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

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5 Photo-CIDNP observed by ^{13}C MAS NMR in isolated membrane fragments of *Heliobacillus mobilis*

Photo-CIDNP has been observed in entire membrane fragments of heliobacteria *Heliobacillus mobilis* by ^{13}C MAS solid-state NMR at magnetic fields of 4.7, 9.4 and 17.6 Tesla. At the highest magnetic field all signals are emissive, while at the lower fields part of the signal is absorptive and two sets of tetrapyrrole cofactors appear. One set, showing the enhanced absorptive signals, is assigned to the BChl *g* donor, while the set of emissive signals is assigned to the acceptor, 8¹-hydroxy Chl *a*. Both donor and acceptor appear to be monomeric. (4- ^{13}C) ALA labelling reveals an isotope effect on the photo-CIDNP intensities.

5.1 Introduction

Heliobacteria are found to be closely related to cyanobacteria and are characterized by the presence of a unique BChl *g* cofactor (Fig. 5.1A) (1-4). The RCs of heliobacteria are less complex in their architecture compared to photosystems of cyanobacteria and purple bacteria, with the antenna pigments and RC bound to a single pigment protein complex which is embedded in the cytoplasmic membrane (5-7). The RCs lack light harvesting antenna complexes like chlorosomes found in green sulphur bacteria and light harvesting complexes LH I, LH II found in purple bacteria, thus having a reduced amount of antenna chlorophylls associated with the RC (8). They are grouped with the type I RCs, along with RCs of green sulphur bacteria, PSI of cyanobacteria and plants. From two members of this category, structural data are now available, namely from PSI of cyanobacteria (9) and of higher plants (10, 11).

The primary electron donor in the RC of heliobacteria is termed either as P798 (12) or P800 (5) and has been reported to be a dimer comprising of two BChl *g* (13) or the 13²-epimers BChl *g* and BChl *g'* (14). On the basis of experimental data the primary electron acceptor is proposed to be a Chl *a* like pigment absorbing at 670 nm (15, 16). Chemical analysis established the structure to be 8¹-hydroxy Chl *a* esterified with a farnesol sidechain (17) (Fig. 5.1B). The pigment composition per RC is about 35-40 molecules of BChl *g* (6), two molecules of BChl *g'* (14) and two molecules of 8¹-hydroxy Chl *a* (17, 18) and about two to three carotenoid molecules (19, 20). Membranes of heliobacteria contain menaquinone, but

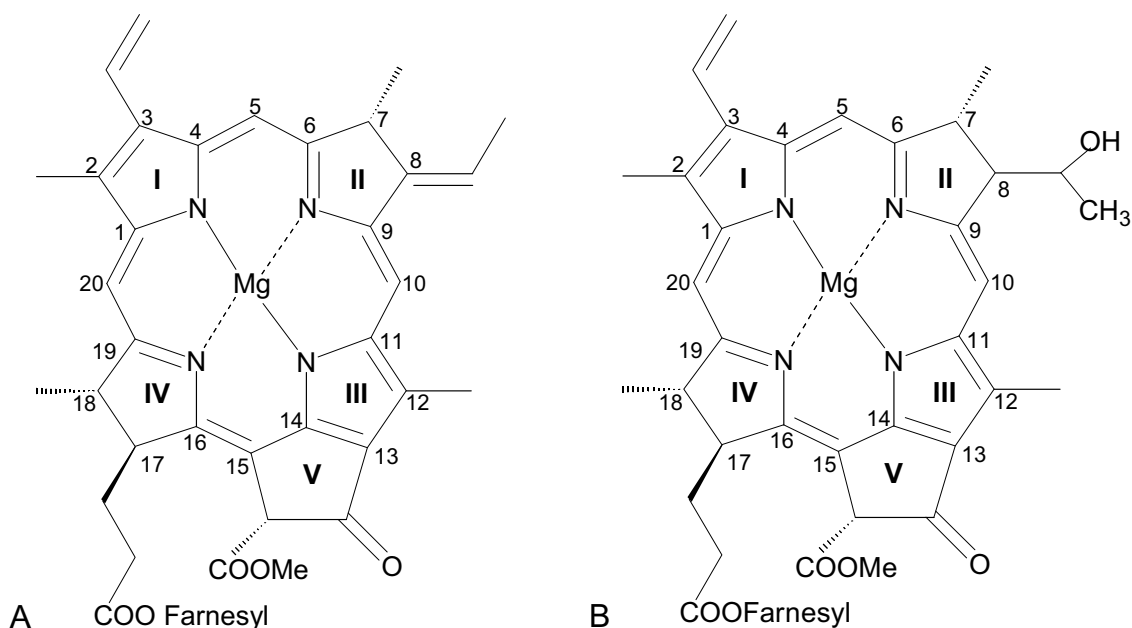


Figure 5.1. The structure of (A) BChl *g* and (B) 8¹-hydroxy Chl *a*, using IUPAC numbering.

there is no clear evidence establishing its role as an intermediate in the forward electron transfer (21-24). EPR and optical spectroscopic data indicate the presence of an iron-sulphur centre similar to F_x (13, 25-28) in PSI where it acts as electron acceptor and recently the presence of F_A and F_B clusters in the RCs have been reported (21).

Photo-CIDNP is an effect well known in liquid NMR and is used for example to explore protein surfaces (30, 31). In solids, photo-CIDNP has been observed for the first time in 1994 by MAS solid-state NMR, in quinone-blocked frozen samples of RCs of *Rb. sphaeroides* R-26 under illumination (32-35). Since then this technique has been employed in investigating RCs ranging from purple bacterial RCs from *Rb. sphaeroides* WT (36), plant PSI (Chapter 2), PSII (37, 38) and RCs from green sulphur bacterium *C. tepidum* (Chapter 4). NMR signals were detected in entire membrane bound photosynthetic units and even whole cells of *Rb. sphaeroides* (35, 39). Until now, the observation of the solid-state photo-CIDNP effect is limited to natural photosynthetic RCs.

In this chapter isolated membrane fragments of *Hba. mobilis* are investigated using photo-CIDNP. Photo-CIDNP MAS NMR provides information of the electronic ground-state structures of the electron donor and acceptor forming a correlated radical pair. In addition, photo-CIDNP MAS NMR intensities are related to the local electron spin densities in the electron donor and the electron acceptor forming the correlated radical pair state (40, 41). Selective ¹³C isotope labelling at various cofactor positions in bacterial RCs provided insight into the ground-state electronic structure of the special pair (42, 43). The origin of the photo-CIDNP observed in the solid-state in photosynthetic RCs has been explained by the occurrence of three mechanisms, called the TSM, DD, and DR (35, 44-46).

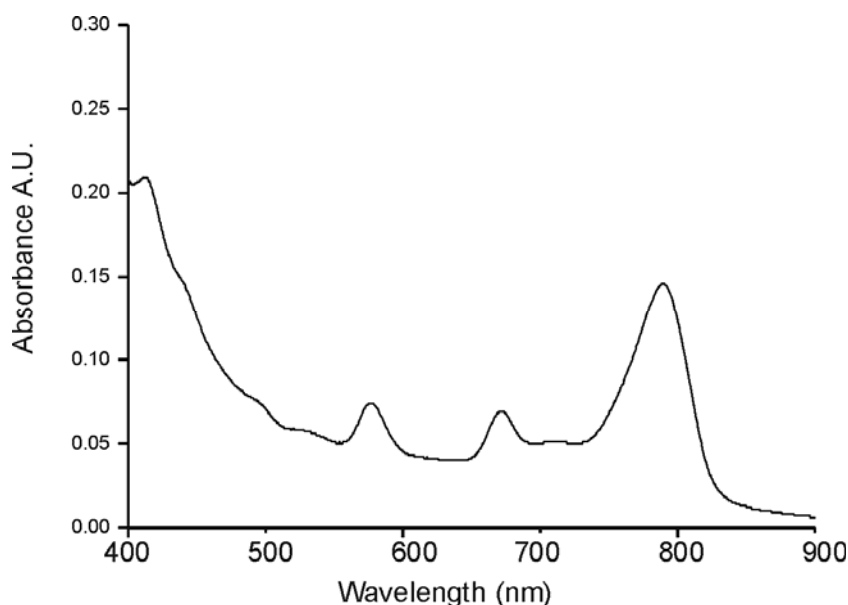


Figure 5.2. Absorption spectrum of *Hba. mobilis* membrane fragments.

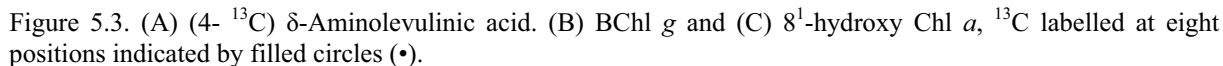
5.2 Materials and Methods

5.2.1 Sample preparation

Hba. mobilis cells were grown in medium no. 1552 as described by van de Meent *et al.* (6). The cells were harvested after a period of seven days by centrifugation and re-suspended in a buffer containing 20 mM Tris-HCl and 10 mM sodium ascorbate (pH 8.0). All buffers used were thoroughly degassed. All the preparation was performed in the dark and care was taken to minimise the exposure of the samples to oxygen. The membrane fragments were prepared by sonication for 35 min followed by a 15 min centrifugation step at 40,000 *g* to remove unbroken cells and large fragments. The resulting supernatant was ultra centrifuged for 2 h at 200,000 *g* at a temperature of 4°C. The pellet containing the membrane fragments was re-suspended in buffer containing 50 mM glycine and 0.02% SB-12 (pH 10.8). The absorbance spectrum of the isolated membrane fragments is shown in Fig. 5.2. The spectrum shows a BChl *g* peak at 790 nm and a peak at 690 nm from Chl *a* like pigments (5). For photo-CIDNP experiments the sample was reduced by 50 mM sodium dithionite under nitrogen air flow.

5.2.2 Preparation of selectively ¹³C labelled membrane fragments

Selective isotope enrichment of (B)Chl in *Hba. mobilis* was done by growing the bacterial cultures (80 mL) anaerobically in the presence of 1.0 mM [4-¹³C]- δ-aminolevulinic acid (Fig. 5.3A) ([4-¹³C]-COOHCH₂CH₂¹³COCH₂NH₂·HCl, 99% ¹³C-enriched) purchased from Cambridge Isotope Laboratories (Andover, USA). ALA is a precursor of naturally occurring tetrapyrroles, including (B)Chl (47). The incorporation of [4-¹³C]-ALA produces (B)Chl labelled at the C-1, C-3, C-6, C-8, C-11, C-13, C-17 and C-19 (Fig. 5.3). The preparation of isolated membranes was done as described above.



BChl *g* is highly sensitive to light and oxygen, while its product of pheophytinization, BPhe *g*, is considerably more stable (4). Since the formation of BPhe is by the loss of Mg from BChl it can be assumed that the isotopic incorporations of the tetrapyrrole moiety of BChl *g* and BPhe *g* are identical, and the isotope enrichment was determined from the pheophytin form (48).

The mass spectrometry measurements were performed by diluting a small fraction of the purified pigment in methanol containing 1% ammonium acetate. The mass spectrum was acquired in positive ion mode by direct infusion (5 μ L/min) using a LTQ FT hybrid mass spectrometer (ThermoFischer, Bremen, Germany) equipped with an electrospray ionization

source. The capillary was typically held at 3.5 kV, the transfer capillary was maintained at 280 °C and the tube lens was set to 240 V. For each experiment, 15 scans were accumulated.

5.2.4 MAS-NMR measurements

The NMR experiments were performed by using AV-750, DMX-400 and DMX-200 NMR spectrometers (Bruker-Biospin GmbH, Karlsruhe, Germany). The samples were loaded into optically transparent 4 mm sapphire rotors. The illumination setup has been specially designed for a Bruker MAS probe (41, 50). The light and dark spectra were obtained with a Hahn echo pulse sequence and TPPM proton decoupling (51).

5.3 Results and Discussion

5.3.1 Field-dependence of the strength of the photo-CIDNP

All ^{13}C MAS NMR spectra obtained from natural abundance sample of *Hba. mobilis* membrane fragments in the dark (Fig. 5.4) show similar features. Strong signals are observed between 0 and 50 ppm and are characteristic for a ^{13}C -MAS NMR spectrum of a large protein (52). Weak resonances from aromatic cofactors and amino acids appear between 120 and 140 ppm. The signal at 174.5 ppm arises mainly from the buffer. Dark spectra were obtained at different magnetic fields, (A) 17.6 Tesla, (B) 9.4 Tesla and (C) 4.7 Tesla. The signals obtained with the highest field (Fig. 5.4A) appear slightly better in terms of signal to noise, while several signals are not resolved at 4.7 Tesla (Fig. 5.4C). The ^{13}C photo-CIDNP MAS NMR spectrum obtained with continuous illumination (Fig. 5.5) shows both strong emissive (negative) and enhanced absorptive (positive) signals between 80 and 200 ppm. These spectra are obtained at three different magnetic fields, at (A) 17.6 Tesla, (B) 9.4 Tesla and (C) 4.7 Tesla. The strongest photo-CIDNP effect is observed at 4.7 Tesla (Fig. 5.5C), while at higher fields the effect decreases. Using the broad dark signal at about 30 ppm as an internal standard, the photo-CIDNP signal intensity at 9.4 Tesla is found to be by a factor of 3 higher than at 17.6 Tesla while at 4.7 Tesla the relative photo-CIDNP intensity increases further to a factor of 10 relative to the effect at 17.6 Tesla. The same pattern has been observed in bacterial RCs of *Rb. sphaeroides* WT and R-26 (35, 36) and PSII (Chapter 3). In contrast, the field dependence of the photo-CIDNP effect in PSI shows a maximum strength at 9.4 Tesla (Chapter 3). In addition the enhancement observed in the light induced signals from *Hba. mobilis* sample appears very strong, considering that the spectra are obtained from unlabelled RCs in membrane fragments.

5.3.2 Field dependence on the sign of photo-CIDNP effect

At high fields, the photo-CIDNP effect in the sample of *Hba. mobilis* is entirely emissive (Fig. 5.5A) as also observed for RCs of *Rb. sphaeroides* WT (36) and for PSI (Chapter 3). In contrast, at 9.4 Tesla, a mixed pattern of absorptive and emissive signals is observed (Fig. 5.5

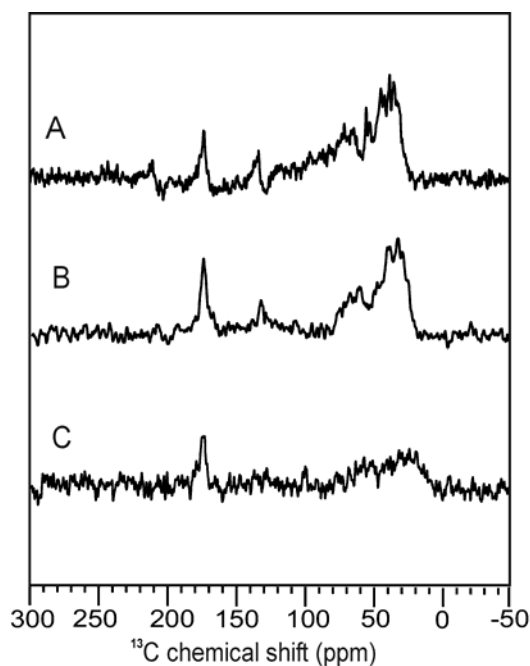


Figure 5.4. ^{13}C MAS NMR spectra of membrane fragments of *Hba. mobilis* obtained in the dark at different magnetic fields, (A) 17.6 Tesla, (B) 9.4 Tesla and (C) 4.7 Tesla, at a temperature of 240 K with a MAS frequency of 8 kHz.

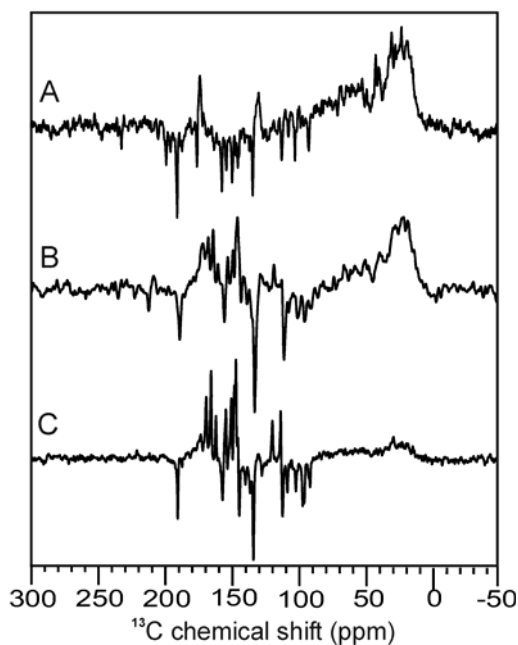


Figure 5.5. ^{13}C MAS NMR spectra of membrane fragments of *Hba. mobilis* obtained using continuous illumination with white light in different magnetic fields, (A) 17.6 Tesla, (B) 9.4 Tesla and (C) 4.7 Tesla at a temperature of 240 K with a MAS frequency of 8 kHz.

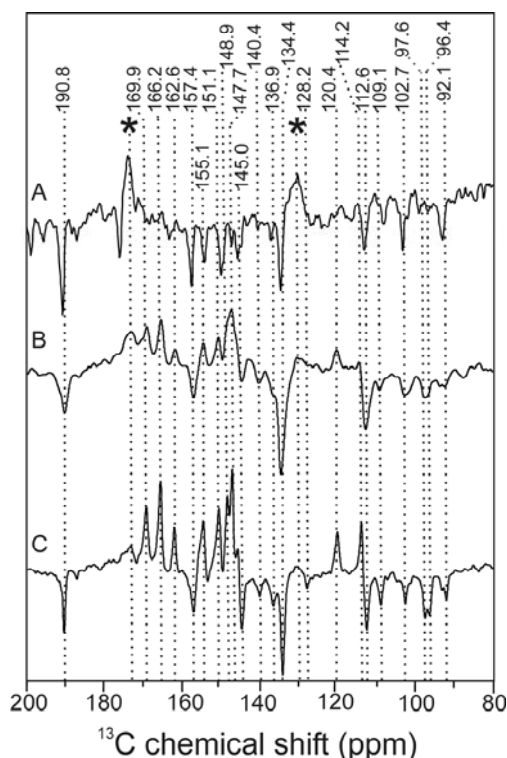


Figure 5.6. Detailed view of the region showing photo-CIDNP at different magnetic fields, (A) 17.6 Tesla, (B) 9.4 Tesla, and (C) 4.7 Tesla. The centerbands are shown in dashed lines and the dark signals are marked by asterisks.

B). A pattern of absorptive and emissive signals are also observed in RCs of *Rb. sphaeroides* R-26 and PSII at all three fields (ref. 35, Chapter 3). At 4.7 Tesla a similar mixed pattern appears (Fig. 5.5C). Hence, there must be an inversion point of the sign of the sub-set of signals between 9.4 and 17.6 Tesla. Such a field-dependent sign change of a sub-set of signals has not yet been observed in any other system. Details can be seen in Fig. 5.6, showing the olefinic and carbonylic regions on an expanded scale. The signals labelled with a star (*) originate from the protein and are not light-induced. All other signals are due to the photo-CIDNP effect. The best spectral resolution is obtained at 4.7 Tesla (Fig. 5.6C), despite the reduced Zeeman splitting and correspondingly less spectral dispersion.

5.3.3 Effect of selective isotope labelling

Selectively [4- ^{13}C]-ALA isotope labelling of *Hba. mobilis* results in label patterns of the tetrapyrrole macrocycles as shown in Fig. 5.3B and C. Since eight molecules of labelled ALA can be used to synthesize one molecule of BChl *g*, a maximum of eight ^{13}C labels can be incorporated in BChl *g* and in BPhe *g* (42). The mass spectra of the unlabelled and labelled BPhe *g* are shown in Fig. 5.7 A and B, respectively. The mass spectrum of the unlabelled BPhe *g* exhibits a molecular peak at $m/z = 797.5$, which corresponds to BPhe *g* ($[\text{M}+\text{H}]^+$, *i.e.* $\text{C}_{50}\text{H}_{60}\text{N}_4\text{O}_5$) as well as three peaks resulting from the ^{13}C , ^{15}N and ^{18}O in natural abundance.

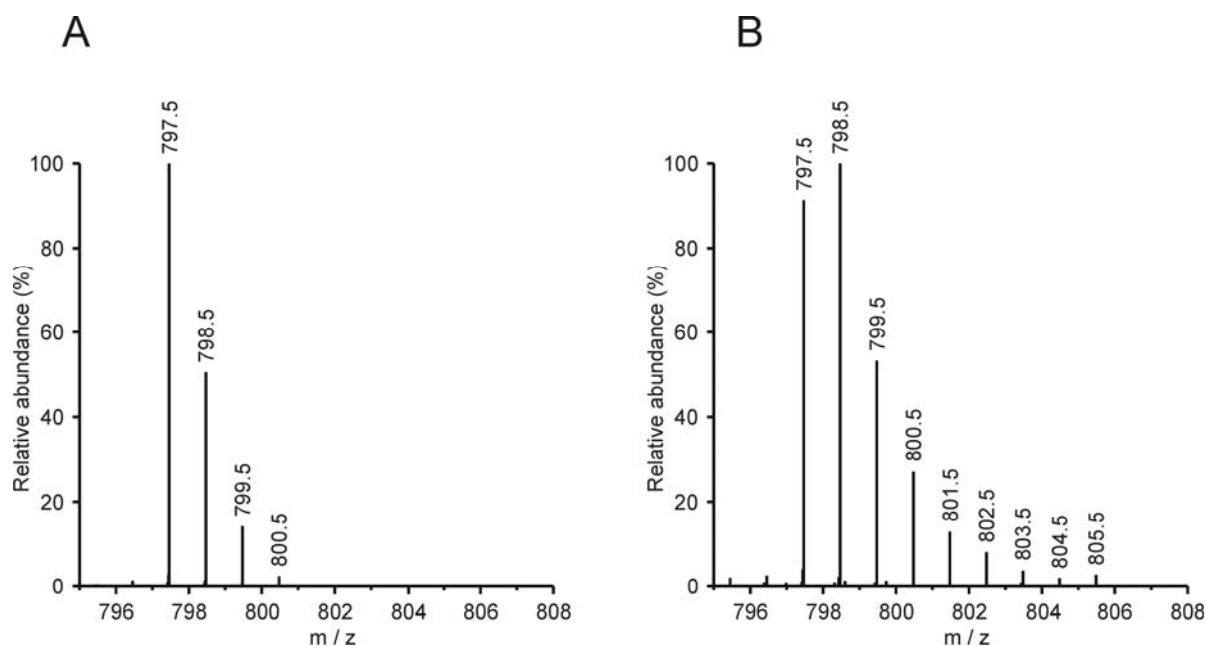


Figure 5.7. Mass spectra of (A) natural abundance BPhe g and (B) 4-Ala labelled $^{13}\text{C}_8$ BPhe g.

The mass spectrum of the labelled sample shows the peak due to unlabelled BPhe g as well as peaks resulting from the incorporation of ^{13}C isotopes ($M+1$ to $M+8$). The isotopic pattern of the unlabelled sample was used to calculate the intensities of the labelled BPhe g (53). The total incorporation of the ^{13}C was calculated to be 12%. The statistical analysis suggests that 50% of the BChl g cofactors in the *Hba. mobilis* sample were not labelled. The ^{15}N and ^{18}O contributions were not taken into account for the calculations of the isotope enrichment.

5.3.4 Effect of isotope labelling

The BChl g molecule (Fig. 5.3B) contains eight ^{13}C labelled positions from which one is aliphatic (C-17). In the 8¹-hydroxy Chl *a* cofactor (Fig. 5.3C), also eight positions are labelled, however two carbons are aliphatic. ^{13}C photo-CIDNP spectra were measured at 4.7 Tesla (Fig. 5.8A) and 9.4 Tesla (Fig. 5.8B). Both spectra show a pattern of absorptive and emissive photo-CIDNP signals, similar to the unlabelled sample. At 4.7 Tesla, five strongly absorptive signals dominate the spectrum, while the emissive signals are much weaker. Comparing the averaged intensity ratios of positive and negative signals, the relative intensity of the positive signals (Spectrum 5.7A) is a factor of 5 stronger than for the unlabelled samples (Spectrum 5C). Assuming that the TSM causes emissive signals, while the DD generates positive signals, as found in bacterial RCs (36), it appears that the isotope labelling affects the outcome of the two mechanisms to a different extent. Hence, it may be that the TSM is weakened or that the DD is enhanced. Magnetic isotope effects have been shown to affect the intersystem crossing frequency (54), and it appears reasonable that this generates positive signals at the expense of the negative TSM by isotope labelling, with little effect on

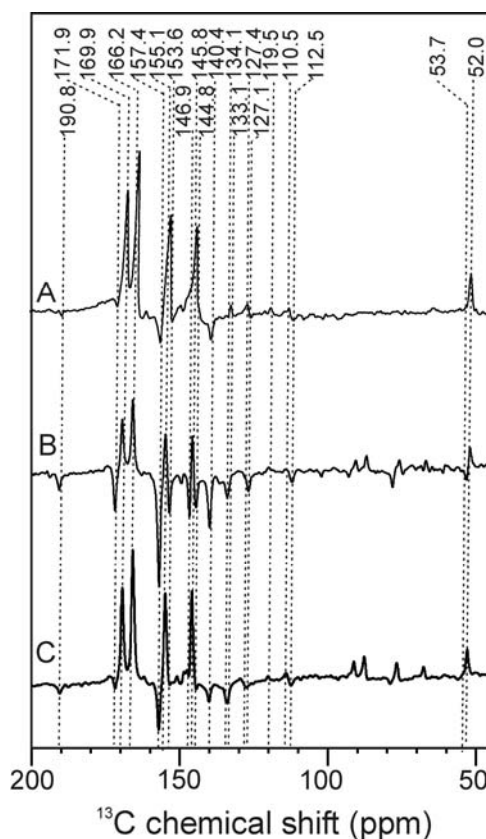


Figure 5.8. ^{13}C Photo-CIDNP MAS NMR spectra obtained in the dark and with continuous illumination with white light at (A) 4.7 Tesla using a cycle delay of 4sec (B) 9.4 Tesla using a cycle delay of 4sec and (C) 9.4 Tesla using a cycle delay of 0.4sec. A spinning frequency of 8 kHz and a temperature at 240 K were used for the experiments.

the DD. Since mass spectrometry data analysis shows an isotope label concentration of 12%, compared to the natural abundance concentration of 1%, a signal enhancement by a factor of 12 due to isotope labelling would be expected. Comparing signal intensities of labelled and unlabelled signals (discussed in following paragraphs), an enhancement factor of about 6 has been found upon labelling for both emissive and enhanced absorptive signals. The loss of signal may be due to spin diffusion, or due to an effect of the isotope labelling on the spin-chemical machinery producing photo-CIDNP.

In the unlabelled sample, no photo-CIDNP is observed in the aliphatic region, while in the 4-ALA labelled sample, a signal appears at 52.0 ppm (Figs. 5.5 and 5.8). Hence an assumption can be made that this aliphatic signal is build-up by spin diffusion. Similar intensity equilibration of photo-CIDNP signals under the steady-state conditions of continuous illumination experiment has been observed previously in RCs of *Rb. sphaeroides* WT (39). This would imply that also the intensities of the aromatic photo-CIDNP signals have been equilibrated by spin diffusion and the small difference in intensity reflects different local relaxation properties.

5.3.5 Effect of fast scanning

Relaxation MAS NMR studies on the RC of *Rb. sphaeroides* WT demonstrate that the T_1 relaxation times of carbon atoms of the more rigid parts of the donor cofactor are around 17 seconds (41). All spectra presented until now have been measured at long cycle delays allowing for sufficient relaxation. Unlabelled samples were measured at 12 seconds, and for labelled samples 4 seconds cycle delay has been applied. As shown for various RCs, this difference in cycle delay has very little effect on the spectral pattern (55). However, the use of very fast cycle delays may prevent complete relaxation, and signals of cofactors having a long T_1 may be quenched. The spectrum in Fig. 5.8C is obtained using a high cycle frequency of 0.4 seconds favouring signals of carbons having a short T_1 relaxation rate. Evidently the set of emissive signals decays dramatically compared to the spectrum measured at the same field with longer cycle delay (Fig. 5.8B). Recently it has been shown that the active role of the triplet involves fast enhanced recovery of the donor signals by relaxation mechanisms (55). If relaxation channels open up when the triplet is present the assignment of the positive signals to the donor would be more reasonable and may indicate the possibility of cross relaxation. Alternatively, when only TSM and DD are present, it is reasonable to assume that the emissive signals arise from the donor side, which is generally known to be very rigid (ref. 56 and Chapter 2).

5.3.6 Signal assignment

The single aliphatic photo-CIDNP signal appears at 52.0 ppm in the 4-ALA labelled sample (Fig. 5.8A) and can be unambiguously assigned to the C-17, the only aliphatic ^{13}C labelled carbon in BChl *g* (Table 5.1). Such an assignment would imply that the positive signals originate from the donor and are caused by the DR mechanism. If the aliphatic response would originate from the acceptor, two signals would be expected, from the hydroxyl moiety at the 8¹ position and the C-17, however, there is no indication for a second signal in the spectrum (Fig. 5.8A). Hence this corroborates the assignment of the enhanced absorptive signals to the donor side, associating the positive signals to the BChl *g* (Fig. 5.1A). This would imply that the emissive signals originate from the acceptor cofactor, which means that the negative signals would be related to the modified plant Chl *a* (Fig. 5.1B).

Upon 4-ALA labelling, four strong signals at 169.9, 166.2, 155.1 and 145.8 appear in the aromatic region (Fig. 5.8A). Additionally, four small signals are observed at 133.1, 127.4, 119.5 and 110.5 ppm. Together with the aliphatic signal at 52.0 ppm, which is assigned to the C-17, nine positive signals can be identified which arise presumably from the donor. The ^{13}C BChl *g* chemical shifts have not yet been reported. However, except for the pyrrole ring II and the esterifying alcohol, the chemical composition of the tetrapyrrole ring of BChl *g* is identical to the well studied plant Chl *a*. The chemical shift of the C-6 may be quite close to the C-6 in BChl *a*, which is expected at 168.9 ppm (Table 5.1).

Chl <i>a</i>		Carbon No.	BChl <i>a</i>		<i>Hba. mobilis</i>	
$\sigma_{\text{liq}}^{\text{a}}$	$\sigma_{\text{ss}}^{\text{b}}$		$\sigma_{\text{liq}}^{\text{c}}$	$\sigma_{\text{ss}}^{\text{d}}$	Positive signals	Negative signals
189.3	190.6	13¹	199.3	188.2		190.8
172.7	175.3	17³	173.4	174.0		
171.0	171.2	13³	171.6	171.4		
167.4	170.0	19	167.3	168.9	166.2	171.9
161.4	162.0	14	160.8	160.7	162.6	
154.0	155.9	1	151.2	153.5	155.1	157.4
155.8	154.4	6	168.9	170.2	169.9	153.6
151.4	154.0	16	152.2	150.1	151.1	
148.0	150.7	4	150.2	152.2	148.9	
147.7	147.2	11	149.5	147.2	145.8	145.0
146.1	147.2	9	158.5	158.0	147.7	
144.1	146.2	8	55.6			
139.0	137.0	3	137.7	136.1		140.4
135.5	136.1	2	142.1	140.7		
134.2	134.0	12	123.9	119.9		
134.0	133.4	7				
131.5	126.2	13	130.5	124.1	133.1	134.4
131.5	126.2	3¹	199.3	194.5	127.4	
118.9	113.4	3²			119.5	
107.1	108.2	10	102.4	100.0	110.5	109.1
106.2	102.8	15	109.7	105.8		102.7
100.0	98.1	5	99.6	98.8		97.6, 96.4
92.8	93.3	20	96.3	93.7		92.1
51.6	51.4	17	50.4		52.0	53.7

Table 5.1. Tentative ^{13}C chemical shifts assignment of the observed negative and positive photo-CIDNP signals in *Hba. mobilis* membrane fragments when compared to published chemical shift data for Chl *a* and BChl *a*. (a) Ref. 57, the liquid NMR data (b) Ref. 58, the solid-state NMR data, which have been obtained from aggregates. (c) Ref. 59 (d) Ref. 59.

The two strong signals appearing at 169.9 and 166.2 ppm can be assigned convincingly to C-6 and C-19, respectively. Since for a Chl *a*, only a single carbon signal is expected downfield, the assignment of the positive signals to the BChl *g* donor is plausible. The signals at 155.1 and 145.8 ppm match well with C-1 and C-11 respectively. The labelled carbon C-13 may be assigned to the weak signal at 133.1 ppm. The other three small positive signals arise from positions that were not labelled. The signals at 127.4, 119.5 and 110.5 may be assigned to the C-3¹, C-3² and C-10 carbons or could originate from histidine, which has a response in this region (60). There is no evidence for a positive signal from C-3 and C-8, which are labeled, while the nearby C-1 and C-6 yield strong signals. In the unlabelled sample, nine enhanced absorptive signals are observed at 169.9, 166.2, 162.6, 155.1, 151.1, 148.9, 147.7, 120.4, 114.2 ppm (Fig. 5.6C). The signal at 162.6 ppm can be assigned to the C-14, and the signal at

151.1 ppm to the C-16. The signals at 148.9 and 147.7 ppm match the NMR signals from carbons C-4 and C-9 for Chl *a* in the solid-state and in solution. The weak signals at 120.4 and 114.2 ppm are in the range of the histidine resonances and occur with similar intensity as for the labelled sample.

In the 4-ALA labelled sample, the negative signals are most pronounced at 9.4 Tesla (Fig. 5.8B). Signals appear at 190.8, 171.9, 157.4, 153.6, 146.9, 144.8, 140.4, 134.1, 127.1, 112.5 and 53.7 ppm. Assuming that the emissive signals originate from the acceptor, which is 8¹-hydroxy Chl *a*, an assignment of the signal at 190.8 ppm to the 13¹ carbonyl is reasonable. A 13¹ carbonyl signal has also been observed from PSI (Chapter 2) and the RC of *C. tepidum* (Chapter 4). However, it is remarkable that an unlabelled carbon shows such a strong, signal intensity. The signal at 171.9 ppm could be assigned to the C-19, which is labelled. The strongest emissive signal for the labelled sample appears at 157.4 ppm, which matches quite well to the response of the C-1 position for Chl *a* in the solid-state and in solution. The signal at 153.6 ppm in the labelled sample overlaps with the strong positive signal at 155.0 ppm, and can be attributed to the C-6 carbon. The two emissive minima at 146.9 and 144.8 for the labelled sample cannot be separated because of overlap with the positive signal at 145.9 ppm. Both may originate from a single emissive signal of the C-11. The shifts of the signals at 140.4 and 134.1 ppm match the shifts expected for the labelled C-3 and C-13, respectively. The two signals at 127.1 and 112.5 ppm again may be assigned to histidines (60). In the aliphatic region, an emissive signal appears at 53.7 ppm and can be assigned to C-8 or C-17. Due to overlap with a positive signal, the exact shift is difficult to determine.

For the unlabelled sample, the negative signals are best resolved at 4.7 Tesla (Fig. 5.6C). Five strong signals appear at 190.8, 157.4, 145.0, 134.4 and 112.6 ppm. The signal at 190.8 ppm, which originates from the C-13¹ carbonyl, is also clearly observed in the labelled sample. The resonance at 157.4 ppm is the strongest for the labelled sample and has been assigned to the C-1. This signal can be detected without spectral overlap and has similar intensity to the carbonyl signal. The sharp negative features at 146.9 and 144.8 ppm in the spectrum of the labelled compound may originate from C-11 which gives a response at 145.0 ppm in the unlabelled sample. The signal at 134.4 ppm may be assigned to the C-13. The signal at 112.6 ppm, which does not change its intensity upon 4-ALA labelling, can be from histidine. In addition, five weak signals appear in the methine region at 109.1, 102.7, 97.6, 96.4 and 92.1 ppm. Assuming that both signals at 97.6 and 96.4 ppm originate from a C-5, the other signals can be assigned to the methine carbons C-10, C-15, C-5 and C-20. The origin of the small splitting of the C-5 signal is not clear. The intensities of the signals of the four methine carbons are roughly similar, suggesting a homogeneous distribution of the electron spin density over the acceptor. This suggests that both donor and primary acceptor are monomeric.

5.4 Conclusions

Strong photo-CIDNP signals have been observed by ^{13}C MAS NMR in isolated membrane fragments of *Hba. mobilis*. A single complete set of positive signals, assigned to the BChl *g* donor, is detected, demonstrating a monomeric character. The emissive signals are assigned to the monomeric acceptor. The shifts suggest that histidines may be carrying electron spin density, probably at both the donor and the acceptor site. The ratio of positive to negative signals is strongly magnetic field dependent. At high fields, the donor signals turn to be emissive. In addition, isotope labeling affects the ratio of positive to negative signals suggesting an involvement of magnetic isotopes into the spin-chemical photo-CIDNP process in the solid-state.

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