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Solid-State Photo-CIDNP Effect Observed in Phototropin LOV1-C57S by ^{13}C Magic-Angle Spinning NMR Spectroscopy

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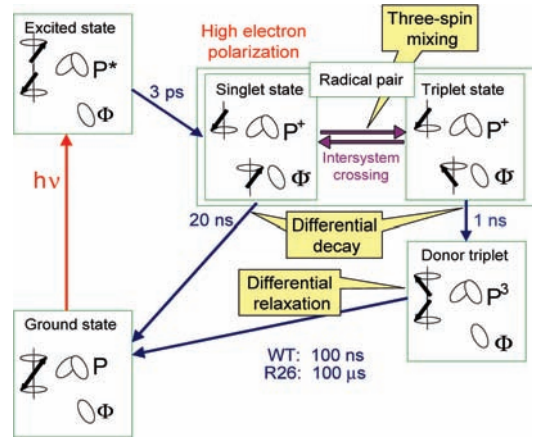
Abstract: Until now, the solid-state photo-CIDNP effect, discovered in 1994 by Zysmilich and McDermott, has been observed selectively in photosynthetic systems. Here we present the first observation of this effect in a nonphotosynthetic system, the blue-light photoreceptor phototropin LOV1-C57S using ^{13}C magic-angle spinning (MAS) NMR.

The solid-state photochemically induced dynamic nuclear polarization (photo-CIDNP) effect was observed for the first time in 1994 by McDermott's group in frozen and quinone-blocked bacterial reaction centers (RCs) of ^{15}N-labeled *Rhodobacter* (*Rb.*) *sphaeroides* R26 by magic-angle spinning (MAS) NMR.[1] NMR signals can be enhanced by a factor of more than 10 000 due to non-Boltzmann nuclear polarization.[2,3] Since then, the effect has also been shown in various other RCs including those of algae[4] and plants,[5,6] and even at a cellular level.[3,4] Following unsuccessful attempts to observe polarization in nonphotosynthetic systems, it has been discussed whether the effect might be confined to natural photosynthetic RCs.[7] Here we demonstrate that the solid-state photo-CIDNP effect can also be observed in a rather different, nonphotosynthetic protein, a mutant of the light-, oxygen-, or voltage-sensitive (LOV) domain of the blue-light photoreceptor phototropin. Signals were recorded from the flavin cofactor without isotopic enrichment.

Photo-CIDNP is well-known in solution NMR and was explained by the radical pair mechanism (RPM)[8] soon after its discovery in 1969.[9] The RPM is based on molecular diffusion and thus cannot be the proper explanation for the solid-state photo-CIDNP effect. This phenomenon is interpreted by up to three mechanisms running in parallel, three-spin mixing (TSM),[10] differential decay (DD),[11] and differential relaxation (DR)[12] (Scheme 1),[13] which transfer the initial electron-spin order of the spin-correlated radical pair[14] to nuclear spins. In illuminated RCs, an electron is transferred from the excited electron-donor P* to a primary acceptor Φ forming an electron-spin-polarized singlet radical pair $^1(P^{+\bullet}\ \Phi^{-\bullet})$ undergoing intersystem crossing (ISC) to a triplet radical pair $^3(P^{+\bullet}\ \Phi^{-\bullet})$. The electron polarization is transferred by TSM to nuclear polarization during ISC via the anisotropic parts of the hyperfine interactions and the coupling between the electrons. The different lifetimes of singlet and triplet states in the radical pair contribute to the nuclear polarization by the DD mechanism. The DR mechanism occurs in RCs such as R26 that have a long-lived donor triplet state.

Phototropin is a member of the family of flavin-containing blue-light photoreceptors and regulates key responses of plants to light, such as phototropic movement and chloroplast relocation.[15] Phototropin comprises two LOV domains, each binding noncovalently a flavin mononucleotide (FMN), and a kinase domain. Upon illumination, the triplet excited state of the flavin reacts with a nearby cysteine residue to form a covalent adduct as the signaling state.[16] Mutation of the reactive cysteine to serine or alanine abolishes this adduct formation. Instead, a less efficient competing pathway of electron transfer from a tryptophan leads to transient accumulation of a flavin anion radical on illumination[17,18] and finally to formation of a flavin neutral radical.[19] The radical is reoxidized by oxygen. We investigated the mutant, C57S, of the phototropin-LOV1 domain from the green alga *Chlamydomonas reinhardtii* (Figure 1).[20]

Scheme 1. Kinetics and Spin Dynamics of Solid State Photo-CIDNP in *Rb. sphaeroides* R26 and Wild-Type (WT) RCs (for Details, See Text)



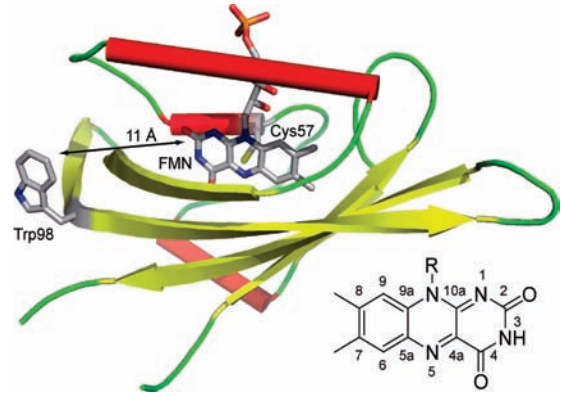


Figure 1. Structure of the phototropin LOV1 domain in the dark (PDB 1N9L).[21] Cysteine 57 was replaced by serine in this study. The numbering of the flavin chromophore is included.

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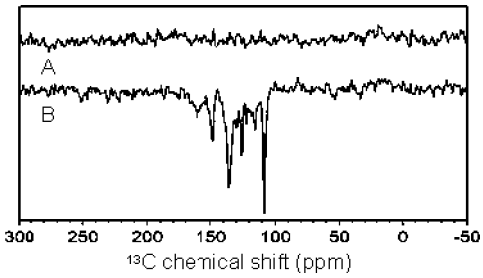

Figure 2. ^{13}C MAS NMR spectra of phototropin LOV1-C57S obtained with 8 kHz MAS at a magnetic field of 2.3 T in the dark (A) and under continuous illumination with white light (B).

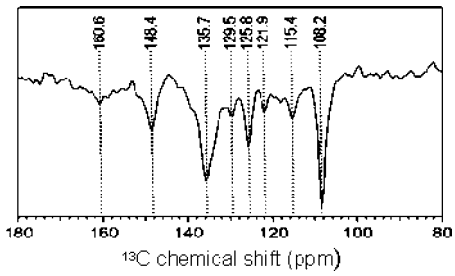

Figure 3. Expanded view on the aromatic region of the ^{13}C MAS NMR spectrum of phototropin LOV1-C57S showing the solid-state photo-CIDNP effect (spectrum 2B).

In Figure 2, the ^{13}C MAS NMR spectra of phototropin LOV1-C57S obtained in the dark (A) and under illumination (B) are shown. Both spectra were measured at 2.3 T (i.e., 100 MHz ^1H frequency) using a spinning frequency of 8 kHz. At a set temperature of 235 K, the sample was entirely frozen as monitored by the NMR tuning frequency. A simple Hahn-echo pulse sequence with two-pulse phase-modulation proton-decoupling was used. Continuous illumination was supplied by a 1 kW xenon lamp.[22] The cycle delay was 2 s, and the measurement time was about 12 h. In the dark no resonance signal was detected, but under illumination several strong signals appear in the aromatic region. An enlarged view of the aromatic region of the solid-state photo-CIDNP spectrum is presented in Figure 3. All light-induced ^{13}C NMR peaks are emissive (negative). This pattern is reminiscent of the photo-CIDNP MAS NMR spectra obtained from RCs of *Rb. sphaeroides* WT[2] and of photosystem I[5] and contrasts with the mixed absorptive/emissive enhancement pattern observed by liquid-state photo-CIDNP of a LOV2 sample.[18] A preliminary assignment of these peaks can be obtained by comparison with the ^{13}C chemical shifts of FMN in solution and in the LOV2 domain of *Avena sativa* phototropin obtained by ^{13}C liquid-state NMR (Table S1).[17] Six of the eight light-induced signals can be assigned to the ten aromatic carbons in the FMN cofactor, with four pairs of overlapping resonances (C2 and C4, C5a and C9a, C6 and C9, C7 and C8). The two additional signals at 108.2 and 115.4 ppm appear upfield of the others and probably do not arise from FMN. As known from solid-state photo-CIDNP studies on RCs, polarized signals are observed from both the electron donor and the electron acceptor. Hence, we assume that at least these two signals arise from the electron donor in the spin-correlated radical pair. In fact, the two peaks can be assigned to C^{γ} and $C^{\zeta 2}$ of a tryptophan residue (Trp) (Table S2),[23] while no match for the resonance at 108.2 ppm would be expected from the two other possible electron donors, histidine and tyrosine. Hence, we conclude that we observed nuclear polarization originating from a light-induced [$FMN^{-\bullet}$ $Trp^{+\bullet}$] radical pair. Trp at position 98 (Figure 1), the only Trp in the protein, is at an about 11 Å edge-to-edge distance from FMN and, thus, at a suitable distance for electron transfer.

To conclude, we have demonstrated that the solid-state photo-CIDNP effect is not a peculiarity of photosynthetic systems but can arise in at least one other photoactive protein. In the same way that photo-CIDNP MAS NMR has provided detailed insights into photosynthetic electron transport in RCs,[1−7] we anticipate a variety of applications in studies of the functionality of blue-light photoreceptors. For example, it may be possible to characterize in detail the photoinduced flavin and tryptophan radicals in cryptochrome, the flavoprotein that has been proposed as the radical pair magnetoreceptor for the avian magnetic compass.[24]

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Supporting Information Available: LOV1 expression and purification. NMR measurement details. Table S1: ^{13}C assignment to FMN. Table S2: ^{13}C assignment to Trp. This material is available free of charge via the Internet at http://pubs.acs.org.

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