6

The Nature of the Special-pair Radical Cation Produced by Primary Charge Separation During Photosynthesis

Jeffrey R. Reimers and Noel S. Hush

6.1

Introduction

Today’s photovoltaic devices are designed around the use of semiconductor materials made either from inorganic semiconductors such as silicon or from organic polymers. These devices harvest light, initiate primary charge separation, and provide carrier diffusion to external electrodes. Photosynthesis in plants and bacteria involves in a key step an analogous photovoltaic mechanism that oversees the harvesting of optical energy and its conversion into electrical energy, en route to long-term storage as chemical energy. This naturally occurring process operates using single-molecule, nominally insulating devices rather than the 100-nm or larger semiconducting objects found in applications photovoltaics. A major goal of the current research of the Molecular Electronics Group at the University of Sydney is to understand the processes of the natural light-harvesting systems. Special emphasis is placed on the mechanisms of primary and secondary charge separation, with the long-term goal being the design of new biomimetic photovoltaic technologies that directly emulate elements of natural photosynthetic systems. To this end, artificial antenna systems and reaction centers have been designed by Crossley [1–3], while details of the mechanism of primary charge separation have been elucidated by Reimers and Hush [4–17]. Herein, this latest work is reviewed, building a thorough picture of the central role of the primary electron donor.

6.2

The Special Pair

During natural photosynthesis, light energy is harvested in manifold types of antenna systems and funneled to the special pair dimer P of chlorophyll or bacteriochlorophyll molecules that initiate primary charge separation, ejecting an electron to become a dimer radical cation. Significant differences in this process
occur between the major classes of photosynthetic proteins – those found in purple bacterial systems as well as in the PSI and PSII systems of cyanobacteria and green plants. To date, very much more spectroscopic and structural information is available concerning the reaction centers of purple bacteria. While our studies have concentrated mainly on the purple bacteria *Rhodobacter (Rb.)* *sphaeroides*, we believe that the results obtained can readily be generalized to account for the differences observed in PSI and PSII. Much more research is required in order to establish this, however, and the story told here is largely that of understanding the nature of the special pair in bacterial photosynthesis.

The most interesting feature of the special pair is actually that it is a dimer. The two halves of the dimer, known as $P_L$ and $P_M$, interact with each other through an electronic coupling $J$, and this coupling is very important in determining the chemical and spectroscopic properties of the system and hence is central to the charge-separation process. It takes on slightly different values before and after charge separation, but this variation is small and not of concern here. Hence, by examining the properties of the final charge-separated state $P^+$, much is learned about the processes that control the charge separation. $P^+$ is a dimer radical cation, an unusual chemical species whose properties are unlike those of typical organic or biological molecules, being in fact more similar to those of inorganic charge-transfer complexes such as the famous Creutz-Taube ion [18].

A characteristic and very striking feature of $P^+$ is the appearance [19–21] of dominant transitions in the vibrational infrared spectrum that do not correspond to transitions observed in either the infrared spectrum of the neutral or radical-anion state of the bacteriochlorophyll monomers or the spectrum of the neutral special pair. Shown in Fig. 6.1 is the observed [21] infrared difference spectrum of the cation radical of *Rb. sphaeroides* less the spectrum of the neutral species. Only vibrational modes whose spectrum differs upon oxidation appear in this difference spectrum. Some modes simply change in frequency, giving rise to sharp positive peaks (at the vibration frequency of the cation radical) with close-lying neighboring sharp negative peaks (at the vibration frequency of the neutral species). However, most of the signal observed in the difference spectrum below 1800 cm$^{-1}$ is associated with new transitions that occur only in the cation-radical species.

Vibrational infrared absorptions are associated with molecular motions that rearrange the internal charge distribution, hence producing a fluctuating dipole moment that interacts with electromagnetic radiation. The new transitions in $P^+$ arise from vibrations that cause the +ve charge (the *electron hole*) to be moved between the two monomers, producing large changes in dipole moment and hence very intense infrared absorptions. They are known in the inorganic electron transfer literature as *phase phonon* lines [22–27]. Our calculations [5] have shown that the most intense of these lines are associated with antisymmetric combinations of local motions on each bacteriochlorophyll, like those shown in Fig. 6.2, that involve expansion and contraction of the macrocyclic rings. These motions are activated when a monomeric bacteriochlorophyll is oxidized. Bacteriochlorophylls are related to porphyrin, a molecule that has a
Fig. 6.1 Observed [21] FTIR difference spectrum (£ is the molar extinction coefficient, ν is frequency) of P⁺ minus P for Rb. sphaeroides showing a broad electronic absorption among the sharp vibrational transitions.

Fig. 6.2 Two monomeric bacteriochlorophyll vibrational modes whose antisymmetric combinations are strongly coupled to the hole-transfer process in the special-pair radical cation, as obtained from density-functional calculations [5].
center of symmetry allowing its vibrations to be classified as of type \( g \) (symmetric) or type \( u \) (antisymmetric). It is well known that only \( u \) modes can give rise to infrared absorptions, and hence the absence of these types of modes in the spectrum of monomers and neutral special pair is readily understood. The most active mode involves the local motion at 1652 cm\(^{-1}\), shown in Fig. 6.2, and is of \( g \) type; that such a mode should dominate the spectrum of the radical cation is quite remarkable.

Another striking feature of the spectrum of \( P^+ \) shown in Fig. 6.1 is the appearance of the broad electronic transition centered at 2700 cm\(^{-1}\) among the vibrational infrared spectrum. The lowest-energy electronic transition in most organic molecules, including chlorophylls and bacteriochlorophylls, occurs either in the visible region of the spectrum or beyond into the ultraviolet, and it is typical to scan for such spectra in the 800–200 nm region. For \( P^+ \), however, the lowest-energy electronic transitions occur in the 5000–2000 nm region, more usually depicted as the infrared wavenumber range of 2000–5000 cm\(^{-1}\). Further intense transitions are also found in the 1250 nm range near 8000 cm\(^{-1}\). All of

![Image of absorption spectrum]

**Fig. 6.3** Observed absorption spectrum from the IR region to the visible region for *Bl. viridis* (\( c \) is the molar extinction coefficient, \( v \) is frequency, \( \lambda \) is wavelength). The Q bands are distributed over various chromophores, while the hole-transfer and trip-doublet bands are localized on the special pair (solid line) from FTIR difference spectroscopy [19] (with normalization taken from [28]), with the noise around 3400 cm\(^{-1}\) attributable to modulation of water OH-stretch vibrations (dashed line) (from [28]).
these transitions are unprecedented in the spectroscopy of monomeric or neutral dimeric species. Some characteristic spectra are shown in Fig. 6.3. In this figure, the spectrum is not shown in the typical fashion as a plot of the extinction \( \varepsilon \) as a function of wavelength \( \lambda \) but rather as a plot of \( \varepsilon/\nu \) as a function of frequency \( \nu \). In this fashion, all influences of the properties of light on the measured absorption are removed, revealing the inherent molecular properties. The areas and bandwidths apparent in this representation provide true indications of the relative intensities and the vibrational relaxation processes that dominate the spectrum and reveal the photophysical and chemical properties of \( \text{P}^+ \).

The absorption at 8000 cm\(^{-1}\) was first observed [29] in 1969 but was never assigned. We have shown that this absorption is due to a \textit{trip-doublet} transition [14]. This is in itself a rather unique transition that corresponds not to the excitation of a single electron, as is the norm in molecular spectroscopy, but rather to the synchronous excitation of two electrons. In effect, the transition can be described as a singlet-to-triplet transition on one of the halves of the dimer cation radical assumed to be in its neutral state, intensified by the presence of the nearby other half, assumed to be in its cation-radical state. This process is analogous to the well-known effect caused by the presence of molecular oxygen in facilitating singlet-to-triplet absorptions in organic molecules. While two-electron excitations are formally forbidden in single-photon spectroscopy, they become allowed due to mixing with singly excited states facilitated by the intermolecular coupling. As the two halves of the special pair are close together and strongly coupled, the intensity of the trip-doublet bands in bacterial photosynthetic systems becomes substantial, making prominent these rather exotic transitions.

### 6.3 The Hole-transfer Band

The broad absorption in the 2000–4000 cm\(^{-1}\) region derives its intensity from the \textit{intervalence hole transfer} band of the dimer cation radical. In one limit known as the \textit{localized diabatic} description [4, 5, 30], this transition can be thought of as taking the electron hole from one of the monomers in the dimer and transferring it to the other. This band is characteristic of mixed-valence inorganic complexes and provides critical information concerning the nature of the system, in this case the nature of the interactions within the special pair as well as key properties associated with the asymmetry of the dimer.

Three different ways in which the hole-transfer absorption may be perceived are shown in Fig. 6.4, a figure that depicts molecular potential energy surfaces for the two lowest-energy electronic states as a function of a generalized nuclear coordinate. The relative intensities of the phase-phonon lines discussed previously are indicative of the projections of this generalized coordinate onto the normal modes of vibration of the radical cation. One way of perceiving the problem is via the localized diabatic picture referred to previously. This involves
Fig. 6.4 Three different representations of the ground-state and first-excited-state electronic potential-energy surfaces for the special-pair radical cation as a function of a generalized antisymmetric nuclear coordinate. Solid lines: localized diabatic surfaces; dots: delocalized diabatic surfaces depicting \( C_2 \) symmetry; dashed lines: Born-Oppenheimer adiabatic surfaces (named GS and HT) obtained by parametric diagonalization of either set of diabatic states. \( \lambda \) is the reorganization energy, \( \delta \) is related to the geometrical displacements between monomeric neutral and cation-radical bacteriochlorophylls, and \( J \) is the electronic coupling between the two chromophores.

states described as \( P^+_l P^*_m \) and \( P^+_l P^*_m \), representing assemblies of neutral and cationic individual dimer halves. It is a simplistic picture as it ignores the effects of the electronic coupling \( J \) that acts between the dimer halves. The effect of this coupling is to attempt to enforce symmetry on the electronic wavefunctions, in much the same way that bonding and antibonding molecular orbitals arise from atomic orbitals for, e.g., the hydrogen molecule. For bacterial photosynthesis, calculations indicate that the bonding orbital has \( B \) symmetry in the pseudo \( C_2 \) point group of the dimer, while the antibonding orbital has \( A \) symmetry. Opposing the electronic coupling, the reorganization energy \( \lambda \) shown in Fig. 6.4 is associated with the desire of the neutral and cationic monomers to adopt different molecular geometries as a function of the generalized nuclear coordinate. Hence the states \( P^+_l P^*_m \) and \( P^+_l P^*_m \) are modeled as (hyper)parabolic surfaces with different equilibrium geometries. Ignoring completely the reorganization energy allows surfaces for the A and B states to be constructed: this is known as the delocalized diabatic picture of the interaction, and these are also shown in Fig. 6.4. In this picture, the charge is equally shared (or delocalized) over both halves of the dimer rather than localized on one half only.

The final way of perceiving the coupling is through the introduction of the Born-Oppenheimer adiabatic approximation [31], as is nearly universally applied in chemistry, to eliminate all direct electronic interactions by, in this case, diago-
nalizing the Hamiltonian obtained using either pair of diabatic states parametrically as a function of the nuclear coordinates. This produces the adiabatic surfaces shown in Fig. 6.4. These are highly anharmonic and it is thus quite difficult to determine the vibrational frequencies and intensities required in order to predict absorption spectra. Further, the key element of the Born-Oppenheimer approximation is the separation of nuclear and electronic motions. Basically, this approach is sensible as the light electrons travel much faster than the heavy nuclei; thus it is possible to determine the nuclear motion in an averaged field of the electrons. However, from Fig. 6.3 it is clear that the energies involved in vibrational excitation are equivalent to those involved in hole-transfer electronic motion, and hence the nuclear and electronic motions are tightly coupled. As a result, the Born-Oppenheimer approximation is a poor starting point for understanding intervalence transfer problems, and Born-Oppenheimer breakdown effects must explicitly be included in any spectral simulation. Born-Oppenheimer-based methodologies are thus poorly suited, with the electronic-nuclear (vibronic coupling) interactions being more readily treated using either diabatic approach.

In the diabatic approaches, one starts by ignoring either the electronic coupling $J$ or the reorganization energy $\lambda$ in order to generate a basis set of mixed vibrational and electronic states that are used to depict the full Hamiltonian of the dimer cation radical. This Hamiltonian must then be constructed and its spectrum determined and compared to the observed absorption spectrum. Naively, the most efficient computational approach is the one whose initial assumptions most closely resemble the system being studied – i.e., if the charge in reality is delocalized, then the delocalized diabatic basis should be used to expand the full Hamiltonian operator, etc.

A third key molecular property is the asymmetry parameter $E_0$. Physically, asymmetry arises as the geometries of the two halves of the dimer are not identical owing to asymmetric interactions with the surrounding protein, while asymmetric electric fields also create a preference for the positive charge to be localized on one side; in the case of bacterial photosynthesis, this side is $P_L$. In our treatment, these manifold effects are incorporated into one parameter that simply indicates the difference in redox potential for oxidation of charge-localized dimer halves. By considering electrochemical measurements for heterodimer mutants in which one bacteriochlorophyll in the special pair is replaced with a bacteriopheophytin, we have recently argued [4] that the experimental value for this quantity is $E_0 = 0.058 \pm 0.020$ eV, though a very wide range of prior estimates have been made. This redox asymmetry aids the reorganization energy in favoring charge localization rather than delocalization.
6.4 Initial Investigations of the Hole-transfer Band

The first efforts [13, 19] made at interpreting the observed intervalence hole-transfer spectrum in terms of molecular properties led to the conclusion that the charge is 95–97% localized on P_L. These analyses were based on analytical formulae rather than full spectral simulation. However, spin-density measurements made at around the same time depicted the hole in Rb. sphaeroides as being actually localized 68% on P_L, in the region midway between full localization (100%) and full delocalization (50%). This provided a major disparity that required explanation as the two scenarios depicted really quite different chemical species. We argued [30] that the analytical expressions used in interpreting the spectra were inadequate in this application as they involved the use of one-electron theory to depict the observed transition moment. According to simple one-electron theory, trip-doublet bands should be forbidden as they correspond to two-electron excitations, and so the very observation of intense trip-doublet absorptions demonstrates the inadequacy of this approximation. Using the localized diabatic approach, we performed [30] numerical spectral simulations to map out likely solution spaces for J, λ, and E_0, concluding that, provided intensity information was ignored, interpretations with as little as 68% of the charge localized on P_L were feasible. The challenge then became to perform quantitative simulations from which accurate values of J, λ, and E_0 could be extracted.

The next approach was to make a priori predictions of the spectrum, performing full spectral simulations. This required independent determination of the values of J, λ, and E_0, as well as the vibrational frequencies and the projections of the generalized nuclear coordinate used in the sketch of Fig. 6.4 onto all of these modes. Alternatively [4, 30], these projections may be represented as vibronic coupling constants, and this is the approach that was finally adopted. All of the vibrational parameters were evaluated [5] using density-functional calculations either for the bacteriochlorophyll monomers, in their neutral and cation-radical states, or for the dimer cation radical, in its ground and first-excited states. The relative values of the vibronic coupling constants were obtained by analysis of the calculated infrared transition moments, which were expressed as an expected term, taken from the calculated spectrum of the neutral dimer, plus a vibronic contribution originating from the coupling between the ground state and hole-transfer excited state of the dimer cation radical. Finally, the absolute values of the vibronic coupling constants were obtained by constraining the total reorganization energy to the previously determined value of λ.

Various attempts were made at the calculation of the main controlling parameters J, λ, and E_0, with varying degrees of success. The reorganization energy λ relates to geometry changes on oxidation and is the easiest quantity to determine reliably. However, quantitative error estimates [4, 5] indicated that the likely accuracy in these calculations is on the order of 0.02 eV or about 10%. The electronic coupling J is difficult to calculate because it is very sensitive to the spacing between the bacteriochlorophylls in the special pair, and the cou-
pling contains a significant dispersive component that, at the present time, is not computationally feasible to evaluate accurately [10]. Early computations appeared to underestimate $J$ by a factor of two, although in light of our more authoritative estimates of the experimental quantity, this factor now appears to be only on the order of 30%. Finally, the redox asymmetry $E_0$ is extremely difficult to calculate, as it is sensitive to long-range, variably screened electrostatic potential influences that require detailed knowledge of the protein structure well beyond that provided by X-ray analysis tools. While local modulation of $E_0$ due to site-directed mutagenesis can be modeled [8], a full, accurate, a priori calculation of the intrinsic quantity is not feasible.

We hence sought to determine $J$, $\lambda$, and $E_0$ from analysis of experimental data. A method for extracting $J$ and $E_0$ from the combination of observed electron-spin distributions and redox potentials for a series of mutants of $Rb. sphaeroides$ had been generated by Allen et al. [32–34], but the results obtained were inconsistent with the observed hole-transfer band maximum. We improved [9] the theory used to interpret this data and added to it allowance for the reorganization energy $\lambda$. Unfortunately, the available experimental data was insufficient to allow for the determination of $\lambda$, but we determined the somewhat realistic values of $J=0.18\pm0.03$ eV and $E_0=0.136\pm0.030$ eV.

It was clear that only couplings at the lower limit of the deduced range could possibly be consistent with the observed FTIR spectra, and hence we performed

---

**Fig. 6.5** Observed [21] difference spectrum and the predicted [7] hole-transfer electronic band contour and phase-phonon line distribution. The predictions cannot account for the feature observed at 2200 cm$^{-1}$. 
preliminary spectral simulations [7] using \( J = 0.15 \text{ eV} \), \( E_0 = 0.14 \text{ eV} \), and the B3LYP-calculated value [5] of \( \lambda = 0.20 \text{ eV} \). It is necessary to restrict the number of vibrational modes used in the simulations, and we initially chose the 91 most active modes of the bacteriochlorophyll dimer. It was clear [7] that the calculations had converged as a function of the number of modes included and the degree of excitation allowed in each mode. The calculated spectrum (normalized to match the observed intensity) is shown in Fig. 6.5, where it is compared to the observed FTIR difference spectrum. Major success was achieved in that the location of the band maximum near 2700 cm\(^{-1}\), the width of the band, and the details of the high-frequency tail were well reproduced by these a priori calculations. Indeed, all of these properties are very sensitive to \( J \), \( \lambda \), and \( E_0 \) as well as the nature of the partitioning of the coupling among the vibrational modes, and it was clear that, for the first time, an authoritative interpretation of the spectrum could be advanced. However, this preliminary simulation provided no indication of the origin of the shoulder observed near 2200 cm\(^{-1}\) to the red of the main hole-transfer absorption band. Proper quantitative analysis of the spectrum demanded a proper explanation of this feature.

6.5 Identification of the SHOMO to HOMO Band

Previously [12], we had argued that the 2200 cm\(^{-1}\) shoulder was not part of the same spectral system as the main hole-transfer band. This conclusion was based on the observation that as the chromophore is varied and as mutations are introduced in the surrounding protein, the spacing between the shoulder and main band, as well as the relative intensity ratio, changes erratically. Initial suggestions [12] as to the origin of this band proved [6] incorrect. Recently [6], we have assigned this shoulder to a second-highest occupied molecular orbital (SHOMO) to highest-occupied molecular orbital (HOMO) transition. This assignment is based on ab initio and density-functional calculations of the energy of this transition as a function of chromophore leading to qualitative interpretations of the shape of the intervalence hole-transfer bands for a variety of bacterial, PSI, and PSII photosystems [6], in conjunction with quantitative calculations of the band intensity [4]. The possibility of such a transition contributing to the spectrum had long been considered, but the possibility had been eliminated, as this transition would also occur at about the same energy in monomeric cation radicals, yet absorption is registered only in dimeric cation radicals. Our interpretation of this is that the SHOMO to HOMO transition is intrinsically very weak and hence is not observed in monomeric cation radicals, but in the special pair it is vibronically coupled to the hole-transfer excited state (with which it is nearly degenerate) and its entire energy originates from this coupling. Subsequently [5], we evaluated the vibronic coupling constants between these states and showed [4] that the predicted values were consistent with the observed stolen intensity.
6.6 Full Spectral Simulations Involving all Bands

A further complication demanded by this picture is that there must exist a fourth low-lying electronic state obtained by combining the SHOMO to HOMO excitation with the hole-transfer excitation, and this state must be strongly coupled to the other states. Hence, quantitative analysis of the FTIR difference spectrum of $P^+$ requires at least the inclusion of these four electronic states. Also, our preliminary calculations [7] included explicitly only antisymmetric modes, the modes that are actively coupled to the electron transfer, but the shape of the spectrum is affected also by symmetric modes as these broaden the spectrum due to the Franck-Condon principle. We thus generated a revised computational procedure [4] that included, at a mutually consistent level, four electronic states, 50 antisymmetric modes, and 20 symmetric modes. A new representation of the vibronic Hamiltonian for electron transfer problems was then introduced [4] using the delocalized diabatic representation, and a feasible computational scheme based on time-dependent quantum mechanics was developed to evaluate the absorption spectrum. In all, up to $4 \times 10^9$ individual vibrational levels were included in these spectral simulations. The calculated spectrum was expressed as a function of seven adjustable parameters, and the observed spectrum was fitted using this model.

Only one region of the parameter space gave acceptable solutions, and the final calculated spectrum is compared to the observed one in Fig. 6.6. Note that in this figure both spectra have been broadened to a resolution of 300 cm$^{-1}$ in order to eliminate from the observed FTIR difference spectrum the contributions from vibrational modes whose frequency simply changes upon oxidation of the special pair. In this way, all of the intensity observed at frequencies below 1800 cm$^{-1}$

![Graph](image_url)

**Fig. 6.6** Observed [21] FTIR difference spectrum $P^+$ minus $P$ for *Rb. sphaeroides* compared to the fitted spectrum [4], all broadened to 300 cm$^{-1}$ resolution.
can be attributed to phase-phonon activity, allowing for direct comparison with the calculated enhancement of the vibrational spectrum upon oxidation. Very good agreement is found between the calculated and observed spectra.

The fitted parameters are given in Table 6.1, where they are compared to alternate estimates. In all cases, the fitted parameters appear to be highly realistic estimates. This indicates that the solution obtained does not arise from some overdetermined fit but actually does depict the physical reality.

For the most important parameters \( J \), \( \lambda \), and \( E_0 \), the best current alternate estimates come from the recent analysis of extensive ENDOR and redox data of Müh et al. [35]. This work takes our equations [9] for the analysis of data of this type and applies them to a vastly enhanced experimental dataset. Unfortunately, unique values for \( J \), \( \lambda \), and \( E_0 \) still cannot be obtained, and in Table 6.1 two indicative solutions are provided, labeled No. 1 and No. 2. Our fitted values for \( J \) and \( \lambda \) are within the range suggested by the ENDOR/redox analysis, while our value of \( 0.069 \pm 0.002 \) eV is outside the expected range of \( 0.09-0.13 \) eV. However, by analyzing [4] redox data [36] for some heterodimer mutants, we have obtained the much more reliable alternative estimate of \( E_0 = 0.058 \pm 0.020 \) eV, in good agreement with the spectroscopically fitted value.

<table>
<thead>
<tr>
<th>Property(^a)</th>
<th>Fitted</th>
<th>ENDOR/redox (^b)</th>
<th>Calculated (^c)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. 1</td>
<td>No. 2</td>
<td>B3LYP</td>
</tr>
<tr>
<td>( J / \text{eV} )</td>
<td>( 0.126 \pm 0.002 )</td>
<td>( 0.12 )</td>
<td>( 0.16 )</td>
<td>( 0.098 )</td>
</tr>
<tr>
<td>( \lambda / \text{eV} )</td>
<td>( 0.139 \pm 0.003 )</td>
<td>( 0.1 )</td>
<td>( 0.2 )</td>
<td>( 0.17 )</td>
</tr>
<tr>
<td>( E_0 / \text{eV} )</td>
<td>( 0.069 \pm 0.002 )</td>
<td>( 0.09 )</td>
<td>( 0.13 )</td>
<td>( 0.042 )</td>
</tr>
<tr>
<td>( \lambda^2 / \text{eV} )</td>
<td>( 0.0027 \pm 0.0011 )</td>
<td>( )</td>
<td>( )</td>
<td>( 0.014 )</td>
</tr>
<tr>
<td>( \lambda^4 / \text{eV} )</td>
<td>( 0.071 \pm 0.004 )</td>
<td>( )</td>
<td>( )</td>
<td>( 0.054 )</td>
</tr>
<tr>
<td>( E_{\text{SHOM}} / \text{eV} )</td>
<td>( 0.180 \pm 0.001 )</td>
<td>( )</td>
<td>( )</td>
<td>( 0.40 \pm 0.25 )</td>
</tr>
<tr>
<td>( M / \text{Å} )</td>
<td>( 1.53 )</td>
<td>( )</td>
<td>( )</td>
<td>( )</td>
</tr>
<tr>
<td>( \rho_1 / e )</td>
<td>( 0.674 \pm 0.007 )</td>
<td>( 0.678 )</td>
<td>( 0.678 )</td>
<td>( )</td>
</tr>
</tbody>
</table>

\(^a\) In addition to the primary parameters \( J \), \( E_0 \), and \( \lambda \), the remaining adjustable parameters are: \( \lambda^2 \): the total reorganization energy associated with symmetric vibrations of wavenumber \( > 200 \text{ cm}^{-1} \); \( \lambda^4 \): the total antisymmetric-mode reorganization energy associated with the vibronic coupling between the hole-transfer and SHOMO to HOMO electronic states; \( E_{\text{SHOM}} \): the energy of the SHOMO to HOMO state relative to the ground state; and \( M \): the allowed electronic transition moment connecting the ground state to hole-transfer state. \( \rho_1 \) is the charge density localized on \( P_1 \).

\(^b\) From ENDOR and redox midpoint potential data [35] supporting a range of solutions varying from No. 1 to No. 2.

\(^c\) From [5] and [6].

\(^d\) DFT calculations from [5] and [16].

\(^e\) From [4] H1.(M202)+LH(L131) mutant [36].

\(^f\) From INDO/S calculations [14].
The other parameters that are adjusted to fit the spectroscopic data are all fitted to values that are close to those obtained from independent estimates. These parameters are the symmetric-mode reorganization energy $\lambda^S$, the reorganization energy that reflects the total magnitude of the vibronic coupling between the SHOMO to HOMO and hole-transfer electronic states $\lambda^{HTSH}$, the energy offset of the SHOMO to HOMO state $E_{SH}$, and the hole-transfer transition moment $M$.

As we have previously reasoned [30], the value of the transition moment is much smaller than one would expect for a transition involving significant delocalization (full delocalization is naively expected to produce $M \sim 8 \text{eÅ}$) due to configuration interaction between the hole-transfer and trip-doublet states. Hence, simple theories [14, 19], when applied to interpret the observed spectrum in terms of the degree of charge delocalization, have produced qualitatively incorrect results. The fraction of the charge localized on $P_L$ as deduced from the full spectral simulation is $0.674 \pm 0.007 \text{e}$, a value that is in excellent agreement with the experimental spin-density localization deduced from ENDOR data [32] of 0.678 e. Our spectral simulations have thus resulted in the development of a unified picture that describes a wide range of the observed physical, chemical, and spectroscopic properties of the photosynthetic reaction centers of purple bacteria.

6.7 Predicting Chemical Properties Based on the Spectral Analysis

The parameters extracted from the spectral analysis are all possibly subject to modification through mutation of the reaction centre, and provided a means is available for monitoring these changes, the changes in chemical properties of the mutants can be predicted from the model. Shown in Fig. 6.7 is the observed redox midpoint potential for the special pair as a function of the observed ENDOR fractional charge on $P_L$ obtained for four related series of mutant reaction centers of *Rh. sphaeroides* [35]. As the location of the mutation site are known, our four-state model can be used to predict these correlations, and the results are also shown in the figure. Indeed, a different correlation is expected for each mutant series owing to the differing relative location of the perturbation to the two halves $P_L$ and $P_M$. In this approach [9, 33, 34], the sole effect of mutation is assumed to be the independent, electrostatically induced variation of the midpoint potentials of the two halves of the dimer, with the difference in this variation thus acting to modulate $E_0$ and hence the charge distribution and intervalence hole-transfer spectrum. What is thus of most importance is the ratio $\eta$ of the modulation of the midpoint potential of the distant bacteriochlorophyll to that of the nearer one [9, 35]. As all of the mutations depicted in Fig. 6.7 arise from variations at either the symmetrically related M160 or L131 sites, we assume that the ratio is the same for all of the mutant series. Hence $\eta$, along with the redox midpoint potential of the wild type, is used as an adjustable parame-
Fig. 6.7 The observed midpoint potential $E_m$ as a function of the observed charge density on $P_L$ for the M160, L131, LH(M160)+L131, and LH(L131)+M160 series of mutants of Rb. sphaeroides [35] is compared to the expected correlation curves for each series determined [17] from our four-state interaction model. Experimental error bars are shown, while species marked $\times$ belong to each of two mutant series.

In order to fit the predicted correlations to the observed ones in Fig. 6.7. The optimized value of $\eta$ for these mutant series is 0.18, in excellent agreement with prior independent estimates [35] of $\sim$0.2. Also shown in Fig. 6.7 are the experimental error bars in the observed midpoint potentials [35]; the root mean square error between the observed and calculated data points of 0.008 V is of similar magnitude to these. Also, from the figure it is clear that some of the experimental data points do not smoothly correlate with the others. These deviations are most likely associated with effects of mutation outside of the simple electrostatic perturbations assumed on the interpretive model. Because simulations of the structures of some of these and other reaction centers indicate that typically the effects of site-directed mutagenesis are quite complex [8], the agreement between the predicted and observed data in Fig. 6.7 is as good as can be expected. We have also investigated other mutant series [37], obtaining similar results [17].

From the previous analysis of the ENDOR/redox data, values are obtained for the redox asymmetry $E_0$ of each mutant reaction center. Our four-state model for the properties of the special-pair radical cation, again assuming that the only effect of mutation is the modulation of electrostatic interactions, can be used to predict the vertical transition energy of the hole-transfer band [37]. This vertical transition energy is formally defined as the average excitation energy of the
band, a quantity that, for high-energy, symmetric-shaped bands, is readily approximated by the energy of maximum absorption [38]. For low-energy, strikingly asymmetric bands such as those observed for the hole-transfer band of photosynthetic special-pair radical cations, this approximation is invalid, and hence we explicitly evaluate [38] the actual average transition energy from the observed hole-transfer band contours for the wild type and a variety of mutant reaction centers [19, 37, 39]. The observed change in this vertical transition energy [17] is plotted against the observed [32–34, 37] charge distribution on $P_L$ in Fig. 6.8. Also shown in the figure is the correlation expected based on our four-state model of the special-pair radical cation. The observed and calculated data both show a minimum in the vertical excitation energy associated with mutants for which the charge is completely delocalized over both halves of the dimer. For such mutants, $E_0 \sim 0$ so that symmetry is maximized; symmetry reduction always acts to increase the transition energy. Quantitatively, the observed correlation falls somewhat outside the predicted one; this is most likely due to breakdown of the assumption used in this analysis that the sole effect of mutation is variation of $E_0$. One point that is clearly established, however, is that the mini-

![Graph](image)

Fig. 6.8 The observed [19, 37, 39] change from the wild type of *Rb. sphaeroides* in vertical excitation energy of the hole-transfer band of the special-pair radical cation for various mutants is correlated with the observed [32–34, 37] spin density on $P_L$. The solid line indicates the correlation expected from our four-state analysis [4] of the hole-transfer absorption band of the wild type.
mum hole-transfer band energy lowering that can be achieved by electric field-induced Stark modulation is on the order of 100 cm\(^{-1}\). Hence, this effect cannot account for the observation of the 2200 cm\(^{-1}\) shoulder shown in Figs. 6.1 and 6.6, as the required lowering would be on the order of 500 cm\(^{-1}\).

The response of the hole-transfer absorption band to small changes in the electric field across the special pair has been determined directly by Treynor and Boxer [40] and is shown in Fig. 6.9 as the fractional change in absorption observed for an applied external electric field of magnitude 0.6 MV cm\(^{-1}\). For the Stark modulation of spectra not dominated by vibronic coupling, the theory of Liptay [41, 42] is usually used to interpret this. Applied to intervalence spectroscopy [43, 44], qualitatively this theory stipulates that if the charge is localized on one half of the special pair, then the response will be strong and contain two nodes, while if the charge is delocalized, then the response will be weak and contain only one node. As the spectrum of the special-pair radical cation is dominated by vibronic coupling effects, simple Liptay theory is inapplicable and full spectral simulation is required [45]. The observed spectrum is shown as recorded and after broadening to 100 cm\(^{-1}\) resolution along with that predicted directly based on our four-state model analysis [4] of the absorption spectrum [17].

Fig. 6.9 Observed [40] Stark electroabsorption spectrum of the hole-transfer band of the special-pair radical cation of *Rh. sphaeroides*, additionally broadened to 100 cm\(^{-1}\) resolution, and that as calculated [17] from our four-state model [4].
The observed and calculated spectra are strikingly similar, with both being of the same strength, both containing just one node, and both displaying long, high-frequency tails and a shoulder at 2200 cm\(^{-1}\). Quantitatively, the locations of the node differ somewhat, while the observed band is significantly more asymmetric about the node than is the calculated one. Such differences are common even for small molecules [44], for which very accurate calculations are possible.

6.8 Conclusions

A comprehensive assignment of the spectrum of the special-pair radical cation of \textit{Rhodopseudomonas sphaeroides} in the 2000–5000 cm\(^{-1}\) region has been obtained using a four-state model [4]. The parameters in this model have been fitted to the observed infrared difference spectrum and shown to be adequate for the description of the complex redox, charge-distribution, and Stark-effect properties of the system [17]. This verifies the correctness of the basic model and the appropriateness of the quantitative solution obtained. The influence of the special pair on all aspects of the overall photosynthetic charge-separation process can be understood from the results of our analysis, with one of the most influential properties being the degree of charge localization within the dimer radical cation. In addition, the analysis obtained here for bacterial photosynthesis will prove to be central to the development of a proper understanding of the role of the special pair in algal and plant photosynthetic systems and to the design of artificial photo voltaic devices that mimic natural photosynthesis.

Acknowledgments

We thank the Australian Research Council for providing 10 years of support for this project through Research Fellowships for J.R.R. and a variety of Large Research Grants; we also thank the Australian Partnership for Advance Computing for provision of computational resources.

References