Selective Detection of the Solid-state NMR Signals from the Bacteriochlorophyll *a* Dimers in a Reconstituted Light-harvesting 1 Complex

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High resolution solid-state ^{13}C NMR spectra have been obtained for bacteriochlorophyll (BChl) *a* in a light-harvesting (LH) 1 complex utilizing a reconstitution method. The LH 1 complexes were reconstituted with ^{13}C-enriched BChl *a* and the apo-LH1 polypeptide from *Rhodospirillum rubrum*. Measurement of the 2-dimentional ^{13}C–^{13}C dipole correlation NMR spectra enabled the selective assignment of the ^{13}C resonance from the BChl *a* molecules in the reconstituted LH1.

High resolution X-ray crystal structures have been performed for the reaction center (RC)[1] and peripheral light-harvesting 2 (LH2) complex,[2] while light-harvesting 1 (LH1) complex has not been determined to atomic resolution because of its instability and the difficulty of the high-quality crystals. LH1 of photosynthetic bacteria generally comprises two small polypeptides, α, and β, with a ratio of 1:1, along with two BChl *a* and one or two carotenoid molecules per $\alpha\beta$ pair. The BChl *a* dimers in the LH1 complexes from a purple nonsulfur bacterium *Rhodospirillum* (*R.*) *rubrum* absorb at 873 nm (Q_y band); the Q_y band is red-shifted about 110 nm from that of its monomeric form. To elucidate the electronic mechanism of the red-shift for BChl *a* in vivo has been one of the major topics in recent studies of LH1 complexes.

The LH1 complex is capable of forming a stable intermediate species with a Q_y absorption at approximately 820 nm (referred to as B820), by dissociation of the LH1 with detergent *n*-octyl-D-glucopyranoside (OG).[3] The B820 species is considered to be a structural subunit of the LH1 complex. Recently, high-resolution solution NMR spectra have been measured for BChl *a* dimers in the B820 species.[4] The replacement of natural abundant BChl *a* to ^{13}C-enriched one in the complex enabled the significant enhancement of the carbon resonance and ^{1}H–^{13}C correlation signals from BChl *a*. The achievement for the chemical shift assignment would open a way to the structural analysis at atomic level.

Here, we reconstituted highly concentrated B870 species with a Q_y absorption at ~870 nm from the B820 species with ^{13}C-enriched BChl *a*, and selective ^{13}C chemical assignments from BChl *a* dimers in the B870 species were performed by high resolution solid-state NMR instead of solution NMR. The B870 species can be considered to correspond to the native LH1 complex, but the high molecular weight and its instability make it difficult to analyze the structure at atomic level. The combination of the solid-state NMR analysis and the reconstitution of stable B870 species enabled the selective detection of the ^{13}C resonance from BChl *a* in the B870 species.

The reconstituted B870 species with ^{13}C-enriched BChl *a* was formed at high concentration by incorporation of the reconstituted B820 species with ^{13}C-enriched BChl *a* into liposome. The B820 species with ^{13}C-BChl *a* was reconstituted according to previous report.[4] That is, ^{13}C-enriched BChl *a* in acetone was added to the lyophilized apo-LH1 (where BChl *a* and carotenoid are removed from LH1 with benzene and methanol) containing OG detergent and phosphate. The BChl *a*-apo-LH1 solution was freeze-dried and then dissolved by distilled water (final concentration of OG was 0.9%). We, firstly, tried to dilute the concentration of OG detergent by the addition of a phosphate buffer; however, the Q_y band was only red-shifted to 855 nm and half of the B820 species remained (data not shown). In order to insert B820 species into liposome, the reconstituted B820 species solution was mixed with a liposome solution which is made from phosphatidylcholine and cholesterol according to Szoka et al.,[5] and then the mixture solution was incubated for 1 h. The OG detergent was removed by the addition of polystyrene beads (Bio-Beads SM2, Bio-Rad, Richmond, California).[6] Figure 1 shows the absorption spectra for the B820-liposome mixture solution. The insertion of B820 species into the liposome induced the red-shift of Q_y to 870 nm, and especially, the addition of the liposome solution (Phosphatidylcholine: 430 mM, cholesterol: 34 mM) at the fifteenth volume of the B820 species solution (apo-LH1: 47 μM) resulted in little monomeric form of BChl *a*. This implied that B870 species was formed by the incorporation of B820 species into a liposome, and that the increase of the concentration of B820 species in a liposome stabilized B870 species because of concentration effect on self-assembly. The liposome with ^{13}C-BChl *a*-B870 species was centrifuged, and the pellet was used for solid-state NMR measurement. The absorption spectrum of the precipitated B870 species was identical to that in the suspension solution.

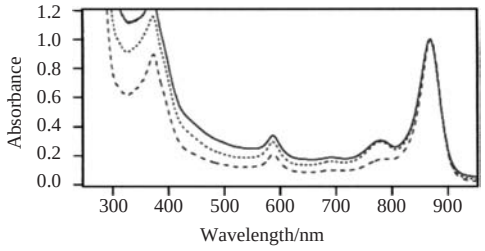

Figure 1. Absorption spectra for the B820-liposome mixture solution. The volume ratio of added liposome solution to B820 solution was changed to 1/7 (solid line), 1/10 (dotted line), and 1/15 (dashed line). The concentrations of the ap-LH1, phosphatidylcholine, cholesterol were 47 μM, 430 mM, and 34 mM.

In order to assign the ^{13}C chemical shifts from the ^{13}C-BChl *a*-B870 species in liposome, 2-dimensional (2-D) homonuclear ^{13}C–^{13}C dipolar correlation NMR spectra were measured. The 2-D ^{13}C–^{13}C dipolar correlation using the technique of radio fre-

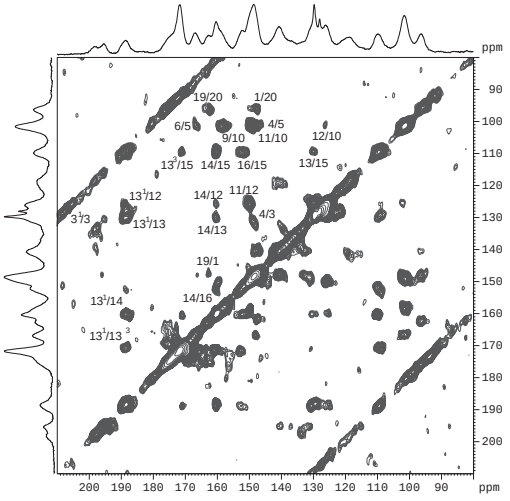

Figure 2. Contour plot of the 2-D RFDR ^{13}C–^{13}C dipolar correlation spectrum for the ^{13}C-BChl *a*-B870 species in a magnetic field of 9.5 T. The MAS rotation frequency and the mixing time were 8 kHz and 4 ms, respectively. The numbering in the plot corresponds with Figure 3.

Table 1. Assignments of ^{13}C chemical shifts of the the ^{13}C-BChl *a*-B870 species

Position	$B870_{solid}$		Position	$B870_{solid}$	
3^1	195.3	(1)	P2	119.8	(1)
13^1	189.4	(0.5)	15	110.5	(0.5)
17^3	171.8	(0.5)	10	102.1	(0.5)
13^3	171.5	(0.3)	5	101.7	(0.5)
6	167.0	(0.5)	20	96.6	(0.5)
19	162.7	(1)	13^2	64.7	(1)
14	160.7	(0.4)	P1	61.6	(0.6)
9	159.3	(0.3)	8	55.4	(0.5)
16	152.7	(0.5)	13^4	53.0	(0.5)
1	148.3	(0.3)	17	50.3	(0.5)
4	148.7	(0.5)	18	48.9	(0.5)
11	149.7	(0.5)	7	47.6	(1)
P3	141.5	(1)	3^2	31.2	(0.5)
	139.8	(1)	8^1	32.4	(0.5)
2	140.7	(0.5)	7^1	24.0	(1)
3	132.0	(0.5)	2^1	12.7	(0.5)
13	130.5	(0.5)	12^1	8.0	(0.5)
12	126.0	(0.5)	8^2	12.6	(0.5)

The estimated errors for the solid state shifts are in parenthesis.

quency-driven dipolar recoupling (RFDR) or proton-driven spin diffusion (PDSD) has been applied for various chlorophyll solid aggregates and the assignment for the ^{13}C resonance from chlorophyll has been achieved.[7] Figure 2 shows the RFDR dipolar correlation NMR spectra for the ^{13}C-BChl *a*-B870 species in liposome. With the short mixing time 4 ms, many clear 2-D cross peaks appeared, revealing transfer of coherence between olefinic carbons in a macrocycle ring. Further, we measured the 2-D correlation spectra at various mixing times from 2 ms to 8 ms; the longer mixing time more easily enabled the transfer of coherence. We also applied the proton-driven spin diffusion (PDSD) correlation spectra because polarization transfer between aliphatic carbons was easier with straightforward spin diffusion techniques than with RFDR. Table 1 shows the assignment of ^{13}C chemical shifts for BChl *a* in B870 species inserted in a liposome. Most of the carbons in a bacteriochlorin ring were assigned; however, the carbons in the peripheral substituents

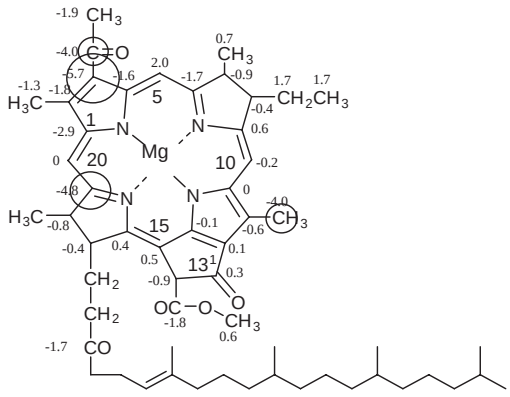

Figure 3. Schematic map showing the ^{13}C chemical shift difference, $\Delta\delta = \delta_{B870} - \delta$ monomer. All values were obtained using Table 1. The ^{13}C chemical shifts of monomeric BChl *a* are collected in acetone.[4] The carbons with the $\Delta\delta > \pm 4.0$ ppm are marked with a circle in the map. Open circles correspond with negative $\Delta\delta$. The sizes of the circles reflect the magnitude of the $\Delta\delta$.

attached to ring IV (17^1-C, 17^2-C, and 18-C) and phytyl ester chain could not be assigned probably because of low cross polarization from proton as a result of higher mobility.

Figure 3 represents a schematic map of $\Delta\delta = \delta_{B870} - \delta_{monomer}$. Significant upfield shifts > 4 ppm were detected for 3-C, 3^1-C, 12^1-C, and 19-C. For 3^1-C, the hydrogen bonding to the NH group in the side chain of the tryptophan residue (α: Trp + 11, β: Trp + 9) has been observed in LH1 and B820 species by Raman[8] and solution NMR studies,[4] respectively; the formation of the hydrogen bond would result in a downfield-shift for 3^1-C. The solid-state NMR result might indicate that some factors causing significant upfield shift have an influence on the ring I. Elucidating the upfield shifts around ring I might provide us the reason of the red-shift of Q_y absorption to 870 nm.

In conclusion, we succeeded in assigning the ^{13}C chemical shift for BChl *a* in the reconstituted LH1 by measuring 2-D ^{13}C–^{13}C dipolar correlation spectra for the ^{13}C-enriched BChl *a* in a natural abundant apo-LH1. The reconstitution method using liposome was much effective for the preparation of the LH1 with only BChl *a* ^{13}C-enriched. The assignment of ^1H chemical shift for BChl *a* in LH1 is in progress by multi dimensional solid-state NMR techniques.

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