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CP/MAS ^{13}C-NMR Studies on the Structure of Bacteriochlorophyll c in Chlorosomes from Chloroflexus aurantiacus

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High resolution solid state NMR spectra have been obtained for chlorosomes by suitable choice of observation conditions, and their comparison with those of monomeric and oligomeric solid BChl c showed the presence of a hydrogen bond between the 9-carbonyl group, and the 2a-hydroxyl group.

Green photosynthetic bacteria are characterized by a unique antenna complex known as a chlorosome, an ellipsoidal structure with approximate dimension of 100x30x10 nm that is attached to the cytoplasmic side of the cell membrane.[1] Chlorosomes contain approximately 10000 molecules of bacteriochlorophyll (BChl) c (Fig. 1a) as the major pigment and also carotenoids and small amounts of BChl a. About a half (by weight) of chlorosomes is composed of proteins.[1] BChl c of chlorosomes isolated from a thermophilic green bacterium Chloroflexus aurantiacus has an absorption maximum at 740 nm which is greatly red-shifted from 665 nm of BChl c in polar organic solvents.[2] BChl c in non-polar solvents forms an oligomer which has many properties similar to BChl c in chlorosomes. The oligomer has been studied by several techniques and it has been proposed that the Mg ion in BChl c in oligomer and chlorosomes is coordinated by 2a-hydroxyl and/or 9-keto carbonyl groups.[3-5] We have employed CP/MAS ^{13}C NMR spectroscopy to clarify the structure of BChl c in chlorosomes.

Chloroflexus aurantiacus was grown and its chlorosomes were isolated by using methods similar to those previously reported.[1] BChl c was extracted with methanol and purified as previously described.[2] Monomeric solid BChl c which showed an absorption maximum at 671.5 nm, was prepared by solidification of BChl c from a methanol solution. Solid aggregated BChl c was prepared by dissolving BChl c in dichloromethane and precipitating it in large excess of hexane. This sample showed an absorption maximum at 740 nm which indicates the formation of the aggregate. We called this sample oligomeric solid BChl c.[2]

CP/MAS ^{13}C NMR spectra were recorded on a Bruker MSL 400 FT NMR spec-

trometer equipped with a double air bearing type CP/MAS probe. Samples (ca. 500 mg) were spun at a spinning rate of 4000 Hz. To eliminate spinning side bands, a TOSS (total elimination of spinning side bands) program[6] was applied for all experiments. In some experiments, a dipole dephasing technique[7] was employed to enhance the quaternary carbon signals. Recycle delays and contact times were varied from 4 s to 60 s, and 0.1 ms to 8 ms, respectively. 90 degree pulses were set to 4 μs. About 1000 transients were accumulated. Chemical shift was referred to TMS by setting the carbonyl signal of solid glycine at 176.03 ppm.

CP/MAS ^{13}C NMR spectra for lyophilized chlorosomes which have near infrared absorption spectra identical to those of intact chlorosomes (data not shown) were taken in several measuring conditions. Resonances from proteins, lipids and carotenoids were dominant in the spectra observed with shorter recycling times (4-5 s) and with shorter contact times (0.5-1 ms) (data not shown). In the experiments with longer contact times (3-5 ms), and with longer recycle delay times (15-60 s), signals which can be attributed to BChl c became predominant especially in the aromatic carbon region. This indicates that hydrogen atoms interacting with the aromatic carbons in BChl c are small in number, far in distance and long in relaxation time. These results are consistent with our previous observations in antenna bacteriochlorophyll complexes from purple photosynthetic bacteria.[8] Figure 1b shows CP/MAS ^{13}C NMR spectrum obtained by a TOSS pulse with a dipole dephasing technique with a long contact time (5 ms) and a long recycle delay (15 s) for lyophilized chlorosomes. CP/MAS ^{13}C NMR spectrum of chlorosomes was compared with those of oligomeric solid BChl c in Fig. 1c, in the low field region. They resemble each other except for

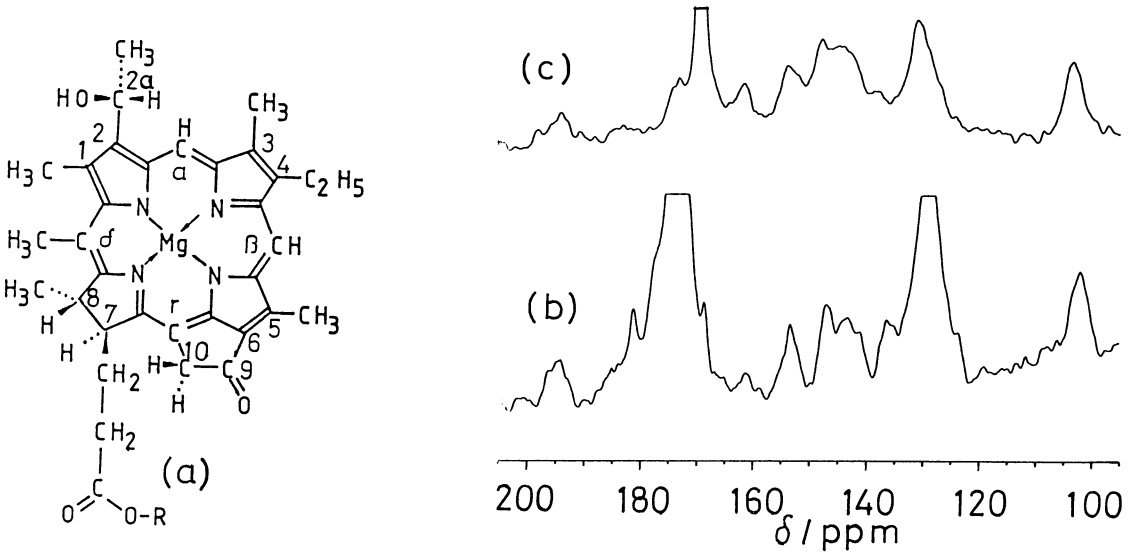

Fig. 1. Structures of BChl c (a), and CP/MAS ^{13}C NMR spectra for chlorosomes (b) and oligomeric solid BChl c (c).

some signals due to proteins, lipids, and carotenoids coexisted, hence most of signals in Fig. 1b can be ascribed to BChl c. Comparison of Fig. 1b with Fig. 1c indicates that oligomeric solid BChl c has structures similar to those of BChl c in chlorosomes.

CP/MAS ^{13}C NMR spectra of monomeric solid BChl c, and solution ^{13}C NMR spectra of BChl c in methanol and acetone were also observed (data not shown). BChl c in methanol and acetone showed absorption peaks at 668.5 and 663.0 nm, respectively, which indicate no aggregate formation in these conditions. It is clear that in every spectra the lowest field signal is assignable to the 9-keto carbonyl signal.[9,10] In solvents which form no hydrogen-bond with the 9-keto carbonyl, this group is observed at 196.0 ppm (in acetone), while it appeared at 199.6 ppm in methanol which forms a hydrogen-bond with the 9-carbonyl group. Since no aggregate formation occurred in BChl c in acetone judged from the absorption position (663.0 nm), the low field shift of the 9-carbonyl carbon in methanol as compared to that in acetone is attributable to the hydrogen bond formation. In the monomeric and oligomeric solid BChl c's, the 9-carbonyl group was observed at 195.8 and 194.8 ppm, respectively (Figs. 1 and 2). These chemical shifts can be interpreted with and without hydrogen bonds in the complexes.[11]

The contact time dependence of the 9-carbonyl signal magnitude was observed, and the results were shown in Fig. 2. In the monomeric solid BChl c, the magnitude of ^{13}C NMR signal of the 9-carbonyl group gave its maximum at the contact time of 2 ms. In the oligomeric solid BChl c, the magnitude of 9-carbonyl signal showed its maximum at the contact time of 0.5 ms.

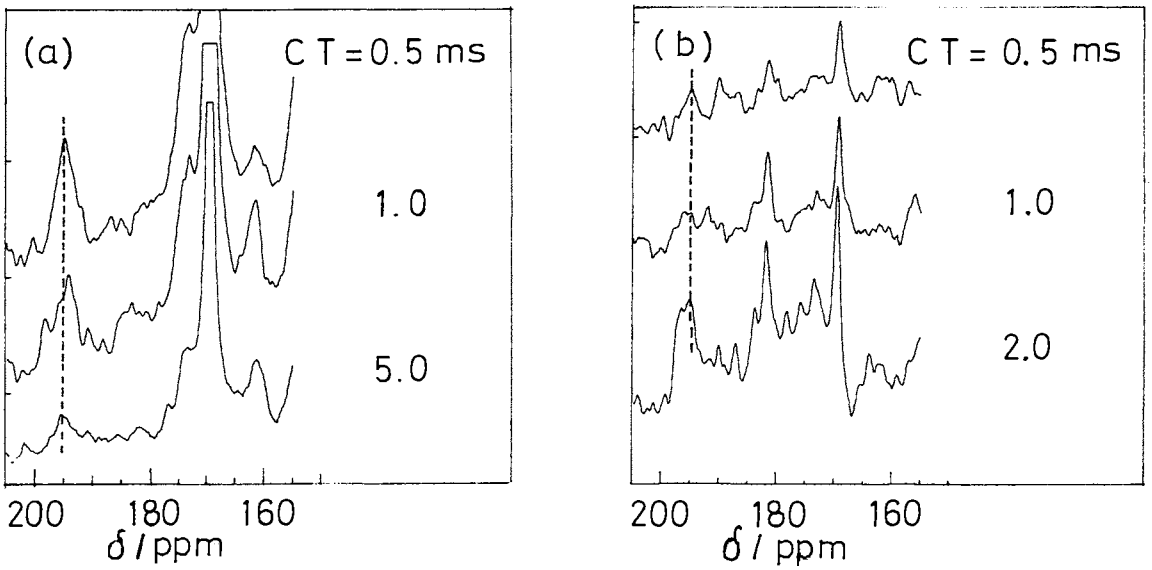

Fig. 2. The contact time (CT) dependence of the signal magnitude in the 9-carbonyl group in oligomeric solid BChl c (a) and monomeric solid BChl c (b) when compared with different contact times in keeping other conditions same. Recycle delay time is 15 s, and accumulation times are 1000.

These data indicate that the 9-carbonyl carbon in oligomeric solid BChl c has a hydrogen in the vicinity and in rigid structures. In the monomeric solid state the contact time dependence of the 9-carbonyl signal intensity indicates that, if the hydrogen bond exists, it may not be in rigid structures as that in the aggregate.[8] In the oligomeric BChl c there is no OH group except for the 2a-hydroxyethyl group, hence a possible hydrogen bond of the 9-carbonyl group may be with 2a-hydroxyethyl group.

For native chlorosomes, the 9-carbonyl group gave its resonance at around 194.3 ppm. This chemical shift as well as a contact time dependence of its signal magnitude similar to that for oligomeric solid BChl c (data not shown) shows that the 9-keto carbonyl of BChl c's in chlorosomes are similar in structure to one in solid oligomeric BChl c. Brune et al. have proposed structures in which the 2a-hydroxyl group ligates the Mg ion of one BChl c molecule while simultaneously hydrogen bonding to the 9-carbonyl group of another BChl c,[12] being consistent with the present NMR results.

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11) If the monomeric solid BChl c has a hydrogen bond and resonating in the chemical shift similar to that in the solution state (199.6 ppm), the solid effect (the shift when BChl c becomes solid) would be a high field shift of 3.8 ppm and the high field shift due to the aggregate formation becomes 1 ppm (the chemical shift difference in those between monomeric and oligomeric BChl c's). If the monomeric solid BChl c has no hydrogen bond, the high field shift due to the aggregation becomes 4.8 ppm, because no high field shift due to the solid effect is expected judged from the fact that the acetone solution (196.0 ppm) and the monomeric solid (195.8 ppm) have similar 9-carbonyl chemical shift values. In any cases the shifts (the solid effect shift in the former case and the aggregation effect shift for the latter case) are much larger than those normally expected, hence the hydrogen bonded 9-carbonyl carbon in solid state might have the resonance at field higher than that in methanol. Calculation of ring current shift by using proposed models (Refs. 2,7,12) and by the method of Abraham et al. (J. Am. Chem. Soc., **104**, 4332 (1982)) predicted 1 to 3 ppm high field shift.
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