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Near-infrared-excitation resonance Raman spectra of bacterial photosynthetic reaction centers

Implications for path-specific electron transfer

David F. Bocian, Nancy J. Boldt, Barry W. Chadwick+ and Harry A. Frank+

Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA 15213 and +*Department of Chemistry, University of Connecticut, Storrs, CT 06268, USA*

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The first near-infrared (Q_y)-excitation resonance Raman spectrum of photosynthetic reaction centers (*Rb. sphaeroides* wild type) is reported. This spectrum exhibits features which are not observed with excitation into either the Q_x or Soret absorption bands. The spectral data indicate that the partial double-bond character is induced in the C_9C_{10} bond of the isocyclic ring of one of the pigments via interactions with the protein. It is proposed that this modified pigment is the L-subunit bacteriopheophytin and that the preference for electron transfer to this molecule could be in part due to the change in electronic structure induced by the site-specific pigment-protein interaction.

Photosynthesis; Reaction center; Resonance Raman spectrum; Electron transfer; Pigment-protein interaction

1. INTRODUCTION

The bacterial photosynthetic reaction center is comprised in part of four bacteriochlorophyll (BChl) and two bacteriopheophytin (BPheo) molecules and one iron-quinone complex [1]. All of these pigments are noncovalently bound to two polypeptides denoted L and M [1]. The characterization of the structure of the bacterial photosynthetic reaction center pigment-protein complex by X-ray crystallographic analysis has revealed that the pigments are arranged with an approximate two-fold rotation symmetry (C_2) relating the L and M subunits [2–4]. Two of the BChl molecules, one bound to each polypeptide, are closely associated with each other and constitute the primary electron donor (the 'special pair', $BChl_2$). The other two BChls and the two BPheos, one of which is thought to be the primary electron acceptor [1], reside in close proximity to the primary donor and are symmetrically positioned about the C_2 symmetry axis [3]. The macromolecular arrangement of the pigments is highly suggestive of parallel paths for electron transfer across the photosynthetic membrane.

Optical absorption experiments performed on the reaction-center complex from *Rb. sphaeroides* have shown that one of the BPheos absorbs at 763 nm (Q_y) and 546 nm (Q_x) whereas the other BPheo absorbs at 753 nm (Q_y) and 531 nm (Q_x) [5,6]. Picosecond transient absorption measurements have shown that at temperatures below 100 K, only the long-wavelength absorbing BPheo undergoes spectral changes consistent with the formation of a radical anion species [6]. This indicates that only the long-wavelength absorbing BPheo is functioning as an electron acceptor. An analysis of the polarized optical absorption bands of the

Correspondence address: D.F. Bocian, Dept of Chemistry, Carnegie-Mellon University, Pittsburgh, PA 15213, USA

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crystals indicates that this BPheo is bound in the L subunit [7–9]. Thus, substantial asymmetry exists in the reaction-center complex despite the symmetrical macromolecular arrangement of the various pigments. The X-ray crystallographic data obtained for reaction centers from *Rb. viridis* [2,3] and *Rb. sphaeroides* [4] lend some insight into the structural basis of the asymmetry. These data indicate that the protein residues in proximity to the BPheo in the L subunit are different from those in proximity to the BPheo in the M subunit. It has been suggested that this difference in local structure is the origin of the difference in the electronic and functional properties of the two BPheos [10]. However, it is not clear precisely how the differential pigment-protein interactions in the two subunits exert control over these properties.

Resonance Raman (RR) spectroscopy has proven to be a useful probe of the structure of chlorophyll molecules both in solution and in photosynthetic proteins [11–19]. Studies by Lutz and co-workers have elucidated the coordination number of the central magnesium ion and the extent of the coordination of the various ring-carbonyl groups in both model and natural systems [12,13]. All of these RR studies were performed with excitation into either the Soret or Q_x absorption bands. No RR studies have been reported which probe the photophysically important, lowest-energy Q_y states of photosynthetic pigments. This is due in part to the low Raman scattering efficiency in the red but, more importantly, to the interference from fluorescence emission. Recently Q_y-excitation RR spectra of metallochlorins [20,21] and chlorophyll derivatives [22] have been obtained by examining systems which contain fluorescence-quenching open-shell metals such as Fe(III) and Ni(II). These model-compound studies have demonstrated that the RR intensity-enhancement patterns observed with excitation into the Q_y state are quite different from those observed with excitation into either the Soret or Q_x states.

In this letter we report the first near-infrared (Q_y)-excitation RR spectrum of photosynthetic reaction centers. The acquisition of this spectrum demonstrates that the photophysically-relevant electronic states of reaction centers can be probed directly by using conventional RR techniques and that it is not necessary to employ more complicated non-linear methods such as coherent anti-Stokes Raman spectroscopy (CARS). The data presented here provide new information concerning the electronic structure of the pigments in the reaction centers and provide new insight into the structural basis of path-specific electron transfer in the photosynthetic system.

2. MATERIALS AND METHODS

The reaction centers were prepared as described in [23] and were solubilized in 0.01 M Tris (pH 8.0), 0.15% Triton X-100 and 0.5 M NaCl. The RR spectra were recorded with the spectrometer described in [21] except that an extended-red photomultiplier tube (Hamamatsu R758) was employed. The excitation wavelength (λ_{ex} = 752.8 nm) was provided by a krypton ion laser (Coherent Radiation K2000). The spectral conditions are given in the figure legend. The RR spectra were obtained at room temperature of sodium dithionite-reduced reaction centers (A_{800} = 15) contained in a sealed capillary tube. The absorption spectrum of the sample was recorded after various time intervals of laser irradiation in order to monitor its photostability. It was found that the reduced reaction centers are stable for 2–3 h at room temperature under low levels (20 mW or less) of infrared laser irradiation. This situation can be contrasted with that observed upon excitation with comparable levels of visible or UV radiation. Under these conditions, the reaction centers exhibit photodamage after only few minutes of illumination at room temperature.

3. RESULTS AND DISCUSSION

The high-frequency (900–1800 cm^{-1}) near-infrared-excitation (λ_{ex} = 752.8 nm) RR spectrum of sodium dithionite-reduced reaction centers from *Rb. sphaeroides* wild type is shown in fig.1. In general, we find that Q_y-excitation RR spectra of chemically-reduced intact reaction centers can be obtained relatively free of fluorescence provided that the protein is scrupulously purified to remove adventitious BChl and BPheo. The RR spectrum shown is obtained at an excitation wavelength which is nearly coincident with the system origins of the Q_y absorptions of the two BPheos in the reaction center [6]; consequently, strong RR scat-

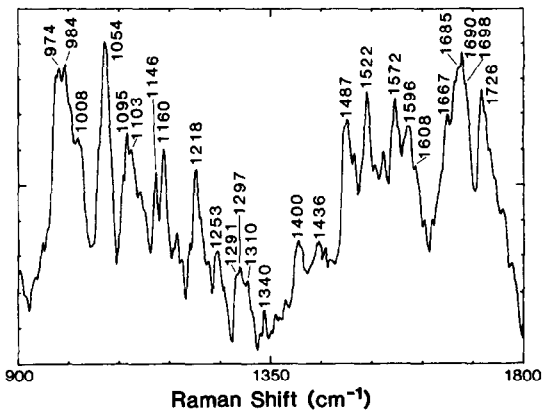

Fig.1. The high-frequency (900–1800 cm^{-1}) near-infrared-excitation (λ_{ex} = 752.8 nm) RR spectrum of sodium dithionite-reduced reaction centers from *Rb. sphaeroides* wild type. The RR spectrum was obtained at room temperature and is an average of three scans recorded at 2 cm^{-1} intervals at a rate of 1 s/point. The spectral slit width was 3 cm^{-1} and the incident power was less than 20 mW.

tering is expected to be observed from these pigments. It should be noted, however, that the excitation wavelength coincides with vibronic transitions of the $BChl_2$ unit and the two accessory BChls; thus, RR scattering is also expected from certain vibrations of these molecules. Although the RR intensity pattern observed with Q_y excitation is quite different from that observed with either Q_x or Soret excitation, some bands are common to the Q_y- and higher-energy-excitation RR spectra. Comparison of the spectrum shown in fig.1 with those reported [12–15,18] reveals that RR bands due to BChl and BPheo are observed in the near-infrared-excitation spectrum. This is evidenced by the multiplicity of bands in both the 1290–1310 cm^{-1} and 1630–1700 cm^{-1} regions. The former group of bands is due primarily to methine hydrogen deformations whereas the latter group is due to the carbonyl stretches of the C_9 keto groups and the C_2 acetyl groups [12,13].

The most striking feature in the Q_y-excitation RR spectrum is the strong band at 1726 cm^{-1}. Bands due to the carbonyl stretches of the C_{10} carbomethoxy and C_7 propionic ester modes are observed in this region of the infrared spectrum [24]. No other fundamental vibrations of the phorbin skeleton or the substituent groups occur in this frequency region. Bands due to ester carbonyl vibrations are not observed in either Q_x- or Soret-excitation RR spectra [11–19]. Similarly, these modes are not observed in Q_y-excitation RR spectra of Ni(II)-substituted chlorophylls in solution ([22]; Boldt, N. and Bocian, D., unpublished). The lack of intensity enhancement of the carbomethoxy and propionic ester carbonyl modes has been attributed to the fact that these carbonyl groups are out of the π-conjugation pathway of the macrocycle [13]. The significant RR intensity of the 1726 cm^{-1} band in the Q_y-excitation spectrum of the reaction centers, along with its lack of enhancement in model complexes, indicates that the protein matrix induces some degree of conjugation of an ester carbonyl group on one of the pigments into its phorbin π-system. The C_{10} carbomethoxy carbonyl group can conjugate into the macrocycle if some double-bond character is induced in the C_9C_{10} bond of ring V via a partial redistribution of charge from the C_9 keto group. A complete redistribution of charge would represent enol formation. Enolized forms of chlorophyll *a* have been postulated to play a role in green plant photosynthesis [25–27]. The presence of a partial double-bond character in C_9C_{10} is supported by the observation of the C_{10} ester carbonyl stretch at 1726 cm^{-1}, which is lower than the frequency observed in solution infrared studies of BChl where C_9C_{10} is a formal single bond (1736 cm^{-1}) [25]. On the other hand, the downshift of the carbonyl stretch which occurs in the reaction-center pigment is less than that observed for chlorophyll *a* enol model compounds where a formal C_9C_{10} double bond results in a shift from 1738 to 1720 cm^{-1} ([28]; Wasielewski, M., personal communication). It should be emphasized that it is not possible to infer the extent of C_9C_{10} double-bond character in the reaction-center pigment by simply comparing the frequency observed for its C_{10} carbonyl stretch with that observed for the analogous mode of model compounds in solution. The frequency of this carbonyl vibration is expected to be a complicated function of a number of factors including the extent of hydrogen bonding to both the C_9 and C_{10} carbonyl groups, the conformation of ring V and the dielectric constant of the medium. The fact that there must be some degree of C_9C_{10} double-bond character in the reaction-center pigment in both the ground and excited electronic

states is dictated by the unusually low frequency of the C_{10} carbonyl vibration and by the fact that it is observed at all in the RR spectrum.

The crystal structure of *Rb. sphaeroides* has been reported at 3.7 Å resolution [4]. The analysis of the structural data (Chang, C.-H. et al., personal communication) indicates that the C_9 carbonyl group of the BPheo in the active L subunit is hydrogen bonded to a nearby glutamic acid residue of the protein. The C_9 carbonyl of the BPheo associated with the inactive M subunit is in proximity to a phenylalanine and two threonines. At the current resolution of the X-ray analysis, neither of the threonine residues is apparently close enough to hydrogen bond to the C_9 keto group of the BPheo. In addition, no hydrogen bonding occurs to the C_9 keto groups of the $BChl_2$ unit or the accessory BChls. The hydrogen bond formation to the C_9 keto group of the BPheo in the L subunit provides a means of redistributing the charge density from the C_9O bond to the C_9C_{10} bond in this pigment. This result strongly suggests that the ester carbonyl stretching mode observed in the RR spectrum is that of the BPheo in the L subunit. The partial double-bond character in C_9C_{10} for this BPheo could account for the differences in both the optical spectra and the electron-transfer activities of the two BPheos in the reaction center. In this connection, studies of pheophytin *a* enol model compounds indicate that complete double-bond formation via enolization results in a large red shift of the Q_y absorption band and a much less-negative reduction potential (Wasielewski, M., personal communication).

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REFERENCES

[1] Okamura, M.Y., Feher, G. and Nelson, N. (1982) in: Photosynthesis: Energy Conversion by Plants and Bacteria, vol.1, pp.195–272, Academic Press, New York.
[2] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398.
[3] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[4] Chang, C.-H., Tiede, D., Tang, J., Smith, U., Norris, J. and Shiffer, M. (1986) FEBS Lett. 205, 82–86.
[5] Dutton, P.L., Prince, R.C. and Tiede, D.M. (1978) Photochem. Photobiol. 28, 939–949.
[6] Kirmaier, C., Holten, D. and Parson, W.W. (1985) Biochim. Biophys. Acta 810, 49–61.
[7] Zinth, W., Knapp, E.W., Fischer, S.F., Kaiser, W., Deisenhofer, J. and Michel, H. (1985) Chem. Phys. Lett. 119, 1–4.
[8] Knapp, E.W., Fischer, S.F., Zinth, W., Sandler, M., Kaiser, W., Deisenhofer, J. and Michel, H. (1985) Proc. Natl. Acad. Sci. USA 82, 8463–8467.
[9] Breton, J. (1985) Biochim. Biophys. Acta 810, 235–245.
[10] Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.
[11] Robert, B. and Lutz, M. (1986) Biochemistry 25, 2303–2309.
[12] Lutz, M. and Robert, B. (1985) in: Antennas and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.E. ed.) pp.138–145, Springer, Berlin.
[13] Lutz, M. (1984) in: Advances in Infrared and Raman Spectroscopy (Clark, R.J.H. ed.) vol.11, pp.211–300, Wiley, New York.
[14] Lutz, M., Hoff, A.L. and Brehanet, L. (1982) Biochim. Biophys. Acta 679, 331–341.
[15] Lutz, M. and Kleo, J. (1976) Biochem. Biophys. Res. Commun. 69, 711–717.
[16] Lutz, M. (1974) J. Raman Spectrosc. 2, 497–516.
[17] Cotton, T.M. and Van Duyne, R.P. (1981) J. Am. Chem. Soc. 103, 6020–6026.
[18] Cotton, T.M., Parks, K.D. and Van Duyne, R.P. (1980) J. Am. Chem. Soc. 102, 6399–6407.
[19] Cotton, T.M. and Van Duyne, R.P. (1978) Biochem. Biophys. Res. Commun. 82, 424–433.
[20] Andersson, L.A., Loehr, T.M., Sotiriou, C., Wu, W. and Chang, C.K. (1986) J. Am. Chem. Soc. 108, 2908–2916.
[21] Andersson, L.A., Sotiriou, C., Chang, C.K. and Loehr, T.M. (1987) J. Am. Chem. Soc., in press.
[22] Boldt, N.J., Donohoe, R.J., Birge, R.R. and Bocian, D.F. (1987) J. Am. Chem. Soc., in press.

[23] McGann, W.J. and Frank, H.A. (1985) Biochim. Biophys. Acta 807, 101–109.
[24] Katz, J.J., Shipman, L.L., Cotton, T.M. and Janson, T.R. (1978) in: The Porphyrins (Dophin, D. ed.) vol. V, pp.401–458, Academic Press, New York.
[25] Franck, J. and Livingston, R. (1941) J. Chem. Phys. 9, 184–190.
[26] Fetterman, L.M., Galloway, L., Winograd, N. and Fong, F.K. (1977) J. Am. Chem. Soc. 99, 653–655.
[27] Wasielewski, M.R., Norris, J.R., Shipman, L.L., Lin, C.-P. and Svec, W.A. (1981) Proc. Natl. Acad. Sci. USA 78, 2957–2961.
[28] Hynninen, P.H., Wasielewski, M.R. and Katz, J.J. (1979) Acta Chem. Scand. B 33, 637–648.