Stark effect spectroscopy of *Rhodobacter sphaeroides* and *Rhodopseudomonas viridis* reaction centers

*(photosynthesis/electron transfer/electroabsorption/electrochromism)*

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**ABSTRACT** The nature of the initially excited state of the primary electron donor or special pair has been investigated by Stark effect spectroscopy for reaction centers from the photosynthetic bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* at 77 K. The data provide values for the magnitude of the difference in permanent dipole moment between the ground and excited state, $[\Delta \mu]$, and the angle $\xi$ between $\Delta \mu$ and the transition dipole moment for the electronic transition. $[\Delta \mu]$ and $\xi$ for the lowest-energy singlet electronic transition associated with the special pair primary electron donor were found to be very similar for the two species. $[\Delta \mu]$ for this transition is substantially larger than for the $Q_s$ transitions of the monomeric pigments in the reaction center or for pure monomeric bacteriochlorophylls, for which Stark data are also reported. We conclude that the excited state of the special pair has substantial charge-transfer character, and we suggest that charge separation in bacterial photosynthesis is initiated immediately upon photoexcitation of the special pair. Data for *Rhodobacter sphaeroides* between 340 and 1340 nm are presented and discussed in the context of the detection of charge-transfer states by Stark effect spectroscopy.

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Fig. 1. Absorption (A) and Stark effect (B) spectra for \textit{Rps. viridis} RCs in a PVA film in the Q\textsubscript{y} region at 77 K (F\textsubscript{ext} = 3.14 \times 10^5 V/cm).

An oriented immobilized sample is given by

$$\Delta A(\nu) = \frac{C_x}{30 h^2} F_{\text{ext}}^2 \frac{d^2(\Delta A/\nu)}{d\nu^2},$$

where $C_x = 5 \Delta \mu^2 + (3 \cos^2 \chi - 1)[3(p \Delta \mu^2 - \Delta \mu^2)]$, $\chi$ is the angle between the applied electric field direction and the polarization vector of the probing beam, $h$ is Planck's constant, and $p$ is a unit vector in the direction of the transition dipole moment being probed at frequency $\nu$. $F_{\text{int}}$ is the actual field felt by the molecules under investigation, which is different from the applied field because of the dielectric properties of the environment. $F_{\text{int}}$ is related to the applied electric field, $F_{\text{ext}}$, by the local field correction: $F_{\text{int}} = \frac{F_{\text{ext}}}{\epsilon}.$

Fig. 2. Absorption (A) and Stark effect (B) spectra for \textit{Rb. sphaeroides} RCs in a PVA film in the Q\textsubscript{y} region at 77 K (F\textsubscript{ext} = 2.59 \times 10^5 V/cm).

Fig. 3. Absorption (A), Stark effect (B), and second-derivative (C) spectra for the Q\textsubscript{x} and Soret region of \textit{Rb. sphaeroides} RCs at 77 K. The Stark effect spectrum was recorded with $F_{\text{ext}} = 4.43 \times 10^5$ V/cm; the spectrum has been scaled (Eq. 1) to $F_{\text{ext}} = 2.59 \times 10^5$ V/cm to facilitate comparison with the Q\textsubscript{y} region (same sample as used for Fig. 2). (Inset) Appropriate first derivative (arbitrary units) of the monomeric BPho \textit{a} Q\textsubscript{x} absorption bands.

Fig. 4. Dependence of the experimental value of the change in absorption measured for the experimental angle $\chi$ over the change in absorption for $\chi = 90^\circ$ for the Q\textsubscript{x} transition of the special pair in \textit{Rps. viridis} and \textit{Rb. sphaeroides} RCs (■; data for the two species are indistinguishable within the size of the symbols), for six-coordinate BChl \textit{b} (○), and six-coordinate BChl \textit{a} (△) at 77 K. The solid lines are best fits of the data to the angle dependence in Eq. 1 for the values of the angle $\zeta$ indicated on the left; theoretical curves are also included for $\zeta = 0^\circ$ and 50° to indicate the sensitivity of the measurements to this angle.
The value of \( f \) is typically on the order of 1.2–1.4 for a dielectric constant of 2, depending on the model used for the electrostatic field (10). We express the value of the dipole moment difference as the product of \( f^{-1} \) and the observed value of \( \Delta \mu \) assuming \( F_{\text{ext}} = F_{\text{ext}} \) to clearly separate the experimental uncertainties from assumptions used to treat the local field.

The value of \( \Delta \mu \) was calculated from \( \mu (A/\rho)/d\rho^2 \), \( \zeta \) (Fig. 4), and \( \Delta A \) obtained near the peak of the absorption band, since the effect due to \( \Delta \mu \) is large and any polarizability (first-derivative) effects can be ignored. The application of Eq. 1 to obtain \( \Delta \mu \) is straightforward when the electronic transition is well resolved and has a simple absorption lineshape. This is not the case for the RC absorption spectrum; consequently a variety of approximate approaches have been used. For the pure monomeric chromophores in PMMA and for the monomeric BChl bands in the RC, \( \mu (A/\rho)/d\rho^2 \) was determined by numerically differentiating the absorption spectrum. For the special pair \( Q_s \) absorption band of the band that does not overlap with other bands was fit to an exponential of a fourth-order polynomial function, which was numerically differentiated. Quantitative analysis of the data leads to the values summarized in Table 1.

**DISCUSSION**

**Survey of Features in the Stark Spectrum.** At a qualitative level, it is apparent from the Stark effect spectra of both species that the dipole moment difference for the lowest energy band in the RC absorption spectrum, whose character is predominately determined by the dimeric primary electron donor or special pair, is much larger than for other features in the spectrum. The quantitative analysis summarized in Table 1 demonstrates that \( \Delta \mu \) for the \( Q_s \) transition of the special pair is likewise much larger than for pure BChl and that the values at both room temperature and 77 K are very similar for \emph{Rps. viridis} and \emph{Rb. sphaeroides} RCs. Furthermore, the angle \( \zeta \) between \( \Delta \mu \) and the transition dipole moment for the special pair \( Q_s \) transition is identical for both species within experimental error, but this angle differs from that determined for the monomeric chromophores. The remarkable similarity of both \( \Delta \mu \) and \( \zeta \) for the \( Q_s \) special pair absorption band of both species is consistent with the gross similarity of the special pair structures seen by x-ray diffraction (1–4). On the other hand, there is a significant chemical difference between BChl \( a \) and BChl \( b \), and amino acids in the vicinity of the special pair are quite different for the two species (2). Apparently neither of these differences is sufficient to perturb the values of \( \Delta \mu \) or \( \zeta \), both of which are expected to be very sensitive to variations in electronic structure.

For the purpose of discussion, we will use the conventional assignment of absorption bands to specific chromophores, recognizing that there is substantial electronic mixing. Because the absorption bands for the monomeric chromophores overlap substantially and one expects vibronic bands from the special pair in this region, it is difficult to quantitate \( \Delta \mu \) and \( \zeta \) for the monomer bands. For \emph{Rb. sphaeroides}, the values of \( \Delta \mu \) for the partially resolved BPhes \( a \) bands are approximately equal and about a factor of 2 smaller than \( \Delta \mu \) for the special pair \( Q_s \) band; in \emph{Rps. viridis}, the value of \( \Delta \mu \) for the monomer BPhes \( b \) band is about a factor of 3 smaller than for the special pair \( Q_s \) band. Note that the values of \( \Delta \mu \) for pure monomeric BPhes \( a \) and BPhes \( b \) in PMMA are of comparable magnitude (Table 1). \( \Delta \mu \) was estimated for the monomeric BChl bands; the value of \( \Delta \mu \) in \emph{Rb. sphaeroides} (2.4 ± 0.2 D at 77 K) is slightly larger than in \emph{Rps. viridis} (1.8 ± 0.6 D at 77 K) for the monomer BChl bands.

**Table 1.** Experimental values for the magnitude of the change in dipole moment, \( \Delta \mu \), and the angle \( \zeta \), between \( \Delta \mu \) and the transition dipole moment for the \( Q_s \) transition of the special pair in \emph{Rps. viridis} and \emph{Rb. sphaeroides} RCs for the special pair in the monomeric BChls and BPhes.

| Chromophore | \( T \) | \( |\Delta \mu|, \text{ D}^* \) | \( \zeta^* \) |
|-------------|--------|-----------------|------|
| \emph{Rps. viridis} | 298 | (10.5 ± 0.7)/f | 36.7 ± 2 |
| special pair | 77 | (6.5 ± 0.4)/f | 37.1 ± 2 |
| \emph{Rb. sphaeroides} | 298 | (9.6 ± 0.7)/f | 39.5 ± 2 |
| special pair | 77 | (7.0 ± 0.5)/f | 38.1 ± 2 |
| BChl \( b \) (6-coordinate) | 77 | (2.9 ± 0.17)/f | 22.1 ± 2 |
| BChl \( a \) (6-coordinate) | 77 | (2.4 ± 0.14)/f | 11.6 ± 2 |
| BPhes \( b \) | 77 | (2.6 ± 0.15)/f | 23.8 ± 2 |
| BPhes \( a \) | 77 | (2.6 ± 0.15)/f | 9.5 ± 2 |

*D, debyes (3.34 x 10^-30 C m). The value of the local field correction \( f \) may be different for each chromophore in its particular environment and at different temperatures (see text).
The variations in the monomer $Q_x$ region can be further accentuated by measuring the Stark effect spectrum as a function of the angle $\chi$. The spectra for Rb. sphaeroides BChl a and BChl b in PMMA at 77 K (Table 1).

Experimentally, the Stark effect is characterized by the change of the absorption spectrum with an applied electric field. The Stark effect in dimeric chlorophyll complexes can generally be matched with absorption features, this is not the case for the monomer band at 22800 cm$^{-1}$. The signal is positive, suggesting that a transition at this energy gains oscillator strength in the presence of the field due to increased mixing with more intense transitions (the bump in the monomer spectrum). However, in the Stark spectrum, it is more clearly visible that the transition is assigned to a broad band, though there is no evidence for such a feature in the first or second derivative of the absorption.

For Rb. sphaeroides, the Stark effect lineshape in the region of the $Q_x$ bands of the BPho monomers appears as the first derivative, suggesting that the effect due to a polarizability difference dominates that due to $|\Delta \mu|$, as is true for pure monomeric Bpheo a and BChl a (10). The Stark effect lineshape around 600 nm consists of first- and second-derivative components as well as features that cannot be accounted for by either derivative, notably the positive feature at 17700 cm$^{-1}$ and the negative feature at 16000 cm$^{-1}$.

The value of $Q_x$ measured for the monomer Bpheo bands is substantially smaller than for the corresponding pure Bpheo a or b (Table 1), whereas the value of $Q_x$ measured for the monomer BChl bands is greater than for the corresponding pure BChl a or b. The difference in relative Stark enhancements and values of $Q_x$ for each band in the monomer $Q_x$ region presumably reflects subtle variations in the mixing of various electronic states and stands as a challenge for theoretical analysis.

Charge-Transfer Bands. We were especially interested to see whether there was any evidence for new bands in the Stark spectrum that do not appear in the absorption spectrum. The electronic absorption bands of pure charge-transfer transitions are likely to have small extinction coefficients and to be quite broad; however, the large value of $|\Delta \mu|$ could enhance their detectability in the Stark spectrum. At least three charge-transfer states are of interest: ones involving charge separation within the dimer (sometimes called $P^+P^-$), charge separation between the special pair and a monomeric BChl ($P^+B^-$), and charge separation between the special pair and a monomeric Bpheo ($P^+H^-\gamma$). Estimates for the energy of the $P^+P^-$ state range from several thousand cm$^{-1}$ above the lowest $\pi\pi^*$ excitation state (8), to nearly degenerate with the $\pi\pi^*$ state (17, 18). Some investigators have observed a shoulder on the red side of the special pair $Q_x$ band, especially in Rps. viridis RCs (19, 20), and this shoulder has been ascribed by some to the $P^+B^-$ charge-transfer state (20).

Measurements of delayed fluorescence (21) and the temperature dependence of the $P^0$ decay rate (22) for Rb. sphaeroides RCs bracket the $P^+B^-$ state between 0.05 (21) and 0.3 eV (22) below the lowest $\pi\pi^*$ state of the special pair at 77 K, placing the $P^+H^-\gamma$ charge-transfer state 1–1.3 eV above the ground state. Since we are interested in estimating the energy for the vertical (Franck–Condon) transition, we must consider the displacement and shape of the potential surfaces; essentially no information is available on this point.

We have searched for new bands in the visible and near-infrared regions (340–1340 nm) of the Stark effect spectrum of Rb. sphaeroides. The Stark effect spectrum in the Soret region contains a number of prominent features. The individual Soret bands of the chlorophores overlap significantly in the 340- to 420-nm region (the Soret band for each pigment consists of two bands, $B_2$ and $B_3$; this region is even more congested for Rps. viridis, which contains bound polycyclic aromatic compounds). Second-derivative features are observed for each partially resolved band at about 24800, 25800, 27000, and 29000 cm$^{-1}$; each feature has a comparable width. It is difficult to reliably compute $|\Delta \mu|$ for these bands because of the uncertainty in their absorption amplitudes and widths. By comparison with the appropriately weighted second derivative of the absorption spectrum (Fig. 3C) it is evident that the redmost feature at 24800 cm$^{-1}$ has the largest value of $|\Delta \mu|$. Using the zero-crossing points of the Stark spectrum to estimate a linewidth for the underlying transitions, we crudely estimate that $|\Delta \mu|$ is about 2.5 D/J. $|\Delta \mu|$ for the Soret bands of pure BChl a and Bpheo a monomers (data not shown) is about 2–2.5 D/J. Although the features in the Stark effect spectrum can generally be matched with absorption features, this is not the case for the unusual band at 22800 cm$^{-1}$. The signal is positive, suggesting that a transition at this energy gains oscillator strength in the presence of the field due to increased mixing with more intense transitions (the bump in the monomer spectrum).
the special pair Qₘ bands. Although such calculations lead to specific values for |Δμ| and ξ that are comparable with what we have obtained experimentally for the special pair Qₘ band (W. W. Parson, personal communication), the molecular orbitals require further refinement before quantitative comparisons are warranted.

We have never observed an electronic absorption band on the red side of the Qₘ special pair band in either species, and it would likely be easily detected, if present, in the Stark effect spectrum due to the expected differences in |Δμ|, ξ, and the linewidth. We have measured the Stark effect lineshape as a function of applied electric field strength for the Qₘ transitions of Rb. sphaeroides in an attempt to change the relative energies of the states and the mixing between them sufficiently to produce an observable effect on the Stark effect lineshape. The predicted quadratic dependence on applied field strength (Eq. 1) was observed between 0.74 × 10⁵ and 4.64 × 10⁶ V/cm at 77 K to within 5% with no detectable change in lineshape throughout the Qₘ region (data not shown). Furthermore, no change in ξ was measured for the special pair Qₘ band over this range of applied field strength. Thus, there is no evidence for spectral changes in the Stark spectrum induced by a change in the energy of or coupling to other states.

The value of f(Δμ) for the Qₘ transition of the special pair in both species decreases substantially when the temperature is decreased, while that for the other bands decreases considerably less. The value of f(Δμ) for BPheo a in PMMA was temperature independent within the experimental uncertainty, while the value of f(Δμ) for a chlorophyll derivative in apomyoglobin embedded in PVA decreased by about 20% between room temperature and 77 K (unpublished data). The low-frequency dielectric constant of PVA decreases by about a factor of 2 between room temperature and 77 K (26). Since the local field correction f depends on the dielectric constant of the matrix, the observed value of f(Δμ) will also be temperature dependent. The exact form of the dependence of f on the dielectric constant is highly sensitive to the method used to model the dielectric environment of the chromophore; however, at least part of the difference in f(Δμ) for the special pair as a function of temperature can be accounted for by the change in dielectric constant. The change in f(Δμ) with temperature for the model chlorophyll in apomyoglobin system is comparable with the change observed for the monomer bands in the RC. The variation with temperature for the special pair Qₘ bands is considerably larger, and it may reflect more subtle local variations in the dielectric or electronic properties of this component.

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