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Characterization by FTIR spectroscopy of the photoreduction of the primary quinone acceptor Q_A in photosystem II

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Molecular changes associated with the photoreduction of the primary quinone acceptor Q_A of photosystem II have been characterized by Fourier transform infrared spectroscopy. This reaction was light-induced at room temperature on photosystem II membranes in the presence of hydroxylamine and diuron. A positive signal at 1478 cm^{-1} is assigned to the C...O stretching mode of the semiquinone anion, and can be correlated to the negative C=O mode(s) of the neutral Q_A at 1645 cm^{-1} and/or 1630 cm^{-1}. Analogies with bacterial reaction center are found in the amide I absorption range at 1672 cm^{-1}, 1653 cm^{-1} and 1630 cm^{-1}. The stabilization of Q_A^- does not result from a large protein conformation change, but involves perturbations of several amino acid vibrations. At 1658 cm^{-1}, a negative feature sensitive to ^1H-^2H exchange is tentatively assigned to a δNH_2 histidine mode, while tryptophan $D_2$252 could contribute to the signal at 1560/1550 cm^{-1}.

Fourier transform infrared difference spectroscopy; Photosystem II; Primary quinone acceptor; Plastoquinone

1. INTRODUCTION

In photosystem II (PS II), the charge separation occurs between the chlorophyllic primary electron donor P_{680} and the primary electron acceptor Q_A (plastoquinone-9, PQ-9) via a pheophytin. This electron pathway presents strong similarities with that characterized in reaction centers (RCs) of purple bacteria [1]. Q_A is in both systems a single electron carrier tightly bound to the protein. Q_A reduction leads to an unprotonated semiquinone anion Q_A^-. In both bacterial RCs and PS II, the electron transfer between Q_A and the secondary electron acceptor Q_B can be blocked by lowering the temperature or by addition of specific inhibitors. Similarities are also found between the amino acid sequences of the polypeptides L and M of bacterial RC and the two homologous polypeptides D_1 and D_2 present in the smallest unit of isolated PS II. In particular, most of the amino acids of the polypeptide M present around Q_A in bacteria are conserved in the polypeptide D_2 in PS II, suggesting structural similarities of the Q_A environment both in bacterial and in plant RCs [2,3]. The three-dimensional picture of the hydrophobic binding pocket of Q_A in bacterial RCs has been provided by X-ray crystallography studies [4,5].

In previous work, structural changes associated with photochemical reactions (primary donor photooxidation and intermediary acceptor photoreduction) have been studied by light-induced Fourier transform infrared (FTIR) difference spectroscopy in bacterial RCs [6-10] and plant photosystems [11-13]. The sensitivity of the method is high enough to detect molecular changes at the level of single bonds against a large absorption background. In the present work, we have investigated the changes in the interactions of Q_A with its binding site and more distant perturbations of aminoacids, which occur upon photoreduction of Q_A in PS II.

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Abbreviations: FTIR, Fourier transform infrared; PS II, photosystem II; RC, reaction center; *Rps., Rhodopseudomonas; Rb., Rhodobacter*; Q_A, primary quinone acceptor; P_{680}, primary electron donor; BBY, PS II-enriched membranes; Mes, 2-(*N*-morpholino)ethanesulphonic acid; Tris, tris(hydroxymethyl)aminomethane; DCMU, diuron, 3-(3,4dichlorophenyl)-1,1-dimethylurea; Chl, chlorophyll; Tyr_Z, secondary electron donor tyrosine $D_1$161; PQ-9, plastoquinone-9; 3-MBQ, trimethylbenzoquinone; THF, tetrahydrofuran

2. MATERIALS AND METHODS

Spinach PS II-enriched membranes (BBY) were prepared according to [14] with modifications described in [15] and resuspended in 10 mM Mes pH 6.5, 10 mM NaCl, 7 mM NH_2OH and 0.1 mM DCMU, incubated for 10 min at 4°C and centrifuged for 30 min at 30 000 × *g*. The FTIR sample consisted in a fraction of the final pellet pressed between two CaF_2 windows. For deuteration experiments, the pellet was resuspended in ^2H_2O with 7 mM NH_2OH and 0.1 mM DCMU at a Chl concentration of about 2 mg/ml, then incubated at 6°C for 15 h and centrifuged for 40 min at 30 000 × *g*. All preparation steps were performed in complete darkness. FTIR spectra were recorded on a Nicolet 60 SX FTIR spectrometer equipped with an MCT-A detector. A germanium filter placed before the sample prevented the actinic effect of the He-Ne laser colinear with the IR beam. Single beam spectra were recorded at 15°C before and after illumination (1 s) at $\lambda > 645$

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nm. The light-induced difference spectra were calculated from the ratio of these two single beam spectra. Maximum absorbance in the amide I region was ≈0.9 (H_2O samples) and ≈0.7 (^2H_2O samples) absorbance units. In order to improve signal to noise ratio, difference spectra obtained from several samples were averaged.

3. RESULTS

The light-induced FTIR difference spectra of dark-adapted BBY membranes in the presence of NH_2OH and DCMU are presented in Fig. 1a (H_2O) and 1b (^2H_2O) and are denoted Q_A^-/Q_A spectra. Under these experimental conditions, DCMU inhibits the electron transfer from Q_A^- to Q_B, while the exogenous electron donor NH_2OH, which reduces P_{680}+via its physiological donor tyrosine Tyr_Z, excludes any contribution of Tyr_Z^+ and P_{680}^+ [16] to the light-induced FTIR difference spectra. Although no relaxation was observed one hour after illumination, a slow relaxation process was taking place for samples maintained at 6°C in the dark overnight.

In the Q_A^-/Q_A spectrum, negative bands are related to the neutral disappearing Q_A state, the positive ones to the semiquinone anion Q_A^- state. The small magnitude of the signals observed in the amide I region indicates that no large conformation change of the protein backbone occurs upon photoreduction of Q_A in PS II. All the features observed in the Q_A^-/Q_A spectra are also present in other spectra obtained under experimental conditions where photoreduction of Q_A occurs in the absence of NH_2OH. More specifically, all the bands that will be discussed are present in the Cyt

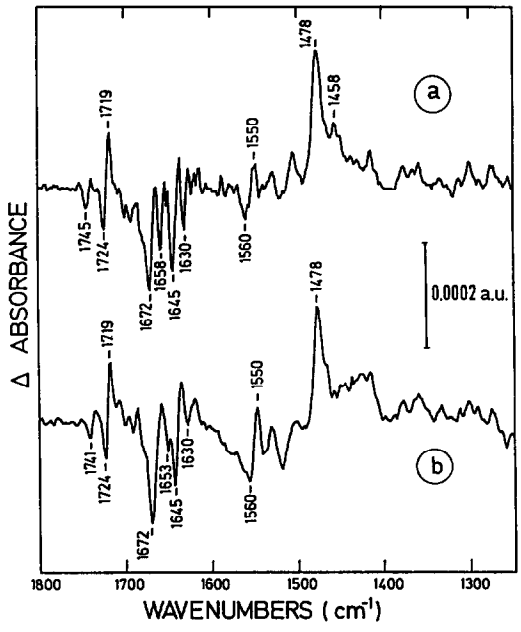

Fig. 1. Light-induced FTIR difference spectra of BBY particles in the presence of NH_2OH and DCMU, (a) in H_2O and (b) in ^2H_2O. For each single beam spectrum, 512 scans (90 s) were accumulated. Each spectrum results from the averaging of 10 different samples. 4 cm^{-1} resolution, 15°C.

$b559^+Q_A^-$/Cyt b559Q_A [17] and $Chl^+Q_A^-$/ChlQ_A (C. Berthomieu, unpublished data) spectra obtained at low temperature as well as in $S_3Q_A^-/S_2Q_A$ spectra obtained at ambient temperature on Ca^{2+}-depleted BBY (C. Berthomieu and A. Boussac, unpublished data). We thus exclude any contribution of NH_2OH to the signals which will be discussed in the following.

The majority of the features observed in Fig. 1a are unchanged upon deuteration as shown in Fig. 1b, in particular at 1724/1719 cm^{-1}, 1672 cm^{-1}, 1653 cm^{-1}, 1645 cm^{-1}, 1630 cm^{-1} and 1478 cm^{-1}. The main difference after deuteration consists of the disappearance of the negative band at 1658 cm^{-1}. A frequency downshift is also observed for the signal at 1745 cm^{-1} (to 1741 cm^{-1}) and for the positive band at 1458 cm^{-1} (to ≈1430 cm^{-1}).

4. DISCUSSION

The Q_A^-/Q_A spectrum reveals the effects that Q_A reduction produces both on the vibrational modes of the quinone itself and of the protein backbone and/or amino acid sidechains. Tentative assignments are made by comparison with IR studies of model compounds of quinones and semiquinone anions and with data obtained on bacterial RCs.

4.1. *Comparison of quinone modes in vitro and in vivo*

Infrared absorption spectra (Fig. 2) of PQ-9 and its closely related model compound trimethylbenzoquinone (3-MBQ) show that the C=O stretching mode appears around 1650 cm^{-1} for quinones, in tetrahydrofuran and in ethanol (Table I). For asymmetrically substituted quinones, a splitting of the C=O band has been previously observed [18–20]. For PQ-9, an asymmetry is induced by the isoprenoid chain (and for 3-MBQ, by a methyl group) and a splitting of the C=O absorption band is expected and could correspond to the shoulder observed at ≈1636 cm^{-1} (Fig. 2, Table I) in the different solvents. The C=C absorption mode of the quinones is observed between 1616 cm^{-1} and 1619 cm^{-1}, with an absorption coefficient corresponding to about one third of the C=O band absorption coefficient. From IR and Raman studies of semiquinone anion radicals, an absorption mode in the 1510–1475 cm^{-1} range has been reported as characteristic of the semiquinone C...O stretching mode [21–23]. More specifically, IR spectroelectrochemical studies of ubiquinone-0 [24], show that upon formation of the semiubiquinone anion radical, the C=O (at 1660 cm^{-1}) and C=C (at 1606 cm^{-1}) absorption bands of the neutral form disappear while positive contributions related to C...O and C...C modes of the semiquinone anion appear at 1500–1440 cm^{-1}.

If we assume the same effect of the reduction of quinones on their vibrational modes in vitro and in vivo, we would expect in Fig. 1 negative Q_A bands

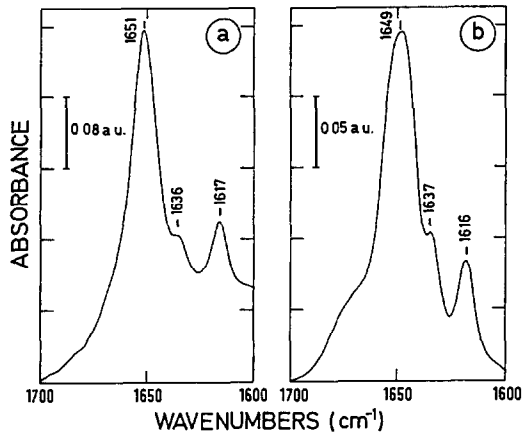

Fig. 2. FTIR absorption spectra of (a) plastoquinone 9 and (b) trimethylbenzoquinone in tetrahydrofuran, 4 cm^{-1} resolution.

around 1650–1630 cm^{-1} (C=O), 1620–1610 cm^{-1} (C=C) and positive contributions from Q_A^- at 1510–1440 cm^{-1}. In the 1620–1610 cm^{-1} range, only very small signals are observed that could be related to the Q_A C=C stretching mode. One of the largest signals of the Q_A^-/Q_A spectra is a positive one at 1478 cm^{-1} (Fig. 1). Comparison with model compounds leads us to assign this signal to the C...O stretching mode of Q_A^- in PS II. However, it should be kept in mind that the structural properties of the neutral quinone and of the semiquinone anion might be very different in vivo and in vitro, for example with a different charge repartition on the aromatic cycle.

In the 1690–1630 cm^{-1} C=O absorption region, which also corresponds to the amide I region, four negative bands are observed at 1672 cm^{-1}, 1658 cm^{-1}, 1645 cm^{-1} and 1630 cm^{-1} as well as a shoulder at 1653 cm^{-1} (Fig. 1a). The 1658 cm^{-1} band, which disappears upon ^1H-^2H exchange (Fig. 1b), is the only band affected by deuteration in this region. Deuteration is not expected to affect quinone carbonyls, since their frequencies are comparable in both deuterated and non-deuterated solvents (Table I, see also [24]). A contribution of Q_A C=O at 1658 cm^{-1} is thus excluded. The 1645 cm^{-1} and 1630 cm^{-1} bands observed in vivo are close to the C=O frequencies obtained in vitro for PQ-9 and 3-MBQ (Table I). These bands are thus good candidates for the Q_A C=O mode(s). A unique mode for the two C=O of Q_A at 1645 cm^{-1} or at 1630 cm^{-1} might be envisioned. However, the splitting of the C=O mode due to the intrinsic asymmetry of Q_A could be further enhanced by the anisotropy of its environment in vivo. Such an effect could lead to contributions from the two quinone C=O at 1645 cm^{-1} and 1630 cm^{-1} in the Q_A^-/Q_A FTIR difference spectrum. The 1672 cm^{-1} band (Fig. 1) has a significantly high frequency compared to the PQ-9 C=O modes observed in vitro. If we cannot rule out that the peculiar environment of Q_A could explain such an extreme upshift of the quinone C=O frequency, a more likely possibility is that this signal corresponds to the peptide C=O of an amino acid.

4.2. *Comparison between plants and bacteria*

Several approaches have been developed to investigate the vibrational contributions of the quinones and their binding sites in bacterial RCs, including steady state [25–27] and time resolved [28] light-induced FTIR spectroscopy as well as spectroelectrochemistry of the cofactors in vitro [24] and in vivo [29]. The three signals observed in the C=O region at 1670 cm^{-1}, $\approx$1650 cm^{-1} and 1630 cm^{-1} for bacterial RCs have been correlated to the Q_A state while signals at around 1470–1420 cm^{-1} were correlated to the Q_A^- state. Signals assigned to amino acid contributions were also observed in the amide II region at 1555 cm^{-1} and at 1533 cm^{-1}. However, definite assignment of the C=O modes of the neutral Q_A has not been achieved yet [24,26–28].

Analogies are found between the light-induced difference spectra of PS II and bacterial RCs, in particular, in the C=O frequency region where negative signals are observed for PS II at 1672 cm^{-1}, 1653 cm^{-1} and 1630 cm^{-1} (Fig. 1) and for bacterial RCs at 1670 cm^{-1}, $\approx$1650 cm^{-1} and 1630 cm^{-1} [27–29]. Contributions at these frequencies of amino acids conserved in plants and bacteria appear thus probable, X-ray data on both *Rb. sphaeroides* and *Rps. viridis* RCs have shown that one of the C=O of Q_A is within hydrogen bonding distance to the peptide NH group of Ala M260 (*Rb. sphaeroides*) or Ala M258 (*Rps. viridis*). The negative signal at 1650 cm^{-1} observed in the

Table I

FTIR absorption frequencies (cm^{-1}) of plastoquinone-9 (PQ-9) and trimethylbenzoquinone (3-MBQ) in different solvents and in the solid state, at 4 cm^{-1} resolution. *str.*, stretching; *sh*, shoulder; *w*, weak.

Quinone		THF	T^2HF	$CH_3CH_2O^2H$	Solid
PQ-9	C=O *str.*	1652	1651	1650	1643
	sh	1636	1636	1634	1632*w*
	C=C *str.*	1617	1618	1616	1614
3-MBQ	C=O *str.*	1649	1650	1651	
	sh	1637	1635	1634	
	C=C *str.*	1619	1619	1619	

$P^+Q_A^-/PQ_A$ difference spectra of *Rb. sphaeroides* and *Rps. viridis* RCs has been tentatively assigned to a localized conformational change of the protein backbone at this conserved alanine residue [26,27]. Alignment of the amino acid sequences of M involved in the Q_A binding pocket with homologous fragments of D_2 [2,3] as well as modelling experiments also suggest interactions between one of the PQ-9 carbonyl groups and a peptide NH group of either Ala $D_2$261 (equivalent of Ala M258 in *Rps. viridis*) [2] or Phe $D_2$262. The small negative band at 1653 cm^{-1} in the Q_A^-/Q_A spectrum of PS II (Fig. 1a and 1b) which is even better resolved in other difference spectra where Q_A reduction occurs (see Results) could correspond to this conserved interaction between Q_A and the protein.

The presence of an additional band at 1645 cm^{-1} in PS II marks a difference between bacterial RCs and PS II spectra (Fig. 1). The well-defined positive band at 1478 cm^{-1} in the Q_A^-/Q_A spectra of PS II is also absent in data obtained for Q_A^- in *Rb. sphaeroides* RC [28,29]. We thus tentatively assign this signal to the C...O mode of Q_A^- and the negative band at 1645 cm^{-1} to the correlated C=O mode of Q_A in PS II.

X-ray studies on bacterial RCs also show that several sidechains of amino acid residues interact with Q_A, such as Trp M250 and Phe M249 in *Rps. viridis* [4] (Trp M252 and Phe M251 in *Rp. sphaeroides* [5]) and thus may be affected by Q_A reduction. In *Rps. viridis*, the second quinone carbonyl is within hydrogen bonding distance with the imidazole side chain of His M217 [4] (and in *Rp. sphaeroides* with Thr M222 side chain). These residues (conserved in PS II as His $D_2$215, Phe $D_2$253 and Trp $D_2$254) may lead to amino acid signals in the Q_A^-/Q_A spectrum. The 1658 cm^{-1} band, which disappears in the Q_A^-/Q_A spectrum in ^2H_2O could reflect the change of a primary amide NH_2 scissoring mode such as δNH_2 of an histidine residue [30]. A negative signal at 1658 cm^{-1} also appears during Q_A photoreduction in *Rps. viridis* RC [25,27]. This negative band could reflect a change in the Q_A-His M217 interaction due to Q_A reduction. This interaction appears conserved between Q_A and His $D_2$215 in PS II [2,3]. It is interesting to note that according to X-ray data, this interaction does not seem to occur in *Rb. sphaeroides* [5], in agreement with the absence of a negative band at 1658 cm^{-1} in the spectra obtained for *Rb. sphaeroides* RCs [26–29].

Above 1700 cm^{-1}, two signals at 1724/1719 cm^{-1} and 1745 cm^{-1} lay in the frequency range of the protonated carboxylic group of aspartic and glutamic side chains. X-ray data on both *Rps. viridis* and *Rb. sphaeroides* show that such residues are absent within a distance of 8 Å from Q_A. However we cannot exclude that perturbations of amino acids at rather long distances from Q_A could also contribute to the FTIR Q_A^-/Q_A spectrum. Still another possibility would be the involvement of the 10_a C=O ester of the intermediatry acceptor pheophytin [12], as suggested for signals observed in the same range concomitant with Q_A reduction in bacterial RCs [29].

A differential feature is observed at 1560/1550 cm^{-1} in Fig. 1, where contribution of the quinone itself is not expected [18–24]. This signal could originate from the NH peptide bending mode of an amino acid in interaction with Q_A and would then be correlated to the change in the amide I region previously discussed for the 1672 cm^{-1} and 1653 cm^{-1} bands. Another possibility is the contribution of an amino acid side chain such as the indole ring vibration of a tryptophan residue (1545–1557 cm^{-1}, [31]). X-ray data for bacterial RCs have shown that Trp M250 in *Rps. viridis* (Trp M252 in *Rb. sphaeroides*) bridges the π electron system of the bacteriopheophytin H_A and of Q_A [4,5]. Its aromatic ring is approximately parallel to the quinone ring, at a distance of $\approx$3 Å and makes van der Waals contacts to both Q_A and Phe M249 [3,5]. A superexchange mechanism involving Trp M250 has been proposed for the electron transfer between H_A and Q_A [32]. These tryptophan and phenalanine residues are conserved in PS II suggesting the conservation of interactions between the delocalized electrons of Trp $D_2$254 side chain and the quinone aromatic cycle [2]. The presence of a negative charge on the Q_A ring is likely to alter the tryptophan indole ring mode, which could in turn lead to the appearance of the 1560/1550 cm^{-1} FTIR difference signal (Fig. 1). The use of site-directed mutagenesis on this residue may help clarify this interpretation.

In conclusion, the relative complexity of the Q_A^-/Q_A spectra compared to reduction of model compounds demonstrates that the reduction of Q_A affects the character of several bonds. In fact, we observe not only contributions from the neutral quinone carbonyls at 1645 cm^{-1} and/or 1630 cm^{-1} and the semiquinone anion C...O stretching at 1478 cm^{-1}, but also from several amino acid peptide and sidechain modes. The stabilization of the semiquinone anion does not result from a large protein conformation change, since the signals observed in the amide I region could correspond to the localized alteration of at most 2–3 peptide bonds, but results from several small perturbations in the Q_A binding pocket. The presence of negative signals at 1672 cm^{-1} and $\approx$1650 cm^{-1} in both bacteria and plants suggests a strong structural similarity between the Q_A pocket of both organisms.

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