Light-Induced Charge Separation in *Rhodopseudomonas viridis* Reaction Centers Monitored by Fourier-Transform Infrared Difference Spectroscopy: The Quinone Vibrations

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ABSTRACT: Static FTIR light-induced difference spectra have been recorded for reaction centers from *Rhodopseudomonas viridis* in the following charge-separated states: P\(^+\)QA\(^-\) - PQ\(_A\), P\(^+\)QB\(^-\) - PQ\(_B\), \(\Gamma\)\(^-\) - I, \(\Gamma\)QA\(^-\) - IQ\(_A\), and \(\Gamma\)QA\(^-\) - relativistic effects on the electron-transfer reaction. This has been achieved by averaging the difference spectra obtained from the primary donor, P, to the secondary acceptor, QB, via the intermediate acceptor, I, and the primary acceptor, QA. The difference spectra are dominated by absorbance changes of prosthetic groups, with very few identifiable contributions from amino acids and little overall structural change in the protein backbone, involving only one or two residues for the various charge-separated states. Oxidation of the primary donor in the reaction center shows the characteristic absorbance changes of the 9-keto and 10-ester carbonyl groups observed upon oxidation of bacteriochlorophyll \(b\) in a non-hydrogen-bonded environment [Ballschmiter, K. H., & Katz, J. J. (1969) *J. Am. Chem. Soc.* 91, 2661-2677]. Reduction of the quinones in the reaction center yields absorbance changes of the carbonyls observed during reduction of quinones in a hydrogen-bonded environment [Bauscher, M., Nabedryk, E., Bagley, K., Breton, J., & Mântele, W. (1990) *FEBS Lett.* 261, 191-195]. It is thus concluded that the protein acts as an optimized solvent to facilitate electron transfer from the primary donor to the secondary quinone acceptor.

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5 Abbreviations: FTIR, Fourier-transform infrared; P, primary donor (bacteriochlorophyll special pair); I, intermediate acceptor (bacterio-phyophytin); QA, primary quinone acceptor; QB, secondary quinone acceptor; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; HEPES, N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid; BC\(_{L,A}\), accessory bacteriochlorophyll in the L branch; BP\(_L\), bacteriopheophytin in the L branch; BP\(_{L,A}\), bacteriopheophytin in the M branch; LDAO, N-lauryl-N,N-dimethylammonio; HPLC, high-pressure liquid chromatography; BC\(_{B\beta}\), bacteriochlorophyll \(b\); BP\(_{B\beta}\), bacteriopheophytin \(b\); QA, ubiquinone 0.

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action centers frozen during illumination (Kleinfeld et al., 1984), the low-temperature recombination kinetics of *Rb. sphaeroides* and *Rhodospirillum rubrum* reaction centers frozen in the dark (Parot et al., 1987), and the changes in linear dichroism spectra upon charge separation in *Rps. viridis* reaction centers (Vermeglio & Paillotin, 1982). Recently, Robert and Lutz (1988) used resonance Raman spectroscopy to provide specific evidence of light-induced conformational changes in *Rb. sphaeroides* reaction centers occurring upon photooxidation of P. However, protein changes occurring upon quinone reduction have not yet been investigated.

In *Rps. viridis* reaction centers, reduction of either of the two quinone acceptors can be distinguished by a variety of spectroscopic methods: UV--visible spectra of QA and QB differ substantially in the near-IR, indicating that QA induces an electrochromic effect on the bacteriopheophytin in the L subunit while QB predominantly affects the bacteriopheophytin in the M subunit (Shopes & Wraight, 1985). EPR measurements show that both QA and QB interact magnetically with the Fe(II) atom but that these interactions are distinctly different for the two quinones (Rutherford & Evans, 1979). Furthermore, the Fe--quinone interactions indicate a role for the Fe(II) atom in electron transfer from QA to QB, but studies on metal-substituted *Rb. sphaeroides* reaction centers (Debus et al., 1986; Buchanan & Dismukes, 1987) have revealed that the Fe(II) atom plays no electronic role in electron transfer nor does it mediate the redox potential of QA (Buchanan et al., 1988). The function of the Fe(II) atom is not yet fully understood nor are specific details known concerning the role of the protein in electron transfer from QA to QB.

It has been shown that the technique of FTIR difference spectroscopy can reveal changes on the order of a single bond in chromophores, protein side groups, and the protein backbone (for a review, see Braimann and Rothchild (1988)). Static difference spectra are taken between a ground state and an activated state stabilized through continuous illumination, resulting in difference spectra where all peaks arise from protein and chromophore changes due to photochemical activation. In photosynthetic reaction centers, because the electron-transfer reactions are all several orders of magnitude slower than the forward reactions (Wraight et al., 1977), it is possible to stabilize several of the charge-separated states with continuous illumination. *Rps. viridis* reaction centers in which the cytochrome subunit is oxidized yield P+QA or P+QQA, depending upon preparation conditions, with steady-state illumination (Prince et al., 1977). When reaction centers are reduced with dithionite, all four hemes in the cytochrome subunit and both quinones are reduced, allowing the photochemical trapping of PF (Shuvalov & Klimov, 1976; Trosper et al., 1977; Prince et al., 1977). In contrast, ascorbate reduces only the two high-potential hemes (Dracheva et al., 1986); illumination under these conditions initially produces the state P=QA and with continued illumination the state P=QA+ (van Wijck et al., 1986).

FTIR difference spectroscopy has been used previously to assign changes in the primary donor occurring upon photooxidation in reconstituted reaction centers (Mantele et al., 1985) and in reaction center crystals (Gerwert et al., 1988). Specifically, H-bond changes for the ester and C=O keto groups were identified (Mantele et al., 1985; Gerwert et al., 1988). A comparison with structural data (Deisenhofer et al., 1983, 1985) has shown that the observed non-hydrogen-bonded C=O keto carbonyl group can most likely be assigned to the bacteriopheophytin b molecule of the special pair situated in the M subunit (Gerwert et al., 1988). In these experiments, however, the contributions from reduced quinone molecules were not specifically characterized. Here we report FTIR difference spectra of reconstituted reaction centers for the charge-separated states P+QA−, P+QA−, P+QA−, I−, I−QA− and I−QA2−. Through the static difference spectra, the electron-transfer pathway can be followed from P to the secondary electron acceptor, QB, via the intermediate acceptor, I, and QA. A detailed analysis of the results obtained allows the determination of the roles of the protein and chromophores in charge stabilization.

**Experimental Procedures**

Reaction centers from *Rps. viridis* (strain DSM 133) were isolated and purified according to Michel (1982), except that the purified reaction centers in 0.1% LDAO (Fluka, Neu-Ulm)/20 mM phosphate buffer, pH 8, were bound to a small anion-exchange column, washed with 0.7% CHAPS (Serva, Heidelberg)/5 mM HEPES (pH 8), and eluted with 0.7% CHAPS/5 mM HEPES/200 mM KCl. Reaction centers were incorporated into soybean phosphatidylcholine vesicles (Cook et al., 1986), with a final lipid concentration of 10 mg/mL and a reaction center concentration of 34 μM. For samples containing only QA, 5 mM terbutryn (Riedel-de Haen, Seelze) was routinely added. For samples containing QB, ubiquinone 0 (Sigma, Deisenhofen) was dissolved in CHAPS and added in 5X excess according to the method of Okamura et al. (1975). This method has been shown to reconstitute 95% QA in *Rps. viridis* (Sinning, 1989). Ascorbate reduction was performed by adding 5 mM sodium ascorbate to reaction centers prior to incorporation into lipid vesicles and subsequent dialysis against 5 mM HEPES/5 mM ascorbate. Dithionite reduction was performed by adding 10 μL of 100 mM HEPES (pH 9)/50 mM Na2S2O4 directly to a N2-dried lipid film.

Reaction center samples were analyzed for photochemical activity with a Zeiss UMSP 80 microspectrophotometer using actinic illumination λ > 700 nm. Infrared spectra were taken on a Bruker IFS 88 instrument. A total of 1024 scans were averaged for each spectrum. The home-built sample holder was thermostabilized at 275 K for all measurements. The difference was taken between absorbance spectra recorded in the dark and during steady-state illumination with actinic light λ > 700 nm. Control experiments using 960-nm excitation provided by an interference filter yielded identical difference spectra. Subtraction of difference spectra was performed as described earlier (Gerwert et al., 1989). This takes into account differing amounts of reacting material in the two measurements and base line drifts between the two difference spectra.

**Results**

Figure 1 compares the light-induced difference spectra (A) without and (B) with QB reconstituted, arising from the transitions PQA− → P+QA and PQB → P+QB. The high reproducibility of the difference spectra can be seen not only in the large absorbance bands at 1753, 1712, 1552, 1477, 1306, and 1196 cm−1 but also in the smaller absorbances between 1412 and 1193 cm−1. Similar difference spectra recorded for reaction center crystals with and without QB reconstituted have been reported (Buchanan et al., 1990a) and compare well with the difference spectra for reaction centers reconstituted into lipids vesicles (Figure 1). In the crystal difference spectra, deviations between the two types of difference spectra are seen at 1477/1456 cm−1 and could be caused by vibrations of the quinone radical (Tripathie, 1981). The difference spectra for reconstituted reaction centers have an improved signal-to-noise ratio as compared to the crystal difference spectra, thus in-
FIGURE 1: FTIR light-induced difference spectra for Rps. viridis reaction centers in H2O. (A) Difference spectrum for reaction centers containing only QA; the difference is taken between PQA and P+QA− states. (B) Difference spectrum for reaction centers reconstituted with QB; the difference is taken between PQAQB and P+QAQB− (T = 275 K; resolution = 4 cm−1).

In Figure 1A, the band shifting from approximately 1740 to 1753 cm−1 represents the characteristic absorbance change of a C=C carbomethoxy ester carbonyl vibration for bacteriochlorophyll b upon cation formation in a non-hydrogen-bonded environment (Mántele et al., 1988). The band shifting from 1674 to 1712 cm−1 represents the characteristic absorbance change of a C9 keto carbonyl vibration for bacteriochlorophyll b upon cation formation also in a non-hydrogen-bonded environment (Mántele et al., 1988). The absorbance change at 1674/1712 cm−1 is assigned to the 9-keto group of BCMP (special-pair bacteriochlorophyll situated more in the M subunit) because the corresponding group of the L subunit is most likely hydrogen-bonded and would absorb at lower frequencies (Gerwert et al., 1988).

Although some of the absorbance bands observed between 1700 and 1600 cm−1 could be caused by vibrations of the primary donor, vibrations from the protein backbone, water, and the quinones would also be expected in this frequency range. Therefore, band assignments in this range are only possible with isotopic labeling or site-directed mutagenesis. The bands at 1552 and 1523 cm−1 are also observed in bacteriochlorophyll b during cation formation (Mántele et al., 1988) and are therefore tentatively assigned to primary donor C=C stretching vibrations. In the range from 1500 to 1400 cm−1 only very small absorbance changes are detected upon oxidation of bacteriochlorophyll b; therefore, absorbance changes in this spectral region are most likely not caused by bacteriochlorophyll vibrations.

A comparison of P+QA− − PQA and P+Qb− − Pqb difference spectra should yield agreement for absorbance changes caused by oxidation of the primary donor and differences for absorbance changes caused by reduction of the quinones. A comparison of QA− and Qb− containing samples was made for difference spectra showing similar intensities for the 1306-cm−1 band. The absorbance change at 1306 cm−1 is indicative of P+ formation. FTIR difference spectra of reaction centers containing reduced cytochromes show light-induced absorbance changes due to cytochrome oxidation in the amide I region around 1640 cm−1 and in the amide II region around 1550 cm−1, as well as the disappearance of the 1306-cm−1 band (Brudler and Gerwert, private communication). The P+Qb− − Pqb difference spectra deviate from the P+QA− − PQA difference spectra at 1743 cm−1, at 1726 cm−1, in a broader negative band around 1650 cm−1 leading to decreases at 1657 and 1651 cm−1, and at increases in intensity at 1577, 1477, 1454, and 1394 cm−1 (Figure 1B). The absorbance change at 1211 cm−1 (Figure 1A) is due to strong buffer absorbance and therefore is not specific to QA or Qb vibrations.

In order to visualize the deviations between the P+QA− − PQA and P+Qb− − Pqb difference spectra, a subtraction was performed (Figure 2). The absorbance changes caused by oxidation of the primary donor should be canceled, and only
the amide I and amide I_1 regions must be interpreted with caution. Clear effects are the difference band at 1735/1722 cm^{-1}, the negative band at 1675 cm^{-1}, the band pattern around 1530/1540 cm^{-1}, and the three bands at 1477, 1436, and 1394 cm^{-1}.

To more accurately search for quinone C=O contributions in the 1700–1600 cm^{-1} region and to verify the small differences discussed above, H_2O exchange was performed in hydration chambers for samples containing only QA and for samples reconstituted with QB. 2H_2O does not absorb strongly at 1600 cm^{-1}, an additional negative band at 1529 cm^{-1}, and in an intense negative band at 1675 cm^{-1}. Again a difference band around 1540 cm^{-1} and the three positive bands at 1479, 1442, and 1395 cm^{-1} are observed. The absorbance changes caused by quinone reduction, especially the bands at 1735, 1722, 1675, 1479, 1442, and 1395 cm^{-1}, agree quite well in H_2O (Figure 2) and D_2O (Figure 4). A second approach to identify absorptions belonging to Q_κ involves the selective reduction of the high-potential cytochromes (of the tightly bound cytochrome subunit) with ascorbate. In ascorbate-reduced samples containing only Q_κ, an electron will be transferred to Q_κ in approximately 200 ps (Woodbury et al., 1985). Before this charge-separated state can recombine (time constant of about 1 ms at pH 8; Shopes & Wraith, 1985), the highest potential cytochrome reduces P^+ in 0.32 μs (Dracheva et al., 1988), allowing a second electron to be transferred to I (about 3 ps; Fleming et al., 1988). Thus, steady-state illumination of ascorbate-reduced reaction centers initially produces PI-Q_κ (van Wijk et al., 1986), which can be compared to dithionite-reduced samples showing light-induced absorptions arising from PI^+ (Trosper et al., 1977; Prince et al., 1977). Continued illumination of dithionite- or ascorbate-reduced samples eventually produces the semiquinone dianion, PI^+Q_κ^−; this process has been estimated to occur in 95% of the reaction center population in Rps. viridis (van Wijk et al., 1986). Steady-state illumination at room temperature of dithionite-reduced reaction centers has also been shown to reduce the bacteriopheophytin in the M subunit (Thorner et al., 1981; Schenck et al., 1981). Although the extent of bacteriopheophytin reduction through continuous illumination of ascorbate-reduced reaction centers is not known, it is likely that BP_κ vibrations will contribute to the difference spectra of both dithionite- and ascorbate-reduced reaction center samples. Nevertheless, a subtraction of the dithionite-reduced reaction center difference spectrum from the difference spectrum obtained from ascorbate-reduced reaction centers should cancel bacteriopheophytin absorbance changes and emphasize vibrations arising from Q_κ / Q_κ.

Figure 5 shows the UV-visible light-induced difference spectra for reaction centers in which the cytochrome subunit is oxidized (Figure 5, top panel) and for reaction centers with the high-potential hemes reduced by ascorbate (Figure 5, bottom panel). Both spectra were obtained by continuous illumination with λ > 700 nm, in a manner analogous to the FTIR difference spectra. In the oxidized preparation (Figure 5, top), illumination stabilizes the charge-separated state P^+Q_κ.
as indicated by a complete bleaching of the 960-nm absorbance band as well as a bleaching of the Q_a (BChl_a) absorbance at 610 nm. The band with a negative lobe at about 850 nm and a positive lobe at 810 nm is thought to be due to a Stark shift of the accessory bacteriochlorophyll monomers and is typical for a P^+Q^- difference spectrum [see, e.g., Knapp et al. (1985)]. In contrast, continuous illumination of the ascorbate-reduced sample yields a bleaching of the BPh_b bands at 545 and 790 nm as well as a large blue shift of the 830-nm band; these changes are indicative of the photochemical trapping of I^- (Buchanan, 1990).

The difference spectra in Figure 6 indicate that in MB-containing reaction centers, the I^- difference spectrum published by Mantele et al. (1988) and the corresponding I^- and I-QA^- difference spectra are in complete agreement except between 1500 and 1400 cm^-1; in this region, positive bands arise at 1477 and 1429 cm^-1 which likely are due to Q_a^- vibrations. This indicates that the I^- - I(Q_a^- - IQ_a^-) difference spectrum is largely dominated by absorption changes due to reduction of the intermediate electron acceptor.

The difference spectra in Figure 6 indicate that in Mantele et al. (1988) a mixture of an I^- - I and I-QA^- - IQ_a^- is obtained and explains the discrepancies cited above.

The model compound spectra of BPh_b (Mantele et al., 1988) show the same absorbance changes between 1420 and 1330

![Figure 5: UV-visible light-induced difference spectra for Rps. viridis reaction centers.](image)

![Figure 6: FTIR light-induced difference spectra for chemically reduced Q_a-containing reaction centers.](image)
negative carbonyl bands are observed at 1703 and 1743 cm\(^{-1}\) in the difference spectrum. The difference spectrum is very similar to the \(I^-\) and \(I^-\)-shifted compared to model compounds, either could represent the reaction center \(I^-\) in the inhibited complex or the \(I^-\) state upon anion formation in an aprotic solvent as are observed in the \(I^-\) - \(I^+\) difference spectra. The absorbance band at 1714 cm\(^{-1}\) upon anion formation in an aprotic solvent is most likely caused by reduction of the intermediate electron acceptor. Upon anion formation of BPh\(_B\) in an aprotic solvent, negative carbonyl bands are observed at 1703 and 1743 cm\(^{-1}\) and a positive band at 1727 cm\(^{-1}\). This deviates from the band pattern observed upon reduction of the intermediate electron acceptor; the \(I^-\) - \(I^+\) difference spectra reveal two different carbonyl vibrations at 1745 and 1732 cm\(^{-1}\). In contrast to the \(P^+\) - \(P^\) difference spectra, no upshifted ester absorbance band is observed in the BPh\(_B\) state for either the model compound difference spectra or the \(I^-\) - \(I^+\) reaction center difference spectra. The absorbance band at 1714 cm\(^{-1}\), which is downshifted compared to model compounds, either could represent a downshifted ester carbonyl vibration (indicating hydrogen bonding of this group) or, as is found for the oxidation of the primary donor, could represent a 9-keto carbonyl vibration upshifted from 1682 cm\(^{-1}\), indicating a hydrophobic environment for the keto group. The absorbance changes below 1420 cm\(^{-1}\) are not perturbed by pigment-protein interactions in the reaction center complex since the same absorbance changes are observed during BPh\(_B\) reduction in aprotic solvents. However, band patterns are observed in the carbonyl region of the reaction center \(I^-\) - \(I^+\) difference spectra which deviate from model compound BPh\(_B\) - BPh\(_B\) difference spectra in an aprotic solvent, indicating more extensive interaction with the protein environment.

Figure 7A shows an FTIR difference spectrum for ascorbate-reduced reaction centers, arising from the transition PIQ\(_X\) → PI\(^+\)Q\(_X\) (subsequently referred to as an \(I^+\)Q\(_X\) - IQ\(_X\) difference spectrum). The difference spectrum is very similar to the \(I^-\) - \(I^+\) difference spectrum (Figure 6A); the characteristic pattern of four negative peaks at 1745, 1734, 1684, and 1655 cm\(^{-1}\) and one broad positive peak at 1716 cm\(^{-1}\) indicates the photochemical trapping of \(I^-\). A comparison of the difference spectra in Figures 6A and 7A reveals the same absorbance changes between 1800 and 1500 cm\(^{-1}\) except for the increased intensity at 1633 cm\(^{-1}\) for the ascorbate-reduced sample (Figure 7A); this intensity change could be caused by phase errors leading to shifts in intensity. Significant deviations are observed between 1500 and 1300 cm\(^{-1}\). In Figure 7A, additional negative bands are seen at 1535 and 1500 cm\(^{-1}\). Larger positive absorbance bands are also observed at 1477, 1441, and 1390 cm\(^{-1}\). Because the only new species stabilized by continuous illumination with ascorbate reduction as compared to dithionite reduction is Q\(_X^+\), the additional positive absorbances in Figure 7A must represent absorbance changes arising from the semiquinone anion radical or affected amino acid residues.

A subtraction of the \(I^-\) - \(I^+\) difference spectrum from the \(I^+\)Q\(_X\) - IQ\(_X\) difference spectrum was performed to more clearly visualize vibrations due to Q\(_X^+\)/Q\(_X^\) in this difference spectrum is shown in Figure 8. The similarity of Figure 8 to the subtractions of difference spectra in Figures 2 and 4 is remarkable: absorbance changes in the carbonyl region, now revealed by subtraction of the larger bacteriopheophytin absorbances in this region, are seen at 1745, 1477, 1440, and 1390 cm\(^{-1}\). A negative absorbance at 1562 cm\(^{-1}\) is apparent, and the absorbance changes at 1477, 1440, and 1390 cm\(^{-1}\) are distinctly revealed through the subtraction and agree with Figures 2 and 4. This is surprising because in Figures 2 and 4 absorbance changes caused by reduction of Q\(_X^\) should also be present, yet no clear deviations can be identified.

Although the infrared spectra of quinones have not been extensively studied, some data exist for neutral ubiquinones and menaquinones (Pennock, 1965) which are useful for the analysis of reaction center difference spectra. Neutral ubiquinones and menaquinones generally show an intense band at approximately 1650 cm\(^{-1}\) which is attributed to the quinone C=O stretch. The C=C stretch of the quinone ring occurs at about 1610 cm\(^{-1}\), and aromatic vibrations occur at about 1590 cm\(^{-1}\). Additionally, menaquinones show absorbance frequencies characteristic of the napthoquinone nucleus at 1667, 1587, 1299, and 714 cm\(^{-1}\). Ubiquinones show bands at 1259 and 1094 cm\(^{-1}\) which have been suggested to arise from the two methoxy groups in the quinone ring (Pennock, 1965). Spectroelectrochemical model compound studies of ubiquinone 0 (Bauscher et al., 1990) show upon anion formation the disappearance of two bands at 1660 and 1606 cm\(^{-1}\), independent of solvent, and the appearance of a larger band at 1500 cm\(^{-1}\) and a smaller band at 1473 cm\(^{-1}\) in an aprotic solvent, or a larger band at 1476 cm\(^{-1}\) with shoulders at 1438 and 1394 cm\(^{-1}\) in a protic solvent. In contrast, BPh\(_B\) model
compound studies (Mäntele et al., 1988) show upon anion formation almost no absorbance changes between 1500 and 1400 cm\(^{-1}\) with the exception of a small band at 1419 cm\(^{-1}\). Therefore, the additional positive bands observed at 1477, 1441, and 1390 cm\(^{-1}\) in the \(\Gamma Q_A^- - IQ_A\) difference spectrum can most likely be assigned to \(\cdots-O\) vibrations of the semiquinone anion radical due to the good agreement in frequency and intensity with quinone absorbance changes observed in aprotic solvent upon anion formation. A comparison of the \(\Gamma^- - I\) (Figure 6A) and \(\Gamma Q_A^- - IQ_A\) (Figure 7A) difference spectra reveals some dissimilarities in the 1700-1600 cm\(^{-1}\) region, but specific assignments require isotopic labeling.

Extended illumination of the ascorbate-reduced sample results in the difference spectrum depicted in Figure 7B. A comparison with Figure 7A shows intensity decreases at 1473, 1466, 1439, and 1410 cm\(^{-1}\). The same spectral features are observed during the formation of \(Q_2^+\) in aprotic solvent (Bauscher et al., 1990), indicating the accumulation of \(Q_{2}^-\) in the ascorbate-reduced reaction center sample. Contributions from \(BP_{M^-}\) are probably also present (Thorner et al., 1981). Model compound spectra for \(Q^2^-\) in an aprotic solvent show different spectral features which do not correspond to those observed in reaction center samples. The agreement of the spectral features in Figure 7B with model compound studies for \(Q^2^-\) formation in a protic solvent supports the assignment of the quinone vibrations and the conclusion that \(Q_{2}^-\) is hydrogen-bonded to its environment.

**DISCUSSION**

FTIR light-induced difference spectra have been recorded in *Rps. viridis* for the following charge-separated states: \(P^+Q_A^- - P^+Q_B^-\), \(P^+Q_A^2^- - P^+Q_B^2^-\), \(I^- - I\), \(I Q_A^- - IQ_A\), and \(I Q_{2}^- - IQ_A\). The \(P^+Q^- - PQ\) difference spectra are dominated by chromophore absorbances. Spectroelectrochemical studies of bacteriochlorophyll model compounds indicate that only the keto and ester carbonyl vibrations have differing absorbances when oxidation takes place in a hydrogen-bonded as compared to a non-hydrogen-bonded environment. The absorbance changes in the \(P^+Q^-\) charge-separated state show the characteristic absorbance changes of the ester and keto carbonyl vibrations of the primary donor in a non-hydrogen-bonded environment.

A lack of specific protein absorbance changes could have two explanations: (1) if the extinction coefficients are lower for reactive side groups than for reactive chromophores, the protein absorptions could be masked by chromophore bands or be too small to be resolved; or (2) the protein does not participate extensively in this charge separation. It has been shown (Gerwert et al., 1989) that extinction coefficients for participating protein groups, in this case an aspartate carbonyl, are of the same order of magnitude as chromophore extinction coefficients. Therefore, it is reasonable to expect that similar protein interactions in the reaction center should be observable if they are significant. Furthermore, the protein backbone undergoes a conformational change involving only one or two residues upon \(P^+Q^-\) stabilization, as estimated by the absorbance changes of the amide I and II bands (1647, 1545 cm\(^{-1}\)) during photooxidation.

A comparison of the \(\Gamma^- - I\) reaction center difference spectra with \(BP_{H} - BP_{B}^2\) difference spectra generated photochemicaly (Mäntele et al., 1988) reveals the same band patterns below 1420 cm\(^{-1}\) for reaction center difference spectra as for \(BP_{H} - BP_{B}\) recorded in an aprotic solvent. Above 1420 cm\(^{-1}\), however, notable deviations between the two types of difference spectra are observed. Electron transfer to \(BP_{B}\) has been measured in *Rps. viridis* under conditions where \(BP_{B}\) is prereduced; an electron-transfer branching ratio of approximately 200 was measured for \(k_{e}/k_{M}\) (Tiede et al., 1990). At room temperature with continuous illumination, the accumulation of \(BP_{M^-}\) cannot be excluded (Thorner et al., 1981) but should contribute no more than 25% to the \(BP_{B^-}\) vibrations.

The \(\Gamma^- - I\) difference spectra reveal two different carbonyl vibrations at 1745 and 1732 cm\(^{-1}\), as compared to the single carbonyl vibration at 1743 cm\(^{-1}\) seen in the model compound spectra. Two explanations could be that (1) the absorbance at 1745 cm\(^{-1}\) represents a protonated carboxylic acid (most likely Glu-L104; Nadebyk et al., 1988; Michel et al., 1986) and the absorbance at 1732 cm\(^{-1}\) arises from a hydrogen-bonded ester carbonyl group or (2) the bands at 1745 and 1732 cm\(^{-1}\) represent non-hydrogen-bonded and hydrogen-bonded ester carbonyl groups, respectively. The former explanation is strengthened by near-IR resonance Raman spectra (Bocian et al., 1987) which also provide evidence of Glu-L104 interaction with the \(C_{9}\) keto group of \(BP_{B}\); a strong band at 1726 cm\(^{-1}\) was attributed to a \(C_{2}\) carboxemethoxy carbonyl group which most likely becomes Raman-active by induced partial double bond character in the \(C_{9}C_{10}\) bond caused by a partial charge redistribution of the \(C_{9}=O\) keto group. However, an absorbance band of a protonated carboxylic acid should shift in \(^2H_2O\) by 10 cm\(^{-1}\) as shown in bacteriochlorophylls (Engelhard et al., 1985); such a pronounced shift was not observed by Nadebyk et al. (1990), making the assignment ambiguous. Once again, a small conformational change involving one or two residues takes place upon \(I^-\) charge stabilization as monitored by the intensities of the amide I and II bands.

The \(\Gamma Q_A^- - IQ_A\) reaction center difference spectra compare well with the \(\Gamma^- - I\) difference spectra above 1500 cm\(^{-1}\), indicating that most bands in this spectral region arise from the bacteriochlorophyll vibrations. The intensities of the amide I and II bands again indicate conformational changes involving one or two residues upon charge stabilization. It is possible to identify \(\cdots-O\) vibrations of \(Q_A\) at 1477, 1440, and 1390 cm\(^{-1}\) by comparison with \(\Gamma^- - I\) difference spectra. The identified \(\cdots-O\) vibrations of \(Q_{2}^-\) indicate clearly that the carbonyls of \(Q_{2}^-\) are hydrogen-bonded to their environments in the charge-separated state. No distinguishable carbonyl vibrations for the neutral \(Q_{2}\) or for \(Q_B/Q_{B^-}\) can be identified. It is possible that \(Q_{2}^-\) vibrations occur at about the same positions as the \(Q_{2}^-\) vibrations or that the \(Q_{2}^-\) vibrations are broadened by more extensive hydrogen bonding to the protein matrix and are masked by \(Q_{2}^-\) vibrations.

Because few specific protein vibrations are seen in all of the difference spectra, it appears that many protein residues must make small contributions to charge solvation rather than large contributions from a few select protein residues. No large conformational changes are observed in any of the charge-separated states stabilized by continuous illumination, indicating that the protein matrix surrounding the chromophores remains rigid throughout electron transfer from \(P\) to \(Q_{B}\). It can be excluded that larger structural rearrangements contribute to the fast forward electron-transfer rates [for a review, see Friesner and Won (1989)]. This conclusion is confirmed by X-ray diffraction experiments comparing dark and illuminated *Rps. viridis* crystals containing \(Q_{B}\), where no significant differences were seen between data sets collected to 3-A resolution (Buchanan et al., 1990b).

A molecular dynamics simulation of electron transfer from \(P\) to \(I\) implies similar conclusions: the \(P^+I^-\) charge separation appears to be stabilized by many small, rapid adjustments in the protein, largely accomplished by electrostatic interactions...
between partial charges localized on the chromophores and the surrounding protein matrix (Treutlein et al., 1991; Nonnella & Schulten, 1990). This would explain the relatively small protein contributions to the FTIR difference spectra; many small contributions from different types of amino acids would yield smaller absorptions than those found for the chromophores.

The difference spectra of the various charge-separated states suggest that the protein matrix of the reaction center behaves like an optimized solvent, largely hydrophobic in nature surrounding the special pair, still hydrophobic but with some polar protein–pigment interactions around the intermediate acceptor, and hydrophilic in the regions of QA and QB. The good solvation of QA- and Qe- can explain the relatively long lifetimes of the QA- and Qe- charge-separated states.

Interestingly, similar results to those presented here for Rps. viridis were recently reported for Rb. sphaeroides using a different experimental approach (Thibodeau et al., 1990). In these studies, the quinone vibrations in Rb. sphaeroides were assigned using time-resolved FTIR spectroscopy; the different recombination times of P+QA- and P+QB- allow selection of the quinone vibrations by subtraction at different time intervals. In agreement with our findings in the Rps. viridis difference spectra, correspondingly small quinone absorbance changes are observed for Rb. sphaeroides as compared to the absorbance changes of the primary donor (P) during charge separation. The similarities between the FTIR studies on Rps. viridis and Rb. sphaeroides reaction centers indicate very similar intermolecular reactions occurring in both types of reaction centers during charge separation.

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REFERENCES


A $^{31}$P NMR Study of Mitochondrial Inorganic Phosphate Visibility: Effects of Ca$^{2+}$, Mn$^{2+}$, and the pH Gradient

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ABSTRACT: The effects of external pH, temperature, and Ca$^{2+}$ and Mn$^{2+}$ concentrations on the compartmentation and NMR visibility of inorganic phosphate (P) were studied in isolated rat liver mitochondria respiring on succinate and glutamate. Mitochondrial matrix P is totally visible by NMR at 8°C and at low external concentrations of P. However, when the external P concentration is increased above 7 mM, the pH gradient decreases, the amount of matrix P increases, and the fraction not observed by NMR increases. Raising the temperature to 25°C also decreases the pH gradient and the P fraction observed by NMR. At physiologically relevant concentrations, Ca$^{2+}$ and Mn$^{2+}$ do not seem to play a major role in matrix P NMR invisibility. For Ca$^{2+}$ concentrations above 30 nmol/mg of protein, formation of insoluble complexes will cause loss of P signal intensity. For Mn$^{2+}$ concentrations above 2 nmol/mg of protein, the P peak can be broadened sufficiently to preclude detection of a high-resolution signal. The results indicate that mitochondrial matrix P should be mostly observable up to 25°C by high-resolution NMR. While the exact nature of the NMR-invisible phosphate in perfused or in vivo liver is yet to be determined, better success at detecting and resolving both P pools by NMR is indicated at high field, low temperature, and optimized pulsing conditions.

The phosphorylation potential [ATP]/[ADP][P] is frequently used as a measure of the available free energy of the cellular adenine nucleotide pool and thus is an important means of evaluating cell metabolism. In various tissues, a considerably higher phosphorylation potential is calculated using phosphorus-31 nuclear magnetic resonance ($^{31}$P NMR) measurements (Iles et al., 1985; Cunningham et al., 1986; Desmoulin et al., 1987) than by using biochemical data (Siess et al., 1982; Soboll et al., 1978; Aw et al., 1987; Kingenberg et al., 1987).