A protein conformational change associated with the photoreduction of the primary and secondary quinones in the bacterial reaction center

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A comparison is made between the $PQ_A \rightarrow P^+Q_A^-$ and $PQ_AQ_B \rightarrow P^+Q_AQ_B^-$ transitions in *Rps. viridis* and *Rb. sphaeroides* reaction centers (RCs) by the use of light-induced Fourier transform infrared (FTIR) difference spectroscopy. In *Rb. sphaeroides* RCs, we identify a signal at 1650 cm^{-1} which is present in the P^+Q_A-minus-PQ_A spectrum and not in the $P^+Q_AQ_B^-$-minus-PQ_AQ_B spectrum. In contrast, this signal is present in both $P^+Q_A^-$-minus-PQ_A^- and $P^+Q_AQ_B^-$-minus-PQ_AQ_B spectra of *Rps. viridis* RCs. These data are interpreted in terms of a conformational change of the protein backbone near Q_A (possible at the peptide C=O of a conserved alanine residue in the Q_A pocket) and of the different bonding interactions of Q_B with the protein in the RC of the two species.

Fourier transform infrared spectroscopy; Bacterial reaction center; Photosynthesis; Primary quinone; Secondary quinone

1. INTRODUCTION

In the photosynthetic bacterial RC, the electron transfer reaction proceeds from the primary electron donor P, a dimer of bacteriochlorophyll, via an intermediate acceptor (a bacteriopheophytin molecule) to a primary quinone Q_A and a secondary quinone Q_B. In *Rb. sphaeroides* RCs, both quinones are ubiquinone while in *Rps. viridis* RCs, Q_A is a menaquinone and Q_B is a ubiquinone. After flash excitation, charge recombination between P^+ and Q_A^- or P^+ and Q_B^- proceeds faster in *Rps. viridis* (≈1 ms and ≈100 ms, respectively [1]) than in *Rb. sphaeroides* (≈100 ms and a few s respectively [2]) RCs. The X-ray three-dimensional structures of both RCs have provided details of the cofactor-protein interactions. In particular, amino-acid residues in the Q_A and Q_B binding pockets have been identified and structural differences between the Q_A and Q_B environments have been demonstrated [3–5]. Moreover, differences in the interactions of the protein with the quinones in *Rb. sphaeroides* [4] and *Rps. viridis* [5] have been described.

While X-ray crystallography provides the detailed structure of an essentially static state of the neutral RC, molecular changes of the complex at the level of individual chemical groups of the protein and the cofactors can be, in principle, monitored by vibrational spectroscopy. Indeed, molecular changes concomitant with charge stabilization in bacterial RCs and plant photosystems have been probed by light-induced FTIR difference spectroscopy [6–10]. From these studies, specific changes in the IR absorption of BChl or bacteriopheophytin carbonyl groups of the primary donor [6,8,9] and intermediate acceptor [7,8,10] have been characterized and the absence of any large conformational change of the RC protein backbone has been demonstrated. However, the quinone absorption bands in the light-induced FTIR spectra have proven much more elusive [8,11–13].

By the use of FTIR difference spectroscopy, we report here the comparison between the $PQ_A \longrightarrow P^+Q_A^-$ and $PQ_AQ_B \longrightarrow P^+Q_AQ_B^-$ transitions in RCs from both *Rb. sphaeroides* and *Rps. viridis*.

2. EXPERIMENTAL

Light-induced FTIR difference spectra between the charge-separated state ($P^+Q_A^-$ or $P^+Q_AQ_B^-$) and the relaxed state (PQ_A or PQ_AQ_B), designated $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra, respectively, were obtained as previously described [11]. FTIR measurements were performed under steady-state illumination [6] with a Nicolet 60SX FTIR spectrometer equipped with a MCT-A detector. Excess of ubiquinone was added to isolated RCs. Air-dried films of RCs were rehydrated for FTIR measurements. For all samples, spectra were recorded at 290 K and 100 K. At 100 K, the electron transfer from Q_A to Q_B is known to be blocked [14]. Films were cooled in the dark and then illuminated to produce the charge-separated state. Interferograms (n = 128) were recorded before and during continuous illumination with saturating actinic light (715 nm

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Abbreviations: FTIR, Fourier transform infrared; Q_A (Q_B), primary (secondary) quinone; P, primary electron donor; BChl, bacteriochlorophyll; RC, reaction center

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$< \lambda < 1100$ nm). The light intensity necessary to achieve maximal yield of $P^+Q_A^-$ in the experiments at 100 K was at least 20 times higher than that used to produce the $P^+Q_AQ_B^-$ state at room temperature, because of the much slower decay time characteristic of $P^+Q_AQ_B^-$. Each light-minus-dark cycle was repeated several hundred times separated by a dark time sufficient to ensure full return to the ground state.

3. RESULTS

The $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ FTIR difference spectra are shown for *Rb. sphaeroides* RC in Fig. 1 and for *Rps. viridis* RC in Fig. 2. In these spectra, negative bands arise from vibrations of the neutral species, i.e., P and Q_A or Q_B while positive bands are associated with the radicals, i.e. P^+ and Q_A^- or Q_B^-. In the carbonyl stretching frequency region (1760–1620 cm^{-1}), contributions may be anticipated from BChl, protein (the amide I band), quinone and lipid C=O groups as well as from the OH bending vibration of water. The largest signals observed between 1760 cm^{-1} and 1680 cm^{-1} have been previously related to the contribution of 10a ester and 9 keto C=O groups of P and P^+ [6,8]. For each RC species, these ester and keto C=O bands appear very similar in both $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra.

3.1. Rb. sphaeroides *RC*

In the 1680–1600 cm^{-1} frequency region, several reproducible differences are observed between the $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra of *Rb. sphaeroides* RC (Fig. 1): four negative bands are detected at 1664 cm^{-1}, 1650 cm^{-1}, 1634 cm^{-1} and 1603 cm^{-1} in the $P^+Q_A^-/PQ_A$ spectrum (Fig. 1a) while only three bands are observed at 1664 cm^{-1}, 1638 cm^{-1} and 1618 cm^{-1} in the $P^+Q_AQ_B^-/PQ_AQ_B$ spectrum (Fig. 1b). The frequency of these bands suggests that they most probably arise from either the C=O or the C=C of the neutral quinone, or the acetyl C=O groups of P, or peptide C=O of the RC protein backbone (amide I). We have previously reported that the 1664 cm^{-1} band could be due to P [8]. The 1603 cm^{-1} signal in Fig. 1a is assigned to the C=C vibrational mode of Q_A since (i) it disappears in the $P^+Q_A^-/PQ_A$ spectra of RC which have been reconstituted with [^{13}C]ubiquinone at the Q_A site and (ii) it is still present in the spectra of RC containing [^{18}O]ubiquinone or duroquinone [11].

The clearest difference between the $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra is a well-defined band at 1650 cm^{-1} in the $P^+Q_A^-/PQ_A$ spectrum (Fig. 1a) which does not appear in the $P^+Q_AQ_B^-/PQ_AQ_B$ spectrum (Fig. 1b). Temperature effects can be ruled out since the $P^+Q_A^-/PQ_A$ spectra in the 1700–1600 cm^{-1} frequency range are nearly identical at both temperatures for *Rb. sphaeroides* RC containing quinone only in the Q_A site (Fig. 1a, inset, see also [11]). In addition, the 1650 cm^{-1} band was consistently found in all spectra

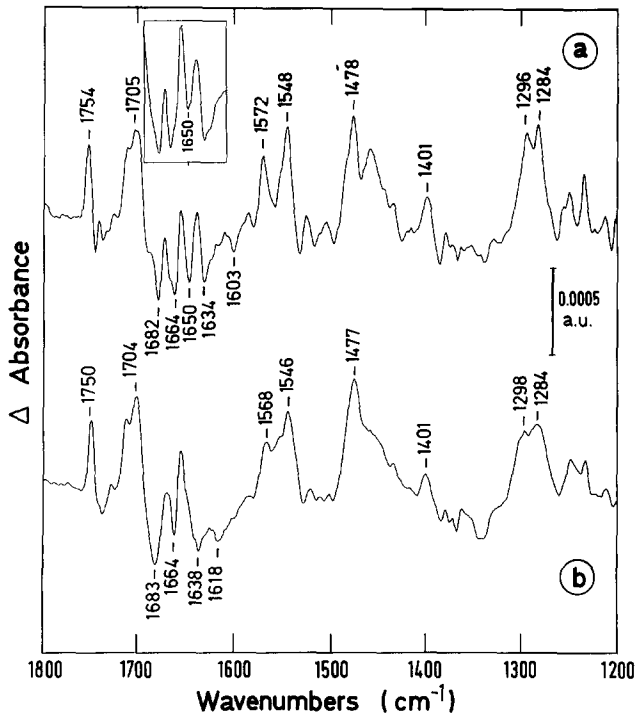

Fig. 1. Light-induced FTIR difference spectra of *Rb. sphaeroides* RC between (a) $P^+Q_A^-$ and PQ_A, 100 K, 76800 interferograms coadded. Inset: $P^+Q_A^-/PQ_A$ spectrum at 250 K in the 1700–1600 cm^{-1} region for RC containing quinone only in Q_A (from [11]). (b) $P^+Q_AQ_B^-$ and PQ_AQ_B, 290 K, 30720 interferograms coadded.

obtained with various RC samples of different amide I absorbance, thus excluding that this band could be caused by phase errors in a region of strong absorption like the amide I region.

The position of the 1650 cm^{-1} band lies in the expected frequency range for carbonyls of ubiquinone model compounds (1660–1650 cm^{-1}) studied in vitro ([11,12] and refs cited therein). However, the 1650 cm^{-1} band is unaffected by isotopic substitution on the ubiquinone or reconstitution of RC with duroquinone [11]. For isolated duroquinone, the C=O stretching frequency is downshifted with respect to ubiquinone by $\approx$15 cm^{-1} [11]. The 1650 cm^{-1} band is also absent in $P^+Q_AQ_B^-/PQ_AQ_B$ spectra of RC reconstituted with [^{13}C]- or [^{18}O]ubiquinone at both Q_A and Q_B sites [11]. It thus appears that the 1650 cm^{-1} band does not arise from a change in either the C=O or the C=C stretching vibration of Q_A but most probably arises from some change in a peptide C=O vibration (amide I band) presumably belonging to an amino acid of the Q_A binding pocket. This point will be further discussed in the *Rps. viridis* section. In correlation with the observed change in the amide I region, it can be noticed that the amide II region (60% peptide NH bending vibration) between 1545 cm^{-1} and 1570 cm^{-1} appears different in the $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra. These differences cannot be

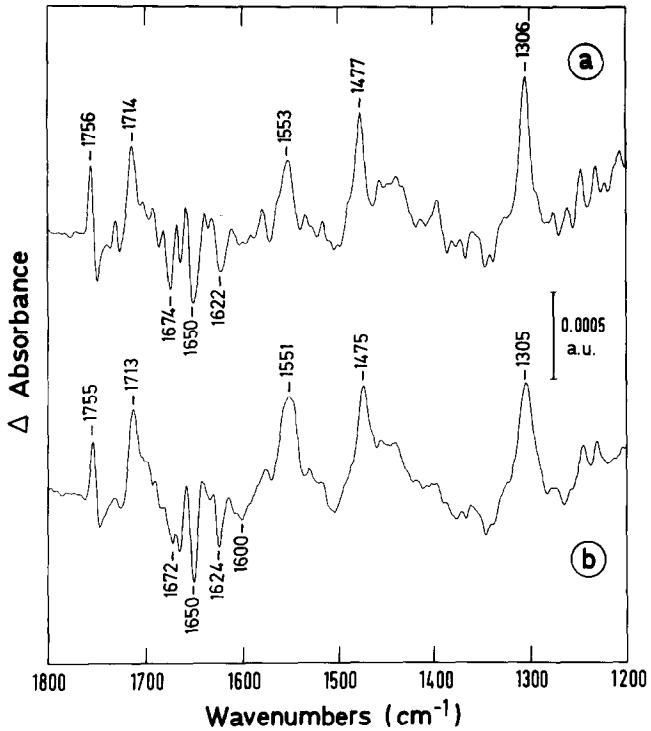

Fig. 2. Light-induced FTIR difference spectra of *Rps. viridis* RC between (a) $P^+Q_A^-$ and PQ_A, 100 K, 112640 interferograms coadded. (b) $P^+Q_AQ_B^-$ and PQ_AQ_B, 290 K, 61440 interferograms coadded.

related to temperature effects since the $P^+Q_A^-/PQ_A$ spectra obtained at both temperatures for RCs containing quinone only in the Q_A site display the same shape in this spectral domain (data not shown).

3.2. Rps. viridis *RC*

In contrast to *Rb. sphaeroides*, light-induced FTIR difference spectra of *Rps. viridis* RC display a negative band at 1650 cm^{-1} which is not unique to the $PQ_A \longrightarrow P^+Q_A^-$ transition (Fig. 2a); the same band appears in the $P^+Q_AQ_B^-/PQ_AQ_B$ spectrum (Fig. 2b, see also [6]). In vitro IR studies of quinone model-compounds in methanol show the C=O band located at 1665 cm^{-1} for menaquinone (M. Bauscher, unpublished data) and at 1659 cm^{-1} for ubiquinone [12]. However, in vivo, although Q_A and Q_B are different chemical species in *Rps. viridis* RC, comparable features are observed in the quinone C=O frequency range for both $P^+Q_A^-/PQ_A$ and $P^+Q_AQ_B^-/PQ_AQ_B$ spectra (Fig. 2). It therefore appears that the 1650 cm^{-1} band is insensitive to the chemical nature of the quinone in both *Rps. viridis* and *Rb. sphaeroides* RCs (as mentioned earlier, the 1650 cm^{-1} band is still present in *Rb. sphaeroides* RC reconstituted with duroquinone in the Q_A site [11]). In good agreement with these data, the 1650 cm^{-1} signal is clearly visible in light-induced FTIR difference spectra obtained at 275 K by Buchanan et al. ([13], see also [15]) on *Rps. viridis* RC containing only Q_A or both Q_A and Q_B. Moreover, this 1650 cm^{-1} signal is also observed in chromatophore spectra at both temperatures (data not shown). By the use of *Rps. viridis* chromatophores, we have also investigated the possibility that the 1650 cm^{-1} band might arise from a contribution of the membrane-bound cytochrome (tightly associated with RC) which acts as electron donor to P^+. When chromatophore films are illuminated in the presence of 100 μM ferricyanide, the 1650 cm^{-1} band is still observed, thus demonstrating that cytochrome is not responsible for this band.

4. DISCUSSION

X-ray structure analysis of *Rps. viridis* and *Rb. sphaeroides* RCs has provided a detailed picture of the quinone-protein interactions [3–5]. While Q_A is bound in a hydrophobic pocket, the binding site of Q_B appears more polar. The residues proximal to C=O groups of Q_A and Q_B are shown in Table I. In *Rb. sphaeroides* RC [4], the two carbonyl oxygens of Q_A are within hydrogen-bonding distance to the peptide nitrogen of Ala M260 and the hydroxyl side chain of Thr M222, respectively. From in situ midpoint potential measurements, it has also been shown that the interaction strength of the semiquinone at the Q_A site is considerably enhanced over that of the quinone [16]. The hydrogen-bond lengths determined by ENDOR spectroscopy for Q_A^- are 1.55 Å and 1.78 Å [4]. In *Rps. viridis* RC [3,5], the carbonyl oxygens of Q_A seem hydrogen-bonded to the peptide NH of Ala M258 (equivalent to Ala M260 of *Rb. sphaeroides*) and the imidazole ring of His M217 (equivalent to His M219 of *Rb. sphaeroides*). Consequently, in both RCs, the binding site of Q_A most likely involves a hydrogen-bond between one C=O of Q_A and a peptide nitrogen of the RC protein backbone. We therefore propose that the

Table I

Groups proximal to Q_A and Q_B carbonyls in *Rb. sphaeroides* and *Rps. viridis* reaction centers

		Rb. sphaeroides	*Rps. viridis*
Q_A	C=O	Ala M260 (peptide NH as Q_A ligand)	Ala M258
	C=O	Thr M222 (hydroxyl OH as Q_A ligand)	His M217 (imidazole NH as Q_A ligand)
Q_B	C=O	His L190 (imidazole NH as Q_B ligand)	His L190
	C=O	Ser L223 (hydroxyl OH as Q_B ligand)	Ser L223 (hydroxyl OH as Q_B ligand) Gly L225 (peptide NH as Q_B ligand)

From [3–5]

1650 cm^{-1} band associated with the $PQ_A \longrightarrow P^+Q_A^-$ transition could arise, in both RCs, from a conformational change of the protein matrix near Q_A and possibly at the peptide C=O of the conserved Ala residue in the Q_A binding pocket. Within this hypothesis, the corresponding change observed in the amide II region could originate from the NH peptide group of the Ala residue in interaction with Q_A. The amplitude of the 1650 cm^{-1} band (less than 0.2% of the amide I band absorbance) corresponds to changes at the level of one (or possibly two) peptide bonds. Furthermore, the absence of the 1650 cm^{-1} band in the $P^+Q_AQ_B^-/PQ_AQ_B$ spectrum of *Rb. sphaeroides* is consistent with the X-ray structure which shows that Q_B, unlike Q_A, does not bind directly to the protein backbone. In *Rb. sphaeroides* RC, the two carbonyl oxygens of Q_B are within hydrogen-bonding distance to the imidazole ring of His L190 and the hydroxyl side chain of Ser L223 (a conserved residue in all bacterial and plant RCs). In contrast, in *Rps. viridis* RC, it seems that Q_B, just like Q_A, forms a hydrogen-bond with an NH of the peptide backbone: one C=O of Q_B is bound to His L190, as in *Rb. sphaeroides*, the other C=O forms bidented hydrogen bonds to both the hydroxyl side chain of Ser L223 and the NH peptide of Gly L225 (Table I). The 1650 cm^{-1} band detected in the $P^+Q_AQ_B^-/PQ_AQ_B$ spectrum of *Rps. viridis* RC could thus be associated with a conformational change of the protein near Q_B, and possibly at the peptide C=O of Gly L225.

The present FTIR work on *Rps. viridis* and *Rb. sphaeroides* RCs reveals that molecular vibrational changes associated with the photoreduction of Q_A or Q_B in vivo can be interpreted in terms of a conformational change of the protein occurring at the Q_A binding site (in both *Rps. viridis* and *Rb. sphaeroides* RCs) and at the Q_B site (only in *Rps. viridis* RC). Moreover, our FTIR data correlate well with X-ray structural models of RC which, at the present stage of refinement, show differences in the interactions of the protein with Q_A and Q_B in *Rps. viridis* [5] and *Rb. sphaeroides* RCs [4]. In addition, while the X-ray approach has not yet provided a picture of a charge-separated state, FTIR difference spectroscopy has proven to be able to detect small changes of structure. These highly localized conformational changes which probably play a key role in assisting the stabilization of the separated charges, may constitute a general process in primary reactions of photosynthesis.

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