



Universiteit
Leiden
The Netherlands

Biological diversity of photosynthetic reaction centers and the solid-state photo-CIDNP effect

Roy, E.

Citation

Roy, E. (2007, October 11). *Biological diversity of photosynthetic reaction centers and the solid-state photo-CIDNP effect*. Solid state NMR group/ Leiden Institute of Chemistry (LIC), Faculty of Science, Leiden University. Retrieved from <https://hdl.handle.net/1887/12373>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12373>

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

2 Photo-CIDNP observed in photosystem I from plants

Photo-CIDNP has been observed in photosystem I of spinach by ^{13}C magic angle spinning solid-state NMR under continuous illumination with white light. All the light-induced ^{13}C NMR signals appear to be emissive. An almost complete set of chemical shifts of the aromatic ring carbons of a single Chl *a* molecule has been obtained.

2.1 Introduction

Photosynthesis in plants is driven by light-induced electron transfer in the two RCs, PSI and PSII. The oxidised primary electron donor of PSII, is a very powerful oxidising agent (1), even enabling the oxidation of water, while the electronically excited primary electron donor of PSI, is a strong reducing agent (2). The X-ray structure at a resolution of 2.5 Å of PSI from the thermophilic cyanobacterium *Synechococcus elongatus* shows the arrangement of cofactors. They are in two branches called A and B corresponding to the protein subunits that comprise the core (Fig. 2.1) (3). The three dimensional structure shows that P700 is a heterodimer formed by one Chl *a* molecule and one Chl *a'* molecule, which is the C13²-epimer of Chl *a*. Due to their 5-coordination, both Chl macrocycles are domed. The interplanar distance between both macrocycles is 3.6 ± 0.3 Å. Chl *a'* forms hydrogen bonds to its environment (Fig. 2.2) (3). There are no hydrogen bonds found on the Chl *a* side. In comparison to the special pair of purple bacterial RCs (4), P700 is a heterodimer, having a shorter distance between the chlorophylls. In addition there is partial overlap of rings I and II while in purple bacterial RCs the rings I have a more perfect overlap (5). The electronic structure of P700 remains under discussion (2). The available spectroscopic data are mainly from vibrational and electron paramagnetic resonance methods. The observation of a broad mid-IR transition (6, 7) in the oxidised and paramagnetic P700⁺⁺ is generally interpreted as proof for charge repartition over two Chl cofactors, called P1 and P2. As concluded from the C=O stretching vibrations, P1 is hydrogen-bonded on both keto-functions and can be assigned to the Chl *a'*. It carries all the triplet character of ³P700, while the carbonyl groups of P2 are free from hydrogen bonding interaction (8). Mutant studies provide evidence for electronic coupling between the two halves of the dimer (9). Data from different electron paramagnetic resonance spectroscopies, such as EPR (10-12), ENDOR (12-19) and ESEEM (20-24), have been interpreted quite differently. Originally, a symmetric dimer (10, 15) and a Chl monomer

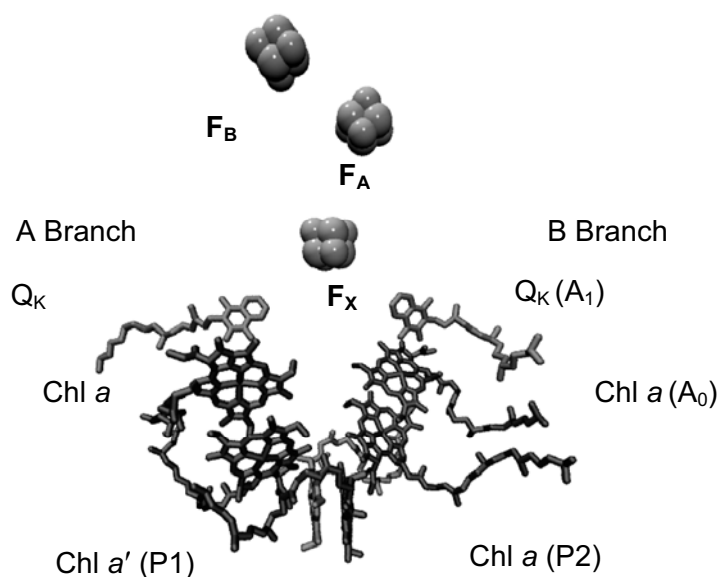


Figure 2.1. The arrangement of cofactors in the electron transfer chain in RC of PSI. The figure was made using VMD molecular graphic programme (<http://www.ks.uiuc.edu/Research/vmd/>).

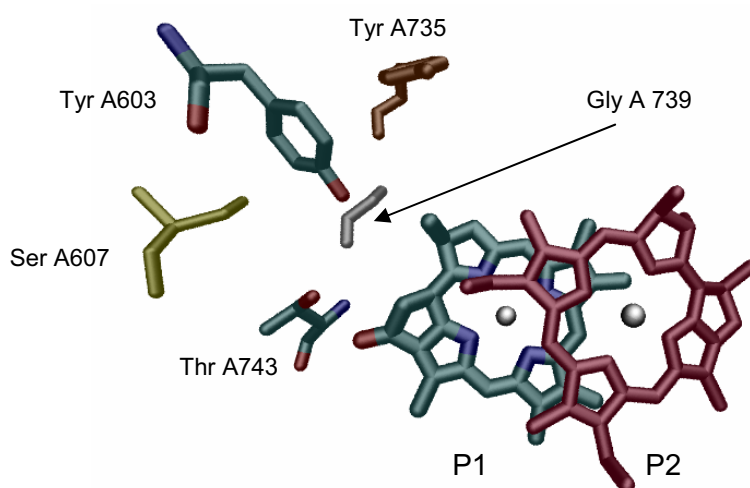


Figure 2.2. Structure of P700 showing residues in the environment involved in formation of hydrogen bond on the Chl *a'* side. The figure was made using VMD molecular graphic programme.

(14) were proposed. More recently, an asymmetric dimer has been proposed (14, 18), in which the second Chl (P1) carries about $\leq 15\%$ of the spin density (19). A very recent molecular orbital study based on the $2.5\text{\AA}$ structure indeed described P700 as dimer with an asymmetric electron spin density distribution in favour of the monomeric Chl *a* (P2) half by a spin density ratio of almost 5:1 (25).

NMR chemical shift information allows for the exploration of spatial, protonic and electronic structures with atomic selectivity in the electronic ground state. Such an analysis can provide detailed insight into the functional mechanisms of proteins. For several

photosynthetic RCs of bacteria and plants it has been shown that photo-CIDNP can overcome the intrinsic insensitivity and non-selectivity of NMR spectroscopy by photochemical induction of a non-Boltzmann population of nuclear spin states. Photo-CIDNP has been observed in quinone-blocked bacterial RCs from *Rb. sphaeroides* R-26 (26-29) and WT (30) and PSII complexes from plants (31). The strong enhancement by the combination of selective ^{13}C -isotope labelling at several cofactor positions allows obtaining two-dimensional photo-CIDNP MAS NMR spectra (32). This has demonstrated that the electron density of the two BChl molecules of the special pair is already different in the electronic ground state of the bacterial RC. In addition, NMR signals were detected in entire membrane-bound bacterial photosynthetic units (>1.5 MDa) (33). In the D1D2 complex of PS II of plants, the observation of the pronounced electron spin density on rings III and V by photo-CIDNP MAS NMR was taken as an indication for a local electric field, leading to a hypothesis about the origin of the remarkable strength of the redox potential of the primary electron donor (31).

In PSI, light-induced electron spin polarisation has been observed for the first time in 1975 (37, 38). Photo-CIDNP solid-state NMR intensities are linked to the local electron spin densities occurring in the radical-pair state (34- 36). Photo-CIDNP intensities are proportional to the nuclear polarisation, and thus depend strongly on the anisotropy of the hyperfine coupling. The exact link between the local electron-spin densities and the photo-CIDNP intensities, however, remains a topic for further studies. The photo-CIDNP effect in solid-state is explained by three mechanisms, TSM, DD and DR (35, 36). This chapter investigates the photo-CIDNP data of PSI observed by ^{13}C MAS NMR.

2.2 Materials and Methods

2.2.1 PSI particle preparation

The PSI complex containing ~110 Chl/P700, termed the PSI-110 particles, was prepared from spinach according to Mullet *et al.* (39). The chloroplasts were isolated by grinding excised leaves in 0.4 M Sorbitol and 50 mM Tricine buffer (pH 7.8) as previously described (40). The isolated chloroplasts were then washed with 10 mM Tricine buffer (pH 7.8) containing 50 mM Sorbitol and 5 mM EDTA, and re-suspended in buffer containing 10 mM Tricine (pH 7.8) to obtain a final concentration of 0.8 mg Chl/mL. The membranes were solubilised with Triton X-100 to a final concentration of 0.8% w/v for 30 minutes at room temperature in the dark with continuous slow stirring. These solubilised membranes were centrifuged at 39,000 *g* for 20 min at a temperature of 4 °C and the supernatant fraction was loaded onto a linear sucrose gradient (0.1 - 1.0 M sucrose, 10 mM Tricine, 0.02% Triton X-100, pH 7.8) which was prepared on a 2 M sucrose cushion followed by ultracentrifugation at 150,000 *g* for 18 h at 4 °C. PSI-110 particles appeared as a dark green non fluorescent band just above the 2 M sucrose cushion. After collecting this band, the PSI-110 particles were

dialysed overnight against 10 mM Tricine and concentrated by ultracentrifugation at 150,000 g for 16 h. PSI-110 particles were finally suspended in 5 mM Tricine buffer (pH 7.8) containing 50 mM Sorbitol. The chlorophyll content of PSI-110 was determined by the method of Arnon *et al.* (41). PSI-110 particles equivalent to ~2 mg Chl/mL were used for NMR measurements.

CPI particles (PSI particles containing ~40 Chl/P700 and lacking the ferredoxin acceptors F_X , F_B , F_A) were prepared using a modification of the method of Rutherford and Mullet.(42) In brief, the PSI-110 particles (1 mg Chl/mL) were incubated with 2% lithium dodecyl sulphate for one hour at 4 °C. Subsequently, the particles were loaded on a linear sucrose gradient (0.1-1 M sucrose, 10 mM Tricine, 0.1% sodium cholate, pH 8) and centrifuged at 150,000 g for 16 h. The CPI particles appeared as a dark green band approximately 2 cm from the bottom of the centrifuge tube. This band containing CPI particles was dialysed overnight against 10 mM Tricine and concentrated by centrifugation at 150,000 g for 16 h. The purity of the PSI-110 particles and CPI particles was analysed by SDS-PAGE. PSI-110 was resolved into 12 clearly distinguishable bands (68, 66, 24.5, 24, 22, 22.5, 21, 17, 16.5, 11.5, 11 and 10.5 kDa). This pattern is similar to the SDS-PAGE data published earlier by Mullet *et al.* (39). CPI particles showed two bands at 66kDa and 68kDa corresponding to PsaA and PsaB polypeptides (43).

2.2.2 MAS-NMR Measurements and DFT computations

The NMR experiments have been performed using a DMX-400 NMR spectrometer (Bruker GmbH, Karlsruhe, Germany) equipped with a triple-resonance MAS light probe working at 396.5 MHz for protons and 99.7 MHz for ^{13}C specially designed for using the illumination set up (29). The samples were loaded into optically transparent 4 and 7 mm sapphire rotors. Reduction of ferredoxin acceptors F_B and F_A in PSI-110 particles was performed by addition of an aqueous solution of 10 mM sodium dithionite solution and 40 mM glycine buffer (pH 9.5) in an oxygen free atmosphere. Immediately following the reduction, slow freezing of the sample was performed directly in the NMR MAS probe inside the magnet with liquid nitrogen-cooled gas under continuous illumination with white light (29). In order to ensure a homogeneous sample distribution against the rotor wall a low spinning frequency ~600 Hz of the sample was used during this slow freezing. Photo-CIDNP ^{13}C MAS NMR spectra were obtained at a temperature of 223 K with continuous illumination. To distinguish the centrebands from the spinning sidebands, photo-CIDNP MAS NMR spectra were recorded at different spinning frequencies, 3.6, 4.0, 5.0, 6.4, 8.0 and 9.0 kHz. The light and dark spectra have been collected by a straightforward Bloch decay followed by a Hahn echo and TPPM proton decoupling (44). A recycle delay of 12 seconds was used and a total number of 14,000 scans per spectrum were collected over a period of 48 h.

Density functional computations were performed using ADF 2002.01 (45). Three different Chl structures were tested, i) a structure obtained from X-ray data (46) which was used without further optimisation, ii) a structure based on standard bond angles and bond lengths (47) and iii) an optimized starting structure of a BChl *a* which was edited in Titan 1.0 (Wavefunction Inc., Irvine, California, USA) to give the structure of Chl *a* in PSI shown in Fig. 2.6, with residue R substituted by a methyl group to save computation time. Further optimisation of this structure was done within ADF. A structure of an analogous Phe *a* was then obtained by deleting the Mg^{2+} ion and adding two hydrogens in Titan 1.0 allowing for a simple comparison of the principal axis frames of *g* tensors, which were computed for the optimized structure of the Chl *a* anion radical and the analogous pheophytin anion radical within the spin-restricted zeroth order relativistic approximation formalism with all-electron basis sets DZP for all atoms (48, 49). A non-relativistic spin-unrestricted computation with an all electron TZ2P basis set on all atoms was used to calculate the hyperfine tensors of Chl *a* cation and anion radicals.

2.3 Results

Fig. 2.3 shows ^{13}C MAS NMR spectra of natural abundance PSI-110 particles in the dark (A) and under continuous illumination with white light (B). Spectrum 2.3A shows the characteristic aliphatic features of a ^{13}C -MAS NMR spectrum of a protein, which is a broad signal between 0 and 50 ppm. The sharp signals at 175.7 and 41.9 ppm arise from glycine. The relatively broad signal at 179 ppm contains intensity of the protein carbonyl groups. In spectrum 2.3B, several strong emissive (negative) signals appear upon illumination. It is indeed remarkable to observe NMR signals of such intensity from the active site of a large membrane protein complex containing 110 Chls. Photo-CIDNP has been observed only in pre-reduced PSI-110 and PSI-CPI particles. The difference spectrum 2.3C shows that all the light-induced signals appear exclusively in the aromatic region.

In the spectra of the PSI-110 preparation, a total of twelve, centrebands have been identified (Fig. 2.4A-C). Using the chemical shifts reported for monomeric or aggregated Chl *a* these centrebands can be tentatively assigned to 17 carbon atoms of a single Chl *a* cofactor (Table 2.1). There is no evidence for signal doubling in the spectrum. In the carbonyl region, the carbon C-13^1 is detected as a relatively broad signal at 190.6 ppm.

The strongest signals are observed in the aromatic region between 120 and 170 ppm. The signal at 154.8 ppm shows a shoulder and can be assigned to both C-1 and C-6. The strong signal at 147.2 ppm is assigned to C-9 and C-11, which is in line with previous MAS NMR experiments on precipitated Chl *a* molecules, where these two signals are also not separated. Also the three carbons C-2, C-4 and C-8 can be detected. The broad signal at ≈ 132 ppm can be assigned to the carbons C-7, C-12 and C-13. The response at ≈ 105.4 ppm, can be assigned

Chl <i>a</i>		Carbon	PSII	PSI
$\sigma_{\text{liq}}^{\text{a}}$	$\sigma_{\text{ss}}^{\text{b}}$	no.	σ^{c}	σ^{d}
189.3	190.6	13 ¹		~190.6 E
172.7	175.3	17 ³		
171.0	171.2	13 ³		
167.4	170.0	19	166.9 A	167.1 E
161.4	162.0	14	162.3 A	160.4 E
154.0	155.9	1	156.0 A }	154.8 E
155.8	154.4	6		
151.4	154.0	16	151.7 A	152.6 E
148.0	150.7	4		149.9 E
147.7	147.2	11	147.7 A }	147.2 E
146.1	147.2	9		
144.1	146.2	8		144.2 E
139.0	137.0	3	137.5 A	138.6 E
135.5	136.1	2		~136 E
134.2	134.0	12		
134.0	133.4	7	133.9 A }	~132 E
131.5	126.2	13		
131.5	126.2	3 ¹		
118.9	113.4	3 ²		
107.1	108.2	10	104.6 E }	105.4 E
106.2	102.8	15		
100.0	98.1	5		
92.8	93.3	20		

Table 2.1. ^{13}C chemical shifts of the photo-CIDNP signals obtained at 9.4 Tesla when matched to published chemical shift data for Chl *a* lead to a first assignment of NMR signals. Abbreviations: σ = chemical shift, A = absorptive signal, E = emissive signal. (a) Ref. (58), the liquid NMR data have been obtained in tetrahydrofuran. (b) Ref. (59), the solid-state NMR data have been obtained from aggregates. (c) Ref. (31). (d) this work.

to both the C-10 and C-15 methine carbons.

No light induced signal is observed in the region of the aliphatic carbons. In bacterial RCs, emissive signals at about 118.5 and 134 ppm have been assigned to an axial histidine ligand of the special pair (50, 51). This is in contrast with the data for PSI, since all twelve centrebands can be conveniently assigned to a single Chl *a* cofactor.

The intensity of the photo-CIDNP signals of PSI-110 is very strong relative to the dark background. The strongest photo-CIDNP signals have about three times the intensity of the maximum of the aliphatic signals at 30 ppm. This is similar to the ratio observed from the best preparations of RCs of bacteria and of PSII in D1D2. The molecular mass of the PSI-110 preparation (≈ 300 kDa) is approximately a factor three larger. This means that PSI-110 shows the most intense photo-CIDNP signals ever observed in an unlabelled RC. This effect can be partially, but not exclusively attributed to the relatively narrow linewidth of 60-65 Hz, which is less than the linewidths of 80 to 100 Hz that are observed for PSII.

The spectrum obtained from the PSI-CPI preparation shows the same centrebands with a similar intensity pattern as found in PSI-110 at the same spinning frequency (Fig. 2.5). The signal at 154.8 ppm, which is assigned to both C-1 and C-6, however, is clearly reduced.

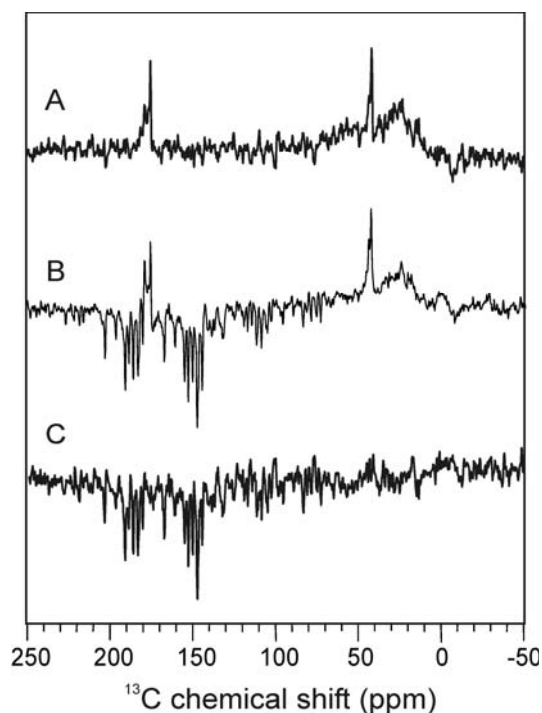


Figure 2.3. ^{13}C MAS NMR spectra of PSI-110 particles measured at 223K with a MAS frequency of 3.6 kHz. Spectra are obtained (A) in the dark, (B) under continuous illumination with white light, (C) by subtraction B – A.

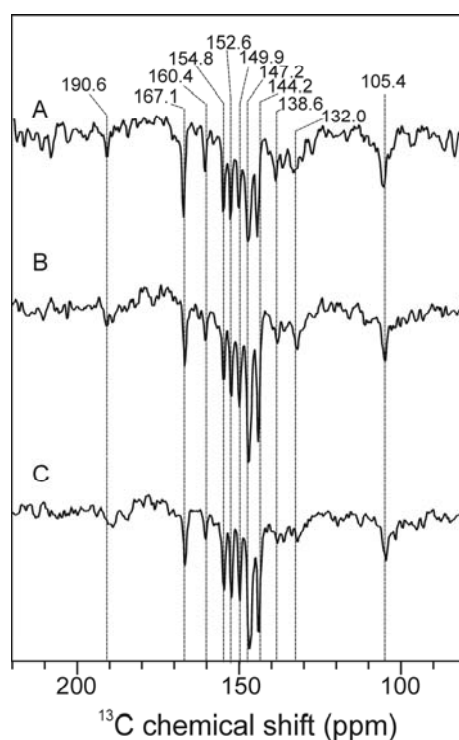


Figure 2.4. ^{13}C MAS NMR spectra of PSI-110 particles obtained under continuous illumination with white light using a MAS frequency of (A) 6.4 kHz, (B) 8.0 kHz and (C) 9.0 kHz. The assigned centerbands are shown by the dashed lines.

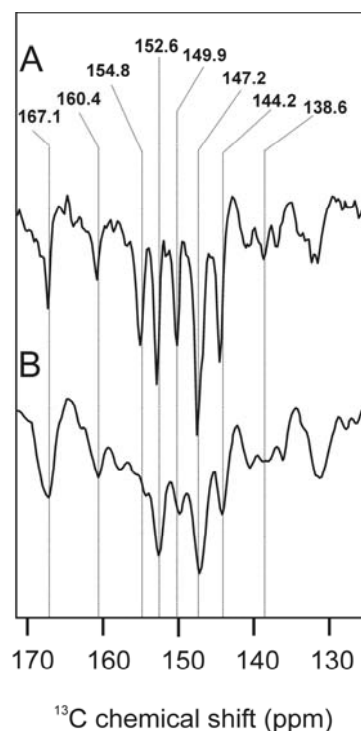


Figure 2.5. ^{13}C MAS NMR spectra of (A) PSI-110 and (B) PSI-CPI particles obtained with continuous illumination with white light at a temperature of 223K and using a MAS frequency of 3.6 kHz. In both spectra, a line-broadening of 50 Hz has been applied. The assigned centerbands are visualised by the dashed lines.

In addition, the linewidth of all signals is significantly increased. These effects indicate increased heterogeneity of the sample compared to the PSI-110 preparation. Probably the removal of the surrounding antenna apparently destabilises the RC in the PSI-CPI preparation.

2.4 Discussion

2.4.1 The radical pair and the sign

In the illumination experiments, photo-CIDNP enhancement can be observed. In reduced PSI-110 and PSI-CPI particles a $\text{P}_{700}^{+\bullet} \text{A}_1^{-\bullet}$ radical pair is formed. The ferredoxins are removed in CPI-particles, which suggests that the quinone needs to be reduced in order to obtain photo-CIDNP. The radical pair $\text{P}_{700}^{+\bullet} \text{A}_1^{-\bullet}$, produced upon illumination in the samples without pre-reduction by sodium dithionite does not produce photo-CIDNP, presumably because the electron-electron coupling is too weak. Under strong permanent illumination, the Chl *a* of the second pair of Chl *a* molecules next to P700 can also become photo-reduced (52). Since this radical pair is tightly coupled and does not produce electronic triplets, no photo-CIDNP can be expected from such an electronic structure. Therefore, it is reasonable to assume that the observed photo-CIDNP enhancement originates from the radical pair $\text{P}_{700}^{+\bullet} \text{A}_0^{-\bullet}$.

The difference between a Chl *a* radical anion in PSI and a Phe *a* radical anion in PSII may

also be responsible for change of the sign of the photo-CIDNP enhancement, the most obvious difference between both RCs.

Recent EPR data on PSI suggest that the isotropic g value of the Chl a acceptor anion radical (53, 54) is closer to the isotropic g value of the P700 donor cation radical (55-57) rather than for the corresponding donor and acceptor in PSII and in bacterial RCs. A smaller Δg causes a smaller contribution of the DD mechanism to the nuclear polarisation and simultaneously a larger contribution of the TSM mechanism. Hence, it is possible that the TSM contribution dominates for PSI, which would explain why all signals have the same sign. For the DD contribution, the sign depends on the sign of several parameters and may even depend on orientation, while for the TSM contribution the sign depends only on the sign of the coupling between the two electron spins (35).

Earlier work demonstrated that DFT computations of the g tensor of the BPhe acceptor anion radicals within the ZORA formalism were in good agreement with experimental values (60). Such computations can also help to estimate differences between the g tensors of Chl a and Phe a anion radicals (Table 2.2). Since DFT predicts rather minor differences both in the principal values and in the principal axes directions, a sign change of the g tensor of the acceptor radical anion appears unlikely.

Alternatively, a change of the sign of the photo-CIDNP enhancement might be explained on the basis of the anisotropy of photo-CIDNP (35). In entire bacterial photosynthetic units containing selectively isotope labelled cofactors also a sign change occurred which has been tentatively explained by self-orientation of the membrane bound proteins induced by sample spinning around the magic angle before freezing (33). Due to the strong anisotropy of photo-CIDNP, oriented RCs are expected to show an enhancement pattern that is different from randomly oriented samples. However, the observation of a similar enhancement pattern in the smaller PSI-CPI sample makes this explanation unlikely.

	g_{11}	g_{22}	g_{33}	g_{iso}
Chl $a^{\bullet-}$	2.00461	2.00317	2.00206	2.00328
Phe $a^{\bullet-}$	2.00415	2.00308	2.00211	2.00311
$\Delta\theta$	4.2°	2.6°	3.3°	-

Table 2.2. Deviations between the g tensors of a Chl a anion radical Chl $a^{\bullet-}$ and a pheophytin anion radical Phe $a^{\bullet-}$ with analogous geometric structure from DFT computations with ADF ZORA. The directions of the principal axes deviate by $\Delta\theta$.

Photo-CIDNP sign rules (35) suggest that the difference between PSI and PSII could then be related either to a substantial difference in the electron-electron coupling, which would also shift the balance between the DD and TSM mechanisms, or to a difference in the hyperfine tensors of those nuclei for which non-equilibrium polarisation is observed.

Due to the broad similarity in the geometry of the RCs, the dipole-dipole coupling only differs slightly between the electron spins. DFT computations suggest that the SOMOs of the acceptor radical anions are rather similar, but given the lower symmetry of the donor in PSII, the SOMOs of P700 and P680 are likely to be different. If the P700 SOMO would have a strong overlap with its acceptor SOMO, this would result in a large exchange coupling and thus in a large TSM contribution. As discussed above, a larger TSM contribution would explain the uniform sign of the photo-CIDNP enhancements in PSI. As the spatial and electronic structure of the radical pair state of the whole RCs cannot be modelled precisely enough with current quantum-chemical approaches, these considerations however remain somewhat speculative. Finally, differences in the hyperfine couplings can give rise to photo-CIDNP sign and intensity changes. This point will be further elaborated after discussing the assignment of the NMR lines.

2.4.2 Linewidth and chemical shifts

The narrow linewidth of ≈ 60 Hz provides evidence for a rather rigid ordered as well as structurally and electrostatically stable donor site. Previous MAS NMR studies revealed similar properties of the donor site in bacterial RCs (61). It appears to be a general property of RCs to have a rigid donor side, keeping reorganisation energies of electron transfer low.

The photo-CIDNP signals of PSI appear considerably stronger than for unlabelled RCs of bacteria and PSII. In addition to the narrower lines, the photo-CIDNP signals may appear to be stronger due to a modified proportion of the two mechanisms producing nuclear enhancement. The predominant effect of the TSM over the DD mechanism in the stronger photo-CIDNP of PSI, as proposed here, would imply that both mechanisms cause opposite effects under current conditions, which is well in line with the model computations in ref (35).

The observed twelve photo-CIDNP signals appear in between 200 and 90 ppm. In this region, a comprehensive set of initial assignments can be obtained. The data are in agreement with previously measured photo-CIDNP spectra of unlabelled RCs of bacteria and of PSII, where no aliphatic carbons have been observed. The moderately high spinning frequency achieved here allows for the first time for an unequivocal detection of a carbonyl response of the aromatic macrocycle in a photo-CIDNP MAS NMR spectrum. The absence of aliphatic carbons is attributed to a weak pseudosecular coupling to the electron pair. There are no signals that can be attributed to amino acids of the surroundings.

The photo-CIDNP data can be assigned to a single Chl *a* cofactor. Since in PSI both the donor and the primary acceptor are Chl *a* cofactors, the possibility that the spectrum contains

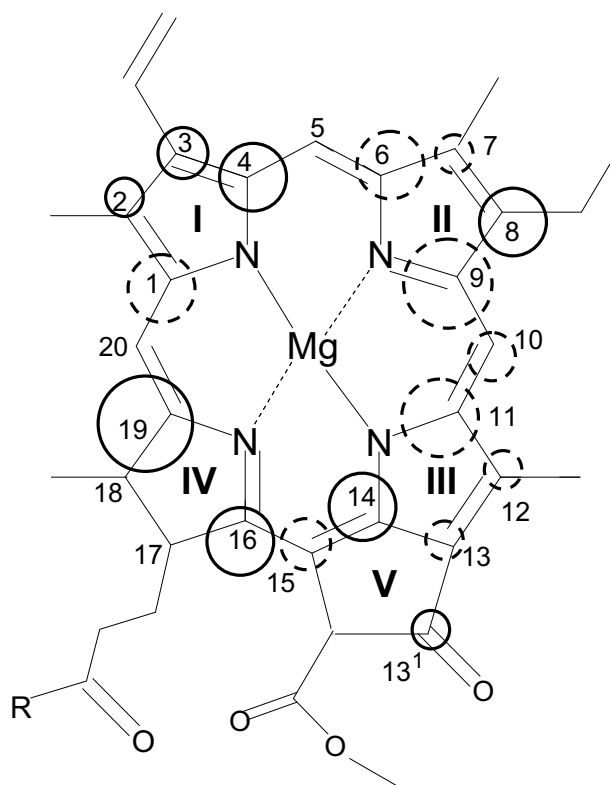


Figure 2.6. ^{13}C Photo-CIDNP patterns of Chl *a* molecule observed in PSI. The size of the circles is semi-quantitatively related to the signal intensity. All observed photo-CIDNP enhanced NMR signals are negative (emissive). The solid circles indicate a clear assignment; the dashed circles rely on signals assigned to two or three carbons (Table 2.1). The numbering of the carbons is according to IUPAC.

contributions from both the donor and the acceptor cofactors on the basis of only the chemical shifts cannot be completely excluded. However, the calculations suggest the appearance of stronger ^{13}C photo-CIDNP NMR signals from the donor than the acceptor as discussed in the following paragraph. This spectral predominance has also been observed in selectively isotope labelled bacterial RCs, for which an unambiguous assignment was possible (32).

2.4.3 Assignment of the cofactors

The assignment of observed carbon resonances allows for a semi-quantitative reconstruction of the electron-spin density pattern of π radical ions from the photo-CIDNP intensities of the observed Chl *a* (Fig. 2.6), as these intensities scale with the anisotropy of the hyperfine coupling (34). Since both the strong signals at 154.8 (C-1 and C-6) and 147.2 ppm (C-9 and C-11) are assigned to two carbons each, some uncertainty remains in the pattern. The pattern appears slightly asymmetric mainly due to the absence of photo-CIDNP intensities on the methine carbons 5 and 20. Comparison of the photo-CIDNP patterns in PSI and PSII (31) shows enhancement for mainly the same atoms. Especially the strong signals of C-4 and C-8 in PSI represent significant differences with PSII. Such an electron-spin density pattern correlates the electronic structure in the radical pair state of the RC to the chemical shift information that pertains to the ground state (35). As the electronic structures of the donor and

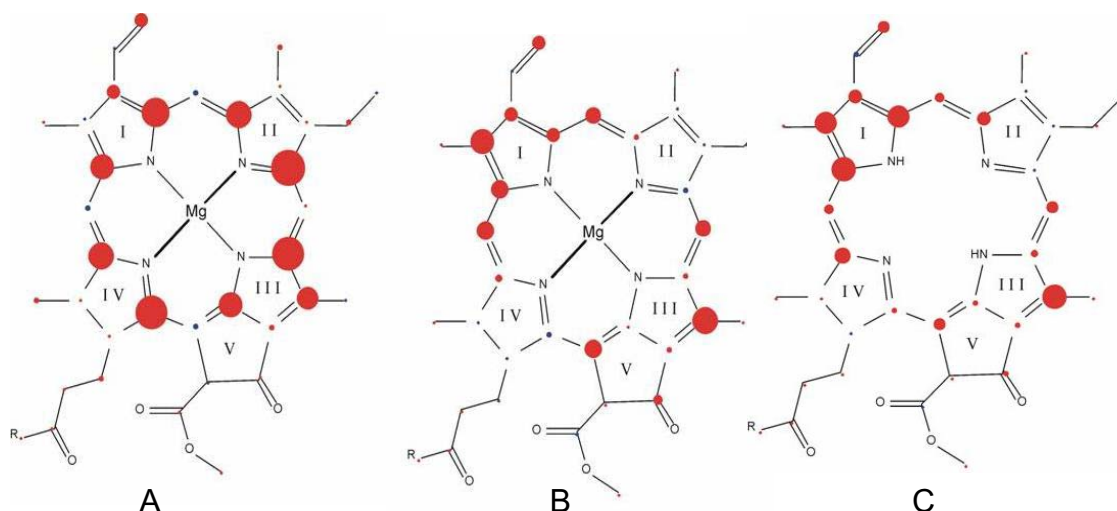


Figure 2.7. Hyperfine anisotropy of ^{13}C nuclei in radical species related to PSI and PSII by DFT computations. (A) Chl *a* radical cation as a model for the donor. (B) Chl *a* radical anion as a model for the acceptor in PSI. (C) Phe radical anion as a model for the acceptor in PSII.

acceptor Chl *a* are similar in the ground state but different in the radical pair state, the intensity pattern provides additional information with respect to the assignment of the carbons to the donor or acceptor.

To utilize this information, computed ^{13}C hyperfine anisotropies of the Chl *a* cation radical as a simple model of the donor are compared with the Chl *a* anion radical as a model of the acceptor (Fig. 2.7). Photo-CIDNP enhancement is strongly correlated to hyperfine anisotropy, but not simply proportional to it, as isotropic hyperfine coupling and the relative orientation of both the *g* and the hyperfine tensor play a minor role (35). Despite the latter complication, it may be concluded from a comparison of Figs. 2.6 and 2.7 that most of the signals very likely originate from the donor. The alternative assignment to the anion radical (Fig. 2.7B) is not convincing. The absence of spin density particularly on C-14, C-16 and C-19 cannot be reconciled. Hence, the assignment of most of the signals to the donor is reasonable. The possible exceptions are the methine carbons C-10 and C-15 and the signal of C-2 which may originate from the acceptor. If these signals are assigned to the acceptor, the common sign would suggest that the TSM mechanism dominates, as the sign for the DD mechanism depends on the sign of the *g*-value difference (35), which is different for the two constituent radicals. The signal at C-8 would not be expected for either the donor or the acceptor, but note that a Chl *a* cation radical may be only a rather crude model for the donor. It also cannot be ruled out that the signal at 144.2 ppm originates from an aromatic amino acid.

There are no significant differences recognised in the chemical shift patterns of P700 and P680 within the limits of the preliminary assignments. The largest difference is observed at the carbon C-14 of 1.9 ppm. This carbon is located on rings III and V, which suggests that the differences between both primary donors are located on this moiety of the Chl cofactor.

Differences on that part of the Chl *a* are expected to be involved into the main changes of the electronic structure causing the shift of the redox potential in P680 to 1.2 V (31). In PSI, the resonance of the carbonyl C- ^{13}C appears at about 189 ppm, which suggests that there is no hydrogen-bond or chemical modification on that carbonyl function in PSI (Table 2.1). The signal appears to be relatively broad which may be due to some heterogeneity.

Similar to the photo-CIDNP response for PSII, in PSI only a single resonance has been observed from the methine carbons at 105.4 ppm. In view of its position and relative broadness, it has been assigned to both methine carbons C-10 and C-15. In the photo-CIDNP MAS NMR spectrum of PSII, the signal at 104.6 ppm is clearly the signal with the highest absolute intensity in the spectrum. In PSI, the signal at 105.4 ppm is weaker than several signals of other aromatic carbons. This observation may be linked to a stronger localisation of electron spin density in P680 while it appears broader distributed over P700. Such an interpretation may also explain the differences between the photo-CIDNP pattern and the pattern of ^{13}C hyperfine anisotropies. The DFT computations also suggest that differences in the electronic structure of the acceptors in a similar environment are rather minor (see Table 2.2 and Fig. 2.6 B, C).

2.5 Conclusions

In the photo-CIDNP data of PSI all ^{13}C NMR signals appear to be emissive. A rational picture emerges in the discussion: (i) The TSM, causing emissive signals, dominates over the DD mechanism. Since both mechanisms cause opposite sign of photo-CIDNP, the predominance of the TSM can also be responsible for the remarkable strength of the photo-CIDNP in PSI whereas in the RCs of bacteria and PSII both mechanisms are of comparable intensity. (ii) In PSI the origin of the predominance of the TSM seems to be due the differences in the hyperfine coupling and not decreased Δg -value. A stronger overlap of the SOMOs of donor and acceptor can be related to less symmetry of electron spin density distribution on P700 compared to P680. (iii) The photo-CIDNP signals can be assigned to a single Chl *a* molecule. The predominance of the donor over the acceptor in the ^{13}C photo-CIDNP NMR spectrum is in line with our calculations and analogue to a clear assignment obtained in the bacterial RCs.

References

1. van Gorkom, H.J.; Schelvis, J.P.M., *Photosynth. Res.* **1993**, 38, 297-301.
2. Webber, A.N.; Lubitz, W., *Biochim. Biophys. Acta* **2001**, 1507, 61-79.
3. Jordan, P.; Fromme, P.; Witt, H.T.; Klukas, O.; Saenger, W.; Krauss, N., *Nature* **2001**, 411, 909-917.
4. Hoff, A.J.; Deisenhofer, J., *Phys. Rep.* **1997**, 287, 2-247.
5. Fromme, P.; Jordan, P.; Krauss, N., *Biochim. Biophys. Acta* **2001**, 1507, 5-31.
6. Breton, J., *Biochim. Biophys. Acta* **2001**, 1507, 180-193.
7. Hastings, G.; Ramesh, V.M.; Wang, R.L.; Sivakumar, V.; Webber, A., *Biochemistry* **2001**, 40, 12943-12949.
8. Breton, J.; Nabedryk, E.; Leibl, W., *Biochemistry* **1999**, 38, 11585-11592.
9. Witt, H.; Schlodder, E.; Teutloff, C.; Niklas, J.; Bordinon, E.; Carbonera, D.; Kohler, S.; Labahn, A.; Lubitz, W., *Biochemistry* **2002**, 41, 8557-8569.
10. Norris, J.R.; Uphaus, R.A.; Crespi, H.L.; Katz, J.J., *Proc. Natl. Acad. Sci. U. S. A.* **1971**, 68, 625-628.
11. Norris, J.R.; Druyan, M.E.; Katz, J.J., *J. Am. Chem. Soc.* **1973**, 95, 1680-1682.
12. Norris, J.R.; Scheer, H.; Druyan, M.E.; Katz, J.J., *Proc. Natl. Acad. Sci. U. S. A.* **1974**, 71, 4897-4900.
13. Hoff, A.J., *Phys. Rep.* **1979**, 54, 75-200.
14. O'Malley, P.J.; Babcock, G.T., *Proc. Natl. Acad. Sci. U. S. A.* **1984**, 81, 1098-1101.
15. Norris, J.R.; Scheer, H.; Katz, J.J., *Ann. New York Acad. Sci.* **1975**, 244, 260-280.
16. Lendzian, F.; Lubitz, W.; Scheer, H.; Hoff, A.J.; Plato, M.; Trankle, E.; Möbius, K., *Chem. Phys. Lett.* **1988**, 148, 377-385.
17. Rigby, S.E.J.; Nugent, J.H.A.; Omalley, P.J., *Biochemistry* **1994**, 33, 10043-10050.
18. Krabben, L.; Schlodder, E.; Jordan, R.; Carbonera, D.; Giacometti, G.; Lee, H.; Webber, A.N.; Lubitz, W., *Biochemistry* **2000**, 39, 13012-13025.
19. Käss, H.; Fromme, P.; Witt, H.T.; Lubitz, W., *J. Phys. Chem. B* **2001**, 105, 1225-1239.
20. Käss, H.; Rautter, J.; Bönigk, B.; Hofer, P.; Lubitz, W., *J. Phys. Chem.* **1995**, 99, 436-448.
21. Käss, H.; Lubitz, W., *Chem. Phys. Lett.* **1996**, 251, 193-203.
22. Käss, H.; Fromme, P.; Lubitz, W., *Chem. Phys. Lett.* **1996**, 257, 197-206.
23. Mac, M.; Tang, X.S.; Diner, B.A.; McCracken, J.; Babcock, G.T., *Biochemistry* **1996**, 35, 13288-13293.
24. Käss, H.; Lubitz, W.; Hartwig, G.; Scheer, H.; Noy, D.; Scherz, A., *Spectrochim. Acta A* **1998**, 54, 1141-1156.
25. Plato, M.; Krauss, N.; Fromme, P.; Lubitz, W., *Chem. Phys.* **2003**, 294, 483-499.
26. Zysmilich, M.G.; McDermott, A., *J. Am. Chem. Soc.* **1994**, 116, 8362-8363.
27. Zysmilich, M.G.; McDermott, A., *J. Am. Chem. Soc.* **1996**, 118, 5867-5873.
28. Zysmilich, M.G.; McDermott, A., *Proc. Natl. Acad. Sci. U. S. A.* **1996**, 93, 6857-6860.
29. Matysik, J.; Alia; Hollander, J.G.; Egorova-Zachernyuk, T.; Gast, P.; de Groot, H.J.M., *Indian J. Biochem. Biophys.* **2000**, 37, 418-423.

30. Matysik, J.; Alia; Gast, P.; Lugtenburg, J.; de Groot, H.J.M. In *Perspectives on solid state NMR in biology*; Kiihne, S., de Groot, H.J.M., Eds.; Kluwer: Dordrecht, 2001; p 215-225.
31. Matysik, J.; Alia; Gast, P.; van Gorkom, H.J.; Hoff, A.J.; de Groot, H.J.M., *Proc. Natl. Acad. Sci. U. S. A.* **2000**, 97, 9865-9870.
32. Schulten, E.A.M.; Matysik, J.; Alia; Kiihne, S.; Raap, J.; Lugtenburg, J.; Gast, P.; Hoff, A.J.; de Groot, H.J.M., *Biochemistry* **2002**, 41, 8708-8717.
33. Prakash, S.; Alia; Gast, P.; Jeschke, G.; de Groot, H.J.M.; Matysik, J., *J. Mol. Struct.* **2003**, 661, 625-633.
34. Jeschke, G., *J. Am. Chem. Soc.* **1998**, 120, 4425-4429.
35. Jeschke, G.; Matysik, J., *Chem. Phys.* **2003**, 294, 239-255.
36. Daviso, E.; Jeschke, G.; Matysik, J. In *Biophysical techniques in photosynthesis*; Aartsma, T.J., Matysik, J., Eds.; Springer: Dordrecht, 2007, p 385-399.
37. Blankenship, R.; McGuire, A.; Sauer, K., *Proc. Natl. Acad. Sci. U. S. A.* **1975**, 72, 4943-4947.
38. van der Est, A., *Biochim. Biophys. Acta* **2001**, 1507, 212-225.
39. Mullet, J.E.; Burke, J.J.; Arntzen, C.J., *Plant Physiol.* **1980**, 65, 814-822.
40. Arntzen, C.J.; Ditto, C.L., *Biochim. Biophys. Acta* **1976**, 449, 259-274.
41. Arnon, D.I., *Plant Physiol.* **1949**, 24, 1-15.
42. Rutherford, A.W.; Mullet, J.E., *Biochim. Biophys. Acta* **1981**, 635, 225-235.
43. Gast, P.; Swarthoff, T.; Ebskamp, F.C.R.; Hoff, A.J., *Biochim. Biophys. Acta* **1983**, 722, 163-175.
44. Bennett, A.E.; Rienstra, C.M.; Auger, M.; Lakshmi, K.V.; Griffin, R.G., *J. Chem. Phys.* **1995**, 103, 6951-6958.
45. Velde, G.T.; Bickelhaupt, F.M.; Baerends, E.J.; Guerra, C.F.; van Gisbergen, S.J.A.; Snijders, J.G.; Ziegler, T., *J. Comput. Chem.* **2001**, 22, 931-967.
46. Chow, H.C.; Serlin, R.; Strouse, C.E., *J. Am. Chem. Soc.* **1975**, 97, 7230-7237.
47. Käss, H.; Bittersmannweidlich, E.; Andreasson, L.E.; Bonigk, B.; Lubitz, W., *Chem. Phys.* **1995**, 194, 419-432.
48. van Lenthe, E.; Snijders, J.G.; Baerends, E.J., *J. Chem. Phys.* **1996**, 105, 6505-6516.
49. van Lenthe, E.; van der Avoird, A.; Wormer, P.E.S., *J. Chem. Phys.* **1998**, 108, 4783-4796.
50. Matysik, J.; Schulten, E.; Alia; Gast, P.; Raap, J.; Lugtenburg, J.; Hoff, A.J.; de Groot, H.J.M., *Biol. Chem.* **2001**, 382, 1271-1276.
51. Alia; Matysik, J.; Soede-Huijbregts, C.; Baldus, M.; Raap, J.; Lugtenburg, J.; Gast, P.; van Gorkom, H.J.; Hoff, A.J.; de Groot, H.J.M., *J. Am. Chem. Soc.* **2001**, 123, 4803-4809.
52. Bonnerjea, J.; Evans, M.C.W., *FEBS Lett.* **1982**, 148, 313-316.
53. MacMillan, F.; Hanley, J.; van der Weerd, L.; Knupling, M.; Un, S.; Rutherford, A.W., *Biochemistry* **1997**, 36, 9297-9303.
54. Rigby, S.E.J.; Muhiuddin, I.P.; Santabarbara, S.; Evans, M.C.W.; Heathcote, P., *Chem. Phys.* **2003**, 294, 319-328.
55. Kamlowski, A.; Zech, S.G.; Fromme, P.; Bittl, R.; Lubitz, W.; Witt, H.T.; Stehlik, D., *J. Phys. Chem. B* **1998**, 102, 8266-8277.
56. Berthold, T.; Bechtold, M.; Heinen, U.; Link, G.; Poluektov, O.; Utschig, L.; Tang, J.; Thurnauer, M.C.; Kothe, G., *J. Phys. Chem. B* **1999**, 103, 10733-10736.
57. Bratt, P.J.; Poluektov, O.G.; Thurnauer, M.C.; Krzystek, J.; Brunel, L.C.; Schrier, J.; Hsiao, Y.W.; Zerner, M.; Angerhofer, A., *J. Phys. Chem. B* **2000**, 104, 6973-6977.

58. Abraham, R.T.; Rowan, A.E. In *Chlorophylls*; Scheer, H., Ed. CRC Press: Boca Raton FL, 1991; p 797-834.
59. Boender, G.J. Ph.D. thesis, University of Leiden, 1996.
60. Dorlet, P.; Xiong, L.; Sayre, R.T.; Un, S., *J. Biol. Chem.* **2001**, 276, 22313-22316.
61. Fischer, M.R.; de Groot, H.J.M.; Raap, J.; Winkel, C.; Hoff, A.J.; Lugtenburg, J., *Biochemistry* **1992**, 31, 11038-11049.