AN INTERNAL CONSISTENCY TEST AND ITS IMPLICATIONS FOR THE INITIAL STEPS IN BACTERIAL PHOTOSYNTHESIS

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Comparison of theoretical expressions for superexchange and chemical intermediate mechanisms for electron transfer from the bacteriochlorophyll dimer to a pheophytin reveals a common factor influencing their rates. If the former mechanism is to dominate the latter an internal consistency test becomes possible and is derived. Consequences are investigated for matrix elements, singlet–triplet splitting of the $\mathrm{BChl}_2^+\mathrm{BPh}^-$ radical pair and other properties. Two possible alternative mechanisms are also considered. To discriminate among some of the possibilities the study of the effect of an applied electric field on BChl depletion and on the initial rates is suggested.

1. Introduction

Several mechanisms have been postulated for the early electron transfer steps in bacterial photosynthetic reaction centers and discussed [1–14]. In each of these mechanisms a bacteriochlorophyll monomer BChl plays a role. In the initial electron transfer, which is from an electronically excited dimer BChl_2^* to a pheophytin BPh, the BChl monomer serves in one postulated mechanism as an intermediate anion BChl^-. In another mechanism it is a participant in a superexchange electron transfer, and in still another it is an intermediate cation, BChl^+. There is also the possibility to be considered, and discussed later, that BChl may not be involved at all.

Recently, two observations have been made which have been used to argue against mechanisms possessing a chemical intermediate: (1) in quinone-free reaction centers the singlet–triplet splitting of the $\mathrm{BChl}_2^+\mathrm{BPh}^-$ radical pair is temperature independent [3,8,14], and (2) when the excitation wavelength is chosen so as to excite the BChl_2 directly, no depletion of BChl is detected spectroscopically during the reaction at room temperature [4], and not even a 2% depletion at 10 K [13]. The <2% result would imply such a high rate constant for the subsequent transfer of an electron from BChl^- to BPh (or a hole transfer from BChl^+ to BChl_2) as to be unlikely [#1]. While the actual numerical figures resulting from argument (1) are open to some question (cf. section 4), argument (2) is, assuming the experimental detectability estimates given in ref. [13], compelling. (Other recent work on the proposed formation of a BChl^- [5] has been discussed in ref. [4]. There seems to be a real bleaching of the BChl band when a higher energy photon is used for the excitation [10,13].)

It might be natural to conclude then that the superexchange mechanism is thereby confirmed. However, a more detailed analysis given in the present paper, based on one given earlier [6], reveals that there is an internal consistency test to be considered (section 2) and that it has interesting consequences. These consequences lead to relatively large individual electron transfer matrix elements (section 3). In turn, using the energetics (section 4), the latter has implications for the predicted singlet–triplet splitting of the radical pair $\mathrm{BChl}_2^+\mathrm{BPh}^-$ (section 5) (too large) and for a possible charge transfer band $\mathrm{BPh}^- \rightarrow \mathrm{BChl}$ (section 6). Two potential alternative

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[#1] The relation between the maximum transient concentration of the intermediate and the relative rate constants is given in eq. (13) of ref. [6].

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mechanisms, one depicted later in fig. 2 and one not involving BChl, are also discussed (section 7), together with an analysis of whether there might be an effect of an applied electric field on BChl depletion and whether its effect on the rate constant for the initial step might discriminate among the possibilities (section 8).

The number of a priori unknown quantities involved with electron transfers in the BChl$_2^*$–BChl–BPh system is so large, compared with the number of knowns, that the finding of an internal consistency test becomes highly desirable. Such a test, which places a severe constraint on the variation of the unknown parameters, is described in section 2 and is applied to the mechanism in the subsequent sections.

2. The internal consistency test

The rate constant k_{12} of the first step of the two-step mechanism, BChl$_2^*$BChl→BChl$_2^+$BChl$^-$, BChl$^-$BPh→BChlBPh$^-$, is given at room temperature T in terms of non-adiabatic electron transfer theory (classical treatment) by [15]

$$k_{12}=\frac{2\pi}{\hbar}\frac{|H_{12}|^2}{(4\pi\lambda_{12}RT)^{1/2}}\exp\left(-\frac{(\Delta G_{12}^0+\lambda_{12})^2}{4\lambda_{12}RT}\right), \tag{1}$$

where ΔG_{12}^0 is the standard free energy of reaction for the first step, λ_{12} is the reorganizational parameter and H_{12} the electron transfer matrix element. The internal consistency test arises because the $\Delta G_{12}^0+\lambda_{12}$ in eq. (1) will be seen later to occur also in an energy denominator in the matrix element for the competitive activationless superexchange mechanism.

We denote several relevant electronic configurations by the numbers 1 to 3:

1. BChl$_2^*$–BChl–BPh;
2. BChl$_2^+$–BChl$^-$–BPh;
3. BChl$_2^+$–BChl–BPh$^-$.

The diagonal elements of the Hamiltonian, which includes the effect of the protein environment, are denoted by H_{ii}, $i=1,2,3$. The matrix elements for electron transfer between these electronic configurations are denoted by H_{ij}, with $i\neq j$.

Experimentally, the initial electron transfer reaction in the bacterial photosynthetic reaction center is known to be activationless [1,4,13]. More precisely, the reaction is even faster at low temperature than at room temperature [1,4,13]. When the mechanism is one of superexchange and is activationless, the free energy versus reaction coordinate curves for BChl$_2^*$–BChl–BPh (state 1) and BChl$_2^+$–BChl–BPh$^-$ (state 3), plus environment, are as depicted in fig. 1a. Also indicated is the position of the free energy curve for BChl$_2^+$–BChl$^-$–BPh (state 2) from the point of view of the superexchange theory. The transition state for an *activationless* superexchange mechanism 1→3 occurs at the equilibrium nuclear configuration for state 1, namely at the intersection of curves 1 and 3 in fig. 1a, while that for the 1→2 transition in an activationless chemical intermediate mechanism occurs at the intersection of curves 1 and 2 in fig. 1b.

A classical expression for the rate constant k_{13}^{super} for this activationless superexchange mechanism at room temperature T, is given by [6]

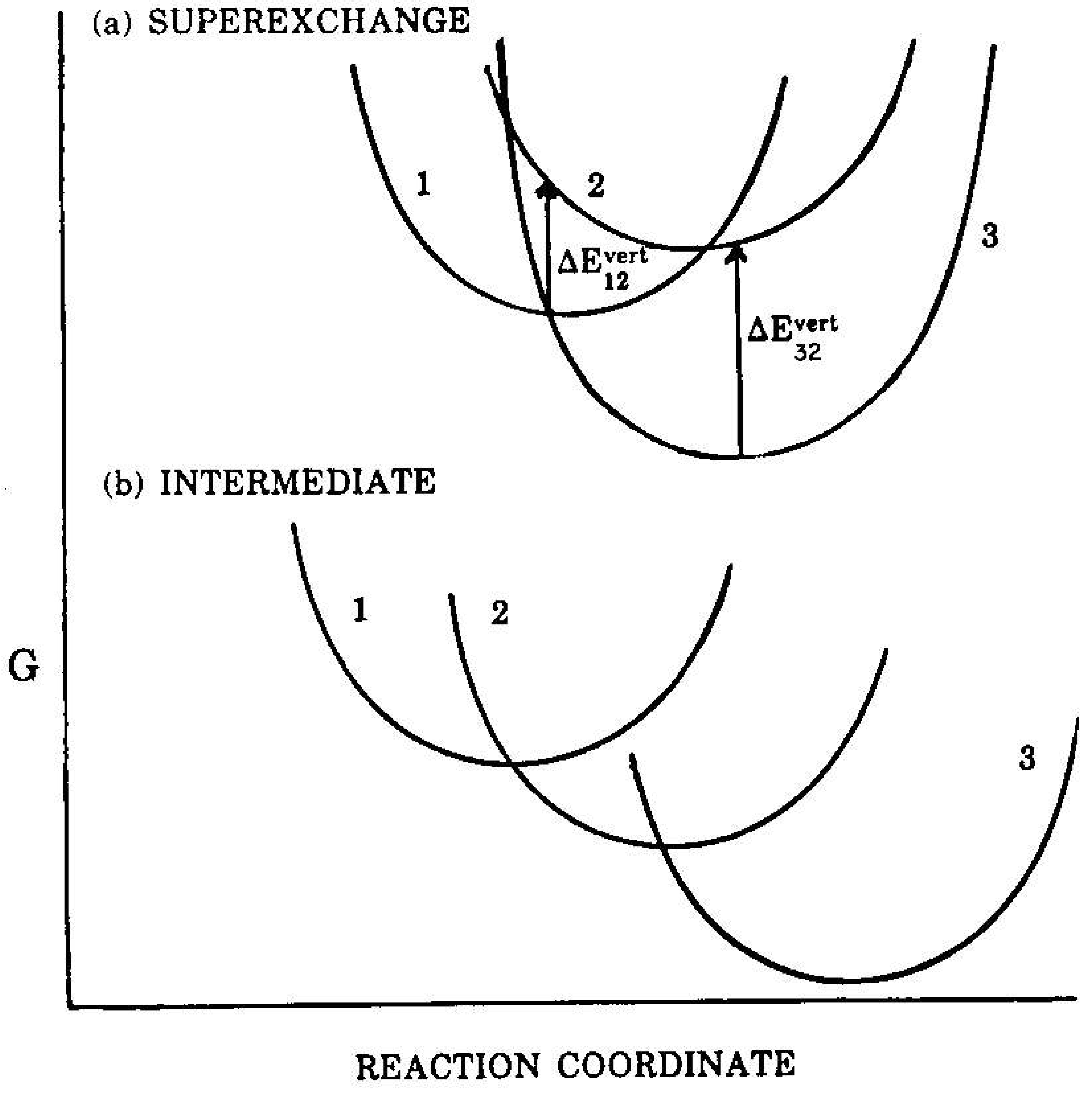

Fig. 1. Plot of free energy G versus reaction coordinate for the various electronic configurations and mechanisms. Curves 1, 2 and 3 refer to the BChl$_2^*$–BChl–BPh, BChl$_2^+$–BChl$^-$–BPh and BChl$_2^+$–BChl–BPh$^-$ states of the protein system, respectively. (a) is appropriate to a superexchange mechanism and (b) to a chemical intermediate mechanism (BChl$^-$). The reaction coordinate in (a) may differ from that in (b) and fig. 2. In (a) the minimum for curve 2 is not necessarily a global minimum in the space of all reaction coordinates.

$$k_{13}^{\mathrm{super}} = \frac{2\pi}{\hbar}\,\frac{|\bar{H}_{13}|^{2}}{(4\pi\lambda_{13}RT)^{1/2}}\,, \tag{2}$$

where the classical value [15] has been used for the Franck–Condon factor in ref. [6]. The effective superexchange matrix element $\bar{H}_{13}$ is given later by eq. (13). In our case the H_{13} there is negligible and, introducing the appropriate value for E into eq. (13), eq. (13) reduces to

$$\bar{H}_{13} = H_{12}H_{23}/(H_{22}-H_{11})\,. \tag{3}$$

Here, $H_{22}-H_{11}$ is a vertical energy difference of states 1 and 2 at the activationless superexchange transition state. More rigorously, the various terms in eqs. (2) and (3) should be suitably averaged over the set of nuclear configurations constituting the transition state. We shall treat the denominator in eq. (3) as an average energy difference $\langle H_{22}-H_{11}\rangle$, a thermodynamic energy difference. It is also, then, a free energy difference of states 1 and 2 at the superexchange transition state, since the entropy of the two electronic configurations is the same at any given value of the abscissa in fig. 1 [16]. This $\langle H_{22}-H_{11}\rangle$ is denoted by $\Delta E_{12}^{\mathrm{vert}}$ in fig. 1a and equals the $\Delta G_{12}^{0}+\lambda_{12}$ appearing in eq. (1) [16]. With this use of the thermodynamic average we have

$$H_{22}-H_{11} \approx \langle H_{22}-H_{11}\rangle \equiv \Delta E_{12}^{\mathrm{vert}} = \Delta G_{12}^{0}+\lambda_{12}\,. \tag{4}$$

From eqs. (1)–(4) we have

$$\frac{k_{13}^{\mathrm{super}}}{k_{12}} = \frac{|\bar{H}_{13}|\,r\exp(\Delta G_{12}^{*}/RT)}{2(\lambda_{13}\,\Delta G_{12}^{*})^{1/2}}\,, \tag{5}$$

where

$$\Delta G_{12}^{*} = (\Delta G_{12}^{0}+\lambda_{12})^{2}/4\lambda_{12} \tag{6}$$

and

$$r = |H_{23}/H_{12}|\,. \tag{7}$$

Assuming a superexchange mechanism, the $\bar{H}_{13}$ in eq. (5) can be expressed in terms of the observed rate constant k_{obs} using eq. (2) thus yielding

$$\frac{k_{13}^{\mathrm{super}}}{k_{12}} = \left(\frac{k_{\mathrm{obs}}\hbar}{\Delta G_{12}^{*}}\right)^{1/2}\left(\frac{RT}{\pi\lambda_{13}}\right)^{1/4}\frac{r\exp(\Delta G_{12}^{*}/RT)}{2}\,. \tag{8}$$

To have the ratio $k_{13}^{\mathrm{super}}/k_{12}$ greater than some quantity it follows that the right-hand side of eq. (8) must exceed this quantity. Since the value of λ_{13} is fixed by other data (sections 3 and 4) while the ratio r is estimated as in section 3, eq. (8) places a condition on ΔG_{12}^{*} and hence on the quantities appearing in eq. (6), and thereby on $H_{22}-H_{11}$ in eq. (4) and so on H_{12} and H_{23} in eq. (3). This result is a consistency test. It is applied in section 3.

As has been pointed out by Jortner [17], the above test assumes a non-adiabatic expression for k_{12} and a more general situation should be considered if the application of the test yields a large value for $|H_{12}|$. If the 1→2 step were adiabatic, eq. (1) would be replaced by a classical adiabatic expression [15]

$$k_{12} = \nu\exp(-\Delta G_{12}^{*}/RT)\,, \tag{9}$$

where ν is the vibration frequency for motion along the reaction coordinate (100 cm^{-1} according to estimates given later). Correspondingly, eq. (8) would be replaced by the simpler expression

$$\frac{k_{13}^{\mathrm{super}}}{k_{12}} = \frac{k_{\mathrm{obs}}}{\nu}\exp(\Delta G_{12}^{*}/RT) \quad (\text{adiabatic } k_{12})\,. \tag{10}$$

The rate constant k_{12} is given by eq. (1) for small values of $|H_{12}|$ and by eq. (9) for large values. Whenever, for the given $|H_{12}|$, the k_{12} calculated by the former exceeds the latter, eq. (9) should be used. The two expressions can be bridged by a formalism such as Landau–Zener (cf. its approximate use in ref. [6]). However, eqs. (8) and (10) serve to bracket the estimate of ΔG_{12}^{*} and are applied next. To anticipate the results, it will be seen that in the present instance the imposition of eq. (10) does not alter the basic conclusions that will be reached from eq. (8).

3. Numerical results

Assuming a superexchange mechanism, a magnitude for $\bar{H}_{13}$ of about 24 cm^{-1} is deduced from eq. (2) (cf. also refs. [6,7]), using the observed rate constant k_{obs} of $3.6\times10^{11}\ \mathrm{s}^{-1}$ at room temperature (taken here as 20°C) for electron transfer from BChl_2^{*} to BPh [4,13] and using $\lambda_{13}\approx0.17$ eV (section 4). (Use of a somewhat higher value of λ_{13} (section 4) would have only a minor effect on the calculated $\bar{H}_{13}$.)

We consider the condition that $k_{13}^{\rm super}/k_{12}$ be greater than, say, 5 in order that any concentration of the intermediate BChl$^-$ at room temperature be small. The ratio r in eq. (7) is not known experimentally. However, Plato has estimated the sum S of the overlap integrals between the various reactants [18] and we consider the postulate [17–19] that the electron transfer matrix elements are proportional to these overlap integrals. Assuming Plato's results we have $r\approx 7$ for transfer from one of the members of the BChl$_2$ pair to BChl, and $r\approx 6$ for transfer from both. We have used $r\approx 7$ ($r\approx 6$ yields only slightly different results) and, as noted below, we have also used $r\approx 2$ for comparison.

Upon solving eq. (8) with these numbers, and $r\approx 7$ it is found from eq. (8) that for $k_{13}^{\rm super}/k_{12}$ to equal or exceed 5, ΔG^{*}_{12} should equal to exceed 850 cm^{-1}. Examples of the infinite set of values of the ($\lambda_{12}, \Delta G^{0}_{12}$) pair which satisfy eq. (6) with $\Delta G^{*}_{12} = 850$ cm^{-1} are (1200, 820) (850, 850), and (300, 710), all in cm^{-1}. The first of these is in the "normal region" ($\lambda_{12} > |\Delta G^{0}_{12}|$) for the 1→2 electron transfer and the last is in the "inverted region" ($\lambda_{12} < |\Delta G^{0}_{12}|$). For these pairs, the $|H_{23}|$'s calculated from eqs. (3) and (4) using the given $\bar{H}_{13}$ and r are 595, 545, and 420 cm^{-1} respectively, or greater if $k_{13}^{\rm super}/k_{12} > 5$. All are rather large.

An alternative choice might be to set $r \equiv H_{23}/H_{12} \approx 2$, as assumed, for the sake of concreteness, in ref. [6]. In this case the use of eq. (8) shows that ΔG^{*}_{12} should equal or exceed an even larger value, 1125 cm^{-1}, if $k_{13}^{\rm super}/k_{12}$ is to equal or exceed 5. Examples of the continuous set of ($\lambda_{12}, \Delta G^{0}_{12}$) values which satisfy eq. (6) with $\Delta G^{*}_{12} = 1125$ cm^{-1} are (1400, 1110), (1125, 1125) and (800, 1100). With them, for the given $\bar{H}_{13}$ and for $r=2$, eqs. (3) and (4) yield $|H_{23}| = 355$, 335 and 310 cm^{-1}, respectively, or larger if $k_{13}^{\rm super}/k_{12}$ at 20°C exceeds 5.

At 20°C, eq. (1) yields higher k_{12}'s than eq. (9) when $|H_{12}| \geqslant 69$ cm^{-1}. With the large H_{12}'s estimated above (597/7, 545/7, 420/7 cm^{-1}, respectively, for $r=7$) one sees that the k_{12} calculated from eq. (1) is comparable with or slightly more than that calculated from eq. (9). Use of eq. (10) instead of eq. (8) yields $\Delta G^{*}_{12} = 760$ cm^{-1} instead of the 850 cm^{-1} found above. Examples of the infinite number of ($\lambda_{12}, \Delta G^{0}_{12}$) pairs consistent with this ΔG^{*}_{12} are found from eq. (6) to be (1200, 710), (760, 760) and (300, 655), all in cm^{-1}. The results for $\Delta E^{\rm vert}_{12}$, given as $\Delta G^{0}_{12} + \lambda_{12}$ in eq. (4), are seen to be virtually unchanged from those arising from eq. (8). The $|H_{12}|$'s obtained from eq. (8) for $r=2$, namely, 355/2, 335/2 and 310/2 cm^{-1}, respectively, all exceed the "critical" value of 69 cm^{-1}, and so require that eq. (10) be used instead of (8). The estimate of ΔG^{*}_{12} based in eq. (10), and the resulting estimates of ($\lambda_{12}, \Delta G^{0}_{12}$) and of H_{23}, are independent of r, since the latter is absent in eq. (10). The new $|H_{23}|$'s corresponding to the above ($\lambda_{12}, \Delta G^{0}_{12}$) pairs are 580, 515 and 410 cm^{-1}, respectively. They are close to those obtained earlier from eq. (8) for $r=7$.

4. Energetics

In order to construct a free energy level scheme for the quinone-free *Rb. sphaeroides* reaction center we use the following data, where the superscript T denotes a triplet→triplet reaction: From delayed fluorescence data the free energy change ΔG^{0}_{13} from an excited state ^{1}BChl$_2^*$ to the radical pair BChl$_2^+$BPh$^-$ is −0.26 eV [20]. The decay of ^{3}BChl$_2^*$BPh proceeds via formation of the triplet BChl$_2^+$BPh$^-$, followed by intersystem crossing to the singlet BChl$_2^+$BPh$^-$ and then via an internal electron transfer it forms the ground state BChl$_2$BPh. From high-field magnetic data and with this mechanism the free energy $\Delta G^{0\rm T}_{13}$ of formation of the triplet BChl$_2^+$BPh$^-$ from ^{3}BChl$_2^*$BPh is found to be 0.17 eV [21]. From the temperature dependence it was also concluded that this $\Delta G^{0\rm T}_{13}$ is essentially also equal to $\Delta H^{0\rm T}_{13}$ [21]. A study of the temperature dependence of the recombination dynamics at low magnetic fields [22], in conjunction with related data [23], yielded the value, $\Delta G^{0\rm T}_{13} = 0.15$ to 0.17 eV [22], depending on the role played by nuclear spin polarization. This $\Delta G^{0\rm T}_{13}$ was found to be almost temperature independent between 77 K and room temperature [22]. The singlet–triplet radical pairs have essentially the same energy, the splitting being very small. There is an entropic difference, due to the 1:3 ratio of the degeneracies of the pairs, giving rise to a free energy difference of $RT \ln 3 = 0.03$ eV.

From these results the free energy of the singlet $^1\mathrm{BChl}_2^*$ minus that of the triplet state $^3\mathrm{BChl}_2^*$ is 0.46 eV. This value may be compared with another result:

Absorption and fluorescence data for $^1\mathrm{BChl}_2^*$ [24] and phosphorescence data [25] for $^3\mathrm{BChl}_2^*$ yield an energy difference for $^1\mathrm{BChl}_2^*$ minus $^3\mathrm{BChl}_2^*$ equal to 0.43 eV [21]. Upon adding the $RT \ln 3$ term for the triplet, there results a contribution of 0.46 eV to the corresponding free energy difference. This result is the same as the value of 0.46 eV obtained above for the free energy difference. Thus, there is little (if any) *configurational* contribution to this singlet–triplet free energy difference of the two BChl_2^* states.

There are further details, which depend on the interpretation of the data on delayed fluorescence: The fluorescence of $^1\mathrm{BChl}_2^*$ shows non-exponential behavior, there being at least three lifetimes [20,24,26,27]. In one interpretation of the non-exponential behavior [20] it is assumed that a second BPh participates. In another [24,26,27] it is assumed, instead, that there is a successive formation of an "unrelaxed" and a "relaxed" state of $\mathrm{BChl}_2^+\mathrm{BPh}^-$ from $^1\mathrm{BChl}_2^*\mathrm{BPh}$. In this case ΔG_{13}^0 is found to be about -0.19 eV for the formation of an "unrelaxed" $\mathrm{BChl}_2^+\mathrm{BPh}$ in the quinone-free system [#2] [26]. Subsequent formation of a "relaxed" state adds about -0.05 eV to this ΔG_{13}^0 [24]. The resulting value of about -0.24 eV for the quinone-free system is close to the -0.26 eV obtained in ref. [20]. A marked temperature effect was reported in ref. [24] for the ΔG_{13}^0 for the formation of the "unrelaxed" state, its value at 80 K being only -0.05 eV for the quinone-reduced system, as compared with a value of -0.16 eV at 295 K for the same system [24].

The formation of this $^3\mathrm{BChl}_2^*$ from the radical pair triplet was seen above to have a $\Delta G_{3\to 1}^{0\mathrm{T}}$ of about 0.17 eV [21,22]. Since this reaction is also activationless [7], we have $\lambda_{13} \approx 0.17$ eV. (It may be added, however, that for a reaction to be activationless it is not essential that $-\Delta G^0 = \lambda$, but rather that these quantities be fairly close.)

The temperature independence of the singlet–triplet splitting of the radical pair $\mathrm{BChl}_2^+\mathrm{BPh}^-$ in quinone-free systems has been used to infer that the free energy of formation $\Delta G_{32}^0 (= -\Delta G_{23}^0)$ of $\mathrm{BChl}^-\mathrm{BPh}$ from $\mathrm{BChl}\,\mathrm{BPh}^-$, is at least 0.23 eV [8]. This result assumes a chemical intermediate BChl^- and also that the exchange integral J for the BChl_2^+–BChl^- interaction is 0.5 cm^{-1} [8,18,28]. The actual value of J_c is not really known, only estimated [18,28]. If it were 0.1 cm^{-1} the ΔG_{32}^0 estimate would be about 0.035 eV smaller. Inasmuch as ΔG_{13}^0 is -0.26 eV [20] it can be seen from these results that ΔG_{12}^0 could be $\geqslant -0.07$ eV, depending on the value of J_c. There is a further complication in such lower bound calculations of $-\Delta G_{13}^0$: If the situation is as depicted later in fig. 2, there is no BChl^- as such in the vicinity of the avoided intersection of curves 2 and 3 in fig. 2, and so some modification of the lower bound calculation would be required.

We shall need in section 5 for the superexchange mechanism an estimate of $\Delta G_{23}^0 + \lambda_{23}$, where $\Delta G_{23}^0 = -\Delta G_{32}^0$ and ΔG_{23}^0 is for the formation of the "relaxed" $\mathrm{BChl}_2^+\mathrm{BPh}^-$. We saw above, using a superexchange mechanism and solving eq. (5) for ΔG_{12}^*, that with a ratio $k_{13}^{\mathrm{super}}/k_{12} = 5$, a range of values of the $(\lambda_{12}, \Delta G_{12}^0)$ pair was possible. For $r \approx 7$ a mean value of $\lambda_{12} + \Delta G_{12}^0$ pairs cited earlier based on eqs. (8) and (10), is about 1500 cm^{-1}, if the superexchange were dominant, and a mean of λ_{12} is about 750 cm^{-1} and that of ΔG_{12}^0 is also about 750 cm^{-1}. ΔG_{13}^0, i.e. $\Delta G_{12}^0 + \Delta G_{23}^0$, equals -0.26 eV [20] for the relaxed state. If λ_{23} is approximately the mean of λ_{13} (roughly 0.17 eV) and λ_{12}, then the relevant $\Delta G_{32}^0 + \lambda_{23}$ is estimated from such results to be roughly 4000 cm^{-1}. This quantity will be denoted by $\Delta E_{32}^{\mathrm{vert}}$, and is indicated in fig. 1a.

5. Effect of large H_{23} on singlet–triplet splitting

The singlet–triplet splitting of the radical pair $\mathrm{BChl}_2^+\mathrm{BPh}^-$ is experimentally found to be about 10^{-3} cm^{-1} [3,14]. In a superexchange interpretation of this splitting the energy of the singlet or triplet state of the pair can be obtained using a partitioning argument [6]. Namely, for either the singlet or the triplet state we have

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[#2] Based on the initial amplitudes of the two slowest components (table 2 of ref. [26]). Using all three initial amplitudes, $\Delta G_{13}^0 \approx -0.15$ eV (table 2 of ref. [26]). An interesting pertinent discussion is given in ref. [26].

$$(\bar{H}_{11}-E)(\bar{H}_{33}-E)=\bar{H}_{13}^{2}\,, \qquad (11)$$

where

$$\bar{H}_{ii}=H_{ii}-H_{i2}^{2}/(H_{22}-E)\,, \quad i=1,3 \qquad (12)$$

and

$$\bar{H}_{13}=H_{13}+H_{12}H_{23}/(H_{22}-E)\,. \qquad (13)$$

Each of the H_{ii} terms in these equations varies with the nuclear configuration (the H_{ij} terms with $i\neq j$ vary only weakly so). The energy E in eq. (11) is obtained for the radical pair in the first approximation by replacing $\bar{H}_{11}-E$ by $\bar{H}_{11}-\bar{H}_{33}$ when $\bar{H}_{11}-\bar{H}_{33} \gg \bar{H}_{13}$[#3]:

$$E\approx\bar{H}_{33}-\bar{H}_{13}^{2}/(\bar{H}_{11}-\bar{H}_{33})\,. \qquad (14)$$

In eq. (14) $\bar{H}_{33}$ may be a relatively small contributor [29] to the singlet–triplet splitting within the present framework of a three-state approximation. (Still another contribution to the singlet–triplet splitting may arise from a BChl* [14].) We focus our attention on the last term in eq. (14). For the singlet–triplet splitting the $\bar{H}_{13}$ needed for the singlet state is obtained from eq. (13) with $\bar{H}_{22}-E=\bar{H}_{22}-\bar{H}_{33}$, evaluated at the equilibrium configuration of the radical pair (rp). The latter is the $\Delta E_{32}^{\rm vert}$ in fig. 1a and in section 3. This $\bar{H}_{13}$ is denoted by $\bar{H}_{13}^{\rm rp}$. For the transition state of the superexchange mechanism $\bar{H}_{13}$ is the $\bar{H}_{13}$ in eq. (2), denoted now by $\bar{H}_{13}^{\rm super}$, and is obtained from eq. (13) by setting $\bar{H}_{22}-E=\bar{H}_{22}-\bar{H}_{11}$, evaluated at the equilibrium configuration for state 1. The latter is the $\Delta E_{12}^{\rm vert}$ in section 3 and fig. 1a. H_{13} in eq. (13) is treated as negligible and the dependence of H_{12} and H_{23} on energy is also negligible [6]. Thereby, $\bar{H}_{13}^{\rm rp}/\bar{H}_{13}^{\rm super}\approx \Delta E_{12}^{\rm vert}/\Delta E_{32}^{\rm vert}$. Values for $\Delta E_{12}^{\rm vert}$ in section 3 based on eqs. (8) and (10) varied from 1000 to 2000 cm^{-1}. A value of 1500 cm^{-1} is used in eq. (15) below. The $\Delta E_{32}^{\rm vert}$ was estimated in section 4 to be roughly 4000 cm^{-1}.

We thus have

$$\frac{\bar{H}_{13}^{\rm rp}}{\bar{H}_{13}^{\rm super}}\approx\frac{\Delta E_{12}^{\rm vert}}{\Delta E_{32}^{\rm vert}}\approx\frac{1500}{4000}\,. \qquad (15)$$

An $\bar{H}_{13}^{\rm super}$ of about 25 cm^{-1} was needed in section 3 to fit the kinetic data to a superexchange mechanism and so from eq. (15) $\bar{H}_{13}^{\rm rp}$is readily estimated.

The $\bar{H}_{11}-\bar{H}_{33}$ in eq. (14) is evaluated at the equilibrium nuclear configuration for the relaxed radical pair and equals $\Delta G_{31}^{0}+\lambda_{13}$, evaluated at that relaxed configuration. Using the estimates of ΔG_{13}^{0} and λ_{13} in section 3 and 4 ($\Delta G_{31}^{0}=-\Delta G_{13}^{0}$) this $\bar{H}_{11}-\bar{H}_{33}$ ($=\Delta G_{13}^{0}+\lambda_{13}$) is about 0.4 eV or 3200 cm^{-1}.

One finds in this way that for the singlet state of the radical pair at its equilibrium configuration the $\bar{H}_{13}^{2}/(\bar{H}_{11}-\bar{H}_{33})$ term in eq. (14) is about 0.03 cm^{-1}. In calculating the corresponding term for the triplet state we note that $\bar{H}_{11}-\bar{H}_{33}$ is of opposite sign, about -0.17 eV [21], when computed at the equilibrium geometries of each state. Its value at the equilibrium geometry of the radical pair (state 3) is expected either to be somewhat negative or somewhat positive in order to be consistent with an activationless [7] recombination of the radical pair to form the ^{3}BChl$_{2}^{*}$. Thus, even if $\bar{H}_{13}$ for the triplet state were somewhat less than for the singlet state, we infer from the above results that the singlet–triplet splitting predicted by the superexchange mechanism is at least comparable to the energy shift of the singlet state, i.e. about 0.03 cm^{-1}. (We discount the likelihood of an accidental cancellation of two large numbers, but do not rule out this possibility.) This value of 0.03 cm^{-1} may be contrasted with the experimental value [3,7] for the splitting of about 10^{-3} cm^{-1}.

This connection between the postulated superexchange rate and the singlet–triplet splitting is seen to be rather direct. The rate supplies a value of $\bar{H}_{13}$ for the equilibrium configuration 1 and, with a change only of the vertical energy, as indicated above, one obtains via eq. (14) a minimal value for the resulting predicted singlet–triplet splitting of the radical pair, BChl$_{2}^{+}$BPh^{-}.

The value in eq. (15) of a factor of only about 2.5 for the ratio of energy denominators $\Delta E_{32}^{\rm vert}/\Delta E_{12}^{\rm vert}$ is a result of the consistency test given by eqs. (8) and (10). It contrasts with a large value which might have been (and has been) postulated in the literature.

We have seen above an apparent inconsistency between the superexchange mechanism and the observed singlet–triplet splitting. Of course, the interpretation of the singlet–triplet splitting may be

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[#3] In eq. (7) of ref. [6] the sign of the last term, and in eq. (10) there that of the rhs, should be changed. The discussion there is unaffected since only absolute values were considered.

more complicated than that considered here or in refs. [6–8,18,28]. BChl* and other excited states and miscellaneous vibronic effects could contribute. Nevertheless, within the framework of the models in refs. [6–8,18,28] there is a real discrepancy in the superexchange case.

6. Possible $BPh^- \rightarrow BChl$ charge transfer band

The charge transfer band arising from an electron transfer from BPh^- to BChl would have a maximum absorption $h\nu_{max}$ at

$$h\nu_{max} = \Delta G^0_{32} + \lambda_{23} \equiv \Delta E^{vert}_{32} \ . \qquad (16)$$

Using the estimate in section 4, $h\nu_{max}$ would be ≈ 4000 cm^{-1}, if a superexchange explanation for the initial electron transfer were valid.

The typical H_{23} in section 3 was, assuming the superexchange mechanism, about 500 cm^{-1}, which is large, giving rise thereby to a significant intensity of a charge transfer band.

7. Alternative mechanism: non-adiabatic/adiabatic mechanism

One possible alternative mechanism for the initial step is depicted in fig. 2. In it the transition at the first intersection is rate controlling and non-adiabatic, while that at the second intersection is adiabatic. In this mechanism $BChl^-$ does not exist (cf. fig. 2) in the way it did for the chemical intermediate mechanism (fig. 1b), and so might not be observed spectrally. We have made some numerical quantum mechanical wavepacket calculations for this case [29], introducing an approximation to allow for the multidimensionality of the motion, to avoid erroneous oscillations that would be found in a purely one-dimensional conservative system. Some results are discussed later.

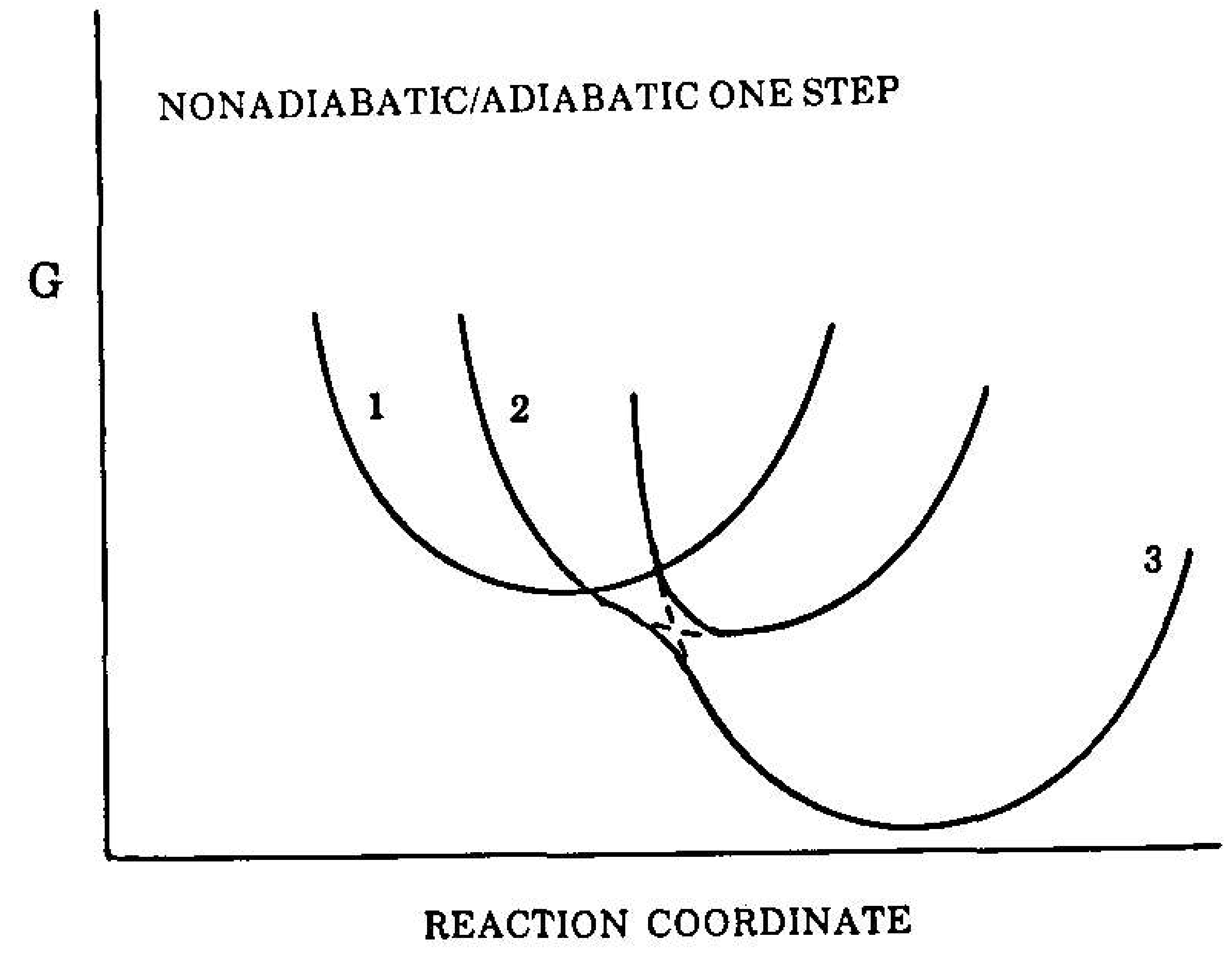

Fig. 2. Plot of free energy G versus reaction coordinate for the various electronic configurations described in the caption to fig. 1. The plot is for a single-step non-adiabatic/adiabatic mechanism in which there is a transition from state 1 to state 3. There is no formation of $BChl^-$ with any appreciable temporal existence. At the "intersection" of curves 2 and 3, the dashed lines are the "diabatic curves" and the solid lines the adiabatic ones.

We have noted in section 4 that the current energetics are such that ΔG^0_{12} may be slightly negative. If $\Delta G^0_{12} \approx -150$ cm^{-1} and $\lambda_{12} \approx 600$ cm^{-1}, the value of ΔG^*_{12} in eq. (6) is 85 cm^{-1}. The actual effective barrier is lowered from this value by two quantities: (a) the zero-point energy (a value for $h\nu \approx 100$ cm^{-1} is a common assumption [13,30,31] to account for some temperature-dependent data), and (b) one-half the splitting at the intersection, H_{12}, the effects (a) and (b) together amounting to roughly 65 cm^{-1}. The net classical free energy barrier is then seen to be very small. The latter contrasts with the value 250–500 cm^{-1} inferred in ref. [8], the difference being due to several factors, including the constraint imposed by the consistency test and an inclusion of the zero-point energy and splitting effects. The difference is significant, since the new value would permit the above alternative non-adiabatic/adiabatic mechanism to be activationless. Indeed, using values of λ and ΔJ consistent with the low-temperature rate constant, the wave packet calculations [29] just mentioned yield a negative temperature coefficient for the reaction.

Elsewhere [32], we have shown that the various energetics and matrix elements used in this mechanism lead to reasonable results for the rate of the low-temperature formation of $^3BChl_2^*$ from the $BChl_2^+ BPh^-$ radical pair and also for the singlet–triplet splitting of $BChl_2^+ BPh^-$.

There is yet another possible mechanism which should be considered: If, for reasons not yet clear the dependence of the electron transfer rate on separation distance were considerably smaller than that typically [15] found, there would be a possibility of an electron transfer from $^1BChl_2^*$ to BPh through the intervening material, with no role for the BChl. However, the small singlet–triplet splitting of the BChl$_2^+$BPh$^-$ appears to eliminate this possibility also: Due to the presence of the ^{1}BChl$_2^*$ state the singlet BChl$_2^+$BPh$^-$ state would be shifted by an amount $\bar{H}_{13}^2/(\bar{H}_{11}-\bar{H}_{33})$, evaluated at the equilibrium geometry of the radical pair. But $\bar{H}_{13} \approx 25$ cm^{-1} to account for the initial rate, and $\bar{H}_{11}-\bar{H}_{33}=\lambda_{13}-\Delta G_{13}^0 \approx 0.43$ eV (i.e. 0.17+0.26). Thereby, this shift is approximately 0.2 cm^{-1}. The triplet BChl$_2^+$BChl$^-$ is shifted by the ^{3}BChl$_2^*$BChl state, but once again we consider it unlikely that there is an accidental cancellation of two large numbers. Since the observed splitting is 10^{-3} cm^{-1} this mechanism which does not involve BChl seems to be eliminated.

8. Effect of applied electric fields on the initial steps

Dutton and co-workers have studied the effect of applied electric fields of as much as 0.15 eV/nm or more on close-packed reaction centers in Langmuir–Blodgett monolayer films [33]. The effect on the quantum yield was studied and pronounced effects were obtained [33]. (Interesting electric field effect studies on randomly oriented reaction centers are described in ref. [34].) It would be useful to determine, in addition, the effect of the field on both the rate and extent of the *initial* electron transfer. Assuming for concreteness the energetics and mechanisms discussed earlier, we consider the implications for such an effect, and the possibility that it might distinguish among the superexchange, the non-adiabatic/adiabatic one-step mechanism, and the non-BChl mechanism. The effects in each case tend to be in the same direction (inhibition), so any difference would tend to be of a quantitative nature. We shall suppose that the C_2 axis of each close-packed reaction center is oriented parallel to and antiparallel to the field [33].

Along the C_2 axis of the reaction center the component of the distance from the center of the BChl$_2$ (or indeed, essentially of either member of the pair) to that of the BChl is about 0.41 nm [35]. The corresponding distance from BChl$_2$ to the (L side) BPh is 1.48 nm. The imposition of an adverse field of 0.15 eV/nm parallel to the C_2 axis makes an adverse contribution to ΔG_{12}^0 of 0.06 eV and to ΔG_{13}^0 of 0.22 eV. (However, the value depends on the actual value of the dielectric constant and dielectric properties of the reaction center monolayer system [33,36,37]. An interesting study of the effect of an electric field on the recombination rate of BChl$_2^+$ and the quinone anion has been reported by Feher et al. [36].)

The expected electric field effect depends on the free energy changes and hence on the interpretation of the delayed fluorescence data, for example on the possible existence of an unrelaxed state of BChl$_2^+$BPh$^-$. If the ΔG_{13}^0 for an unrelaxed BChl$_2^+$BPh$^-$ at room temperature were about -0.19 eV in the quinone-free system [26], its value in the presence of this applied field would now become $+0.03$ eV. If $\Delta G_{12}^0 \approx -0.02$ eV in the absence of the field, as assumed earlier $\Delta G_{12}^0 \approx 0.04$ eV in the presence of an adverse field of 0.15 eV/nm, and so now $\Delta G_{12}^0 \approx \Delta G_{13}^0$. Thus, with this applied field there would now be a possibility of observing a depletion of BChl. If, the superexchange mechanism or the mechanism having no BChl involvement prevailed, a depletion of BChl would still not be observed. Incidentally, a difficulty in explaining the electric field effect on the various data [33,34] assuming a superexchange mechanism has been reported [18,28], though the reason has not yet been established. The electric field effect for the one-step non-adiabatic/adiabatic mechanism depends in part on the presently uncertain value of ΔG_{12}^0.

If the unrelaxed/relaxed interpretation [24,26,27] of the delayed fluorescence were correct, so that $\Delta G_{13}^0 \approx -0.05$ eV at 80 K [24], the ΔG_{13}^0 in the presence of an adverse electric field of 0.15 eV/nm would be $+0.17$ eV. The effect of the electric field on the reaction rate and on BChl depletion would be dramatic. Even smaller fields would have a large effect at 80 K.

9. Conclusions

We have seen that to satisfy the consistency test the superexchange mechanism requires large individual electron transfer matrix elements, and that there is a discrepancy between the superexchange-predicted and the experimental singlet–triplet splitting for the $BChl_2^+ BPh^-$ radical pair. Either a more elaborate treatment of the latter than that previously given in refs. [6,8,18,28] or some other mechanism, such as the single-step non-adiabatic/adiabatic mechanism depicted in fig. 2, may prevail. We have also seen that a mechanism in which there is no BChl involvement leads to much too large a singlet–triplet splitting for the $BChl_2^+ BPh^-$ pair. If the single-step non-adiabatic/adiabatic mechanism is correct the application of a strong adverse electric field might permit the observation of a depletion of BChl in experiments such as those in refs. [4,13]. In any event the effect of such an adverse electric field on the rate of the initial step can provide valuable additional information, particularly at low temperatures.

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