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Towards photo-CIDNP MAS NMR as a generally applicable enhancement method

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Chapter 5

Magnetic field dependence of the solid-state ^{13}C photo-CIDNP effect in phototropin LOV1-C57S

5.1 *Abstract*

Recently, the experimental observation of a solid-state photo-CIDNP effect in the C57S mutant of the LOV1 domain of the blue light photoreceptor phototropin was reported [chapter 4]. It was the first description of this effect in a non-photosynthetic photoactive protein. The magnetic field dependence of the effect in the range between 1.4 and 9.6 T is studied here and shows a different range of signal enhancement with maximum at 2.4 T than for photosynthetic RCs. A future theoretical simulation of this field-dependence will test whether the mechanism for origin of solid-state ^{13}C photo-CIDNP effect in phototropin LOV1-C57S can be described in terms of the same mechanisms known to operate in photosynthetic systems.

5.2 *Introduction*

The solid-state photo-CIDNP (photochemically induced dynamic nuclear polarization) effect was discovered in 1994 by Zysmilich and McDermott[1] by studying quinone-blocked photosynthetic reaction centers (RCs) of the purple bacterium *Rhodobacter (Rb.) sphaeroide*s with solid-state magic-angle spinning (MAS) NMR spectroscopy while illuminating the sample. Although the classical radical-pair mechanism (RPM)[2,3] requires diffusion for symmetry breaking, the possibility to observe photo-CIDNP in solids was predicted by Goldstein and Boxer.[4] They recognized that a mechanism known from liquid-state photo-CIDNP can also be operative in solids. This mechanism, called 'cyclic reactions'[5,6] in the liquid-state and 'differential relaxation' (DR) in solid-state NMR,[7,8] the symmetry breaking relies on the difference in the nuclear relaxation kinetics of the chemical product originating from either the singlet or the triplet decay branch of the radical pair. The DR mechanism, however, did not fully explain the experimental observations. Also signals of the acceptor cofactor obtain enhancement, while the difference in relaxation occurs only on the donor during its molecular triplet state. Signal enhancement by optical nuclear polarization (ONP)[9,10] involves selective population of the molecular triplet states (T_0, T_+ and T_-) that leads to electron polarization that is transferred to nuclei. This requires matching the applied magnetic fields with the hyperfine fields in the mT range. Although ONP has been reported for solids, the high field of the NMR magnet provides little prospect for ONP as a possible source of the enhanced polarization.

Therefore, two other mechanisms have been proposed, transferring under solid-state conditions the initial electron-spin order (pure singlet or pure triplet state), occurring upon generation of the radical pair in a non-stationary state, into nuclear polarization. Both mechanisms, called three-spin mixing mechanism (TSM)[11,12] and differential decay (DD)[13] have been described in detail in Chapter 1.

Analysis of the field-dependence[7,14][Chapter 3] and time-evolution[15] in RCs of *Rb. sphaeroides* wild type (WT) and the carotenoid-less mutant R26 allowed for recognition of the enhancement mechanisms. In particular, in Chapter 3, we studied the full field range of the effect from 1.4 to 17.6 T, demonstrating that the effect is

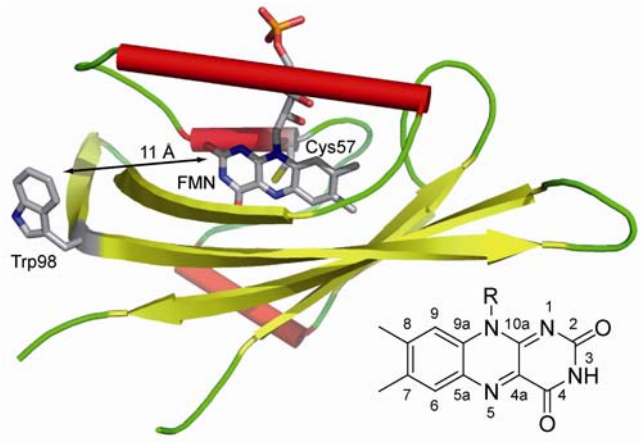
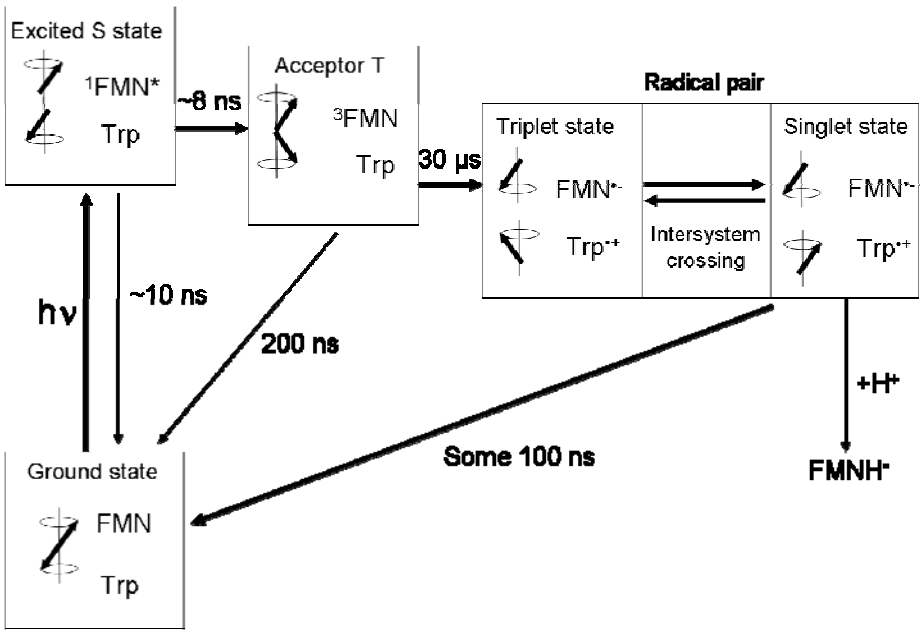


Figure 5.1: Structure of the LOV1 domain of the blue-light receptor phototropin. The flavin cofactor is shown at the bottom.

indeed a matching effect as expected for a TSM and DD based origin. Hence, in WT RCs, the all-emissive (*i.e.* negative) ^{13}C NMR envelope is explained by the dominance of the TSM over the DD in absence of another mechanism.[14] In R26 RCs, the DR occurs in addition to TSM and DD, and allows also for positive signals, mainly from donor signals that are affected by the long-living donor triplet state[8] [Chapter 3].

The effect is also observed in a variety of other natural RCs,[16-19] and the strong enhancement allows for observations directly in membranes[17] and cells.[8,20] The short radical-pair lifetime in RCs, ~20 ns or less, leads to a very limited number of coherent evolution cycles within the radical pair and to broad matching windows[22] which makes photo-CIDNP MAS NMR studies on these systems rather straightforward. The radical pair lifetime of phototropin LOV1-C57S (Figure 5.1) from the alga *Chlamydomonas reinhardtii* can be more than ~100 ns.[21] This leads to a larger number of coherent evolution cycles within the radical pair than for photosynthetic RCs. Assuming that the broad enhancement window as observed in photosynthetic RCs is due to lifetime broadening, the short life times in photosynthetic RCs of ~10 ns translate into a frequency window of ~100 MHz.[22] The field-dependent experiments on the blue-light photoreceptor are expected to show a smaller matching window ~10 MHz corresponding with the longer life time of ~100 ns. In this respect, many radical-pair systems as artificial RCs or other



Scheme 5.1: Tentative photocycle in phototropin LOV1-C57S. The initial excited singlet state of the FMN converts into an acceptor triplet state, which is followed by slow electron transfer, on a time scale of ~30 µs to form the correlated radical pair in its non-stationary T_0 state.[21] The radical pair cannot decay via its triplet state but can convert into the singlet state by coherent evolution of the electron-electron zero quantum coherence. The singlet state can decay back to the ground state, which can take more than 100 ns, or it can form a product state $FMNH^+$ by uptake of a proton.

blue-light photoreceptors[23] have correlated radical-pair lifetimes in the µs range, leading to sharp matching windows that make it difficult to detect the photo-CIDNP effect with standard NMR equipment.

The long radical pair lifetime might be the reason why until now, despite extensive of effort, only a single non-photosynthetic system has been discovered that shows the solid-state photo-CIDNP effect [see chapter 4]. A homologous domain, LOV2-C250S from *Avena sativa* phototropin, has shown a CIDNP effect in solution as well.[24,25]

In the wild type LOV domains, illumination of oxidized flavin leads to formation of the triplet excited state. Its reaction with a nearby cysteine residue results in a covalent adduct as the signaling state. In the C57S or C250A mutants, the reactive cysteine has been replaced, which abolishes adduct formation and strongly increases triplet lifetime.[21] Instead, a much less efficient competing pathway of electron transfer from a tryptophan leads to transient accumulation of a

flavin anion radical on illumination[24,25] and finally to formation of a flavin neutral radical[26,27] (Scheme 5.1).

Due to the concomitant presence of CIDNP effects in solution and in the solid, the question arises whether the solid-state photo-CIDNP effect can also explained in this system by a solid-state photo-CIDNP mechanism. In an attempt to answer this question, the field-dependent photo-CIDNP MAS NMR data presented here will provide the basis of future analysis.

5.3 *Materials and Methods*

5.3.1 *LOV1 expression and purification*

The C57S mutant of the LOV1 domain (amino acids 16-133) of *Chlamydomonas reinhardtii* phot was expressed in *E. coli* strain BL21(DE3) carrying an N-terminal 10x His-tag as described before.[21] The cells were disintegrated using a French Press and the protein was purified via affinity chromatography using His-bind resin (Novagen) loaded with copper sulphate. Protein was transferred into 50 mM potassium phosphate, pH 8, containing 300 mM NaCl by repeated ultrafiltration using an Amicon Ultra-15 filter device with a 10 kDa cutoff (Millipore). The final solution was concentrated to an optical density of ~20 at 450 nm (optical path length, 1 cm).

5.3.2 *MAS NMR experiment*

MAS NMR spectroscopy: The DMX-400, DMX-200, AV-100 and AV-60 MAS NMR spectrometers equipped with a 4-mm MAS probe (Bruker, Karlsruhe, Germany) were used for all MAS NMR experiments at different magnetic fields. The sample was packed into a 4-mm sapphire rotor and inserted into the MAS probe. For a homogeneous sample distribution against the rotor wall, the sample was frozen at a very low spinning frequency of 500 Hz.[28] The temperature setting was 235 K. The spinning frequency was increased to 8 kHz after the sample was completely frozen. This frequency and set temperature were used for all ^{13}C MAS NMR measurements at different magnetic fields. A simple Hahn echo pulse sequence[29] with TPPM (two-pulse phase-modulation) proton decoupling[30] was used for all NMR measurements at the magnetic fields of 9.6, 4.7 and 2.4 T. The

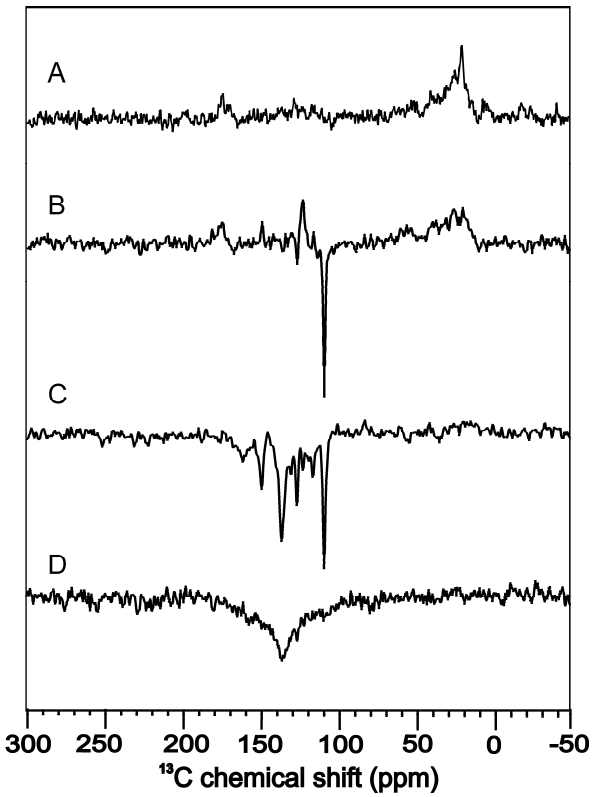


Figure 5.2: The solid-state photo-CIDNP effect in phototropin LOV1-C57S at 9.4 (A), 4.7 (B), 2.4 (C) and 1.4 T (D) observe by ^{13}C MAS NMR. Spectrum D is measured without ^{1}H decoupling. Spectra were obtained under continuous illumination with white light. Temperature was in all cases 235 K and MAS frequency 8 kHz.

hardware of the spectrometer does not allow for proton decoupling in magnetic fields less than 2.4 Tesla. Data collected in magnetic fields below 2.4 T were measured with an anti-ringing pulse sequence.[31] Continuous illumination with white light supplied by a 1 kW xenon lamp was used for all ^{13}C photo-CIDNP MAS NMR experiments.[29,32] For the four magnetic fields of 1.4, 2.4, 4.7 and 9.6 T, artificial line broadening of 10 Hz, 10 Hz, 20 Hz and 50 Hz, respectively, was applied prior to the Fourier transformation. A cycle delay of 2 s was used for all NMR experiments and signals were accumulated for 12 h. ^{13}C MAS NMR spectra were referenced with respect to the $^{13}COOH$ chemical shift of solid glycine at 176.04 ppm. The same phase-correction parameters required to properly phase a glycine spectrum were used for both the dark and photo-CIDNP spectra.

5.4 *Results and discussion*

Figure 5.2 shows ^{13}C MAS NMR spectra of phototropin LOV1-C57S that are CIDNP enhanced by continuous illumination with white light. The data show a solid-state photo-CIDNP effect between about ~7 and ~1 T. Light-induced signals occur at 4.7, 2.4 and 1.4 T (Spectra B-D), while essentially no induction is observed at 9.4 T (Spectrum A). The strongest effects are observed at 4.7 and 2.4 T, and the enhancement towards lower field appears to decrease, while the lack of decoupling contributes to the loss of spectral quality. The corresponding dark spectra, showing no signal, are presented in the Figure 5.3. This matching window is narrower than for the RCs of *Rb. sphaeroides* WT, which also shows significant enhancement at 17.4 and 9.4 T [chapter 3].[8,14] This is well in line with the difference in radical pair lifetime between the two species.

Figure 5.4 presents the light-induced signals in more detail. Apparently in phototropin LOV1-C57S each carbon signal has its own characteristic enhancement function. This observation contrasts previous studies on photosynthetic reaction

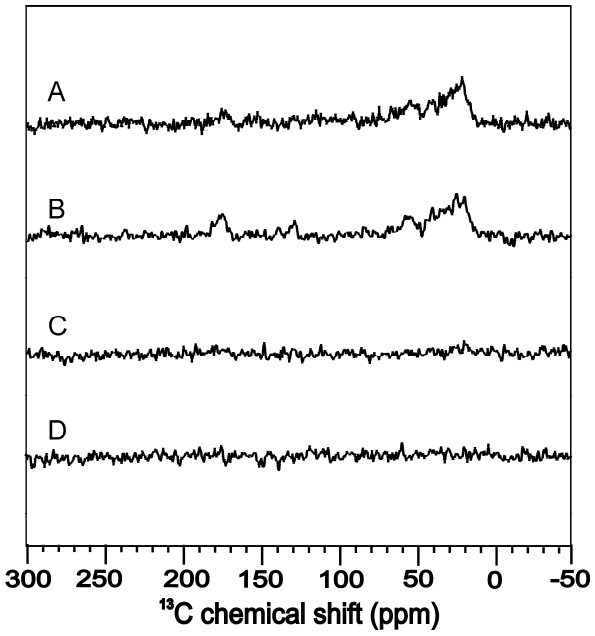


Figure 5.3: The ^{13}C MAS NMR spectra of phototropin LOV1-C57S at 9.4 (A), 4.7 (B), 2.4 (C) and 1.4 Tesla (D) under dark condition. Spectrum D is measured without ^{1}H decoupling. Spectra were obtained under continuous illumination with white light. Temperature was in all cases 235 K and MAS frequency 8 kHz

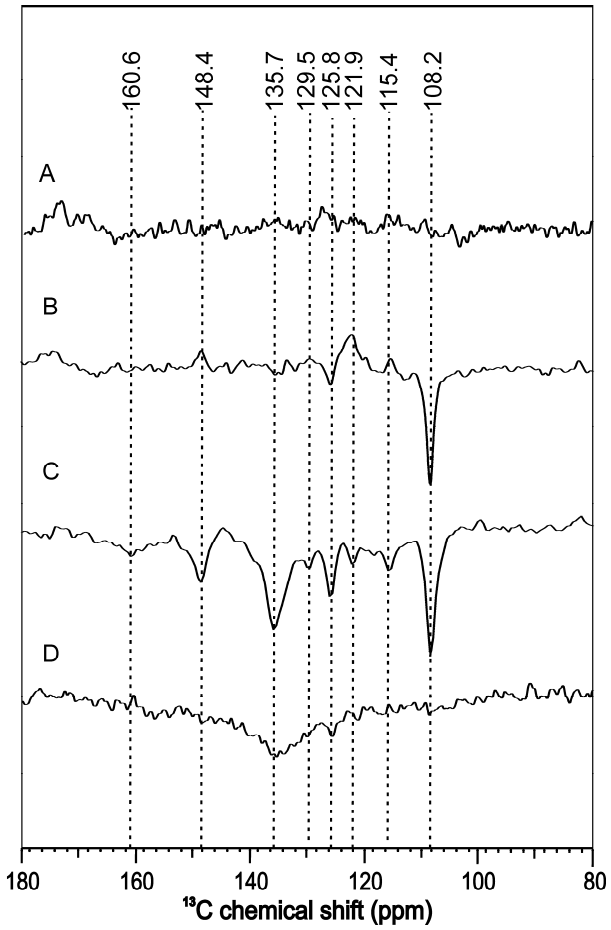


Figure 5.4: Expanded view on the spectra presented in Figure 5.2. The solid-state photo-CIDNP effect in phototropin LOV1-C57S at 9.4 (A), 4.7 (B), 2.4 (C) and 1.4 T(D) observed by ^{13}C MAS NMR. Spectrum D is measured without ^{1}H decoupling. Spectra were obtained under continuous illumination with white light. Temperature was in all cases 235 K and MAS frequency 8 kHz.

centers showing very similar enhancement factors across the carbons contributing to the ^{13}C photo-CIDNP response. The cofactors in blue-light photoreceptors that support the radical pair are significantly smaller than the chlorophylls in photosynthetic RCs and are structurally rather asymmetric. Probably substantial differences in the hyperfine parameters and their anisotropies lead to differences in field dependency for the involved nuclei.

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