Photochemically Induced Dynamic Nuclear Polarization in the Solid-State ^{15}N Spectra of Reaction Centers from Photosynthetic Bacteria *Rhodobacter sphaeroides* R-26

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We present here a novel application of solid-state nuclear magnetic resonance (SSNMR) to bacterial photosynthesis: we have observed photochemically induced dynamic nuclear polarization (photo-CIDNP) in the ^{15}N-SSNMR-magic-angle spinning (MAS) spectra of reaction centers from photosynthetic bacteria. In nonspecifically ^{15}N-labeled reaction centers, when forward electron transfer was blocked either by removal (Q-dep) or prereduction (Q-red) of the quinone acceptor, strongly emissive ^{15}N signals were observed (Figure 1). As elaborated below, we attribute these signals to the tetrapyrrole nitrogens of the ground state of the special pair P. CIDNP is a well-known effect in solution NMR and is utilized for studying the mechanisms of photochemical reactions. Most CIDNP signals have been explained by the radical pair mechanism,[1] which postulates that if the outcome of a photochemical reaction depends on the extent of singlet–triplet mixing in some intermediate and if the mixing is partly driven by the hyperfine coupling, then the products could have strongly polarized NMR signals. The polarization for one product is generally emissive, and the other is enhanced absorptive. In contrast to the large number of mechanistic studies by solution-state CIDNP, to our knowledge, this is the first observation of S–T_0 CIDNP in the solid state and is certainly the first application for studying membrane biophysics. SSNMR is a rapidly growing technique in the study of membrane proteins and has already been applied to bacteriorhodopsin and rhodopsin,[2,3] gramicidin,[4] and bacterial photosynthetic reaction centers (RCs).[5,6]

Reaction centers from *Rhodobacter sphaeroides* R-26 contain two bacteriochlorophylls $(BChl)_2$ forming the so-called special pair (P), two accessory bacteriochlorophylls, two bacteriopheophytins ($BPhe_L$ and $BPhe_M$), two quinones (Q_A and Q_B), and a non-heme divalent iron. Although these cofactors are arranged almost C_2 symmetrically in the protein,[7] light-driven electron transfer normally proceeds from P through $BPhe_L$ (frequently referred to as I, the primary acceptor) to Q_A, so early electron transfer steps involve electron acceptor cofactors on one side of the protein only. The electron transfer kinetic scheme for reaction centers in which the quinones are removed or prereduced is shown in Scheme 1. The state $P^{\bullet+}$ $I^{\bullet-}$ that would normally undergo forward electron transfer from I to Q_A must choose between two normally unimportant processes: recombination to form the ground state and mixing to triplet states, which is described by the magnetic field dependent singlet–triplet mixing parameter ω.[8] The triplet also decays relatively slowly back to the ground state. Of all the species in this kinetic scheme, only the diamagnetic and long-lived ground states P and I are expected to be observable by NMR, and in the following we discuss the origin of nuclear polarization in the ground state of a photocycling system.

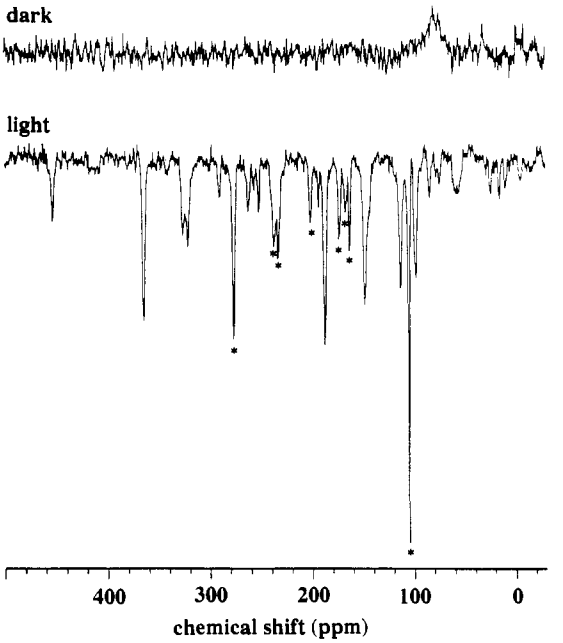
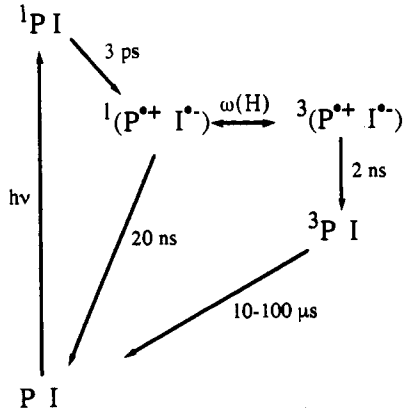


Figure 1. ^{15}N-SSNMR spectra of ^{15}N-labeled, Q-depleted reaction centers at −45 °C in the dark (upper) and under illumination (lower). Spinning speed, 3600 Hz. Q-removal was performed, and final quinone occupancy was measured by time-resolved photobleaching[19] and was found to be about 5%. Centerbands are marked with asterisks (see text). Chemical shifts are relative to an external reference of 1 M $^{15}NH_4Cl$ in 2 M HCl. The spectra (1280 transients) were acquired in a Chemagnetics CMX 400 spectrometer (Chemagnetics Otsnka Electronics, Ft. Collins, CO) operating at 40.176 MHz for ^{15}N using a single 90° (7.5 μs) pulse and rotor-synchronized echo detection with proton decoupling. Solid protein samples were placed inside a transparent sapphire rotor, and illumination was accomplished using the visible radiation from a 1000-W Xe lamp (Oriel Corp., Stratford, CT) passed through a copper sulfate solution and an IR absorbing filter and continuously delivered by means of a glass fiber optic installed inside the spectrometer probe.

Scheme 1. Kinetic Scheme for the Initial Photochemistry in Q-Depleted (or Q-Reduced) RCs[a]



[a] *Rhodobacter sphaeroides* R-26 (carotene-less mutant) bacteria were photosynthetically grown in a medium containing malic acid and ^{15}N-ammonium chloride (Icon Isotopes, Mt. Marion, NY) as the sole sources of carbon and nitrogen, respectively. Nonspecifically ^{15}N-labeled RCs were isolated by standard procedures[18] and precipitated by dialysis against a suspension of Bio-Beads SM-2 (Bio-Rad, Richmond, CA) in water. Purity was checked by means of UV–visible spectroscopy ($A_{280}/A_{802} < 1.35$) and polyacrylamide gel electrophoresis.

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(2) For example, see Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Courtin, J. M. L.; Lugtemburg, J.; Herzfeld, J.; Mathies, R. A.; Griffin, R. G. *Biochemistry* **1985**, *24*, 6955–6962.
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This reaction scheme shows the critical ingredient for the CIDNP effect, namely that two distinct photochemical pathways are in kinetic competition and that at the branching point a singlet–triplet mixing involving hyperfine couplings controls the relative yields of the two processes. Thus the nuclear spin state in part dictates the decision that a molecule makes at that branch point. The prompt recombination pathway could be associated with a large nuclear polarization, while the triplet pathway could be associated with a large and opposite polarization. More specifically, for the acceptor I, an excess population of a particular nuclear spin state occurs for the singlet intermediates, while triplet intermediates are correspondingly enriched in the opposite nuclear spin state. However, at the end of the reaction, both pathways produce the NMR-observable ground state I, and the emissive and absorptive signals should approximately cancel, so actually no net observable polarization is expected for the ground state. The situation for P is significantly different: relatively efficient nuclear spin relaxation in the long-lived triplet state ^{3}P would be expected to obliterate the nuclear polarization in those molecules that react via triplets. In that case, the strongly polarized signal for the prompt singlet recombination pathway should be observed. In solution CIDNP experiments involving cyclic reactions, strong polarization has been observed which has been attributed to such a mechanism.[9]

For reactions in solution where the radical pair mechanism is operative, the sign of CIDNP signals (enhanced absorption or emission) can be predicted by a simple multiplicative sign rule.[10] Even if it is not clear that these rules are applicable in our case, it is noteworthy that the emissive phase of the signals can be rationalized with them when the negative magnetogyric ratio of ^{15}N is considered.[11-14] The experimental intensity is approximately −300 times thermal. A crude estimate of the expected intensities (assuming the CIDNP is proportional to the ratio of the hyperfine coupling and the difference in the *g* factors) predicts a polarization on the order of −1000 times the thermal polarization, in reasonable agreement with the observation. Due to the anisotropy of the system under study, a more detailed description of the polarization mechanism should take into account both the tensorial nature of the hyperfine couplings and the anisotropy of the magnetic field-unpaired electron interactions (*g* anisotropy). Computer simulations that take these factors under consideration are in progress.

Dark and light SSNMR spectra of Q-depleted ^{15}N-RCs are shown in Figure 1. The spectrum taken under illumination shows signals due to at least eight species whose chemical shift values (centerbands) are marked with asterisks. The unmarked peaks are spinning sidebands which do not correspond to different chemical species but result normally in SSNMR from the magic-angle spinning methods utilized to narrow the lines. Centerbands were identified on the basis of their consistent appearance in spectra taken at several spinning speeds. Further experiments should be performed for an unambiguous identification.

Only amide signals from the protein backbone are observed in the dark spectrum. The polarized spectra include strong signals centered at 105, **167**, **169**, **174** , 201, **233**, **237**, and 276 ppm, while the reported signals for chlorophyll *a* in solution are at 163.6, 166.4, 183.5, and 224.0 ppm,[15] relative to aqueous $^{15}NH_4Cl$ in HCl. We tentatively assign the five signals listed in boldface to the ^{15}N nuclei of the special pair P. The remaining signals might be attributed to nitrogens of nearby groups that are polarized by a spin diffusion or rotational resonance process,[16] such as deprotonated histidines (276 ppm), tryptophan, or amide backbone (105 ppm), but some of the observed shifts would indicate substantial environmental perturbations (201 ppm).

The ^{15}N-SSNMR spectrum of Q-containing ^{15}N-labeled RCs with illumination shows no polarized signals, but when Q_A in this sample was then reduced by addition of ascorbic acid and cytochrome *c* and freezing while the sample was being illuminated, strongly polarized emissive signals are also detected (spectra not shown). Compared to the spectrum from the Q-depleted experiment, the same centerbands are observed but with different relative intensities. The difference in the relative intensities of the polarized spectra of Q-dep and Q-red RCs probably results from the fact that the triplet lifetime of Q-red RCs is about 1 order of magnitude shorter than the triplet lifetime of Q-dep RCs.[17] For Q-red RCs, the emissive polarization from the singlet pathway is apparently partially canceled by remaining absorptive polarization from the triplet pathway, and this cancelation affects each nucleus to a different extent, dictated by the relaxation phenomena in the triplet state.

Our data could provide information about the electron density in P in the radical pair if the intensities are proportional to hyperfine couplings and the couplings are in turn proportional to unpaired spin densities. These couplings could differ from those for the stable oxidized radical cation ($P^{+\bullet}I$) derived from EPR measurements.[12] Our measurements also provide the chemical shifts of the nuclei of the ground state of the special pair and some of the nearby protein groups so that interactions at the active site could be selectively studied. It is unusual to obtain signals of such intensity from the active site of a large protein.

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