

The Nature of the Near-Infrared Electronic Absorption at 1250 nm in the Spectra of the Radical Cations of the Special Pairs in the Photosynthetic Reaction Centers of *Rhodobacter Sphaeroides* and *Rhodospseudomonas Viridis*

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Abstract: The P⁺ electronic spectra of bacterial photosynthetic reaction centers contain a band at ca. 1250 nm (8000 cm⁻¹). This has received little attention: the possibility that it represents an optical hole transfer between the two bacteriochlorophylls in the special pair is eliminated by the recent discovery of an even lower energy transition which is clearly of this (intervalence) type. Also, we find that suggestions that this band in fact corresponds to SHOMO → HOMO absorption cannot account for the strong intermonomer-coupling dependence of the band intensity. In this work, this band is assigned to a triplet-coupled (“trip-doublet”) Q_y absorption, expected, and found to occur at the triplet absorption energy of the monomer chromophores. As is observed, this band is local to one monomer and hence produces no significant change in the molecular dipole moment, but its intensity is highly dependent on the strength of the electronic coupling between the L and M bacteriochlorophylls of the special pair, as observed, becoming asymptotically spin-forbidden.

1. Introduction

The primary electron donor P in the photosynthetic reaction center is known to be a “special pair” P_L and P_M of bacteriochlorophyll-*b* (Bchl-*b*) molecules in *Rhodospseudomonas (Rp.) viridis* and bacteriochlorophyll-*a* (Bchl-*a*) molecules in *Rhodobacter (Rb.) sphaeroides*; X-ray structure determinations^{1–3} show that the two molecules are situated close together, with their molecular planes nearly parallel and separated by about 3.3 Å. They are in a “slipped” conformation, such that there is effective overlap of only one pyrrole ring of each molecule and are pseudo-related by a noncrystallographic axis of two-fold symmetry. The important question of the nature and extent of electronic interaction between the two molecules P_L and P_M of the special pair, which is of crucial importance in determining electron-transfer properties (see, e.g., ref⁴ and references therein), has not been definitively answered. When the reaction center is excited with light, the excited dimer P* transfers an electron to a bacteriopheophytin on the L pathway in a few picoseconds⁴ and is transformed into a cation radical dimer P⁺. The dimer cation is a mixed-valence species, and examination of its electronic spectrum should thus yield^{5,6} useful information about the nature of the interaction between the P_L and P_M moieties. It can also be generated chemically,⁷ and its spectrum is identical to that obtained photochemically, and hence there can be no specific role for the counterion in the absorption process. In

this work, we are concerned with theoretical prediction and interpretation of the second-lowest electronic excited state.

It has been known for many years that the oxidized reaction center P⁺ has a characteristic very narrow (ca.⁸ 350 cm⁻¹) electronic absorption band in the near infrared at about 8000 cm⁻¹ (1.0 eV), which has been used to monitor the short-time kinetics of the reaction center.⁷ While the ground-state absorption of P has been discussed at considerable length in the literature, the origin of the near infrared band of P⁺ has not been a topic of much interest until the recent electroabsorption measurements of Stocker *et al.*⁹ It is to be noted that the intensity and frequency of this band are close to those observed (see, e.g., refs 10 and 11) in the cofacial mixed-valence “sandwich” porphyrin radical cations of similar mean plane separation, which thus might similarly be interpreted as a delocalized macrocycle to macrocycle charge transfer absorption. In a *delocalized* process, the ground and first excited states of a dimer of the form (A–B)⁺ are taken to have the form A⁺–B ± A–B⁺, whereas for a *localized* process, the ground and excited states are of the form A⁺–B and A–B⁺, respectively. The finding of Stocker *et al.*⁹ that only a very small change of dipole moment on excitation at 8000 cm⁻¹ in P⁺ for *Rb. sphaeroides* is consistent with such an interpretation.

Recently Breton, Nabedryk, and Parson⁸ observed another absorption at even lower frequency, also attributed to an electronic transition, in the mid infrared region in the spectrum of P⁺. Their spectrum as well as the previous spectrum of Clayton and Clayton¹² and Reed^{7,13,14} in the near infrared to red region are shown in Figure 1 for *Rp. viridis* and *Rb.*

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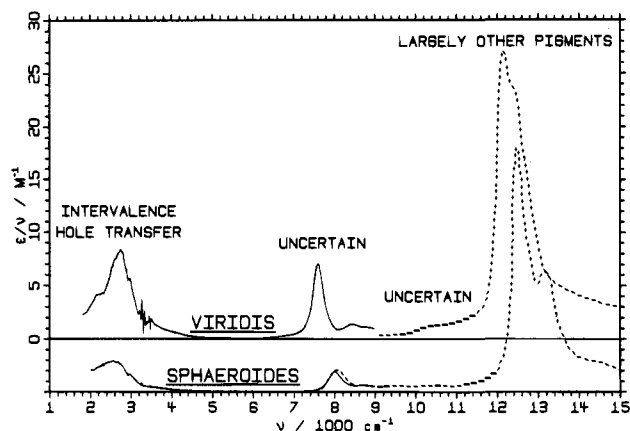


Figure 1. Observed band contour ϵ/ν for the low-lying electronic absorptions of the cations P^+ and other pigments in the oxidized reaction centers of *Rp. viridis* and *Rb. sphaeroides*, including firm and uncertain assignments are indicated. Data shown dashed are taken from Clayton and Clayton¹² (*Rp. viridis*, $T = 300$ K, $\epsilon/\nu \pm 20\%$) and Reed^{7,13} (*Rb. sphaeroides* R-26, $T = 300$ K); solid lines indicate data from Breton *et al.*,⁸ renormalizing their difference spectra to match the observed^{12,14} intensities for the Q_y bands of P and P^+ . In this paper, the unassigned bands just below 8000 cm^{-1} are assigned to trip-doublet absorption.

sphaeroides. This new band occurs at $2600\text{--}2750\text{ cm}^{-1}$ ($0.32\text{--}0.34\text{ eV}$), close to that found by Binstead and Hush¹⁵ for intervalence transitions observed in *weakly coupled* porphyrin dimer radical cations ($2200\text{--}2800\text{ cm}^{-1}$). It is clear⁸ that it is this band which is in fact the intervalence charge-transfer excitation between localized electronic states. The weak interring coupling arises not via a large mean interplanar separation, as is found in other porphyrinic systems,¹⁵ but rather by translation of monomer units to allow overlap of only one of the pyrrole rings of each tetracyclic monomer.

The question thus remains as to the nature of the original absorption near 8000 cm^{-1} , see Figure 1, and a range of possibilities have been suggested (see, e.g. refs 8 and 16–19). Based on orbital calculations, Breton, Nabdryk, and Parson^{8,18} have recently suggested that this band arises from a local excitation on the charged BChl monomer which takes an electron from the second-highest occupied molecular orbital (SHOMO) to the highest (partly) occupied molecular orbital (HOMO). This assignment was advanced based on the calculated absorption energy and dipole moment change, but, as originally indicated, a difficulty arises as the corresponding transition is forbidden by symmetry in porphyrin and was predicted to remain quite weak in P^+ , accounting for only one third of the observed intensity. Some recent INDO/S calculations by Scherer and Fischer¹⁹ predict the existence of an absorption with similar frequency and intensity in the presence of a counterion. The resultant state is highly configurationally mixed and did not give rise to simple interpretation: however significant SHOMO \rightarrow HOMO character was not predicted. We perform INDO/S-CI calculations, implemented in a slightly, though importantly, different method using multiconfigurational

self-consistent field techniques; these allow the questions as to the magnitude of the intensification conceivable for the SHOMO \rightarrow HOMO band and the identity and importance of the excitation found by Scherer and Fischer¹⁹ to be determined. Additional transitions in the infrared region are also predicted, and provisional assignments are made for some weak features of P^+ observed (see Figure 1) in the $8000\text{--}11\,000\text{ cm}^{-1}$ region.

It has been found that semiempirical methods such as INDO/S-CI^{19–27} and CNDO/S-CI²⁸ provide a generally reliable description of the spectroscopy and spin density in porphyrins, chlorins, bacteriochlorophylls, etc. and indeed for entire bacterial photosynthetic reaction centers. We perform calculations for the special pair cation P^+ and related monomeric cations, seeking primarily to identify the nature of the second-lowest energy electronic absorption. Considerable additional experimental evidence supporting the proposed assignment is also discussed.

Computational Method

All INDO/S-CI calculations are performed using the restricted open-shell Hartree-Fock (ROHF) formalism of Zerner,^{29–31} using our own program. For computational reasons, we replace the long phytol side chains on all bacteriochlorophylls and bacterioopheophytins with methyl groups. These calculations are not straightforward for a variety of reasons. Most important, significant charge rearrangements occur in some of the excited states, and these are thus extremely poorly represented in terms of configuration interaction (CI) expansions based on the ground-state determinant. At both *ab initio* (e.g., see refs 32 and 33) and semiempirical levels, it is essential that the orbitals that are not directly involved in an electronic transition are determined unbiased toward either the initial or final electronic states. For porphyrins and related molecules, the key electronic spectroscopy is usually described in terms of the “four orbital [per macrocycle] model” introduced originally by Longuet-Higgins, Rector, and Platt;³⁴ this considers explicitly only excitations from the highest two occupied orbitals (SHOMO and HOMO) into the lowest unoccupied molecular orbital (LUMO) and second-lowest unoccupied molecular orbital (SLUMO). We find this picture to be appropriate to describe qualitatively the spectra for all molecules considered herein, and, so to prevent biasing of the spectra, it is thus necessary to treat the occupied HOMO and SHOMO orbitals *equivalently* during the self-consistent field (SCF) stage of all calculations. To achieve this, we employ a multiconfigurational SCF (MCSCF) technique, implemented using ROHF methodology in which the SCF step is forced to optimize the orbitals so as to minimize the sum of the energies of all electronic

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states obtained by rearranging the orbital assignments of the HOMO and SHOMO levels for all macrocycles. In closed shell calculations such as those for the neutral parents of all cations considered herein, only one such state arises, and this operation is accomplished automatically. For our monomer cations, however, a situation in which three electrons are assigned in all possible ways (of doublet spin) to two orbitals arises, while for the special pair P^+ seven electrons are assigned in all possible ways (of doublet spin) to four orbitals. Alternative approaches such as treating, say, the dimer problem as a closed-shell plus just one open-shell electron in one orbital, or adding counterions to provide the illusion of the system being closed shell,^{19,26} result in very poor convergence of the CI calculation and the need to include many higher doubly and triply excited states. Indeed, a second significant difficulty in the implementation of semiempirical schemes for calculating spectra of open-shell systems is that the semiempirical parameters used are designed to produce reliable answers when only a small number of single excitations are included in the CI; in open shell systems, it is essential that some higher excitations be included, but the arbitrary inclusion of a large number of excitations will usually produce poor results. Based on our MCSCF orbitals, we include in the CI only states which arise as single excitations from any one of the determinants whose energy was included in the MCSCF step: this is known as a singles multireference CI (MRCI-S) calculation and is believed³³ to include all essential higher excitations in a way which does not compromise the small-singles-only CI nature of semiempirical spectroscopic methods. Specifically, we include in the single excitations only ones arising from the highest nine occupied orbitals of porphyrin into the lowest 15 unoccupied orbitals and consider the analogous excitations of up to 50 000 cm^{-1} for other ions. This results in ca. 100 excitations for the monomers and ca. 500 for the dimer P^+ . As an indication of the degree of convergence obtained, the energy of the 1250 nm band of P^+ is calculated to be 9175, 8670, 8256, 8116, and 8269 cm^{-1} when the CI is truncated at 14, 68 (the "four orbitals per macrocycle" model), 150, 250, and 500 configurations, respectively.

The language and notation used to describe the electronic states of P^+ used herein is that which is appropriate to a weakly interacting dimeric system. Indeed, it is found to be rather convenient to localize the molecular orbitals to one-half of the dimer before the CI calculation. This is achieved by first diagonalizing the "local density" matrix independently for the shells of doubly-occupied, partially-occupied, and unoccupied orbitals (using an effective shell-independent assignment of two electrons per orbital) and then rediagonalizing the resultant Fock matrices for each molecular half in each shell. As this transformation does not mix orbitals of different occupancy, the localization is not complete but in practice is found to localize at least 94% of each orbital, typically localizing 99.9%. It is found that no significant change to the CI energies or intensities result, but the eigenvectors thus obtained are found to be dominated by single, easily-described electronic configurations, with the dominant CI coefficient usually accounting for over 70% of the CI state probability.

Another difficulty that arises in these calculations is that they are for gas-phase molecules, while the systems of interest are observed either in solution or in a protein matrix. Significant environmental effects do occur, however, and indeed electrostatic interactions of P^+ with its environment are known to be an essential feature of the reaction center energetics (e.g., see refs 4,24,25,35–40), affecting particularly the relative energies of charge-transfer excited states of P . While it is possible to include environmental effects in semiempirical calculations either discretely²⁸ or implicitly,²⁴ these effects are not of central importance for the band of P^+ considered herein, and a simple empirical scheme which is described later is used to model environmental effects, when necessary.

The SHOMO \rightarrow HOMO transition

This transition is possible in any porphyrinic monocation and calculated energies and oscillator strengths for many such species are given in Table 1, along with the calculated energies of the Q_y band for reference. Comparison of the Q_y band with experimental data, where available,^{41–43} suggests that the calculated Q_y band centers are accurate to $\pm 300 \text{ cm}^{-1}$, the only exception being the dimer P^+ discussed later. This indicates that the INDO calculations are capable of describing the excited states of these cations. The SHOMO \rightarrow HOMO transition will not be easy to observe in the one-photon spectrum of a D_{4h} cation, as in this case the transition is Laporte-forbidden ($u \rightarrow u$); however, it could possibly be observed in a two-photon spectrum. For ions of lower symmetry, a one-photon transition of finite oscillator strength is predicted, however. Table 1 shows that the energy of this band is very sensitive to variation in the nature of the macrocyclic rings as well as the central substituent, but the changes seen simply parallel known substitutional effects on the relative energies of the SHOMO and HOMO levels. Little variation is predicted, however, for the intensity of this band; for P^+ itself, the calculated oscillator strength for *Rp. viridis* is 0.002, only 1/80th of that observed, while the value for *Rb. sphaeroides* it is 1/7th. Note also that the observed intensity ratio of the first to second electronic transitions, shown later in Table 3, increases from 1.0 in *Rp. viridis* to 1.4 in *Rb. sphaeroides*, while the calculated ratio for the SHOMO \rightarrow HOMO band decreases from 36 to 4, respectively. It is thus highly unlikely that this, the second lowest observed band of P^+ is actually the SHOMO \rightarrow HOMO transition, even though the INDO calculations predict this to lie very close in energy to the 1250 nm band. Indeed, no band assigned in a related molecule of a SHOMO \rightarrow HOMO transition has, to our knowledge, ever been made, see refs 8 and 44.

Simulating the Spectrum of P^+

No difficulty is found in localizing the SHOMO, HOMO, LUMO, and SLUMO orbitals calculated by INDO/S onto one-half of the special pair, with the resulting degree of localization being 98.5%, 99.8%, 99.8%, and 99.9%, for *Rb. sphaeroides* and 95.3%, 99.2%, 99.2%, and 99.9% for *Rp. viridis*, respectively. The localization procedure results in nonzero off-diagonal Fock matrix elements connecting within each orbital shell the orbitals localized on P_L and P_M , and the resulting couplings are given in Table 2 for *Rb. sphaeroides* and *Rp. viridis*. As previously concluded,^{8,45} the coupling between the HOMO levels of *Rp. viridis*, 0.115 eV (930 cm^{-1}), is in magnitude much greater than that of *Rb. sphaeroides*, 0.052 eV (420 cm^{-1}), due to increased pyrrole overlap. Couplings between different localized orbital types (e.g., P_L HOMO to P_M SHOMO) are in general much weaker than that between the same orbital type. Note that these calculated coupling are for gas-phase molecules, and it is possible that they could be modified slightly by environmental effects.⁴⁵

For gas-phase P^+ , INDO/S predicts that the SHOMO \rightarrow HOMO state discussed above is actually the fourth lowest energy excited state: in *Rb. sphaeroides*, three lower energy

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Table 1. Calculated Energies ν , in 1000 cm^{-1} , and Oscillator Strengths f_{osc} for the SHOMO \rightarrow HOMO Band Plus Energies for the Q_y Band^a

| cation | SHOMO \rightarrow HOMO | | $Q_y \nu$ |
|-----------------------------------|--------------------------|-----------|------------|
| | ν | f_{osc} | |
| porphyrin ⁺ | 2.0 | 0 | 18.7 |
| TPP ⁺ | -1.1 | 0 | 14.6 |
| Mg-porphyrin ⁺ | 4.3 | 0 | ~17 |
| Mg-TPP ⁺ | -0.2 | 0 | ~16 |
| Zn-TPP ⁺ | 0.6 | 0 | 11.8 |
| BPheo- <i>a</i> ⁺ | 6.4 | 0.003 | 11.8 |
| Mg-BPheo- <i>a</i> ⁺ | 9.0 | 0.003 | 11.7 |
| BPheo- <i>b</i> ⁺ | 6.2 | 0.003 | |
| BChl- <i>b</i> ⁺ (L) | 9.6 | 0.005 | 10.9 |
| P ⁺ <i>sphaeroides</i> | 10.8 | 0.008 | 12.1, 14.3 |
| P ⁺ <i>viridis</i> | 8.2 | 0.002 | 11.5, 14.5 |

^a For the radical cations of porphyrin at PM3 D_{2h} structure;^{62,63} TPP, i.e., mesotetraphenyl porphyrin in D_2 symmetry with the phenyl planes set at 60° to the porphyrin plane; Mg-porphyrin and Mg-TPP, their Mg derivatives; BPheo-*a*, i.e., bacteriopheophytin-*a*;⁶⁴ Mg-BPheo-*a*, its Mg derivative; BPheo-*b* at the structure of BPheo-*a*⁶⁴ with the appropriate bond changed; BChl-*b*(L) is the L half of the reaction center P in *Rp. viridis* on which the charge in P⁺ is known^{21,27,46-48} to reside; and P, the reaction centers from *Rp. viridis*² and *Rb. sphaeroides*.³ Note that phytol groups have been replaced by methyl groups throughout.

Table 2. Calculated INDO/S Fock-Matrix Coupling Elements between Molecular Orbitals Localized on the P_L and P_M Halves of the Special pair, in eV, Obtained from ROHF Calculations for the Cation P⁺^a

| coupling | | <i>Rb. sphaeroides</i> | <i>Rp. viridis</i> |
|----------------|----------------|------------------------|--------------------|
| P _L | P _M | | |
| SHOMO | SHOMO | 0.133 | 0.190 |
| SHOMO | HOMO | 0.037 | -0.008 |
| HOMO | SHOMO | 0.003 | 0.067 |
| HOMO | HOMO | -0.052 | 0.115 |
| LUMO | LUMO | 0.108 | 0.222 |
| LUMO | SLUMO | 0.032 | -0.001 |
| SLUMO | LUMO | -0.011 | 0.047 |
| SLUMO | SLUMO | 0.033 | 0.030 |

^a With phytol groups replaced with methyl groups.

states at $\nu = 800, 3700,$ and 4700 cm^{-1} are predicted. The lowest energy excited state is the intervalence L \rightarrow M hole-transfer (HT) state and corresponds to the absorption observed (see Figure 1) at ca. 2500 cm^{-1} , but the other two states are predicted to occur with significant intensity in a dark region of the spectrum. We consider in detail the origin of these bands, and their actual location in the observed spectra.

The origin of some of the possible spin-allowed (doublet) low-energy excited states involving just the HOMO and LUMO orbitals of interacting P_L and P_M molecules are sketched in Figure 2 and have been described in detail elsewhere.³³ In this figure it is assumed the charge resides on the P_L bacteriochlorophyll^{21,27,46-48} so that in the ground state P_M is assigned two electrons while P_L only one. The intervalence hole-transfer (HT) state is obtained by reversing the HOMO assignments and corresponds to the lowest energy excitation predicted. This band is observed⁸ at $2500-2700 \text{ cm}^{-1}$ in the spectra of *Rb. sphaeroides* and *Rp. viridis*; our gas-phase calculations do not include the inductive effects of the surrounding protein which are known to be important (see, e.g., refs 24,25,35,36,38,45-49) and hence

Figure 2. Important nonspin-adapted localized single determinants in terms of localized BChl HOMO and LUMO orbitals for special pair cations P⁺, including the ground state (assumed P_L⁺-P_M); the lowest-energy hole transfer state, HT (assumed P_L-P_M⁺); asymptotic singlet-coupled and triplet-coupled excitations on P_M, ¹Q_y(M), and ³Q_y(M), respectively; the corresponding states on P_L after hole transfer, HT + ¹Q_y(M) and HT + ³Q_y(L), respectively; the simple excitation of the unpaired electron on P_L, Q_y(L⁺), and the corresponding state on P_M⁺ after hole transfer, HT + Q_y(M⁺). The arrows indicate spin-allowed single-electron promotions from the doublet ground state. These determinants are characteristic of a weakly-interacting dimer, becoming doublet eigenstates asymptotically (i.e., at infinite L-M separation).

underestimate this energy considerably, predicting 800 cm^{-1} for *Rb. sphaeroides* and -1600 cm^{-1} for *Rp. viridis*.

The next band in Figure 2, labeled ³Q_y(M), is indicated to arise as the result of two spin-allowed excitations from the ground state, but the resultant determinant is in fact identical to that which would be produced by a spin-forbidden excitation from the HOMO to the LUMO of P_M. At large P_L-P_M separation, the energy of this band is thus identified to be the Q_y triplet absorption energy of neutral P_M. Also shown in Figure 2 is another excitation labeled ¹Q_y(M) and, at large separation, corresponds to the spin-allowed HOMO to LUMO excitation of neutral P_M. The determinants shown in this figure are, however, not eigenstates of the spin operator and show two of the ways in which three electrons may be assigned to three orbitals. Such an assignment produces three spin-adapted configurations, two of which are doublets and one of which is a quartet. It is the two doublet states which form spin-allowed excitations from the ground state, and calculational methods such as that embodied in the INDO/S program used here automatically generate both spin-allowed states: ³Q_y(M) and ¹Q_y(M) are usually referred to as the *triplet-coupled* and *singlet-coupled* doublet states, respectively, according to their asymptotic limit at large separation. The properties of these states have been considered extensively;^{17,33,50-53} the double-excitation nature of the triplet-coupled excitation ensures that it has low

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Table 3. Cations P⁺ of *Rb. sphaeroides* and *Rp. viridis* and Absorption Band Centers ν , in 1000 cm⁻¹, and Oscillator Strengths f_{osc} ^a

| band | <i>Rb. sphaeroides</i> | | | | | <i>Rp. viridis</i> | | | | |
|--------------------------------------|------------------------|-----------|------------|-----------|-------------|--------------------|-----------|------------|-----------|-------------|
| | observed | | calculated | | $\Delta\mu$ | observed | | calculated | | $\Delta\mu$ |
| | ν | f_{osc} | ν | f_{osc} | | ν | f_{osc} | ν | f_{osc} | |
| HT | 2.49 | 0.039 | 2.63 | 0.028 | 29 | 2.66 | 0.164 | 2.74 | 0.07 | 19 |
| ³ Q _y (M) | 8.04 | 0.028 | 7.99 | 0.007 | 4 | 7.60 | 0.158 | 7.6 | 0.14 | 5 |
| HT + ³ Q _y (L) | 10.8 | 0.03 | 10.8 | 0.11 | 27 | 10.5 | 0.09 | 9.9 | 0.23 | 14 |
| Q _y (L ⁺) | 11.8 | 0.06 | 12.1 | 0.08 | 8 | 11.5 | 0.06 | 11.5 | 0.11 | 4 |
| ¹ Q _y (M) | 13.2 | 0.2 | 14.3 | 0.73 | 7 | 12.7 | 0.2 | 14.7 | 0.55 | 4 |

^a Deduced by fitting Gaussian band shapes to the spectra of Breton *et al.*⁸ ($\nu < 9000$ cm⁻¹, 100 K) and Clayton *et al.*¹² or Reed^{7,13} ($\nu > 9000$ cm⁻¹, 300 K), see Figure 3, along with the modified INDO/S calculated band centers, oscillator strengths, and dipole moment changes $\Delta\mu$, in Debye. Assignments for the last three bands are tentative and indeed the "observed" intensity of ¹Q_y(M) is deduced indirectly from the total intensity difference between this band in P⁺ and in P.

intensity. As the two units come closer together,³³ its intensity rises appreciably, while its energy is not affected until strong interactions are encountered, say interplanar separations of less than 3 Å. We thus expect this band to appear in the spectrum with appreciable intensity at quite close to the frequency of the lowest triplet absorption of the neutral BChl monomer P_M. Bands of this nature are well known in other contexts, eg., the intensification of phthalocyanine $\pi \rightarrow \pi^*$ triplet transitions in Cu porphyrins^{52,53} and Cu phthalocyanine⁵⁴ as a result of the unpaired spin of the Cu²⁺ ion and also the intensification of triplet absorptions in molecules during collisions with molecular oxygen.⁵⁵ The triplet-coupled combination³³ of two Q_y bands in neutral reaction centers is known¹⁹ to contribute significantly to the spectrum^{56,57} of P*.

Another band shown in Figure 2 labeled HT + ³Q_y(L) is shown as a single excitation from the HOMO of P_M to the LUMO of P_L. At large separation of the two molecules, the energy of this excitation is seen to be the sum of the intervalence HT energy and the triplet absorption energy of P_L; indeed, this is another triplet-coupled excitation, whose singlet-coupled partner, ¹Q_y(L), is also shown in the figure. The other two excitations shown therein are Q_y(L⁺), corresponding asymptotically to the HOMO \rightarrow LUMO excitation on isolated P_L, and its analogue HT + Q_y(M⁺) which is generated by first transferring the hole to P_M.

The calculated energies for the ³Q_y(M) and HT + ³Q_y(L) bands are 3700 and 4700 cm⁻¹, respectively. Indeed, these are close to the calculated triplet absorption energies for isolated P_M and P_L molecules (plus the HT energy for P_L) of 5100 and 4100 cm⁻¹, respectively. The implementation of INDO/S adopted herein²⁹ uses the Nishimoto–Mataga–Weiss parameterization for the two-electron integrals which is designed to produce good frequencies for spin-allowed processes but is known to considerably underestimate the energies of spin-forbidden absorptions. For these, alternate formulations such as Ohno–Klopman or Pariser integrals are usually used, but open-shell systems such as weakly-interacting dimers intricately connect the triplet- and singlet-coupled manifolds of the monomers and hence no single formulation is capable of describing adequately all of the spin-allowed transitions. Nevertheless, the intensification of such bands is known experimentally and is believed to be evaluated to the same level of accuracy as are other bands, and hence we expect that these bands should indeed be found in the spectrum of P⁺ but presumably at much higher energy.

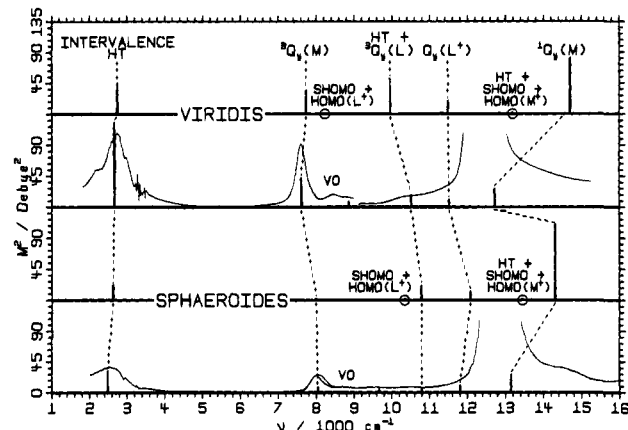


Figure 3. For the cations P⁺ of *Rp. viridis* and *Rb. sphaeroides*, the lower panels show the observed (see Figure 1) band shape and bars indicating the center and squared transition moment M^2 obtained by fitting to Gaussian-shaped bands. The top panels show results and assignments obtained from INDO/S calculations for P⁺, modified by shifting all triplet-coupled states by 3100 and 2400 cm⁻¹ and all charge-transfer states by 3500 and 4400 cm⁻¹ for *Rp. viridis* and *Rb. sphaeroides*, respectively. VO indicates that this band is presumed⁹ to be a vibronic origin based on the indicated ³Q_y(M) allowed origin. Assignments for the upper three bands are tentative.

Experimentally, the triplet phosphorescence frequency of the Q_y state of BChl-*a* is known⁵⁸ to be 8190 cm⁻¹, ca. 4000 cm⁻¹ higher in energy than that calculated by Figure 3/S. This energy is very close to that observed for the second absorption in *Rb. sphaeroides*, 8000 cm⁻¹, and this band is thus assigned to the ³Q_y(M) transition. For a bacteriochlorophyll-*b* monomer, the triplet phosphorescence energy is reduced by 200 cm⁻¹, while the band in P⁺ for *Rp. viridis* is reduced by a similar quantity, 400 cm⁻¹. Other evidence supporting this assignment is discussed in the next section; historically, this assignment was first suggested by Shuvalov and Parson¹⁶ and detailed by Vermeglio and Paillot.¹⁷

These INDO/S calculations thus have two significant shortcomings in quantitatively modelling the spectra of P⁺: the neglect of interactions with the environment and the incorrect treatment of the energy of the triplet-coupled states. In order to describe the entire spectrum, we introduce two empirical corrections to the INDO/S method, both of which are added to the calculated CI matrix before diagonalization. The first corrects for environmental effects and has the appearance of shifting all of the orbital energies of P_M with respect to P_L. This is nearly equivalent to adding an energy correction to each spin-adapted configuration proportional to the dipole moment change from the lowest P_L–P_M state of the dimer. In order to set the energy of the HT state to its observed value, a shift of 3000

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cm^{-1} is used for *Rp. viridis* and 2400 cm^{-1} is used for *Rb. sphaeroides*. The second correction is applied to all triplet-coupled spin-adapted configurations and simply increases their energy by a fixed amount. In order to set the energy of the second transition in P^+ to its observed value, a shift of 3500 cm^{-1} is applied for *Rp. viridis* and 4400 cm^{-1} for *Rb. sphaeroides*; these shifts are approximately the values of ca. 4000 cm^{-1} required to correctly position the BChl monomer triplet bands. Transition energies and intensities calculated using these corrections are compared in Table 3 and Figure 3 to values obtained by fitting Gaussian-shaped bands to the observed spectra. Note that the absolute intensities determined by Clayton and Clayton¹² for *Rp. viridis*, to which the observed intensities are normalized, are listed to only 20% accuracy.

The most significant feature in the spectra of P^+ shown in Figure 1 are the two peaks at ca. $12\text{--}13\,000\text{ cm}^{-1}$. Almost all of this absorption is associated with Q_y transitions on pigments in the reaction center other than the special pair, and hence the determination, *in vitro*, of the spectra in this region is very difficult. $\text{P}^+ - \text{P}$ difference spectra^{12,13} are difficult to interpret as large first-derivative-type components arise due to small shifts in the frequencies on the other chromophores, but a total increase in absorption corresponding to a relatively wide bandwidth transition of magnitude ca. 10% is observed. As shown in Figure 2, two different (possibly interacting) Q_y electronic transitions are expected, one weak band associated with the cationic BChl and one strong band associated with the neutral BChl. As the Figure 3 calculations are unlikely to be completely in error concerning the predictions for the seemingly intense $\text{Q}_y(\text{M})$ band, we attribute the additional intensity observed in the $12\text{--}13\,000\text{ cm}^{-1}$ region to this transition. Note that INDO/S overestimates the energy of the Q_y band in P itself by 1500 and 1000 cm^{-1} for *Rp. viridis* and *Rb. sphaeroides*, respectively, and appears to overestimate the $\text{Q}_y(\text{M})$ band of the cation by similar amounts.

A tentative assignment of the entire spectrum of P^+ up to $16\,000\text{ cm}^{-1}$ is shown in Figure 3 and detailed in Table 3; results for all but the first two states are tentative, however. Indeed, these first two bands at ca. 2600 and 8000 cm^{-1} are the only clearly resolved features in the spectra of P^+ . The calculated band frequencies for these bands have been adjusted to fit the observed band centers, and they are seen to be correctly positioned in Figure 3. The next resolved feature, at ca. 800 cm^{-1} to the blue of the second band, is presumed⁹ to be a vibronic origin. This assignment is supported by the observation that these bands scale and shift in parallel, as evidenced in Figure 3 and elsewhere (e.g., see ref 36), and the observation of similar circular dichroism.⁵⁹ Other partially resolved features are found in the spectra and may correspond to the predicted weak SHOMO \rightarrow HOMO transitions; the strongest features are, however, tentatively assigned to the HT + $^3\text{Q}_y(\text{L})$ and $\text{Q}_y(\text{L}^+)$ states.

Conclusions

We find that the electronic absorption at ca. 8000 cm^{-1} in the near-infrared spectrum of P^+ is due to a triplet-coupled absorption occurring on the neutral P_M monomeric unit of the special pair. This absorption is spin forbidden at large dimer separation but is intensified³³ significantly in the weak interaction region by a process similar to that by which triplet absorptions are promoted in collision complexes with molecular oxygen. It is a transition which is localized on one of the BChl monomers but whose intensity derives entirely from the inter-

BChl coupling. Experimental evidence supporting this assignment includes the following.

(1) The band in P^+ occurs at (as expected³³) just below the triplet phosphorescence frequency observed in the monomers, with both energies for *Rp. viridis* being slightly smaller than those for *Rb. sphaeroides*. Bands of this type appear as a general feature of the spectra of porphyrinic systems coupled to unpaired spins.^{52,53} Also, the μ -oxoiron(III) porphyrin dimers studied by Binstead and Hush¹⁵ exhibit, on oxidation to the π -cation radical dimer state, new weak absorption bands (at $14\,490$ and $15\,150\text{ cm}^{-1}$ for the meso-tetraphenyl and octaethyl derivatives, respectively), are observed in the region of the $^3\pi \rightarrow \pi^*$ absorption in metal porphyrin monomers (ca.⁶⁰ $14\,000\text{--}15\,000\text{ cm}^{-1}$), and could provide another example of this process, but other interpretations are possible in this case.

(2) The intensity of this band scales with the intensity of the hole transfer band at ca. 2600 cm^{-1} : the interBChl coupling is significantly reduced in *Rb. sphaeroides* as compared to *Rp. viridis*, an effect attributed to reduced pyrrole overlap.⁸ Both bands are thus seen to depend on the magnitude of the coupling for their intensity, and indeed no absorption corresponding to either band has been found in any monomeric BChl or related molecule.^{8,44} Also, Boxer's group has observed a four-fold decrease in the intensity of the 8000 cm^{-1} band in the sym1 mutant of *Rhodobacter capsulatus* compared to the wild type³⁵ and has established by other means that the intermonomer coupling is considerably reduced in the mutant.³⁶ The alternate assignment of this band as a localized SHOMO \rightarrow HOMO transition can not account for these observed interBChl-coupling dependencies of the observed intensity, predicting at the simplest level equal intensity for not only all dimers but also all monomers.

(3) The change in dipole moment from the ground state calculated for this band is shown in Table 3 and is just 4 Debye or 10% of that expected if the state involved full electron transfer between the macrocycles. Comparing this to the value of $19\text{--}29$ Debye predicted for the HT state, one sees that the calculations predict that this transition does not involve a significant charge rearrangement. Experimentally,⁹ this band is known to have a very small dipole change of less than 1 Debye, consistent with our predictions.

(4) The widths⁶¹ of the observed monomer phosphorescence bands are 240 cm^{-1} at 20 K or 580 cm^{-1} at 280 K for *Rb. sphaeroides* and 190 cm^{-1} at 77 K for *Rp. viridis*, similar to those found for the ca. 8000 cm^{-1} band of the dimer, 330 cm^{-1} at 100 K or 410 at 260 K for *Rb. sphaeroides* and 360 cm^{-1} at 100 K for *Rp. viridis*.

Evidence supporting this assignment has been sought by Olsen, Trunk, and Sutherland⁵⁹ from circular dichroism studies. They found no evidence to suggest that this band in *Rp. viridis* could be split into two bands with different rotational strengths: two bands are expected if the transition involved participation of both BChl monomers, as required by the trip-doublet mechanism. Hence their experiment found no evidence to support the assignment, and perhaps for this reason early suggestions^{16,17} of a trip-doublet assignment were not pursued but rather alternative explanations^{8,18,19} sought. We see from Table 3, however, that the two trip-doublet components,

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$^3Q_y(M)$ and $HT + ^3Q_y(L)$, are predicted to be split by 2300 cm^{-1} (i.e., essentially the HT energy) and (tentatively) assigned to spectral features 2900 cm^{-1} apart. Hence, in the much narrower clean spectral range available to Olsen *et al.*, only one transition is expected, as they observed.

We note finally that there has been a good deal of interest in cofacial porphyrin "sandwich" complexes of higher transition and actinide metals as, e.g.,¹⁰ [(octaethylporphyrin)₂Eu]⁺, with interring separations of ca. $3.1\text{--}3.3\text{ \AA}$ as models of the photosynthetic special pair. In radical cation oxidation state, they exhibit an absorption band in the region $6000\text{--}10\,000\text{ cm}^{-1}$

which can be assigned with reasonable certainty to a transition between states completely delocalized over the porphyrin rings.^{9,15} The occurrence of a transition close to that of the 8000 cm^{-1} band of P^+ is, however, coincidental, and the strongly-bound cofacial sandwich complexes are inappropriate as synthetic models of the very weakly coupled special pair.

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