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

Roy, E.

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4 Photo-CIDNP in the reaction center of the green sulphur bacterium *Chlorobium tepidum* observed by ^{13}C MAS NMR

Photochemically induced dynamic nuclear polarisation has been observed in RCs of the green sulphur bacterium *Chlorobium tepidum* by ^{13}C magic-angle spinning solid-state NMR using continuous illumination with white light. All light-induced ^{13}C NMR signals appear to be emissive, which is similar to the pattern observed in the RCs of plant PSI and purple bacterial RCs of *Rb. sphaeroides* WT. The donor in RCs of green sulphur bacteria clearly differs from the substantially asymmetric special pair of purple bacteria and appears to be similar to the more symmetric donor of PSI.

4.1 Introduction

Photosynthesis is the process in which light energy is transformed into chemical energy and stored by an organism (1). Photosynthetic RCs are classified into two types on the basis of their early electron acceptors (2-4). The RCs containing membrane bound iron-sulphur centers are called 'Fe-S type RC' (Type-I), while those containing (B) Phe and quinones as 'pheophytin-quinone type RC' (Type-II). Type-I RCs are found in green sulphur bacteria, heliobacteria, cyanobacteria as well as in plants. On the other hand, type-II RCs are found in purple bacteria, cyanobacteria and in plants. Oxygenic photosynthetic organisms, such as plants, algae and cyanobacteria, contain both types of photosystems, namely PSI and PSII. The two photosystems have very different redox potential properties. PSII provides a strong positive redox potential, which enables the oxidation of water and production of molecular oxygen, while PSI generates a strong negative redox potential. The question of what are the determining factors of the redox properties has recently been addressed (5-8).

Anoxygenic photosynthetic bacteria contain a single photosystem, either type-I RCs, as found in green sulphur bacteria and heliobacteria, or type-II RCs, in purple and filamentous green bacteria. Green sulphur bacteria have large light-harvesting antenna complexes known as chlorosomes, which contain BChl aggregates (9) and FMO proteins (10).

Interestingly, in green sulphur bacteria and in heliobacteria a single gene of the RC core protein has been identified (11, 12). Structural analysis of the RC core complex of the green sulphur bacteria *C. tepidum* indicated the presence of a homodimer formed by two 82 kDa PscA proteins (13) which is in contrast to a heterodimer formed by PsaA and PsaB in PSI. In

a single PscA protein, eight BChl *a*, two plant Chl *a* derivatives and between two and eleven carotenoids have been reported per RC (14, 15) which is considerably less than the number of chlorophylls found attached to the heterodimeric core of PSI. Until now, no X ray crystal structure of a RC of green sulphur bacteria has been reported.

The primary donor in the RC of green sulphur bacteria is termed P840, due to the absorption maximum at 840 nm. It has been assigned to two BChl *a* molecules (16, 17), probably two C-13² epimers (18). The RC of green sulphur bacteria also contains a plant Chl *a*, called Chl 670, presumably acting at the primary electron acceptor (A_0) (19). That Chl *a* cofactor, however, is esterified with $\Delta^2,6$ -phytadienol, rather than phytol as in plants and cyanobacteria (18). Based on EPR experiments, a menaquinone cofactor has been proposed to be the secondary electron acceptor (A_1) (20, 21). The putative quinone binding site appears to be partially conserved in PSI, green sulphur bacteria and heliobacteria (22). It has been reported that the RCs of green sulphur bacteria and heliobacteria are active without the presence of quinones (23, 24). The terminal electron acceptors are three iron sulphur centers, F_X , F_A and F_B , as detected by EPR studies on the RCs (25). The structural and functional aspects of RCs of green sulphur bacteria have been probed by several spectroscopic methods (26-30).

A rapidly emerging technique in the study of membrane proteins is MAS NMR (31, 32). The chemical shifts allow the exploration of the electronic and protonic structures in the electronic ground state. In RCs upon illumination, photo-CIDNP has been observed by MAS NMR as modification of signal intensity (33, 34, 35). Photo-CIDNP intensities are related to the local electron spin densities. In purple bacterial RCs of *Rb. sphaeroides* WT and carotenoid-less mutant R-26, the strongest enhancement of NMR signals observed is a factor of 10,000 (36, 37). Until now, photo-CIDNP has been observed in four photosynthetic systems: In purple bacterial RCs of *Rb. sphaeroides* WT (36, 38), R-26 (33, 37, 39-41), D1D2 complex of PS II of plants (6, 8) and from PSI complex of plants (Chapter 2).

Recently, it has been shown that three mechanisms can produce photo-CIDNP in solids (34, 35). In the TSM mechanism (42), the extent of the photo-CIDNP effect is maximum when matching of the nuclear Zeeman frequency to coupling between the two electron and hyperfine interaction occurs in the spin-correlated radical pair. The DD mechanism (43) also requires anisotropic hyperfine coupling, but a net photo-CIDNP effect is caused due to the different lifetimes of the two forms of the spin-correlated radical pair, the singlet and triplet states. This mechanism requires a single matching of the nuclear Zeeman frequency to the hyperfine interaction. In addition, a third mechanism appears active in systems having a long-lived triplet state of the donor, leading to the DR process (44).

4.2 Materials and Methods

4.2.1 Preparation

C. tepidum strain TLS were grown in a medium described by Wahlund et al. (45). The 3FMO-RC particles of *C. tepidum* were isolated as described in ref. (46). The purity of the FMO-RC particles was analysed by SDS-PAGE. The purified FMO-RC particles were then recovered from the sucrose gradient and dialysed against buffer containing 50 mM Tris/HCl and 10 mM sodium ascorbate (pH 8.3), for 3 h and then ultracentrifuged at 200,000 g for 3 h. The pellet containing the particles was dissolved in buffer containing 50 mM glycine and 0.01% Triton X-100 (pH 10.8). For photo-CIDNP studies the sample was reduced by 50 mM sodium dithionite.

4.2.2 MAS-NMR Measurements

The NMR experiments were performed by using a DMX-200 NMR spectrometer (Bruker GmbH, Karlsruhe, Germany). The sample was loaded in an optically transparent 4 mm sapphire rotor. The sample was reduced by addition of an aqueous solution of 50 mM sodium dithionite in an oxygen-free atmosphere. Following the reduction, slow freezing of the sample was performed directly in the NMR probe inside the magnet with liquid nitrogen-cooled gas under continuous illumination with white light (47). The illumination setup was specially designed for a Bruker MAS probe (41). Photo-CIDNP ^{13}C MAS NMR spectra were obtained at a temperature of 240 K with a spinning frequency of 8 kHz. The light and dark spectra were measured with a Hahn echo pulse sequence and TPPM proton decoupling (48).

4.3 Results and Discussion

4.3.1 Dark spectrum

Fig. 4.1 shows the ^{13}C MAS NMR spectra of natural abundance FMO-RC particles of *C. tepidum* in the dark (A) and under continuous illumination with white light (B) in a magnetic field of 4.7 Tesla. Spectrum 4.1A shows the characteristic features of a ^{13}C -MAS NMR spectrum of a protein, *i.e.*, broad responses between 0 and 50 ppm. The sharp signal at 175.7 ppm arises mainly from glycine which is present in the buffer. Additional weak features of aromatic cofactors and amino acids appear between 190 and 80 ppm.

4.3.2 Overall spectral pattern

In spectrum 4.1B, obtained under illumination, several strong emissive (negative) signals appear. A total of ten centrebands has been identified (Table 4.1). These signals appear in the carbonylic region as well as in the aromatic region. The signals observed at lowest frequency arise at about 100 ppm from methine carbons, while no photo-CIDNP is observed in the aliphatic region.

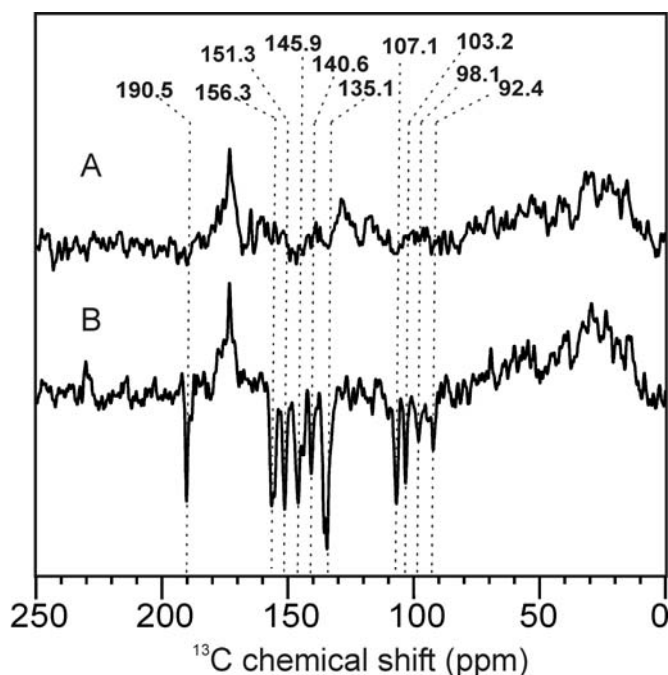


Figure 4.1. ^{13}C MAS NMR spectra of RC complexes of *C. tepidum* at 240 K recorded with a MAS frequency of 8 kHz at 4.7 Tesla. Spectra are obtained: in the dark (A) and under continuous illumination with white light (B). In both experiments, the cycle delay was 12 seconds.

This overall pattern has also been observed in RCs of PSI (Chapter 2) and *Rb. sphaeroides* WT (36) and is in contrast to the pattern of positive aromatic signals combined with negative methine signals as observed in RCs of PSII (6, 8) and *Rb. sphaeroides* R-26 (33, 37, 39-41). In case of the two bacterial RCs of *Rb. sphaeroides*, it has been demonstrated that the difference in the pattern is due to a difference of the lifetime of the donor triplet (37). RCs of *Rb. sphaeroides* WT have a triplet lifetime of 100 ns, while the RCs of the carotene-less mutant R26 have a lifetime of the donor triplet of 100 μs , a time long enough to produce net polarization by the DR effect leading to an inversion of the sign of the donor signals (36, 37). Hence, based on such comparison, we assume that the donor side of the RC of *C. tepidum* contains a carotene which is able to quench efficiently the triplet states of the donor. In fact, similarity observed in the photo-CIDNP pattern in the RCs of *C. tepidum* (49) is also in line with the presence of carotenoids in the RCs.

4.3.3 Assignments

Most of the signals can be assigned to a BChl *a* or Chl *a* cofactor (Table 4.1). In the carbonyl region, the strong and sharp signal at 190.5 ppm is detected and can be assigned directly to the carbonyl carbon C-13¹. Such a strong emissive signal of a carbonyl carbon has been observed in the photo-CIDNP spectrum of PSI (Chapter 2), where it has been assigned to the donor, while it is weak in the spectrum of *Rb. sphaeroides* WT (36). The strongest signals are observed in the aromatic region between 120 and 170 ppm. The signal at 156.3 ppm may be doubled and can be assigned to C-9 of a BChl *a* or C-1 and C-6 of a Chl *a*. The

Chl <i>a</i>		PSI	Carbon No	BChl <i>a</i>		<i>C. tepidum</i>
$\sigma_{\text{liq}}^{\text{a}}$	$\sigma_{\text{ss}}^{\text{b}}$	σ^{c}		$\sigma_{\text{liq}}^{\text{d}}$	$\sigma_{\text{ss}}^{\text{e}}$	σ^{f}
189.3	190.6	~190.6 E	13¹	199.3	188.2	190.5 E
172.7	175.3		17³	173.4	174.0	
171.0	171.2		13³	171.6	171.4	
167.4	170.0	167.1 E	19	167.3	168.9	
161.4	162.0	160.4 E	14	160.8	160.7	
154.0	155.9	154.8 E	1	151.2	153.5	
155.8	154.4		6	168.9	170.2	
151.4	154.0	152.6 E	16	152.2	150.1	
148.0	150.7	149.9 E	4	150.2	152.2	151.3 E
147.7	147.2	147.2 E	11	149.5	147.2	145.9 E
146.1	147.2		9	158.5	158.0	156.3 E
144.1	146.2	144.2 E	8			
139.0	137.0	138.6 E	3	137.7	136.1	135.1 E
135.5	136.1	~136 E	2	142.1	140.7	140.6 E
134.2	134.0			123.9	119.9	
134.0	133.4	~132 E	12			
			7			
131.5	126.2		13	130.5	124.1	
131.5	126.2		3¹	199.3	194.5	
118.9	113.4		3²			
107.1				102.4	100.0	103.2 E
106.2	108.2	105.4 E	10			
	102.8		15	109.7	105.8	107.1 E
100.0	98.1		5	99.6	98.8	98.1 E
92.8	93.3		20	96.3	93.7	92.4 E

^aSee ref. (52), ^bSee ref. (53), ^c(Chapter 2), ^dRef. (41), ^eRef. (54), ^fthis work.

Table 4.1. ¹³C chemical shifts of the photo-CIDNP signals observed in *C. tepidum* in comparison to chemical shift data of BChl *a* and Chl *a*.

peak at 151.3 ppm can be assigned to C-4 or C-16 of either a BChl *a* or Chl *a* cofactor. The signal at 145.9 ppm, having a clear shoulder on its low-frequency wing, can arise from a C-11 of a BChl *a* or from C-8 of a Chl *a*. The signal at 140.6 ppm can be assigned to C-2 of a BChl *a*, while an assignment to a Chl *a* is rather unlikely. The signal at 135.1 ppm shows a shoulder and can be assigned to C-3 of BChl *a* or C-2 of Chl *a*. Also in the region of the methine carbons, most signals may be assigned to either the BChl *a* donor molecule(s) or to the Chl *a*

acceptor. The signal at 107.1 ppm can be assigned to the C-15 of a BChl *a* or a C-10 of a Chl *a*, while the response at 103.1 ppm can arise from the C-10 of a BChl *a* or a C-15 of a Chl *a*. The signals at 98.1 and 92.4 ppm originate from the C-5 and C-20, respectively, from either the BChl *a* or the Chl *a*.

Hence, the chemical shift information is not sufficient to assign the photo-CIDNP signals to either the donor or the acceptor, although the strength of the carbonyl signal and the chemical shift of 140.6 ppm indicate that at least some contribution from the donor exists. In analogy to PSI and the RC of *Rb. sphaeroides* WT, in which the downfield signals with shifts ~ 130 ppm were assigned to the donor based on simulations of donor and acceptor photo-CIDNP intensities, we tend to assign the set of aromatic carbons with shifts greater than 130 ppm to the donor, while there is little evidence for an assignment of the signals of the methine carbons to either the donor or the acceptor.

4.3.4 Line shape and linewidth

Some of the signals that are attributed to the donor appear to be doubled or show a shoulder, namely the signals at 156.3, 145.9 and 135.1 ppm. The signal doubling can be interpreted in terms of a slightly asymmetric dimer. If this is the case, small differences between the two halves exist in the electronic ground state, indicated by the chemical shift differences and for the radical cation, indicated by different signal intensities. This interpretation depends on the assignment of these signals to the donor. First, it implies that the two branches of *C. tepidum* RCs differ much less from each other than in RCs of purple bacteria, where a clear asymmetry in the electronic ground state has been demonstrated for the special pair donor (36, 38, 50, 51) and the radical cation state (36). This is hardly surprising, as *C. tepidum* RCs appear to be scaffolded by a protein homodimer, while a heterodimer is found in purple bacteria. The slight asymmetry, however, indicates that the two branches are not fully equivalent, which in turn implies that the symmetry of the homodimer is broken. Data on RCs of *C. limicola* ENDOR and Special TRIPLE spectroscopies show that P840⁺ has a symmetrical distribution over the two halves of the pair, having approximately a 1:1 distribution of electron spin density (27). This conclusion on the radical-pair state matches with our observation of similar photo-CIDNP intensities of both parts of split signals, making an interpretation of an asymmetric dimer P840⁺ unlikely (30). On the other hand, circular dichroism data on RCs of *C. tepidum* were interpreted in terms of a difference in asymmetry of the P840 donor relative to the special pair in purple bacteria (29). Our chemical shift data do not allow for an interpretation of a strong asymmetry within the P840 donor dimer in the ground state. This contrasts with the photo-CIDNP data for the special pair of RCs of purple bacteria, where the symmetry is already broken in the electronic ground state (36, 38, 50, 51). Hence, the difference observed by CD spectroscopy may have a different origin than

electronic ground-state asymmetry. FTIR data on the primary donor have shown that at least one of the two BChl *a* forming the primary donor is free from hydrogen bonding (30).

The five signals at 190.5, 151.3, 140.6, 103.2 and 106.6 ppm do not indicate any doubling and appear to be remarkably narrow, as indicated by full width at half-height of 54.1, 68.9, 64.0, 56.6 and 73.8 Hz, respectively. These linewidths are similar to those found in PSI (Chapter 2) and reveal a rigid, ordered as well as structurally and electrostatically stable donor side, keeping the reorganization energies of the electron transfer low. Hence, the donor of the RC of *C. tepidum* is probably similar in electronic structure and rigidity to that of PSI, despite the difference in the chemical structure of the cofactors.

4.4 Conclusions

Photo-CIDNP has been observed in RCs of the green sulphur bacterium *C. tepidum*. It appears that photo-CIDNP is an inherent property of all types of natural RCs. In the ^{13}C photo-CIDNP MAS NMR spectrum of the RC of *C. tepidum*, all signals are emissive (negative). The overall photo-CIDNP pattern is similar to that observed in PSI. The carbonylic and aromatic signals can be assigned to the two BChl *a* molecules of the donor side. Doubling of several signals suggests an only slightly asymmetric dimer in both the electronic ground state and radical cation state of the donor side. Hence, the donor in RCs of green sulfur bacteria clearly differs from the substantially asymmetric special pair of purple bacteria and appears to be similar to the more symmetric donor of PSI.

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