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Temperature Dependence of the Reorganization Energy for Charge Recombination in the Reaction Center from *Rhodobacter sphaeroides*†

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ABSTRACT: The rate of charge recombination from the primary quinone to the bacteriochlorophyll dimer of the reaction center from the photosynthetic purple bacterium *Rhodobacter sphaeroides* has been investigated using time-resolved optical spectroscopy. Measurements were performed at temperatures from 293 to 10 K on reaction centers that have specific mutations that result in a range of 425−780 meV for the free energy difference of charge recombination compared to 520 meV for wild type [Lin, X., Murchison, H. A., Nagarajan, V., Parson, W. W., Allen, J. P., & Williams, J. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 10265−10269]. In all cases the rate increased as the temperature decreased, although the details of the dependence were different for each mutant. The observed dependence of the rate upon temperature is modeled as arising principally from a several hundred meV change in reorganization energy. The relationships among the rate, temperature, and free energy differences can be well fit by a Marcus surface using two modes centered near 150 and 1600 cm^{-1} with a total reorganization energy that decreases from 930 to 650 meV as the temperature decreases from 293 to 10 K. In the inverted region, where the driving force is greater than the reorganization energy, the rate is found to be approximately independent of the free energy difference. This is modeled as due to the additional coupling of high frequency modes to the reaction. An alternative model is also considered in which a 140 meV increase in the reorganization energy is matched by a 140 meV increase in the free energy difference as the temperature decreases. The possible role of solvent dipoles in determining this temperature dependence of the reorganization energy and the implications for other electron transfer reactions are discussed.

In photosynthesis, light energy is converted into chemical energy through a series of electron and proton transfer processes. The primary process of bacterial photosynthesis occurs in a pigment−protein complex called the reaction center [for reviews, see Feher et al. (1989), Parson (1991), and Kirmaier and Holten (1993)]. Light absorption results in excitation of the primary donor (P),[1] a bacteriochlorophyll dimer, and an electron is transferred in ∼200 ps to the primary quinone acceptor, Q_A, that is a ubiquinone-10 in *Rhodobacter sphaeroides*. Electron transfer normally proceeds in 150 μs from Q_A^- to the secondary quinone, but this process can be blocked when inhibitors, such as terbutryn, are bound to the reaction center. Under these conditions, the electron is stable on the quinone in the state Q_A^- until it recombines with P^+ with a lifetime of ∼100 ms in wild-type reaction centers.

Detailed models of electron transfer are often difficult to apply to biological systems such as the reaction center due to the complexity of proteins and the consequential large number of parameters that are difficult to determine experimentally (Parson & Warshel, 1987). For example, the critical relationship between the rate and the free energy difference between the final and initial states has been measured for only a limited number of electron transfer processes. An exception is the charge recombination process $P^+Q_A^- \rightarrow PQ_A$ in the reaction center for which several independent studies have been reported. One approach is to alter the free energy difference by chemically removing the ubiquinone and replacing it with quinones that have different reduction potentials (Gunner et al., 1986; Feher et al., 1987; Gunner & Dutton, 1989). Interpretation of these measurements is limited by the alteration of quinone binding for the different quinones and the restriction to low temperatures to prevent the contribution of a secondary activated pathway. A second approach is to orient reaction centers in either bilayers (Feher et al., 1988), Langmuir-Blodgett films (Popovic et al., 1986), or polyvinyl alcohol films (Franzen et al., 1990; Franzen & Boxer, 1993). The free energy difference can then be altered by application of an electric field across the oriented reaction centers. The studies on oriented reaction centers have produced different results for the dependence of rate upon

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[1] Abbreviations: P, bacteriochlorophyll dimer; Q_A, primary quinone; λ, reorganization energy.

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Table 1: Dependence of the $P^+Q_A^-$ Charge Recombination Rate (k) upon Temperature

sample	$-\Delta G°$ [a] (meV)	k (s^{-1})												
		293 K	265 K	250 K	240 K	210 K	195 K	180 K	160 K	140 K	120 K	105 K	90 K	10 K
wild type	520	11	11	13	13	17	20	26	32	35	38	40	43	44
HF(L168)	425	4.8	5.0	5.2	6.0	7.5	13	20	22	23	27	26	27	28
HF(L168)+LH(M160)	500	8.3	10	11	12	15	24	29	31	33	36	39	39	50
LH(M160)	580	12	14	17	18	24	35	39	42	47	51	54	58	69
FH(M197)	645	15	18	21	22	26	31	32	36	40	42	43	45	54
LH(L131)+LH(M160)	650	14	19	20	23	28	30	35	39	43	46	48	50	57
LH(M160)+FH(M197)	715	21	25	29	30	37	40	43	46	49	54	56	59	69
LH(L131)+LH(M160)+FH(M197)	780	24	25	27	28	32	34	36	38	39	42	45	47	54

[a] The value of $-\Delta G°$ for each mutant at 303 K is determined by assuming that the change relative to the wild-type value of 520 meV (Arata & Parson 1981) is due to the change in the P/P^+ midpoint value in the mutant compared to wild type as reported in Lin et al. (1994a).

the free energy difference and have been mostly limited to room temperature measurements. The differences among the studies arise at least partly from the influence of the local environment of the protein [see discussions in Popovic et al. (1986), Feher et al. (1988), and Franzen and Boxer (1993)].

Recently, a series of mutants have been designed to systematically alter the P/P^+ midpoint potential by the changes of hydrogen bonds to P (Lin et al., 1994a). Using a number of spectroscopic techniques, the changes of the hydrogen bonds have been confirmed, and the P/P^+ midpoint potential was found to vary from 410 to 765 mV compared to 505 mV for wild type [reviewed in Allen and Williams (1995)]. The availability of these mutants has allowed another approach toward investigating the dependence of the various electron transfer rates on the free energy difference. For each mutant, the free energy difference of the charge separated state relative to the ground state is altered due to the change in midpoint potential of P. The dependence of the charge recombination rate upon the free energy difference for these mutants at 295 K has been reported (Lin et al., 1994a), where it was shown that the decays proceed through direct recombination and that the measured rate is correlated with the P/P^+ midpoint potential.

A study of the temperature dependence of the $P^+Q_A^-$ charge recombination rate in these mutants is of interest as it should allow the determination of the vibrational modes that are coupled to the electron transfer reaction. The charge recombination is well suited for this type of study because it can occur even at cryogenic temperatures. Moreover, it is well established that the rate increases when the temperature is decreased, a property for which several explanations have been proposed, although none of them is fully satisfactory (Hsi & Bolton, 1974; Hales, 1976; Romijn & Amesz, 1976; Clayton, 1978; Kakitani & Kakitani, 1981; Bixon & Jortner, 1986; Feher et al., 1987; Parot et al., 1987; Sebban, 1988). In this communication, we present the dependence for these mutants of the $P^+Q_A^-$ charge recombination rate on both the free energy difference and the temperature from 10 to 293 K. The results are discussed in terms of theoretical models of electron transfer in biological systems and also compared to the previously reported data. A preliminary report of the 10 K data has been presented elsewhere (Ortega et al., 1995).

MATERIALS AND METHODS

The general construction and initial characterization of the mutants have been described previously (Lin et al., 1994a,b). Reaction centers were isolated from semiaerobically grown cultures following published procedures (Paddock et al., 1989; Williams et al., 1992; Lin et al., 1994a,b). For the spectroscopic measurements, the reaction centers in 15 mM tris(hydroxymethyl)aminomethane HCl, pH 8, 0.025% lauryldimethylamine *N*-oxide, and 1 mM EDTA were mixed with glycerol to a final proportion of 60% per volume, yielding a final concentration of reaction centers of approximately 1 μM. Terbutryn was added at a final concentration of 20 μM for all measurements.

The electron transfer kinetics from Q_A^- to P^+ were determined by monitoring the absorption changes of the reaction center at 1250 nm, where P^+ has a characteristic absorption band (Parson & Cogdell, 1975). The measuring light was provided by a halogen lamp filtered with a Corion band-pass filter centered at 1250 nm with a 20 nm bandwidth. The sample was excited by 8 ns laser pulses obtained by pumping a rhodamin 6G methanol solution with frequency-doubled light from a Q-switch YAG laser. The temperature was varied by placing the sample in a cryostat cooled with helium gas at a controlled temperature (240 to 10 K) or in a cuvette holder cooled with a thermostated bath (293 to 240 K). Further details have been described elsewhere (Ortega & Mathis, 1992).

RESULTS

The kinetics of charge recombination were measured as a function of temperature by monitoring the optical absorption decay of P^+ at 1250 nm following its formation in response to a laser pulse. Detailed measurements were performed on wild type and seven mutant strains. For each sample, measurements were performed at approximately 15 K intervals between 293 and 90 K and then at 10 K. In general, the recombination kinetics were reasonably fitted by a single exponential term. The rates obtained from these fits are summarized in Table 1. Fitting the data to two exponential components did improve the quality of the fit in many cases. The ratio of the rates for the fast and slow components was approximately a factor of 3, which is fairly small. For this reason, the relative amplitude of the fast and slow terms varied considerably. The rates for the two component fits each exhibited similar temperature dependencies as was observed for the single component fit.

For wild type, the recombination rate increased from 11 to 44 s^{-1} as the temperature decreased from 293 to 10 K. This dependence is similar to that previously reported (Hsi & Bolton, 1974; Hales, 1976; Romijn & Amesz, 1976; Clayton, 1978; Feher et al., 1987; Parot et al., 1987). In every mutant, the rate was found to progressively increase as the temperature was decreased (Figure 1). However, the

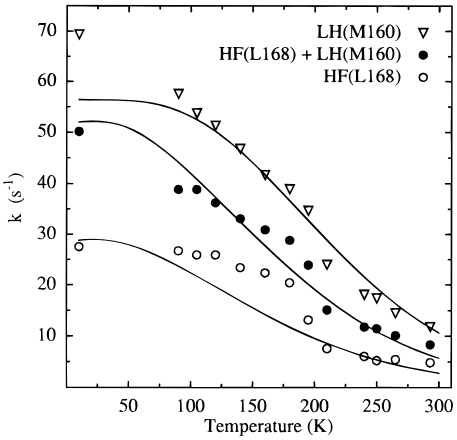

FIGURE 1: Dependence of the rate of $P^+Q_A^-$ → PQ_A upon the temperature for three mutants, LH(M160), HF(L168) + LH(M160), and HF(L168), that have free energy differences of −580, −500, and −425 meV, respectively. The solid lines are fits of the data using eq 3 with modes of frequency 150 and 1600 cm^{-1} and λ decreasing from 930 to 650 meV with temperature.

Table 2: Comparison of the $P^+Q_A^-$ Charge Recombination Rate (k) for Mutants with Similar $-\Delta G°$ Values but Different Spin Densities

sample	$-\Delta G°$ (meV)[a]	spin density on P_L[b]	k (s^{-1}) 293 K	k (s^{-1}) 10 K
LH(M160)	580	0.83	12	69
LH(L131)	600	0.47	11	41
LH(M160)+FH(M197)	715	0.83	21	69
LH(L131)+FH(M197)	725	0.40	15	37

[a] The value of $-\Delta G°$ for each mutant is determined as in Table 1. [b] As reported in Rautter et al. (1995a).

dependence of the rate upon temperature was different for each mutant. For example, for the mutant HF(L168) the rate increased from 4.8 to 28 s^{-1}, while for the mutant LH(L131) + LH(M160) + FH(M197) the rate increased from 24 to 54 s^{-1} (Table 1). For most mutants, the largest increase in rate occurred as the temperature decreased from 225 to 175 K, and the rate was relatively independent of temperature below 100 K. The exceptions are the mutants with the highest P/P^+ midpoint potentials whose rates more gradually increased throughout the entire temperature range. For two mutants, LH(L131) and LH(L131) + FH(M197), rates were measured only at 10 and 293 K and increased by factors of 3.7 and 2.4, respectively, at 10 K compared to 293 K (Table 2).

DISCUSSION

The temperature dependence of the rate of charge recombination from $P^+Q_A^-$ for mutants with a wide range of P/P^+ midpoint potentials allows the testing of different models of electron transfer that are critically dependent upon temperature and the free energy difference. The free energy difference for charge recombination from the primary quinone in wild-type reaction centers has been measured to be 520 meV at 303 K (Arata & Parson, 1981). In each mutant, the properties of the quinones are presumably unchanged and the change in free energy difference is determined by the change in the P/P^+ midpoint potential. Since the measured midpoint potentials range from 410 to 765 mV, compared to 505 mV for wild type, the corresponding free energy differences range from 425 to 780 meV. In this analysis, the rates are those determined from one-exponential fits of the decays. Many groups have reported that, for reasons not understood, the kinetics of electron transfer are not strictly monophasic (Popovic et al., 1986; Feher et al., 1987; Parot et al., 1987; Sebban, 1988; Franzen et al., 1990). We have confirmed these observations and found that, with reaction centers from all strains studied, a one-exponential fit is not fully satisfying and two exponentials give a better fit of the decays. In all cases the ratio of the two rates for a two-exponential fit is between 2 and 5. Since the ratio is relatively small, it is difficult to obtain safe values for the two rates and for their respective contribution. Nevertheless, we have found that, within experimental uncertainty, both rates have similar dependencies upon temperature and free energy difference as shown for the one-exponential analysis.

Modeling Using Electron Transfer Theory. Different theories that relate the electron transfer rate to the free energy difference and the temperature have been developed for biological systems. According to conventional electron transfer theory (Marcus & Sutin, 1985) the rate of electron transfer, k, has an exponential dependence upon the free energy difference between the final and initial states, $\Delta G°$, according to

$$k = (4\pi^2/h)V^2(4\pi\lambda k_B T)^{-1/2} \exp[-(\Delta G° + \lambda)^2/(4\lambda k_B T)] \quad (1)$$

where h is Planck's constant, V is the electronic coupling factor between the initial and final states, k_B is the Boltzmann constant, T is the temperature, and λ is the reorganization energy. In this theory, it is assumed that the electron transfer is coupled only to low frequency vibrations such that $h\nu \ll k_B T$. For this assumption to hold at 10 K, the modes must be of low frequency with energies less than 1 meV (or 8 cm^{-1}). For a more general theory that assumes that the donor and the acceptor are coupled to a single vibrational mode of any frequency ω in a harmonic approximation (Jortner, 1976), the rate is given by

$$k = 8\pi^3/h^2\omega V^2[e^{-S(2n+1)}][(n+1)/n]^{p/2} I_p[2S(n(n+1))^{1/2}] \quad (2)$$

where $n = 1/[\exp(h\nu/k_B T) - 1]$ which is the thermal population of a mode with vibrational frequency ν, $p = -\Delta G°/h\nu$, $I_p()$ is a modified Bessel function of order p, and $S = \lambda/h\nu$, which is the strength of the coupling. For the situation where there is coupling to more than one mode, these relationships are altered by substituting the product of the Franck−Condon factors for each mode [see Jortner (1976), Sarai (1980), and Kakitani and Kakitani (1981)]. For instance, when coupled to both an intermediate mode (ω_M with frequency between 10 and 1000 cm^{-1}) and a high frequency mode (ω_L with a frequency larger than 1000 cm^{-1}), the rate k is given by

$$k = 8\pi^3/h^2\omega_M V^2 \sum_{i=0}^{\infty} \{[e^{-S'} S'^i/i!][e^{-S(2n+1)}] \times [(n+1)/n]^{p/2} I_p[2S(n(n+1))^{1/2}]\} \quad (3)$$

where $S' = \lambda_L/h\nu_L$, $S = \lambda_M/h\nu_M$, n is the population of mode ω_L, and $p = -(\Delta G° + ih\nu_L)/h\nu_M$. In such circumstances, each class of vibrational modes contributes to the total value of

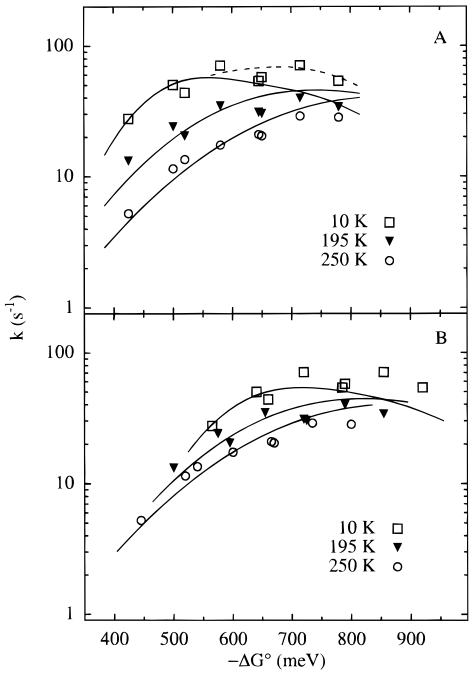

FIGURE 2: Dependence of the rate of $P^+Q_A^- \rightarrow PQ_A$ upon the free energy difference at three temperatures, 250, 195, and 10 K. (A) The solid lines are fits of the data using eq 3 with modes of frequency 150 and 1600 cm^{-1} and λ equal to 890, 780, and 650 meV for temperatures of 250, 195, and 10 K, respectively. The dashed line shown for the 10 K data is generated by incorporating an additional mode with a frequency of 600 cm^{-1}. (B) The solid lines are fits of the data using eq 3 with the same parameters as in (A) but λ equal to 910, 855, and 790 meV and $-\Delta G°$ increased for each strain by 20, 75, and 140 meV for temperatures of 250, 195, and 10 K, respectively.

the reorganization energy according to

$$\lambda = \lambda_M + \lambda_L \quad (4)$$

These theories provide a framework for understanding the experimentally determined dependence of the rate on both temperature and $\Delta G°$. For each relationship the parameters were adjusted to provide the best fit for the rates of all of the mutants at all of the temperatures. This constrains the possible choice of parameters much more severely than the previous models that were based upon either the temperature dependence or the $\Delta G°$ dependence alone. The conventional theory that assumes coupling to only low frequency modes (eq 1) fits the rates very poorly as it predicts a much stronger dependence of the rate upon $\Delta G°$ than was observed. For instance, at 10 K the model predicts that the rate should decrease by a factor of 100 when $\Delta G°$ is greater than or less than λ by 100 meV. However, the rates change only by a factor of 2.5 at 10 K over the entire 350 meV range of $\Delta G°$. The next simplest model is the one-mode model (eq 2) that was used previously to describe the 293 K data (Lin et al., 1994a). For the rates at 293 K, a good fit was obtained using a high frequency mode of 1240 cm^{-1}, and the data could not be fit when the frequency was significantly lowered.

The critical observation for the temperature dependence of the rate for all of the mutants is that the rate reaches a maximal value at lower values of $-\Delta G°$ as the temperature decreases (Figure 2A). In terms of the electron transfer theories, this can be described as a significant decrease in the value of λ as the temperature decreases. In these theories,

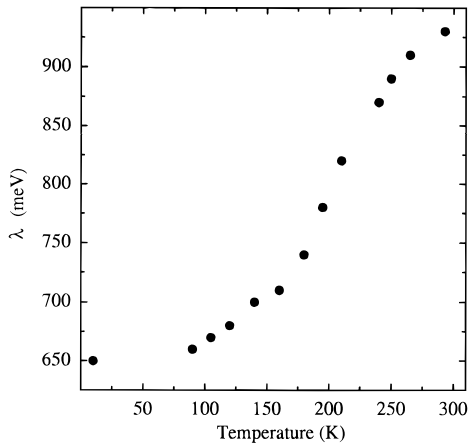

FIGURE 3: Temperature dependence of the total reorganization energy. The total reorganization energy contains contributions from both the medium and high frequency modes (eq 4) but the dependence comes entirely from the 150 cm^{-1} mode as the 1600 cm^{-1} mode has a temperature independent contribution of 120 meV.

a decrease in λ with temperature is expected only when the rate is coupled to medium frequency modes, which are associated with solvent dipoles (Marcus & Sutin, 1985). The contribution of high frequency modes is expected to be independent of temperature unless there is a specific mechanism for a temperature dependence. Therefore, to describe all of the rates including a temperature dependence of λ, it is necessary to incorporate both a medium frequency and high frequency mode using eq 3. With this choice, rates in the region $-\Delta G° < \lambda$ at all temperatures are well fitted when $\nu_M = 150$ cm^{-1}, $V = 5 \times 10^{-8}$ eV, and the rate is weakly coupled ($S = 0.6$) to a high frequency 1600 cm^{-1} mode. The obtained temperature dependence for λ (Figure 3) is entirely due to the 150 cm^{-1} mode as the 1600 cm^{-1} mode has a temperature-independent contribution of 120 meV. This choice of parameters predicts that the rate will decrease with increasing free energy difference in the inverted region, when $-\Delta G° > \lambda$. However, no such decrease is observed for the rates (Figure 2A). To fit the rates in the inverted region, the relationship can be modified to include additional high frequency components that contribute significantly to the rate only in the inverted region. Shown in Figure 2 is a fit using an additional mode with a frequency of 600 cm^{-1} and a coupling factor $S = 2.5$.

The curves shown in Figure 2 represent isothermal sections of a Marcus surface that is shown in Figure 4. With this model the observed increase in rate with decreasing temperature is determined by the temperature dependence of λ since all other parameters, such as the coupling factor V, are held fixed. Comparison of the temperature dependencies for the different mutants shows that the decrease in reorganization energy is the major factor in the observed increases in the rates as the temperature decreases. The largest decrease in λ occurs when the temperature changes from 225 to 175 K. This matches the temperature region in which the rates change by the largest amount (Figure 1). In general, the detailed temperature dependence for each mutant is different but well described by the model. The poorest fit is for the mutants with the highest values of $-\Delta G°$. These mutants undergo a change from the normal region, where $-\Delta G° < \lambda$, to the inverted region where $-\Delta G° > \lambda$ as the temperature is lowered. As evident in Figure 2, this region can be better described with the use of additional modes and

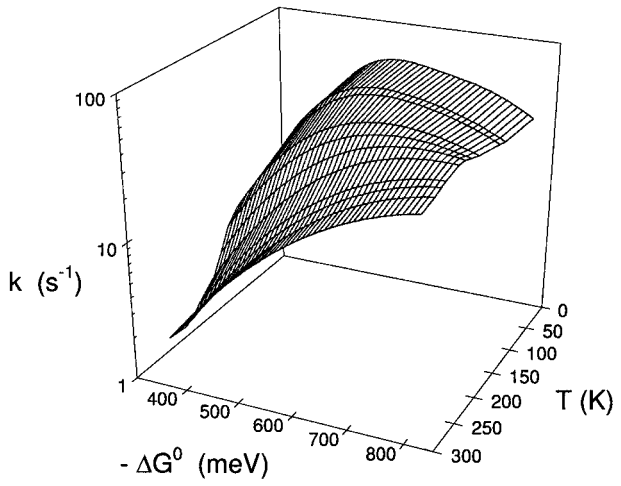

FIGURE 4: Dependence of the rate upon both the temperature and the free energy difference for charge recombination. This "Marcus surface" shows the dependence using the relationship given in eq 3 with modes of frequency 150 and 1600 cm^{-1} and λ decreasing from 930 to 650 meV with temperature.

hence additional parameters. However, the fit using a mode of 600 cm^{-1} is not uniquely determined by the limited amount of data and a wide range of modes with different coupling factors can be used. Therefore, we have elected to model the data using the simpler and better-defined two-mode description.

The model proposed in this report uses two different vibrational modes. The major vibrational mode is a low frequency mode of 150 cm^{-1}. This frequency of the mode should be regarded as simply an average value as alteration of the frequency between 80 and 200 cm^{-1} does not significantly change the fitting or the basic result of a decreasing value for the reorganization energy with temperature. Modes in the 50−200 cm^{-1} region are widely found in protein systems and have been specifically identified as influencing the charge recombination rate in theoretical models of the vibrational spectrum of reaction centers (Warshel et al., 1989). The modeling also includes coupling to a 1600 cm^{-1} mode. Modes with frequencies in the range of 1500−1700 cm^{-1} have been shown to associated with the primary quinone by Fourier transform infrared spectroscopy (Bauscher et al., 1993; Breton et al., 1994; Brudler et al., 1994). It is possible to describe all of the data presented here using a model in which the 1600 cm^{-1} mode contributes a reorganization energy that decreases in temperature by several hundred meV. However, there is no clear physical description of why a mode of this frequency would have such a temperature dependence. In contrast, modes of low frequencies are known to be temperature dependent [discussed in Franzen and Boxer (1993)].

Temperature Dependence of the Free Energy Difference. In the model described above, the decrease in the value of $-\Delta G°$ for which the rate achieves a maximal value is interpreted as arising from a 280 meV decrease in the reorganization energy (from 930 to 650 meV) as the temperature is lowered from 293 to 10 K. This model assumes that $\Delta G°$ does not change with temperature. Since the relationships given in eqs 1−3 predict that a change in either λ or $\Delta G°$ will result in a shift in the maximal rate, an alternate method of fitting the data is to vary $\Delta G°$ and not λ. A 280 meV increase in $-\Delta G°$ as the temperature decreases can explain the apparent shift of the peak position of the Marcus curves, but the resulting fit of the rates at all temperatures is poorer than obtained when λ alone is allowed to change (not shown).

A more general model is to allow both λ and $\Delta G°$ to vary with temperature. Formally, changes in λ and $\Delta G°$ do not have to be coupled; however, if both of these parameters change independently, the data cannot be uniquely fit. A physical description provides an interpretation of why λ and $\Delta G°$ would change with temperature. If it is assumed that interactions that stabilize the presence of charged species are frozen out at low temperatures, a decrease in λ and an increase in $-\Delta G°$ would result. Thus the more general model can be constrained such that the decrease in λ with temperature is matched by a corresponding increase in $-\Delta G°$. The data can be well fit using this alternate model when λ decreases by 140 meV (from 930 to 790 meV) and $-\Delta G°$ for each strain increases by 140 meV as the temperature decreases from 293 to 10 K (Figure 2B) with the largest change occurring between 225 and 175 K. This fit is comparable to the fit achieved when λ alone is varied. Thus, our data are consistent with a net change of ∼280 meV for ($\Delta G° + \lambda$) and cannot be used to distinguish between the relative changes of these two energies.

There are no current experimental measurements for the temperature dependence of either λ or $\Delta G°$ over a wide range of temperatures. Measurements of the delayed fluorescence from reaction centers have been performed from 288 to 308 K (Arata & Parson, 1981) and indicate that an increase in $-\Delta G°$ is correlated with a decrease in temperature. A simple extrapolation of these data to low temperatures predicts a linear increase of ∼100 meV in $-\Delta G°$ as the temperature decreases from 293 to 10 K, but cannot explain the large change found between 225 and 175 K. Thus, the dependence of $\Delta G°$ upon temperature must arise from free energy changes as a result of freezing of the protein.

Comparison with Other Data. In this work we describe the temperature dependence of the $P^+Q_A^-$ charge recombination rate as the free energy difference is also varied by changes in the P/P^+ midpoint potential. These results can be compared to previous measurements in which $\Delta G°$ was varied by two other independent techniques. First, the protein was incorporated into bilayers (Feher et al., 1988), Langmuir−Blodgett films (Popovic et al., 1986) or polyvinyl alcohol films (Franzen et al., 1990; Franzen & Boxer, 1993). The application of an electric field across the oriented reaction center alters $\Delta G°$ by the product of the external field and a scaling constant β. Second, the ubiquinone at the primary quinone site was biochemically replaced with other quinones, and $\Delta G°$ was altered by both the change in Q_A/Q_A^- midpoint potential and any altered interactions with the surrounding protein (Gunner et al., 1986; Gunner & Dutton, 1989). For these different studies, the observed decays were fit to different numbers of exponential components as noted below.

The dependence of the rate upon the free energy difference as measured by several different techniques at 293 K is remarkably consistent (Figure 5). Franzen and Boxer (1993) utilized a model that is very similar to the one presented in this paper and that uses both a low frequency (50 cm^{-1}) and high frequency mode (1510 cm^{-1}) and a total reorganization energy of 1250 meV. In that report (Franzen & Boxer, 1993), the data were fit to two components that had similar dependencies on the free energy difference (only the rates

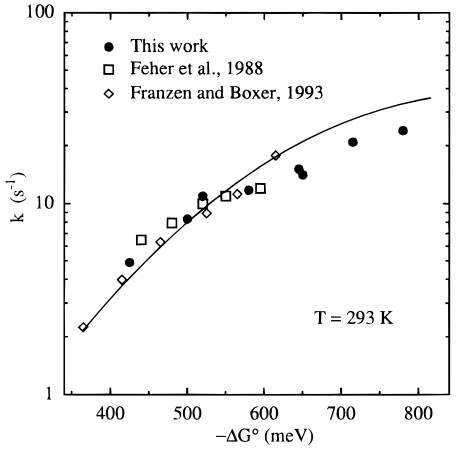

FIGURE 5: Relationship of the charge recombination rate at 293 K and the free energy difference for wild type and hydrogen bond mutants reported here (●), the data of Feher et al. (1988), and the data of Franzen and Boxer (1993). The curve is the fit using eq 3 with modes of frequency 150 and 1600 cm^{-1} and $\lambda = 930$ meV. The data of Feher et al. (1988) and Franzen and Boxer (1993) shown are representative points of more complete data sets. The rates of Franzen and Boxer (1993) are the rates for the fast component of the decay and have been decreased uniformly by a factor of 3.5 in order to overlay for clarity the three data sets. Although the rate was also measured for larger values of $-\Delta G°$, these rates are not shown as they include a contribution from a second activated pathway; thus the observed rates were not a measurement of direct charge recombination. The data of Feher et al. (1988) are unchanged although alteration of a scaling parameter results in an improved fit as discussed in the text.

for the fast component are shown in Figure 5 as diamonds). Feher et al. (1988) fit their data utilizing eq 1, which assumes coupling to only very low frequency modes and obtained a reorganization energy of 640 meV. This model agrees well with their data but the relationship used by Feher et al. (1988) is clearly incompatible with the other data sets. However, the data of Feher et al. (1988) are similar to the data reported here and the data from Franzen and Boxer (1993) (Figure 5). As was noted by Feher and co-authors, the assignment of the change in the $\Delta G°$ value compared to the wild-type value for each applied voltage utilized the scaling parameter β that was regarded as a free parameter and was influenced by the specific relationship used to describe the rates (eq 1). With the choice of the two mode model (eq 3) the scaling parameter would be lowered by a factor of 2−3. This lower value of β would then result in better agreement between the data of Feher et al. (1988) and the model presented here (not shown) and also be more consistent with estimates for β based upon other independent experiments (Feher et al., 1988). Popovic et al. (1986) have reported the dependence of the rate upon the free energy difference for reaction centers embedded in Langmuir−Blodgett films. In contrast to the other measurements, the decays in those experiments were strongly nonexponential for possible reasons that have been noted elsewhere (Popovic et al., 1986; Feher et al., 1988; Franzen & Boxer, 1993). Their decays were fit to three exponential components, each of which exhibited a different dependence on $\Delta G°$; therefore, a direct quantitative comparison with our data is not possible. In summary, the room temperature data presented here are, within experimental limitations, fully compatible with the previous measurements.

At low temperatures (below 90 K), two experiments have been performed that measured the dependence of the rate upon the free energy difference. Gunner and Dutton (1989) altered the free energy difference by substituting different quinones biochemically. Using a one-exponential component to describe the decays, they fit the $\Delta G°$ dependence using eq 3 assuming that $\Delta G°$ was independent of temperature. The resulting parameters are similar to ours with values of 120 cm^{-1} for the medium frequency mode, 1600 cm^{-1} for the high frequency mode, and 600 ± 100 meV for the total reorganization energy. The measurements were limited to below 110 K due to the presence of a second activated process at higher temperatures. Franzen and Boxer (1993) measured the free energy dependence for reaction centers in polyvinyl alcohol films at 80, 160, and 290 K and used two-component fits to describe their decays. At each temperature the dependence of the rates was independently fitted with the assumption that $-\Delta G°$ increased by 80 meV from 293 to 80 K based upon an extrapolation of the data of Arata and Parson (1981). For the fast component of the decay, the dependence of the rate on $\Delta G°$ was fit using frequencies ranging from 33−58 and 1360−1870 cm^{-1} for the medium and high frequency modes, respectively, and the total reorganization energy was lowered by 360−450 meV for the 80 K data compared to 290 K data. Those authors did not fit the temperature dependence of the rate. During the incorporation of the reaction centers in polyvinyl alcohol films, the protein is dehydrated, which causes the temperature dependence to be significantly altered compared to reaction centers in glycerol solutions (Clayton, 1978; Feher et al., 1988). Thus all of these data sets are consistent with the decrease in reorganization energy as the temperature is lowered although only the data presented here show that this factor leads to the observed temperature dependence of the rate.

Models for the temperature dependence of the $P^+Q_A^-$ charge recombination rate have been proposed based upon the measurements of wild-type reaction centers. This dependence can be roughly fitted using a simple one-mode model (Bixon & Jortner, 1986) with improvement using a temperature dependence for the electronic coupling factor V (Hales, 1976; Feher et al., 1987). The variation of V is proposed to be due to thermal contraction of the protein as it freezes, although the extent of the contraction needed to explain the data is larger than expected (Bixon & Jortner, 1986). If V is temperature dependent, but not λ or $\Delta G°$, then all of the relationships predict that the measured dependence of the rate upon $\Delta G°$ would be the same at all temperatures. The observed shift in the maximal rate is not consistent with such an assumption. In addition, the temperature dependence of λ is sufficient to account for the observed temperature dependence of the rate although a temperature variation of V may also contribute at a smaller level than previously modeled.

For some mutants with comparable values of the P/P^+ midpoint potential, the electron transfer rates are similar at 293 K, but differences are apparent at 10 K (Table 2). The LH(M160) and LH(L131) mutants have rates of approximately 11 s^{-1} at 293 K but at 10 K the rates are 69 and 41 s^{-1}, respectively. Likewise, despite similar rates at 293 K, LH(M160) + FH(M197) is faster by a factor of 1.8 than LH(L131) + FH(M197) at 10 K. In both cases, the mutant with the slower rate at 10 K has the P^+ spin density preferentially located on the M-side of P, rather than the L-side as in wild type, as measured by ENDOR spectroscopy (Rautter et al., 1995a). A similar discrepancy has also been

noted for the rate of electron transfer from cytochrome c_2 to P^+ in these mutants (Rautter et al., 1995b). These differences in rates for mutants with the same values of $\Delta G°$ presumably are due to differences in the electron transfer parameters. A difference in the coupling factor V is unlikely as this would predict that the rate difference would be present at all temperatures. This suggests that another factor, such as the reorganization energy, is perhaps different depending upon which side of P has the spin density. Modeling of this effect has not been done due to the limited amount of data available.

Conclusions. A critical feature of the model presented here is the decrease in the reorganization energy with temperature. This model allows reconciliation of the previous studies and demonstrates that the same dependence of the rate upon $\Delta G°$ has been determined by three completely independent physical techniques. The decrease in λ reported here was predicted by Dutton and Moser (1994) on the basis of a comparison of our 293 K (Lin et al., 1994a) and the 10 K data of Gunner and Dutton (1989). The results of the three techniques clearly agree thus confirming the assumption that the variation of the P/P^+ midpoint potential in the mutants directly corresponds to a variation of $-\Delta G°$ without any significant change in any other parameter. The results do not necessarily preclude the possibility that other parameters, such as the electronic coupling factor, have a temperature dependence but suggest that such effects are small compared to that due to the reorganization energy and the free energy difference.

The temperature dependence of the energies associated with the transfer of the electron has implications for the mechanism. Such a dependence would arise if interactions between the primary quinone and either solvent or protein dipoles are frozen out when the temperature is lowered below the glass temperature. A possible role of solvent is suggested by the recent structure determination of the reaction center from *Rb. sphaeroides* that has water molecules located within 5 Å of the quinone (Ermler et al., 1994) and the well-established influence of water on the temperature dependence of the charge recombination rate in wild-type reaction centers (Clayton, 1978). The dipoles also may be contributed by the side chains of amino acid residues; such interactions have been shown to stabilize the secondary quinone (Paddock et al., 1989; Takahashi & Wraight, 1990). This conclusion is also consistent with the observation that the kinetics of charge recombination rate are different when reaction centers are frozen in the light compared to the dark (Feher et al., 1987). A possible interpretation of this experiment is that, when frozen in the light, the dipoles are aligned favoring the charge-separated state, thus resulting in slower kinetics than observed when the protein is frozen in the dark which would result in a random orientation of dipoles and no stabilization of the charge-separated state. In model systems, the influence of solvent on electron acceptors has been shown to cause a decrease in the reorganization energy with temperature (Gaines et al., 1991). Similar interactions may also play a role in the transfer of electrons in other systems, such as photosystem I that exhibits a temperature dependence for the electron transfer rate to the iron−sulfur center that is suggested to be influenced by the solvent (Schlodder et al., 1995).

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