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Cross Polarization Characteristics in Solid State High Resolution ^{13}C NMR of Chlorophyll a and Pheophorbide a

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High resolution ^{13}C NMR spectra in solid states were observed for chlorophyll a and pheophorbide a with a high magnetic field spectrometer. The rate constants of cross polarization (T_{CH}) and ^{1}H spin-lattice relaxation time in the rotating frame ($T_{1\rho}^{H}$) were determined. The rate constants and relaxation times reflected structural differences among them.

In photosynthesis various chlorophylls and bacteriochlorophylls function as antenna or reaction center pigments. In the reaction center, a pigment whose chlorophyll magnesium ion is replaced by two protons also plays important roles. The pigments absorb light energy, transfer to other pigments, and eventually utilize the energy to photoinduced electron transfer.[1] The biological functions of chlorophylls and their derivatives are closely related with their structures and interactions among them. Intact states of these pigments *in vivo* are difficult to investigate. Cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR which gives a high resolution solid state NMR was anticipated to give structural and electronic information of these pigments in intact states.[2] As a basic study to examine chlorophylls *in vivo*, we have investigated chlorophyll a (Chl a), and pheophorbide a (Pheo a) with a CP/MAS ^{13}C NMR technique. Comparison of solid state ^{13}C NMR of Chl a and Pheo a revealed several interesting cross polarization properties, which are critical for the investigation of these systems.

Chl a was extracted from spinach and purified as described previously.[1] Pheo a was obtained from Wako Pure Chemical Industries, Ltd. Solid state ^{13}C NMR spectra were obtained on a Bruker CXP300 FT-NMR spectrometer equipped with a CP/MAS probe Z32. The proton resonance frequency was 300.066 MHz. The observation was done with 90 deg pulse in 5 μs which corresponds to magnetic field of 1.17 mT and 4.65 mT,

respectively, for radio-wave strength of ^{1}H and ^{13}C. Samples were inserted in a 10 mm mushroom type spinner, and spun at the rate of 3.3 kHz. Chemical shift was externally referred to tetramethylsilane by taking the high field signal of adamantane as 28.7 ppm. Home made boron nitride (BN_3) spinners were used to eliminate back ground signals. Dixon's TOSS program was used to suppress spinning side bands.[3] The contact time was 2 ms for all experiments except for ones for contact time variation to obtain cross polarization rate constant (T_{CH}) and spin-lattice relaxation time in the rotating frame ($T_{1\rho}^{H}$). FID was observed for 30 ms, and recycle delay was 5 s.

Figures 1 and 2 show solid state ^{13}C NMR spectra for Chl a and Pheo a. Variations of CP/MAS

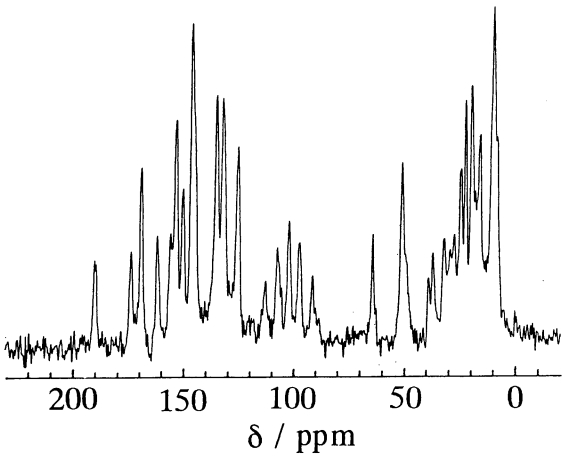

Fig. 1. A CP/MAS ^{13}C NMR spectrum of Chl a.

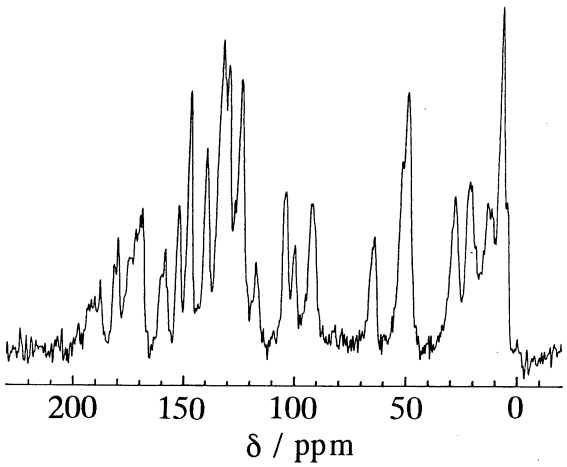

Fig. 2. A CP/MAS ^{13}C NMR spectrum of Pheo a.

^{13}C NMR signal intensities were measured by changing the length of the contact times (data not shown). The signals grow with the increase of the contact time, and pass the maximum, then decrease gradually. From the analysis[4] of the observed results the time constant which expresses the transfer rate of magnetization from ^{1}H to ^{13}C (T_{CH}), and the relaxation time of the spin-locked ^{1}H magnetization along the spin lock axis ($T_{1\rho}^{H}$) can be obtained within + 5% errors as shown in Tables 1 and 2.

The CP/MAS ^{13}C NMR spectrum of Chl a (Fig. 1) at high magnetic field strength (7 T) with the TOSS pulse program yielded the highly resolved signals more than that previously observed in a 3.5 T spectrometer with a normal CP/MAS technique.[2] The results indicate that the TOSS pulse program was effective enough to suppress the spinning side bands due to the larger chemical shift anisotropy under the high magnetic field. They also indicate that the CP/MAS ^{13}C NMR with a higher magnetic field spectrometer using the TOSS technique is more applicable to the structure investigation of Chl derivatives *in vivo*.

Table 1. ^{13}C CP/MAS NMR of Chlorophyll a[a)]

δ/ppm	T_{CH}/ms	$T_{1\rho}{}^{H}$/ms	Assignment
10	0.3	5	C-1a, 5a (12.6, 12.3) C-3a (11.0)
17	0.2	5	C-4b (17.5), P-3a (16.2)
19	0.2	8	C-4a, P-7a, 11a (19.7)
22	0.16	12	C-8a (23.6), P15a, 16 (22.7)
27	0.06	8	C-7a (30.9), C-7b (29.8)
51	0.1	5	C-10b (53.0), C-7 (50.4) C-8 (49.7)
64	0.06	6.4	C-10 (65.3), P-1 (61.5)
91	0.06	7	δ (93.4)
97	0.1	10	α (100.3)
101	1	3	γ (105.8)
107	0.08	9	β (108.2)
125	2	4	C-2b (119.8), P-2 (118.9)
132	1.6	3	C-6 (130.9), C-2a (131.0)
135	0.8	7	C-1 (135.9), C-3 (134.2) C-5 (134.1), C-2 (139.5)
146	1	5	C-14 (146.6), C-4 (144.6)
150	1.8	4	C-12 (148.9), C-15 (148.1)
154	1.4	3.2	C-17 (156.3), C-11 (155.4) C-13 (152.7)
161	2	4	C-16 (162.4)
169	1	3	C-10a (170.7), C-18 (169.4)
173	1.6	3	C-7c (173.1)
189	1.6	3	C-9 (190.0)

Table 2. ^{13}C CP/MAS NMR of Pheophorbide a

δ/ppm	T_{CH}/ms	$T_{1\rho}{}^{H}$/ms	Assignment
8	0.17	20	C-5a, 1a (12.0), C-3a (11.1)
13	0.14	16	C-4a (19.3), C-4b (17.3)
22	0.12	20	C-8a (23.1)
28	0.06	20	C-7b (31.1), C-7a (29.9)
50	0.1	16	C-10b (52.8), C-7 (51.2) C-8 (50.2)
64	0.14	12	C-10 (64.8)
92	0.1	16	δ (93.1)
100	0.12	20	α (97.5)
104	0.6	16	γ (105.3), β (104.3)
124	0.6	12	C-2b (122.6)
133	0.6	22	C-1 (131.8) C-5, 6, 2a (129.0)
140	0.5	21	C-11 (142.0), C-15 (137.8) C-2 (136.5), C-12, 3 (136.2)
148	0.8	16	C-16 (149.7), C-4 (145.1)
153	0.9	17	C-13 (155.6), C-14 (151.0)
160	0.9	7	C-17 (161.2)
172	0.6	12	C-18 (172.0), C-10 (169.6)
180	0.4	20	C-7c (173.3 methylester)
190	1	10	C-9 (189.6)

a) The chemical shifts in the solid state differ by 4-5 ppm as compared with Ref. 2. Since the method for determining the chemical shift was not described in Ref. 2, the reason for the discrepancy is not clear.

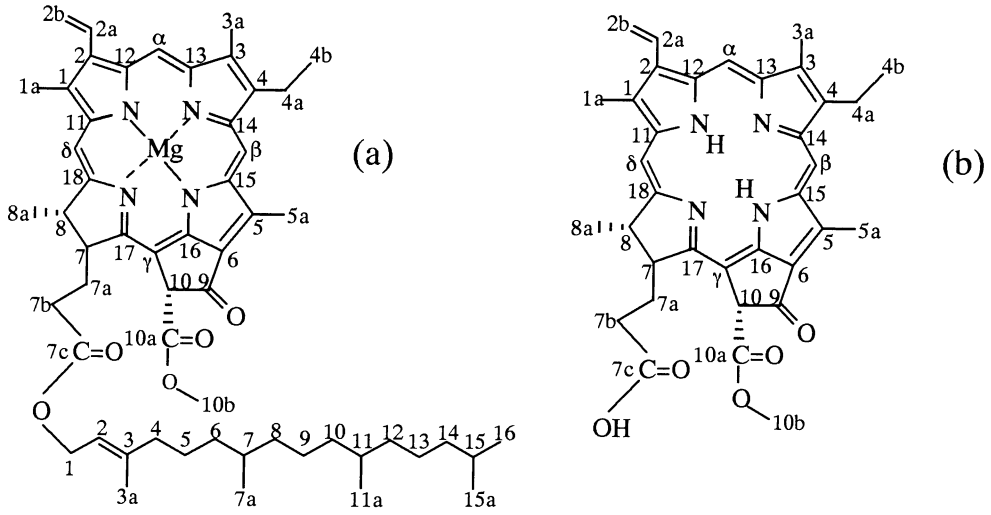

Fig. 3. Conjugation structures for chlorophyll a (Chl a) (a) and pheophorbide a (Pheo a) (b). Numbering of the carbons used in Tables 1 and 2 (C and P stand for Chlorin and Phytol) are labeled in the figures.

Assignments of the solid state ^{13}C NMR signals for Chl a (Fig. 1) and Pheo a (Fig. 2) were accomplished with the aid of the solution NMR assignments in $CDCl_3$ solutions. Some results are summarized in Tables 1 and 2 (for carbon numbering, see Fig. 3). Apart from some differences in line-withs the chemical shift changes in the presence or absence of the central metal ion were clearly observed in the solid state as well as in the solution state.[1] The differences are especially critical for α pyrrole carbons, and are larger for C-11, 12, 15, and 16 than those for C-13, 14, 17, and 18 as in the solution.[1] The result implies that the structure and electronic states are significantly influenced by the presence of the central magnesium ion in the solid state.

Information on conformations and molecular dynamics can be obtained from the cross-polarization rate (T_{CH}) and relaxation rates ($T_{1\rho}^{H}$, T_1^{H}, and $T_{1\rho}^{C}$). In native systems these values reflect interactions of the pigments with surrounding molecules and the mobility of the pigments which should be important for the understanding of their functions. Observed signal intensities which reflect the enhanced ^{13}C magnetization depend both on the level crossing rate and relaxation rate of the magnetizations of ^{1}H and ^{13}C nuclei.[4] In Chl a T_{CH} values for the ^{13}C with bound ^{1}H were around 0.06 to 0.16 ms, while those for the ^{13}C without ^{1}H showed the values from 0.8 to 2 ms, the latters being longer by one order of magnitude than the formers. For Pheo a these values were 0.06–0.17 ms for the ^{13}C with ^{1}H, 0.4–1 ms for ones without ^{1}H. The T_{CH} values for carbons with ^{1}H are smaller than those without ^{1}H. This is reasonable from the fact that the CP efficiency is inversely proportional to the C-H distance. The T_{CH} values for the ^{13}C with ^{1}H showed very little difference among the compounds observed, while the T_{CH} values for the ^{13}C with no ^{1}H gave fairly large differences among compounds. The latter T_{CH} values are in the order of Chl a > Pheo a. The $T_{1\rho}^{H}$ values for carbons with ^{1}H are larger than those without ^{1}H. This indicates that the spin diffusion of ^{1}H is not uniform in these systems. The $T_{1\rho}^{H}$ values for Chl a are in the range of 2-12 ms, while those for Pheo a are 7-22 ms. The values corresponding to respective carbons for Pheo a are several times larger than those of Chl a.

In conclusion the presence or absence of the central metal ion affects critically on the cross polarization characteristics of Chl's. CP/MAS ^{13}C NMR spectra as well as observation of the T_{CH} and $T_{1\rho}^{H}$ values will give valuable information of the structures and functions of the pigments in the intact systems.

References

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