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BBA Report

Effects of pigment–protein interactions on the conformation of the primary electron acceptor in *Rhodobacter capsulatus* reaction centers

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Resonance Raman spectra are reported for RCs from *Rb. capsulatus* in which the L104 glutamic acid is replaced by glutamine. The skeletal modes of the primary electron acceptor, BPh_L, in these RCs undergo temperature-dependent frequency shifts that are identical to those observed for BPh_L in RCs from wild-type. This observation suggests that the strength of the hydrogen bond between the L104 residue and the C_9 keto group of BPh_L is not a determinant of the temperature-dependent conformation of this pigment.

The X-ray crystal structures obtained for the reaction center (RC) proteins from *Rps. viridis* and *Rb. sphaeroides* provide detailed information regarding the arrangement of the photophysically active pigments in the proteins and the nature of the interactions between these pigments and the protein matrix [1–9]. These data indicate that the protein environment in the vicinity of the bacteriopheophytin (BPh) molecule in the L subunit is different from that in the M subunit. In particular, glutamic acid L104 is within hydrogen-bonding distance of the C_9 keto group (ring V) of the primary electron acceptor, BPh_L. Conversely, no amino acid residues are sufficiently close for hydrogen bonding to the inactive pigment, BPh_M [4]. A variety of spectroscopic studies have provided evidence that there is direct interaction between BPh_L and glutamic acid L104 [10–13].

Recently, a number of site-specifically modified mutants have been prepared for RCs from *Rb. capsulatus* [14–17]. Although the crystal structure of RCs from *Rb. capsulatus* wild-type has not been determined, a variety of studies indicate that the structural and photophysical properties of this protein are similar to those of RCs from *Rb. sphaeroides* and *Rps. viridis* [3,14,18–24]. This in turn suggests that the X-ray structures of these latter species serve as an appropriate model for RCs from *Rb. capsulatus*. The crystallographic data in conjunction with the availability of mutant RCs allows a direct investigation of how changes in pigment structural and electronic properties and changes in pigment–protein interactions influence the electron transfer process. In this regard, two mutant *Rb. capsulatus* RCs have been prepared which involve changes in glutamic acid L104 [14]. In the $Glu^{L104} \rightarrow Gln$ mutant, the strongly hydrogen bonding glutamic acid residue is replaced by a much weaker hydrogen bonding glutamine. In the $Glu^{L104} \rightarrow Leu$ mutant, the hydrogen bond is completely elimimated. Optical studies on RCs from these two mutants indicate that much of the red shift of the Q_x absorption band of BPh_L with respect to that of BPh_M in wild-type RCs is due to the presence of the strongly hydrogen-bonding glutamic acid residue [14,18]. Picosecond transient optical measurements on these mutants have shown that altering the hydrogen-bonding characteristics at position L104 also changes the rates of electron transfer [14]. In RCs from both the $Glu^{L104} \rightarrow Gln$ and $Glu^{L104} \rightarrow Leu$ mutants, these rates are slower than those exhibited by RCs from the wild-type [20]. The changes in hydrogen-bonding capability at position L104 do not, however, alter the path specificity of electron transfer (through the L branch [25]).

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Abbreviations: RC, reaction center; BPh, bacteriopheophytin; BChl, bacteriochlorophyll; RR, resonance Raman; C_aC_m, methine bridges of the bacteriochlorin macrocycle.

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Our group has been using resonance Raman (RR) spectroscopy to probe the structure of the bacteriochlorin macrocycles in both wild-type and genetically modified RCs [10,13,19]. In recent studies on RCs from *Rb. sphaeroides* and *Rb. capsulatus* wild-type, we found that the frequencies of certain skeletal modes are different for BPh_L and BPh_M [13,19]. In addition, the conformation of the BPh_L ring skeleton changes with temperature, whereas the conformations of BPh_M and the bacteriochlorophyll (BChl) pigments in the RCs do not. This conformational change involves a decrease in the extent of out-of-plane distortion of the macrocycle at low (< 100 K) versus high (> 180 K) temperatures. The exact origin of the temperature sensitivity of the structure of BPh_L is not certain; however, the RR studies on RCs from the $Glu^{L104} \rightarrow$ Leu mutant of *Rb. capsulatus* clearly indicate that the amino acid residue at position L104 mediates the effect [19]. In particular, substitution of leucine for glutamic acid completely eliminates the temperature sensitivity of the conformation of BPh_L. Furthermore, in RCs from the $Glu^{L104} \rightarrow$ Leu mutant, the skeletal-mode frequencies of BPh_L are identical with those of BPh_M. This latter observation indicates that the conformations of the two BPhs are essentially identical when the interactions between BPh_L and glutamic acid L104 are eliminated.

In order to investigate further the nature of the pigment–protein interactions which give rise to the temperature sensitivity of the conformation of BPh_L, we have examined the RR spectra of RCs from the $Glu^{L104} \rightarrow$ Gln mutant of *Rb. capsulatus*. This system is appealing for several reasons. On the one hand, the hydrogen bonding capabilities of glutamine are poor relative to those of glutamic acid. Thus, from this perspective, the two residues are quite different. On the other hand, the R-groups of glutamine and glutamic acid are similar in size. Accordingly, the steric interactions between BPh_L and either of these two residues are probably similar. The RR data reported herein for RCs from the $Glu^{L104} \rightarrow$ Gln mutant along with those previously reported for RCs from the wild-type and $Glu^{L104} \rightarrow$ Leu mutant provide a new perspective on the nature of the pigment–protein interactions which influence the conformation of the primary electron acceptor.

The RCs from the $Glu^{L104} \rightarrow$ Gln mutant of *Rb. capsulatus* were prepared and purified as previously described [14,15,26]. The RCs were solubilized in 0.01 M phosphate (pH 7.4)/0.05% lauryldimethylamine *N*-oxide. The RR spectra were acquired by using the scattering geometry and instrumentation described in Ref. 13. The samples used in the RR experiments were diluted 1 : 3 in ethylene glycol, sealed in capillary tubes, and maintained at constant temperature throughout the course of data acquisition. The detailed aspects of the experimental procedures and the protocols used to insure the accuracy and reproducibility of the RR data are identical to those given in Ref. 19. Other pertinent spectral parameters are given in the figure legends.

The 1575–1625 cm^{-1} regions of the UV-excitation (λ_{ex} = 363.8 nm) RR spectra of RCs from the $Glu^{L104} \rightarrow$ Gln mutant of *Rb. capsulatus* obtained at 200 K and 30 K are shown in the middle traces of Figs. 1 and 2, respectively. For comparison, the RR spectra of RCs from the wild-type and the $Glu^{L104} \rightarrow$ Leu mutant are presented in the top and bottom traces, respectively, of the two figures. UV excitation (near the Soret band maximum) results in scattering from all of the bacteriochlorin pigments in the RCs. The prominent feature observed near 1610 cm^{-1} is a composite band which is due to scattering from a characteristic C_aC_m vibration of the BChl and BPh ring skeletons [27,28]. This mode is the analogue of the ν_{10} band of porphyrins and is sensitive to the core size and the extent of out-of-plane distortion of the macrocycle [29]. The relatively strong bands in the 1580–1585 cm^{-1} region of the spectra are to due scattering from another C_aC_m mode of the ring skeleton [28]. These bands are strong in BPh and weak or absent in BChl. RR spectra of RCs from the $Glu^{L104} \rightarrow$ Gln mutant were also obtained in the 1625–1750 cm^{-1} region (not shown). A number of bands are observed in this region due to the C = O stretching vibrations of the C_2 acetyl and C_9 keto groups [27,30].

Comparison of the RR spectra of RCs from the $Glu^{L104} \rightarrow$ Gln mutant with those of the wild-type at high and at low temperatures reveals a number of similarities. In particular, at 200 K the BPh bands in the 1580–1585 cm^{-1} region are doubled, with maxima near 1580 and 1585 cm^{-1} (cf. Fig. 1 middle and top). In the mutant, the former band appears to be slightly more intense than the latter, whereas the opposite is the case for wild-type. At 30 K, the 1580 cm^{-1} band of RCs from both the $Glu^{L104} \rightarrow$ Gln mutant and the wild-type is upshifted by at least 3 cm^{-1} such that it cannot be resolved from the 1585 cm^{-1} band (cf. Fig. 2 middle and top). Temperature sensitivity is also observed for the ν_{10}-like modes of these two RCs. At 30 K, the position of the centroid of the composite ν_{10}-like band is several wavenumbers higher than that observed at 200 K (cf. Fig. 2 (middle, top) and Fig. 1 (middle, top)). In contrast, the RR spectra of the RCs from the $Glu^{L104} \rightarrow$ Leu mutant are quite different from those of either the wild-type or the $Glu^{L104} \rightarrow$ Gln mutant. For this RC, the BPh band exhibits a single maximum near 1585 cm^{-1} at both 200 K and 30 K (cf. Figs. 1 and 2 (bottom)). In addition, no shift is observed in the position of the ν_{10}-like band as the temperature is lowered. On the basis of these and other RR data obtained for RCs from both *Rb. sphaeroides* and *Rb. capsulatus* [13,19], we have assigned the bands observed at 1580 and 1585 cm^{-1} in the wild types at low temperatures to analogous vibrations of BPh_L and BPh_M, respectively.

At high temperature, the bands due to the two pigments are significantly overlapped and occur near 1585 cm^{-1}. At low temperatures, the ν_{10}-like bands of BPh_L and BPh_M occur near 1612 and 1609 cm^{-1}, respectively. At high temperatures, both bands are near 1609 cm^{-1}. The higher frequency of the ν_{10}-like mode of BPh_L at low temperatures results in the higher frequency for the centroid of the composite band. Given the similarities between the RR spectra of the RCs from the $Glu^{L104} \rightarrow$ Gln mutant and those of the wild-type, an analogous set of skeletal-mode assignments appears to be reasonable for the the BPh pigments in RCs from this mutant.

Comparison of the carbonyl-region RR spectrum of the RCs from the $Glu^{L104} \rightarrow$ Gln mutant with that of the wild-type reveals various differences at both high and low temperatures. However, due to the complexity of the spectra in this region, no differences could be identified that could be explicitly associated with the change in the hydrogen bonding interaction at position

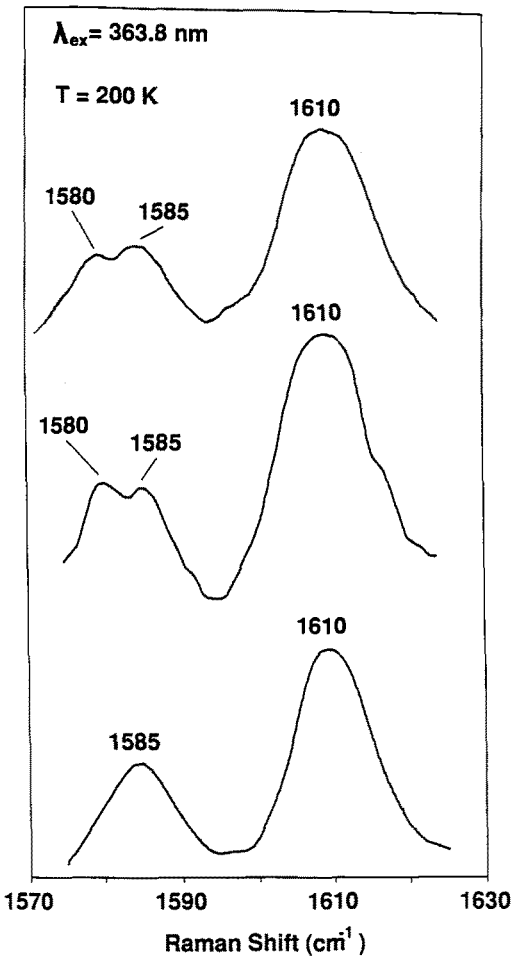

Fig. 1. UV excitation (λ_{ex} = 363.8 nm) RR spectra of RCs from wild-type (top), the $Glu^{L104} \rightarrow$ Gln mutant (middle), and the $Glu^{L104} \rightarrow$ Leu mutant (bottom) of *Rb. capsulatus* in ethylene glycol glasses at 200 K. The spectra are an average of eight scans. The spectral slit width is approx. 4 cm^{-1}. The spectra are normalized such that the intensities of the 1585 cm^{-1} bands are comparable for the three RCs.

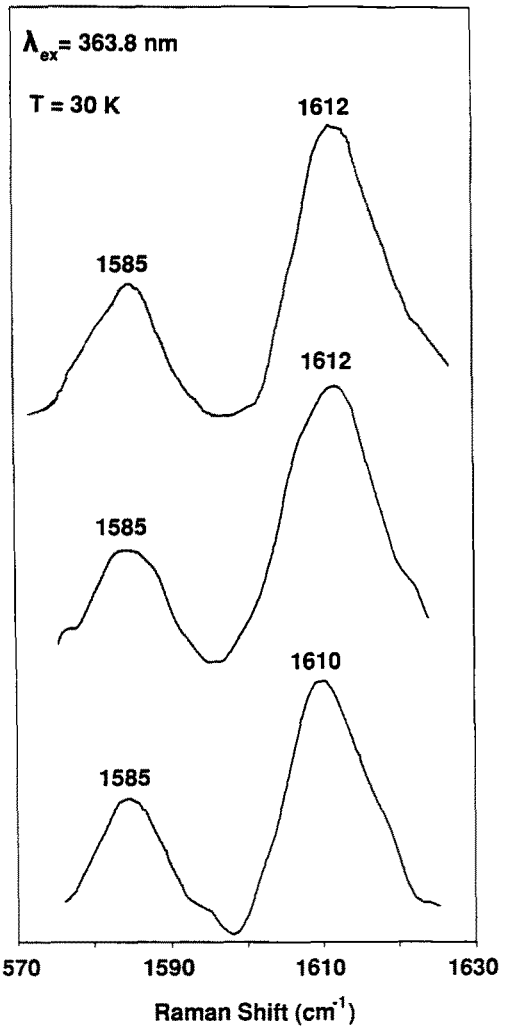

Fig. 2. UV-excitation (λ_{ex} = 363.8 nm) RR spectra of RCs from wild-type (top), the $Glu^{L104} \rightarrow$ Gln mutant (middle), and the $Glu^{L104} \rightarrow$ Leu mutant (bottom) of *Rb. capsulatus* in ethylene glycol glasses at 30 K. The spectra are an average of eight scans. The spectral slit width is approx. 4 cm^{-1}. The spectra are normalized such that the intensities of the 1585 cm^{-1} bands are comparable for the three RCs.

L104. This was also found to be the case in our previous RR studies of RCs from the $Glu^{L104} \rightarrow$ Leu mutant [19]. The potential difficulties in the interpretation of the RR spectra in the carbonyl region are discussed in detail in Ref. 19 and will not be discussed further here.

The RR spectra reported here for RCs from the $Glu^{L104} \rightarrow$ Gln mutant of *Rb. capsulatus* indicate that the BPh_L pigment in these RCs undergoes a temperature-dependent conformational change similar to that which occurs in RCs from the wild-type. In addition, to within experimental error, the magnitude of the RR frequency shifts observed for BPh_L in this mutant are identical to those observed for the pigment in the wild-type. This result suggests that the nature and extent of the conformational change in BPh_L is comparable in these two systems. Collectively, these observations strongly suggest that the strength of the hydrogen bond-

ing interaction (as expected for an O-H versus N-H moiety in the absence of other constraints) between the C_9 keto group of BPh_L and the amino acid at position L104 is not a determining factor in the temperature sensitivity of the conformation of BPh_L. Presuming that the temperature sensitivity is due to direct interactions between BPh_L and the L104 residue, this suggests that the interactions are steric rather than electrostatic in nature. In this picture, interactions with the X-H groups of the L104 glutamic acid or glutamine might push BPh_L into a unique location in the protein matrix. On the other hand, the temperature sensitivity may not be due to direct interactions but rather to a unique structure which is imparted to the protein pocket by the presence of a glutamic acid (or glutamine) at position L104. Either proposal is qualitatively consistent with the observation that the BPh_L pigment in RCs from the $Glu^{L104} \rightarrow$ Leu mutant of *Rb. capsulatus* does not exhibit temperature-dependent structural changes [19]. Perhaps, the R-group of leucine is too small for strong steric interactions with BPh_L or for interactions between other amino acid residues which in turn might alter the structure of the protein pocket.

In order to test further the influence of the L104 residue on the temperature sensitivity of the conformation of BPh_L, it would be useful to examine other mutants in which the characteristic features of the R-groups of the residues are altered in a systematic fashion. For example, residues could be incorporated which are capable of hydrogen bonding but which possess R groups that are much smaller than those of glutamic acid or glutamine (for example aspartic acid). Alternatively, residues might be incorporated which have R-groups comparable in size to those of glutamic acid or glutamine but which are not capable of hydrogen bonding (for example methionine). For any of these genetically modified RCs, it would also be interesting to assess whether the temperature-dependent conformational changes have any connection with the temperature dependence of the electron transfer rates [31–34]. Such studies are currently in progress on RCs from both the the $Glu^{L104} \rightarrow$ Gln and $Glu^{L104} \rightarrow$ Leu mutants of *Rb. capsulatus* (Holten, D., personal communication).

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