Resonance Raman Spectroscopic Evidence for Dielectric Asymmetry in Bacterial Photosynthetic Reaction Centers

Vaithianathan Palaniappan and David F. Bocian*

Department of Chemistry, University of California Riverside, California 92521-0403

Received December 12, 1994

The primary electron transfer processes in bacterial photosynthesis occur in a membrane protein complex known as the reaction center (RC).[1] Bacterial RCs consists of 10 cofactors (four bacteriochlorophylls (BChs), two bacteriopheophytins (BPhs), two quinones, a non-heme iron center, and a carotenoid) located in three polypeptide subunits designated by L, M, and H. The primary donor is a closely associated pair of BChs, known as the special pair (P). The primary acceptor is the BPh pigment in the L subunit.[1] The X-ray crystal structures of RCs reveal that the BCh and BPh pigments are arranged in the L and M subunits such that the macroscopic symmetry is approximately C_2.[2−4] However, this symmetry is broken by subunit-specific pigment−protein interactions. There are also asymmetries in L- versus M-side amino acid residues which do not directly interact with the pigments. The asymmetrical distribution of amino acid residues in the L versus M subunits is presumably responsible for the fact that the primary electron transfer proceeds only along the L side.[5] The possibility that the asymmetrical distribution of protein residues might be responsible for the unidirectionality of electron transfer has led to the characterization of a variety of genetically modified RCs in which the L- versus M-side asymmetries have been altered.[6−8] To date, however, no mutations have been made that alter the unidirectionality of electron transfer.

The fact that alteration of specific protein residues has no effect on the unidirectionality of electron transfer suggests that this property is determined by global environmental differences on the L versus M side of the RC. Recently Boxer and coworkers obtained experimental evidence in support of this hypothesis.[9] These workers showed via Stark effect measurements that the effective dielectric constant (ϵ_{eff}) on the L side is substantial (ϵ_{eff}(L) as high as 11) and considerably higher than that on the M side (ϵ_{eff}(M) ~ 2). The higher dielectric constant on the L side would preferentially stabilize charge-separated states such as $P^+BCh_L^-$ and $P^+BPh_L^-$ versus $P^+BCh_M^-$ and $P^+BPh_M^-$. The results of the Stark effect experiments are consistent with those of theoretical studies which predict that the energies of the former states are lower than those of the latter.[10]

If the dielectric asymmetry in the RC is in fact as large as that measured in the Stark effect experiments, this asymmetry should be manifested in other spectroscopic signatures of the L- versus M-side pigments. In particular, it is known that the frequency of the C_9-keto carbonyl stretching vibration ($\nu_{C_9=O}$) of chlorophyll is extremely sensitive to the dielectric properties of the host medium.[11] In aprotic solvents, the frequency of this mode is described reasonably well by the relationship

$$\nu_{C=O} = \nu_0 - C\frac{\epsilon - 1}{2\epsilon + 1}\frac{n^2 - 1}{2n^2 + 1} \quad (1)$$

where ϵ is the dielectric constant of the medium, n is the refractive index, and ν_0 and C are empirically determined constants.[11b] This relationship predicts that dielectric asymmetry in the RC could result in frequency differences in the $\nu_{C_9=O}$ modes of the L- versus M-side pigments that are as large as 10−20 cm^{-1}.[12]

In this communication, we explore the issue of dielectric asymmetry in RCs via resonance Raman (RR) spectroscopic studies of wild-type and genetically modified RCs from *Rhodobacter capsulatus*. The RR experiments focus on the $\nu_{C_9=O}$ modes of the BPh cofactors. The BPhs were targeted for study because scattering from these pigments can be selectively enhanced with the appropriate choice of excitation wavelength.[14,15] In the present study, $Q_y(1,0)$ excitation was used because the desired selectivity is achieved and the $\nu_{C_9=O}$ modes

(1) (a) Kirmaier, C.; Holten, D. *Photosynth. Res.* **1987**, *113*, 225−260. (b) Deisenhofer, J.; Michel, H. *Science* **1989**, *245*, 1463−1473. (c) Feher, G. *Annu. Rev. Phys. Chem.* **1989**, *58*, 607−663. (d) Boxer, S. G.; Goldstein, R. A.; Lockhart, D. J.; Middendorf, R. T.; Takiff, L. *J. Phys. Chem.* **1989**, *93*, 8280−8294. (e) Friesner, R. A.; Won, Y. *Biochim. Biophys. Acta* **1989**, *977*, 99−122. (f) Breton, J., Verméglio, A., Eds. *NATO ASI Ser., Ser. A* **1992**, *237*, 1−429. (g) Deisenhofer, J., Norris, J. R., Eds. *The Photosynthetic Reaction Center*; Academic: San Diego, 1993; Vol. II.

(2) (a) Deisenhofer, J.; Epp, O.; Miki, R.; Huber, R.; Michel, H. *Nature* **1985**, *339*, 618−624. (b) Deisenhofer, J.; Michel, H. *EMBO J.* **1989**, *8*, 2149−2169.

(3) (a) Allen, J. P.; Feher, G.; Yeates, T. O.; Komiya, H.; Rees, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8487−8491. (b) Yeates, T. O.; Komiya, H.; Chirino, A.; Rees, D. C.; Allen, J. P.; Feher, G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7993−7997.

(4) (a) Chang, C.-H.; Tiede, D.; Tang, J.; Smith, U.; Norris, J.; Schiffer, M. *FEBS Lett.* **1986**, *205*, 82−86. (b) El-Kabbani, O.; Chang, C.-H.; Tiede, D.; Norris, J.; Schiffer, M. *Biochemistry* **1991**, *30*, 5361−5369.

(5) Kirmaier, C.; Holten, D.; Parson, W. W. *Biochim. Biophys. Acta* **1985**, *810*, 49−61.

(6) (a) Bylina, E. J.; Youvan, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7226−7230. (b) Bylina, E. J.; Kirmaier, C.; McDowell, L.; Holten, D.; Youvan, D. C. *Nature* **1988**, *336*, 182−184. (c) Breton, J.; Bylina, E. J.; Youvan, D. C. *Biochemistry* **1989**, *28*, 6423−6430. (d) Coleman, W. J.; Youvan, D. C. *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 333−367. (e) DiMagno, T. J.; Bylina, E. J.; Angerhofer, A.; Youvan, D. C.; Norris, J. R. *Biochemistry* **1990**, *29*, 899−907. (f) Chan, C.-K.; Chen, L. X.-Q.; DiMagno, T. J.; Hanson, D. K.; Nance, S. L.; Schiffer, M.; Norris, J. R.; Fleming, G. *Chem. Phys. Lett.* **1991**, *176*, 182−184. (g) Du, M.; Rosenthal, S. J.; Xie, X.; DiMagno, T. J.; Schmidt, M.; Hanson, D. K.; Schiffer, M.; Norris, J. R.; Fleming, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8517−8521. (h) Taguchi, A. K. W.; Stocker, J. W.; Alden, R. G.; Causgrove, T. P.; Peloquin, J. M.; Boxer, S. G.; Woodbury, N. W. *Biochemistry* **1992**, *31*, 10345−10355. (i) Stocker, J. W.; Taguchi, A. K. W.; Murchison, H. A.; Woodbury, N. W.; Boxer, S. G. *Biochemistry* **1992**, *31*, 10356−10362. (j) Taguchi, A. K. W.; Stocker, J. W.; Boxer, S. G.; Woodbury, N. W. *Photosynth. Res.* **1993**, *36*, 43−58.

(7) (a) Peloquin, J. M.; Bylina, E. J.; Youvan, D. C.; Bocian, D. F. *Biochemistry* **1990**, *29*, 8417−8424. (b) Peloquin, J. M.; Bylina, E. J.; Youvan, D. C.; Bocian, D. F. *Biochim. Biophys. Acta* **1991**, *1056*, 85−88. (c) Nabedryk, E.; Robles, S. J.; Goldman, E.; Youvan, D. C.; Breton, J. *Biochemistry* **1992**, *31*, 10852−10858.

(8) (a) Finkele, U.; Lauterwasser, C.; Zinth, W.; Gray, K. A.; Oesterhelt, D. *Biochemistry* **1990**, *29*, 8517−8521. (b) Nagarajan, V.; Parson, W. W.; Gaul, D.; Schenck, C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7888−7892. (c) Hammes, S. L.; Mazzoli, L. T.; Boxer, S. G.; Gaul, D.; Schenck, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5682−5686. (d) Middendorf, T. R.; Mazzoli, L. T.; Gaul, D. F.; Schenck, C. C.; Boxer, S. G. *J. Phys. Chem.* **1991**, *95*, 10142−10151. (e) Kirmaier, C.; Holten, D. *Biochemistry* **1991**, *30*, 609−613. (f) Kirmaier, C.; Gaul, D.; DeBey, R.; Holten, D.; Schenck, C. C. *Science* **1991**, *251*, 922−927. (g) Williams, J. C.; Alden, R. G.; Murchison, H. A.; Peloquin, J. M.; Woodbury, N. W.; Allen, J. P. *Biochemistry* **1992**, *31*, 11029−11037. (h) Murchison, H. A.; Alden, R. G.; Peloquin, J. M.; Taguchi, A. K. W.; Woodbury, N. W.; Williams, J. C. *Biochemistry* **1993**, *32*, 3498−3505.

(9) Steffan, M.; Lao, K.; Boxer, S. G. *Science* **1994**, *264*, 810−816.

(10) (a) Parson, W. W.; Chu, Z. T.; Warshel, A. *Biochim. Biophys. Acta* **1990**, *1017*, 251−272. (b) Thompson, M. A.; Zerner, M. C. *J. Am. Chem. Soc.* **1991**, *113*, 8210−8215. (c) Treutlein, H.; Schluten, K.; Brunger, A. T.; Karplus, M.; Deisenhofer, J.; Michel, H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 75−79. (d) Marchi, M.; Gehlen, J. N.; Chandler, D.; Newton, M. *J. Am. Chem. Soc.* **1993**, *115*, 4178−4190.

(11) (a) Koyama, Y.; Umemoto, Y.; Akamatsu, A. *J. Mol. Struct.* **1986**, *146*, 273−287. (b) Krawczyk, S. *Biochim. Biophys. Acta* **1989**, *976*, 140−149.

(12) Frequency differences in this range are predicted if $C \sim 250$ cm^{-1} (determined for chlorophyll[11b]) and $n \sim 1.4$ (a reasonable value[13]). For example, if ϵ_{eff} (L) = 9.5 and ϵ_{eff} (M) = 1.5,[9] a ~14 cm^{-1} difference is predicted.

(13) *Handbook of Chemistry and Physics*, 50th ed.; Weast, R. C., Ed.; CRC Publishing: Cleveland, OH, 1969; pp E230−E231.

(14) (a) Palaniappan, V.; Bocian, D. F. In ref 1f, pp 119−126. (b) Palaniappan, V.; Martin, P. C.; Chynwat, V.; Frank, H. A.; Bocian, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 12035−12049.

0002-7863/95/1517-3647$09.00/0

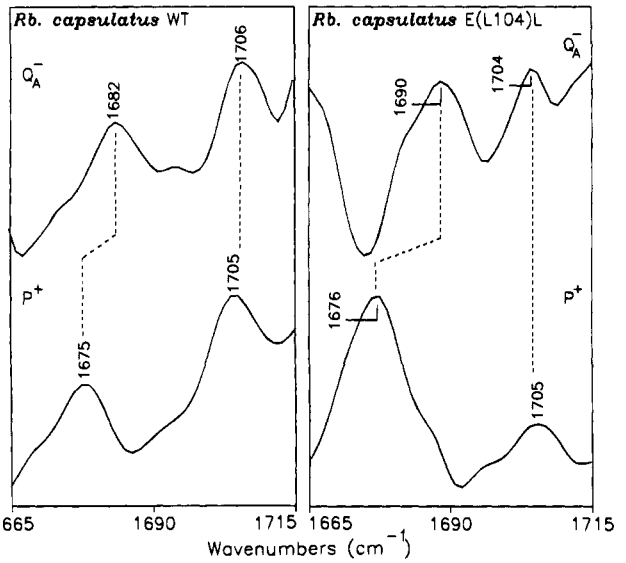

Figure 1. $Q_y(1,0)$ excitation (λ_{ex} = 676.4 nm) RR spectra of wild-type (left panel) and E(L104)L mutant (right panel) RCs from *Rb. capsulatus* observed in the region of the $\nu_{C_9=O}$ modes of the BPhs. The spectra of chemically reduced (Q_A^-) and oxidized (P^+) RCs are shown in the top and bottom traces of each panel, respectively. The laser power was ~1 mW; the temperature was 200 K.

scatter very strongly.[14] The $\nu_{C_9=O}$ modes of the BPhs are also preferred targets because their frequencies lie in a spectral region which allows unambiguous assignments to be made.[14,15] In contrast, there are still uncertainties in the assignments for the $\nu_{C_9=O}$ modes of the BChs (and the C_{2a}=O acetyl stretches of the BPhs and BChs).[14b] The new RR data reported in the present study are consistent with the existence of a significant dielectric asymmetry in the RC. The data are also consistent with the direction and magnitude of the asymmetry measured in the Stark effect experiments.[9]

The $Q_y(1,0)$ excitation (λ_{ex} = 676.4 nm) RR spectra of wild-type and E(L104)L mutant RCs from *Rb. capsulatus* observed in the region of the $\nu_{C_9=O}$ modes of the BPhs (1665−1715 cm^{-1}) are shown in Figure 1.[16] In the mutant, the glutamic acid at position L104, which forms a hydrogen bond to the C_9=O group of BPh_L in wild-type, is replaced by a non-hydrogen-bonding leucine.[6b] The RR spectra of chemically reduced (Q_A^-) wild-type RCs (top left trace) exhibit two prominent bands near 1706 and 1682 cm^{-1}. These bands are assigned to the $\nu_{C_9=O}$ vibrations of BPh_M and BPh_L, respectively, by analogy to previous assignments made for the BPhs in RCs from *Rb. sphaeroides* wild-type.[14,15] The lower frequency of the $\nu_{C_9=O}$ mode of BPh_L relative to that of BPh_M has generally been attributed to a strong hydrogen-bonding interaction between the keto group and the E(L104) residue.[15] In contrast, no residues are in a position to hydrogen bond to the C_9-keto group of BPh_M,[6b] hence the higher frequency of its $\nu_{C_9=O}$ mode. The RR spectrum of Q_A^- RCs from the E(L104)L mutant (top right trace) also exhibits two prominent features in the 1665−1715-cm^{-1} range. One band is at 1704 cm^{-1}, which is within experimental error of the frequency of the $\nu_{C_9=O}$ mode of BPh_M in wild-type RCs. The other band occurs at 1690 cm^{-1}, which is ~8 cm^{-1} higher than that observed for the $\nu_{C_9=O}$ mode of BPh_L in wild-type RCs. The higher frequency for the $\nu_{C_9=O}$ mode of BPh_L in the mutant is consistent with removal of the hydrogen-bonding residue at position L104. Surprisingly, replacement of this residue results in an upshift that only compensates for approximately one-third of the frequency difference observed for the $\nu_{C_9=O}$ modes of BPh_L versus BPh_M in wild-type RCs. This result indicates that a significant asymmetry exists between BPh_L and BPh_M above and beyond that resulting from specific interactions with the E(L104) residue. The frequency difference between the $\nu_{C_9=O}$ modes of BPh_L and BPh_M in the E(L104)L mutant cannot be due to formation of a new hydrogen bond to the former pigment or to other mutation-induced structural differences between the two pigments. Indeed, the E(L104)L mutation eliminates asymmetries in the absorption[6b,c] and ring-skeletal vibrational[7b] spectra of BPh_L and BPh_M that are characteristic of wild-type RCs.[14b] The fact that BPh_L is closer to the negatively charged quinone (Q_A^-) is also not a determinant of the frequency difference between the $\nu_{C_9=O}$ modes of BPh_L and BPh_M. These frequencies are independent of the oxidation state of Q_A in both the E(L104)L mutant and wild-type RCs (data not shown). An alternative explanation for the lower frequency of the $\nu_{C_9=O}$ vibration of BPh_L is that this pigment resides in a region of intrinsically larger ϵ_{eff}. The ~14-cm^{-1} frequency difference observed for the $\nu_{C_9=O}$ modes of BPh_L versus BPh_M in the E(L104) mutants is predicted by eq 1 using values of $\epsilon_{eff}(L)$ and $\epsilon_{eff}(M)$ that are within the range determined in the Stark effect studies.[9] The fact that symmetrization of the structure and local environment of the two BPhs in the RC compensates for only a fraction of the asymmetry between the cofactors explains why the E(L104)L mutation does not affect the directionality of electron transfer and also why the kinetics of this process are only slightly different from those of wild-type.[6b]

In order to further explore the effects of dielectric asymmetry, RR spectra were also recorded for chemically oxidized RCs (P^+) (Figure 1, bottom traces). These spectra reveal that the formation of P^+ results in significant downshifts of the $\nu_{C_9=O}$ modes of the BPh_L in both wild-type and E(L104)L mutant RCs. In contrast, negligible effects are observed on the frequencies of the $\nu_{C_9=O}$ modes of the BPh_M cofactors. These results are consistent with those made in previous RR studies of wild-type RCs from *Rb. sphaeroides*.[14] The lower frequency observed for the $\nu_{C_9=O}$ mode of BPh_L in P^+ versus Q_A^- RCs could arise if $\epsilon_{eff}(L)$ of the former RCs is larger than ϵ_{eff} (L) of the latter. This result is qualitatively consistent with the prediction of the Stark effect experiments.[9] The increase in $\epsilon_{eff}(L)$ would tend to preferentially stabilize charge-separated states on the L side of the RC. It should be stressed, however, that the oxidation-induced RR downshifts are larger (particularly in the mutant) than those expected on the basis of the difference in ϵ_{eff} (L) determined from the Stark effect measurements. This result indicates that BPh_L experiences additional perturbations (besides changes in ϵ_{eff} (L)) upon oxidation of P. Regardless of the origin of the oxidation-induced perturbations, the fact that oxidation affects only the BPh on the active electron-transfer branch suggests that cooperative events occur in the RC which could influence the directionality of electron transfer. We are currently in the process of investigating this issue.

(15) (a) Lutz, M.; Robert, B. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley: New York, 1988; Vol. 3, pp 347−411. (b) Robert, B. *Biochim. Biophys. Acta* **1990**, *1017*, 99−111. (c) Lutz, M.; Mäntele, W. In *Chlorophylls*; Scheer, H., Ed.; CRC Press: Boca Raton, FL, 1991; pp 855−902.

(16) The RCs were prepared as described in ref 7a. The RR spectra were acquired using instrumentation and techniques described in ref 14b.

Acknowledgment. This work was supported by Grant GM39781 (D.F.B.) from the National Institute of General Medical Sciences. We thank Drs. E. J. Bylina, D. C. Youvan, and C. Kirmaier for providing the RCs from *Rb. capsulatus*.

JA944003L