Photo-CIDNP studies on reaction centers of rhodobacter sphaeroides

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Summary

Solid state magic angle spinning (MAS) NMR is fast developing as an important technique for the study of large membrane protein systems. However, it suffers from an inherent problem of low sensitivity and selectivity (**Chapter 1**). Photochemically induced dynamic nuclear polarization (photo-CIDNP) MAS NMR increases NMR intensities by induction of photochemical reactions, which shuffle the nuclear spin system out of its Boltzmann equilibrium. The understanding and development of photo-CIDNP MAS NMR can therefore provide a method to overcome the intrinsic insensitivity and non-selectivity of MAS NMR. Until now, photo-CIDNP in the solid state has been observed only in the natural photosynthetic reaction centers. In the photosynthetic bacterium, *Rhodobacter sphaeroides,* the primary photosynthetic process takes place in the pigment protein complex known as reaction center (RC). Four molecules of bacteriochlorophyll *a* (BChl *a*), two molecules of bacteriopheophytin a (BPhe *a*), two ubiquinone-10 molecules (Q), a non-haem iron (Fe^{2+}) and a carotenoid molecule (C) form the cofactors of the RC. The light induced electron transfer process starts at the primary donor, formed of two BChl molecules P_L and P_M. The special pair, P donates an electron to the BPhe molecule, Φ_A.

Three different mechanisms have been proposed to describe photo-CIDNP in solids.

- The electron-electron-nuclear three spin mixing (TSM) mechanism: According to this mechanism a net nuclear polarization is created due to the presence of both anisotropic hyperfine interaction and coupling between the two electron spins in the spin-correlated radical pair.
- The differential decay (DD) mechanism: In this mechanism a net photo-CIDNP effect is caused due to the anisotropic hyperfine coupling if spin-correlated radical pairs have different lifetimes in their singlet and triplet states.
- The differential relaxation (DR) mechanism: This takes place due to the significant differential relaxation between the nuclear spins in the special pair triplet ^3P and the nuclear spins in the singlet ground state of P.

The reconstruction of local electronic spin densities from photo-CIDNP intensities requires a thorough understanding of the mechanisms involved. Understanding the photo-CIDNP process is thus equivalent to gaining information on the primary step of photosynthesis.

In **Chapter 2**, ^{13}C photo-CIDNP MAS NMR studies from RCs of *Rhodobacter sphaeroides* WT are presented. Photo-CIDNP has been observed at three different magnetic field strengths. At a magnetic filed strength of 4.7 Tesla (200 MHz proton frequency), the

strongest enhancement of more than 10000 above the Boltzmann polarisation is observed. At higher fields, the enhancement factor decreases. The light induced NMR signals at all fields are emissive (negative) and could be assigned to P_L and P_M of the donor and the acceptor Φ_A. The photo-CIDNP MAS NMR spectra at the three different magnetic field strengths have been simulated assuming two competing mechanisms of polarisation transfer from electrons to nuclei, three-spin mixing (TSM) and differential decay (DD) mechanisms. The assignment of the light induced signals has been done with the help of simulations. The simulated spectra agree well with the photo-CIDNP MAS NMR spectra demonstrating that photo-CIDNP effect in the RCs from *Rb. sphaeroides* WT can be interpreted by TSM and DD mechanism.

The photo-CIDNP studies from the mutant strain *Rhodobacter sphaeroides* R26 are shown and discussed in **Chapter 3**. In the R26 RCs as compared to WT, the donor triplet lifetime is longer since no carotenoid molecule is present. The magnetic field effect of photo-CIDNP is similar for both RCs. However, the light induced signals assigned to the donor, P in the RCs of the R26 strain, are absorptive which could not be explained by the TSM and DD mechanism. This led us to consider the DR mechanism to be operative in R26, in addition to the two other mechanisms. Simulations done with inclusion of the DR mechanism have been able to reproduce the sign change in the light induced signals for the donor, P. The polarization pattern of WT RCs has thus been interpreted in terms of the spin density distribution in the radical pair state and the polarization change between the WT and R26 spectra in terms of the spin density distribution in the triplet state of the donor.

The strong photo-CIDNP enhancement at a field strength of 4.7 T has enabled the observation of cofactor molecules at a concentration of ~100 nM inside intact R26 cells without isotope enrichment (**Chapter 3**). The overall photo-CIDNP intensity pattern is in some details distinct from the isolated reaction centres at 4.7 T. The overall similarity between the photo-CIDNP spectrum from the isolated RCs and intact cells suggests that the ground-state electronic structure of the special pair is not strongly influenced by the surrounding protein complexes in the natural environment of an intact cell. Photo-CIDNP MAS NMR is thus established as a method to study the electronic structure of photosynthetic cofactors at the molecular and atomic resolution as well as at cellular concentrations.

^{13}C-^{13}C dipolar correlation photo-CIDNP MAS NMR studies have been performed in **Chapter 4** on RCs of *Rhodobacter sphaeroides* WT, selectively isotope labelled in all the BChl and BPhe cofactors at positions C-4, 5, 9, 10, 14, 15, 16 and 20, to provide a comprehensive map of the ground-state electronic structure of the cofactors involved in the electron transfer process. Three strong components are observed in the ^{13}C-^{13}C dipolar correlation photo-CIDNP MAS NMR spectra. These have been assigned to two BChls, P1 and P2, and one BPhe, Φ_A. In addition a weak component is observed which is assigned to another BChl, denoted as P3. Pronounced upfield shifts are present for P2 in the pyrrole ring I as compared to P1 and P3. The large upfield shifts could be explained by a hydrogen bond to

His L168, located in the proximity of the 3-acetyl group of P_L. Thus P_L is assigned to P2 making it a special BChl in the special pair. Then P_M is assigned to P1 and the weak component P3 is assigned to B_A. The electronic structure of the special pair is thus asymmetric in the ground state.

Photo-CIDNP studies have been further extended to entire photosynthetic unit (PSU) bound to membrane selectively ^{13}C-isotope labelled in all the BChl and BPheo cofactors at positions C-1, 3, 6, 8, 11, 13, 17 and 19 (**Chapter 5**). All the light induced signals for the membrane bound PSU are absorptive. Addition of detergent released intact PSU from the chromatophore membrane and caused significant changes in the sign and intensity pattern of the light-induced MAS NMR spectrum. In contrast, detergent solubilised PSU and detergent solubilised RCs with the same isotope label pattern exhibit essentially the same chemical shifts with only minor differences in the intensity pattern. The pronounced differences between intact membrane bound and detergent solubilized photosynthetic units has been explained by the loss of self-orientation of the membrane-bound samples by solubilization.

Chapter 6 provides a future outlook for photo-CIDNP studies on the RCs of *Rhodobacter sphaeroides*. The first part of the Chapter is devoted to the build up of the photo-CIDNP response. This paves the way for time-resolved photo-CIDNP experiments. The second part describes future developments in four different directions from biophysical studies to magnetic resonance force microscopy. The biophysical studies have provided information towards building artificial photosynthetic devices. Photo-CIDNP can be used as a 'spin torch' to explore the binding pocket and its influence on the cofactors. 'Spin torches' could also be used as contrast agents in Magnetic Resonance Imaging (MRI). Photo-CIDNP in combination with Magnetic Resonance Force Microscopy (MRFM) may allow for detection of single nuclear spin in the near future.