



Universiteit
Leiden
The Netherlands

13C Photo-CIDNP MAS NMR studies on oriented reaction centers

Diller, A.; Alia, A.; Gast, P.; Groot, H.J.M. de; Glaubitz, C.; Jeschke, G.; ... ; Osmond, B.

Citation

Diller, A., Alia, A., Gast, P., Groot, H. J. M. de, Glaubitz, C., Jeschke, G., & Matysik, J. (2008). 13C Photo-CIDNP MAS NMR studies on oriented reaction centers. In J. F. Allen, E. Gantt, J. H. Golbeck, & B. Osmond (Eds.), *Photosynthesis. energy of sun: 14th international congress on photosynthesis research 2007* (pp. 97-100). Dordrecht: Springer.
doi:10.1007/978-1-4020-6709-9_22

Version: Publisher's Version

License: [Licensed under Article 25fa Copyright Act/Law \(Amendment Taverne\)](#)

Downloaded from: <https://hdl.handle.net/1887/3454752>

Note: To cite this publication please use the final published version (if applicable).

¹³C Photo-CIDNP MAS NMR Studies on Oriented Reaction Centers

Anna Diller¹, A. Alia¹, Peter Gast¹, Huub J. M. de Groot^{1,2}, Clemens Glaubitz², Gunnar Jeschke³, and Jörg Matysik¹

Abstract In order to study the orientation dependence of photochemically induced dynamic nuclear polarization (photo-CIDNP) occurring photosynthetic reaction centers of *Rhodobacter sphaeroides*, magic-angle spinning (MAS) NMR has been applied to both, isotropic and oriented RC samples. The sample orientation is obtained by magic-angle oriented sample spinning (MAOSS). In contrast to original expectations, experiments and simulations show that the orientation obtained in MAOSS experiments does not induce significant spectral effects in the polarization pattern.

Keywords Photo-CIDNP, MAS NMR, electron transfer

Introduction

The solid-state photo-CIDNP effect (for review see Daviso et al. 2007), discovered in 1994 (Zysmilich and McDermott 1994), has been observed until

now exclusively in photosynthetic systems with magic-angle spinning (MAS) NMR. Recently, it has been shown for wildtype (WT) RCs of *Rhodobacter (Rb.) sphaeroides* that the photo-CIDNP effect at 4.7 Tesla allows for enhancement of NMR signals of a factor of more than 10,000 above the Boltzmann level (Prakash et al. 2005). The effect is explained in terms of a combination of several spin-chemical mechanisms: (i) In the three-spin mixing (TSM), net nuclear polarization is created in the spin-correlated radical pair due to the presence of both anisotropic hyperfine interaction and coupling between the two electron spins (Jeschke 1998). (ii) In the differential decay (DD) mechanism, a net photo-CIDNP effect is caused by difference in singlet and triplet radical-pair lifetimes (Polenova and McDermott 1999). The original study predicted strong directional effects of the solid-state photo-CIDNP effect. In fact, both the TSM and the DD are coherent mechanisms and are expected to cause high anisotropy. The activity of both mechanisms is sufficient to explain the photo-CIDNP effect in WT RCs (Prakash et al. 2005). (iii) On the other hand, in R26 RCs, having a long lifetime of the triplet donor, nuclear polarization is partially extinguished leading to an excess

¹Leiden Institute of Chemistry, Leiden University, 2300 RA Leiden, The Netherlands.

²Institut für Biophysikalische Chemie, Johann-Wolfgang-Goethe-Universität, 60438 Frankfurt, Germany

³Institut für Physikalische Chemie, Universität Konstanz, 78457 Konstanz, Germany

of opposite nuclear polarization produced by the singlet radical pair (Prakash et al. 2006). This differential relaxation (DR) mechanism (McDermott et al. 1998) is a statistical, non-coherent process and, hence, photo-CIDNP produced by the DR has lost its spatial information, while this information is remained in the TSM and the DD mechanism.

The spatial information which may be obtained by photo-CIDNP MAS NMR experiments of oriented samples may allow for revealing the orientation of the cofactors producing photo-CIDNP. Furthermore, the anisotropy of this effect may provide a chance for spectral editing and allow for assignment of signals to particular cofactors. A method to orient membrane proteins is magic-angle oriented sample spinning (MAOSS) (Glaubitx and Watts 1998; Lopez et al. 2007). In this case, round glass disks carrying the membrane layers are inserted into MAS rotors. Hence, the combination of MAOSS with photo-CIDNP MAS NMR may provide the chance to study the orientation properties of photo-CIDNP and using this information for cofactor determination, for example in complex plant RCs.

Materials and methods

RC preparation. Bacteria of *Rb. sphaeroides* WT strain 2.4.1 have been grown anaerobically at 30 °C, illuminated with incandescent light of intensity of 2,700 lux. For details, see Okamura et al. (1975).

Orientation of RCs. Isolated RCs have been reconstituted into L- α -phosphatidylcholine (egg, chicken; Avanti Polar Lipids) bilayers. The lipid-protein mixture is deposited on the planar surface of a glass disc as used in microscopy (diameter 5.4 mm, size 00, Marienfeld GmbH, Lauda-Königshofen, Germany). Upon hydration-induced self-assembly of lipids, the bilayers adopt the orientation of the surface. For MAOSS experiments, the glass disks were carefully inserted into a 7-mm sapphire rotor.

MAS NMR measurements. The solid-state NMR experiments were performed at either a DMX-200 or a DMX-400 NMR spectrometer (Bruker GmbH, Karlsruhe, Germany). Spectra have been collected with a Hahn-echo pulse sequence and

proton decoupling at a temperature of 240 K. Non-oriented samples were measured in 4-mm sapphire rotors, while MAOSS samples were investigated in a 7-mm sapphire rotor. The spinning frequency has been 4 kHz in both cases.

Simulations. Simulations of photo-CIDNP patterns are based on the approach that was previously introduced for unoriented samples (Prakash et al. 2005). For the simulation of any orientation effects we assume that the RCs in the membrane can freely rotate about their C₂ pseudo-symmetry axis. Orientation selection can then be characterized by a distribution of angles θ between the C₂ pseudo-symmetry axis and the magnetic field vector. For a perfect MAOSS orientation the C₂ axis is parallel to the axis of the sample rotor. It thus includes a magic angle of $\theta_m = 54.74^\circ$ with the magnetic field axis.

To include imperfect orientation we have assumed a Gaussian distribution of the angle $\Delta\theta_m$ between the sample rotor axis and the C₂ axis, which is fully characterized by the standard deviation σ . We have also tested Gaussian distributions with two parameters for which the mean angle between the C₂ axis and the magnetic field vector deviates by a tilt ξ from the magic angle. In both cases, the distribution $P(\theta)$ of angles θ was computed by integrating over a uniform distribution of angles ϕ corresponding to free rotation about the C₂ axis, and over the distribution of angles $\Delta\theta_m$.

The nuclear polarizations are computed as an average over an orientational grid (θ, ϕ) . Compared to a simulation without orientation selection, we multiply the contribution of each grid point with an additional weighting factor $P(\theta)$. The contribution of orientations with a $P(\theta)$ smaller than 0.1% of the maximum value is neglected to speed up computations.

Results and discussion

Assignments

In Fig. 1, photo-CIDNP spectra of non-oriented (A) and oriented (B) WT RCs are shown. The most downfield shift appears at 189.4 ppm and has