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Infrared vibrational modes of histidine ligands of the primary electron donor and of Q_A in purple photosynthetic bacteria

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Abstract: Histidine isotopically labeled with ^{13}C have been incorporated in the RC of *Rb. sphaeroides* and the light-induced FTIR difference spectra for the oxidation of the primary donor P and the reduction of the quinone Q_A have been measured. The isotope effect on the pure Q_A^-/Q_A spectra provides IR signatures of the His modes that are very comparable to those previously reported for Q_A reduction in photosystem II. Comparison with published model compounds spectra indicates that the imidazole ring of the M219 His side chain is strongly hydrogen bonded via its Nπ site, in agreement with the RC crystallographic model. In the $P^+Q_A^-/PQ_A$ FTIR spectra, the contribution of Q_A reduction on the His vibrations is dominated by that of P oxidation. The large isotope signals from the P^+/P species observed in the 1140-1090 cm^{-1} range are assigned for a major part to the perturbation upon P photooxidation of the ring mode of the His serving as axial ligands to the Mg atom of the bacteriochlorophylls in the primary donor.

Introduction

The crystal structure of the reaction center (RC) of photosynthetic purple bacteria provides essential information on the organization of the cofactors within the core of the protein scaffold which is made of the two homologous and highly symmetrical L and M subunits (for reviews, see Deisenhofer and Michel, 1989; Feher et al., 1989; Lancaster et al., 1995; Fyfe and Jones, 2000). For example, the crystal structure of *Rhodopseudomonas* (*Rp*.) *viridis* RCs shows that each of the two overlapping bacteriochlorophyll (Bchl) molecules constituting the primary electron donor (P) has the central Mg atom at the proper distance to have the imidazole ring of a His side chain as axial ligand. In the case of P870, the primary donor of *Rhodobacter* (*Rb*.) *sphaeroides*, His M202 and His L173 have been proposed to be coordinated to the central Mg atom of P_M and P_L, respectively, although the distance appears significantly larger on the L-side than on the M-side. The only other bonding interaction between P870 and the protein described in the crystal structure also involves a His side chain (L168) that would form a hydrogen bond with the 2a-acetyl C=O group of P_L. Similarly, the carbonyls of the primary quinone acceptor Q_A have been reported to be in hydrogen bonding interaction with a peptide NH on one side and a His side chain (M219) on the other. The crystal structure has also been highly instrumental in allowing mutagenesis to be directed at the potentially most interesting sites (for reviews, see Coleman and Youvan, 1990; Woodbury and Allen, 1995; Okamura and Feher, 1995). However, many details regarding the electronic structure, the conformation, and the bonding interactions of the cofactors involved in the charge

separation, as well as of their changes upon electron transfer are generally not directly accessible from the structure. Such parameters are important in order to relate structure and function at a molecular level. Therefore, various techniques of optical and magnetic resonance spectroscopy are currently used in an attempt to retrieve some of this missing information. Among these techniques, light-induced FTIR difference spectroscopy is well suited to probe the vibrational properties, and thus the close molecular environment, of the various chemical groups involved in the charge separation process.
In the case of the charge separation between P870 and the primary quinone acceptor Q_A, the FTIR difference spectra (denoted $P^+Q_A^-/PQ_A$) have been compared to those obtained upon electrochemical oxido-reduction of model Bchl and quinone compounds in solution (for reviews, see Mäntele,1995; Nabedryk, 1996; Breton and Nabedryk, 1996). These spectra appear largely dominated by vibrational modes of the Bchl pigments together with less intense contributions from the quinone and even smaller ones from the protein. IR modes of the carbonyl groups of P870 have been identified by comparison with model compound spectra, by analyzing the effect of mutations directed at sites that could modify the interaction of a side chain with the Bchl, and by measuring the FTIR difference spectra for $P870^+$ and $^3P870$ formation. The quinone vibrational modes have been analyzed in photochemically induced pure Q_A^-/Q_A FTIR difference spectra and identified by the use of isotopic labeling. The contribution from the protein in these spectra, however, has been much more elusive and only very tentative assignments have been proposed so far.
In the present work, we describe the effect of incorporating ^{13}C labeled His amino acids in *Rb. sphaeroides* on the Q_A^-/Q_A and $P^+Q_A^-/PQ_A$ FTIR difference spectra and we identify several His modes contributing to these spectra, notably around 1100 cm^{-1}.

Materials and methods

Cells from the strain GR 0229 of *Rb. sphaeroides* (Choudhary et al., 1996) that is deficient for His synthesis (a kind gift of S. Kaplan) were grown initially under chemoheterotrophic conditions in Siström minimal medium supplemented with unlabeled His (^{12}C His). At the onset of the stationary phase, the cells were transferred to the same medium containing either ^{12}C His or uniformly ^{13}C labeled His ($u^{13}C_6$ His; 97-98% ^{13}C; Cambridge Isotope Laboratories) at a concentration of 10 µg per mL and were grown under photoheterotrophic conditions. Chromatophores were prepared by French press treatment and purified by centrifugation. RCs were isolated with LDAO under standard conditions. Only one culture with either unlabeled His or $u^{13}C$ His was made.
IR films were prepared by drying a suspension of chromatophores in deionized water in the presence of the Q_B inhibitor stigmatellin on CaF_2 windows under a gentle stream of argon. The RCs samples used for measuring the Q_A^-/Q_A FTIR difference spectra at 283K were prepared under standard conditions and were resuspended in Tris buffer (50 mM; pH 7) containing stigmatellin (1 mM), ferrocyanide (250 mM), ascorbate (10 mM), and the mediator TMPD (50 mM).

Results and discussion

Q_A^-/Q_A FTIR difference spectra:
The light-induced Q_A^-/Q_A FTIR difference spectra recorded at 283K on RCs of *Rb. sphaeroides* are shown for samples unlabeled (fig.1a) and $u^{13}C$ His labeled (fig. 1b). The unlabeled-minus-$u^{13}C$ His double-difference spectrum is also presented (fig. 1c).

Pronounced isotopic effects are reproducibly observed, notably around 1670 and 1640 cm^{-1} in the Amide I region and around 1550 cm^{-1} in the Amide II range. These IR signals are to be assigned to peptide modes involving most probably His M219, which

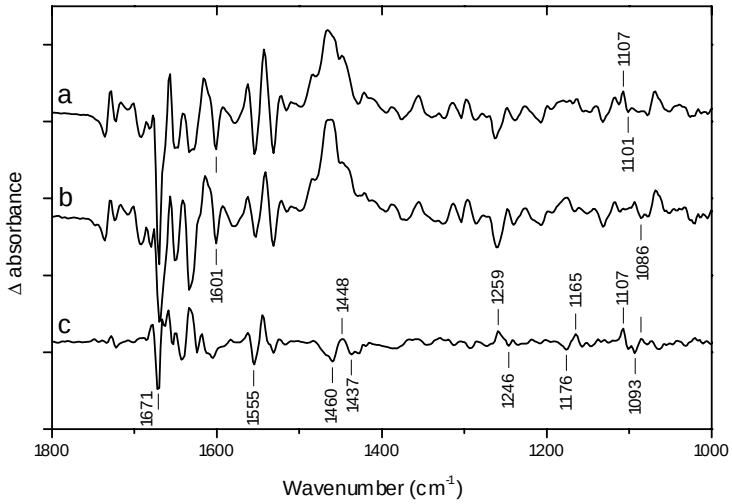

Figure 1. Light-induced Q_A^-/Q_A FTIR difference spectra at 283K of *Rb. sphaeroides* RCs with (a) unlabeled ^{12}C His and (b) u-^{13}C His. (c) double-difference spectrum ^{12}C His minus u-^{13}C His.

is in strong hydrogen bonding interaction with Q_A, and possibly one or more of the three additional His residues the side chains of which are coordinating the non-heme iron atom located between Q_A and Q_B.

Isotopic effects of smaller amplitude are also detected around 1450, 1250, 1170, and 1100 cm^{-1} (Fig. 1). The IR frequency of these bands corresponds to modes that have been previously assigned to imidazole ring vibrations based upon comparison with spectra of model compounds (Berthomieu et al., 1992; Noguchi et al., 1999a), normal mode analysis (Majoube et al., 1995), and isotope labeling. For example, His bands around 1100 cm^{-1}, ascribed predominantly to an imidazole C-N stretching mode (Majoube et al., 1995), have been detected in photosystem II upon (i) oxidation of cytochrome b_{559} and of the related model compound bis(4-methylimidazole) complex of Fe protoporphyrin IX (Berthomieu et al., 1992), (ii) oxidation of the non-heme iron atom (Hienerwadel and Berthomieu, 1995; Berthomieu and Hienerwadel, 2001), (iii) S_1 to S_2 transition of the oxygen–evolving complex (Noguchi et al. 1999b), as well as (iiii) reduction of Q_A (Noguchi et al. 1999a). In the latter case, it is notable that a negative His mode at 1102 cm^{-1} has been found to upshift to 1109 cm^{-1} upon Q_A^- formation in *Synechocystis* 6803 core PSII particles. A differential signal, negative at 1101 cm^{-1} and positive at 1107 cm^{-1} is observed at almost the same frequency in the Q_A^-/Q_A FTIR difference spectra of the unlabeled *Rb. sphaeroides* RC sample (fig.1a). A very large fraction, if not all, of this differential signal downshifts by about 15 cm^{-1} to 1093/1086 cm^{-1} upon labeling (fig.1b) giving rise to the clearest isotopic signature in this spectral range of the double-difference spectrum (fig.1c). We therefore assign the 1101/1107 cm^{-1} differential feature in fig.1a to a predominantly CN stretching mode of His M219 that is perturbed upon Q_A reduction. Several other differential signals in the double-difference spectrum (fig.1c), notably at 1460/1448/1437, 1259/1246, and 1176/1165 cm^{-1}, have clear counterparts in the double-difference spectrum obtained for Q_A reduction in PSII containing His labeled with ^{15}N in the imidazole ring (Noguchi et al.,1999a) although the difference in the isotopic label precludes a more detailed comparison. Nevertheless, the unlabeled His bands observed

at 1101 and 1176 cm^{-1} (fig.1c) are diagnostic of a side chain protonated at the Nπ site and strongly hydrogen bonded (Majoube et al., 1995; Noguchi et al, 1999a). This observation is consistent with the crystallographic model of the RC showing the non-heme iron coordinated at the Nτ site as well as with the evidence for a hydrogen bond with the C_4=O carbonyl of Q_A (Breton et al. 1994).

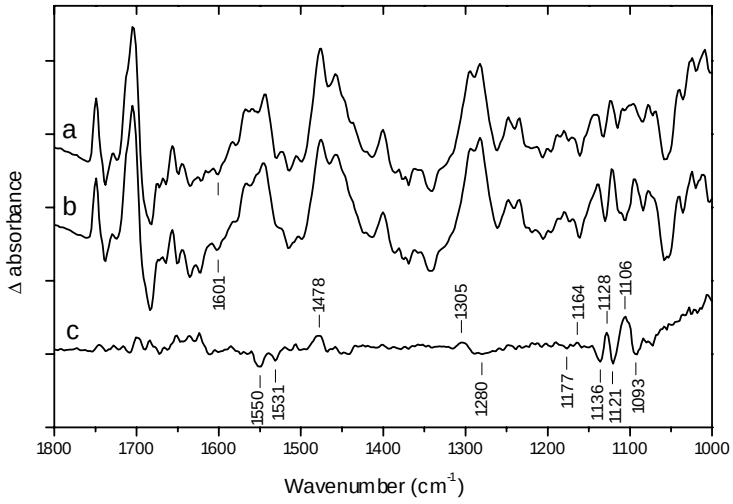

Figure 2. $P^+Q_A^-/PQ_A$ FTIR difference spectra at 283K of *Rb. sphaeroides* chromatophores with (a) unlabeled ^{12}C His and (b) u-^{13}C His. (c) double-difference spectrum ^{12}C His minus u-^{13}C His.

$P^+Q_A^-/PQ_A$ FTIR difference spectra:

The light-induced $P^+Q_A^-/PQ_A$ FTIR difference spectra recorded at 283K on films of *Rb. sphaeroides* chromatophores are shown for samples unlabeled (fig.2a) and u^{13}C His (fig. 2b). For most of the spectral range between 1800 and 1000 cm^{-1}, only small features that could be assigned to isotope effects are reproducibly detected in the unlabeled-minus-u^{13}C His double-difference spectra (fig. 2c) calculated from several pairs of sample. These features include, notably, a double trough at 1550 and 1531 cm^{-1}, a positive band at 1478 cm-1 and a broad differential signal, positive at around 1305 cm^{-1} and negative at about 1280 cm^{-1}. The clearest signature for the His isotope effect is a complex differential signal with three negative lobes at 1136, 1121, and 1093 cm^{-1}, and two positive ones at 1128 and 1106 cm^{-1}. The amplitude of the signals observed below 1200 cm^{-1} in the double-difference spectrum (fig.2c) is comparable to that of the bands directly observed in the parent $P^+Q_A^-/PQ_A$ FTIR difference spectra (fig.2a,b). In contrast, all the other signals observed above 1200 cm^{-1} in the double-difference spectrum correspond to small bands that are buried under much larger ones in the parent spectra. This is notably the case for the region under the Amide I absorption band around 1660 cm^{-1} where no clear signature of His labeling could be recognized in the parent spectra. In order to check whether the signals observed between 1600 and 1200 cm^{-1} in the double-difference spectrum can be confidently interpreted in terms of genuine His isotope effects or have to be ascribed to some fluctuations of the main bands in the $P^+Q_A^-/PQ_A$ spectra, we have changed the state of the sample by performing the light-induced FTIR measurements at low temperature and by comparing dried films of chromatophores and isolated RCs prepared as wet samples. The light-induced $P^+Q_A^-/PQ_A$ FTIR difference spectra recorded at 100K on films of *Rb. sphaeroides* chromatophores are shown for samples unlabeled (fig.3a) and u^{13}C His (fig. 3b) together with the double-difference unlabeled-minus-u^{13}C His (fig. 3c).

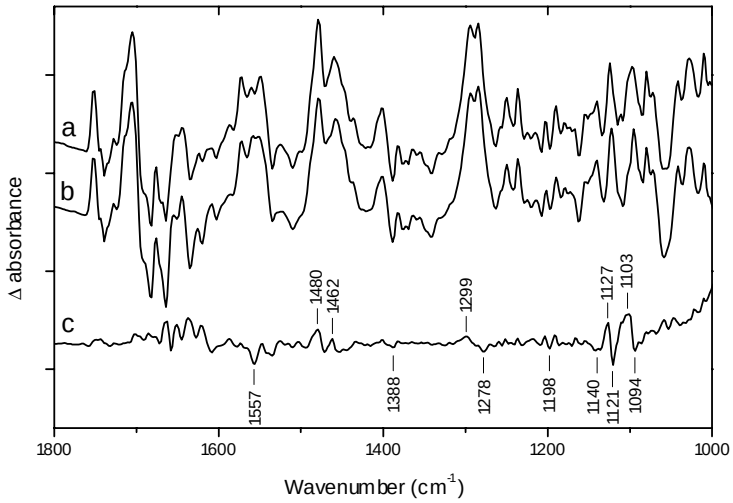

Figure 3. $P^+Q_A^-/PQ_A$ FTIR difference spectra at 100K of *Rb. sphaeroides* chromatophores with (a) unlabeled ^{12}C His and (b) u-^{13}C His. (c) double-difference spectrum ^{12}C His minus u-^{13}C His.

Equivalent light-induced spectra measured for the samples of isolated RCs that had been previously used to record Q_A^-/Q_A spectra at 283K and that had been cooled to 100K in the dark are depicted in figures 4a-c. Although several bands in the $P^+Q_A^-/PQ_A$ spectra are quite different for chromatophores at 283K (fig.2a) and at 100K (fig 3a) or for isolated RCs at 100K (fig 4a), all the double-difference spectra show common features. This is clearly the case for the complex differential signal between 1140 and 1090 cm^{-1} and is also observed for the signals centered around 1280, 1480, and 1550 cm^{-1}. Therefore these bands can be confidently assigned to His modes.

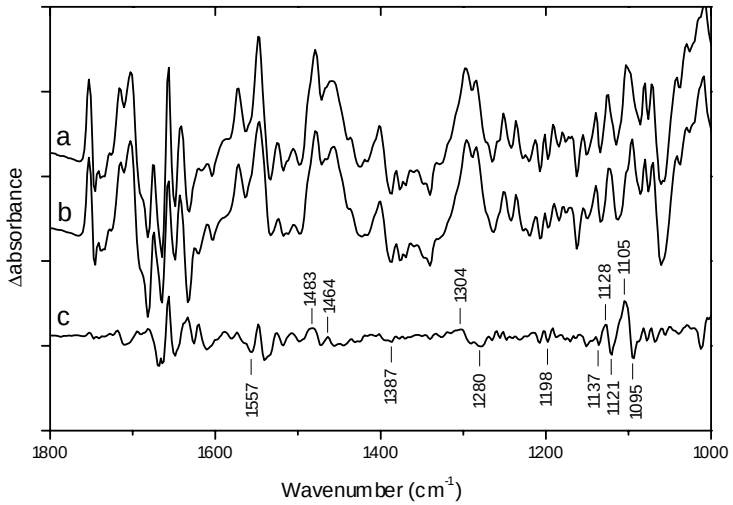

Figure 4. $P^+Q_A^-/PQ_A$ FTIR difference spectra at 100K of *Rb. sphaeroides* RCs with (a) unlabeled ^{12}C His and (b) u-^{13}C His. (c) double-difference spectrum ^{12}C His minus u-^{13}C His.

While the isotope effect on the Q_A^-/Q_A FTIR double-difference spectrum (fig. 1c) should contribute to the isotope effect on the $P^+Q_A^-/PQ_A$ spectrum (fig. 2c), it is not straightforward to separate the two. In order to analyze their relative contribution, the spectra in 1a and 2a were normalized (data not shown) on the isolated band at 1601 cm^{-1}, which has been assigned to one of the C=O modes of Q_A (Breton et al., 1994). Taking this normalization into account, the contribution to the spectrum in 2c of the

His modes affected by the reduction of Q_A can only be detected as small signals at 1670, 1550, and 1177-1164 cm^{-1}. The remaining His bands, and most notably those giving rise to the complex signal between 1140 and 1090 cm^{-1} (fig. 2c, 3c, 4c), have their origin in the photooxidation of P870. While a contribution from the His residue (L168) in hydrogen bonding interaction with the 2a-acetyl group of P_L is possible, the major contribution is expected to arise from the ligation of the central Mg atoms of P_M and P_L by the His side chains M202 and L173, respectively. This result is in line with the observation of a pronounced coupling between Mg-N_{his} stretching modes and doming modes of the Mg in the neutral state of the RC using resonance Raman spectroscopy (Czarnecki et al., 1997). The observation that modes from the axial His ligands of P870 contribute to the P^+/P spectra between 1140 and 1090 cm^{-1} also agrees with a preliminary analysis of the effect of mutations at the M202 and L173 sites on the FTIR difference spectra.

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