Dielectric Asymmetry in the Photosynthetic Reaction Center

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Although the three-dimensional structure of the bacterial photosynthetic reaction center (RC) reveals a high level of structural symmetry, with two nearly equivalent potential electron transfer pathways, the RC is functionally asymmetric: Electron transfer occurs along only one of the two possible pathways. In order to determine the origins of this symmetry breaking, the internal electric field present in the RC when charge is separated onto structurally characterized sites was probed by using absorption band shifts of the chromophores within the RC. The sensitivity of each probe chromophore to an electric field was calibrated by measuring the Stark effect spectrum, the change in absorption due to an externally applied electric field. A quantitative comparison of the observed absorption band shifts and those predicted from vacuum electrostatics gives information on the effective dielectric constant of the protein complex. These results reveal a significant asymmetry in the effective dielectric strength of the protein complex along the two potential electron transfer pathways, with a substantially higher dielectric strength along the functional pathway. This dielectric asymmetry could be a dominant factor in determining the functional asymmetry of electron transfer in the RC.

The solution of the three-dimensional structure of the bacterial photosynthetic RC laid to rest many questions concerning the events that immediately follow absorption of a photon in photosynthesis (1, 2). One of the most surprising and puzzling discoveries was the observation of an approximate twofold axis of symmetry (Fig. 1). In spite of this structural symmetry, the light-driven primary electron transfer reactions take place only along one of the two potential branches of redox-active components (3, 4). This phenomenon has been named unidirectional electron transfer, and understanding its origin has generated much experimental and theoretical interest (3–7). To date, no convincing physical explanation has been provided. The significance of this problem extends beyond its obvious importance in photosynthesis, because there is widespread interest in understanding the factors that determine electron transfer rates in condensed phases (8), including proteins (9, 10). We present experimental evidence that the effective dielectric constant in the vicinity of the functional branch of components is considerably larger than that for the non-functional branch. This difference may be fundamentally related to the functional asymmetry of the RC because the enhanced dielectric screening along the functional pathway would stabilize charge-separatated intermediates relative to those on the nonfunctional side.

The RC is comprised of two principal protein subunits, denoted L and M. Chromophores that are primarily associated with these subunits are labeled with subscripts (Fig. 1). The functional branch of cofactors is along the L side, whereas the nonfunctional branch is along the M side. The secondary and tertiary structures of the L and M subunits are related by the approximate twofold axis of symmetry, and there is a moderately high level of sequence homology between them. Various attempts to increase the sequence homology, and therefore increase the symmetry of the RC, have led to many interesting results (11–16), but none has yet produced any evidence of a deviation from unidirectional electron transfer. The absorption of light initiates the transfer of an electron from two closely interacting bacteriochlorophyll (BChl) molecules, referred to as the special pair and labeled P, to the bacteriochlorophybin monomer (BPheo, in which the central Mg atom of BChl is replaced by 2H), labeled H, with some participation of the intervening BChl monomer B,. The electron then moves from H to the quinone QA, to form the state QA*-H*, which has a lifetime of tens of milliseconds if quinone QA (not shown) is absent from the RC, as in the experiments discussed below.

We used the absorption bands of the BPheo (H, and H,()) and BChl (B, and B,) chromophores as built-in probes of the internal electric field produced by the charge-separated QA*-H* state. We focus principally on the results obtained for H, and H, because the magnitude of excitonic coupling among various chromophores for the monomeric BChls is less well understood (see below). Upon formation of the internal electric field due to QA*-H*, the BPheo absorption bands shift, a phenomenon often referred to as an electrochromic band shift. Although known for many years (17), these shifts have not been quantitatively analyzed. A quantitative analysis requires a determination of the sensitivity of individual absorption features to electric fields and a knowledge of the three-dimensional structure. Calibration of the field sensitivity is provided by a measurement of the Stark effect spectrum, and the distances and orientations of the probe chromophores are obtained from the x-ray structure.

Specifically, the magnitude of the elec-

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The effect of an external applied electric field, \( F_{\text{ext}} \), on the absorption spectrum, also called the Stark effect spectrum (18–22). The magnitude and direction of the internal electric field at any position produced by P" and Q_A" in vacuum (\( \varepsilon = 1 \)), can be calculated from Coulomb’s law by using the x-ray crystal structure coordinates and information on the P" and Q_A" charge distributions from electron-nuclear double resonance (ENDOR) spectroscopy and theory (23):

\[
F_{\text{calc}}(P"Q_A", \varepsilon = 1) = \sum_{i=1}^{n} \frac{q_i}{r_i^2} \hat{r}_i
\]

where \( q_i \) is the partial charge on atom \( i \), \( \hat{r}_i \) is the vector between the charge \( q_i \) and the probe charge, and \( \hat{r}_i \) is a unit vector in the direction of \( \hat{r}_i \). We can use \( F_{\text{calc}}(P"Q_A", \varepsilon = 1) \) to calculate the electrochromic band shift in vacuum, \( \Delta \nu_{\text{calc}}(\varepsilon = 1) \):

\[
\Delta \nu_{\text{calc}}(\varepsilon = 1) = -|\Delta \mu| F_{\text{calc}}(P"Q_A") \cos \theta
\]

The ability of a protein to screen charge decreases the magnitude of the electric field from the value calculated for \( \varepsilon = 1 \), thereby reducing the magnitude of the observed band shift from the value calculated with Eq. 3. This difference can be characterized in terms of an effective dielectric constant, \( \varepsilon_{\text{eff}} \), defined as the ratio of the calculated (\( \varepsilon = 1 \)) to the observed band shift:

\[
\varepsilon_{\text{eff}} = \frac{\Delta \nu_{\text{calc}}(\varepsilon = 1)}{\Delta \nu_{\text{obs}}}
\]

In the experiments discussed below, the band shifts predicted by vacuum electrostatics are compared with the experimentally determined electrochromic band shifts for H_4 and H_4 (see below for a discussion of H_4 and H_4 that result when P" and Q_A" become charged, and the derived value of \( \varepsilon_{\text{eff}} \) is compared for chromophores on the L and M sides of the RC. A similar approach was used recently to measure the screening of charges along synthetic \( \alpha \) helices (24).

**Determination of \( \Delta \nu_{\text{obs}} \).** The absorption and steady-state P"Q_A"-minus-PQ_A" difference spectra (25) of the Q region of wild-type RCs (26) at 1.5 K are shown with solid lines in Fig. 2. A and C, respectively. In the difference spectrum, a small increase in absorption, thought to be due to P" is seen on the low energy side of the spectrum. It appears as a flat positive baseline offset over a wide wavelength region. In order to obtain the absorption spectrum of RCs in the charge-separated state, \( \Delta(P"Q_A") \), the magnitude of the bleach of the P band in the difference spectrum (Fig. 2C) was scaled to equal the maximum of the P absorption (Fig. 2A), the two were added together, and the flat baseline due to P" absorption was subtracted to yield the spectrum in Fig. 2D (27). This procedure assumes that there is no structure in the P" absorption between 740 and 830 nm (12,000 to 13,500 cm\(^{-1}\)); none is observed.

We now focus on the H-band region and assume that the spectral shifts observed when P"Q_A" is formed are due exclusively to electrochromic effects. This assumption is strongly supported by the absence of absorption changes in the H-band region when P is in the triplet state (28, 29). Like P", P has little oscillator strength in the 12,000 to 13,500 cm\(^{-1}\) region; however, P is unchanged so electrochromic shifts are negligible. Thus, the triplet-minus-singlet difference spectrum is specifically sensitive.

**Fig. 2.** (A) Absorption, (B) Stark effect (\( |F_{\text{calc}}| = 9 \times 10^4 \) V/cm, \( \chi = 90^\circ \)), (C) P"Q_A"-minus-P difference absorption, and (D) P"Q_A" absorption spectra in the Q region of wild-type Rb. sphaeroides RCs in a 50 percent (v/v) glycerol/buffer glass at 1.5 K. The solid lines are the data and the circles indicate the fits to the data.
to absorption changes associated with changes in excitonic coupling (28, 29). The absence of changes in the H bands demonstrates that changes in excitonic coupling with P are negligible for H₄ and H₅, and therefore the observed band shifts in the P⁺Q₇⁻ state are likely entirely electrochromic in origin.

Expanded plots of the BPheo and BCHl monomer region are shown in Fig. 3 with band assignments taken from previous studies (30). The two BPheo bands shift to lower energy upon formation of P⁺Q₇⁻, and the two BCHl bands shift to higher energy (depicted by the arrows in Fig. 3). The signs of the spectral shifts imply that the angle between F(P⁺Q₇⁻) and Δμ is <90° for the monomer BPheos, whereas the angle is >90° for the BCHls (assuming the shifts are electrochromic in origin; see below). From the crystal structure, the observed signs of these spectral changes are consistent with the positive end of Δμ being closer to rings C and E of the macrocycle for all of the monomeric chromophores, as has been predicted by calculation (31).

In order to obtain Δν₂₈₀, a method is needed to model quantitatively the absorption in the ground and P⁺Q₇⁻ states in the monomer region between 740 and 775 nm (12,900 to 13,500 cm⁻¹). Because the absorption bands are clearly non-Gaussian, as expected for the absorption of an aromatic molecule, each band was treated simply as a sum of two Gaussians. Both Gaussians for each band were treated identically in subsequent analyses of the band shifts (Δν₂₈₀) and Stark spectra (see below), and the same widths of the Gaussians were used to deconvolute the two absorption spectra (32). Most importantly, the deconvolution of the two absorption spectra was required to fit the Stark spectrum simultaneously (Fig. 2B; see below) so that the deconvolution is consistent with all of the experimental information used in the analysis of the band shifts. The best simultaneous fits to all of the data are shown with the open circles in Fig. 2, and the deduced band shifts are listed in Table 1. The H₅ band exhibits a larger shift than its symmetry-related counterpart on the L side.

Determination of Δμ. For an isotropic, immobilized sample, the line shape of the change in absorbance due to an externally applied electric field ΔA₁₄₀ can be described by a sum of the zeroth, first, and second derivatives of the absorption spectrum (33–36). A second-derivative line shape dominates the Stark effect for the Q⁻ absorption bands of both isolated monomers (37) and the monomer bands in wild-type RCs (Fig. 2B) (38, 39). The open circles in Fig. 2B show the fit to the Stark spectrum that was obtained by using the same spectral decomposition used to fit the ground and P⁺Q₇⁻ absorption spectra.

The absolute directions of Δμ in the molecular axis system are also needed for the analysis of the band shifts. Precise experimental values for the directions in the RC are difficult to obtain for several reasons: first, the determination of θ (35) only provides the projection of Δμ on the transition moment p; second, the direction of p is not precisely known relative to the molecular axes, either for isolated monomeric BCHl and BPheo or for these chromophores in the RC (40); and third, the measurement of the direction of Δμ, although straightforward, can give misleading results for overlapping bands (41). We approach the uncertainty in the direction of Δμ as follows. In order to provide a frame of reference, the direction of p is taken to lie along the line connecting the nitrogen atoms of rings A and C. This direction is close to that obtained by calculation and is consistent with linear dichroism measurements (42). We first fix Δμ to be parallel to p (θ = 0°), with the positive end of the difference dipole in the direction of ring C (consistent with the sign of the electrochromic band shifts). Next, the direction of Δμ was varied ±5° in the plane of each macrocycle by using the apparent value of θ measured for each band in the RC at 77 K. This approach yields the widest reasonable range for the direction of Δμ. Values of θ measured for the isolated BCHl and BPheo monomers in polymer matrices are not complicated by overlapping bands and are smaller [BPheo: θ = 10°; BCHl: θ = 12°, see (21)] than the apparent values of θ obtained for the overlapping bands in the RC, so it is very likely that the actual orientation of Δμ for the monomer bands in the RC is contained within the range of orientations sampled. There is no experimental evidence in any system which suggests that Δμ lies outside of these ranges. As shown below, regardless of the precise orientation of Δμ within this range, the qualitative results for our estimates of e₂₈₀ are unaffected.

Determination of Fcalc (P⁺Q₇⁻, e = 1). The direction and magnitude of the field Fcalc (P⁺Q₇⁻) at the center of each probe chromophore assuming e = 1 (vacuum) can be calculated with Coulomb's law (Eq. 2). The charge density distribution for P⁺ is

<table>
<thead>
<tr>
<th>Parameter</th>
<th>B₄</th>
<th>H₄</th>
<th>B₅</th>
<th>H₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption position (cm⁻¹)</td>
<td>12,271</td>
<td>13,268</td>
<td>12,452</td>
<td>13,113</td>
</tr>
<tr>
<td>A(P⁺Q₇⁻) position (cm⁻¹)</td>
<td>12,447</td>
<td>13,158</td>
<td>12,529</td>
<td>13,011</td>
</tr>
<tr>
<td>Δν₂₈₀ (cm⁻¹)</td>
<td>176</td>
<td>-110</td>
<td>77</td>
<td>-102</td>
</tr>
<tr>
<td>Δμ (D/μ)</td>
<td>2.5</td>
<td>2.7</td>
<td>2.9</td>
<td>3.3</td>
</tr>
<tr>
<td>θ (degrees)</td>
<td>30</td>
<td>36</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>Fcalc (P⁺Q₇⁻) (V/m)</td>
<td>7.6 x 10⁶</td>
<td>4.5 x 10⁶</td>
<td>1.1 x 10⁶</td>
<td>1.2 x 10⁶</td>
</tr>
<tr>
<td>Δνcalc (cm⁻¹)</td>
<td>280</td>
<td>-176</td>
<td>367</td>
<td>-466</td>
</tr>
<tr>
<td>e₂₈₀</td>
<td>1.5</td>
<td>1.6</td>
<td>4.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Range (67)</td>
<td>0.7–1.8</td>
<td>0.9–1.7</td>
<td>3.3–5.9</td>
<td>2.5–5.1</td>
</tr>
</tbody>
</table>

Fig. 3. Expanded view in the monomer region from Fig. 2 of (A) the ground-state absorption and (B) the P⁺Q₇⁻ absorption spectra of wild-type Rb. sphaeroides RCs. The dark solid curves are the data; the thinner lines are a deconvolution of the absorption assuming that only four species contribute to the absorption in this region (monomeric B₄, B₅, H₄, and H₅) and that each chromophore's non-Gaussian absorption profile can be approximated by a sum of two Gaussians (see text). The identical components (amplitudes and linewidths) are shifted in (B) to obtain Δν₂₈₀, which is illustrated with the arrows and vertical lines (see Table 1).
Table 2. Experimental and calculated parameters for the analysis of electrochromic band shifts associated with the formation of the singly charged states P+ and Q-.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P+</th>
<th>Q-</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔH (cm⁻¹)</td>
<td>B_M</td>
<td>H_M</td>
</tr>
<tr>
<td>110</td>
<td>-60</td>
<td>34</td>
</tr>
<tr>
<td>Δμ (D/μ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>θ (degrees)</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>F (V/m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.4 x 10⁸</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (degrees)</td>
<td>143</td>
<td>50</td>
</tr>
<tr>
<td>ΔE (cm⁻¹)</td>
<td>276</td>
<td>-132</td>
</tr>
<tr>
<td>e(ε)</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Range</td>
<td>1.3-3.0</td>
<td>0.2-3.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>B_M</th>
<th>H_M</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (V/m)</td>
<td>5.7</td>
<td>7.2</td>
</tr>
<tr>
<td>6 (degrees)</td>
<td>132</td>
<td>123</td>
</tr>
<tr>
<td>ΔE (cm⁻¹)</td>
<td>276</td>
<td>-132</td>
</tr>
<tr>
<td>e(ε)</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Range</td>
<td>1.3-3.0</td>
<td>0.2-3.2</td>
</tr>
</tbody>
</table>

Based on the analysis of ENDOR spectra and quantum mechanical calculations (23). A simpler alternative is to place a point charge at the geometric center of P; however, this overestimates the field at B_L and B_M by nearly 25 percent, because the entire field originates from a single point in space. If one uses the more realistic distribution of partial positive charges over the two macrocycles, the field is decreased by the canceling of components perpendicular to the final direction of the field. The results are not very sensitive to the details of the charge distribution on the macrocycles, and similar results are obtained if the positive charge is simply distributed evenly over the two macrocycles. The location of the negative charge on Q- is taken as the geometric center of the atoms contributing to the conjugated π system. Relative to the charge on P+, the spatial extent of the charge on Q- is smaller, and its distance from the other chromophores is greater, such that the consequences of approximating the location of the charge at a single point were relatively minor. The values of F(ε) for P+ Q- (ε = 1) range from 4.5 x 10⁸ to 1.2 x 10⁸ V/m and are presented in Table 1 for each probe chromophore.

Band shifts in (M)L214H RCs. Absorption spectra in the monomer region are shown in Fig. 4, A and B, for the neutral ground and P+ Q- states, respectively, of the β mutant RC (LeuM124 → His) (43). The ground-state spectrum shows the improved spectral resolution when a BChl replaces BPho in the H_M binding site. As was the case for wild-type RCs, the two B bands shift to higher energy and the H_M and B_L bands shift to lower energy upon formation of P+ Q-. The shift for the H_M band is somewhat larger than for the B_L band, as illustrated with the vertical lines and arrows. The B_L band shows a large second-derivative Stark signal (Fig. 4C).

Chemical oxidation of P and reduction of Q_. The band shifts that occur when P is chemically oxidized to P+ (44) were investigated to rule out the possibility that any of the observed asymmetry in e(ε) was a consequence of electron transfer occurring down the L side when P+ Q- is formed (45). The absorption spectra of wild-type RCs in the presence of either 400 mM KCl or 400 mM KFe(CN)₆, which oxidizes P to P+, were measured, and the absorption spectra were deconvolved as described above. The spectra are similar to those at higher temperatures (44). The spectral shifts for P+ RCs are summarized in the first four columns of Table 2.

As a complement to the chemical oxidation of P, the band shifts that occur when Q_ is reduced to Q- with sodium dithionite (44) were investigated. Because Q- absorbs in the near ultraviolet, the band shifts in the near infrared due to Q- contain no significant contributions from changes in excitonic coupling. Absorption spectra were obtained in the presence of 20 mM sodium chloride or 20 mM sodium dithionite, again giving spectra similar to those reported previously (44), and the spectra were deconvolved as described above. The only significantly shifted absorption band is that of H_M, as expected given the close proximity of H_M and Q_, summarized in the last column of Table 2.

Calculation of e(ε). The data for RCs from the β mutant provide an exceptionally clear example of the asymmetry in e(ε) by direct inspection. The Stark effect spectrum in Fig. 4C shows that [Δμ] for B_L is considerably larger than for H_M (this result is confirmed by a quantitative analysis). Thus, the B_L band is more sensitive to an electric field than the H_M band, and for identical values of the field the B_L electrochromic band shift should be substantially larger. Furthermore, inspection of the x-ray structure in Fig. 1 shows that the field produced by P+ Q- should be larger at the B_L (or H_M) site than the H_M site because the charges on P+ and Q- are closer [see Table 1 for F(ε) for P+ Q- for the quantitative analysis]. Thus, these factors lead to the prediction that the electrochromic band shift for B_L in the presence of P+ Q- should be substantially larger than for H_M in vacuum. In contrast, comparison of Fig. 4, A and B, shows that the electrochromic band shift for B_L is smaller than the band shift for H_M (this result is confirmed by a quantitative analysis in which the procedure discussed in detail for wild-type RCs is used). The implication is that the protein dielectric screening of the charges on P+ and Q- is considerably larger on the L side than it is on the M side.

Inspection of the data for wild type in Figs. 2 and 3 leads to a similar qualitative conclusion. Because wild-type RCs have been much more thoroughly studied and characterized, we restrict the quantitative analyses to wild-type RCs. Table 1 summarizes the calculation of e(ε) for the optically prepared P+ Q- state (45). Table 2 summarizes the calculation for the chemically prepared P+ and Q- states. The orientation of [Δμ] is treated as discussed in detail above, and e(ε) is calculated according to Eq. 4. As can be seen, the range of estimates for e(ε) is consistently higher on the L side than for the M side.

Analysis of band shifts for the mono- meric BChls. In principle, independent information can be obtained by an analysis of the band shifts in the B band region (12,100 to 12,800 cm⁻¹; 780 to 825 nm). If we adopt the view that the excitonic contribution is small relative to the electrochromic contribution, we can perform the same quantitative analysis as for the H bands (46-49). The results are summarized in Tables 1 and 2 and yield estimates for both the asymmetry in e(ε) and for the absolute magnitudes of e(ε) which are similar to those for the H bands (50). As discussed
elsewhere in detail (51), the absorption spectra of the B and H monomer bands change much less when the absorption spectrum of P is changed drastically, for example, in the heterodimer mutant (Hu1200 → Leu) (52) or upon chemical modification of the B or H chromophores (51), than when P4Q5+ is formed. Furthermore, if the B and P absorption band positions had a significant contribution from exciton interactions between B and P, then the loss of this exciton splitting upon formation of P+ would be expected to shift the absorption of B to lower, not higher, energy because the splitting is removed. Additionally, for identical chromophores at nearly identical distances and relative orientations, excitonic coupling should be nearly equal, producing splittings of similar magnitude. We observe band shifts of very different magnitude for the two sides. These experimental data argue that both the H and B absorption band positions are not strongly affected by exciton interactions with P+, supporting the hypothesis that the band shifts observed for both sets of chromophores upon formation of P4Q5+ and P4Q5- are largely electrochromic in origin. These data contrast with the results of some quantum chemical calculations of the absorption spectrum (53–55), but, as discussed in (51), this conclusion is far from unanimous.

Implications for RC function. All else being equal, the higher effective dielectric constant on the L side implies that the energy of the key charge-separated states, such as P4B5- and P4H5-, will be lower on the L side than on the M side because the ion pairs will be more strongly solvated. This experimental result is consistent with the conclusion of theoretical studies which suggest that the higher energy of charge-separated states on the M side is one of the primary determinants of the unidirectionality of electron transfer in the RC (56–59). The obvious next question is to determine which amino acids are responsible for the dielectric asymmetry. Attempts to symmetrize the environment around the special pair (14, 15), the amino acids in the vicinity of the entire electron transport chain (12), and residues that clearly break the symmetry, such as TyrH2110 (13, 16) and GhuL104 (11), have not shown evidence for electron transport down the M side. We have investigated the electrochromic band shifts for the TyrH2110 → Phe mutant and obtained results that are similar to those of the wild type for the shifts of the two BPhe bands. We conclude that many small contributions, including those from amino acids that are quite distant from the redox-active components, combine to give a substantial dielectric, and therefore functional, asymmetry. A similar view emerges from molecular dynamics simulations, which suggest, in one case, that 20 amino acid residues participate in the solvation of P4H5-. These studies, as well as the relative insensitivity of the initial electron transfer to single-site mutations, suggest that the molecular explanation for the functional asymmetry of the photosynthetic RC is due to collective properties of a large portion of the protein complex. It is unlikely that any individual amino acid residue is alone responsible for the dielectric asymmetry, so the challenge of forcing electrons to transfer down the nonfunctional M side may be difficult to achieve.

An examination of the polarity of the amino acid residues that are within 7 Å of either H1 or H4 (60), as measured by their hydrophobicity (61), reveals a previously unrecognized asymmetry in polarity that mirrors the observed dielectric asymmetry. The asymmetry persists when distances less than 7 Å are used as a cutoff; residues further than 7 Å are also expected to contribute, but were not considered here. The residues that primarily account for the asymmetry in polarity tend to be located near the edges of the macrocycles, whereas the polarity of the residues located above and below the macrocycle tend to be more or less equal. A similar asymmetry in polarity is observed for residues within 7 Å of the two BChl molecules that comprise the special pair, P1 and P2. The differences in polarity are not restricted to a few amino acid residues near the chromophores. Twelve residues are more polar near H4, and nine residues are more polar near P1. No asymmetry of polarity is found for the residues near B and M in Rhodobacter sphaeroides; however, a similar analysis reveals an even more pronounced asymmetry in the polarity of amino acid residues around all of the symmetry-related chromophores in Rhodopseudomonas viridis.

Spatial variation of the dielectric constant and the asymmetry between the two branches as suggested by this study, could also have significant consequences for electron coupling matrix elements. This possibility has received only limited discussion in the photosynthesis field (62) and in electron transfer work in general (63–66). The simple concept is that the barrier height for electron tunneling may decrease with increasing dielectric constant, thereby increasing orbital overlap and enhancing the electronic coupling between reactant and product states (65, 66). If this effect is significant, it implies that, all else being equal, the electronic coupling will be greater along the L pathway, although the quantitative magnitude of the effect needs to be considered theoretically.

The absolute values of eeff support the notion of a low dielectric interior in the RC at 1.5 K; however, they are subject to the greatest cumulative experimental error (67). Most of these uncertainties do not affect the relative value of eeff on the L and M sides, which is the central result of this study. Because of the loss of spectral resolution that accompanies thermal broadening, the individual bands cannot be reliably deconvoluted at higher temperatures (even for the beta mutant (43)), and therefore no information can be obtained regarding dielectric asymmetry at room temperature. However, by treating the monomeric H bands as a unit, we can estimate the average band shift. If we compare the average shift at 1.5, 77, and 298 K, we find little difference between 1.5 and 77 K, but a significant decrease (~45 percent) in the magnitude of the average band shift at 298 K. This result indicates that the dielectric strength of the protein nearly doubles between 77 and 298 K, which is consistent with other measurements on the RC (68). The unidirectionality of electron transfer in the RC is independent of temperature (69), so the conclusions reached here regarding asymmetry can likely be transferred to physiological conditions.

REFERENCES AND NOTES

15. J. C. Williams et al., ibid., p. 11029.
22. M. Losche, G. Feher, M. Y. Okamura, in The Photosynthetic Bacterial Reaction Center—Structure and Dynamics, J. Breton and A. Vermeglio,

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25. Transmission spectra of the samples and a blank at 1.5 K were obtained to yield the absorption spectra. Transmission spectra of the RC samples with excitation from an argon-ion laser (0.5 W/20 cm² at 514 and 488 nm) were used to obtain the F(PO₂)−/F(PO₂)− of the RC absorption spectra.

26. Wild-type (carotenoid-containing) Rhodobacter sphaeroides were grown semiaerobically. The RC in isolated by standard methods, with final purification by chromatography at moderate pressure on DEAE Toyopearl 650S. The RCs, dissolved in 0.025 percent lauryl-dimethylamine-oxide (LDAO) detergent, 10 mM NaCl, pH 8.0, 10 mM NaCl, diluted with glycerol to a final solvent composition of 50% (v/v) glycerol/buffer.

27. Absorption spectra are the more common way of presenting the data, in part, because it emphasizes the changes in the spectra. However, it is misleading to refer to the features at 12,300, 12,450, and 12,500 cm⁻¹ as bands that are disappearing or growing in. Comparison of Fig. 2, A and D, demonstrates that bands already observed change absorption in this region, and that no new bands need be invoked.


33. The long-wave contribution to the change in absorption, ΔA = ωΔrms of an applied electric field is:

- The desired symmetry-related experiment of examining band shifts upon formation of P₅O₃⁻ can unfortunately not be done because electron transfer from the H₁ binding site to P₅O₃⁻ does not occur at cryogenic temperatures.

- The analysis assumes that the upper exciton component of the transition at 12,500 cm⁻¹ (417) has a small integrated oscillator strength relative to the other exciton components. This is supported by the observation that oscillator strength in this wavelength region is very nearly conserved in the ground state and P₅O₃⁻ absorption spectra, and it is consistent with most calculations.


35. The estimates for εₕ suggest that the dielectric reorganization of the excited state of P₂O₃⁻, the probe chromophores, and the pathways connecting them. The estimates should not be thought of as the dielectric response of the protein at the particular location of the probe chromophore. Thus, it would have been surprising if the estimates for changes in the dielectric response of two chromophores (B₅ and H₅) on the L side, because these two chromophores probe very nearly the same region of the protein. Such agreement would not have been unexpected if the shifts for B₅ and P were due predominantly to changes in excitonic coupling, because the coupling between H₅ and P should be much smaller than the coupling between B₅ and P.


52. Uncertainties in the relative values of εₕ are likely to arise from errors in ΔA and ΔAₑₑₑₑ. For overlapping bands, positive and negative features of the Stark spectrum may cancel, so that the values of ΔA that cannot be derived directly from the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis of the Stark spectra and without deconvolution produced values for ΔA that in all cases agreed to within 0.6% of the values obtained without deconvolution. Analysis
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