Photo-Oxidation of P740, the Primary Electron Donor in Photosystem I from *Acaryochloris marina*

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ABSTRACT Fourier transform infrared spectroscopy (FTIR) difference spectroscopy in combination with deuterium exchange experiments has been used to study the photo-oxidation of P740, the primary electron donor in photosystem I from *Acaryochloris marina*. Comparison of ($P740^+$-P740) and ($P700^+$-P700) FTIR difference spectra show that P700 and P740 share many structural similarities. However, there are several distinct differences also: 1), The ($P740^+$-P740) FTIR difference spectrum is significantly altered upon proton exchange, considerably more so than the ($P700^+$-P700) FTIR difference spectrum. The P740 binding pocket is therefore more accessible than the P700 binding pocket. 2), Broad, "dimer" absorption bands are observed for both $P700^+$ and $P740^+$. These bands differ significantly in substructure, however, suggesting differences in the electronic organization of $P700^+$ and $P740^+$. 3), Bands are observed at 2727(−) and 2715(−) cm^{-1} in the ($P740^+$-P740) FTIR difference spectrum, but are absent in the ($P700^+$-P700) FTIR difference spectrum. These bands are due to formyl CH modes of chlorophyll *d*. Therefore, P740 consists of two chlorophyll *d* molecules. Deuterium-induced modification of the ($P740^+$-P740) FTIR difference spectrum indicates that only the highest frequency 13^3 ester carbonyl mode of P740 downshifts, indicating that this ester mode is weakly H-bonded. In contrast, the highest frequency ester carbonyl mode of P700 is free from H-bonding. Deuterium-induced changes in ($P740^+$-P740) FTIR difference spectrum could also indicate that one of the chlorophyll *d* 3^1 carbonyls of P740 is hydrogen bonded.

INTRODUCTION

Acaryochloris marina is a recently discovered oxygenic photosynthetic marine prokaryote (Miyashita et al., 1996). Unlike most oxygenic photosynthetic organisms that utilize Chl *a* for light-induced photochemistry, the major pigment found in *A. marina* is Chl *d* (Miyashita et al., 1997). See Fig. 1 for the structure of Chl *d*. In whole cells, the Chl *a*/Chl *d* ratio is 0.03:0.09 (Miyashita et al., 1997), and cells of the prokaryote display a far-red absorption band at ~714 nm that is due to Chl *d* (Miyashita et al., 1997). Recently a trimeric PS I complex has been isolated from *A. marina*. The PS I complexes contain ~180 Chl *d* molecules and <1 Chl *a* per complex (Hu et al., 1998). The flash-induced spectral properties of the PS I complex are consistent with a dimeric, Chl *d*-containing, primary donor species (called P740) that is similar to P700 of plants (Hastings, 2001; Hu et al., 1998). In addition, the redox properties of the acceptor side appear to be similar to PS I from plants (Hu et al., 1998). It has been suggested that there must be similarities in the structural organization of P700 and P740 (Hu et al., 1998); however, at present, little is known about the structural organization of P740 and its binding site. Very recently, it has been suggested that P740 contains Chl *d′* (Akiyama et al., 2002a,b, 2001). This observation strengthens possible analogies between P700 and P740, since P700 contains Chl *a′* (Fromme et al., 2001; Jordan et al., 2001).

In PS I particles from plants, algae, and cyanobacteria, the oxidized primary donor, $P700^+$, has a lifetime of several tens of milliseconds (ms) (Brettel, 1997; Golbeck and Bryant, 1991). In PS I particles from *A. marina*, the oxidized primary donor, $P740^+$, decays with a similar lifetime (Hastings, 2001; Hu et al., 1998). Therefore, under steady-state illumination, it is possible to photoaccumulate a substantial population of $P700^+$ and $P740^+$. Here we describe the photoaccumulation of $P740^+$ in isolated PS I particles of *A. marina*, and the generation of a ($P740^+$-P740) FTIR difference spectrum (DS).

Upon comparison of the ($P740^+$-P740) FTIR DS with the ($P700^+$-P700) FTIR DS obtained from cyanobacterial PS I particles, we show that P740 has many of the same molecular properties as P700; however, we also show some distinct differences. In particular, deuterium exchange impacts at least one of the ester carbonyl modes of P740 while no such effect is observed on the ester carbonyl modes of P700. This indicates that the P740 binding site is more accessible than the P700 binding site.

MATERIALS AND METHODS

For preparation of PS I particles from *Synechocystis* sp. 6803 (*S. 6803*), a mutant that lacks PS II was used, as described previously (Hastings et al., 1995a,b, 1994a,b, 2001). Growth procedures and preparation of membrane fragments from these PS II-less mutant cells from *S. 6803* have been described (Hastings et al., 1994a). PS I particles were prepared by incubating

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Abbreviations used: *A. marina*, *Acaryochloris marina*; Chl *d*, chlorophyll *d*; Chl *a*, chlorophyll *a*; BChl *a*, bacteriochlorophyll *a*; *C. reinhardtii*, *Chlamydomonas reinhardtii*; C=O, carbonyl; DS, difference spectra, spectrum, or spectroscopy; ET, electron transfer; H-bond, hydrogen bond; P_A, the chlorophyll *a′* molecule of P700 bound to *Psa*A; P_B, the chlorophyll *a* molecule of P700 bound to *Psa*B; PS I, photosystem I; P700, primary electron donor in PS I particles from *S. 6803*; P740, primary electron donor in PS I particles from *A. marina*; *Rb.*, *Rhodobacter*; *S.* 6803, *Synechocystis* sp. PCC 6803.

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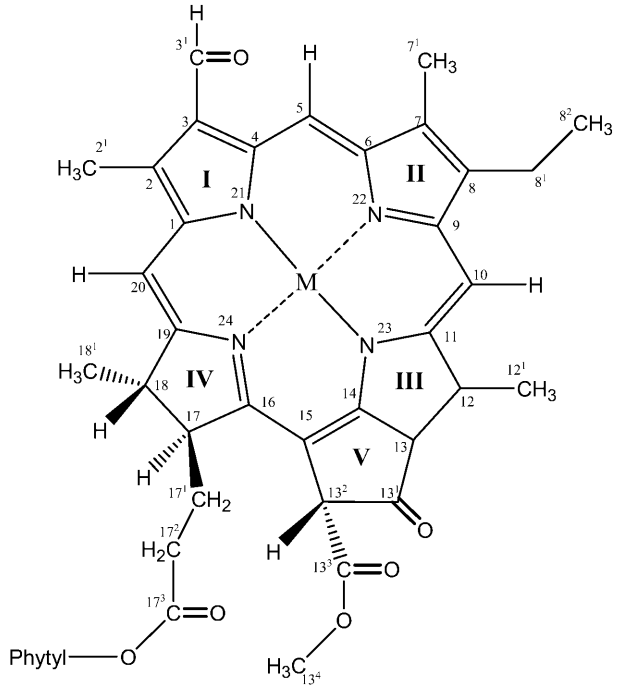

FIGURE 1 Molecular structure and IUPAC numbering scheme for Chl *d*. The 3^1, 13^1, and 13^3 carbonyls are referred to frequently throughout the text. For Chl *a*, the 3^1 formyl group is replaced by a vinyl group. Chl *d′* is a 13^2 epimer of Chl *d*. M = Mg.

membranes in 20 mM tris-HCl buffer (pH 8) containing 1% β-dodecyl maltoside, as described (Hastings et al., 1995a). Membrane debris was removed by centrifugation and the supernatant was loaded onto an 8–32% sucrose gradient containing 0.03% β-dodecyl maltoside and 10% glycerol, as described (Hastings et al., 1995a). After ultracentrifugation two green bands are found, which are associated with monomeric and trimeric forms of PS I. In experiments reported here only the upper green band was used, which contains monomeric PS I particles.

Cells of *A. marina* were grown in K+ESM medium as described previously (Hu et al., 1998). PS I particles from *A. marina* were prepared as follows. Cells were harvested by centrifugation and disrupted in a bead beater using 0.1-mm beads. Cell debris was removed by centrifugation at 6000 $\times$ *g*. The thylakoid-containing supernatant was suspended at ~1 mg/mL Chl *d* (using a Chl *d* extinction coefficient of 77 $\times$ 10^3 $M^{-1}cm^{-1}$ at 697 nm) in buffer containing 50 mM Tris, pH 7, 10 mM $CaCl_2$, 10 mM NaCl, 2 mM EDTA, 20% glycerol w/v, and 2 mM phenazine methosulfate. 0.8% β- dodecyl maltoside was added and the medium was stirred in the dark, on ice, for 1 h. The detergent-incubated mix was then centrifuged at 197,568 $\times$ *g* in 10–32% sucrose gradient for 12 h. The lower green band was enriched in trimeric PS I (Hu et al., 1998). Sucrose was removed and PS I particles were resuspended in the above buffer and frozen.

For FTIR experiments, PS I particles from *S. 6803* or *A. marina* were pelleted and placed between a pair of rectangular CaF_2 windows. For some samples a mixture of ferricyanide and ferrocyanide (in D_2O) were added to the pellet. Otherwise, no redox mediators were added. No effects of mediator addition were observed in the difference spectra, indicating that no spectral signatures associated with reduced iron sulfur clusters or ferricyanide/ferrocyanide are observed in the spectral regions considered here. All experiments described here were performed at room temperature and samples were lightly dried until the amide I absorption band at 1656 cm^{-1} had an optical density <0.7 cm^{-1} (see Fig. 2). Under these conditions the absorbance between 2800 and 2700 cm^{-1} is below 0.3 (see Fig. 6 *A*).

For experiments described here, one of three different filters was placed in front of the sample to select the spectral region of interest and block

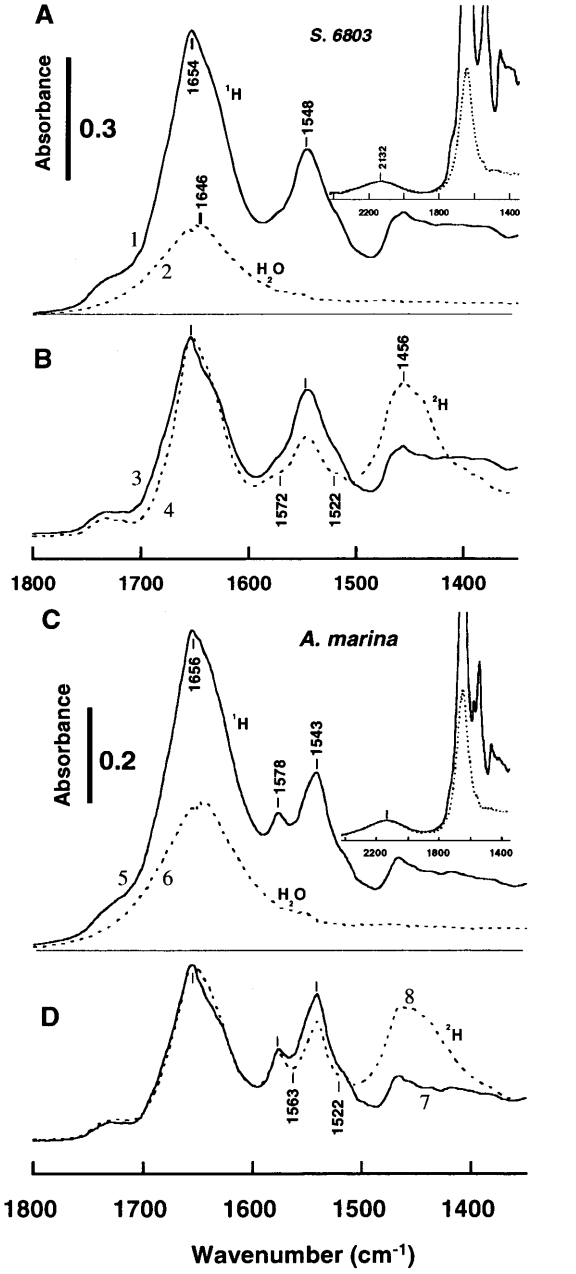

FIGURE 2 Infrared absorbance spectra of PS I samples from *S. 6803* (*A* and *B*) and *A. marina* (*C* and *D*) in the 1800–1450 cm^{-1} region. In *A* and *C*, a spectrum of pure water (^1H_2O) is also shown (*dotted line labeled* H_2O). (*A* and *C*, *insets*) Same spectra as in *A* and *C*, but on an extended scale to show the 2420–1380 cm^{-1} spectral region. Both sample spectra in insets display a band at 2132 cm^{-1} that is due to H_2O. The "pure" H_2O spectra shown in *A* and *C* were normalized to this band. Subtraction of the H_2O spectrum (*A* and *C*, *dotted lines*) from the sample spectra (*solid lines*) in *A* and *C* results in the sample spectra (*solid lines*) shown in *B* and *D*, respectively. The spectra (*solid lines*) in *B* and *D* therefore show the infrared absorbance spectra of PS I samples from *S. 6803* and *A. marina* that have been adjusted to remove absorbance contributions from water. Also shown in *B* and *D* are the infrared absorbance spectra of PS I samples from *S. 6803* (*B*, *dotted line*) and *A. marina* (*D*, *dotted line*) that have been incubated in D_2O. The amide I band in these latter two spectra contain virtually no absorbance contributions from water at 2132 cm^{-1} (data not shown), or in the amide I and II regions.

radiation from the interferometer helium neon laser reaching the sample. Depending on the spectral region of interest, a 5000–1000, 2000–1000, or 3000–2000 cm^{-1} bandpass filter was used. Identical spectra were obtained using different filters in the regions of spectral overlap.

Light minus dark and dark minus dark ($P700^+$-P700) FTIR difference spectra were recorded as described previously (Hastings et al., 2001; Hastings and Sivakumar, 2001). Spectral resolution was set at 4 or 2 cm^{-1}. FTIR spectra were recorded using a Bruker IFS/66 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Sixty-four spectra were collected before, during, and after light excitation from a 5 mW helium-neon laser emitting at 633 nm. The spectra collected before illumination were ratioed directly against the spectra collected during or after illumination. The dark-light-dark cycle was repeated ~200 times and all spectra were averaged (the presented spectra therefore represent the coaddition of ~12,800 interferograms). Such procedures were repeated several times on different samples.

PS I samples were exchanged into D_2O as follows. PS I samples in H_2O buffer were pelleted and resuspended in otherwise identical D_2O buffer. These samples were then concentrated using Centricon centrifugal filters (Millipore, Billerica, MA), or by ultracentrifugation, and resuspended again in D_2O buffer. The mixture was then refrigerated at 4°C for 1–2 days in the dark. Finally, the mixture was pelleted and used immediately. By considering the ratio of the area of the amide II absorbance band (Rath et al., 1998) we were able to estimate the extent of proton exchange upon incubation of the PS I particles in D_2O (see below).

RESULTS

Fig. 2 shows the infrared absorption spectra for the *S. 6803* (*A*, spectrum 1) and *A. marina* (*C*, spectrum 5) PS I samples used in our experiments. These samples contain a considerable amount of water, as evidenced by the intensity of the absorption bands in the 3600–3100 cm^{-1} region (data not shown), and the band at ~2132 cm^{-1} (Fig. 2, *insets*). The spectrum labeled H_2O (spectrum 2) was obtained by subtracting infrared absorption spectra of two PS I samples that had been dried for different amounts of time. Spectrum 2 is typical of pure water (Breton and Nabedryk, 1998).

By comparison of the intensity of the amide II absorption bands of PS I samples incubated in H_2O and D_2O, it is possible to estimate the extent of ^2H incorporation into the PS I particles. However, first the infrared absorption spectra of the two samples (in H_2O and D_2O) must be normalized. For dehydrated samples it is usually sufficient to normalize spectra based on the intensity of the amide I absorption band (Kim et al., 2001; Rath et al., 1998). In our measurements, however, we use only hydrated samples. Under these conditions, an absorption band at ~1646 cm^{-1} that is due to water overlaps the amide I band. This water absorption band makes normalization of infrared absorption spectra difficult. However, if the absorption spectrum of water (Fig. 2, *A* and *C*, *dotted line*) and the PS I sample (in H_2O) are normalized to the water absorption band at ~2132 cm^{-1} (see *insets* in Fig. 2) then it is straightforward to subtract water contributions from the PS I sample infrared absorption spectrum. Fig. 2, *B* and *D* (*solid lines*), then show the infrared (IR) absorption spectra, corrected for water absorption, for PS I samples from *S. 6803* and *A. marina*, respectively.

In Fig. 2, *B* and *D*, the amide II absorption band is considerably more intense in the ^1H spectra, compared to the ^2H spectra. In Fig. 2, *B* and *D*, the ratio of the integrated area ($^2H/^1H$) between 1572–1522 and 1563–1522 cm^{-1} for *S. 6803*/*A. marina* is 0.502/0.679, respectively. This indicates 49.8/32.1% incorporation of deuterium into PS I from *S. 6803*/*A. marina*, respectively. The level of incorporation of ^2H into PS I from *S. 6803* is considerably higher than that reported previously (Kim et al., 2001).

Fig. 3 shows the ($P740^+$-P740)/($P700^+$-P700) FTIR DS obtained using PS I particles isolated from *A. marina*/*S. 6803* in H_2O, in the 4200–1200 cm^{-1} spectral region. For PS I from *S. 6803*/*A. marina*, a broad, positive difference band is observed and is centered near 3300–3000 cm^{-1}. The *A. marina* spectrum also displays a distinct shoulder near 2250 cm^{-1}. In the 3600–3160 cm^{-1} region, sample absorbance considerably exceeds 1.5, due mainly to strong water absorption. Since very little light in this spectral region reaches the detector the noise level is high. Therefore, the difference spectra in this region will not be discussed.

Fig. 4 *A* shows ($P740^+$-P740) and ($P700^+$-P700) FTIR DS obtained using PS I particles incubated in H_2O (*solid line*) and D_2O (*dotted line*), in the 1760–1530 cm^{-1} spectral region. The relatively flat lines in Fig. 4 *A* show the difference spectra measured well after (minutes) illumination. These (dark-dark) difference spectra give a measure of the noise level in our experiments and show that the absorption

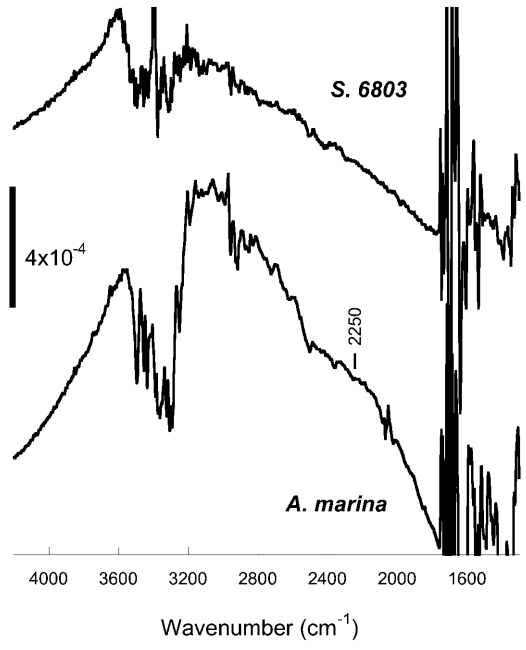

FIGURE 3 ($P700^+$-P700) and ($P740^+$-P740) FTIR DS in the 4400–1600 cm^{-1} spectral region, obtained using trimeric PS I particles from *A. marina* (*bottom*) and monomeric PS I particles from *S. 6803* (*top*) incubated in H_2O-based buffer. A 5000–1000 cm^{-1} bandpass filter was used to select the spectral region of interest. The *A. marina* ($P740^+$-P740) FTIR DS was scaled by a factor of 2.6, and shifted for clarity.

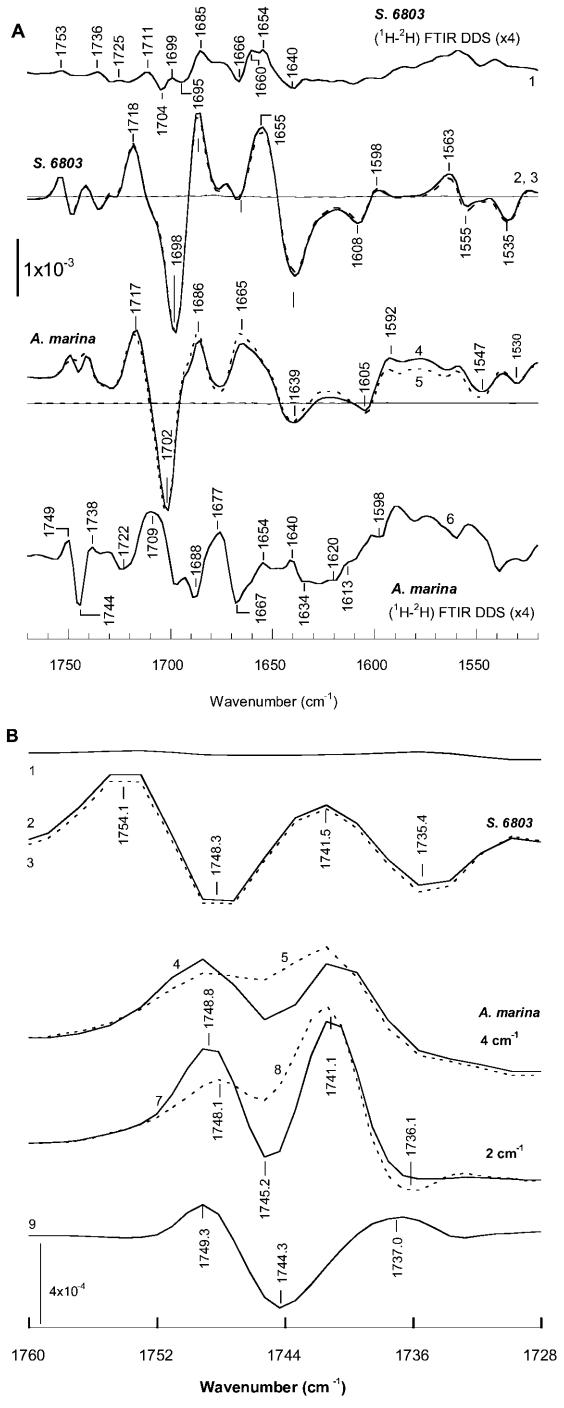

FIGURE 4 (*A*) (P740$^+$-P740) and (P700$^+$-P700) FTIR DS in the 1770–1530 cm^{-1} spectral region, obtained using trimeric PS I particles from *A. marina* and monomeric PS I particles from *S. 6803* incubated in H_2O (*solid line*) or D_2O (*dotted line*). The corresponding dark-dark difference spectra (noise level) are also shown for each experiment. A 2000–1000 cm^{-1} bandpass filter was used. The (P740$^+$-P740) spectra have been scaled by 2.6 to produce a similar bleaching at 1698-1702 cm^{-1}. The *A. marina* "noise" spectra were also scaled by 2.6. The (^{1}H-^{2}H) FTIR DDS for *S. 6803* (*top, spectrum 1*) and *A. marina* (*bottom, spectrum 6*) are also shown and have been multiplied by 4 for ease of viewing. (*B*) Spectra 1–5 are the same as in *A*, except they are shown expanded in the 1760–1728 cm^{-1} region. Also shown are the (P740$^+$-P740) FTIR DS obtained using *A. marina* PS I particles incubated in H_2O (*solid line, spectrum 7*) and D_2O (*dotted line, spectrum 8*), at 2 cm^{-1} spectral resolution. Spectrum 9 shows the (^{1}H-^{2}H) isotope edited double difference spectrum, obtained by subtracting the spectrum collected in D_2O (2 cm^{-1}) from that collected in H_2O (2 cm^{-1}).

changes are completely reversible. The (^{1}H-^{2}H) FTIR double difference spectra (DDS) for *S. 6803* (*top*) and *A. marina* (*bottom*) are also shown in Fig. 4 *A*. In the 1760–1600 cm^{-1} region (Fig. 4 *A*), several difference bands are observed, and the overall band patterns in the (P740$^+$-P740) and (P700$^+$-P700) FTIR DS are remarkably similar. The (P700$^+$-P700) FTIR DS for PS I from *S. 6803* in this spectral region has been described previously (Breton, 2001; Breton et al., 1999; Hastings et al., 2001; Kim et al., 2001). For the (P740$^+$-P740) FTIR DS in Fig. 4 *A*, an intense negative band is observed at 1702 cm^{-1}. Three positive bands are observed at 1717, 1741, and 1749 cm^{-1} (in H_2O). A shoulder is observed at 1692 cm^{-1}, and intense positive peaks at 1686 and 1665 cm^{-1}. Further negative bands are observed at 1639 and 1605 cm^{-1}. For *S. 6803* two difference bands at 1754(+)/1748(−) and 1742(+)/1735(−) cm^{-1} are quite well resolved (Fig. 4 *B*). Corresponding difference bands are observed in the *A. marina* spectra at 1749(+)/1745(−) and 1741(+)/1736(−) cm^{-1} (Fig. 4 *B*).

In the (^{1}H-^{2}H) FTIR DDS for *A. marina*, the amplitudes of the bands are significantly more intense than in the corresponding spectra for *S. 6803*, especially above ~1700 cm^{-1}. This is in spite of the fact that deuterium is incorporated into PS I from *S. 6803* at a much higher level than that from *A. marina*.

Fig. 4 *B* shows an expanded view of the spectra in Fig. 4 *A*, in the 1760–1728 cm^{-1} region. In Fig. 4 *B*, the ^{1}H and ^{2}H FTIR DS for *S. 6803* (*spectra 2* and *3*) are virtually identical, and no changes are observed in the (^{1}H-^{2}H) FTIR DDS (*spectrum 1*, *top*). For the (P740$^+$-P740) FTIR DS collected at 4 cm^{-1} resolution (*spectra 4* and *5*), however, it is obvious that incubation in D_2O strongly impacts the bands in the spectra. In the (P740$^+$-P740) FTIR DS collected at 4 cm^{-1} resolution, there appear to be two partially overlapping difference bands in the 1760–1725 cm^{-1} region. In an attempt to clearly resolve these features, FTIR DS were collected for *A. marina* PS I particles incubated in H_2O and D_2O at 2 cm^{-1} resolution. These spectra are also shown in Fig. 4 *B* (*spectra 7* and *8*, respectively), along with the difference between the two spectra [(^{1}H-^{2}H) FTIR DDS] (*spectrum 9*). The clearest observation in the (P740$^+$-P740) FTIR DS collected at 2 cm^{-1} resolution is that a positive band at 1748.8 cm^{-1} undergoes a ^{2}H-induced downshift of ~0.7 cm^{-1}.

Fig. 5, *A* and *B*, show the (P740$^+$-P740) and (P700$^+$-P700) FTIR DS in the 1500–1250 and 1160–1080 cm^{-1} spectral regions, respectively. Again, the overall band structure of the (P740$^+$-P740) and (P700$^+$-P700) FTIR DS are very similar. In the 1500–1250 cm^{-1} region both the (P740$^+$-P740) and (P700$^+$-P700) FTIR DS are virtually identical for PS I samples incubated in H_2O and D_2O. Of

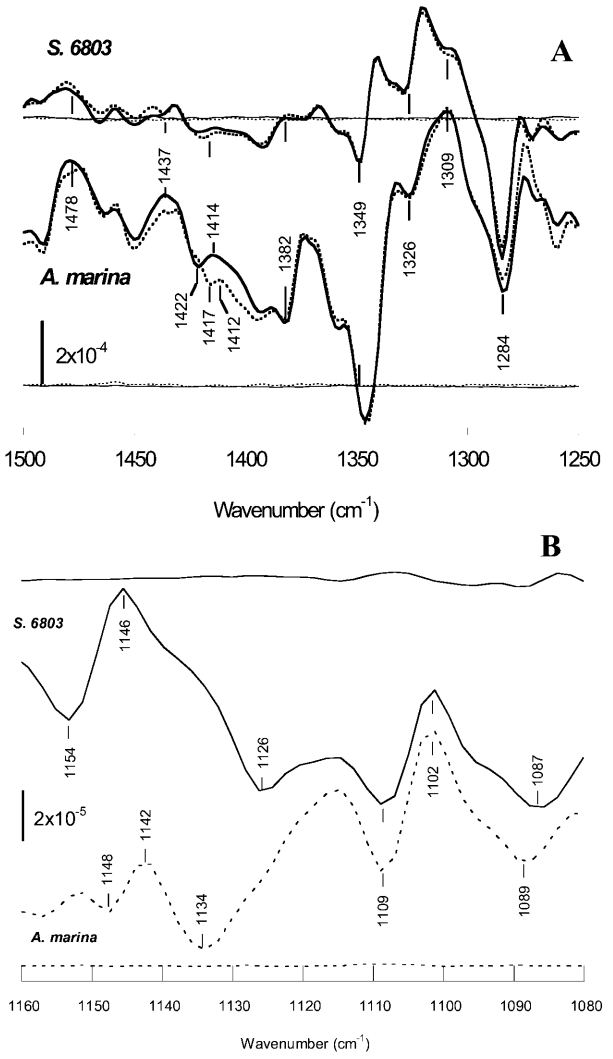

FIGURE 5 (*A*) (P740$^+$-P740) (*bottom*) and (P700$^+$-P700) (*top*) FTIR DS in the 1500–1250 cm^{-1} spectral region, obtained using PS I particles incubated in H_2O (*solid line*) and D_2O (*dotted line*). The corresponding dark-dark difference spectra are also shown for each experiment. A 2000–1000 cm^{-1} bandpass filter was used. The (P740$^+$-P740) spectra have been scaled by 2.6 and shifted for ease of comparison. The ''noise'' spectra have also been scaled and shifted. (*B*) (P740$^+$-P740) (*dotted line*) and (P700$^+$-P700) (*solid line*) FTIR DS in the 1160–1080 cm^{-1} spectral region. The corresponding dark-dark difference spectra are also shown.

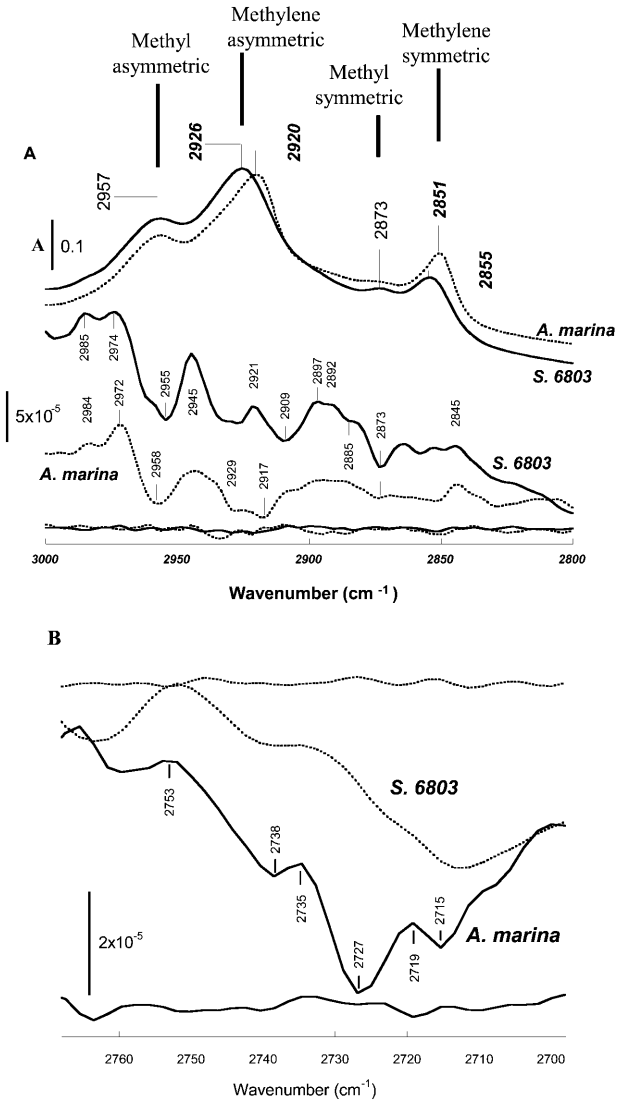

FIGURE 6 (*A, top*) P740 (*dotted line*) and P700 (*solid line*) infrared absorption spectra; (*middle*) (P700$^+$-P700) (*solid line*) and (P740$^+$-P740) (*dotted line*) FTIR difference spectra in the 3000–2800 cm^{-1} spectral region. The corresponding (dark-dark) FTIR difference spectra are also shown (*bottom*). PS I samples were incubated in H_2O. A 3000–2000 cm^{-1} bandpass filter was used. (*B*) (P740$^+$-P740) (*solid line*) and (P700$^+$-P700) (*dotted line*) FTIR DS in the 2780–2698 cm^{-1} region for PS I samples incubated in H_2O. The corresponding (dark-dark) FTIR DS are also shown. A 3000–2000 cm^{-1} bandpass filter was used.

particular interest is the observation of a difference band at 1109(−)/1102(+) cm^{-1} in both the (P740$^+$-P740) and (P700$^+$-P700) FTIR DS (Fig. 5 *B*). For PS I from *S. 6803*, it has recently been suggested that this difference band is due to *both* axial ligand histidines of P700 (Breton et al., 2002).

Fig. 6 *A* (*top*) shows the infrared absorbance spectra for *A. marina* (*dotted line*) and *S. 6803* (*solid line*) PS I samples, incubated in H_2O buffer, in the 3000–2800 cm^{-1} region. Fig. 6 *A* also shows the corresponding (P740$^+$-P740) (*dotted line*) and (P700$^+$-P700) (*solid line*) FTIR DS. The ''dark-dark'' difference spectra are also shown in Fig. 6 *A* (*bottom*). Fig. 6 *B* shows an expanded view of the (P740$^+$-P740) (*solid line*) and (P700$^+$-P700) (*dotted line*) FTIR DS in the 2770–2698 cm^{-1} region. The corresponding ''dark-dark'' FTIR DS are also shown. Generally, CH modes of methyl (CH_3) and methylene (CH_2) groups absorb in the 3000–2800 cm^{-1} region. Absorption bands associated with the CH_2 and CH_3 symmetric and asymmetric stretching modes are labeled in Fig. 6 *A*. Formyl CH modes are generally found without interference from other CH modes in the 2800–2700 cm^{-1} spectral region (Smith, 1999; Socrates, 2001).

Interestingly, in Fig. 6 *A*, the methylene absorption bands are more intense than the methyl absorption bands. However,

in the difference spectra for *S. 6803*, the methyl difference bands appear more intense than the methylene difference bands. In the 2750–2700 cm^{-1} region (Fig. 6 *B*), the (P700$^+$-P700) FTIR DS is featureless, displaying only very broad nonspecific changes. In contrast, in the (P740$^+$-P740) FTIR DS a clear difference band is observed at 2727(−)/2735(+) cm^{-1}. Lower intensity bands are also observed at 2715(−), 2719(+), and 2738(−) cm^{-1}.

DISCUSSION

Identical FTIR DS in all spectral regions were observed when 1, ferricyanide/ferrocyanide was used to accept electrons from the iron sulfur clusters; and 2, no mediators were added. These observations indicate that the FTIR DS do not contain contributions from either reduced iron sulfur clusters or redox mediators, and that all the bands in the spectra are due to P700 and P740 oxidation. In this study we have used monomeric PS I particles from *S. 6803* but trimeric PS I particles from *A. marina*. Previously we have shown that (P700$^+$-P700) FTIR DS are very similar for both the monomeric and trimeric forms of PS I from *S. 6803* (Hastings, 2001; Hastings and Sivakumar, 2001). This indicates that it is quite appropriate to compare spectra obtained using monomeric PS I from *S. 6803* and trimeric PS I from *A. marina*. Finally, very similar absorption changes were also observed in *S. 6803* PS I membrane fragments (data not shown), suggesting that modes associated with the detergent used to prepare the PS I particles do not contribute to the FTIR DS.

(P700$^+$-P700) FTIR DS obtained using PS I from *S. 6803*

(P700$^+$-P700) FTIR DS for PS I particles from *S.6803* have been obtained by several authors (Breton, 2001; Breton et al., 2002; Hastings et al., 2001; Kim et al., 2001), and it has been indicated that (P700$^+$-P700) FTIR DS are identical for PS I particles extensively washed and incubated in either D_2O or H_2O (Breton, 2001; Breton et al., 1999). These indications are in line with our observations that show only very small changes in (P700$^+$-P700) FTIR DS, even after 49% proton exchange. Our observation is similar to that of Kim et al. (2001), who also observed only very small changes in (P700$^+$-P700) FTIR DS with ~30% deuteration of PS I. Given the amplitude of the changes in the (^{1}H-^{2}H) FTIR DDS for *S. 6803* in Fig. 4, *A* and *B*, we estimate that any ^{2}H-induced band shifts are at most 0.1–0.2 cm^{-1}. Thus the changes are unlikely to be associated with exchangeable protons that directly interact with the pigments of P700. From the PS I crystal structure at 2.5 Å resolution (Fromme et al., 2001; Jordan et al., 2001), water molecule 19 is thought to provide a hydrogen bond to the 13^3 ester oxygen of the A-side Chl *a′* of P700 (P_A). Exchange of H_2O-19 would strongly impact (P700$^+$-P700) FTIR DS and it is therefore unlikely that this water molecule is exchanged, even after ~49% proton exchange. It is possible that water molecules distant from P700 are exchanged, however, and it is these distantly located water molecules (for example H_2O-93) that are responsible for the ^{2}H-induced changes in the (P700$^+$-P700) FTIR DS, as discussed by Kim et al. (2001). However, we note that the band pattern in our (^{1}H-^{2}H) FTIR DDS for *S. 6803* is entirely different from that reported by Kim et al. (2001). This is not surprising because the ^{1}H and ^{2}H (P700$^+$-P700) FTIR DS reported here are also very different from those reported by Kim et al. (2001), but are similar to those in previous reports (Breton et al., 1999). In particular, the intense 1639(−)/1655(+) difference band (Fig. 4 *A*, spectra 2 and 3) is considerably diminished in intensity in the spectra reported by Kim et al. (2001)., as is the broad band centered near 3200 cm^{-1} (Fig. 3).

In all experiments reported here we have used only hydrated samples. In contrast, Kim et al. (2001) used PS I samples that were much more dehydrated. We use only hydrated samples because it is well known that the intensity of the light-induced FTIR difference bands can be seriously impacted in dehydrated samples (Morita et al., 1993). For example, in FTIR DS obtained using reaction centers from *Rb. sphaeroides* and *Rhodopseudomonas viridis*, no light-induced signals are observed in dehydrated samples (Morita et al., 1993).

The small ^{2}H-induced band shifts in the (P700$^+$-P700) FTIR DS suggest that the effects of deuteration are not a result of direct interactions of protons with the pigments of P700. This indicates that the ^{2}H-induced band shifts are almost impossible to interpret. Given this, the bands in the (^{1}H-^{2}H) FTIR DDS for *S. 6803* are of little interest, and will not be discussed further.

Methyl and methylene modes of P700 and P740

Fig. 6 *A* shows typical IR absorption spectra for *A. marina* and *S. 6803* PS I particles. Four bands are observed at 2957, 2926–2920, 2873, and 2855–2851 cm^{-1}. As outlined in Fig. 6 *A*, these bands are due to methyl asymmetric, methylene asymmetric, methyl symmetric, and methylene symmetric stretching modes, respectively (Katz et al., 1966, 1978; Smith, 1999), of all of the chlorophylls (including the phytyl chain) and amino acids in the PS I particles. Generally, CH_3 and CH_2 asymmetric modes are more intense than the symmetric modes, while the CH_3 (both asymmetric and symmetric) modes are more intense than the corresponding CH_2 modes (Katz et al., 1966; Smith, 1999). The above observations indicate that more methylene modes (of amino acids and chlorophyll) than methyl modes contribute to the absorbance spectra in Fig. 6 *A*. However, comparing the difference spectra to the absorption spectra in Fig. 6 *A*, the methyl modes appear more pronounced in the difference spectra. From IR absorbance spectra of chlorophylls in vitro, it is found that methylene modes of the phytyl chain

dominate the spectra in the 3000–2800 cm^{-1} region (Katz et al., 1966). The fact that the methyl modes predominate in the difference spectra in Fig. 6 *A* could then indicate that the difference bands are due to methyl and methylene modes of the chlorophyll macrocycle. We cannot rule out, however, that nearby amino acids also contribute to the difference spectra in Fig. 6 *A*.

The observation of only symmetrically distributed positive/negative difference features in Fig. 6 *A* is not consistent with previous observations, where difference bands in the 3000–2800 cm^{-1} region for PS I from *S. 6803* (Kim et al., 2001) or *C. reinhardtii* (Hastings et al., 2001) appear to be dominated by methylene groups, and are about an order of magnitude more intense than the difference bands in Fig. 6 *A*. The previous observation of intense negative difference bands in the 3000–2800 cm^{-1} region appears to be related to the experimental conditions, and is not well understood. However, under the conditions used in Fig. 6 (see Materials and Methods section), we observe highly reproducible, symmetrically distributed positive/negative difference features.

The intensity of the difference bands in the 3000–2800 cm^{-1} region in Fig. 6 *A* is consistent with only a few methyl or methylene groups being impacted by cation formation. The 2955(−) cm^{-1} difference band for *S. 6803* has an amplitude of $\sim 5 \times 10^{-5}$ while the 2957 cm^{-1} absorbance band has an intensity of ~0.15. Therefore, ~1 methyl group in 3000 contributes to the difference spectrum. A typical PS I complex contains ~2300 amino acids, 100 Chl *a*, ~10 β-carotenes, and 2 lipids (Golbeck and Bryant, 1991). From this we estimate that a PS I complex will contain ~2135 methyl groups (assuming 9 methyl groups per 20 amino acids, 10 methyl groups per Chl *a*, and 10 methyl groups per β-carotene). Therefore, each derivative feature in Fig. 6 *A* is likely associated with 1–2 methyl or methylene groups. Similar conclusions can also be drawn for P740.

Does P740 consist of Chl *d* molecules?

IR absorption spectra for Chl *a* and Chl *b*, in the 3000–2700 cm^{-1} spectral region, have been obtained (Katz et al., 1966, 1978). Generally, four distinct bands are observed in the 3000–2850 cm^{-1} region. These bands are due mainly to modes associated with the phytyl chain, as they are considerably diminished in chlorophyllides and pheophorbides that lack a phytyl chain (Katz et al., 1966). In IR absorption spectra of methyl pheophorbides, several bands are still observed in the 3000–2850 cm^{-1} region but these are due to methyl and methylene modes of the Chl macrocycle (Katz et al., 1966). For Chl *b*, but not Chl *a*, a clear infrared absorbance band is observed at ~2727 cm^{-1} (Katz et al., 1978). Chl *b* contains a formyl group at the 7^1 position of ring II (see Fig. 1 for numbering), and the 2727 cm^{-1} band of Chl *b* was assigned to the CH stretch of this formyl group (Katz et al., 1966, 1978). This assignment is based on the well-known fact that aldehydic CH modes generally occur in the 2700–2800 cm^{-1} region, at frequencies considerably lower than that of methyl and methylene modes (Smith, 1999). By considering IR absorption spectra of methyl pheophorbide-*b* in the 3000–2700 cm^{-1} region, it is clear that the absorption band intensity of the CH stretch of the 7^1 formyl group is similar to that of bands associated with the methyl and methylene groups of the macrocycle (Katz et al., 1966). Therefore, given that the bands in the 3000–2850 cm^{-1} region in the ($P740^+$-P740) FTIR DS in Fig. 5 *A* are due to macrocyclic methyl and methylene groups of the Chls of P740, if P740 consists of Chl *d* molecules, then it is likely that we should observe a difference band associated with a CH mode of the 3^1 formyl group of Chl *d* in the 2700–2800 cm^{-1} region. In addition, no such bands should be observed in this region in ($P700^+$-P700) FTIR DS, since P700 consists of Chl *a* molecules that lack a formyl group.

For the ($P740^+$-P740) FTIR DS, the clearest feature is a negative band at 2727 cm^{-1} that appears to upshift to 2735 cm^{-1} upon $P740^+$ formation (Fig. 6 *B*). Importantly, no sharp features are observed in the ($P700^+$-P700) FTIR DS. These observations then suggest that the 2727(−)/2735(+) cm^{-1} difference band can be assigned to an aldehydic C–H stretch of at least one Chl *d* molecule of P740. A further lower intensity difference band is observed at 2715(−)/2719 cm^{-1}, which could suggest the presence of another Chl *d* aldehydic C–H stretch. Thus, comparison of the ($P740^+$-P740) and ($P700^+$-P700) FTIR DS supports the idea that P740 consists of two Chl *d* molecules.

Broad electronic transitions between 2000 and 4000 cm^{-1}

Previously, Breton et al. (1999) observed a broad, positive IR difference band centered near 3300 cm^{-1}, using PS I particles from *S. 6803* at 90 K. They also observed a similar band, centered at slightly lower frequency, using spinach PS I particles at 280 K. Fig. 1 indicates that such a broad, positive IR difference band is clearly present at room temperature for PS I from *S. 6803*. A broad IR difference band is also observed in ($P700^+$-P700) FTIR DS obtained using PS I particles from *C. reinhardtii* (Hastings et al., 2001). Broad FTIR difference bands above 2000 cm^{-1} have also been observed upon donor photo-oxidation in photosynthetic particles from purple bacteria (Breton et al., 1992; Nabedryk, 1996; Nabedryk et al., 1996), heliobacteria (Nabedryk et al., 1996; Noguchi et al., 1997), and green sulfur bacteria (Nabedryk et al., 1996; Noguchi et al., 1996). These difference bands are due to low-frequency electronic transitions associated with the dimeric nature of the primary electron donor (Binstead and Hush, 1993; Breton et al., 1992). The observation of broad, positive absorption bands in the 4000–2000 cm^{-1} spectral region is therefore usually taken as an indication that the species under consideration consists of at least two chlorophyll molecules. Consistent with this is the observation of broad, low-frequency tran-

sitions in cation porphyrin dimers, but not in monomers (Binstead and Hush, 1993). The above discussion therefore indicates that P700 and P740 are not single chlorophyll species, and that the cation radical is, to some degree, delocalized over at least two chlorophylls.

The fact that the broad, positive absorption band(s) observed for *S. 6803* and *A. marina* have different structures could indicate that the delocalized charge distribution in P740 is different from that of P700. It should be pointed out, however, that the underlying structure of the broad, positive absorption band for *A. marina* is similar to that observed in (P700$^+$-P700) FTIR DS obtained using PS I particles from *C. reinhardtii*, especially concerning the presence of a distinct shoulder near 2200 cm^{-1} (Hastings et al., 2001) The distinct shoulder near 2200 cm^{-1} is observed in cation minus neutral FTIR DS for most species. This suggests that it is the *S. 6803* FTIR DS that is quite unusual in that it lacks a band near 2200 cm^{-1}. The differences between the (P700$^+$-P700) FTIR DS for PS I from *S. 6803* and *C. reinhardtii* suggest differences in the structural and/or electronic organization of P700 and/or P700$^+$ from the two species. This could be an important point, especially since the PS I crystal structure (obtained from a cyanobacterial strain) is used as input for calculations aimed at modeling spectroscopic data that were obtained using PS I particles from *C. reinhardtii*.

Dimer "marker modes"

In FTIR DS associated with donor oxidation in *Rb. sphaeroides*, positive FTIR difference bands with high intensity are observed at ~1550, 1480, and 1295 cm^{-1} (Breton et al., 1992). These bands have been related to the dimeric nature of the primary donor in *Rb. sphaeroides* and, thus, are ''marker'' bands for multimeric pigment species. It has also been suggested that similar, high-intensity IR bands are due to the dimeric nature of the donors in heliobacteria (1535 and 1300 cm^{-1}) (Nabedryk et al., 1996; Noguchi et al., 1997) and green sulfur bacteria (1465 and 1280 cm^{-1}) (Nabedryk et al., 1996; Noguchi et al., 1996). In *A. marina* and *S. 6803* PS I particles, no such high-intensity transitions are observed. In PS I particles from *S. 6803* and *A. marina*, all of the bands between 1500–1300 cm^{-1} in Fig. 5 *A* have an intensity that is ~8 and 4 times less than the keto C=O band at 1698 and 1702 cm^{-1}, respectively. Therefore, enhanced intensity bands in the 1550–1300 cm^{-1} region do not appear to be a universal characteristic of dimeric pigment species.

Do histidine residues provide ligands to the Chl *d* molecules of P740?

It is thought that the negative band at 1608 cm^{-1} in the (P700$^+$-P700) FTIR DS (Fig. 4 *A*) is indicative of pentacoordinated Chl *a* (Breton et al., 2002; Fujiwara and Tasumi, 1986). A similar band is observed at 1605 cm^{-1} in (P740$^+$-P740) FTIR DS, and it is likely that this band has the same origin as the 1608 cm^{-1} band in the (P700$^+$-P700) FTIR DS. Therefore, given the interpretation of the 1608 cm^{-1} band in (P700$^+$-P700) FTIR DS, it is likely that the Chl *d* molecules of P740 are pentacoordinated. In addition, we have suggested that the coordinating histidine ligands for P700 give rise to the 1639(−)/1655 cm^{-1} difference band in (P700$^+$-P700) FTIR DS (Fig. 4 *A*). A difference band is also observed at 1639(−)/1665 cm^{-1} in (P740$^+$-P740) FTIR DS. This band is weaker and more asymmetric than the 1639(−)/1655 cm^{-1} difference band in (P700$^+$-P700) FTIR DS. Notwithstanding these differences, it is likely that the same interpretation is valid for these difference bands in both spectra, but other species probably also contribute to the 1639(−)/1665 cm^{-1} in (P740$^+$-P740) FTIR DS.

Finally, a clear difference band at 1109(−)/1102(+) cm^{-1} is observed in both the (P700$^+$-P700) and (P740$^+$-P740) FTIR DS. For PS I from *S. 6803*, it has recently been established that the 1109(−)/1102(+) cm^{-1} difference band is due to side-chain imidazole modes of *both* axial ligand histidines of P700 (Breton et al., 2002). Since a band is observed at identical frequency in (P740$^+$-P740) FTIR DS, the suggestion is that the Chl *d* molecules of P740 could also be ligated to histidine residues. More extensive studies using ^{15}N-labeled PS I from *A. marina* will be required for definitive conclusions.

The 13^3 ester carbonyls

In the (P700$^+$-P700) FTIR DS in Fig. 4 *B*, the 1748(−)/1754(+) and 1735(−)/1742 cm^{-1} difference bands have been assigned to the 13^3 ester C=O of the two Chls of P700 (P_A and P_B) (Breton, 2001; Kim et al., 2001; Nabedryk et al., 1990). The fact that the 13^3 ester C=O absorb at different frequencies indicates different environmental perturbations on each of the 13^3 ester C=O of the Chls of P700. In view of the recent high-resolution crystal structure of PS I (Fromme et al., 2001; Jordan et al., 2001) the 1748(−)/1754(+) cm^{-1} difference band can be assigned to the 13^3 ester C=O of P_B that is free from H-bonding. The 1735(−)/1741(+) cm^{-1} difference band is then assigned to the 13^3 ester C=O of P_A. The downshift of the 13^3 ester C=O of P_A is due to the fact that the ester oxygen is H-bonded to a water molecule (H_2O-19) (Fromme et al., 2001; Jordan et al., 2001). Given this prediction from the crystallographic data, our deuterium exchange experiments indicate that the water molecule, H_2O-19, is not exchangeable, at least in PS I from *S. 6803*. This then suggests that the P700 binding site is quite inaccessible.

In the (P740$^+$-P740) FTIR DS, two positive bands are observed at 1742 and 1749 cm^{-1}. Given the assignments outlined above for (P700$^+$-P700) FTIR DS, we assign the positive 1742 and 1749 cm^{-1} bands to the 13^3 ester C=O of two different Chl *d* molecules of P740$^+$. The 13^3 ester C=O of the Chl *d* molecules of P740 probably absorb at 1745 and 1736 cm^{-1} (Fig. 4 *B*). In analogy to P700, the higher frequency band at 1742 cm^{-1} could be associated with an

ester C=O mode that is free from H-bonding. However, we show below that this hypothesis is unlikely.

The (^{1}H-^{2}H) FTIR DDS for *S. 6803* in Fig. 4 *B* indicate that no protons in the vicinity of the ester C=O of P700 are exchanged, even with ~49% proton exchange. In contrast, the (^{1}H-^{2}H) FTIR DDS for *A. marina* show that at least one ester C=O of P740 is significantly modified with only ~32% proton exchange. In the (^{1}H-^{2}H) FTIR DDS for *A. marina* (Fig. 4 *B*), a second derivative feature at 1749(+)/1744(−)/1737(+) cm^{-1} is consistent with the downshift of a complete difference band at ~1749(+)/1745(−) cm^{-1} by <1 cm^{-1}. The ^{2}H-induced shift of the 1749(+)/1745(−) cm^{-1} difference band suggests either 1, that it is associated with an ester C=O mode that is H-bonded to an amino acid with an exchangeable proton; or 2, that it is associated with an ester mode that is coupled to a second mode that is H-bonded to a species with an exchangeable proton.

Considering the first case, in the ($P740^+$-P740) FTIR DS, the highest frequency difference band in Fig. 4 *B* is ~5 cm^{-1} lower than the corresponding difference band in the ($P700^+$-P700) FTIR DS. This could indicate that the 1749(+)/1745(−) cm^{-1} difference band in the ($P740^+$-P740) FTIR DS is due to an H-bonded 13^3 ester C=O mode of P740, unlike P_B of P700. If the 1749(+)/1745(−) cm^{-1} difference band in the *A. marina* FTIR DS is due to an H-bonded 13^3 ester C=O mode of one of the pigments of P740, then a shift greater than 0.7 cm^{-1} would be expected upon exchange of the proton involved in the H-bond. This then indicates that the mode giving rise to the 1749(+)/1745(−) cm^{-1} difference band is only very weakly H-bonded, or it is coupled to another mode that is H-bonded. The most likely candidate for a mode that could couple to the ester C=O mode is the 13^1 keto C=O mode of the same chlorophyll. If this is the case then this H-bonded keto C=O mode would be strongly perturbed upon ^{2}H exchange. We find no evidence for this (see below). We thus favor the idea that the 1749(+)/1745(−) cm^{-1} difference band is due to a weakly H-bonded 13^3 ester C=O mode of P740. The 1741(+)/1736(−) cm^{-1} difference band could also be due to a (more-strongly) H-bonded 13^3 ester C=O mode of P740. However, in this case, the H-bond proton is not exchangeable in our experiments.

The 13^1 keto C=O modes

In the ($P700^+$-P700) FTIR DS in Fig. 4 *A*, the negative band at 1698 cm^{-1} is due to the 13^1 keto C=O of one (Breton et al., 2002; Witt et al., 2002) or both (Hastings et al., 2001) of the Chls of P700. Part (Hastings et al., 2001) or all (Breton et al., 2002; Witt et al., 2002) of the 1698 cm^{-1} band upshifts to 1718 cm^{-1} upon $P700^+$ formation. In the ($P740^+$-P740) FTIR DS a difference band is observed at 1702(−)/1717(+) cm^{-1} (Fig. 4 *A*). Following the interpretation of the 1698(−)/1718(+) cm^{-1} difference band in ($P700^+$-P700) FTIR DS, we could assign at least part of the 1702(−)/1717(+) cm^{-1} difference band in the ($P740^+$-P740) FTIR DS to a 13^1 keto C=O mode of one of the Chl *d*s of P740.

Although controversial, it has been suggested that the 1639(−)/1655(+) cm^{-1} difference band in the ($P700^+$-P700) FTIR DS in Fig. 4 *A* is due to a strongly H-bonded 13^1 keto C=O mode of P_A (Breton et al., 1999). As discussed above, a similar but less intense difference band is observed at 1639(−)/1665(+) cm^{-1} in the ($P740^+$-P740) FTIR DS in Fig. 4 *A*, and probably has the same origin as the 1639(−)/1655(+) cm^{-1} difference band in the ($P700^+$-P700) FTIR DS. Regardless of the precise interpretation of ($P700^+$-P700) FTIR DS, the similarity in the FTIR DS in the 1720–1600 cm^{-1} region for the two species suggests similar environments for the 13^1 keto C=O of the Chls of P700 and P740, in both the ground and cation radical state.

On the one hand, the overall similarity of the ($P740^+$-P740) and ($P700^+$-P700) FTIR DS in the 1710–1200 cm^{-1} region indicates that the P740 and P700 binding sites are very similar. It suggests that the Chls of P700 and P740 are oriented similarly. It also suggests that the 13^1 keto C=O are in a similar environment and display similar types of bonding interactions. This would indicate that the protein structure in the vicinity of the P700 and P740 binding sites is similar. On the other hand, however, the fact that there are larger ^{2}H-induced changes in the ($P740^+$-P740) FTIR DS compared to the ($P700^+$-P700) FTIR DS indicates that the P740 binding site is more accessible than the P700 binding site.

^{2}H exchange leads to further alterations in the ($P740^+$-P740) FTIR DS in the 1730–1630 cm^{-1} region, as evidenced in the *A. marina* (^{1}H-^{2}H) FTIR DDS (Fig. 4 *A*). Importantly, the amplitude of all the double difference bands in the 1725–1630 cm^{-1} region are small, relative to the amplitude of the bands in the ($P740^+$-P740) FTIR DS. This suggests that the ^{2}H-induced band shifts are very small (<0.2–0.8 cm^{-1}). This in turn indicates that the changes in the 1725–1630 cm^{-1} region are not associated with directly H-bonded 13^1 keto C=O modes with exchangeable protons, as larger amplitude changes would be expected in the (^{1}H-^{2}H) FTIR DDS. The ^{2}H-induced band shifts in the 1725–1630 cm^{-1} region are therefore more likely associated with secondary effects on the keto C=O modes of the Chls of P740, due to coupling with other modes that do have exchangeable protons. It is also possible that some of the features in the (^{1}H-^{2}H) FTIR DDS in the 1725–1630 cm^{-1} region are associated with modification of C=O modes of amino acids (such as Asn or Gln) or due to small changes in the protein backbone near P740. More extensive isotope labeling studies, using PS I particles obtained from *A. marina* cells grown in D_2O, will be required to distinguish definitively between the different possibilities.

Formyl C=O modes of Chl *d*

The negative band at 2727 cm^{-1} in Fig. 4 *B* is at a frequency that is typical for a CH mode of a chlorophyll formyl group

(Katz et al., 1966). In particular, we assign the 2727 cm^{-1} band in Fig. 6 *B* to a CH mode of the 3^1 formyl group of Chl *d*. Given this assignment we could expect to observe a difference band associated with the C=O mode of the 3^1 formyl group of Chl *d*. From IR absorption spectroscopy of Chl *b* and its derivatives (Chl *b* contains a formyl group at the 7^1 position) the 7^1 formyl C=O was found to absorb at 1652–1669 cm^{-1} (Katz et al., 1966, 1978). For BChl *a* and its derivatives (BChl *a* has an acetyl group at the 3^1 position of ring I) the 3^1 acetyl C=O absorbs at 1656–1675 cm^{-1} (Katz et al., 1966, 1978). From FTIR DS of the primary donor in *Rb. sphaeroides* the BChl *a* 3^1 acetyl C=O is H-bonded and absorbs near 1620 cm^{-1} (Mäntele et al., 1988; Nabedryk 1996). Very recently, from Raman spectroscopic studies of solid films of Chl *d* at 77 K, it has been suggested that a medium-intensity Raman band at 1659 cm^{-1} is due to the 3^1 formyl C=O of Chl *d* (Cai et al., 2002).

Given the frequencies outlined above for Chl *d*, Chl *b*, BChl *a* and the H bonded acetyl C=O of BChl *a* in *Rb. sphaeroides*, the 3^1 formyl C=O of Chl *d* is likely to contribute to the (P740$^+$-P740) FTIR DS in the 1665–1620 cm^{-1} region. In the (P740$^+$-P740) FTIR DS large amplitude difference bands are observed in the 1665–1620 cm^{-1} region. The origin of these difference bands have been discussed above. If a Chl *d* formyl C=O partially contributes to the 1639(−)/1665(+) cm^{-1} difference band then only small amplitude changes may be expected in the (^{1}H-^{2}H) FTIR DDS, if indeed the Chl *d* formyl C=O is affected by deuteration. If the formyl C=O is free from H-bonding then it will be very difficult to observe ^{2}H-induced band shifts associated with this mode in the (^{1}H-^{2}H) FTIR DDS. On the other hand, if the Chl *d* formyl C=O is H-bonded then larger ^{2}H-induced *downshifts* could be expected. The only spectral feature in the (^{1}H-^{2}H) FTIR DDS in Fig. 4 *A*, below 1670 cm^{-1}, that could be associated with a downshifting difference band, is the ~1640(+)/1634(−)/1620(−)/1613(+) feature. If this feature is due to formyl C=O modes of P740 then this would indicate a formyl C=O band of P740 at ~1635 cm^{-1}, that upshifts 5 cm^{-1}, to 1640 cm^{-1}, upon P740$^+$ formation. It would also indicate that this ~1634(−)/1640(+) cm^{-1} difference band downshifts ~21 cm^{-1} to 1613(−)/1620(+) cm^{-1} upon ^{2}H exchange. The free 7^1 formyl C=O of Chl *b* absorbs at ~1663 cm^{-1}, so a frequency of 1635 cm^{-1} for the 3^1 formyl C=O of Chl *d* could indicate that it is strongly H-bonded. A ^{2}H-induced downshift of 21 cm^{-1} seems extraordinarily large, but it may also indicate that the formyl C=O must be strongly H-bonded to a species with an exchangeable proton.

It could also be possible that the feature at 1667(−)/1654(+) cm^{-1}, and part of the positive band at 1677 cm^{-1}, are due to a downshifting 3^1 formyl C=O group of Chl *d*. In this case the 3^1 formyl C=O upshifts from ~1667 cm^{-1} to near 1677 cm^{-1} upon P740$^+$ formation. The mode also downshifts ~13 cm^{-1} upon deuteration. A frequency of 1667 cm^{-1} for the 3^1 formyl C=O of Chl *d* could indicate that it is free from H-bonding. A ^{2}H-induced downshift of 13 cm^{-1}, however, would suggest a strongly H-bonded formyl C=O. This latter hypothesis is therefore unlikely.

It could also be the case that the features in the 1700–1620 cm^{-1} region in the (^{1}H-^{2}H) FTIR DDS are associated with protein modes, and that the formyl C=O of Chl *d* is little affected by ^{2}H exchange. Again, more extensive labeling studies of PS I from *A. marina* will be required for more definitive conclusions to be drawn.

CONCLUSIONS

In PS I particles from *A. marina* some of the protons in the vicinity of P740 are exchangeable. This does not appear to be the case for P700. Two spectroscopic signatures associated with formyl group CH modes suggest that P740 is a dimeric Chl *d*-containing species. From the shape of the broad cation absorbance band centered near 3000 cm^{-1} it is likely that the charge over the Chls of P740$^+$ is more symmetrically distributed than that of P700$^+$. At least one of the 13^3 ester C=O modes of one of the Chl *d* molecules of P740 is weakly H-bonded. One of the 3^1 aldehydic C=O modes of one of the Chl *d* molecules of P740 could also be strongly H-bonded. The Chls of P740 are likely ligated to histidine residues. Deuterium exchange experiments on PS I are suggestive but not conclusive, and do not provide a means to directly probe pigment-protein interactions. More extensive labeling studies using PS I particles obtained from cells grown in media containing D_2O, ^{15}N, and/or ^{13}C will be required for definitive FTIR difference band assignments. In addition, spectro-electrochemical studies of isolated Chl *d* should prove useful.

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