FTIR spectroscopy shows weak symmetric hydrogen bonding of the Q_B carbonyl groups in *Rhodobacter sphaeroides* R26 reaction centres

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Received 22 May 1995; revised version received 6 July 1995

Abstract The absorption frequencies of the C = O and C = C (neutral state) and of the C···O (semiquinone state) stretching vibrations of Q_B have been assigned by FTIR spectroscopy, using native and site-specifically 1-, 2-, 3- and 4-^{13}C-labelled ubiquinone-10 (UQ_{10}) reconstituted at the Q_B binding site of *Rhodobacter sphaeroides* R26 reaction centres. Besides the main C = O band at 1641 cm^{-1}, two smaller bands are observed at 1664 and 1651 cm^{-1}. The smaller bands at 1664 and 1651 cm^{-1} agree in frequencies with the 1- and 4-C = O vibrations of unbound UQ_{10}, showing that a minor fraction is loosely and symmetrically bound to the protein. The larger band at 1641 cm^{-1} indicates symmetric H-bonding of the 1- and 4-C = O groups for the larger fraction of UQ_{10} but much weaker interaction as for the 4-C = O group of Q_A. The FTIR experiments show that different C = O protein interactions contribute to the factors determining the different functions of UQ_{10} at the Q_A and the Q_B binding sites.

Key words: Bacterial reaction centre; Ubiquinone; Fourier transform infrared spectroscopy; isotopic labelling; Photosynthesis

1. Introduction

The photosynthetic reaction centre (RC) of the purple non-sulfur bacterium *Rhodobacter (Rb.) sphaeroides* is a transmembrane pigment protein complex whose structure has been determined with up to 2.65 Å resolution [1–3]. Upon light excitation, an electron is transferred from the primary donor P (bacteriochlorophyll *a*-dimer) at the periplasmic side via a monomeric bacterichlorophyll *a* and a bacteriopheophytin *a* molecule to the primary quinone Q_A and finally to the secondary quinone Q_B at the cytoplasmic side of the membrane [4]. Although Q_A and Q_B both are ubiquinone-10 (UQ_{10}) molecules their functions are different. Q_A is tightly bound to the RC. It accepts only one electron and transfers it to Q_B in about 200 s; Q_B is less tightly bound to the protein. It accepts two electrons and two protons and is finally released from the RC as Q_BH_2.

In order to elucidate the protein-cofactor interactions which determine the different functions of UQ_{10} at their binding sites, FTIR difference spectroscopy has been applied [5,6]. By use of UQ_{10}, ^{13}C-labelled at the ring positions 1, 2, 3 and 4, respectively, the 1- and 4-C = O and 2/3-C = C stretching vibrations of UQ_{10} have been specifically assigned in the Q_A^-–Q_A difference spectra [7]. The mode dominated by the 4-C = O vibration is drastically downshifted in the RC as compared to unbound UQ_{10}, indicating unusually strong hydrogen bonding to His M219. In contrast, the 1-C = O group is only weakly bound to the protein. In the charge-separated state, the asymmetric binding is largely maintained. These results are fully supported by similar FTIR studies [8], by EPR [9] and NMR spectroscopy [10]. In the present contribution the interaction of Q_B with its protein environment is investigated using site specifically 1-, 2-, 3- and 4-^{13}C-labelled UQ_{10}. The results and their implications for the different functions of UQ_{10} at the Q_A and the Q_B site will be discussed.

2. Materials and methods

RC protein was purified from *Rb. sphaeroides* strain R26 [11]. UQ_{10}, selectively ^{13}C-labelled at positions 1, 2, 3 and 4 was synthesized as described in [12]. Q_B was selectively removed by incubating RCs with OD_{800} = 2 in 2% LDAO, 1 mM *o*-phenanthroline, 10 mM Tris pH 8 at 25–26°C for 6 h, followed by extensive washing on a DEAE Sephacel column [13]. The Q_A and Q_B content was determined by measuring the amplitude of the fast (100 ms) and slow (1 s) decay component of the photobleaching of P^+ at 865 nm after a light flash [7]. After the selective quinone-removal, the Q_B content was less than 10% while Q_A was present in more than 95% of the RCs. Q_B reconstitution with native and selectively ^{13}C-labelled UQ_{10} was done according to [13], except that the stock solution of UQ_{10} was in 1% Triton X-100. The Q_B content after reconstitution was better than 85% (except 70% for 1-^{13}C-UQ_{10}).

Sample preparation for the IR measurements was performed according to [7]. 45 μl of 40 μM RCs, dissolved in 10 mM Tris/HCl, 1 mM EDTA, 0.025 (w/v) LDAO, pH 8, were pipetted on a CaF_2 window, approximately ten-fold concentrated under a gentle stream of nitrogen and mixed with 5 μl of 10 mM sodium ascorbate, 20 mM diaminodurene (DAD) dissolved in the same buffer as the RCs. After further drying to a final volume of ~1 μl, the sample was sealed with another CaF_2 window and thermostabilized at 295 K in the FTIR apparatus.

IR spectra of free ubiquinones and Q_B^-–Q_B difference spectra were recorded as reported [7], except that the actinic light intensity allowing the saturation of Q_B^- was about ten-fold lower than that for the saturation of Q_A^- [14]. Spectral resolution was 4 cm^{-1}.

Double difference spectra were computed as described [7]. The difference spectra with unlabelled and ^{13}C-labelled Q_B were normalized on the 1800–1700 cm^{-1} region that was unaffected by the labelling.

3. Results

In Fig. 1a the Q_B^-–Q_B difference spectrum of *Rb. sphaeroides* R26 RCs reconstituted with unlabelled UQ_{10} is shown. The

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Abbreviations: RC, reaction centre; Rb., Rhodobacter; P, primary electron donor; Q_A, primary acceptor quinone; Q_B, secondary acceptor quinone; UQ, ubiquinone; ENDOR, electron-nuclear double resonance; EPR, electron paramagnetic resonance; FTIR, Fourier transform infra-red; NMR, nuclear magnetic resonance.

SSDI 0014-5793(95)00805-5

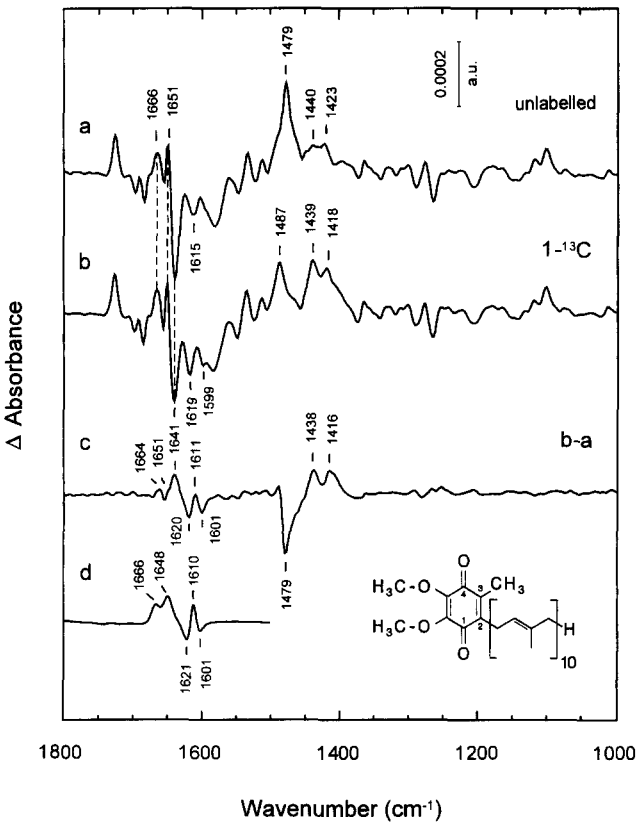

Fig. 1. Q_B^-–Q_B difference spectra of *Rb. sphaeroides* R26 RCs reconstituted with (a) unlabelled and (b) 1-^{13}C-labelled UQ_{10} at the Q_B site. (c) double difference spectrum (b − a). (d) difference spectrum between unbound unlabelled and 1-^{13}C-labelled UQ_{10}. The difference spectrum between unbound unlabelled and 4-^{13}C-labelled UQ_{10} is identical. Inset: structural formula of ubiquinone-10.

difference between the charge-separated and the ground state absorption selectively represents the light-induced absorption changes of the RCs. Positive bands belong to the charge-separated state, negative signals to the ground state.

The Q_B^-–Q_B difference spectrum (Fig. 1a) agrees remarkably well with the one published in [14]. It was shown that other species like Q_B^{2-}, Q_BH and Q_BH_2 do not significantly contribute to the difference spectra [14]. Only minor deviations in band positions and intensities within the experimental error are observed between the Q_B^-–Q_B difference spectra shown here and in [14], which may be caused by different detergent and water contents of the samples and different measuring temperatures (283 K instead of 295 K). Bauscher et al. [15] have also presented a Q_B^-–Q_B difference spectrum, obtained by the subtraction of an electrochemically generated P^+–P difference spectrum from a light-induced $P^+Q_B^-$–PQ_B difference spectrum. In this approach the much larger P^+–P bands have to be subtracted and only the minor Q_B^-–Q_B bands should result. The experimental error of this method and consequently the deviations to the directly measured Q_B^-–Q_B difference spectra shown here and in [14] are larger.

In Fig. 1b the Q_B^-–Q_B difference spectrum of RCs containing 1-^{13}C-labelled UQ_{10} at the Q_B site is displayed. The isotope labelling induces a frequency shift of the labelled group absorption to lower wavenumbers and thereby allows the specific assignment of the quinone vibrations. The C = O and C = C stretching vibrations of neutral UQ_{10} are expected to absorb between 1670–1570 cm^{-1} [7]. In this spectral region the intensity of the strong negative band at 1641 cm^{-1} decreases and two additional negative bands appear at 1619 and 1599 cm^{-1} (compare Fig. 1a and 1b). In addition, the positive bands at 1666 and 1651 cm^{-1} slightly gain intensity (Fig. 1b), indicating shifts of underlying negative bands. The C⋯O and C⋯C stretching vibrations of the semiquinone are found between 1500 and 1400 cm^{-1} [7]. In Fig. 1b the strong band of unlabelled UQ_{10} at 1479 cm^{-1} (Fig. 1a) is shifted and new bands appear at 1439 and 1418 cm^{-1}.

In order to visualize more clearly the deviations between the difference spectra, they have been subtracted as described [7]. The resulting double difference spectrum is shown in Fig. 1c (=Fig. 1b − 1a). In the double difference spectra, the bands of the C = O and C = C stretching vibrations of neutral UQ_{10} (1670–1570 cm^{-1}) are positive for unlabelled UQ_{10} while the corresponding shifted bands of labelled UQ_{10} are negative. Inversely, the signals of the C⋯O and C⋯C stretching vibrations of the semiquinone (1500–1400 cm^{-1}) are negative for unlabelled UQ_{10} and the shifted bands of labelled UQ_{10} are positive. In Fig. 1c the bands of unlabelled UQ_{10} are seen at 1664, 1651, 1641, 1611 and 1479 cm^{-1}. The positive band at 1664 cm^{-1} and the shoulder at 1651 cm^{-1} are small compared to the band at 1641 cm^{-1} but have been reproduced in every measurement. The shifted bands of 1-^{13}C-UQ_{10} can be seen at 1620, 1601, 1438 and 1416 cm^{-1}. For comparison, in Fig. 1d the difference spectrum between unbound unlabelled and 1-^{13}C-labelled UQ_{10} is depicted in the region of the C = O and C = C stretching vibrations. For the spectra of unbound unlabelled and 1-, 2-, 3- and 4-^{13}C-labelled UQ_{10} see [7]. The difference spectrum shown in Fig. 1d is identical to the difference spectrum between unbound unlabelled and 4-^{13}C-labelled UQ_{10} [7]. As in the double difference spectra, the positive bands belong to unlabelled UQ_{10} while the negative signals represent the shifted bands of labelled UQ_{10}. Two bands at 1666 and 1648 cm^{-1} are shifted to 1621 cm^{-1} for 1- and 4-^{13}C-labelled UQ_{10} and are dominated by the 1- and 4-C = O stretching vibrations [7]. A distinction between the vibrations of the 1- and 4-C = O group is not possible due to mixing of the 1- and 4-C = O vibrations (both contribute to the bands at 1666 and 1648 cm^{-1}). A band at 1610 cm^{-1} is shifted to 1601 cm^{-1} (Fig. 1d) and is dominated by the 2/3-C = C stretching vibration [7]. As discussed in [7], isotope labelling of C = O groups shifts also bands of C = C vibrations and vice versa due to extensive coupling of the C = O and C = C vibrations of UQ_{10}. As for unbound UQ_{10}, the two bands at 1664 and 1651 cm^{-1} seem to be shifted to 1620 cm^{-1} for RC bound UQ_{10} (Fig. 1c). They are therefore assigned to C = O stretching vibrations of UQ_{10} at the Q_B site. The largest band in the carbonyl region appears at 1641 cm^{-1} (Fig. 1c). It is not directly seen to which position this band is shifted. The maximal shift for a C = O vibration due to ^{13}C-labelling is 36 cm^{-1} [16]. It seems to be shifted underneath the band at 1611 cm^{-1} (Fig. 1c). The difference band at 1611/1601 cm^{-1} in Fig. 1c represents, as compared to Fig. 1d, the shift of the 2/3-C = C vibration of Q_B. The intensity of the band at 1611 cm^{-1} (Fig. 1c) is reduced as compared to the band at 1610 cm^{-1} (Fig. 1d) in agreement with the assumption that the C = O vibration is shifted from 1641 cm^{-1} underneath the band at 1611 cm^{-1}. This assumption is furthermore supported by the observation of a clear C = C vibration at 1611 cm^{-1} for 2-^{13}C- and

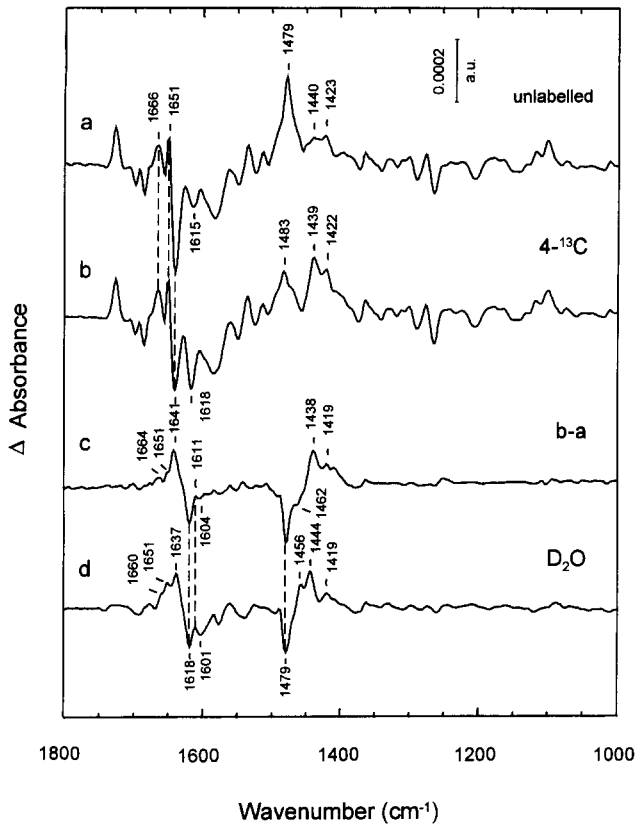

Fig. 2. Q_B^-–Q_B difference spectra of *Rb. sphaeroides* R26 RCs reconstituted with (a) unlabelled and (b) 4-^{13}C-labelled UQ_{10} at the Q_B site. (c) double difference spectrum (b − a). (d) double difference spectrum as in Fig. 2c but measured in D_2O instead of H_2O.

3-^{13}C-labelled UQ_{10} (Fig. 3d,e). In this case the C = O vibration at 1643 cm^{-1} is less shifted to 1621 cm^{-1} but not underneath the band at 1611 cm^{-1}.

Fig. 2b shows the Q_B^-–Q_B difference spectrum of RCs with 4-^{13}C-UQ_{10} at the Q_B site. It is similar to the corresponding spectrum with 1-^{13}C-UQ_{10} at the Q_B site (Fig. 1b): the negative band at 1641 cm^{-1} decreases and the positive bands at 1666 and 1651 cm^{-1} slightly gain intensity. At 1618 cm^{-1} a new negative band appears. The band at 1479 cm^{-1} (Fig. 2a) is shifted and new bands appear at 1439 and 1422 cm^{-1} (Fig. 2b). Again the labelling-induced band shifts can be more clearly seen in the double difference spectrum (Fig. 2c). The signals of unlabelled UQ_{10} (neutral state) appear at 1664, 1651 and 1641 cm^{-1} as in the double difference spectrum with 1-^{13}C-labelled UQ_{10} (Fig. 1c) and are correspondingly assigned to C = O stretching vibrations. As for unbound UQ_{10}, the 1- and 4-C = O vibrations of Q_B cannot be distinguished. The shifted signal of the bands at 1664 and 1651 cm^{-1} is seen at 1618 cm^{-1} as a negative band (compare Fig. 2c with Fig. 1d). The difference band at 1610/1601 cm^{-1} that is observed in the difference spectrum between unbound and 1- or 4-^{13}C-labelled UQ_{10} (Fig. 1d) is missing in Fig. 2c. A ^{13}C-induced shift of the C = O vibration from 1641 cm^{-1} to around 1610 cm^{-1} and of the C = C vibration from 1610 to 1601 cm^{-1} seem to cancel each other more than in the case of 1-^{13}C-labelling (compare Fig. 2c with Fig. 1c). In order to clarify, the samples have been measured in D_2O where the bands shift slightly different (Fig. 2d). The difference band at 1611/1601 cm^{-1} is better resolved than in H_2O. In addition, the bands appearing at 1664, 1651, 1641 and 1618 cm^{-1} in H_2O (Fig. 2c) are observed in D_2O as well at 1660, 1651, 1637 and 1618 cm^{-1}, respectively (Fig. 2d). They are also found in the double difference spectrum between the difference spectra with 1-^{13}C-UQ_{10} and unlabelled UQ_{10} at the Q_B site, measured in D_2O (not shown). Their presence in D_2O excludes that they are caused by absorption of water. In D_2O, the O-H group is replaced by the O-D group and the corresponding bending vibration is shifted from 1645 to 1215 cm^{-1}. For the semiquinone state the double difference spectra (Fig. 2c,d) show a band at 1479 cm^{-1} of unlabelled UQ_{10}. Shifted bands of 4-^{13}C-UQ_{10} are found at 1438 and 1419 cm^{-1} in H_2O (Fig. 2c) and at 1456, 1444 and 1419 cm^{-1} in D_2O (Fig. 2d). The band at 1456 cm^{-1} has a counterpart at 1462 cm^{-1} in H_2O (Fig. 2c). It seems that the broad band at 1479 cm^{-1} decreases the intensity of the band at 1462 cm^{-1} in H_2O but has less effect on the band at 1456 cm^{-1} in D_2O.

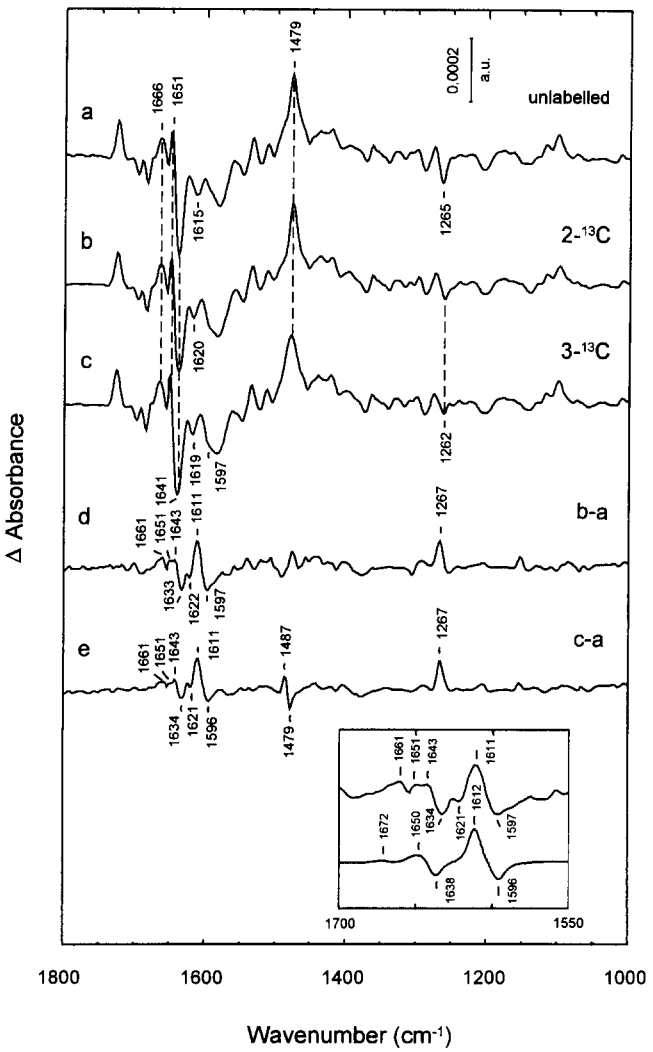

Fig. 3. Q_B^- − Q_B difference spectra of *Rb. sphaeroides* R26 RCs reconstituted with (a) unlabelled, (b) 2-^{13}C-labelled and (c) 3-^{13}C-labelled UQ_{10} at the Q_B site. (d) double difference spectrum (b − a). (e) double difference spectrum (c − a). Inset: double difference spectrum of Fig. 3d (upper trace) in comparison with the difference spectrum between unbound unlabelled and 2-^{13}C-labelled UQ_{10} (lower trace). It is identical to the difference spectrum between unbound unlabelled and 3-^{13}C-labelled UQ_{10} (not shown).

In Fig. 3 the Q_B^-–Q_B difference spectra of RCs reconstituted with 2-^{13}C- (Fig. 3b) and 3-^{13}C-UQ_{10} (Fig. 3c) at the Q_B site are displayed. They are very similar. The corresponding double difference spectra agree therefore nicely and show small positive bands at 1661, 1651 and 1643 cm^{-1} and a stronger band at 1611 cm^{-1} (Fig. 3d,e). Negative bands are found at 1633/1634, 1622/1621 and 1597/1596 cm^{-1} (Fig. 3d,e). An expanded view of Fig. 3d is shown in the inset of Fig. 3 in the upper trace. Interestingly, no band shifts are found for the semiquinone vibrations (1500–1400 cm^{-1}; Fig. 3d,e). For 3-^{13}C-labelled UQ_{10} (Fig. 3e) a positive band at 1487 cm^{-1} is shifted to 1479 cm^{-1}. It cannot be assigned to semiquinone vibrations since a semiquinone band should be negative and the shifted signal positive. The inset of Fig. 3 shows the difference spectrum between protein unbound unlabelled and 2-^{13}C-labelled UQ_{10} in the lower trace. It is identical to the difference spectrum between unbound unlabelled and 3-^{13}C-labelled UQ_{10} [7]. The strongest band of this difference spectrum at 1612 cm^{-1} is shifted to 1596 cm^{-1} and is assigned to the C = C stretching vibration [7]. These bands have counterparts in the double difference spectra at 1611 and 1597 cm^{-1} (inset of Fig. 3, upper trace). The band at 1611 cm^{-1} is therefore assigned to the C = C stretching vibration of UQ_{10} at the Q_B site. Due to extensive coupling of the C = C and C = O vibrations ^{13}C-labelling at the 2/3-C = C positions shifts also the C = O vibrations. The difference spectrum in Fig. 3 (inset, lower trace) shows also shifts of C = O vibrations from 1672 and 1650 cm^{-1} to 1638 cm^{-1}. These bands have counterparts in the double difference spectra at 1661, 1651 and 1634 cm^{-1} (inset, upper trace) and therefore are assigned to C = O stretching vibrations. As for 1- and 4-^{13}C-labelled UQ_{10} (Figs. 1c and 2c) an additional band appears in the region of the C = O stretching vibrations at 1643 cm^{-1} (inset, upper trace) but is less pronounced as compared to 1- and 4-^{13}C-labelled UQ_{10} (Figs. 1c and 2c). It seems to be shifted to 1621 cm^{-1} and is assigned to a C = O stretching vibration of Q_B as discussed above.

4. Discussion

The isotope shifts and the assignments of the dominant vibrations for UQ_{10} bound at the Q_B site are summarized in Table 1. As for unbound UQ_{10} the C = O and C = C stretching vibrations are strongly coupled but the dominant vibrations can be assigned.

Surprisingly, three carbonyl vibrations are observed for UQ_{10} at the Q_B site (Table 1) instead of two as for unbound UQ_{10}, indicating heterogeneous binding to the protein. The two minor bands at 1664 and 1651 cm^{-1} agree with the frequencies of unbound UQ_{10}. The band at 1641 cm^{-1} is slightly downshifted and indicates a stronger interaction of the 1-and 4-carbonyl groups with the Q_B site. Comparison of the integral intensity of the carbonyl bands results in roughly 25 % for the fraction absorbing at 1664 and 1651 cm^{-1} and roughly 75% for the fraction absorbing at 1641 cm^{-1}. Both fractions represent reacting UQ_{10} because only bands of functionally active groups appear in the difference spectra. It is not clear, whether both fractions have physiological relevance.

The absorption frequencies of the two bands at 1664 and 1651 cm^{-1} (Table 1) are almost unaltered as compared to unbound UQ_{10} (1666 and 1648 cm^{-1}). They can be attributed to a fraction of Q_B with the carbonyl groups not specifically hydrogen bonded to the protein in the ground state. The C = O vibration of the other Q_B fraction, absorbing at 1641 cm^{-1}, indicates symmetric hydrogen bonding to the protein in the ground state. In contrast, the FTIR difference spectra of Q_A^-–Q_A have revealed an asymmetry between the two carbonyls of Q_A. The 1-C = O group (absorbing at 1660 cm^{-1}) is only very weakly bound, whereas the 4-C = O group (absorbing at 1601 cm^{-1}) is much stronger hydrogen-bonded to the protein than the carbonyl groups of Q_B. The strong hydrogen bonding binds Q_A tightly to the protein and governs the fast electron transfer via His M219 to Q_B [7,8,17].

In agreement with the heterogeneous binding of UQ_{10} at the Q_B site, seen in FTIR experiments, also in X-ray crystallography experiments different positions of Q_B have been observed. Eight possible binding positions have been reported for UQ_1 (ubiquinone with one isoprene unit) at the Q_B site of *Rhodopseudomonas viridis* RCs [18]. For *Rb. sphaeroides* RCs two different binding positions of Q_B are proposed. Ermler et al. [3] have positioned the Q_B molecule at the more hydrophobic entrance of the Q_B binding pocket, whereas Allen et al. [19] and El-Kabbani et al. [20] have located it deeper in the protein. Consequently, different hydrogen bond donors to the carbonyl groups of Q_B have been suggested. In [19] and [20] the 1-C = O group is in hydrogen bonding distance to Ser L223, the 4-C = O group to His L190. In [3] only the 1-C = O group could form a hydrogen bond with the peptide nitrogen of Ile L224. The differently bound fractions of UQ_{10} at the Q_B site seen in the IR experiments may explain the high temperature factor for the Q_B site [3] and the different positions for UQ_{10} at the Q_B site

Table 1
Absorption frequencies (cm^{-1}) and assignments of UQ_{10} IR bands at the Q_B site of *Rb. sphaeroides* R26 RCs in the neutral (above) and in the semiquinone state (below)
The frequency shifts of the labels are indicated in brackets.

Q_B	1-^{13}C	4-^{13}C	2-^{13}C	3-^{13}C	Assignment of dominant vibration
1641	~1610 (−31)	~1610 (−31)	1622 (−19)	1621 (−20)	1/4-C = O
1664	1620	1618	1633	1634	1/4-C = O
1651	(−44/−31)	(−46/−33)	(−31/−18)	(−30/−17)	
1611	1601 (−10)	1604 (−7)	1587 (−14)	1596 (−15)	2/3-C = C
Q_B^-	1-^{13}C	4-^{13}C	2-^{13}C	3-^{13}C	Assignment of dominant vibration
1479	1438; 1416 (−41/−63)	1438; 1419 (−41/−60)	–	–	1/4-C···O

of *Rb. sphaeroides* RCs [3,19,20]. Furthermore, Giangiacomo and Dutton [21] have shown that the Q_B site is not highly specific for the native UQ_{10} and functions as well with various substituted quinones, including quinones with the ortho-carbonyl configuration.

In the semiquinone spectral region (1500–1400 cm^{-1}) [22] the strong positive band at 1479 cm^{-1} is shifted by the 1- and 4-^{13}C-labels to 1438 and 1416/1419 cm^{-1}. The band at 1479 cm^{-1} is therefore assigned to the 1- and 4-C⋯O stretching vibration of Q_B^- (Table 1). Similar band shifts have been observed for uniformly ^{13}C-labelled UQ_{10}, dissolved in CH_2Cl_2. A semiquinone band at 1483 cm^{-1} is shifted to 1442 and 1412 cm^{-1} and has been assigned to the C⋯O stretching vibrations [23]. Since no band shifts of the band at 1479 cm^{-1} are seen in the spectra of 2-^{13}C- and 3-^{13}C-labelled UQ_{10} bound to the protein (Fig. 3d, 3e), the band is assigned to mixed 1- and 4-C⋯O vibrations without C⋯C contributions. The close similarity of the 1- and 4-^{13}C-induced shifts of the band at 1479 cm^{-1} (Fig. 1b,2b and 1c,2c) indicates that the carbonyl groups of Q_B^- are nearly equivalent and equally hydrogen bonded to the protein. This observation is in agreement with results of ENDOR measurements [24,25]. In contrast, at the Q_A site the asymmetry of the carbonyl groups is maintained as well in the semiquinone state [9,7]. The frequency of the 1- and 4-C⋯O vibrations of Q_B^- at 1479 cm^{-1} indicates weaker hydrogen bonding than for the 4-C⋯O group of Q_A^-, which absorbs at 1466 cm^{-1}. The weak symmetric binding in the charge separated state indicates an equally delocalized π electron distribution and thereby seems to support the stabilization of the negative charge on Q_B^- until a second electron is accepted.

In conclusion, the Q_A^-–Q_A and the Q_B^-–Q_B FTIR difference spectra demonstrate two strongly differing binding sites, which tune the different functions of the same prosthetic group by specific protein interactions.

Acknowledgements: We thank A.H.M. de Wit and S.J. Jansen for culturing the cells and isolating the RCs, and W.F. Steggerda and R. Esmeijer for ^{13}C-labelling of UQ_{10}. P.G. is a recipient of a Royal Netherlands Academy of Arts and Sciences (KNAW) career fellowship. This work was supported by the Foundation for Chemical Research (SON), financed by the Netherlands Organization of Pure and Applied Research (NWO). K.G. acknowledges the DFG for financial support (Ge-599/7–1).

References

[1] Feher, G., Allen, J.P., Okamura, M.Y. and Rees, D.C. (1989) Nature 339, 11–116.
[2] Chang, C.-H., El-Kabbani, O., Tiede, D., Norris, J. and Schiffer, M. (1991) Biochemistry 30, 5352–5360.
[3] Ermler, U., Fritzsch, G., Buchanan, S. and Michel, H. (1994) Structure 2, 925–936.
[4] Norris, J.R. (1992) Israel J. Chem. 32, 418–422.
[5] Gerwert, K. (1993) Curr. Opin. Struct. Biol. 3, 769–773.
[6] Mäntele, W. (1993) in The Photosynthetic Reaction Center (Deisenhofer, J., Norris, J., eds.), pp. 239–283, Academic Press, New York, Vol. 2.
[7] Brudler, R., de Groot, H.J.M., van Liemt, W.B.S., Steggerda, W.F., Esmeijer, R., Gast, P., Hoff, A.J., Lugtenburg, J. and Gerwert, K. (1994) EMBO J. 13, 5523–5530.
[8] Breton, J., Boullais, C., Burie, J.-R., Nabedryk, E. and Mioskowski, C. (1994) Biochemistry 33, 14378–14386.
[9] van den Brink, J.S., Spoyalov, A.P., Gast, P., van Liemt, W.B.S., Raap, J., Lugtenburg, J. and Hoff, A.J. (1994) FEBS Lett. 353, 273–276.
[10] van Liemt, W.B.S., Boender, G.J., Gast, P., Hoff, A.J., Lugtenburg, J. and de Groot, H.J.M. (1995) Biochemistry, in press.
[11] Feher, G. and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K., Sistrom, W.R., eds.), pp. 349–386, Plenum Press, New York.
[12] van Liemt, W.B.S., Steggerda, W.F., Esmeijer, R. and Lugtenburg, J. (1994) Recl. Trav. Chim. Pays-Bas 113, 153–161.
[13] Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.
[14] Breton, J., Berthomieu, C., Thibodeau, D.L. and Nabedryk, E. (1991) FEBS Lett. 288, 109–113.
[15] Bauscher, M., Leonhard, M., Moss, D.A. and Mäntele, W. (1993) Biochim. Biophys. Acta 1183, 59–71.
[16] Engelhard, M., Gerwert, K., Hess, B., Kreutz, W. and Siebert, F. (1985) Biochemistry 24, 400–407.
[17] Bosch, M.K., Gast, P., Hoff, A.J., Spoyalov, A.P. and Tsvetkov, Yu.D. (1995) Chem. Phys. Lett., in press.
[18] Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.
[19] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 8487–8491.
[20] El-Kabbani, O., Chang, C.-H., Tiede, D., Norris, J. and Schiffer, M. (1991) Biochemistry 30, 5361–5369.
[21] Giangiacomo, K. and Dutton, P.L. (1989) Proc. Natl. Acad. Sci. USA 86, 2658–2662.
[22] Buchanan, S., Michel, H. and Gerwert, K. (1992) Biochemistry 31, 1314–1322.
[23] Bauscher, M. and Mäntele, W. (1992) J. Phys. Chem. 96, 11101–11108.
[24] Feher, G., Isaacson, R.A., Okamura, M.Y. and Lubitz, W. (1985) in Antennas and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.E., ed.), pp. 174-189, Springer, Berlin.
[25] Lubitz, W., Abresch, E.C., Debus, R.J., Isaacson, R.A., Okamura, M.Y. and Feher, G. (1985) Biochim. Biophys. Acta 808, 464–469.