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# Two sets of nuclear magnetic resonances in the CH<sub>2</sub>Cl<sub>2</sub>-treated (3<sup>1</sup>R)-type bacteriochlorophyll *c* solid aggregates under ultra high magnetic field

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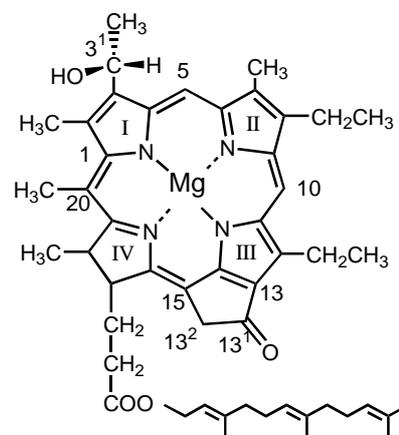
Key words: solid-state NMR, (3<sup>1</sup>R)-type bacteriochlorophyll *c*, chlorosome

### Introduction

Chlorosome in green sulfur and filamentous bacteria is a special light harvesting entity with an absorption maximum around 740 nm. Bacteriochlorophyll (BChl) *c* is well-known to form rod-like large aggregates in the chlorosome without influence of protein. BChl *c* is characterized by the hydroxyl group at 3<sup>1</sup>-position, which brings about a unique self-aggregation of BChl *c*, and the difference in stereochemistry is considered to influence the behavior of self-aggregation. The (3<sup>1</sup>R)-type [E,E]BChl *c*<sub>F</sub> with a *R*-type chiral center at the 3<sup>1</sup>-position and ethyl groups at the 8-/12-positions (Fig.1) is the most abundant component in the chlorosome of green bacteria *Chlorobium tepidum*. Recently, we could form the solid aggregates with an absorption maximum at 734 nm by drying the (3<sup>1</sup>R)-[E,E]BChl *c*<sub>F</sub>-CH<sub>2</sub>Cl<sub>2</sub> solution and the CH<sub>2</sub>Cl<sub>2</sub>-treated solid aggregates gave rise to a resolved <sup>13</sup>C NMR spectrum. In this study, we have measured the solid-state <sup>13</sup>C- and <sup>15</sup>N- NMR spectra for the CH<sub>2</sub>Cl<sub>2</sub>-treated solid aggregates of (3<sup>1</sup>R)-[E,E]BChl *c*<sub>F</sub> at an ultra high magnetic field and can assign all the <sup>13</sup>C and <sup>15</sup>N chemical shifts from 2-dimensional experiments.

### Materials and methods

(3<sup>1</sup>R)-[E,E]BChl *c*<sub>F</sub> was extracted with methanol from *Chlorobium tepidum* dry cells and purified by a reverse-phase HPLC column. For the <sup>13</sup>C, <sup>15</sup>N-labeled BChl *c*, cells were grown in a medium containing sodium bicarbonate (NaHCO<sub>3</sub>) and ammonium chloride (NH<sub>4</sub>Cl) as carbon and nitrogen sources. All the carbons and nitrogens in BChl *c* were uniformly <sup>13</sup>C, <sup>15</sup>N-labeled with NaH<sup>13</sup>CO<sub>3</sub> and <sup>15</sup>NH<sub>4</sub>Cl (<sup>13</sup>C, <sup>15</sup>N >98 % atom, Isotec Inc.). For the magic angle spinning (MAS) NMR measurements, about 20 mg of <sup>13</sup>C, <sup>15</sup>N-labeled (3<sup>1</sup>R)-[E,E]BChl *c*<sub>F</sub> was dissolved in neat CH<sub>2</sub>Cl<sub>2</sub>, and then the BChl *c*-CH<sub>2</sub>Cl<sub>2</sub> solution was dried spontaneously in a spinner in dry air and further evaporated in vacuo.

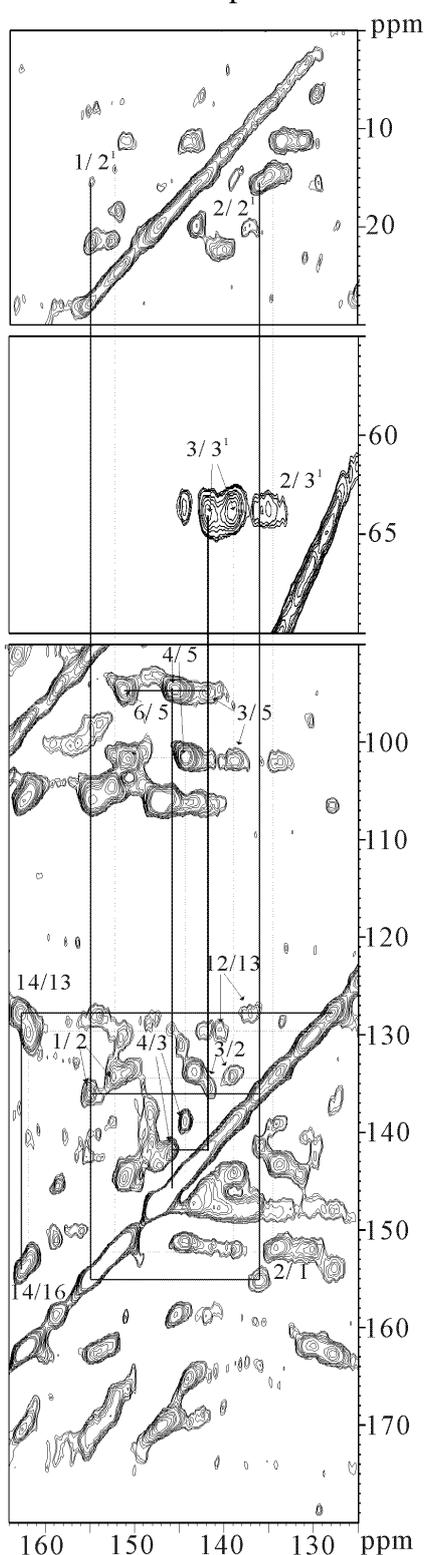


**Figure 1.** Molecular structure of (3<sup>1</sup>R)-[E,E]BChl *c*<sub>F</sub>

Solid NMR spectra were collected at room temperature under a magnetic field of 17.6 T. The rate of MAS rotation around the magic angle was 10-13 kHz and the frequency was kept stable within a few Hz. In all solid NMR experiments, the two pulse phase-modulation (TPPM) was used for decoupling protons. The ramp sequence was applied for the heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  polarization transfer at high MAS rates during contact time. The 2-D frequency-driven dipolar recoupling (RFDR) homonuclear ( $^{13}\text{C}$ - $^{13}\text{C}$ ) dipolar correlation spectra were measured with the XY-8 pulse sequence in the mixing period, and the 2-D heteronuclear ( $^{15}\text{N}$ - $^{13}\text{C}$ ) correlated spectra were acquired with double cross polarization (CP).

## Results and Discussion

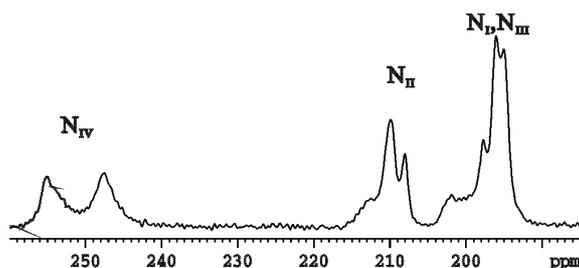
Fig.2 shows the contour plot of the 2-D CP/MAS RFDR  $^{13}\text{C}$  dipolar correlation NMR



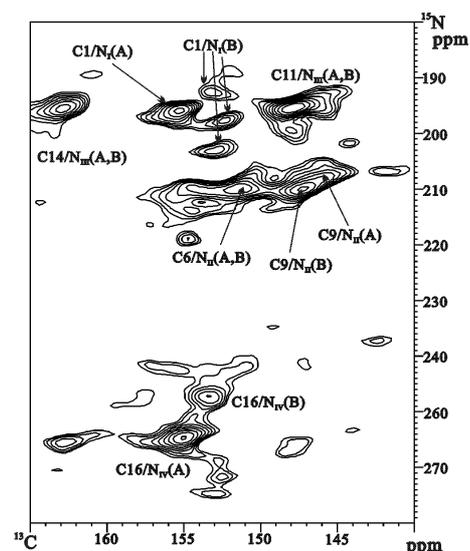
spectrum for the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregates of uniformly  $^{13}\text{C}$ -labeled ( $3^1R$ )-[E,E]BChl  $c_F$  at the magnetic field of 17.6 T. With the short mixing time ( $\tau_m$ ) of 2.7 ms, there appeared many clear 2-D cross peaks revealing transfer of coherence between olefinic carbons in a macrocycle ring. The two resonances were observed for the C5 meso-carbon at 94.8 ppm and 101.5 ppm and each of the C5 resonances

**Figure 2.** Contour plot of the 2-D RFDR  $^{13}\text{C}$ - $^{13}\text{C}$  correlation spectrum for the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregate of  $^{13}\text{C}$ -labeled ( $3^1R$ )-[E,E]BChl  $c_F$ . The MAS rotation rate and mixing time were 12 kHz and 2.7 ms, respectively.

made an individual correlation network with C3, C4 and C6 (solid and dotted lines in Fig.2). The two different networks reveal that the C3, C4 and C6 give rise to two sets of resonances in the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregates. Further, the two resonances of C3 led us to the observation of the splitting of C1 and C2, which were confirmed by the two resonances of C2<sup>1</sup>. For the carbons in the tail region of BChl  $c$  (C11-19), two resonances were also observed for C13, C14 and C16 from the cross peaks of C12/13 and C14/16. Although the C19 resonance can not be recognized in Fig 2 because of a series of spinning side bands, the C16/19 and C19/20 correlations were confirmed and each of C19 and C20 showed two resonances at another MAS rotation frequency. Consequently, the resonances of the carbons in a chlorin ring could be connected by the two correlation networks (networks-A and -B: solid and dotted lines in Fig.2, respectively). The two correlation networks-A and -B could be spread on the whole



**Figure 3.** 1-D CP/MAS  $^{15}\text{N}$  spectrum for the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregate of  $(3^1R)$ -[E,E]BChl  $c_F$ . The MAS rotation rate was 12 kHz.



**Figure 4.** Contour plot of the 2-D heteronuclear  $^{15}\text{N}$ - $^{13}\text{C}$  correlation spectrum for the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregate of  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeled  $(3^1R)$ -[E,E]BChl  $c_F$ . The MAS rotation rate was 12 kHz. The contact times for  $^1\text{H}$ - $^{15}\text{N}$  and  $^{15}\text{N}$ - $^{13}\text{C}$  were 4 ms and 2 ms, respectively.

BChl  $c$  molecule, however, some carbons in a chlorin ring (C3, C7, C8 and C12) and peripheral substitutes ( $\text{C2}^1$  and  $\text{C18}^1$ ) showed multiple resonances in the network- $B$ . Therefore, two different solid states (types- $A$  and - $B$ ) basically exist in the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregates of  $(3^1R)$ -type BChl  $c$  and there may be structural heterogeneity in the type- $B$  solid state. For the assignment of the farnesyl ester chain, 2-D CP/MAS spin diffusion dipolar correlation spectra were also additionally applied. The coherence between the carbons in the farnesyl group was more easily transferred with the spin diffusion technique than RFDR. Differently from the macrocycle carbons, the  $^{13}\text{C}$  resonances for the farnesyl chain showed only a broad cross peak. This may indicate that the structural organization of the farnesyl chains is moderately heterogeneous.

Fig.3 shows the 1-D  $^{15}\text{N}$  NMR spectrum for the  $\text{CH}_2\text{Cl}_2$ -treated solid aggregates of uniformly  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeled  $(3^1R)$ -BChl  $c_F$  at the magnetic field of 17.6 T. Two resonances were observed for each of the  $\text{N}_{\text{II}}$  and  $\text{N}_{\text{IV}}$  atoms in the solid aggregates as well as carbon. Whereas, the  $\text{N}_{\text{I}}$  resonance is hardly distinguished from the  $\text{N}_{\text{III}}$  resonance in the 1-D spectrum due to the up-field-shift of  $\text{N}_{\text{I}}$ , and the 2-D heteronuclear ( $^{13}\text{C}$ - $^{15}\text{N}$ ) correlation experiment was necessary for the complete assignment of the nitrogen resonances (Fig.4). The two resonances for each of the  $\text{N}_{\text{II}}$  and  $\text{N}_{\text{IV}}$  atoms can be connected with the correlation networks- $A$  and - $B$  of carbon by the 2-D heteronuclear correlation spectrum. The  $\text{N}_{\text{I}}$  atom seems to have multiple resonances. The 155.5 ppm-resonance of C1 which belongs to the network- $A$  is correlated with only the 196 ppm-resonance of  $\text{N}_{\text{I}}$ , while the C1 resonance in the network- $B$  (152.2 ppm) seems to show three cross peaks with  $\text{N}_{\text{I}}$  (196 ppm, 199 ppm, and 203 ppm). This may support the structural heterogeneity in the type- $B$  solid state.

In conclusion, all the  $^{13}\text{C}$  chemical shifts could be assigned by the combination of the RFDR and spindiffusion homonuclear correlation experiments at the high magnetic field, and two sets for resonances for each carbon in a chlorin ring were observed. For the  $^{15}\text{N}$  resonances, two resonances were also observed for  $\text{N}_{\text{II}}$  and  $\text{N}_{\text{IV}}$ . The 2-D heteronuclear ( $^{15}\text{N}$ - $^{13}\text{C}$ ) correlation experiment enables us to assign the complicated  $\text{N}_{\text{I}}$  resonances and link the  $^{15}\text{N}$  resonances with the two correlation networks of carbon.

## References

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