moves from $I^{\dot{-}}$ to a quinone (denoted Q) [11,12]. When this latter reaction is blocked by removal or prior reduction of the quinone, the radical pair decays by charge recombination to re-form ^{1}PI, the ground state, or ^{3}PI, the excited triplet state of the donor (see Fig. 1).

The driving force for the initial electron transfer reaction is a question of considerable interest. Extensive theoretical studies and experimental tests with model systems [13] demonstrate a strong relationship between rate and driving force in charge transfer reactions. It is widely believed that studying this relationship can provide insight into the mechanism by which photosynthetic systems optimize the efficiency of forward reactions and minimize back reactions. In contrast to most model

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Abbreviations: I, primary electron acceptor; P, primary electron donor; Q, quinone; RC, reaction center; TT, 10 mM Tris/0.1% Triton X-100 (pH 8.0) buffer.

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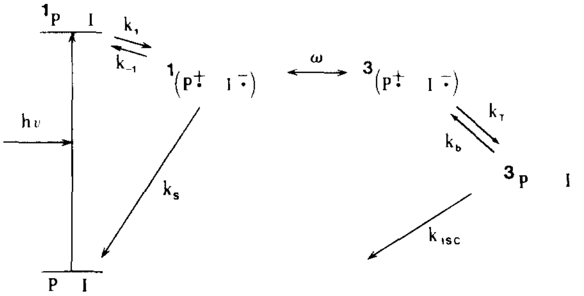

Fig. 1. Reaction scheme used to analyze reaction dynamics in RCs in which electron transfer to the quinone has been blocked.

electron transfer systems, where the driving force can be estimated accurately by measurements of the excitation energy and the reversible one-electron reduction potentials of the donor and acceptor, the energetics of the initial charge separation in RCs must be estimated by indirect methods. In this paper we consider the energy difference between the ^{3}PI and $P^{\dot{+}}I^{\dot{-}}$ states which, when combined with the energies of the ^{1}PI and ^{3}PI states, gives an estimate for the energetics of the initial charge separation reaction, $^1\mathrm{PI} \rightarrow P^{\dot{+}}I^{\dot{-}}$.

As shown in Fig. 1, in blocked RCs the radical pair is initially formed from ^{1}PI in a singlet spin configuration, denoted $^1(P^{\dot{+}}I^{\dot{-}})$, with rate constant k_1. $^1(P^{\dot{+}}I^{\dot{-}})$ can decay to the ground state, PI (rate constant k_S), reform ^{1}PI (rate constant k_{-1}), or undergo coherent electron spin evolution to form the triplet spin configuration of the radical pair $^3(P^{\dot{+}}I^{\dot{-}})$. $^3(P^{\dot{+}}I^{\dot{-}})$ either decays by recombination to generate the excited triplet state of P, ^{3}PI (rate constant, k_T), or it evolves back to $^1(P^{\dot{+}}I^{\dot{-}})$. ^{3}PI can decay by intersystem crossing (rate constant, k_{isc}) or reform $^3(P^{\dot{+}}I^{\dot{-}})$ (rate constant, k_b). The rate of interconversion between the singlet and triplet radical pair states is dependent on external magnetic field strength. At zero magnetic field, the nuclear hyperfine interaction causes interconversion of the singlet radical pair state and all three triplet radical pair states. When the magnetic field is increased from 0 to about 1 kG, the rate of interconversion decreases because of loss of the near degeneracy of the singlet and two of the triplet radical pair states. As the field is further increased the rate of interconversion increases as the g-factor difference between $P^{\dot{+}}$ and $I^{\dot{-}}$ starts to become the dominant mechanism for interconversion [14]. Because of the dependence of the singlet-triplet mixing rate on applied magnetic field, the quantum yield of formation and the decay kinetics of the states represented in Fig. 1 are affected by magnetic field strength [15].

We have shown that in quinone-depleted RCs from *Rb. sphaeroides* the observed rate of ^{3}PI decay (k_{obs}) depends on the strength of an applied magnetic field [16]. We interpreted this result to mean that ^{3}PI decay at room temperature proceeds largely through thermal reformation of $^3(P^{\dot{+}}I^{\dot{-}})$, spin evolution to $^1(P^{\dot{+}}I^{\dot{-}})$, and decay through the k_S path. Using the reaction scheme shown in Fig. 1 and making a series of assumptions about the temperature and magnetic field dependence of the rate constants and the rates of nuclear and electron spin-lattice relaxation (see below), we derived the expression:

$$k_{obs} = k_{isc} + \tfrac{1}{3} k_S \Phi_{^3\mathrm{PI}}\, e^{-\Delta G^0 \beta} \qquad (1)$$

where $\Phi_{^3\mathrm{PI}}$ is the magnetic-field dependent quantum yield of ^{3}PI formation, ΔG^0 is the standard free energy difference between the ^{3}PI and $^3(P^{\dot{+}}I^{\dot{-}})$ states, and $\beta = 1/kT$, where k is the Boltzmann constant and T is the absolute temperature. In the original derivation [16], entropy changes between the $^3(P^{\dot{+}}I^{\dot{-}})$ and ^{3}PI states were considered negligible and the free-energy difference was equated with the standard enthalpy difference ΔH^0; this assumption is not required in the present analysis.

Although Eqn. 1 is qualitatively followed, detailed measurements between 0 and 50 kG revealed discrepancies with the predictions of Eqn. 1 [16]: at room temperature, k_{obs} was observed not to vary linearly with $\Phi_{^3\mathrm{PI}}$. As has been explained in detail elsewhere [17], this deviation is likely due to nuclear spin polarization at low fields where the singlet-triplet mixing is dominated by nuclear hyperfine interactions. The presence of nuclear spin polarization effects can especially complicate the analysis of the temperature dependence of k_{obs}, since the nuclear spin relaxation rate is likely to be temperature dependent.

At very high magnetic fields, singlet-triplet mixing is dominated by the difference in precession rates of the two unpaired electrons (Δg effect) rather than by nuclear hyperfine interactions, and the reaction dynamics are insensitive to the effects of nuclear spin polarization. For this reason we have investigated the magnetic field and temperature dependence of k_{obs} for applied fields up to 135 kG. Our previous analysis indicated that nuclear spin polarization effects should not be significant at this field [17]. Our results demonstrate that k_{obs} does vary linearly with $\Phi_{^3PI}$ within experimental error at room temperature in the high field regime (magnetic field greater than 20 kG). The analysis of k_{obs} at high field gives a larger calculated value for the $^3(P^+I^-) - {}^3PI$ energy difference than obtained from earlier analyses using lower field data.

Experimental

Quinone-depleted RCs from *Rb. sphaeroides* R-26 mutant were prepared by standard procedures [18,19] and were suspended in aqueous buffer (10 mM Tris/0.1% (v/v) Triton X-100 (pH 8.0); TT buffer) or viscous buffer (glycerol/TT buffer (2:1, v/v) or ethylene glycol/TT buffer (2:1, v/v)). The sample was placed in a 3.3 mm pathlength variable-temperature cell (temperature control ±1° C, measured by a platinum resistance thermometer) with a total absorbance in the cell of approx. 0.3 at 860 nm at room temperature. The sample cell was placed in a split-bore superconducting magnet (magnetic field accuracy ±1 kG) at the Francis Bitter National Magnet Laboratory. Zero-field values were obtained with the magnet quenched (residual magnetic field of less than 1 G).

The relative concentration of ^{3}PI was monitored by observing the change in ground-state absorption at 868 nm following subsaturating 8 ns excitation flashes (532 nm, 10 Hz, less than 1.0 mJ/cm^2 per pulse). Data were taken with the polarization of the probe beam parallel, perpendicular, and at the magic angle to the magnetic field direction. The data reported represent measurements with magic angle polarization unless otherwise indicated. The observed absorbance change was linear with excitation power and independent of probe light intensity. Relative quantum yields and decay rates of ^{3}PI were calculated by fitting the time evolution of the absorbance change to a single exponential plus a small baseline and to a gaussian distribution of exponentials plus a small baseline [20]. Triplet quantum yield anisotropies were calculated as described elsewhere [21].

Results

The values of k_{obs} obtained by averaging the decay rates over distributions of exponentials were generally slightly higher (approx. 10%) than those obtained by fits to single exponentials. The second moment of the distribution in k_{obs} was, at all fields and temperatures, approx. 30% of the average value. The fits to a distribution were significantly better and resulted in a smaller error in resulting calculated parameters; the values of k_{obs} thus obtained were used in the following analysis. Both sets of k_{obs} values yielded essentially identical values for ΔH^0 and ΔG^0. This heterogeneity in observed decay rates will be discussed further below.

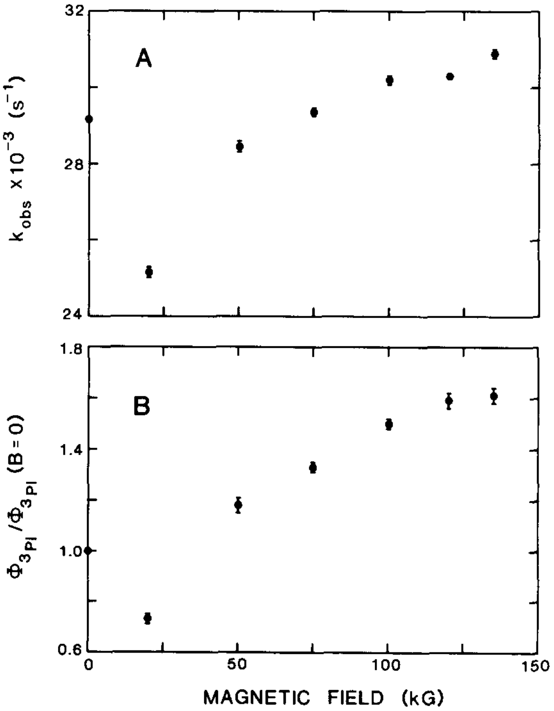

Fig. 2. Dependence of (A) k_{obs} and (B) $\Phi_{^3PI}/\Phi_{^3PI}(B=0)$ on magnetic field between 0 and 135 kG at 15 ° C for quinone-depleted RCs in aqueous buffer, with probe beam polarized at the magic angle with respect to the applied magnetic field. k_{obs} is the observed rate of ^{3}PI decay, $\Phi_{^3PI}$ is the quantum yield of ^{3}PI formation, and $\Phi_{^3PI}(B=0)$ is the ^{3}PI quantum yield at zero applied field.

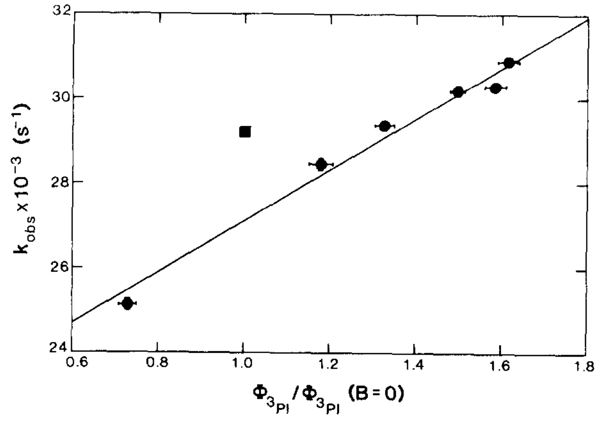

Fig. 3. k_{obs} plotted as a function of relative triplet yield at 15 ° C from 0 G to 135 kG. The sample was in aqueous buffer. The data from 20 kG to 135 kG (●) are fit to a straight line with intercept $21.09 \cdot 10^3$ s^{-1} and slope $6.02 \cdot 10^3$ s^{-1}; the datum at 0 G (■) deviates significantly from this line.

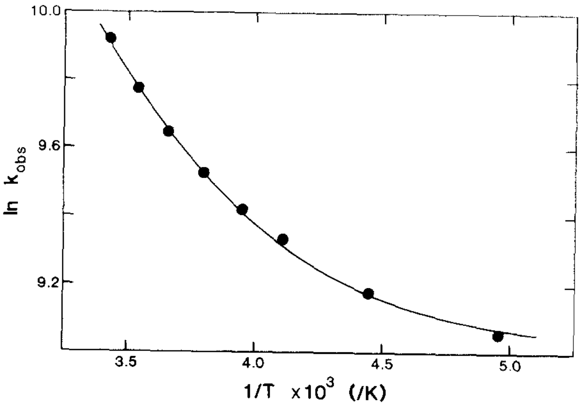

Fig. 4. Temperature dependence of k_{obs} at 135 kG for RCs in viscous buffer. Experimental errors were smaller than the size of the symbols. The probe beam polarization was at the magic angle with respect to the magnetic field direction. The solid line is a fit to an Arrhenius expression plus constant with a low-temperature asymptote of $8.2 \cdot 10^3$ s^{-1}, a slope of 1450 cm^{-1}, and a pre-exponential of $15 \cdot 10^6$ s^{-1}.

Values of k_{obs} and the quantum yield of ^{3}PI normalized to the yield at zero field as a function of magnetic field strength at room temperature in TT buffer are presented in Fig. 2. At higher magnetic fields, k_{obs} parallels $\Phi_{^3PI}$ as a function of applied field, in agreement with Eqn. 1. This is shown clearly in Fig. 3, where the observed triplet decay rate is plotted vs. relative triplet yield. The high field data ($\geq$ 20 kG) were fit to a straight line with y-intercept = $(21.09 \pm 0.07) \cdot 10^3$ s^{-1} and slope = $(6.02 \pm 0.06) \cdot 10^3$ s^{-1}. The datum at zero field deviates significantly from the fit. Similar data for an ethylene glycol sample obtained at 20 ° C at fields up to 37.5 kG were also fit to a straight line with y-intercept $(10.60 \pm 0.10) \cdot 10^3$ s^{-1} and slope $(5.70 \pm 0.14) \cdot 10^3$ s^{-1} (data not shown). The data at zero field again deviated from the fit. The temperature dependence of k_{obs}, measured with a glycerol buffer sample at an applied field of 135 kG, is shown in Fig. 4; at room temperature, $k_{obs} = (20.3 \pm 0.1) \cdot 10^3$ s^{-1}. The data were fit to an Arrhenius expression plus a constant with a low-temperature asymptote of $(8.2 \pm 0.2) \cdot 10^3$ s^{-1}, a slope of 1450 ± 70 cm^{-1}, and a pre-exponential of $(15 \pm 5) \cdot 10^6$ s^{-1}. The large difference in ^{3}PI decay rates between samples in aqueous and viscous solution has been noted previously [16]. k_{obs} as a function of temperature at 135 kG with the probe beam polarization parallel and perpendicular to the magnetic field direction is compared with magic angle data in Fig. 5. Note the large anisotropy in k_{obs} at intermediate temperatures. The anisotropy of the ^{3}PI yield was measured at − 20 ° C in viscous buffer. The anisotropy was independent of magnetic field within experimental error above 50 kG, with a value at 135 kG of -0.10 ± 0.01 (data not shown).

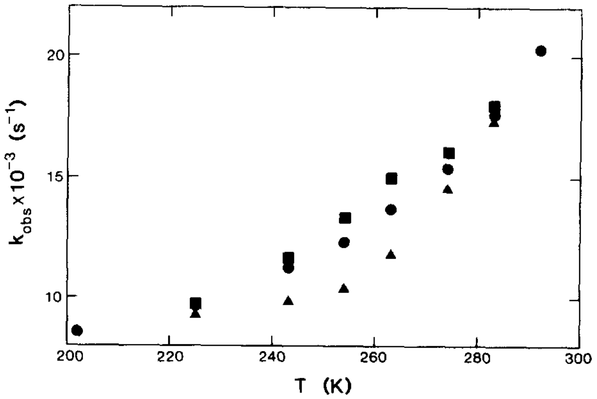

Fig. 5. Temperature dependence of k_{obs} at 135 kG for RCs in viscous buffer, as observed with the probe light polarization perpendicular (■), parallel (▲), and at the magic angle (●) to the magnetic field. Experimental errors were smaller than the size of the symbols. Data at 202 K and 292 K are not resolvable within the experimental error. Note that the anisotropy in k_{obs} goes to zero at low temperatures where ^{3}PI decay is via the magnetic field independent k_{isc} path, and at higher temperatures as the RC rotational correlation time becomes short relative to the ^{3}PI lifetime.

Discussion

Approaches to data analysis

Eqn. 1 provides two methods for obtaining the $^3(P^+I^-) - {}^3PI$ energy difference. If k_{obs} as a function of relative ^3PI quantum yield is fit to a straight line, the y intercept is equal to k_{isc}, while the slope is $\frac{1}{3}k_S\ \Phi_{^3PI}(B=0)\ e^{-\Delta G^0\beta}$, where $\Phi_{^3PI}(B=0)$ is the quantum yield of ^3PI at zero applied field (the field strength is symbolized with a B instead of H to avoid confusion with the enthalpy). k_S and $\Phi_{^3PI}(B=0)$ have been measured previously; substitution allows calculation of ΔG^0.

Alternatively, Eqn. 1 can be rewritten as an Arrhenius-type expression in the form

$$k_{obs} = k_{isc} + \alpha\ e^{-\Delta H^0\beta} \qquad (2)$$

where

$$\alpha = \tfrac{1}{3}k_S\Phi_{^3PI}\ e^{\Delta S^0/k} \qquad (3)$$

and ΔS^0 is the standard entropy difference between $^3(P^+I^-)$ and ^3PI. If α and k_{isc} are assumed to be temperature independent, then fitting the temperature dependence of k_{obs} to Eqn. 2 yields ΔH^0; the small temperature dependence of α and k_{isc} are discussed further below. Comparison of the derived values for ΔH^0 and ΔG^0 yields ΔS^0.

Assumptions behind Eqn. 1

A series of assumptions is involved in the derivation of Eqn. 1. Three assumptions in particular bear close scrutiny. The first assumption is that the scheme in Fig. 1 is correct. The consequences of possible modifications of the reaction scheme are discussed briefly below and will be discussed in much greater detail elsewhere in the analysis of the ^3PI quantum yield at very high fields. The second assumption is that nuclear spin-lattice relaxation in ^3PI is fast relative to k_{obs}, so the nuclear spins in ^3PI are always at equilibrium. We believe that this assumption is not valid at low fields, and that this explains the discrepancy noted in the introduction. A third assumption is that the RC rotational correlation time is short relative to the ^3PI lifetime so that anisotropic interactions can be neglected. We have shown previously that RCs rotate rapidly relative to k_{obs} in aqueous solution at room temperature [21], but this is not the case in viscous solutions at low temperature.

Nuclear spin polarization

A quantitative analysis of the effect of nuclear spin polarization on the magnetic field dependence of k_{obs} has been presented earlier [17]. The hyperfine-induced singlet-triplet mixing in the P^+I^- state at low field, which leads to formation of ^3PI, enriches ^3PI in nuclear spin states which generate the largest hyperfine fields (nuclear spin polarization). Subsequent decay of ^3PI, again via the singlet-triplet mixing process, will then be faster than if the nuclear spins in ^3PI were at thermal equilibrium. The result is a higher value of k_{obs} than predicted by Eqn. 1, at temperatures at which the radical-ion pair is thermally accessible from ^3PI.

The degree of nuclear spin polarization will change with temperature due to the temperature dependence of the nuclear spin relaxation rate. As the temperature of the sample is lowered, the nuclear spin relaxation rate of ^3PI will decrease; as a result, the assumption of fast spin relaxation will become worse at lower temperatures, increasing the degree of nuclear spin polarization. In addition, nuclear spin polarization effects will go away at low temperature as the quantum yield of ^3PI approaches unity [23] and the probability of the triplet state being formed becomes independent of nuclear spin state. It is not clear which of these two effects will dominate. Since the spin polarization increases the value of k_{obs} above that expected for no spin polarization, if this polarization increases as the temperature is lowered, k_{obs} will decrease more slowly with decreasing temperature than predicted by Eqn. 2. If k_{obs} is fit to Eqn. 2, the fit value of ΔH^0 will be lower than the actual enthalpy difference between $^3(P^+I^-)$ and ^3PI. Conversely, if spin polarization decreases with temperature, the effect will increase the fit value of ΔH^0. In order to distinguish the slope of the Arrhenius plot from the true ΔH^0, we denote the experimental slope ΔH^0_{app} or apparent enthalpy change. As mentioned above, the nuclear spin polarization effects can be avoided by performing the measurements in the high field regime.

Anisotropic interactions

The spin evolution of $P^{\ddagger}I^{\overline{\cdot}}$ is influenced by anisotropic nuclear hyperfine, electron–electron dipole–dipole and electronic spin–orbit (g-tensor) interactions [21]. As a result, the rate of singlet-triplet mixing for a particular RC depends upon its orientation with respect to the external magnetic field. In samples with distributions of orientations, ^{3}PI is formed preferentially in RCs with orientations that favor singlet-triplet mixing. If this orientation is preserved during the decay of the ^{3}PI state, i.e., the rotational correlation time is long relative to the ^{3}PI lifetime, k_{obs} will be larger than predicted by Eqn. 1. This effect will vanish at lower temperatures where the ^{3}PI quantum yield approaches unity and the ^{3}PI decay proceeds only through k_{isc}.

The rotational correlation time can be monitored by observing the ^{3}PI quantum yield anisotropy decay. The assumption of rapid RC rotation is valid at room temperature in both aqueous and viscous buffer, but becomes invalid in viscous buffer as the temperature of the sample decreases below 270 K. A breakdown in this assumption will cause an increase in k_{obs} above the value calculated from Eqn. 1 in a manner similar to the consequences of slow nuclear spin relaxation. As discussed above for the case of nuclear spin polarization, the temperature dependence of the anisotropy will cause the analysis of the temperature dependence of k_{obs} to underestimate ΔH^0. Unfortunately, these effects cannot easily be avoided by going to higher magnetic field. At infinite field the singlet-triplet mixing is so rapid for all RC orientations that $\Phi_{^3\mathrm{PI}}$ and k_{obs} should become independent of field strength and orientation. However, we have found that $\Phi_{^3\mathrm{PI}}$ is still anisotropic at 135 kG in viscous buffer (anisotropy $= -0.10 \pm 0.01$), and the data in Fig. 5 show that observed values of k_{obs} at 135 kG are likewise dependent on orientation.

Analysis of the room-temperature data: determination of ΔG^0

The assumption of rapid rotation is valid for the data represented in Fig. 3. The fact that a straight line fits the data, including at very high field where nuclear spin polarization effects are not important, indicates that spin polarization effects are not significant down to 20 kG at room temperature in aqueous buffer. The high value of k_{obs} at zero magnetic field is consistent with nuclear spin polarization effects at this low field. Since the nuclear spin relaxation is likely mediated by RC rotation, nuclear spin polarization effects may still be present at higher fields at lower temperature or in viscous solution. The slope of the fit line ($(6.02 \pm 0.06) \cdot 10^3\ \mathrm{s}^{-1}$) is equal to $\frac{1}{3}k_{\mathrm{S}}$ $\Phi_{^3\mathrm{PI}}(B=0)\ \mathrm{e}^{-\Delta G^0 \beta}$; this number can be combined with values of k_{S} ($(4.9 \pm 0.4) \cdot 10^7\ \mathrm{s}^{-1}$) and $\Phi_{^3\mathrm{PI}}(B=0)$ (0.32 ± 0.04) obtained from independent measurements [24] to yield $\Delta G^0 = 1370 \pm 30$ cm^{-1}. The y-intercept of the fit line is equal to k_{isc}: $k_{\mathrm{isc}} = (21.09 \pm 0.07) \cdot 10^3\ \mathrm{s}^{-1}$ for an aqueous sample at 15 °C.

ΔG^0 was calculated from the viscous sample data in the same manner as for the aqueous sample data. At room temperature the assumption of rapid RC rotation is valid (Fig. 5). While nuclear spin polarization effects may be present at fields up to 37.5 kG, the fact that all of the non-zero field values of k_{obs} as a function of $\Phi_{^3\mathrm{PI}}$ fit a straight line suggests that such effects are small. Assuming that k_{S} and $\Phi_{^3\mathrm{PI}}(B=0)$ are independent of sample viscosity, the slope of this fit line ($(5.70 \pm 0.14) \cdot 10^3\ \mathrm{s}^{-1}$) gives a value of $\Delta G^0 = 1390 \pm 30$ cm^{-1}, in close agreement with the value of $\Delta G^0 = 1370 \pm 30$ cm^{-1} calculated with the aqueous sample data. The fact that the two values of ΔG^0 closely agree indicates that the radical pair reaction energetics do not depend upon the viscosity of the sample; the difference in the observed ^{3}PI decay rates between aqueous and viscous samples can be explained by the difference in k_{isc}: $k_{\mathrm{isc}}(\text{aqueous sample}) = (21.09 \pm 0.07) \cdot 10^3\ \mathrm{s}^{-1}$, $k_{\mathrm{isc}}(\text{viscous sample}) = (10.60 \pm 0.10) \cdot 10^3\ \mathrm{s}^{-1}$. Because the calculation of ΔG^0 using the viscous sample data requires assumptions not required of the calculation using the aqueous sample data, the latter will be used below.

Analysis of the temperature dependence of k_{obs}: estimation of ΔH^0

Assuming for the moment that only the exponential term in Eqn. 2 is temperature dependent, the slope of the Arrhenius curve of k_{obs} at any magnetic field should yield the enthalpy difference between $^3(\mathrm{P}^{+}\mathrm{I}^{-})$ and $^3\mathrm{PI}$. Prior analysis of data obtained at zero and moderate fields (less than 50 kG) using this assumption yielded a calculated enthalpy difference of $\Delta H^0 = 950 \pm 50$ cm^{-1} [16]. As discussed above, this result could be affected by nuclear spin polarization effects. These effects can be avoided by measuring k_{obs} at very high magnetic field where the Δg effect dominates singlet-triplet mixing in the radical pair state. Our analysis predicts that the value of $\Delta H^0_{\mathrm{app}}$ obtained at high field may be different from that derived at zero field. This prediction is born out by the results presented in Fig. 4, where the data were fit to Eqn. 2 using a least-squares fit. The value obtained for k_{isc} obtained from the low temperature asymptote at 135 kG is $(8.2 \pm 0.2) \cdot 10^3$ s^{-1}, in good agreement with the value obtained previously at low field with a viscous sample ($7.0 \cdot 10^3$ s^{-1}) [16]. The fit value of ΔH^0 is 1450 ± 70 cm^{-1}, significantly higher than the value of 950 ± 50 cm^{-1} obtained at lower fields [16]. Because of the effects of nuclear spin polarization, the value of $\Delta H^0_{\mathrm{app}}$ obtained at 135 kG is likely most representative of the actual $^3(\mathrm{P}^{+}\mathrm{I}^{-}) - {}^3\mathrm{PI}$ enthalpy difference.

In the preceding analysis of the temperature dependence of k_{obs}, it was assumed that k_{S}, $\Phi_{^3\mathrm{PI}}$, and k_{isc} are temperature independent. It is known that k_{S} and $\Phi_{^3\mathrm{PI}}$ depend on temperature, k_{S} decreasing by about a factor of 4 as the temperature is lowered to 200 K [25], while $\Phi_{^3\mathrm{PI}}$ increases by about a factor of 3 [26]. The two contrasting temperature dependences at least partially offset each other, since the two terms enter Eqn. 1 as a product. If we separate out the temperature dependence of α and write:

$$\alpha = \zeta(B)\,\mathrm{e}^{-\Lambda\beta} \tag{4}$$

Eqn. 2 then becomes

$$k_{\mathrm{obs}} = k_{\mathrm{isc}} + \zeta(B)\,\mathrm{e}^{-(\Delta H^0 + \Lambda)\beta} \tag{5}$$

Schenck et al. [18] measured $\Phi_{^3\mathrm{PI}}$ as a function of magnetic field and temperature in RCs where electron transfer from I^{-} to the quinone had been blocked by prior reduction of the quinone. k_{S} can be obtained from our analysis of the radical pair lifetime data [24]. The product of k_{S} and $\Phi_{^3\mathrm{PI}}$ can be fit to an Arrhenius curve with $\Lambda = 120$ cm^{-1} at zero applied field over the temperature range of interest. While this measurement is for a different sample at a different applied field, it indicates the likely magnitude of Λ. Since the slope of the Arrhenius curve is equal to the sum of ΔH^0 and Λ, a value of $\Lambda = 120$ cm^{-1} would decrease the obtained value of $\Delta H^0_{\mathrm{app}}$ to 1330 cm^{-1}. Because $\Phi_{^3\mathrm{PI}}$ depends in a complex way on temperature and magnetic field, Λ will in principle be magnetic field dependent. Separating this effect from the nuclear spin polarization effect would require more knowledge of the temperature dependence of $\Phi_{^3\mathrm{PI}}$ as a function of magnetic field.

A temperature dependence to k_{isc} in Eqn. 5 would introduce another source of error in the calculation of ΔH^0. In fact, the value of k_{isc} at room temperature in viscous buffer calculated from the magnetic field dependence of k_{obs} ($(10.60 \pm 0.10) \cdot 10^3$ s^{-1}) does differ slightly from the low temperature asymptote of Fig. 4 ($(8.2 \pm 0.2) \cdot 10^3$ s^{-1}). We can model this weak temperature dependence of k_{isc} with an Arrhenius expression with activation energy $\Delta H^0_{\mathrm{isc}} = 20 \pm 10$ cm^{-1}. This value is consistent with the slight temperature dependence of k_{isc} observed previously at temperatures below 200 K [16]. Accounting for the temperature dependence of k_{isc} in this way causes a small increase in $\Delta H^0_{\mathrm{app}}$ of 100 cm^{-1}.

The presence of anisotropic interactions will cause the temperature dependence of k_{obs} to underestimate ΔH^0 due to the temperature dependence of the rotational correlation time. Since the change from rapid to slow RC rotation relative to k_{obs} occurs over the narrow temperature range from 290 K to 270 K in glycerol/water mixtures, the fact that k_{obs} fits an Arrhenius expression over the whole temperature range of the experiment indicates that anisotropic interactions do not cause a significant perturbation.

The factors discussed above introduce only minor and somewhat offsetting corrections into the value of ΔH^0 derived from the data in Fig. 4. Because these corrections are small and the exact values are unknown, we will use the uncorrected value of $\Delta H^0_{\mathrm{app}}$ in further discussion. The similarity between the value of ΔH^0 obtained from the slope of the Arrhenius plot at 135 kG and the values of ΔG^0 obtained by substitution into Eqn. 1 indicates that the entropy change between $^3(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ and $^3\mathrm{PI}$ is small.

Heterogeneity of ^3PI decay rates

As discussed above, the $^3\mathrm{PI}$ decay process exhibits a measurable heterogeneity in decay rates. The fits to a gaussian distribution of exponentials showed a reduced χ^2 (χ^2 divided by the number of degrees of freedom) significantly lower (average value = 1.0) than the reduced χ^2 for fits to single exponentials (average value = 1.4; with approx. 62 degrees of freedom, this represents a change of about 25 standard deviations.) In contrast, simulated single exponential decays with normally distributed noise showed best fits to sharp distributions of exponential decay rates (second moment less than 0.07 of the average value), insignificantly better than fits to single exponentials. A distribution of decay rates is expected due to the distribution of orientations and nuclear spin states in the sample; under conditions when the RC orientation or nuclear spin state is preserved during the lifetime of $^3\mathrm{PI}$, RCs with orientations and nuclear spin states that favor singlet-triplet mixing will have a faster rate of $^3\mathrm{PI}$ decay. The fact that the second moment of the distribution is an almost constant fraction of the average value, even at low temperatures where the radical pair state is thermally inaccessible and decay is only through k_{isc}, in aqueous solution where RC rotation is rapid, and at high field where nuclear polarization is not important, indicates that there are other sources of heterogeneity in the sample large enough to obscure any temperature or magnetic field dependent heterogeneity. There is precedence for non-exponential decay kinetics in these systems: a distribution in reaction rates for $\mathrm{P}^{\dot{+}}\mathrm{IQ}^{\dot{-}} \rightarrow \mathrm{PIQ}$ has been observed previously in quinone-containing RCs at low temperatures [27]; this distribution becomes wider when the RCs are frozen under illumination [28].

Initial charge separation energetics

The energy of the $^1\mathrm{PI}$ state is known quite accurately from absorption and fluorescence spectra to be 11 220 cm^{-1} above the ground state [29]. The energy of the $^3\mathrm{PI}$ state has been recently measured by observing the phosphorescence from this state; based on reasonable assumptions about the $^3\mathrm{P}$ Stokes shift, it is calculated to be 7730 cm^{-1} above the ground state [30]. There is little information available about the entropy changes involved in these transitions; however, since as observed above, the re-formation of the dipolar radical pair state from $^3\mathrm{PI}$ involves only a small entropy change, it is reasonable to assume entropy changes accompanying changes in the electronic state of P would be small. There would be a slight reduction of the free energy of $^3\mathrm{PI}$ of $kT \ln 3$ relative to its energy, due to the spin multiplicity of the triplet state. This would reduce the free energy of $^3\mathrm{PI}$ at room temperature to 7500 cm^{-1} above the ground state. The same spin multiplicity factor would increase the free energy of $^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ to 230 cm^{-1} above $^3(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$. Using the aqueous sample value of $\Delta G^0 = 1370 \pm 30$ cm^{-1}, these factors give a free energy for $^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ of 9100 cm^{-1} above the ground state at room temperature. Combined with the free energy of $^1\mathrm{PI}$ obtained from the absorption and emission measurements, this results in a free energy difference of 2120 cm^{-1} (0.263 eV) for the initial $^1\mathrm{PI} \rightarrow {}^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ electron transfer step ($\Delta G^0_{^1\mathrm{PI}\rightarrow{}^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})}$).

Likewise, combining the energies of the $^1\mathrm{PI}$ and $^3\mathrm{PI}$ states with the estimate of ΔH^0 obtained here of 1450 ± 70 cm^{-1} yields an enthalpy difference of 2040 cm^{-1} (0.253 eV) between $^1\mathrm{PI}$ and $^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ ($\Delta H^0_{^1\mathrm{PI}\rightarrow{}^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})}$). Comparisons of this value with $\Delta G^0_{^1\mathrm{PI}\rightarrow{}^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})} = 2120$ cm^{-1} derived above indicates only a small change in entropy accompagnies the initial charge transfer step.

Comparison with other methods

Two other methods have been used to determine the $^1\mathrm{PI} - {}^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ energy difference: measurements of the ambient redox potential at which reactions are blocked by prior reduction or oxidation of reactants and measurements of delayed fluorescence due to back electron transfer from $^1(\mathrm{P}^{\dot{+}}\mathrm{I}^{\dot{-}})$ to $^1\mathrm{PI}$. P can be oxidized in the dark with a midpoint redox potential of approx. + 450 mV vs. NHE [31]. The reduction potential of I in *Rb. sphaeroides* RCs has not been measured to the best of our knowledge. The reduction potential of bacteriopheophytin *a* in organic solvents is − 550 mV vs. NHE [32]. Combining these numbers with the energy of the ^{1}PI state above the ground state (11220 $cm^{-1} = 1.39$ eV) gives a value of $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})}$ of 0.39 eV, or 3150 cm^{-1}. This type of analysis suffers from three problems. First, the redox potentials of P and I are affected by the charge of the other; the free energy of $P^{\dot{+}}I$, as measured by redox titration, may not be simply related to the free energy of $P^{\dot{+}}I^{\dot{-}}$. Second, the redox potential of the bacteriopheophytin may be affected by the protein environment. Third, the redox states probed by redox titration are equilibrium states of the RC. It is not clear how these states compare to the transient states formed during RC photochemistry.

The principle of the delayed fluorescence measurements is that the initial amplitude of the delayed fluorescence relative to the amplitude of the prompt fluorescence is a measure of the relative concentrations of the first intermediate state in the reaction scheme and the fluorescing state, assumed to be the $^1(P^{\dot{+}}I^{\dot{-}})$ state and the ^{1}PI state, respectively. With the reasonable assumption that the ^{1}P and $^1(P^{\dot{+}}I^{\dot{-}})$ states are in thermal equilibrium several hundred ps after excitation, the equilibrium constant for the $^1PI \rightleftarrows {^1(P^{\dot{+}}I^{\dot{-}})}$ reaction can be obtained giving $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})}$. Woodbury and Parson [33] obtained the result that for Q^- containing *Rb. sphaeroides* RCs $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})}$ equals 1370 cm^{-1} at room temperature and decreases to 400 cm^{-1} at 100 K. Similar results were also obtained for quinone-depleted RCs [34]. Possible reasons for the discrepancy between the delayed fluorescence result and the conclusions of our work are discussed below.

Effect of modification of the reaction scheme

As discussed above, a major assumption of this analysis is that the reaction scheme illustrated in Fig. 1 is correct. A variety of modifications have been proposed by a number of authors. For instance, Woodbury and Parson [33], in their analysis of the delayed fluorescence measurements, explained their observation of non-exponential decay kinetics by proposing that the initial state formed by decay of ^{1}PI relaxes by hundreds of cm^{-1} through a series of intermediate states during the first few ns. There are other indications of the existence of intermediate states en route to formation of $^1(P^{\dot{+}}I^{\dot{-}})$. Results from Reaction Yield Detected Magnetic Resonance (RYDMR) experiments and the magnetic field dependence of $\Phi_{^3PI}$ indicate that the state that undergoes singlet-triplet mixing cannot be within 1370 cm^{-1} of ^{1}PI at room temperature. These experiments are sensitive to dephasing processes that interrupt the singlet-triplet spin evolution in the radical pair state, such as reformation of ^{1}PI, and can be used to put limits on k_{-1}. The width of the RYDMR resonance indicates that $k_S + k_T + k_{-1} < (0.4\text{–}7.0) \cdot 10^8\ s^{-1}$ [22,35]. This width is relatively insensitive to temperature [22]. Assuming that $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})}$ is mostly enthalpic, k_{-1} should show a strong temperature dependence. The temperature independence of the RYDMR linewidth then argues that k_{-1} must be relatively small compared with k_S and k_T. A value of $k_{-1} < 1.0 \cdot 10^8\ s^{-1}$ combined with the observed value of $k_1 = 3.0 \cdot 10^{11}\ s^{-1}$ [1–3] requires that $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})}$ at room temperature be greater than 1700 cm^{-1}. Analysis of the magnetic field dependence of the ^{3}PI quantum yield gives similar results. Comparison of these results with the results from the delayed fluorescence measurements indicate that the state formed within a few ps is not the state that undergoes singlet-triplet mixing, but rather is an intermediate state en route to the formation of $^1(P^{\dot{+}}I^{\dot{-}})$. A difference between the radical pair state probed by the delayed fluorescence measurement on the ns time scale and the radical pair state repopulated by ^{3}PI on the μs time scale could explain the discrepancy between the values of $\Delta G^0_{^1PI \to ^1(P^{\dot{+}}I^{\dot{-}})} = 1370\ cm^{-1}$ calculated by Woodbury and Parson and 2120 cm^{-1} calculated here. The effect of intermediate states in the initial electron transfer process on the reaction dynamics has important consequences for the reaction mechanism and the analysis of the magnetic field dependence of $\Phi_{^3PI}$ (Goldstein, R.A. and Boxer, S.G., unpublished results).

Schenck et al. [18] and Chidsey et al. [24] proposed that a fraction of $^3(P^{\dot{+}}I^{\dot{-}})$ might decay to the ground state without forming ^{3}PI. This path would lead to a temperature-dependent, but field-independent component to k_{obs}. As we will discuss elsewhere in detail, no set of rate constants can be found that match experimental values of k_{obs}, $\Phi_{^3PI}$, the RYDMR linewidth, and the radical pair lifetime with such a branching in the decay of

$^3(P^+I^-)$. Moreover, the experimental observations that led Schenck et al. [18] and Chidsey et al. [24] to propose such a branching path can be explained by the existence of an intermediate state en route to $^1(P^+I^-)$ formation (Goldstein, R.A. and Boxer, S.G., unpublished results).

Conclusion

We have obtained information on the energy difference between the radical pair and ^{3}PI states in quinone-depleted *Rb. sphaeroides* RCs by three methods: the magnetic field dependence of the triplet decay rate and the quantum yield of ^{3}PI formation in an aqueous sample at room temperature and moderate to very high field ($\Delta G^0 = 1370 \pm 30$ cm^{-1}); the magnetic field dependence in a viscous sample at room temperature and moderate to high field ($\Delta G^0 = 1390 \pm 30$ cm^{-1}); and the temperature dependence of k_{obs} at 135 kG in a viscous sample ($\Delta H^0 = 1450 \pm 70$ cm^{-1}). Combining the results of the first and third methods with values for the energies of the ^{1}PI and ^{3}PI states obtained from absorption and emission measurements indicates that the initial electron-transfer reaction, $^1\mathrm{PI} \rightarrow {^1(P^+I^-)}$ has a driving force of approx. 2120 cm^{-1} (0.263 eV) with little change in entropy. This conclusion is in contrast to those based on previous measurements of delayed fluorescence [33]. Because we can find no obvious fault with the delayed fluorescence measurements, we are forced to conclude that the radical pair intermediate being monitored during the decay of the triplet state is different from that being sampled in the delayed fluorescence measurements. The present experiment cannot distinguish whether the difference is due to a shift in the energy of the P^+I^- ion-pair state with time (called nuclear relaxation in Ref. 33, not to be confused with nuclear spin relaxation), or whether the states are actually chemically distinct species. We note that the energy of the radical pair obtained in our measurements is consistent with the energy of the intermediate needed to explain the temperature dependence of the decay of the P^+Q^- state in RCs substituted with different quinones [34].

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