Solution and Solid State High Resolution ^{13}C NMR Studies on Tetraphenylchlorins

Tsunenori NOZAWA,* Mitsushi NISHIMURA,† Masahiro HATANO,† and Mitsuo SATO††
Department of Biochemistry and Engineering, Faculty of Engineering, Tohoku University, Sendai 980
† Institute for Chemical Reaction Science, Tohoku University, Sendai 980
†† Biophysics Division, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko-cho, Tsukui-gun, Kanagawa 199-01

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High-resolution ^{13}C NMR spectra in solid and solution states were observed for tetraphenylchlorin (TPC), tetraphenylbacteriochlorin (TPBC), and tetraphenylisobacteriochlorin (TPIBC). Chemical-shift assignments of the ^{13}C NMR signals for TPBC were completed by a comparison of the ^{1}H decoupled solution ^{13}C NMR spectra with non-decoupled and solid state ones. The absence of signal number changes by temperature in the solution ^{13}C NMR spectra and their comparison with those of the solid state ones suggested the absence of an NH tautomerism in the chlorin ring for TPC and TPBC, indicating the predominant stability of one tautomer over the other. On the other hand, for TPIBC the presence of temperature-dependent signal number changes in the solution ^{13}C NMR spectra were observed. The rate constants of cross polarization (CP) (T_{CH}) and the ^{1}H spin-lattice relaxation time in the rotating frame ($T_{1\rho}^{H}$) were determined based on the dependence of the NMR magnitude on the contact time.

In plant and bacterial photosynthesis various chlorophylls and bacteriochlorophylls function as antenna or reaction center pigments. The pigments absorb light energy, transfer it to other pigments, and eventually utilize the energy to photoinduced electron transfer.[1] Chlorophyll and bacteriochlorophyll comprise, respectively, a chlorin ring (i.e., a porphyrin ring with one saturated pyrrole ring) and a bacteriochlorin ring (a porphyrin ring with two saturated pyrrole rings). The biological functions of chlorophylls and bacteriochlorophylls are closely related to their structures and interactions among them. The structures of these pigments in vivo are difficult to investigate. Cross-polarization/magic angle spinning (CP/MAS)^{13}C NMR, which provides high-resolution solid state NMR, was anticipated to give both structural and electronic information concerning these pigments in intact states.[2—4] As a basic study used to examine chlorophylls and bacteriochlorophylls in vivo, we investigated various synthesized chlorin and bacteriochlorins (i.e., tetraphenylchlorins (TPC), tetraphenylbacteriochlorin (TPBC), and tetraphenylisobacteriochlorin (TPIBC)) using the cross-polarization/magic angle spinning (CP/MAS) ^{13}C NMR technique. Comparisons of the solution and solid state ^{13}C NMR of TPC, TPBC, and TPIBC revealed several interesting results concerning the dynamic structures of these molecules, as well as the cross-polarization properties.

Experimental

Materials. Tetraphenylporphyrin (TPP), TPC, TPBC, and TPIBC were synthesized and purified as described previously.[5] The $CDCl_3$ used for solution NMR was purchased from Merck & Co., Inc. The other chemicals were obtained from Wako Pure Chemical Industries, Ltd.

Methods. The solution ^{13}C NMR spectra were recorded at 75.46 MHz on a Bruker CXP300 FT-NMR spectrometer equipped with a broad-band probe which covered the 30—125 MHz range. Sample tubes with 10 mm diameters were used for observations in solution states. The observation spectral width was 30 kHz in 8 kW data mem-

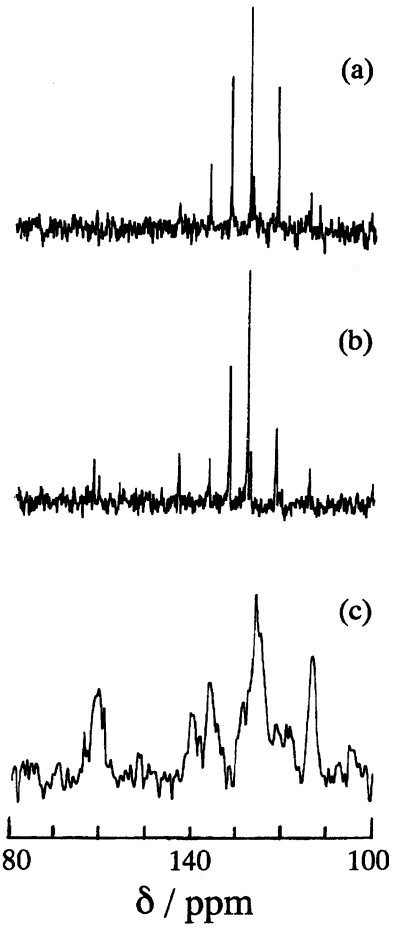

Fig. 1. Solution and solid state ^{13}C NMR spectra of tetraphenylbacteriochlorin (TPBC). In $CDCl_3$ at 298 K (a) and 213 K (b), and the CP/MAS spectrum in the solid state (c).

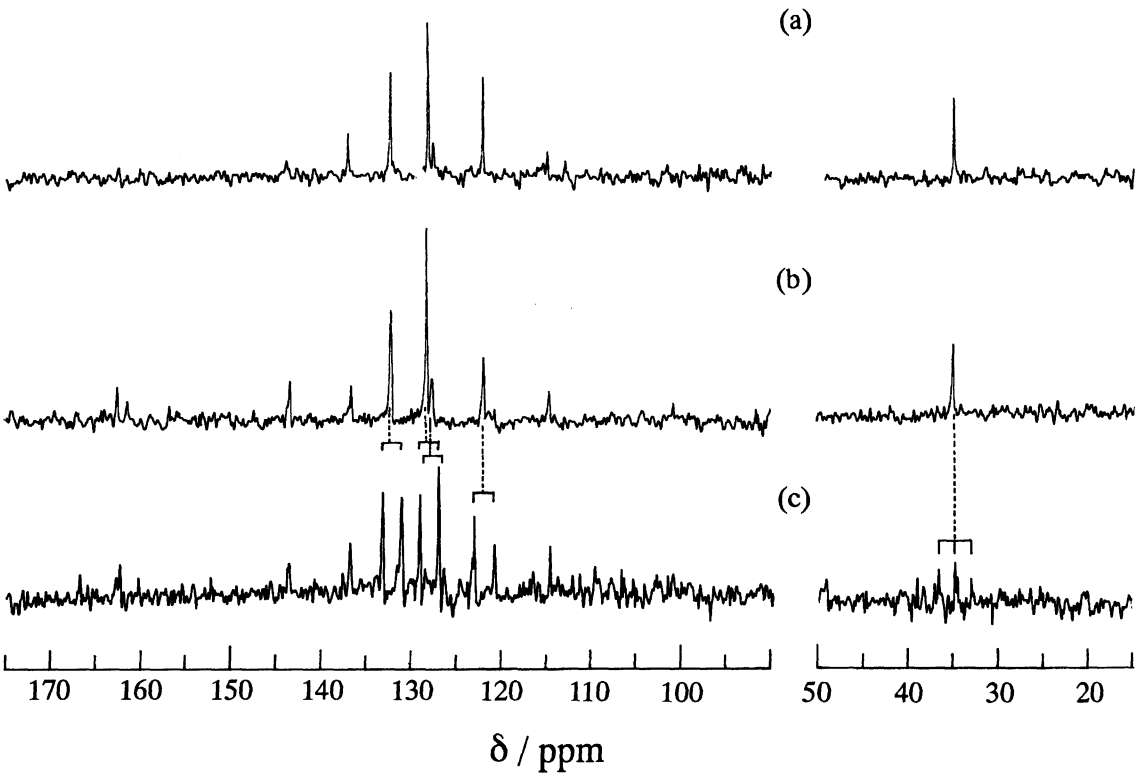

Fig. 2. Solution ^{13}C NMR spectra for tetraphenylbacteriochlorin (TPBC). In $CDCl_3$ ^{1}H decoupled spectra at 298 K (a) and 213 K (b) and ^{1}H non-decoupled spectrum at 213 K (c).

ory. A 16-μs pulse (ca. a 40-deg pulse) and a recycle delay of 2 s were used. The decoupling power was 3 W, and line broadening of 2 Hz was applied. The sample temperature was regulated by a Bruker B-VT-1000 autotemperature regulator. Solid state ^{13}C NMR spectra were obtained on the same machine, but equipped with a CP/MAS probe Z32. The proton resonance frequency was 300.066 MHz. The homogeneity of the magnetic field was adjusted by using FID of benzene, and Hartmann–Hahn conditions were obtained with adamantane. The observation was carried out with a 90-deg pulse (5 μs) which correspond to magnetic fields of 11.7×10^{-4} and 46.5×10^{-4} T, respectively, for the radio-wave strength of ^{1}H and ^{13}C. Samples were inserted in a 10-mm mushroom-type spinner, and spun at a rate of 3.3 kHz. The 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 any background signal. Dixon's TOSS program was used to suppress spinning side bands.[6] The contact time was 5 ms for all of the experiments, except for those concerning the contact time variation, to obtain the cross-polarization rate constant (T_{CH}) and spin-lattice relaxation time in the rotating frame ($T_{1\rho}^{H}$).[7] FID was observed for 30 ms, and the recycle delay was 5 s.

Results

Solution and Solid State ^{13}C NMR. Figure 1 shows the ^{1}H decoupled solution ^{13}C NMR spectra (at 298 and 213 K) and a solid state CP/MAS ^{13}C NMR spectrum for TPBC. The solution ^{1}H decoupled ^{13}C NMR spectra (at 298 K (Fig. 2a), and at 213 K (Fig. 2b)) were compared with the ^{1}H non-decoupled ^{13}C NMR spectrum (Fig. 2c) in an expanded scale in order to aid the signal assignment. The number of signals in the ^{1}H decoupled solution ^{13}C NMR spectra of TPBC did not show any temperature-dependent change at these temperatures (Fig. 2). This is in contrast with that of TPP, which showed clear temperature-dependent changes in the signal numbers in these temperature ranges.[8]

For TPC the solution ^{1}H decoupled ^{13}C NMR spectra at 298 and 213 K (Figs. 3a and 3b) were compared with that in the solid state CP/MAS ^{13}C NMR spectrum (Fig. 3c). The solution ^{13}C NMR spectra at 298 and 213 K (Figs. 3a and 3b) did not show any change in the number of signals. The observed intensity changes of signals may be attributable to various relaxation effects.

Figure 4 shows the ^{1}H decoupled solution ^{13}C NMR spectra (at 298 and 213 K) and a solid state CP/MAS ^{13}C NMR spectrum for TPIBC. Figure 5 shows the expanded solution ^{13}C NMR spectra at 298 and 213 K for TPIBC. It is noted that in the case of TPIBC, signal changes with temperature were observed, especially at 120.4, 126.5, and 152.8 ppm, which may be assigned to the β and α carbons of the pyrrole ring (see Discussion).

Cross Polarization. Variations of the CP/MAS ^{13}C NMR signal intensities were measured by changing the length of the contact times for TPP and TPC. A typical result is shown in Fig. 6 for two signals at 125

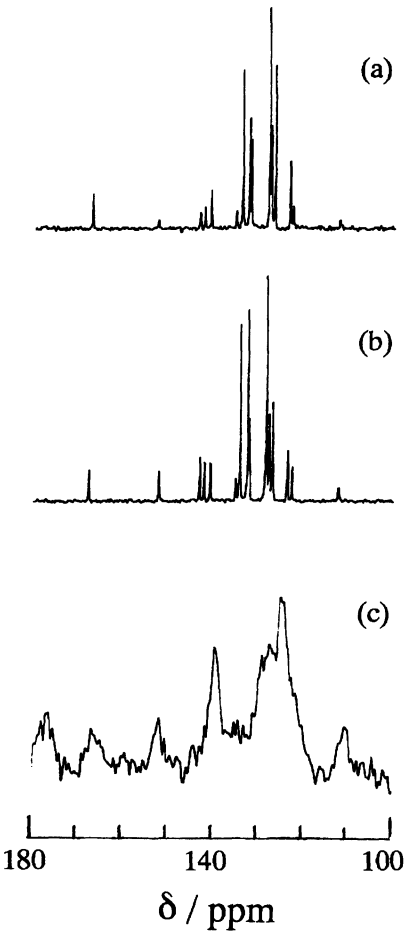

Fig. 3. Solution and solid state ^{13}C NMR spectra of tetraphenylchlorin (TPC). In $CDCl_3$ at 298 K (a) and 213 K (b), and the CP/MAS spectrum in the solid state (c).

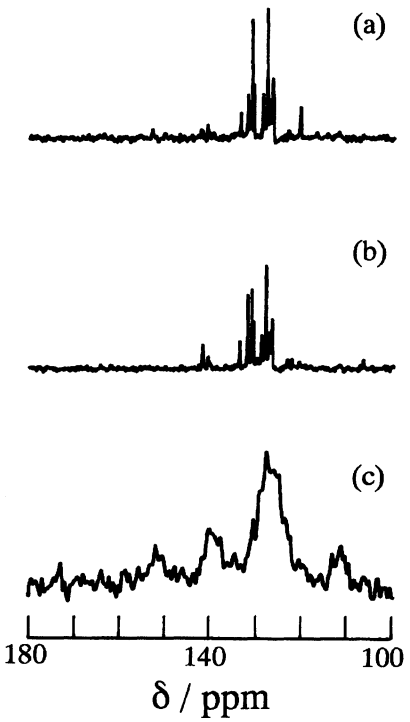

Fig. 4. Solution and solid state ^{13}C NMR spectra of tetraphenylisobacteriochlorin (TPIBC). In $CDCl_3$ at 298 K (a) and 213 K (b), and the CP/MAS spectrum in the solid state (c).

and 139 ppm in TPC. The signals grow along with an increase in the contact time, pass a maximum, and then gradually decrease. From an analysis of the observed results (described in Discussion), the time constant which expresses the transfer rate of magnetization from ^1H to ^{13}C (T_{CH}), and the relaxation of the spin-locked ^1H magnetization along the spin lock axis ($T_{1\rho}^{H}$) can be obtained for TPP and TPC, as described later.

Discussion

Assignment of Signals. The assignment of all the ^{13}C NMR signals for TPBC was accomplished with the aid of the ^1H non-decoupled spectra (Fig. 2c) and solid state NMR (Fig. 1c), as well as the NMR assignment of similar compounds.[8,9] The results are summarized in Table 1. The signal at 35 ppm can be assigned to the β'-1 carbons in the rings II and IV (see Fig. 7), because they are the only aliphatic carbons and split into triplets due to the two bound protons (Fig. 2c). The singlet signal at 114.6 ppm should be attributed to *meso* carbons, since it should be in the highest field, as aromatic carbons of this chlorin ring.[8,9] From the relative intensity, the weaker doublet at 127.1 ppm should be assigned to phenyl (Ph)-4 (see Fig. 7), the stronger doublet at 127.7 ppm to Ph-3,5, the doublet at 131.9 ppm to Ph-2,6, and the singlet at 143.3 ppm to Ph-1. Since the pyrrole α carbon with ^1H at its nitrogen in TPP is around 135 ppm, the signal at 136.5 ppm can be attributed to α-2 carbons. The signal at 161.9 ppm can be assigned to α'-1 carbons, because the α pyrrole ring carbon in saturated pyrrole is at the lowest field in chlorophyll derivatives. The signal at 121.8 ppm should be assigned to the remaining pyrrole ring β-2 carbons in rings I and III, based on the relative chemical shifts.

The assignment of the ^{13}C NMR resonances of TPC (Table 2) is not so unequivocal, as that for TPBC. The resonance at 35.7 ppm (data not shown) can be assigned to β'-1 in ring IV (see Fig. 8), since this is the only type of aliphatic carbon. There are two types of meso carbons; the signal at 112.0 ppm may be assigned to them. The signals at 122.3 and 123.2 ppm may be assigned to β-2 and β-3 in the pyrrole ring with the NH group from a comparison with the assignment for TPBC. Similarly, the resonances at 126.5, 127.3, and 128.0 ppm can be assigned to the Ph para (Ph-4) and meta (Ph-3, 5) carbons. Two of the three resonances at 131.7, 132.0, and 133.7 ppm may be grouped to the Ph ortho (Ph-2, 6) carbons. Two of the three resonances at 140.3, 141.6, and 142.6 ppm may be assigned to the Ph carbons attached directly to the meso carbon (Ph-1). The

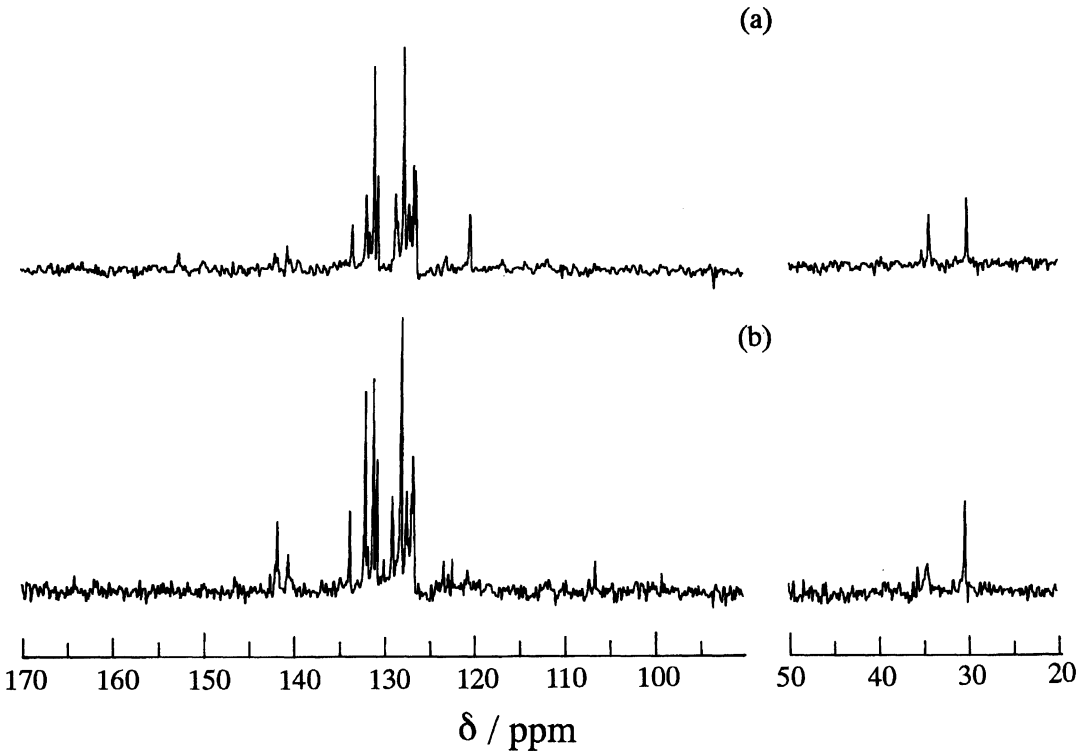

Fig. 5. Solution ^{13}C NMR spectra of tetraphenylisobacteriochlorin (TPIBC) at 298 K (a) and 213 K (b).

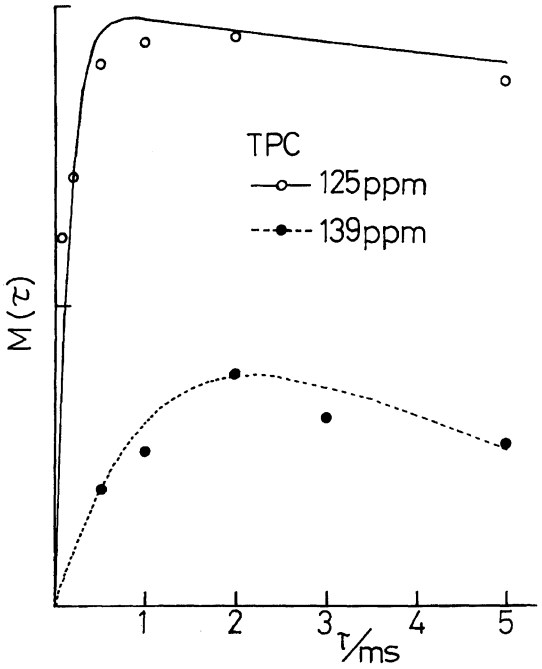

Fig. 6. Contact time (τ) dependence of the ^{13}C magnetization ($M(\tau)$) for signals at 125 and 139 ppm of tetraphenylchlorin (TPC).

pyrrole β carbon β'-4 may be one of the resonances at 131.7, 132.0, and 133.7 ppm. From a comparison of the chemical shifts with those in TPP[8,9] and TPBC, the α carbon in the saturated pyrrole ring (α'-1) may resonate at 167.5 ppm, and the α carbon in the pyrrole ring with

Table 1. Observed Chemical Shifts and Their Assignment of ^{13}C NMR for Tetraphenylbacteriochlorin (TPBC)

CP/MAS ^{13}C NMR δ/ppm	^{13}C NMR in $CDCl_3$[a] δ/ppm	Assignment
161	161.9(s)	Cα'-1
141	143.3(s)	Ph-1
137	136.5(s)	Cα-2
	131.9(d)	Ph-2,6
127	127.7(d)	Ph-3,5
	127.1(d)	Ph-4
121	121.8(d)	Cβ-2
114	114.6(s)	meso
35	35.0(t)	Cβ'-1

a) The symbols in the parentheses, s, d, and t stand for singlet, doublet, and triplet.

N, having no attached H, α'-4, at 151.7 ppm. Further, from knowledge concerning the ^{13}C NMR of chlorins, one of the three resonances at 140.3, 141.6, and 142.6 ppm can be assigned to α-3, and the resonance at 134.6 ppm may be assigned to α-2 carbons.

NH Tautomerism and Dynamic Structure. As shown in Fig. 7 for TPBC, there are, respectively, four types of α and β pyrrole carbons, one with ^{1}H at its nitrogen (designated as α-1, α-2 and β-1, β-2) and one without ^{1}H at its nitrogen (designated as α'-1, α'-2 and β'-1, β'-2). Therefore, eight ^{13}C resonances are expected to appear for TPBC. Experimentally, only four

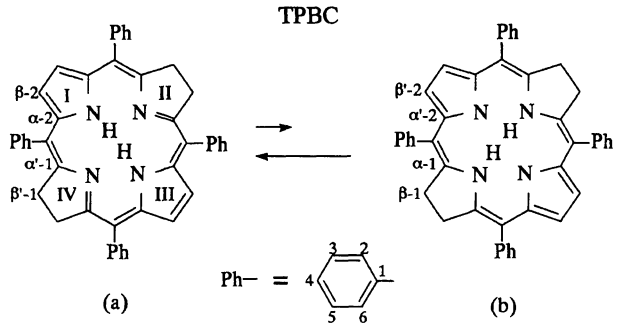

Fig. 7. Conjugate structures for tetraphenylbacteriochlorin (TPBC), and its possible tautomerism reactions. The numbering of rings I to IV, and the pyrrole α, β carbons used in the text are indicated on the Figures. Pyrrole carbons with N (nitrogen without proton) are indicated by a prime.

Table 2. Observed Chemical Shifts and Their Assignment of ^{13}C NMR for Tetraphenylchlorin (TPC)

CP/MAS ^{13}C NMR δ/ppm	^{13}C NMR in $CDCl_3$ δ/ppm	Assignment
165	167.5	Cα'-1
152	151.7	Cα'-4
139	142.6	Cα-3
	141.6	1Ph-1
	140.3	2Ph-1
134	134.6	Cα-2
	133.7	1Ph-2,6
	132.0	2Ph-2,6
	131.7	Cβ'-4
	128.0	1Ph-3,5
	127.3	2Ph-3,5
	126.5	1Ph-4
		2Ph-4
125	123.2	Cβ-3
	122.3	Cβ-2
111	112.0	1meso
		2meso
36	35.7	Cβ'-1

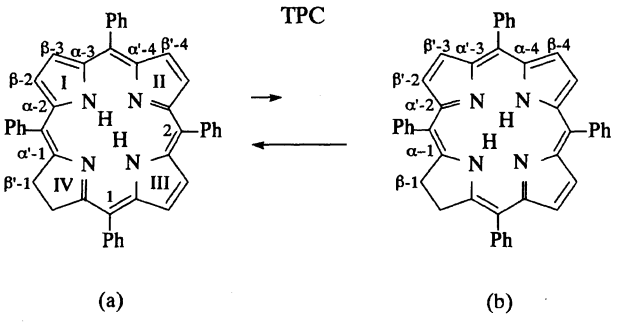

Fig. 8. Conjugate structures for tetraphenylchlorin (TPC), and its possible tautomerism reactions. The numbering of rings I to IV, (pyrrole α, β carbons) and the numbering of the phenyl groups used in the text are indicated on the Figure.

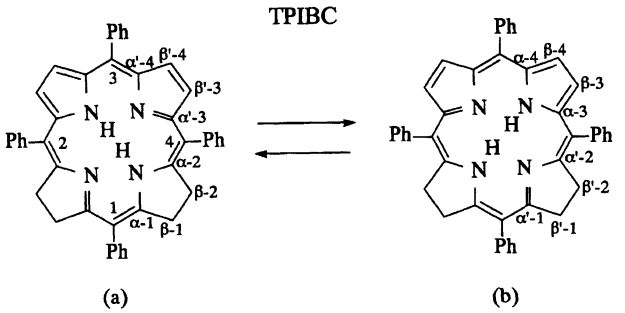

Fig. 9. Conjugate structures for tetraphenylisobacteriochlorin (TPIB), and its tautomerism reactions. The numbering of rings I to IV, and pyrrole α, β carbons and numbering of phenyl groups are labeled on the Figure.

Table 3. Time Constant for Cross-Polarization (T_{CH}) and Spin-Lattice Relaxation Time in Rotating Frame ($T_{1\rho}^{H}$) of Tetraphenylporphyrin (TPP) and Tetraphenylchlorin (TPC)

	Chemical Shift/ppm	T_{CH}/ms	$T_{1\rho}^{H}$/ms	Assignment
TPP	127	0.2	50	Ph-3,4,5 Cβ
	152	2	10	Cα'
TPC	125	0.2	50	Ph-3,4,5 Cβ
	139	2	4	Ph-1

pyrrole carbon resonances were observed at 35.0, 121.8, 136.5, and 161.9 ppm, both at 298 and 213 K (Figs. 2a and 2b). These α and β carbon signals showed no temperature-dependent change in numbers and have corresponding signals in the solid states at similar chemical shifts (see Fig. 1). Since the NH tautomerism is frozen in the solid state, the results indicate that the NH tautomerism is also frozen in one tautomeric state in the solution state. The structure shown in Fig. 7a is considered to be one for the existing tautomers based on the chemical-shift assignment described above. This may be consistent with the consideration that the tautomeric structure shown in Fig. 7a is expected to be more stable than that of the other tautomeric structure (Fig. 7b), due to the extended conjugate systems, more than the other.

For TPC, no temperature-dependent number changes were observed for solution NMR signals which correspond to the solid NMR signals at around 36, 125, 134, 139, 152, and 165 ppm (Fig. 1 and Table 2). These results indicate that no tautomeric equilibrium occurs within these temperature ranges. Since the tautomeric structure shown in Fig. 8a has more extended conjugate systems than the other (Fig. 8b), the tautomer shown in Fig. 8a is expected to exist.

As shown in Fig. 5 for TPIBC, solution ^{13}C NMR exhibited signal changes with temperature, especially at 120.4, 126.5, and 152.8, which may be assigned to β and

α carbons in the pyrrole ring. This fact may indicate the presence of an NH tautomerism in TPIBC. This may be consistent with the tautomeric structures shown in Fig. 9. Thus, since in this case both tautomeric structures have the same conjugate systems, both structures are expected to exist in a similar way.

Cross Polarization Rate. The observed signal intensities which reflect the induced ^{13}C magnetization depend on both the induced rate and relaxation rate of the magnetizations of ^{1}H and ^{13}C nuclei.[7,10] Thus magnetization induced at the cross-polarization time (contact time) (τ) can be described as

$$M(\tau) = M_0\lambda^{-1}[1 - \exp(-\lambda\tau/T_{CH})]\exp(-\tau/T_{1\rho}^{H}), \quad (1)$$

where $\lambda=1+(T_{CH}/T_{1\rho}^{C})-(T_{CH}/T_{1\rho}^{H})$. The equilibrium carbon magnetization (M_0) is determined by the proton spin temperature. For large radio-frequency fields, $M_0=(\gamma_H/\gamma_C)M_0^C$, where M_0^C is the ordinary carbon thermal magnetization appropriate for the static field. Eq. 1 allows a simple interpretation: The carbon magnetization increases at a rate of T_{CH}^{-1} while being depleted at $(T_{1\rho}^{H})^{-1}$. Since a quantitative analysis of Fig. 6 shows that $T_{CH} \ll T_{1\rho}^{H}(T_{1\rho}^{C})$ in these systems, λ can be set to 1. Then Eq. 1 becomes

$$M(\tau) = M_0[1 - \exp(-\tau/T_{CH})]\exp(-\tau/T_{1\rho}^{H}), \quad (2)$$

From this equation, T_{CH} and $T_{1\rho}^{H}$ can be evaluated by a simulation method from the observed results given in Fig. 6. The typical simulation results are indicated by solid and broken lines in Fig. 6 for TPC. The obtained time constants (T_{CH} and $T_{1\rho}^{H}$) are shown in Table 3, along with the data for TPP. The signal assignments given in Table 3 indicate that the T_{CH} values for the carbons with ^{1}H are smaller than those without ^{1}H. This is reasonable from the fact that the CP efficiency is proportional to the inverse of the C–H distance. The $T_{1\rho}^{H}$ values for carbons with ^{1}H are larger than those without ^{1}H. This indicates that ^{1}H spin diffusion is not uniform along the chlorin ring.

In conclusion, the ^{13}C NMR signals of various tetraphenylchlorins have been characterized. The results indicate the predominance of one tautomeric structure for TPC and TPBC. Reasonable T_{CH} and $T_{1\rho}^{H}$ values were obtained for TPC and TPP.

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