Photochemically Induced Nuclear Spin Polarization in Bacterial Photosynthetic Reaction Centers: Assignments of the ^{15}N SSNMR Spectra[†,‡]

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Abstract: We report proposed chemical assignments of polarized nitrogen NMR signals from photosynthetic reaction centers of *Rb. sphaeroides.* These signals, which we previously described, are observed with solid state NMR methods in samples of Q-blocked reaction centers that are enriched in ^{15}N. The CIDNP is excited by CW illumination with a Xenon arc lamp; we presume that they result from a radical pair mechanism (RPM) involving mixing of the electronic triplet and singlet spin states of $P^{\bullet+}I^{\bullet-}$. In this work selective labeling and comparison with chemical shifts of model compounds were used to assign the signals and were also used to distinguish directly and indirectly polarized signals. Signals at isotropic shifts of 163, 173, 232, and 236 ppm (relative to 1 M $^{15}NH_4Cl$ in 2 N HCl) were assigned as arising from the tetrapyrrole nitrogens of the special pair P865, and all appear to be directly polarized from the RPM. An additional small peak at 167 ppm appear to be another bacteriochlorophyll species, either the monomeric "B" or the "other half" of P865. Signals at 105, 113, and 276 ppm arise from the tetrapyrrole nitrogens of the bacteriopheophytin acceptor ("I"), and some of these signals (particularly the nitrogens in rings II and IV) seem to be directly polarized, while others are polarized by homonuclear spin diffusion involving a neighboring directly polarized nitrogen. Signals at 147 and 201 ppm arise from the δ and ϵ nitrogens of histidine, presumably from the ligand of P865, and are indirectly polarized. The intensities of the bacteriopheophytin signals are sensitive to the lifetime of ^{3}P, consistent with a RPM mechanism in which ^{3}P acts as the nuclear relaxant for I; this was concluded from comparisons of samples in which the acceptor Q_A was prereduced and samples in which is was chemically extracted, which are known from previous work to differ strongly in the lifetime of ^{3}P. Many of the signals have chemical shift values that closely correspond to related model compounds, but moderate deviations (ca. 10 ppm) are seen for a few including the histidine resonances.

Introduction

In an earlier communication we reported the observation of photochemically induced dynamic nuclear polarization (CIDNP) in the ^{15}N-solid-state nuclear magnetic resonance (SSNMR) spectra of photosynthetic reaction centers in which the forward electron transfer was blocked.[1] Recently conducted experiments involving various sample labelings, pulse sequences, and types of sample preparations that have helped to accomplish a more definite chemical assignment of the observed polarized signals are presented here.

Reaction centers from photosynthetic bacteria *Rb. sphaeroides* R-26 are three-subunit−nine cofactor proteins with several reported 3D crystal structure.[2] The cofactors, arranged in an almost C_2 symmetric configuration (see inset on Scheme 1), participate in a light initiated electron transfer chain: light excites the bacteriochlorophyll dimer (special pair, P) which transfers an electron to the bacteriopheophytin acceptor (I) which in turn delivers it to a quinone (Q_A) involved in the final electron transfer to another quinone (Q_B). Despite the symmetrical arrangement of the cofactors, only one branch participates in the electron transfer sequence. If the forward electron transfer is blocked by removing or chemically reducing Q_A, the charged separated state $^1(P^{\bullet+}I^{\bullet-})$ can recombine toward the ground state, a process that competes with a magnetic field and nuclear spin state dependent intersystem crossing to a triplet state $^3(P^{\bullet+}I^{\bullet-})$. This short-lived triplet decays to a molecular triplet ^{3}P which lives between 10 and 100 μs (depending on the presence and oxidation state of Q_A) before returning to the ground state[3] (Scheme 1).

The origin of the directionality in the electron flow in reaction centers has long been a matter of great interest.[4,5] It has recently been proposed that an unusual electric field generated by the protein environment is the driving force for this directionality and is also important for the stabilization of charge separation. Recent theoretical calculations show an asymmetry in the electrostatic potential between the two branches in reaction centers from *Rhodopseudomonas viridis*;[6] Stark effects found on the electronic absorption spectra of the cofactors are, so far, the principal direct experimental evidence.[7]

It is known that nonbonded environmental factors can have substantial effects on NMR chemical shifts. In particular,

[†] Keywords: nuclear polarization, magnetic resonance, photosynthesis, reaction centers, solid-state NMR, CIDNP.

[‡] Abbreviations: CIDNP: chemically induced dynamic nuclear polarization, I: primary acceptor, MAS: magic angle spinning, NMR: nuclear magnetic resonance, P: special pair, Q: quinone, Q-dep: quinone-depleted, Q-red: quinone-reduced, *Rb.: Rhodobacter*, RC: reaction center, RPM: radical pair mechanism, SELTICS: spinning sideband elimination by temporary interruption of the chemical shift, SSNMR: solid-state nuclear magnetic resonance.

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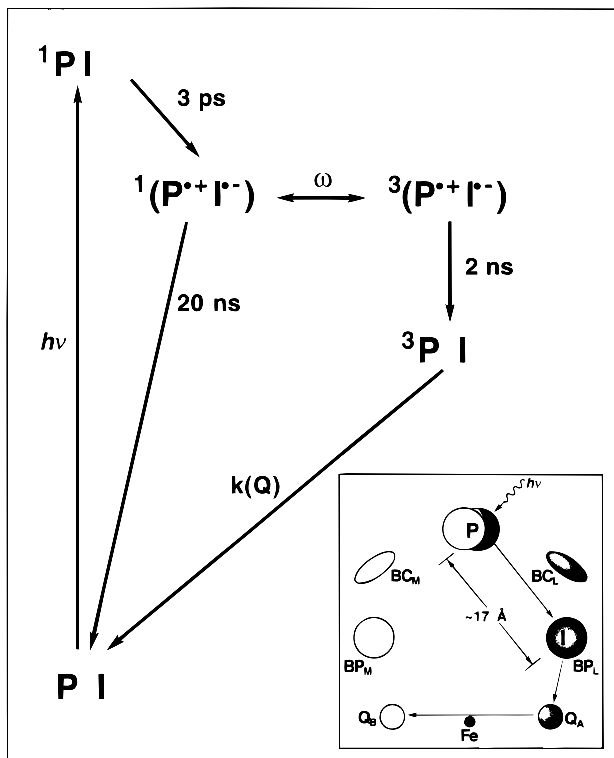
Scheme 1. Kinetic Scheme for the Photoinduced Electron Transfer in Blocked (Q-dep or Q-red) RCs[a]



[a] The electronic singlet−triplet mixing parameter ω is a function of the nuclear spin states and the strength of the external magnetic field. The triplet relaxation lifetime k(Q) depends on the presence and oxidation state of Q_A. Inset: Arrangement of the cofactors in the photosynthetic reaction center from *Rb. sphaeroides* R-26 showing the direction of electron flow in unblocked reaction centers. BC and BP stand for bacteriochlorophyll and bacteriopheophytin, respectively, while the subindexes L and M refer to the light and medium subunits of the protein, respectively.

contributions to the chemical shifts from electrostatic fields, hydrogen bonding, and local conformation in proteins have been analyzed.[8] The polarized signals that we observe come directly from the chromophores involved in the electron transfer chain and some of the surrounding amino acids, and their chemical shifts should provide an intrinsic probe for the characteristics of protein environment. Subsequent studies will be aimed at interpretation of these shifts.

Low sensitivity is one of the most important drawbacks in SSNMR of large proteins such as the photosynthetic reaction centers. Furthermore, site specific labeling of cofactors or nearby amino acids with NMR observable nuclei is normally required to overcome the problem of complexity and of spectral overlap. Nevertheless, the labeling methods can be tedious, expensive, and sometimes impossible. In spite of these complications, SSNMR has found applications in membrane biophysics; gramicidin,[9] bacteriorhodopsin, rhodopsin,[10−12] and bacterial photosynthetic reaction centers[13] can be counted among the targets. With rather simple and relatively inexpensive labeling techniques, through the polarization mechanism we obtained exceptionally strong NMR signals directly from the active site of the protein.

CIDNP is observed as emissive or enhanced absorptive lines in NMR spectra taken during chemical reactions that involve radical intermediates. The radical pair mechanism (RPM) adequately describes much of the solution phase work that has been reported and is likely to be operative here. This mechanism is relevant when nuclear spin states influence the mixing rates of electronic spin states which in turn affect the outcome of the radical reaction. A more detailed explanation and review can be found elsewhere.[14] It is noteworthy that the chemical shifts observed in these experiments correspond to the diamagnetic, ground-state species (PI), unless the steady-state population of the excited paramagnetic species is large enough to give rise to paramagnetic shifted signals.

In our last communication we suggested that the RPM, known to be responsible for the electron spin polarization in reaction centers, might also be relevant as the operative mechanism for the nuclear spin polarization. This was proposed with the knowledge that the two critical kinetic elements in the RPM for cyclic reactions are present in blocked photosynthetic RCs.[15] Namely, the singlet−triplet electronic spin mixing step can sort the nuclear spin states through the two possible reaction pathways, and the molecular triplet ^{3}P can act as an efficient nuclear spin relaxant (Scheme 1). However, we are reporting here the observation of nuclear spin polarization in the bacteriopheophytin acceptor which might appear to be in conflict with this proposed model. Rationalization of all the recent observations will require further elaboration of the polarization mechanism and the kinetic model.

Materials and Methods

Sample Preparation. Photosynthetic bacteria *Rhodobacter sphaeroides* R-26 were grown anaerobically under illumination in a medium containing NH_4Cl and malic acid as the only source of nitrogen and carbon, respectively, along with the necessary metals and vitamins, but no casamino acids were used. ^{15}N labeled reaction centers (100%) were obtained using $^{15}NH_4Cl$ (Cambridge Isotopes Laboratories, Andover, MA and Icon Isotopes, Mt. Marion, NY). The bacterial strain was a generous gift of Dr. Mel Okamura. The 20% ^{15}N labeled reaction centers were obtained from bacteria grown with a 4+1 mixture of natural abundance NH_4Cl and $^{15}NH_4Cl$ as the nitrogen source. The reverse labeling of the histidines was achieved by adding 0.20 g/L of naturally abundant histidine to the 100% ^{15}N growth medium. Reaction centers were isolated by standard procedures.[16] All the samples used for the experiments were quinone-depleted (Q-dep) unless specified. Quinone depletion was accomplished by fixing the purified RCs on a DEAE-cellulose column and washing them at room temperature during 10 h with approximately 1 L of a 15 mM Tris buffer, 1 mM in EDTA, 4% in LDAO, and 10 mM in *o*-phenanthroline (pH 8.0).[17] The protein was precipitated by exhaustive dialysis against detergent free buffer and then water. Quinone reduction was performed *in situ* by addition of a 100 mM ascorbic acid and 5 mM cytochrome *c* solution buffered at pH 8.0 with 1 M Tris and freezing under illumination. These conditions are comparable to those described previously in which a combination of sodium dithionate and cytochrome *c* is used and result in the formation of Q_A^{2-}.[18] It is noteworthy that in our hands the use of dithionate without any mediator failed to yield CIDNP signals.

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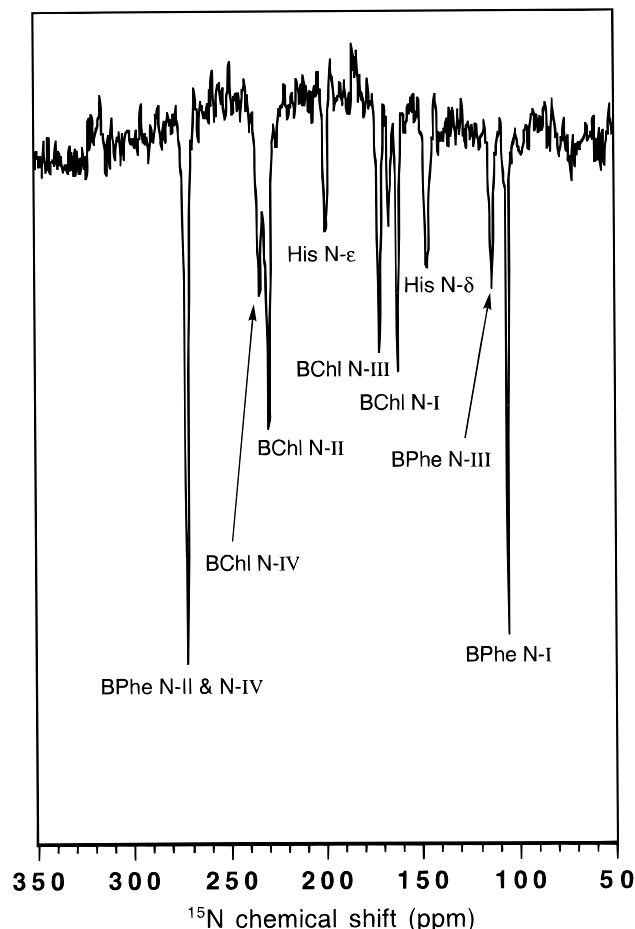

Figure 1. Light-induced polarized ^{15}N-SSNMR spectrum of 100% ^{15}N labeled quinone-depleted RCs acquired with a SELTICS pulse sequence. Only centerbands are obtained with this pulse sequence and the proposed assignments are indicated. Temperature: −45 °C. Spinning speed: 3.6 kHz.

Solid State NMR Experiments. All the experiments were carried out at −45 °C using a Chemagnetics CMX 400 spectrometer (Chemagnetics Otsuka Electronics, Ft. Collins, CO) equipped with a double resonance MAS probe, working at 396.5 MHz for proton and 40.2 MHz for nitrogen-15. Solid samples were placed inside clear sapphire rotors and continuously irradiated through a glass fiber optic with visible light from a 1000 W Xenon arc lamp (Oriel Corp., Stratford, CT), filtered through a copper sulfate solution and IR absorbing filters. Roughly 1 to 5 W of actinic light arrived at the sample under these conditions. Spectra consisting of 1000 to 15 000 transients were collected using a single 90 degree pulse (typically 5−7 μs) with rotor synchronized echo detection, proton decoupling and a pulse delay of 15 s. Sideband suppressed spectra were acquired employing a SELTICS pulse sequence.[19] $^{15}NH_4Cl$ (1 M) in 2 N HCl was used as an external chemical shift reference.

Results and Discussion

The light- induced polarized spectrum of the quinone-depleted 100% ^{15}N- labeled RCs obtained with a sideband suppression pulse sequence (SELTICS) is shown in Figure 1. The observed peaks correspond only to the centerbands. All of the proposed assignments indicated in the following are based on available chemical shift data and comparison with previously characterized systems,[20] and some are confirmed by additional experiments described below. The peaks at 163, 173, 232, and 236 ppm strongly resemble reported chemical shifts obtained with cross-polarization MAS from a solid sample of bacteriochlorophyll[20] as well as those from bacteriochlorophyll in solution.[21] We tentatively assign these peaks to the nitrogen nuclei on one of the bacteriochlorophylls that form the special pair, most likely the one that carries the larger electron density, although additional bacteriochlorophyll species in the reaction center could also contribute. The peak at 167 ppm is also in the range expected for bacteriochlorophyll but is not directly polarized (see below) and could be assigned to a nitrogen nucleus in an additional tetrapyrrole ring in the special pair or in the monomeric bacteriochlorophyll. The centerbands at 105, 113, and 276 ppm are attributed to the nitrogens on the bacteriopheophytin acceptor, in consideration of the similarity of their chemical shifts to those of a sample of bacteriopheophytin in THF.[20] The two remaining signals at 147 and 201 ppm are assigned to the δ and ϵ nitrogens of one of the histidines coordinating the magnesium of the bacteriochlorophyll in the special pair. Table 1 summarizes these assignments and compares them to the existing literature values.

Table 1. Assignments of ^{15}N-SSNMR Polarized Signals

proposed species[e]	lit. chemical shift (ppm)		obsd centerbands chemical shift (ppm)[f]
BChl (N-I)	167[a]	166−171[b]	163 (m[g],d[h]) & 167 (w,i)
BChl (N-II)	234[a]	234−241[b]	232 (m,d)
BChl (N-III)	175[a]	166−174[b]	173 (m,d)
BChl (N-IV)	243[a]	235−240[b]	236 (m,d)
BPhe (N-I)	110[c]		105 (m,i)
BPhe (N-II)	279[c]		276 (s,d)
BPhe (N-III)	115[c]		113 (w,i)
BPhe (N-IV)	289[c]		276 (s,d)
histidine (N-δ)	147−166 (H-bonded)[d]		147 (m,i)
histidine (N-ϵ)	211 (H-bonded)-228[d]		201(m,i)

[a] Bönigk, B. Thesis, Technische Universität Berlin, 1994. The ^{15}N chemical shifts reproduced correspond to a solid sample of bacteriochlorophyll-a, collected with CP-MAS and given here relative to 1M $^{15}NH_4Cl$ in 2 N HCl. [b] ^{15}N Chemical shifts for bacteriochlorophyll in different solvents: Limantara, L.; Kurimoto, Y.; Furukawa, K.; Shimamura, T.; Utsumi, H.; Katheder, I.; Scheer, H.; Koyama, Y. *Chem. Phys. Lett.* **1995**, *236*, 71−77. [c] Same source as *a*. The ^{15}N chemical shift values originate from a solution of bacteriopheophytin-*a* in THF relative to a 5 M solution of $^{15}NH_4Cl$ in 2 N HCl. [d] Smith, S. O.; Farr-Jones, S.; Griffin, R. G.; Bachovchin, W. W. *Science* **1989**, *244*, 961−964. Please note that ^{15}N shift data for Mg-Imidazole compounds have not been located; these data are for imidazole in proteins and organic H-bonded environments. [e] BChl: bacteriochlorophyll; BPhe: bacteriopheophytin. [f] Relative to 1 M $^{15}NH_4Cl$ in 2 N HCl. [g] w, m, s: weak, medium and strong intensity. [h] d, i: directly and indirectly polarized (N−N homonuclear polarization transfer).

Even allowing for cases with strong hydrogen bonding, electrostatic fields, and polarizable molecules, the match of our shifts with the characteristic range of chemical shift values for bacteriochlorophyll and bacteriopheophytin is unambiguous. It is highly improbable, assuming diamagnetic complexes, that other functional groups would be so perturbed as to appear in these ranges, given that typical environmental perturbations are maximally of the order of 10 ppm. For example, the dependence of ^{15}N chemical shifts in bacteriochlorophyll on the polarizability of the solvent showed a maximum deviation of only about 7.5 ppm.[21] This modest dependence of shift on environmental factors may also preclude resolution of the two bacteriochlorophylls on the special pair, regardless of the asymmetry of the environment that surrounds them.

In this regard it is worthwhile considering the effects of a finite steady-state population of paramagnetic species in rapid

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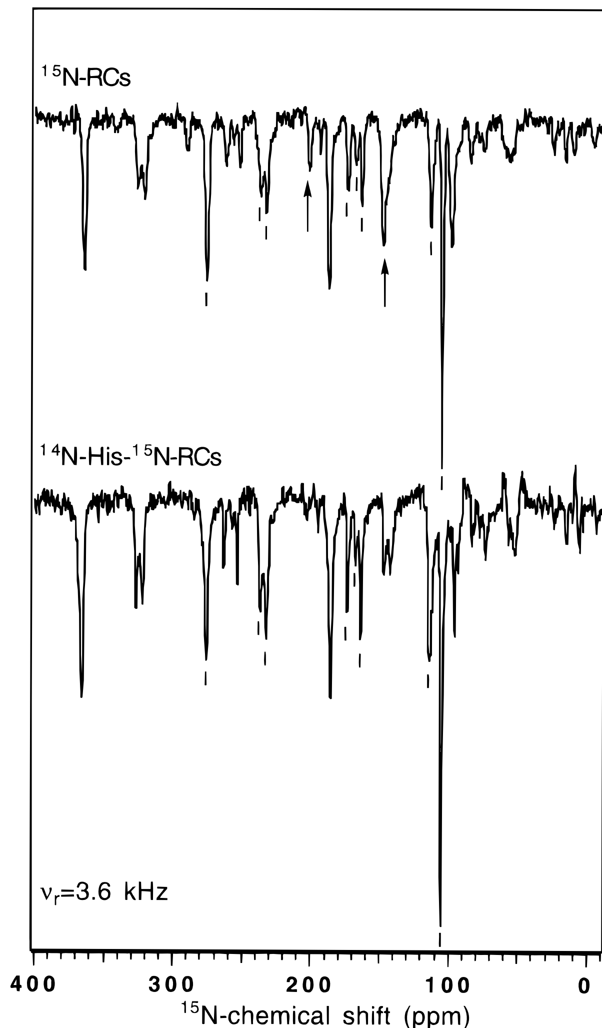

Figure 2. Light-induced polarized ^{15}N-SSNMR spectrum of the ^{14}N-His-^{15}N-Q-dep RCs (bottom) compared to the nonspecifically labeled sample (top). Centerbands are indicated with small vertical bars. The missing signals in the polarized spectrum of the reverse labeled sample are indicated with arrows in the spectrum of the nonspecifically labeled sample. Temperature: −45 °C. Spinning speed: 3.6 kHz.

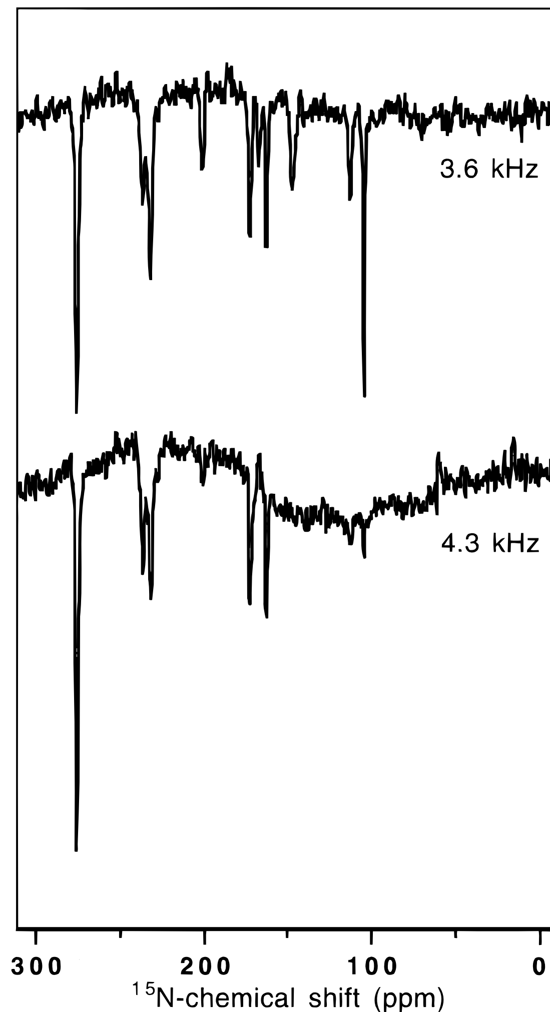

Figure 3. Sideband-suppressed polarized ^{15}N-SSNMR spectra of 100% ^{15}N labeled quinone-depleted reaction centers at two different spinning speeds: 3.6 kHz (top) and 4.3 kHz (bottom). Temperature: −45 °C.

chemical exchange with the ground state species. In consideration of the kinetic parameters involved in this photochemical process, an estimate of the steady-state concentration of the paramagnetic species ^{3}P provides a value much less than 1% of total P with 5 W of incident light. Together with the hyperfine coupling constant for ^{15}N in the special pair in the order of 2 MHz[22] and the estimate for the paramagnetic shift ($\Delta\nu_{app}$) in the NMR spectrum of pure ^{3}P ($\Delta\nu_{app} \approx Ag\beta H/4kT$; with A the hyperfine coupling constant, β the Bohr magneton, H the magnetic field, k the Boltzman constant, T the absolute temperature, and $g \approx 2$),[23] they indicate that the kinetically averaged (apparent) paramagnetic shift of the signals, if any, would be less than 2 ppm. In addition k(Q) in Scheme 1 is 10^4 s^{-1}, so the averaging of ^{3}P and P is not a fast-limit process. Any ^{3}P NMR lines could be simply broadened and undetected.

Verification of the histidine assignments was achieved by conducting an experiment with a selectively reverse-labeled sample. In the preparation of this sample, the sources of nitrogen for the bacteria were $^{15}NH_4Cl$ and natural abundance histidine, yielding a protein that was ^{15}N labeled on all the nitrogens with the exception of those on the histidines which remained as ^{14}N. Thus, signals associated with histidine would be edited out of the ^{15}N NMR spectrum. The polarized spectrum of the ^{14}N-histidine-^{15}N RCs appears in Figure 2 where it is compared to the polarized spectrum of the nonspecifically labeled sample. The arrows on the spectrum corresponding to the nonspecifically labeled sample indicate those peaks that we assign as histidine since they do not show up on the spectrum of the reverse-labeled sample. These peaks also coincide with those assigned to histidine nitrogens based on chemical shifts.[24] The one at 147 ppm corresponds to a weakly hydrogen bonded nitrogen in the δ position of the histidine (bearing a hydrogen), while the other at 201 ppm arises from the nitrogen in the ϵ position (not bearing a hydrogen but presumably coordinated to the magnesium). The signal at 201 ppm is far below the value for a N-ϵ participating in a strong hydrogen bond, and the coordination to the magnesium cation on the bacteriochlorophyll or other perturbations from the protein environment could be responsible for this effect.

As we pointed out in our previous communication, the use of decoupling from protons does not have a dramatic effect in the appearance of most of the polarized signals. The major difference (data not shown) between proton-decoupled and undecoupled experiment is experienced by the bacteriopheo-

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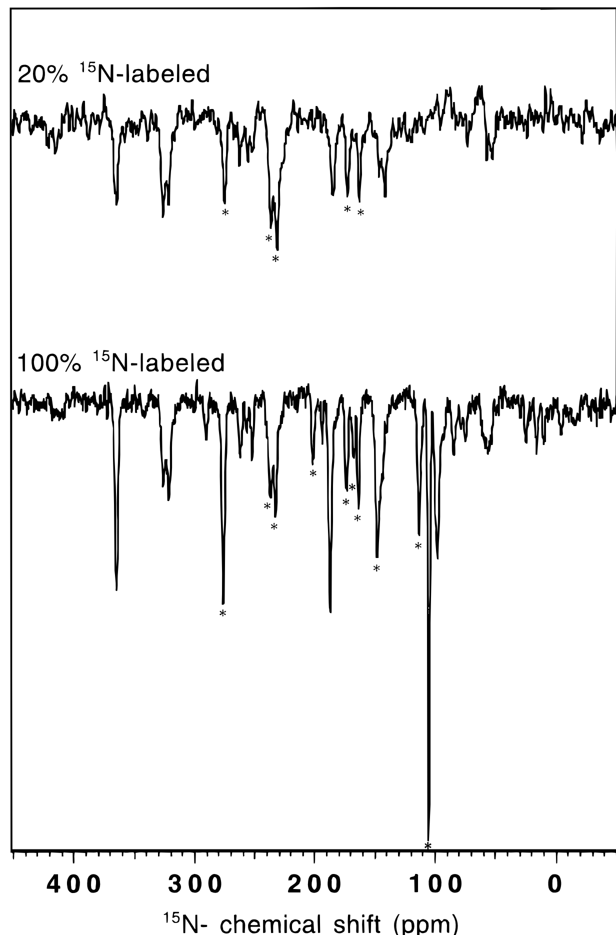

Figure 4. Light-induced polarized ^{15}N-SSNMR spectra of 100% ^{15}N labeled RCs (bottom) and 20% ^{15}N labeled RCs (top). Centerbands are denoted with stars. Only 5 out of 10 polarized signals are observed in the partially labeled sample which indicates that the missing peaks arise from indirectly polarized nuclei, such as the peaks from histidine and bacteriopheophytin (see main text and Table 1). Temperature: −45 °C. Spinning speed: 3.6 kHz.

phytin peak located at 113 ppm, which completely disappears in the absence of decoupling. A decrease in the intensity of the bacteriopheophytin at 105 ppm is also observed, while the remaining bacteriopheophytin signal at 276 ppm is unaffected. It is noteworthy that the signals affected by the absence of proton decoupling, those of N-I and N-III, have a proton directly attached. The corresponding information for the histidine peaks is unavailable at the moment.

Some of the observed signals are from nuclei that are *directly* polarized in the RPM due to the strong couplings with unpaired electrons. It is conceivable that others are *indirectly* polarized, meaning that they obtain polarization from neighboring directly polarized nuclei via a spin diffusion process. Two experiments helped to distinguish directly and indirectly polarized nitrogen nuclei (Figures 3 and 4).

When light-induced polarized spectra were collected at different spinning speeds, severe changes in the intensities of some of the centerbands and even disappearance of some of them was observed (data not shown). Since certain changes could be hidden under spinning sidebands, we appealed to the sideband suppression pulse sequence. Figure 3 shows the comparison of sideband suppressed polarized spectra recorded at two different spinning frequencies. The peaks at 105, 113, 147, 167, and 201 ppm that are present at a spinning speed of 3.6 kHz either disappear or are severely weakened at a higher speeds, which might indicate that these peaks originate from

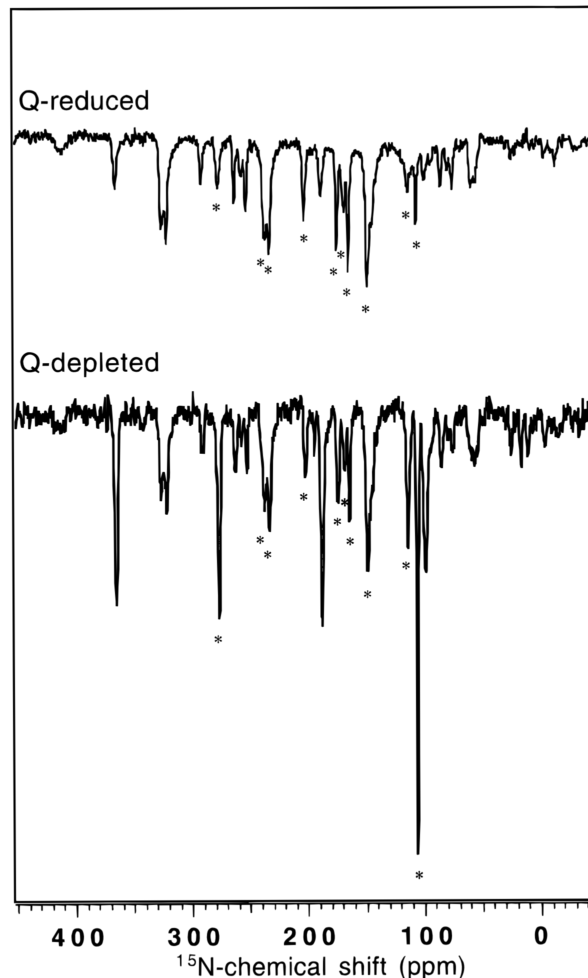

Figure 5. Light-induced polarized ^{15}N-SSNMR spectra of quinone-reduced (top) and quinone-depleted (bottom) RCs. Centerbands are denoted with stars. Note the dramatic intensity changes for the lines associated with bacteriopheophytin at 110 and 276 ppm. These samples differ mainly in the triplet lifetime of the special pair: ∼10−50 μs in Q-red RCs and ∼100 μs in Q-dep RCs. Temperature: −45 °C. Spinning speed: 3.6 kHz.

homonuclear polarization transfer. In order to corroborate this observation an experiment was carried out on a sample in which only 20% of the nitrogens in the protein were labeled with ^{15}N. Under these conditions the likelihood that a ^{15}N nucleus has another ^{15}N as a close neighbor is dramatically reduced, and homonuclear polarization transfer is suppressed. The polarized nuclear magnetic resonance spectrum obtained with this sample is shown in Figure 4 and is contrasted to the polarized spectrum of the nonspecifically labeled sample. The same peaks that vanished as the spinning frequency was increased are also absent in the spectrum of the 20% ^{15}N labeled sample, thus strengthening the statement that these peaks arise from homonuclear polarization transfer. The ^{15}N nuclei that give rise to the signals at 147, 167, and 201 ppm are most likely polarized from the ^{15}N on one of the bacteriochlorophylls of the special pairs. The nitrogen on the bacteriopheophytin that gives rise to the peak at 276 ppm could be the origin for the polarization on the nitrogens corresponding to the peaks at 105 and 113 ppm. We envision the magnetization transfer as a "rotational-resonance" phenomenon.[25] Simulations of the expected rates of magnetization transfer (including linewidth effects) show that such a

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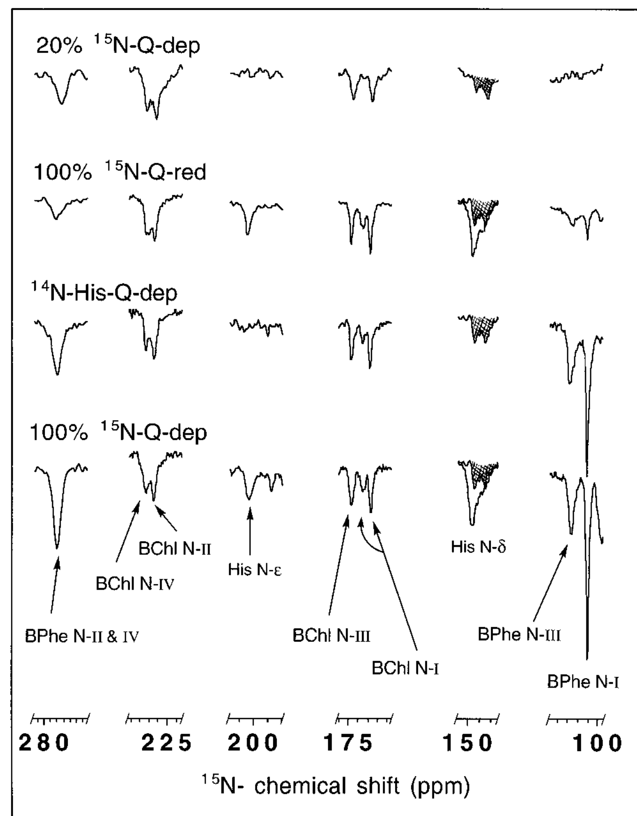

Figure 6. Summary of the results from four different samples showing only the centerbands with the proposed assignments. From top to bottom: partially ^{15}N labeled, totally ^{15}N labeled quinone-reduced, histidine reverse labeled, and totally ^{15}N labeled quinone-depleted RCs. The shadowed peaks under the signal at 147 ppm are spinning sidebands of the signals at 232 and 236 ppm. Normalization was based on the centerband intensities for bacteriochlorophyll in the special pair (which can be expected to increase in the case of the 20% labeled sample for which polarization transfer to bacteriopheophytin and histidine has been suppressed). Also note that the signals whose centerbands appear at around 230 and 276 ppm have large chemical shift anisotropies (nitrogens II and IV in the macrocycles) which would increase their size by about a factor of 4 if the spinning sidebands were included in the integration. All the experiments showed in this figure were collected at −45 °C, have a spinning speed of 3.6 kHz, and were referenced to 1 M $^{15}NH_4Cl$ in 2 N HCl. For the numbering of the nitrogens in the heterocycles see Figure 7.

process would be feasible. Curiously, the peak at 276 ppm is also somewhat attenuated in the 20% ^{15}N labeled sample when compared to the nonspecifically labeled sample. If this peak is also due to a polarization transfer process, we still do not have an appropriate candidate for the partner nucleus, since the other nitrogens that are directly polarized are too far away from the bacteriopheophytin to transfer polarization. It must be noted however that the spectra are somewhat arbitrarily normalized to the bacteriochlorophyll peaks, and unfortunately absolute integrals are unavailable. These bacteriochlorophyll peaks could be poor intensity standards since they also participate in a polarization transfer process in some of the samples and not in others. In consideration of this possibility we suggest the bacteriopheophytin peak at 276 ppm is larger than it appears in the "normalized" spectrum of the 20% labeled sample.

Figure 5 shows a comparison of the polarized spectra of the quinone-depleted (Q-dep) nonspecifically labeled sample and another nonspecifically labeled sample in which the quinone acceptor is present but chemically reduced (Q-red). The polarized signals from these two samples have identical chemical

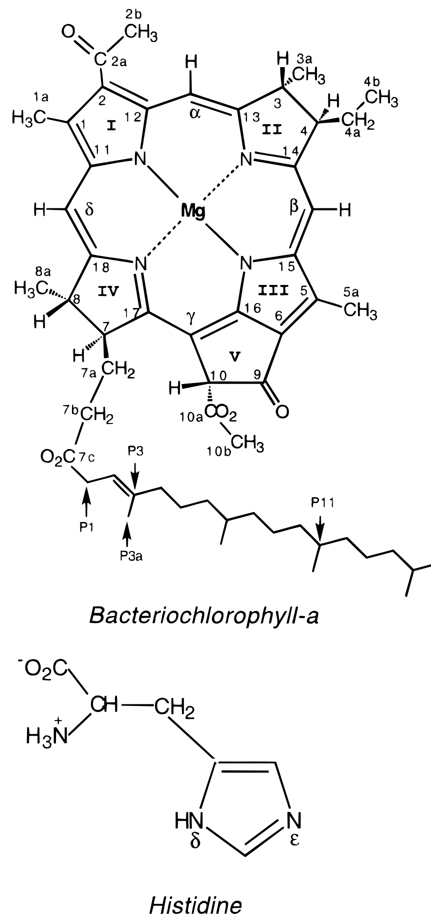

Figure 7. Structures of bacteriochlorophyll-*a* (top) and histidine (bottom). Bacteriopheophytin-*a* has the same structure and numbering as bacteriochlorophyll-*a* except that the magnesium is replaced by two protons on nitrogens I and III.

shifts and those arising from the bacteriochlorophylls and the histidine appear with unchanged intensity, while those coming from the bacteriopheophytin are strongly attenuated. The fact that the chemical shifts are unchanged indicates the the quinone-depletion procedure has no considerable effect on the environment of the primary reactants.

All these observations concerning the effects of the experimental conditions and sample preparations on the polarized spectra are summarized in Figure 6 where only the centerbands are shown. In this figure all the signals are normalized relative to the intensities of the bacteriochlorophyll centerbands. It should be noted that the integrated intensities of the signals should include the spinning sidebands which would substantially increase the size of the peaks corresponding to N-II and N-IV in the macrocycles (approximately by a factor of 4).

It is of clear interest to establish whether the bacteriochlorophyll and histidine signals arise from both sides of the dimer and also whether any signals arise from the bacteriochlorophyll monomer species. If the signals originate from both halves of the special pair they appear to be unresolved, with the exception of the small peak at 167 ppm. Unfortunately, as pointed out before, the data are presently equivocal on these points. In this respect, our assignments are not "site specific" but are indicative of chemical species.

We previously proposed that these signals arise through a radical pair mechanism in which the cancellation of polarization normally caused by the cyclic electron transfer reaction would be avoided by the relatively fast nuclear spin relaxation in the molecular triplet localized on the special pair. In that case, peaks

from the nitrogens in the bacteriochlorophylls that form P would be expected, but those in the bacteriopheophytin would not. In consideration of the new results the proposed mechanism should be modified to account for the bacteriopheophytin signals observed. A possible explanation for the appearance of the ^{15}N signals of the bacteriopheophytin is that the paramagnetic molecular triplet ^{3}P can also act as a nuclear relaxant for the nuclei on the bacteriopheophytin despite the long distance between P and I. The enhancement factor previously reported[1] of −300 times Boltzman is still a lower limit. In a forthcoming paper we will discuss the mechanism of nuclear spin polarization in blocked reaction centers as well as the intensities of the polarized signals in more detail.

With some of the chemical assignments in hand, it is possible to focus on interpretation of chemical shifts and further clarification of the polarization mechanism.

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