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Ultrahigh Field MAS NMR Dipolar Correlation Spectroscopy of the Histidine Residues in Light-Harvesting Complex II from Photosynthetic Bacteria Reveals Partial Internal Charge Transfer in the B850/His Complex

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Abstract: Low-temperature ^{15}N and ^{13}C CP/MAS (cross-polarization/magic angle spinning) NMR has been used to analyze BChl–histidine interactions and the electronic structure of histidine residues in the light-harvesting complex II (LH2) of *Rhodospseudomonas acidophila*. The histidines were selectively labeled at both or one of the two nitrogen sites of the imidazole ring. The resonances of histidine nitrogens that are interacting with B850 BChl *a* have been assigned. Specific ^{15}N labeling confirmed that it is the τ -nitrogen of histidines which is ligated to Mg^{2+} of B850 BChl molecules (β -His30, α -His31). The π -nitrogens of these Mg^{2+} -bound histidines were found to be protonated and may be involved in hydrogen bond interactions. Comparison of the 2-D MAS NMR homonuclear (^{13}C – ^{13}C) dipolar correlation spectrum of [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-histidines in the LH2 complex with model systems in the solid state reveals two different classes of electronic structures from the histidines in the LH2. In terms of the ^{13}C isotropic shifts, one corresponds to the neutral form of histidine and the other resembles a positively charged histidine species. ^{15}N – ^{13}C double-CP/MAS NMR data provide evidence that the electronic structure of the histidines in the neutral BChl *a*/His complexes resembles the positive charge character form. While the $\text{Mg}\cdots^{15}\text{N}$ isotropic shift confirms a partial positive charge transfer, its anisotropy is essentially of the lone pair type. This provides evidence that the hybridization structure corresponding to the neutral form of the imidazole is capable of “buffering” a significant amount of positive charge.

Introduction

The LH2 complex is a peripheral photosynthetic antenna complex. It serves to absorb light and to transfer the excited-state energy to the LH1-reaction center.^{1,2} The structures of LH2 from *Rhodospseudomonas (Rps.) acidophila* strain 10050 and *Rhodospirillum rubrum* have been resolved by X-ray crystallography down to 2.5 and 2.4 Å resolution, respectively.^{3,4} The crystal structure of the LH2 of *Rps. acidophila* shows that the complex consists of two concentric cylinders of helical protein subunits called α -polypeptides and β -polypeptides. Eighteen BChl *a* molecules (BChl = bacteriochlorophyll; B850 = 850 absorbing bacteriochlorophyll *a*) are sandwiched between the α - and β -polypeptides and form a continuous overlapping ring. Another ring of BChl *a* (B800 = 800 nm absorbing

bacteriochlorophyll *a*) is positioned between the subunits of the outer cylinder with the bacteriochlorin rings perpendicular to the transmembrane helix axis. The entire LH2 complex has 9-fold rotational symmetry.

Each α -polypeptide contains 2 histidines (α -His 31 and α -His 37) while each β -polypeptide contains 3 histidines (β -His 12, β -His 30, and β -His 41) (Figure 1A). The histidine residues His 31 on the α - and His 30 on the β -polypeptides support the eighteen B850 BChl *a* molecules by ligating to the Mg^{2+} in the center of the bacteriochlorin.^{3–5} According to the X-ray structure, the second N atom of the imidazole ring of these histidines is within hydrogen-bonding distance, at ~ 3.0 Å, from the C13¹-keto carbonyl of the adjacent B850 BChl *a*.⁶ However, there is no experimental evidence for the existence of this H-bond.⁶ In *Rhodobacter sphaeroides*, no LH2 complex was formed when histidine residues located in the hydrophobic phase and coordinating with the central magnesium atom of a BChl *a* molecule were changed to Asn by site-directed mutagenesis.⁷ This experiment suggests that these histidines are essential for the formation of the LH2 complex. The tertiary structure of

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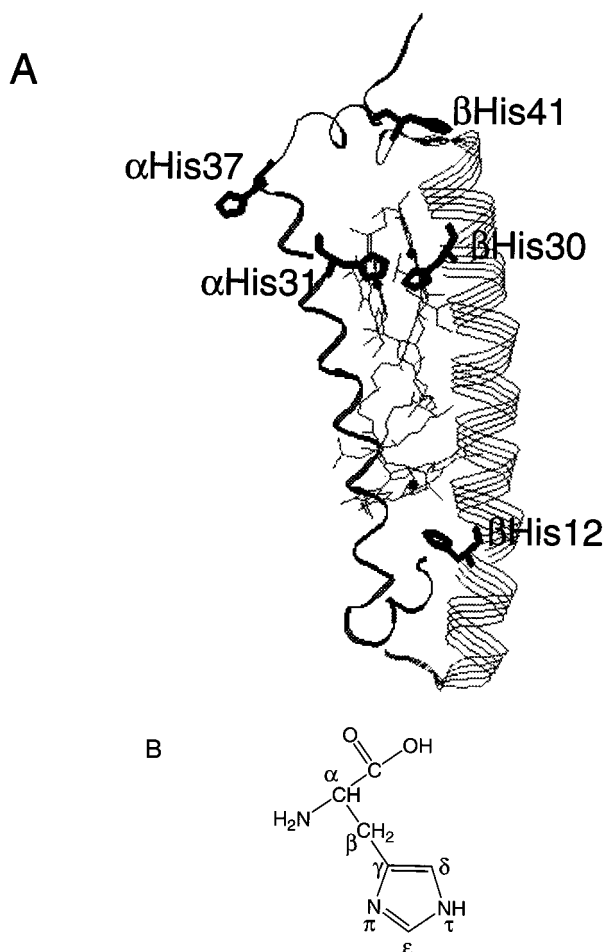


Figure 1. (A) The arrangement of histidine residues (bold) in one protomer of LH2 from *Rhodospseudomonas acidophila*. The helices are represented by ribbons. (B) The nomenclature of the histidine.

LH2 shows that the terminal histidine of the β -apoprotein (β -His 41) donates a hydrogen bond to the main-chain carbonyl oxygen of β -Thr 37.⁶

Interaction of histidine with Mg^{2+} has been suggested in all bacteriochlorophyll–protein complexes with known structures.^{6,8,9} However, the assignment of ^{15}N chemical shifts of histidines in pigment–protein complexes is difficult. In particular, the ^{15}N chemical shifts for imidazole nitrogens of histidines coordinating with Mg^{2+} of chlorophylls are not known. Solid-state NMR in conjunction with selective isotope labeling provides a powerful arsenal which can resolve electronic structure down to the atomic level even in large-membrane proteins. In the present study, low-temperature ^{15}N and ^{13}C MAS NMR was used to investigate the charge state of the histidines in the LH2 complex of *Rps. acidophila*, in which histidines were labeled with ^{15}N at both or one of the two nitrogen sites of the imidazole ring. The results provide for the first time a conclusive ^{15}N chemical shift assignment of histidine nitrogens coordinating with Mg^{2+} in a neutral BChl *a*/histidine complex in a large-membrane protein assembly. It reveals an electronic structure with distinct positive charge character of formally neutral imidazole side chains that are interacting on one side with B850 BChl *a* and on the other side protonated, pointing to an internal charge-transfer state of the form BChl $a^{\delta-}/His^{\delta+}$ for the B850.

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Materials and Methods

Media Preparation and Culturing of *Rps. acidophila*. *Rps. acidophila* strain 10050 was grown at 30 °C and a light intensity of 2000 lux (incandescent lamps) in sterile liquid medium. For each liter of culture medium, 20 mL of 1.0 M ammonium malate solution (pH 6.9), 20 mL of 1.0 M phosphate buffer (pH 6.9), 20 mL of trace elements, 4 μ L of a solution of thiamine (25 g/L), and biotine (0.5 g/L) was added. The trace elements were prepared as described earlier.¹⁰ For incorporation of [$^{13}C_6, ^{15}N_3$]-L-histidine, [π - ^{15}N]-L-histidine, or [τ - ^{15}N]-L-histidine (see Figure 1B for nomenclature), 10 mL of a solution containing the labeled histidine (8 g/L) together with other 19 (unlabeled) amino acids at concentrations described by Raap et al.¹⁰ was added. [$^{13}C_6, ^{15}N_3$]-L-Histidine was purchased from Cambridge Isotope Laboratories (Andover, MA) while [π - ^{15}N]-L-histidine and [τ - ^{15}N]-L-histidine were synthesized as described elsewhere.¹¹

Preparation of LH2 Complex. The LH2 complex of *Rps. acidophila* strain 10050 was prepared as described elsewhere.¹ In brief, chromatophores were prepared and incubated for 2 h in a 2% LDAO solution at 4 °C and subsequently ultracentrifuged overnight with use of a discontinuous sucrose gradient. The purified LH2 was dialyzed against 30 mM Tris/EDTA buffer (pH 8.0) containing 0.3% LDAO for 24 h and concentrated to an OD_{800} of 330 with use of a Filtron 30-kDa filter.

The ^{15}N -isotope enrichment of the histidine residues in LH2 was measured by gas chromatography and electron impact mass spectrometry (GC-MS). First the proteins in the sample were hydrolyzed¹⁰ followed by the derivatization of histidines by using the method of Husek.¹² The GC-MS was performed by using a GC Chrompack 25 m fused silica column (CP-sil-5CB 0.25 mm id.; MS ITD 700, Finnigan MAT). Incorporation of [$^{13}C_6, ^{15}N_3$]-L-histidine, [π - ^{15}N]-L-histidine, or [τ - ^{15}N]-L-histidine in LH2 complex was more than 95%.

Detergent-solubilized LH2 was used for all measurements presented in this paper. Before and after taking the CP/MAS NMR data on the LH2 preparations, electronic absorption spectra were measured with a Shimadzu UV-160 A spectrometer. No change in absorption spectrum was detected, which confirms that the integrity and quality of the sample were maintained during the MAS NMR measurement procedure. For the titration experiments on LH2, the pH of the sample was adjusted to different values by adding small aliquots of 1 M HCl or 1 M NaOH while monitoring the pH with a microelectrode.

CP/MAS NMR Measurements. For ^{15}N CP/MAS NMR experiments, 0.3 mL of an LH2 sample with an OD_{800} of 330 was loaded into a 7 mm MAS rotor. ^{15}N CP/MAS NMR spectra were obtained by using an MSL-400 NMR spectrometer (Bruker, Karlsruhe, Germany) with a spinning frequency $\omega_r/2\pi = 4$ kHz, a mixing time of 2 ms, and at a temperature of 225 K. The sample was frozen slowly over a period of 10 min with liquid nitrogen-cooled bearing gas at a low rotor spinning frequency of 1 kHz to ensure a homogeneous sample distribution against the rotor wall.¹³ A home-built spinning speed controller was used to keep the spinning rate constant.¹⁴ Cross-polarization and CW decoupling with a nutation frequency of ~ 80 kHz in the proton channel were used. The data were recorded over a period of 48 h. Prior to Fourier transformation the data were zero filled to 8K points and an exponential apodization of 100 Hz was applied. All ^{15}N data were referenced to liquid ammonia with use of an external standard of ^{15}N -acetyl valine (123.2 ppm). The chemical shift anisotropy was estimated as described by de Groot et al.¹⁵ For accurate measurement of the chemical shift anisotropy the spectrum was recorded at a spinning frequency of 3.3 kHz. This allows observation of at least two sets of sidebands for the

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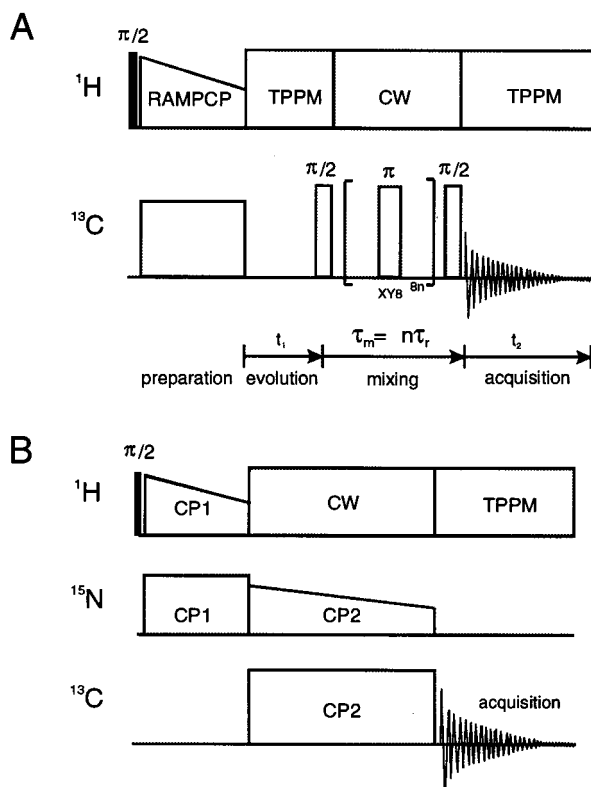


Figure 2. Schematic representation of the (A) phase-sensitive RFDR pulse sequence used for the 2-D MASNMR homonuclear (^{13}C – ^{13}C) dipolar correlation experiment and (B) the pulse sequence used for the ^{15}N – ^{13}C double-CP/MAS NMR experiments. Relevant nutation angles are depicted on top of the corresponding pulses.

signal from the N–Mg while overlap between sidebands and other peaks in the spectrum is avoided.

1-D ^{13}C CP/MAS NMR spectra and 2-D homonuclear (^{13}C – ^{13}C) dipolar correlation spectra of [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2 complex were measured at 220 K by using a mixing time of 2 ms with a wide bore 750 NMR spectrometer in a 4 mm MAS probe (Bruker, Karlsruhe, Germany). 1-D ^{13}C CP/MAS data were recorded with a spinning rate of 12 kHz. 2-D homonuclear (^{13}C – ^{13}C) dipolar correlation spectra were recorded with the broad-banded RFDR (radio frequency-driven dipolar recoupling) technique with phase-sensitive detection in ω_1 by using the pulse sequence shown in Figure 2A. This pulse sequence is similar to the RFDR pulse sequences proposed earlier.^{16,17} Following the ^1H 90° preparation pulse, RAMP-amplitude CP is applied to transfer magnetization from ^1H to ^{13}C . The RAMP-amplitude CP was varied from 100% to 50% during this preparation step. The protons were decoupled from the carbon by use of the two-pulse phase modulation (TPPM)¹⁸ decoupling scheme during ^{13}C evolution and detection, while continuous wave (CW) decoupling was applied during mixing in the homonuclear (^{13}C – ^{13}C) experiments. An XY8 train of rotor-synchronized π pulses during a mixing time $\tau_m = 8n\tau_r$ with phases $(xyxyxyxy)_n$ was applied to favor polarization transfer via the homonuclear ^{13}C – ^{13}C dipolar couplings.¹⁶

^{15}N – ^{13}C double CP/MAS spectra of [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2 complexes were measured in a magnetic field of 17.6 T by using a wide bore NMR spectrometer with a 4 mm MAS probe operating at 220 K with a spinning rate of 12 kHz and the pulse sequence shown in Figure 2B. The first cross-polarization step (CP1) consists of RAMP-amplitude cross-polarization, from 100% to 50% on the ^1H -channel, whereas the second step (CP2) consists of RAMP-amplitude cross-

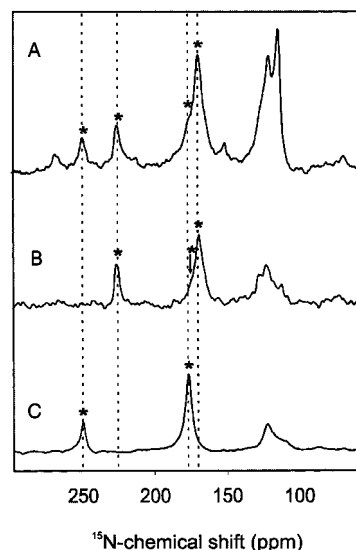


Figure 3. CP-MAS ^{15}N NMR spectra of the LH2 complex containing labeled histidines. (A) [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2, (B) τ - ^{15}N -His labeled LH2, and (C) π - ^{15}N -His labeled LH2 measured in a magnetic field of 9.4 T at 220 K. Each spectrum represents about 15 000 scans collected with an acquisition time of 20 ms and a recycle time of 1 s. The spinning frequency around the magic angle was 4.0 kHz for spectra A and B and 10 kHz for spectrum C. The asterisks indicate the position of the center band signals from imidazole nitrogens of histidines.

polarization, from 100% to 70% on the ^{15}N channel. Radio frequency fields and mixing time were chosen to ensure broad-band, heteronuclear polarization transfer across one peptide bond.¹⁹ Proton decoupling during the heteronuclear contact time CP2 was optimized by adjusting the radio frequency carrier frequency under continuous wave (CW) irradiation.¹⁹ Two-pulse phase modulation (TPPM) was employed for decoupling during acquisition.

Results

One-dimensional ^{15}N NMR spectra of *Rps. acidophila* LH2 complex, in which histidines are selectively labeled at both or one of the τ and π nitrogen sites of the imidazole ring, are presented in Figure 3. The ^{15}N spectrum from [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2 shows distinct centerband signals at 123, 170, 225, and 250 ppm. The response around 123 ppm is attributed to labeled and natural abundance nitrogen in the peptide backbone (Figure 3A). The signals at 170 and 225 ppm in Figure 3A are attributed to the τ -nitrogen in the imidazole ring of the histidines. This is confirmed by the ^{15}N spectra of τ - ^{15}N His labeled LH2 (Figure 3B), while signals at 178 and 250 ppm are attributed to the π -nitrogen of the imidazole ring (Figure 3C). The signal at 170 ppm in Figure 3A comprises a shoulder at 178 ppm.

It is well established that the ring nitrogens of a histidine side chain that is not coordinated to an Mg^{2+} can be divided into three main types, namely, (i) protonated nitrogen in a neutral imidazole ring, $>\text{NH}$ (type α), (ii) nonprotonated nitrogen in a neutral imidazole ring, $>\text{N}$ (type β), and (iii) protonated nitrogens in a cationic imidazole ring, $+\text{>NH}$ (type $\alpha+$), which generally resonate around 170, 250, and 180 ppm, respectively (Scheme 1). Hydrogen bonding induces an 8 to 10 ppm change in chemical shift of these nitrogens, moving the hydrogen bond donor $>\text{NH}$ and $+\text{>NH}$ type responses upfield to ~ 178 and ~ 188 ppm, respectively, while moving the $>\text{N}$ type hydrogen bond acceptor signal downfield to ~ 240 ppm.^{20,21} Thus, the

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Scheme 1

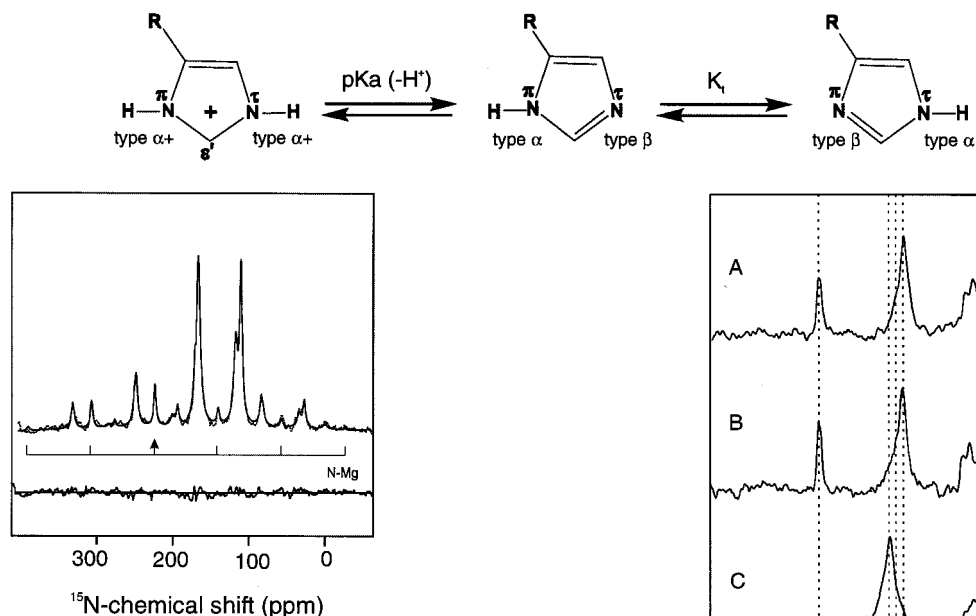


Figure 4. Results of the theoretical fit analysis of the CP-MAS ^{15}N NMR spectrum of the $^{13}\text{C}_6, ^{15}\text{N}_3$ -His labeled LH2 complex measured at a spinning speed of 3.3 kHz. The upper part compares the experimental NMR spectrum (dotted lines) with the theoretical fit (thick lines). The lower part gives the residue, i.e., the difference between data and theory. The centerband and two sets of sidebands of peaks corresponding to N-Mg are indicated.

signal at 170 ppm corresponds to the $>\text{NH}$ form of the $\tau\text{-}^{15}\text{N}$, while the signal at 178 ppm, corresponds either to the $>\text{NH}$ form of the $\pi\text{-}^{15}\text{N}$ if it is involved in hydrogen bonding or to the $+>\text{NH}$ form in the cationic species. The signal at 250 ppm in Figure 3A,C is assigned to the π -position $>\text{N}|$ type nitrogens. The remaining centerband signal at 225 ppm in Figure 3A,B is then assigned to an $\text{N}\cdots\text{Mg}$ type nitrogen response from the τ -position, i.e., a nitrogen not bearing a hydrogen but coordinated to an Mg^{2+} in a bacteriochlorophyll ring. Recently by using a CIDNP (photochemically induced dynamic nuclear polarization) technique on ^{15}N labeled bacterial reaction centers, Zysmilich and McDermott²² reported a signal ~ 25 ppm downfield from the $>\text{N}|$ response, i.e., at 225 ppm on our scale. This signal was tentatively assigned to a nitrogen coordinated to Mg^{2+} .

Figure 3A also shows weak spinning sidebands at integral multiples of the rotational frequency, relative to the centerband. These sidebands contain information about the chemical shift anisotropy, i.e., the asymmetry of the diamagnetic susceptibility associated with the ground-state electron density distribution around the atom. The chemical shift anisotropy is estimated to be $\delta \sim 4.5$ kHz with $\eta \sim 1$ for the signals at 170 ppm and $\delta \sim 8.7$ kHz with $\eta \sim 0.4$ for signal at 250 ppm. These anisotropy values of protonated and deprotonated nitrogens of the imidazole ring of histidine are in agreement with the values reported earlier.²³ The anisotropy for the signal from $\text{N}\cdots\text{Mg}$ type nitrogen has not been reported in the literature. Figure 4 shows the theoretical fit of the ^{15}N CP/MAS spectra of $^{13}\text{C}_6, ^{15}\text{N}_3$ -His labeled LH2 recorded at a spinning speed of 3.3 kHz. This allows the analysis of at least two sidebands at the sides of the

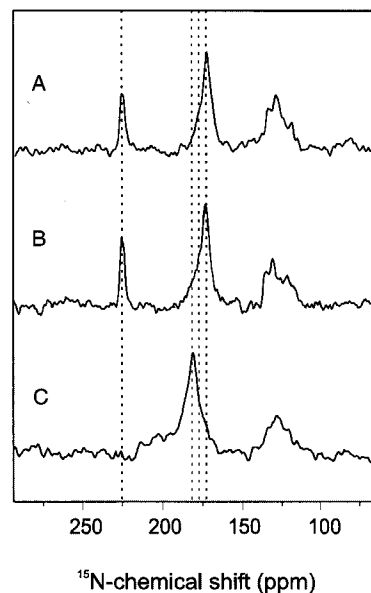


Figure 5. CP-MAS ^{15}N NMR spectra of $\tau\text{-}^{15}\text{N}$ -His labeled LH2 complexes at 220 K. Each spectrum represents about 15 000 scans collected with an acquisition time of 20 ms and a recycle time of 1 s. The spinning rate around the magic angle was 4.0 kHz. For each data set the pH was adjusted at room temperature prior to the experiment: (A) pH 8.0, (B) pH 6.0, and (C) pH 4.0.

MAS centerband response corresponding to $\text{N}\cdots\text{Mg}$. The anisotropy for the signal at 225 ppm was estimated to be $\delta \sim 8.4$ kHz with $\eta \sim 0.4 \pm 0.15$. Interestingly, both δ and η of the $\text{N}\cdots\text{Mg}$ type nitrogen closely match the value of ~ 8.7 kHz and 0.4, found for the lone pair type nitrogen.

The $\tau\text{-}^{15}\text{N}$ signals of histidines were examined in $\tau\text{-}^{15}\text{N}$ His labeled LH2 at various pH values (Figure 5). No change in the spectra was observed at pH 6.0 as compared to pH 8.0. Since the response at 225 ppm arises from histidines interacting with Mg of B850, these data suggest that the ligation is largely unaffected at pH 6.0. In contrast, at pH 4.0 a single peak at 180 ppm is detected, while the peaks at 170, 176, as well as at 225 ppm were not observed (Figure 5C). These results suggest that at pH 4.0 all histidines are positively charged and fully protonated. In particular, the disappearance of the response at 225 ppm at low pH suggests that the $\text{N}\cdots\text{Mg}$ interaction of $\beta\text{-His30}$ and $\alpha\text{-His31}$ with B850 is broken at such low pH and that these histidines are protonated. Disappearance of the peak at 176 ppm also suggests that the H-bond interaction is broken at low pH. The optical spectrum of the LH2 complex incubated at pH 4.0 for 6 h shows a significant loss ($\sim 80\%$) of its absorbance at 850 nm (corresponding to bound BChl-850). These optical changes also indicate that the $\text{N}\cdots\text{Mg}$ interaction of $\beta\text{-His30}$ and $\alpha\text{-His31}$ with B850 is broken at pH 4.0.

Figure 6 shows an ultrahigh field (17.6 T) $^{13}\text{C}\text{--}^{13}\text{C}$ homonuclear dipolar correlation spectrum from $^{13}\text{C}_6, ^{15}\text{N}_3$ -His labeled LH2 complexes recorded with the RFDR technique. The spectrum clearly reveals separate correlation networks corresponding to two different types of histidines in the LH2 complex. On the basis of cross-peaks a full assignment of all

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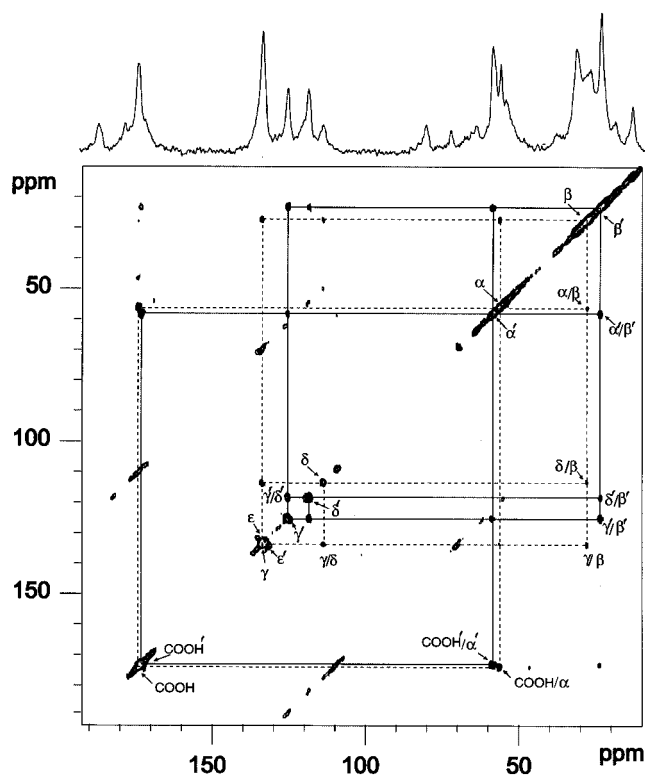


Figure 6. Contour plot of a 2-D MAS NMR homonuclear (^{13}C – ^{13}C) dipolar correlation spectrum of [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-histidine labeled LH2 complex collected at a field of 17.6 T. The spectrum was recorded with a spinning rate of 12 kHz at a temperature of 230 K and a homonuclear polarization transfer time of 3 ms. ^{13}C signals from cationic histidines are indicated with a prime. Cross-peaks from similar histidine residues are connected with lines.

Table 1. Comparison of Solid-State ^{13}C Chemical Shifts of the Imidazole Ring of Histidine in the Neutral and Cationic State in Model Systems with Those of Histidines in the LH2 Complex from *Rhodospseudomonas acidophila*

carbon type	chemical shifts (ppm)			
	model system		LH2	
	neutral His	cationic His	Type 1 His	Type 2 His
C γ	137.0	128.0	133.7	125.1
C δ	113.5	119.3	113.5	118.3
C ϵ	138.6	136.4	133.5	133.5

^a All ^{13}C data are relative to the chemical shift of TMS.

^{13}C resonances of the two types of histidines can be obtained (Table 1). To model the system, salts of neutral and positively charged [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-histidine were used and ^{13}C – ^{13}C homonuclear dipolar correlation spectra were recorded. The ^{13}C chemical shifts of the imidazole ring carbons of the neutral and positively charged histidines as a model system are listed in Table 1. A downfield shift of 9 ppm of C γ and 2 ppm of C ϵ and an upfield shift of 6 ppm of C δ were observed in the cationic histidine relative to the neutral histidine. These shifts appear considerably larger in the solid than for histidine in solution.²⁴

Comparison of the ^{13}C -chemical shifts of the imidazole ring of histidine in LH2 with the side chain responses in the model system shows that the shifts for the type 1 histidines in LH2 with ring carbons designated as γ , δ , and ϵ resemble the shifts observed for the neutral solid-state model, while the type 2 histidines with ring carbons designated as γ' , δ' , and ϵ' appear

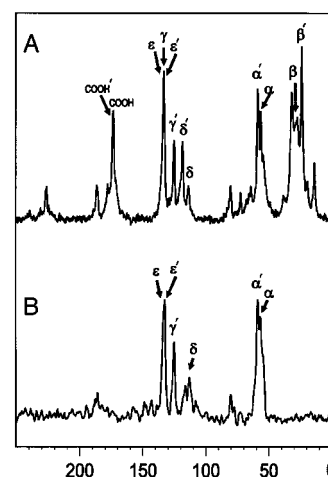


Figure 7. (A) CP-MAS ^{13}C NMR spectrum and (B) ^{15}N – ^{13}C double-CP/MAS NMR data of the [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2 complex, measured at 220 K by using a wide-bore 750 NMR spectrometer. The spinning rate around the magic angle was kept at 12 kHz. Each spectrum represents about 156 000 scans collected with an acquisition time of 8 ms and a recycle time of 2 s. The spectra are normalized at the α' peaks.

to exhibit positive charge character (Table 1). The intensity of the cross-peaks of the type 2 histidines in the ^{13}C dipolar correlation spectrum is systematically ~ 1.5 times the intensity for the signals collected from the type 1 histidines (Figure 6). This observation suggests that 3 out of 5 histidines in LH2 may have positive charge character.

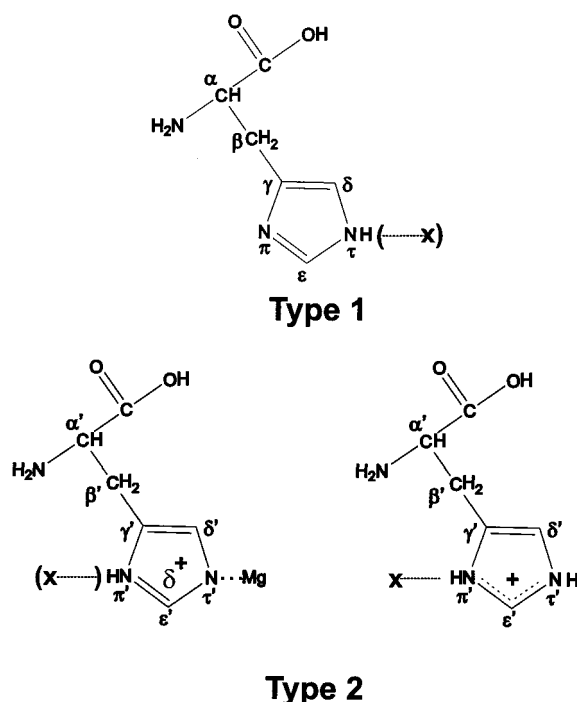
To arrive at an unambiguous assignment a correlation of ^{15}N and ^{13}C signals is essential. Thus, 1D ^{13}C CP/MAS NMR spectra and ^{15}N – ^{13}C double CP/MAS NMR spectra of LH2 are shown in Figure 7, spectra A and B, respectively. For a ^1H , ^{15}N contact time of 2 ms, protonated nitrogens are predominantly excited in the CP1 step of the double CP/MAS NMR experiment in high field. This was verified with the 1D ^{15}N CP MAS experiment performed prior to the double CP/MAS experiment (data not shown). During the CP2 period, magnetization is transferred from a protonated ^{15}N to an adjacent ^{13}C . Among the imidazole ring carbons of neutral histidines, the γ carbon appears partially suppressed (Figure 7B). Therefore, the π -nitrogens of the neutral histidines are most likely unprotonated while their τ -nitrogens appear protonated. These results demonstrate that the τ -nitrogens of the type 1 histidines are not bound to B850 (Chart 1). On the other hand, among the ring carbons of type 2 histidine, the δ' carbon is significantly suppressed in the double CP/MAS NMR spectrum. This observation is crucial for the assignment and the interpretation of the results. In particular, it provides convincing evidence that most of the type 2 histidine is not a regular doubly protonated species with a full positive charge, like the structure depicted in the left panel of Scheme 1. The double CP/MAS data are consistent with the picture that most of the τ -nitrogens of the type 2 histidines are not protonated while their π -nitrogen is protonated. Since the type 2 histidines correspond to the positive charge character species, the coordination to Mg of B850 should be responsible for imparting a partial positive charge on these histidines (Chart 1). In this way, the double CP/MAS NMR data are consistent with the ^{15}N chemical shift data presented in Figure 3.

Discussion

^{15}N CP/MAS NMR data obtained from specific ^{15}N labeled LH2 samples (Figure 1) demonstrate that the imidazole nitrogen

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Chart 1



of histidines at the τ -position is either protonated or bound to Mg^{2+} . In contrast, the nitrogen of histidine at the π -position can be unprotonated or protonated with or without taking part in a H-bond interaction. In the following paragraph we compare our ^{15}N and ^{13}C CP/MAS NMR data with the information available from the crystal structure to arrive at a tentative assessment of the electronic structure of the various histidines in the LH2 complex.

β -His 30 and α -His 31. In the crystal structure the conserved histidines β -His30 and α -His31 are the ligands to the central Mg atom of the B850 BChl *a*. Our results provide converging evidence that the τ -nitrogen of the histidines takes part in this interaction. The π -nitrogens of these histidines are protonated and resonate most likely at 178 ppm (Figure 3). The 2-D homonuclear (^{13}C – ^{13}C) dipolar correlation spectrum (Figure 6) and the ^{15}N – ^{13}C double-CP/MAS NMR spectrum (Figure 7) from [$^{13}\text{C}_6$, $^{15}\text{N}_3$]-His labeled LH2 confirm that the π -nitrogens of these histidines are protonated. If the chemical shift value is indeed 8 ppm higher than the characteristic value of a $>\text{NH}$ type nitrogen, then the π -nitrogens of β -His30 and α -His 31 should be involved in H-bond interactions. Crystal structure data show that the π -N atoms of the imidazole rings of β -His30 and α -His31 are within hydrogen-bonding distance (3.5 Å) of the C13¹-keto carbonyl of the adjacent B850.⁶ The presence of a H-bond could not be established unambiguously by X-ray structural data, and it is therefore emphasized that our results are in favor of such H-bonds.

The isotropic ^{15}N shift of the $\text{N}\cdots\text{Mg}$ response confirms a stabilization of positive charge in the imidazole ring. This is in contrast with the anisotropy parameters δ and η , which suggest that the hybridization of the $\text{N}\cdots\text{Mg}$ type nitrogen is similar to the electronic structure of the lone pair type nitrogen. The positive charge character of neutral BChl *a*/His complexes points to considerable internal charge transfer of the form BChl *a*/His in B850. It shows that the imidazole ring can “buffer” a considerable amount of positive charge and deplete its electron density without changing the hybridization of its constituent atoms and the molecular orbital structure. According to the X-ray model, the B850 are arranged in pairs. The α -His31 and

β -His30 coordinate to the Mg of one of the B850 and are within hydrogen bonding distance of the C13¹ keto group of the other B850. The data for the LH2 contrast with recent model studies on chlorophyll/imidazole complexes in an apolar solvent that revealed a stable negatively charged species, with the imidazole deprotonated.²⁵ In the LH2 antenna, the presence of the keto group may be important to stabilize the neutral form with partial positive charge transfer to the His observed with the NMR. Protonation of the keto group is considered unlikely. It would either involve deprotonation of the coordinating His, which is consistent with NMR results, or it would involve a H-bond with two protons, like a positive Bjerrum defect. This is probably energetically less favorable.

β -His 41. The X-ray structure data show that the π -nitrogen of β -His 41 donates a H-bond to β -Thr 37.⁶ It means that in β -His 41, the π -nitrogen should be of the $>\text{NH}$ type taking part in the H-bond interaction. ^{15}N MAS/NMR data show that the τ -nitrogens of all histidines that are not bound to B850 are protonated. Thus, the τ -nitrogen in β -His 41 is protonated, and we conclude that β -His 41 is positively charged, with both nitrogens protonated and the π -nitrogen taking part in a H-bond interaction.

These results are consistent with the ^{15}N data in Figure 3A and the signal intensities in the 2-D homonuclear (^{13}C – ^{13}C) dipolar correlation data, which indicate that 3 of the 5 histidines in LH2 have positive charge character (Figure 6). It follows that β -His 41 as well as β -His 30 and α -His 31 exhibit positive charge character with remarkably similar ^{13}C -chemical shift, although β -His 41 has a proton on both nitrogens of the imidazole ring while β -His 30 and α -His 31 have a proton only on the π -nitrogen and τ -nitrogen is interacting with Mg. The observation of similar ^{13}C -chemical shifts is remarkable, since ring current shifts from the BChl *a* in β -His 30 and α -His 31 are expected for the $\text{C}\delta'$ and $\text{C}\epsilon'$. According to the X-ray structure, the distances between $\text{C}\epsilon$ and $\text{C}\delta$ of β -His 30 and the center of the bound BChl are 3.31 and 3.38 Å, respectively.³ For these positions, an upfield shift of about –5 ppm is expected due to a ring current effect.²⁶ The contribution from the other nearby BChl should be negligible because of the much larger distance to the His. For example, the $\text{C}\epsilon$ of β -His 30 is 8.4 Å away from the BChl plane. Since there is no evidence for ring current shifts from the BChl *a* for β -His 30 and α -His 31 in the MAS NMR data, this would indicate that the ring currents are affected by the overlap in the superstructure of a complete ring of 18 B850 BChl *a* molecules in the LH2 complex. If this interpretation is right, the absence of ring currents may be indicative for the electronic structure of the BChl *a* aggregate. Further research in this direction is currently underway.

β -His 12 and α -His 37. On the basis of the ^{15}N resonances arising from the τ - and π -nitrogen of histidines, we suggest that the β -His 12 and α -His37 are neutral with protonated τ -nitrogens that may or may not be involved in H-bond interaction, while their π -N is unprotonated. This result is consistent with the signal intensities in the 2-D homonuclear (^{13}C – ^{13}C) dipolar correlation data (Figure 6), which provide evidence that two of the histidines in LH2 are neutral, and the ^{15}N – ^{13}C double-CP/MAS NMR spectrum (Figure 7), which shows that neutral histidines in LH2 have a proton at their τ -N position while their π -N is unprotonated.

β -His 12 is present in the binding pocket of B800 BChl *a*.^{4,6} The τ -N can be of the $>\text{NH}$ type, resonating at 176 ppm with

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Table 2. ^{15}N Chemical Shifts Assignment of the Imidazole Ring Nitrogens of Histidines in the LH2 Complex from *Rhodopseudomonas acidophila*

		chemical shifts (ppm)	assignment ^a
β -His 12	N τ	170 or 176	>NH or >NH...X
	N π	250	>N
β -His 30	N τ	225	>N...Mg
	N π	178	>NH...X
β -His 41	N τ	170	>NH
	N π	178	>NH...X
α -His 31	N τ	225	>N...Mg
	N π	178	>NH...X
α -His 37	N τ	170	>NH
	N π	250	>N

^a Tentative.

the π -N of the >N| type and resonating at 250 ppm (Figures 1 and 3). A 6 ppm upfield shift of a τ -NH response suggests a hydrogen bond interaction for one of the histidines. In the crystal structure of LH2 from *Rps. acidophila*, a water molecule has been found close to α -fMet 1 and β -His 12 and the X-ray structure of *Rs. molischianum* shows that β -His17 (which is comparable to β -His 12 in *Rsp. acidophila*) makes contact with a water molecule at a distance of 2.94 Å.^{3,4} This correlates with

our inference that β -His12 in LH2 of *Rsp. acidophila* can be neutral with the τ -nitrogen protonated and hydrogen bonded and the π -nitrogen unprotonated and not participating in a H-bond interaction.

In summary, our results provide invaluable information on the charge state of histidines and the hydrogen-bonding status of imidazole rings in the LH2 complex (Table 2 and Chart 1). Two different environments around the histidines in LH2 were observed: (1) one corresponding to a neutral form of histidine and (2) another in which histidines either appear positively charged or exhibit partial positive charge character due to the presence of a proton on the π -nitrogen and Mg on the τ -nitrogen in an overall neutral BChl *a*/His complex.

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