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Rapid report

Characterization of the photoreduction of the secondary quinone Q_B in the photosynthetic reaction center from *Rhodobacter capsulatus* with FTIR spectroscopy

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Abstract

The photoreduction of the secondary quinone acceptor Q_B in reaction centers (RCs) of the photosynthetic bacteria *Rhodobacter* (*Rb.*) *capsulatus* has been investigated by light-induced FTIR difference spectroscopy in ^1H_2O and ^2H_2O. The Q_B^-/Q_B FTIR spectra reflect reorganization of the protein upon electron transfer, changes of protonation state of carboxylic acid groups, and (semi)quinone–protein interactions. As expected from the conservation of most of the amino acids near Q_B in the RCs from *Rb. capsulatus* and *Rb. sphaeroides*, several protein and quinone IR bands are common to both spectra, e.g., the 1728 cm^{-1} band is assigned to proton uptake by a carboxylic acid residue, most probably Glu L212 as previously proposed for *Rb. sphaeroides* RCs. However, noticeable changes are observed at 1709 cm^{-1} (deprotonation of a Glu or Asp residue), 1674 and 1659 cm^{-1} (side chain and/or backbone), around 1540 cm^{-1} (amide II), and in the semiquinone absorption range. This FTIR study demonstrates that the environment of the secondary quinone in *Rb. capsulatus* is close but not identical to that in *Rb. sphaeroides* suggesting slight differences in the structural organization of side chains and/or ordered water molecules near Q_B.

Keywords: Photosynthetic reaction center; Secondary quinone; Fourier transform infrared spectroscopy; (*Rhodobacter capsulatus*)

Coupling between electron and proton transfer reactions is a key mechanism in a number of functionally important membrane proteins. In the photosynthetic bacterial reaction center (RC), proton uptake is coupled to light-induced electron transfer leading to the double reduction of the secondary quinone Q_B and the formation of the dihydroquinone Q_BH_2, which then dissociates from the RC [1]. Although the first electron transfer to Q_B does not involve the direct protonation of Q_B^-, substoichiometric proton uptake by the protein following reduction to Q_B^- has been experimentally measured [2,3] and also predicted from electrostatic calculations [4–7] based on the X-ray structures. In the most recent high-resolution structures of the RCs from two purple bacteria, *Rhodobacter* (*Rb.*) *sphaeroides* [8–11] and *Rhodopseudomonas* (*Rp.*) *viridis* [12], a large number of bound internal water molecules can be identified which, together with ionizable amino acid side chains of the L, M, and H protein subunits, can form hydrogen bonded networks that might be used for the transfer of the protons to the (doubly) reduced quinone.

The influence of specific residues in coupled electron/proton transfer reactions comes from experi-

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mental measurements of electron and proton transfer rates in RCs that have been genetically modified (for reviews, see [13,14]). Initially, site-directed mutagenesis has been most developed for a bacterial RC for which the structure is not yet solved, that of *Rb. capsulatus* [15] and a considerable amount of biophysical data on native and mutant RCs has been documented (for reviews, see [16,17]) and is still actively reported. A structural model for the L and M subunits and the associated cofactors in the *Rb. capsulatus* RC has been calculated [18] based on sequence homology. Indeed, the *Rb. capsulatus*, *Rp. viridis*, and *Rb. sphaeroides* RCs are structurally homologous with a sequence identity of 78%, 76%, and 64%, for the L, M, and H subunits, respectively, between *Rb. capsulatus* and *Rb. sphaeroides*, and of 59%, 50%, and 38%, respectively, between *Rb. capsulatus* and *Rp. viridis* RCs [19–21]. Hydropathy plots of the *Rb. capsulatus* RC [19] strongly suggest the same pattern of transmembrane helices as is present in the two other RCs. Thus, it is generally assumed that the three-dimensional structure of the RC from *Rb. capsulatus* should be closely related to that of the two known RCs. Notably, on the basis of the conservation of the residues around Q_B (ubiquinone-10) between RCs from *Rb. capsulatus* and *Rb. sphaeroides* (sequence identity is greater than 90%), the structure of the Q_B binding site of *Rb. capsulatus* is expected to resemble that of *Rb. sphaeroides*. In *Rb. sphaeroides*, six carboxylic acids, two arginines, and one lysine form an electrostatically interacting cluster near Q_B, each residue being within 4.5 Å of a neighboring carboxylic acid or a bridging water molecule [10]. This cluster may be important for fast protonation of the reduced quinone. From sequence alignments [19–21], it appears that the position and the identity of these acid/basic residues is equivalent in *Rb. capsulatus*. Moreover, site-directed mutagenesis developed for *Rb. capsulatus* and *Rb. sphaeroides* suggests the similar functional involvement of specific amino acid residues in the electron/proton coupled transfer reactions (for reviews, see [13,14,16,17]). Notably, the importance of Ser L223, Asp L213, and Glu L212 for rapid coupled electron/proton transfer has been established in both RCs. It has been shown that the mutation Asp L213→Asn almost completely blocks the first proton transfer to reduced Q_B [22,23] while the mutation Glu L212→Gln [24–27] prevents rapid delivery of the second proton to reduced Q_B. However, in both *Rb. capsulatus* and *Rb. sphaeroides* RCs, several second-site mutations which compensate for the loss of Asp L213 and/or Glu L212 have been obtained, suggesting that other residues can substitute for Asp L213 or Glu L212 to restore proton transfer [27–34].

On the other hand, some differences have been reported between the two types of RCs. From the faster Q_A^- to Q_B electron transfer rate in *Rb. capsulatus* than in *Rb. sphaeroides*, it has been suggested that the Q_A to Q_B distance should be smaller by 0.7 Å in *Rb. capsulatus* than in *Rb. sphaeroides* [35]. Furthermore, the pattern of the proton uptake upon Q_B reduction is somewhat different in the two RCs: for example, at pH 7, about 0.6–0.8 H^+/RC has been reported in *Rb. capsulatus* [3,17] compared to 0.4 H^+/RC in *Rb. sphaeroides* [2]. The pH dependence of H^+ binding upon formation of the Q_B^- state also involves a group or a cluster with a high pK_a value of about 10.1 for *Rb. capsulatus* [28,36] and 9.5 for *Rb. sphaeroides* [24,25]. This group was associated with Glu L212, either directly or indirectly, suggesting that Glu L212 has an unusual titration behavior and is essentially protonated at neutral pH. Measurements of the pH dependence of proton uptake in mutant RCs from *Rb. capsulatus* lacking Glu L212 support this assignment [27]. From detailed electrostatic calculations based on the X-ray structures of *Rb. sphaeroides*, it has been proposed that Glu L212 is fully [4] or partially [5] ionized over the whole pH range when Q_B is neutral becoming protonated following Q_B reduction. However, the latest calculations indicate that Glu L212 is protonated at physiological pHs in Q_B (M.R. Gunner, E.G. Alexov, personal communication). On the other hand, Fourier transform infrared (FTIR) difference spectroscopy, which is a highly suited experimental method to analyze the changes in the protonation state of carboxylic acids, has shown that Glu L212 in *Rb. sphaeroides* RCs is partially ionized at pH 7 in the Q_B state becoming more protonated in the Q_B^- state [37,38]. In the IR absorption region of the C=O stretching vibration of protonated carboxylic acid groups (1770–1700 cm^{-1}), the Q_B^- minus Q_B FTIR difference spectrum (Q_B^-/Q_B) corresponding to the photoreduction of Q_B in native RCs shows a positive band at 1728 cm^{-1} that is sensitive to $^1H/^2H$ isotopic

exchange, as expected for a carboxylic acid group. This 1728 cm^{-1} signal has been attributed to substoichiometric proton uptake by Glu L212 upon Q_B^- formation based on its absence when Glu L212 was replaced with Gln and its presence in mutants constructed at different sites of the carboxylic acid cluster, e.g., at Asp L213, Asp L210, or Glu H173 [37,39,40]. In view of the large number of mutants constructed around Q_B in *Rb. capsulatus* RC that would be of interest for FTIR investigations, we have performed a study of the light-induced FTIR absorption changes associated with the photoreduction of Q_B in the native RC from *Rb. capsulatus* in order to investigate possible changes in the protonation state of carboxylic acid residues and in the protein–quinone interactions.

RCs from *Rb. capsulatus* were isolated and purified following published procedures [41,42]. The RC samples (~1 mM) for FTIR experiments were prepared at pH 7 (Tris–HCl, 50 mM) essentially as reported previously for *Rb. sphaeroides* [37,40], i.e., in the presence of sodium ascorbate (20 mM) and 2,3,5,6-tetramethyl-*p*-phenylenediamine (45 mM), and of an excess of ubiquinone. $^1H/^2H$ isotopic exchange was performed as described in [40]. The Q_B^- state was generated under single saturating flash excitation (Nd:YAG laser, 7 ns, 530 nm). Light-induced FTIR difference spectra were acquired at 15°C with a Nicolet 60SX spectrometer, as described in [37].

Fig. 1a shows the light-induced Q_B^-/Q_B FTIR difference spectrum corresponding to the Q_B to Q_B^- transition in RCs from *Rb. capsulatus* in ^1H_2O. In this spectrum, negative bands originate from the neutral Q_B state and positive bands arise from the Q_B^- state. Prominent positive peaks are seen at 1728, 1651, and 1480 cm^{-1} while negative bands appear at 1709, 1685, 1674, 1659, 1641, 1614, 1290, and 1265 cm^{-1}. This spectrum presents a large number of common features with that of *Rb. sphaeroides* RCs (Fig. 1d) which displays comparable positive peaks at 1728, 1651, and 1479 cm^{-1}, and negative ones at 1685, 1640, 1617, 1290, and 1265 cm^{-1} [37,43,44]. It must be noticed that the amplitude of the absorption changes associated with the photoreduction of Q_B is about 4-fold smaller in *Rb. capsulatus* than in *Rb. sphaeroides* when the RC samples are normalized to the amide I absorption (~0.7 a.u). On the basis of

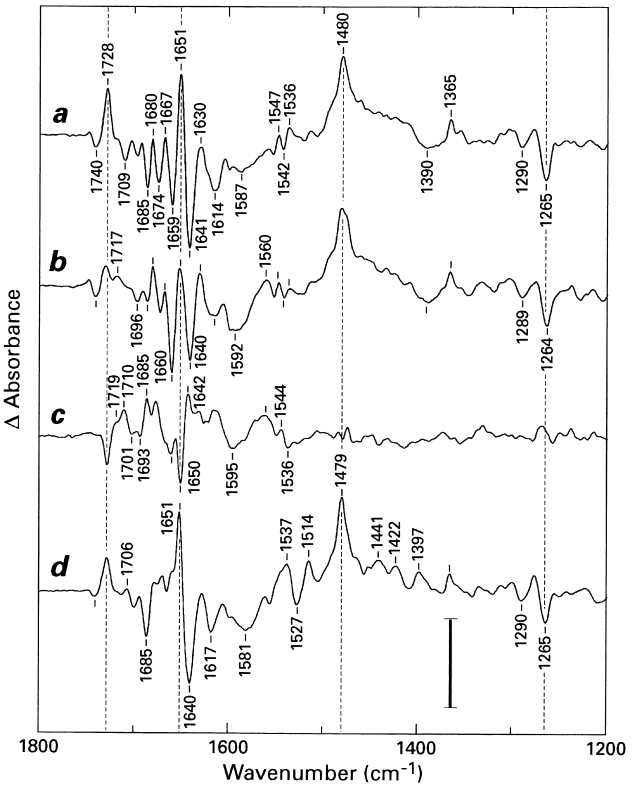

Fig. 1. Light-induced Q_B^-/Q_B FTIR spectra of *Rb. capsulatus* RCs at pH 7. (a) ^1H_2O (nine samples averaged). (b) ^2H_2O (three samples averaged). (c) ^2H_2O minus ^1H_2O double-difference spectrum. (d) Q_B^-/Q_B spectra of *Rb. sphaeroides* [37]. Q_B^-/Q_B spectra were normalized based on the semiquinone (at 1480 cm^{-1}) and methoxy (1265 and 1290 cm^{-1}) bands. About 50 000 interferograms at 4 cm^{-1} resolution were coadded. The bar represents 10^{-4} and 4 10^{-4} absorbance units for *Rb. capsulatus* and *Rb. sphaeroides* spectra, respectively.

studies of *Rb. sphaeroides* RCs reconstituted with isotope-labeled ubiquinones [44,45] and of in vitro model quinone compounds [46], assignments of the neutral and anion quinone modes in the Q_B^-/Q_B spectrum have been established. The 1480 cm^{-1} peak in the $Q_B^-Q_B$ spectrum of *Rb. capsulatus* RCs (Fig. 1a) can be similarly assigned to the semiquinone (C...O and C...C) modes. The 1641 and 1614 cm^{-1} bands most probably correspond to the bands observed at 1640 and 1617 cm^{-1} in *Rb. sphaeroides* which arise at least partly from the C=O and the C=C modes of Q_B. Similarly, the 1290 and 1265 cm^{-1} bands observed in both spectra of *Rb. capsulatus* and *Rb. sphaeroides* RCs contain contributions from the methoxy groups of Q_B. A positive band at 1651 cm^{-1} lies in the amide I region mainly due to the C=O

stretching vibration of peptide groups. In the amide II region (NH bending and CN stretching modes), several small signals are observed at 1547 (+), 1542 (−), and 1536 (+) cm^{-1} in the Q_B^-/Q_B spectrum of *Rb. capsulatus* in contrast to the positive band peaking at 1537 cm^{-1} in *Rb. sphaeroides*. Also, additional negative bands are seen in the spectrum of *Rb. capsulatus* at 1674 and 1659 cm^{-1} in the absorption range of peptide groups and/or of amino acid side chains. The C=O stretching region of protonated Asp and Glu residues in the Q_B^-/Q_B spectrum of *Rb. capsulatus* RCs displays a positive band at 1728 cm^{-1} and two negative signals at 1709 cm^{-1} and 1740 cm^{-1} (Fig. 1a). The 1728 and 1740 cm^{-1} peaks are also present in the spectrum of *Rb. sphaeroides* (Fig. 1d) with, however, a slightly smaller intensity than in *Rb. capsulatus*. In contrast, the 1709 cm^{-1} band is unique to *Rb. capsulatus*. Corresponding COO^- stretching modes of carboxylate groups could contribute to the broad negative signals at around 1590 (antisymmetric) and 1390 (symmetric) cm^{-1}.

Fig. 1b shows the Q_B^-/Q_B spectrum of *Rb. capsulatus* RCs in ^2H_2O in order to identify bands that are sensitive to isotopic exchange. This spectrum also exhibits several features similar to those found in *Rb. sphaeroides* RCs [37]. Notably, the quinone bands do not shift significantly upon $^1H/^2H$ exchange; e.g., the main anion band of Q_B^- peaks at 1480 cm^{-1}, the Q_B modes at 1640 and 1614 cm^{-1}, and the methoxy groups of Q_B at 1289 and 1264 cm^{-1}. As previously observed for *Rb. sphaeroides* [37], the most noticeable changes occur in the spectral region between 1735 and 1645 cm^{-1}. The amplitude of the positive band at 1651 cm^{-1} is reduced. In the C=O stretching region of protonated carboxylic groups, the main changes occur at 1728 cm^{-1} where the intensity of the band is strongly decreased, at 1717 cm^{-1} where a new positive signal appears, and at 1709 cm^{-1} where the negative band present in ^1H_2O is not seen in ^2H_2O. The negative signal at 1740 cm^{-1} is not affected in ^2H_2O, suggesting that it does not arise from a carboxyl group. These changes are best seen in the Q_B^-/Q_B double-difference spectrum shown in Fig. 1c, calculated from the Q_B^-/Q_B spectrum obtained in ^2H_2O (Fig. 1b) minus the Q_B^-/Q_B spectrum obtained in ^1H_2O (Fig. 1a). Fig. 1c shows negative signals at 1728, 1650, and 1595 cm^{-1} and positive ones at 1710, 1685, 1642, and 1560 cm^{-1}. In the carboxylic acid region, a negative band at 1728 cm^{-1}, a positive band at 1710 cm^{-1} with a shoulder at 1719 cm^{-1}, and a trough at 1701 cm^{-1} reflect the frequency downshifts of protonated carboxylic groups from ^1H_2O to ^2H_2O. The signal at 1728 cm^{-1} in ^1H_2O, which is thus downshifted to ~1718 cm^{-1} in ^2H_2O is assigned to the C=O stretching mode of an Asp or Glu side chain becoming protonated upon Q_B reduction. The negative signal observed at 1709 cm^{-1} in ^1H_2O is assigned to the C=O mode of a carboxylic acid becoming deprotonated in the Q_B^- state. It is most probably downshifted to ~1701 cm^{-1} in ^2H_2O. Such ~8–10 cm^{-1} deuteration-induced downshifts are indeed characteristic of protonated carboxylic acid groups accessible to the solvent [47].

The present FTIR data on the photoreduction of Q_B in the RC from *Rb. capsulatus* show that the protonation of a carboxylic acid group occurs (at 1728 cm^{-1}) together with the deprotonation of another carboxylic group (at 1709 cm^{-1}). The amplitude of the band at 1728 cm^{-1} is larger (about 3-fold) than that at 1709 cm^{-1} which does not favor a coupled protonation/deprotonation event. Assuming that the extinction coefficient of the two carbonyls absorbing at 1728 and 1709 cm^{-1} are comparable, the greater amplitude of the band at 1728 cm^{-1} compared to that at 1709 cm^{-1} indicates a net proton uptake by the carboxylic acid groups of the RC upon Q_B reduction. The low-frequency position of the ν (C=O) vibration at 1709 cm^{-1}, close to that of carboxylic acids in solution [48] would correspond to a protonated carboxylic acid involved in a strong hydrogen bond and/or in a very polar environment. The carbonyl absorbing at 1728 cm^{-1} would be weakly hydrogen bonded. Thus, the question is raised: which residues protonate and deprotonate upon Q_B reduction in the RC from *Rb. capsulatus*?

The Q_B binding pocket of *Rb. sphaeroides* is taken as a model for that of *Rb. capsulatus* on the basis of the conservation of most of the residues in the surrounding of Q_B in both RCs. In *Rb. sphaeroides*, the side chains of His L190, Ser L223, and Ile L224 (Val in *Rb. capsulatus*) are in proximity to the carbonyls of Q_B, and a cluster of interacting acid and basic residues located near Q_B has been described [10]. Such a cluster probably also exists in *Rb. capsulatus*,

since identical residues are found at the same position in this RC [19,20], i.e., Glu L212, Asp L213, Asp L210, Glu H175 (Glu H173 in *Rb. sphaeroides*), Asp H127 (Asp H124 in *Rb. sphaeroides*), Arg L217, Lys H133, and Arg H179 (Lys H130 and Arg H177 in *Rb. sphaeroides*). The only change is the replacement of Asp M17 in *Rb. sphaeroides* by Glu in *Rb. capsulatus*. In agreement with this high homology of the residues around Q_B in both RCs, the Q_B^-/Q_B spectra of *Rb. capsulatus* and *Rb. sphaeroides* bear a number of similar spectral features. Notably, the 1728 cm^{-1} band is present in both spectra and exhibits the same $\sim$10 cm^{-1} downshift in ^2H_2O. In RCs from *Rb. sphaeroides*, this band has been assigned to proton uptake by Glu L212 following Q_B reduction [37–40]. It is thus probable that the 1728 cm^{-1} band in *Rb. capsulatus* can be also assigned to the C=O mode of Glu L212 becoming more protonated upon Q_B^- formation. Studies of the effect of mutations at Glu L212 in *Rb. capsulatus* are necessary to ascertain this tentative assignment. Note that the intensity of the 1728 cm^{-1} signal in *Rb. capsulatus* is slightly larger ($30 \pm 10\%$) than in *Rb. sphaeroides*, suggesting a greater proton uptake by Glu L212 in *Rb. capsulatus* than in *Rb. sphaeroides* at pH 7. Such an increased proton uptake by Glu L212 upon Q_B^- formation has been previously observed in several mutant/revertant RCs from *Rb. sphaeroides* compared to native RCs and has been interpreted in terms of electrostatic (i.e., a more positive electrostatic potential around Q_B) and/or structural changes [37,39,40,49]. Furthermore, the Q_B^-/Q_B spectra of *Rb. sphaeroides* RCs in ^1H_2O (Fig. 1d) and ^2H_2O [37] do not show evidence for changes of protonation state of carboxylic groups other than Glu L212 [37,39,40]. Since the residues near Q_B are conserved in both RCs, a similar pattern could be expected in *Rb. capsulatus*. However, a major difference is observed in the Q_B^-/Q_B spectrum of *Rb. capsulatus* RCs where a new signal appears at 1709 cm^{-1} and corresponds to a deprotonation of a carboxylic acid. Nevertheless, it should be noted that the overall proton uptake by carboxylic groups in *Rb. capsulatus* RCs, as reflected by the positive band at 1728 cm^{-1} and the negative band at 1709 cm^{-1}, is roughly equivalent to the proton uptake measured at 1728 cm^{-1} in *Rb. sphaeroides*.

How does one explain the differences observed between the FTIR spectra of RCs from *Rb. capsulatus* and *Rb. sphaeroides*? One possibility is that the organization and/or the number of bound water molecules near Q_B might not be conserved in *Rb. capsulatus* and thus some of the interactions between polar side chains and water molecules within the network could be different in the two RCs. In *Rb. sphaeroides*, three fixed water molecules are in contact with several carboxylic side chains belonging to the cluster [10]. In *Rb. capsulatus*, water molecules could be located in different places. Such a perturbation of water molecules near Q_B in *Rb. capsulatus* could affect the electrostatic interactions between the residues forming the cluster or between residues located at more distant sites, and consequently induce pK_a shifts of carboxylic acids within the Q_B pocket.

More specifically, the latest crystal RC structure from *Rb. sphaeroides* [10] shows three possible proton transfer pathways named P1, P2, and P3. They connect the three functionally important residues Ser L223, Asp L213, and Glu L212 to the cytoplasmic exposed surface, via internal water molecules. P1 connects Glu L212 with the cytoplasmic surface near Glu H224 and Asp M240, and thus is divided in two branches. P2 connects Asp L213 with the surface at Tyr M3 via Glu H173. P3 connects Asp L213 to the surface at Asp M17 via a single water molecule. In *Rb. capsulatus*, most of the connecting residues located near the surface of the protein are conserved and thus similar proton transfer pathways might exist except for a major change at the outset of one of the P1 branch where a Val residue (Val H226 in the *Rb. capsulatus* sequence) is substituted to Glu. Thus, in *Rb. capsulatus*, the P1 proton pathway could be affected. Only the detailed three-dimensional structure of this RC would reveal possible channels for proton transfer. The carboxylic acid that deprotonates upon Q_B reduction in *Rb. capsulatus* and gives rise to the negative signal at 1709 cm^{-1} might be either a residue from the cluster or a residue located at a more distant site. It has been documented that long range electrostatic effects occur in RCs and affect the proton/coupled electron transfer rates [28–32,34,49,50]. Studies of the effects of mutations near Q_B in the RC from *Rb. capsulatus* are required to attribute the observed signals in the carboxylic acid region of the Q_B^-/Q_B spectrum to individual side chains. FTIR data on *Rb. sphaeroides*

mutant RCs have shown that the ionization state of Asp L213, Asp L210, and Glu H173 does not change in response to the formation of Q_B^- at pH 7 [37,39,40]. It would be of interest to know if these carboxylic acids behave differently in *Rb. capsulatus*. In *Rb. sphaeroides*, we had previously proposed that, in addition to proton uptake by Glu L212 visualized at 1728 cm^{-1}, the peptide C=O of Glu L212 is also affected by the photoreduction of Q_B and gives rise to the negative band at 1685 cm^{-1} in the Q_B^-/Q_B spectra of native RCs and of mutants that do not contain the Glu L212→Gln substitution [37,39,40]. A negative signal at 1685 cm^{-1} is also observed in the Q_B^-/Q_B spectrum of *Rb. capsulatus* RC (Fig. 1a). In addition, the similar differential signal observed at 1651/1641 cm^{-1} in both RCs can be attributed in part (contribution from the quinone carbonyls occurs at 1641 cm^{-1}) to a conformational change or a movement of a backbone C=O or of a side chain following Q_B reduction.

The Q_B^-/Q_B spectra of *Rb. capsulatus* RCs also exhibit a broad continuum band at ~2600 cm^{-1} in ^1H_2O shifting to 2100 cm^{-1} in ^2H_2O (data not shown). Comparable bands have been previously observed in *Rb. sphaeroides* at identical frequencies as well as in *Rp. viridis* at ~2800 cm^{-1} in ^1H_2O and at ~2200 cm^{-1} in ^2H_2O, respectively [51]. These IR continua have been interpreted in terms of highly polarizable hydrogen bonds [52] in a large web involving carboxylic acid groups and/or polar residues and ordered water molecules [51]. Such bands could reflect a net increase of the proton concentration or an increase of the polarizability of the protons in the network upon quinone photoreduction.

It is striking that the Q_B^-/Q_B FTIR spectra of the RCs from *Rb. capsulatus* and *Rb. sphaeroides* display a number of common features both in the protein (side chain and backbone) and the quinone/semiquinone absorption regions, as well as at 2600 cm^{-1}. In contrast, it is worth noting that they are significantly different from the Q_B^-/Q_B spectrum of *Rp. viridis* RCs [43,44], at least in several absorption regions of the protein (amide I, amide II, side chains). Differences seen notably in the carboxylic acid region of the FTIR spectra between *Rb. sphaeroides* and *Rp. viridis* have been extensively discussed in previous reports [44,49,51]. Although the semiquinone band is observed at 1480–1479 cm^{-1} in both *Rb. capsulatus* and *Rb. sphaeroides* RCs (Fig. 1), the band is slightly broader in *Rb. capsulatus* than in *Rb. sphaeroides* showing a shoulder at around 1493 cm^{-1}. This is most probably indicative of slight differences in the interactions between the protein and the quinone anion. Such a larger width of the main semiquinone peak and the presence of a shoulder at higher energy have been previously observed in several mutant/revertant RCs from *Rb. sphaeroides* [37,39,40,49,53]. Also, the 1440–1390 cm^{-1} region of the Q_B^-/Q_B spectra of *Rb. capsulatus* shows small differences in shape and intensity of the bands, possibly resulting from altered interactions between the methoxy groups of Q_B^- and their binding site.

The Q_B^-/Q_B spectra of *Rb. capsulatus* and *Rb. sphaeroides* RCs further display several distinct features, notably at 1709 cm^{-1}, 1674 cm^{-1}, 1659 cm^{-1}, and around 1540 cm^{-1}. Interestingly, the 1680 to 1655 cm^{-1} region (amide I and/or side chains) of *Rb. sphaeroides* Q_B^-/Q_B spectra has been observed to be sensitive to point mutations of carboxylic residues from the cluster near Q_B [37,39,49]. For example, the mutant RCs containing the Asn L213→Asn substitution (DN L213) as well as several revertants of the DN L213 mutation show additional negative signals at around 1657 and 1676 cm^{-1} with respect to native RCs [37,49]. Comparable signals are indeed observed at 1659 and 1674 cm^{-1} in the RC from *Rb. capsulatus* (Fig. 1a). The 1659 cm^{-1} signal is not significantly sensitive to $^1H/^2H$ exchange, suggesting that it arises from a buried peptide C=O group while the 1674 cm^{-1} band that is downshifted in ^2H_2O could arise from either an accessible peptide group or a side chain.

On the basis of the present FTIR study on *Rb. capsulatus*, and of previous data obtained on native and mutant/revertant RCs from *Rb. sphaeroides* [37,39,40,49], it appears that the differences seen by FTIR between *Rb. capsulatus* and *Rb. sphaeroides* are comparable to those found in *Rb. sphaeroides* between native RCs and several mutants like DN L213 or EQ H173 (Glu H173→Gln). Upon formation of Q_B^-, the *Rb. capsulatus* RC similarly undergoes small changes in the conformation of peptide/side chain groups and in the interactions of some of the groups of the semiquinone with the protein. Despite of the conserved residues found near Q_B in both RCs from *Rb. capsulatus* and *Rb. sphaeroides*, the

non-identity of the two Q_B^-/Q_B spectra points to a slightly different behavior of the protein involved in conformational change and proton uptake upon Q_B reduction. Importantly, and in contrast to native RCs and most of the studied mutants/revertants from *Rb. sphaeroides* [37,39,40,49,53], it appears that (at least) two carboxylic acids are involved in proton events in the RC from *Rb. capsulatus*. Thus, the structural organization of some of the side chains and/or the ordered water molecules along the proton transfer pathways may be slightly different in *Rb. capsulatus* and *Rb. sphaeroides*.

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References

[1] G. Feher, J.P. Allen, M.Y. Okamura, D.C. Rees, Nature 339 (1989) 111–116.
[2] P.H. McPherson, M.Y. Okamura, G. Feher, Biochim. Biophys. Acta 934 (1988) 348–368.
[3] P. Maróti, C.A. Wraight, Biochim. Biophys. Acta 934 (1988) 329–347.
[4] M.R. Gunner, B. Honig, in: J. Breton, A. Verméglio (Eds.), The Photosynthetic Bacterial Reaction Center II, Plenum Press, New York, 1992, pp. 403–410.
[5] P. Beroza, D.R. Fredkin, M.Y. Okamura, G. Feher, Biophys. J. 68 (1995) 2233–2250.
[6] C.R.D. Lancaster, H. Michel, B. Honig, M.R. Gunner, Biophys. J. 70 (1996) 2469–2492.
[7] B. Rabenstein, G.M. Ullmann, E.-W. Knapp, Biochemistry 37 (1998) 2488–2495.
[8] U. Ermler, G. Fritzsch, S.K. Buchanan, H. Michel, Structure 2 (1994) 925–936.
[9] M.H.B. Stowell, T.M. McPhillips, D.C. Rees, S.M. Soltis, E. Abresch, G. Feher, Science 276 (1997) 812–816.
[10] E.C. Abresch, M.L. Paddock, M.H.B. Stowell, T.M. McPhillips, H.L. Axelrod, S.M. Soltis, D.C. Rees, M.Y. Okamura, G. Feher, Photosynth. Res. 55 (1998) 119–125.
[11] G. Fritzsch, L. Kampmann, G. Kapaun, H. Michel, Photosynth. Res. 55 (1998) 1–6.
[12] C.R.D. Lancaster, H. Michel, Structure 5 (1997) 1339–1359.
[13] E. Takahashi, C.A. Wraight, in: J. Barber (Ed.), Advances in Molecular and Cell Biology: Molecular Processes in Photosynthesis, JAI Press, Greenwich, 1994, pp. 197–251.
[14] M.Y. Okamura, G. Feher, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic, Dordrecht, 1995, pp. 577–594.
[15] D.C. Youvan, S. Ismail, E.J. Bylina, Gene 38 (1985) 19–30.
[16] W.J. Coleman, D.C. Youvan, Annu. Rev. Biophys. Biophys. Chem. 19 (1990) 333–367.
[17] P. Sebban, P. Maróti, D.K. Hanson, Biochimie 77 (1995) 677–694.
[18] N. Foloppe, M. Ferrand, J. Breton, J.C. Smith, Proteins Struct. Func. Genet. 22 (1995) 226–244.
[19] D.C. Youvan, E.J. Bylina, M. Alberti, H. Begusch, J.E. Hearst, Cell 37 (1984) 949–957.
[20] J.C. Williams, L.A. Steiner, G. Feher, Proteins Struct. Func. Genet. 1 (1986) 312–325.
[21] H. Komiya, T.O. Yeates, D.C. Rees, J.P. Allen, G. Feher, Proc. Natl. Acad. Sci. USA 85 (1988) 9012–9016.
[22] E. Takahashi, C.A. Wraight, Biochim. Biophys. Acta 1020 (1990) 107–111.
[23] M.L. Paddock, S.H. Rongey, P.H. McPherson, A. Juth, G. Feher, M.Y. Okamura, Biochemistry 33 (1994) 734–745.
[24] M.L. Paddock, S.H. Rongey, G. Feher, M.Y. Okamura, Proc. Natl. Acad. Sci. USA 86 (1989) 6602–6606.
[25] E. Takahashi, C.A. Wraight, Biochemistry 31 (1992) 855–866.
[26] D.K. Hanson, Y.-L. Deng, P. Sebban, M. Schiffer, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, vol. I, Kluwer Academic, Dordrecht, 1995, pp. 859–862.
[27] J. Miksovska, L. Kálman, M. Schiffer, P. Maróti, P. Sebban, D.K. Hanson, Biochemistry 36 (1997) 12216–12226.
[28] D.K. Hanson, L. Baciou, D.M. Tiede, S.L. Nance, M. Schiffer, P. Sebban, Biochim. Biophys. Acta 1102 (1992) 260–265.
[29] D.K. Hanson, D.M. Tiede, S.L. Nance, C.-H. Chang, M. Schiffer, Proc. Natl. Acad. Sci. USA 90 (1993) 8929–8933.
[30] M. Schiffer, C.-K. Chan, C.-H. Chang, T.J. DiMagno, G.R. Fleming, S. Nance, J. Norris, S. Snyder, M. Thurnauer, D.M. Tiede, D.K. Hanson, in: J. Breton, A. Verméglio (Eds.), The Photosynthetic Bacterial Reaction Center II, Plenum Press, New York, 1992, pp. 351–361.
[31] J. Miksovska, M. Valerio-Lepiniec, M. Schiffer, D.K. Hanson, P. Sebban, Biochemistry 37 (1998) 2077–2083.
[32] M. Valerio-Lepiniec, J. Miksovska, M. Schiffer, D.K. Hanson, P. Sebban, Biochemistry 38 (1999) 390–398.
[33] S.H. Rongey, M.L. Paddock, G. Feher, M.Y. Okamura, Proc. Natl. Acad. Sci. USA 90 (1993) 1325–1329.
[34] M.L. Paddock, M.E. Senft, M.S. Graige, S.H. Rongey, T. Turanchik, G. Feher, M.Y. Okamura, Photosynth. Res. 55 (1998) 281–291.
[35] L. Baciou, E.J. Bylina, P. Sebban, Biophys. J. 65 (1993) 652–660.
[36] P. Maróti, D.K. Hanson, L. Baciou, M. Schiffer, P. Sebban, Proc. Natl. Acad. Sci. USA 91 (1994) 5617–5621.
[37] E. Nabedryk, J. Breton, R. Hienerwadel, C. Fogel, W. Mäntele, M.L. Paddock, M.Y. Okamura, Biochemistry 34 (1995) 14722–14732.
[38] R. Hienerwadel, S. Grzybek, C. Fogel, W. Kreutz, M.Y. Okamura, M.L. Paddock, J. Breton, E. Nabedryk, W. Mäntele, Biochemistry 34 (1995) 2832–2843.
[39] E. Nabedryk, J. Breton, R. Hienerwadel, C. Fogel, W. Mäntele, M.L. Paddock, M.Y. Okamura, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, vol. I, Kluwer Academic, Dordrecht, 1995, pp. 875–878.

[40] E. Nabedryk, J. Breton, M.Y. Okamura, M.P. Paddock, Biochemistry 37 (1998) 14457–14462.
[41] R.C. Prince, D.C. Youvan, Biochim. Biophys. Acta 890 (1987) 286–291.
[42] E.J. Bylina, D.C. Youvan, Proc. Natl. Acad. Sci. USA 85 (1988) 7226–7230.
[43] J. Breton, C. Berthomieu, D.L. Thibodeau, E. Nabedryk, FEBS Lett. 288 (1991) 109–113.
[44] J. Breton, C. Boullais, G. Berger, C. Mioskowski, E. Nabedryk, Biochemistry 34 (1995) 11606–11616.
[45] R. Brudler, H.J.M. de Groot, W.B.S. van Liemt, P. Gast, A.J. Hoff, J. Lugtenburg, K. Gerwert, FEBS Lett. 370 (1995) 88–92.
[46] M. Bauscher, E. Nabedryk, K. Bagley, J. Breton, W. Mäntele, FEBS Lett. 261 (1990) 191–195.
[47] F. Siebert, W. Mäntele, W. Kreutz, FEBS Lett. 141 (1982) 82–87.
[48] S.Yu. Venyaminov, N.N. Kalnin, Biopolymers 30 (1990) 1243–1257.
[49] E. Nabedryk, J. Breton, M.Y. Okamura, M.P. Paddock, Photosynth. Res. 55 (1998) 293–299.
[50] P. Sebban, P. Maróti, M. Schiffer, D.K. Hanson, Biochemistry 34 (1995) 8390–8397.
[51] J. Breton, E. Nabedryk, Photosynth. Res. 55 (1998) 301–307.
[52] G. Zundel, Trends Phys. Chem. 3 (1992) 129–156.
[53] E. Nabedryk, J. Breton, M.Y. Okamura, M.P. Paddock, in: G. Garab (Ed), Photosynthesis: Mechanisms and Effects, Kluwer Academic, Dordrecht, 1998, 845–848.