Self-Assembly of Synthetic Bacteriochlorophyll-*f* Analogues Having C8-Formyl Group

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3^1-Epimerically pure C8-formyl analogues Zn–**3*R*/*S*** of zinc methyl bacteriopheophorbide-*f* (Zn–MBPhe-*f*) were synthesized. Their spectroscopic and self-aggregation properties were compared to those of Zn–MBPhe-*f* possessing the C7-formyl group (Zn–**2*R*/*S***) as a model compound of bacteriochlorophyll-*f*. Movement of a formyl group from the 7- to 8-position as in Zn–**2** → Zn–**3** caused large spectral changes in both monomeric and oligomeric forms. Monomeric Zn–**3*R*/*S*** in THF showed a more red-shifted Q_y peak (650 nm) than Zn–**2*R*/*S*** (628 nm), but both afforded the same Soret maximum at 448 nm. Self-aggregates of Zn–**3*R*/*S*** in 6% (v/v) THF–H_2O gave a more red-shifted Q_y peak accompanied by a large diastereomeric control in the oligomeric Q_y peaks.

Chlorosome is a unique light-harvesting apparatus of green photosynthetic bacteria, where bacteriochlorophylls (BChls)-*c*, -*d*, and -*e* self-aggregate to form antennas without any assistance from proteins.[1] The structural characteristics of BChls-*c*/*d*/*e* are defined by their C7- and C20-substituents (see R^7/R^{20} in Fig. 1a). Namely, the difference between BChl-*c* and BChl-*d* lies on the C20-substituent; the methyl group as in BChl-*c* and hydrogen atom as in BChl-*d*. BChl-*e* is regarded as a 7-formyl analogue of BChl-*c*. This relationship is the same as in naturally occurring chlorophylls-*a*/*b*. Furthermore, the term BChl-*f* is reserved for the 7-formyl derivative of BChl-*d*, although it has not yet been found in natural resources.[2]

We recently reported the synthesis and spectral characteristics of the model compound of BChl-*f* (Zn–**2*R*/*S*** in Fig. 1b) possessing 7-formyl group (R^7 = CHO).[3] It is also known that BChl-*d* consists of several homologues having different alkyl groups at the 8- and 12-positions (R^8/R^{12} in Fig. 1a). To investigate the effects of C8-substituents on BChl-*d*, we earlier synthesized BChl-*d* analogues possessing a series of alkyl/alkenyl substituents at the 8-position.[4] Here, we report the effects of the 8-formyl group in synthetic BChl-*d* analogue. Newly synthesized 3^1-epimerically pure zinc complexes (Zn–**3*R*/*S*** in Fig. 1b) can also be regarded as a BChl-*f* analogue. It was demonstrated that the spectroscopic and self-aggregation properties of

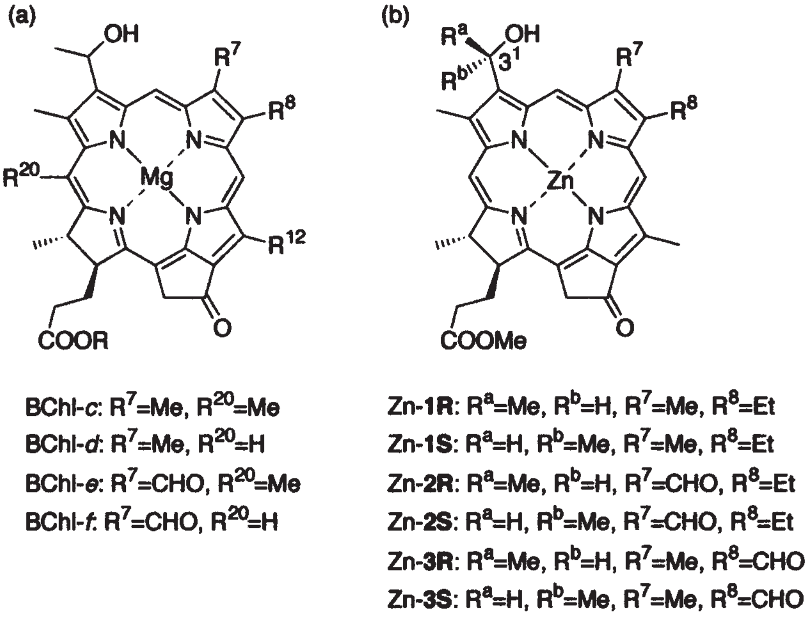

Fig. 1. Molecular structures of (a) magnesium chlorin, BChls-*c*/*d*/*e*/*f* and (b) their model compounds, zinc methyl $3^1R/S$-8-ethyl-12-methyl-bacteriopheophorbides-*d* and *f* (Zn–**1*R*/*S*** and Zn–**2*R*/*S***), and synthetic BChl-*f* analogues having C8-formyl group Zn–**3*R*/*S***.

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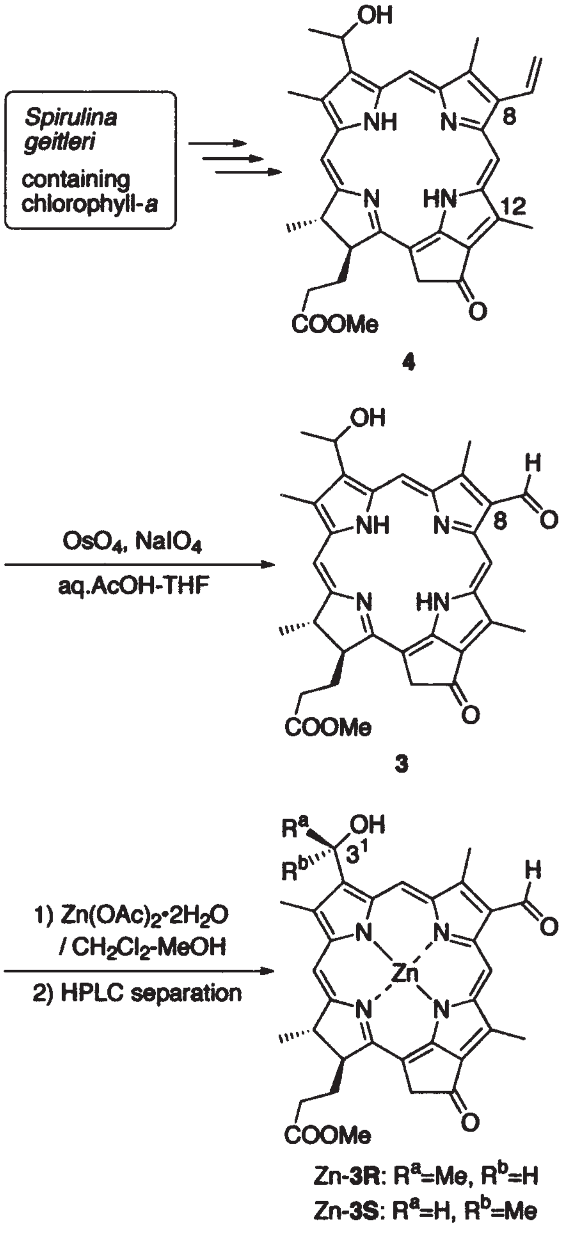
Zn–**3*R*/*S*** (7-Me/8-CHO) are quite different from those of the corresponding analogues Zn–**1*R*/*S*** (7-Me/8-Et)[5] or Zn–**2*R*/*S*** (7-CHO/8-Et).[3]

Results and Discussion

The synthesis of BChl-*f* analogues Zn–**3*R*/*S*** is outlined in Scheme 1. Methyl 8-vinyl-12-methyl-bacteriopheophorbide-*d* (**4**)[4b] was prepared by modification of chlorophyll-*a* extracted from *Spirulina geitleri* (a cyanobacterium) according to the reported procedures.[6] The 8-vinyl group of **4** was oxidatively cleaved by OsO_4 and $NaIO_4$[6,7] to give 8-formyl-chlorin **3** in 61% yield. Subsequent zinc-metallation was examined as previously reported:[5b,6,7] only half of free-base chlorin **3** was zinc-metallated after stirring at room temperature for 12 h, while **4** was completely metallated within an hour. Thus reflux in CH_2Cl_2 for 5 h is required for the complete zinc insertion to **3**. The low reactivity in **3** is ascribed to the electron-withdrawing 8-formyl group. The resulting 3^1-epimeric mixture Zn–**3** was separated by reversed-phase HPLC to give epimerically pure Zn–**3*R*** and Zn–**3*S***. The first and second fractions were assigned to 3^1R and 3^1S epimers, respectively, because it has been shown that all the 3^1-epimers of the related compounds including Zn–**1*R*/*S*** and Zn–**2*R*/*S*** are eluted in the order of 3^1R and 3^1S under such HPLC conditions.[2b,3,5b,8]



Scheme 1.

Absorption spectra of Zn–**3*R*/*S*** were measured in THF, where zinc chlorins existed as their monomeric forms due to the coordination of a THF molecule to the central zinc. Their observed spectra are shown in Fig. 2a, and the results are summarized in Table 1 together with those of Zn–**1*R*/*S*** and Zn–**2*R*/*S*** for comparison. Monomeric Zn–**3*R*** gave almost the same absorption spectrum as the corresponding epimer Zn–**3*S***, indicating that the 3^1-stereochemistry did not affect visible spectra of the monomeric species as reported in Zn–**1/2**.[3,5] In THF, the Q_y and Soret peaks of Zn–**3** are shifted to longer wavelengths (650 and 448 nm) than those of Zn–**1** (645 and 424 nm) due to the effect of the 8-formyl substituent. Compared to the 7-formyl analogue Zn–**2**, the Q_y peak of zinc 8-formyl-chlorin Zn–**3** red-shifted to 650 nm, while retaining the position of the Soret peak at 448 nm. Considering the reported result that oxidation of the 3-ethyl group to the 3-formyl group induced red-shifts in the Soret and Q_y peaks,[9] we can conclude that the position of a formyl group on a peripheral position of the chlorin π-system greatly affected the absorption spectra.

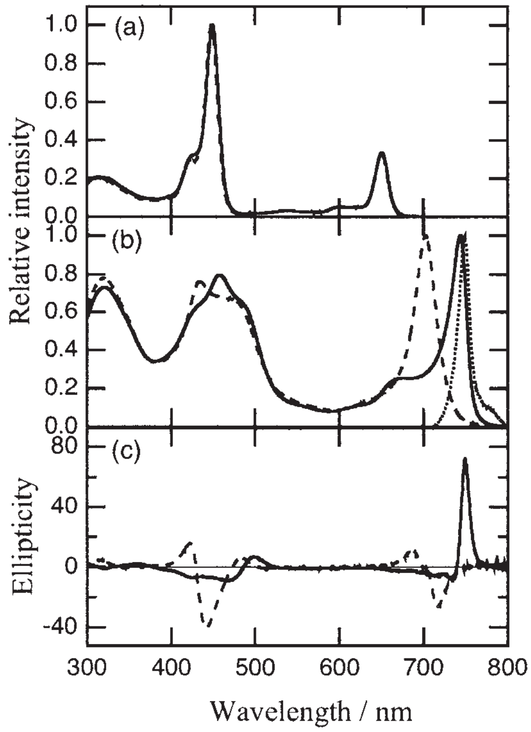


Fig. 2. Absorption spectra of 3^1-epimeric Zn–**3** in (a) THF and (b) 6% (v/v) THF–water, and (c) their CD spectra in 6% (v/v) THF–water. Dashed and solid lines represent the spectra of Zn–**3*R*** and Zn–**3*S***, respectively. The dotted line in Fig. 2b represents fluorescence spectrum of Zn–**3*S*** in 6% THF–water excited at 500 nm. The spectra in Fig. 2b are normalized at their longest wavelength peaks.

Table 1. Absorption Maxima (λ_{max}/nm) of Epimeric Zinc Chlorins Zn–**1**–**3*R*/*S***, Ratio of the Observed Peak Intensities, and Red-Shifts Δ (cm^{-1}) of the Q_y Peaks Caused by Self-Aggregation

	THF			6% (v/v)		
	λ_{max}		Int(Soret)	THF–H_2O		
Compound	Soret	Q_y	/Int(Q_y)	λ_{max}(Q_y)	Δ[a]	
Zn–**1*R*** (R^7 = Me, R^8 = Et)	424	645	0.75	699	1200	this work
Zn–**1*S*** (R^7 = Me, R^8 = Et)	424	645	0.76	699, 655	1200	this work
Zn–**2*R*** (R^7 = CHO, R^8 = Et)	448	628	0.33	671	1020	Ref. 3
Zn–**2*S*** (R^7 = CHO, R^8 = Et)	448	628	0.33	671	1020	Ref. 3
Zn–**3*R*** (R^7 = Me, R^8 = CHO)	448	650	0.34	702	1140	this work
Zn–**3*S*** (R^7 = Me, R^8 = CHO)	448	650	0.33	744, 668(sh)	1940	this work

a) $\Delta = [1/\lambda_{max}(Q_y \text{ peak of monomer}) - 1/\lambda_{max}(\text{oligomer})] \times 10^7$.

The Q_y peak position of the monomeric species was predictable by the molecular modeling study. The longest wavelength absorption maxima were calculated by successive MM+ and ZINDO/S (HyperChem 6.0) for THF-coordinating zinc complexes.[3,10] The estimated Q_y maxima of THF•Zn–**2** and THF•Zn–**3** were 625 nm and 648 nm, respectively, which turned out to be coincident with the observed 628 and 650 nm values. Thus, it is apparent that the position of the formyl group (R^7 or R^8) clearly affected the Q_y peak maxima but not the Soret ones. On the contrary, the ratio of peak intensities Int(Q_y)/Int(Soret) was independent of the position: 0.33 for Zn–**2**/**3**. The value is less than half of the ratio in Zn–**1**. Considering the reported result that substitution of ethyl with other alkyl/alkenyl groups at the 8-position gave almost no changes of their ratios,[4b] we find it noteworthy that oxidation of the 7- or 8-alkyl to the formyl group (Zn–**1** → Zn–**2** or Zn–**3**) greatly suppressed the ratio: 0.75 → 0.33.

Upon dilution of the monomeric THF solution with water, Zn–**3*R*/*S*** self-aggregated to give oligomers possessing red-shifted absorption bands (Figs. 2a/b), similar to the previous reports.[3] It is interesting to note that the spectra of oligomeric Zn–**3*R*/*S*** in 6% (v/v) THF–water are quite different from each other. The red-shift of Q_y peak of Zn–**3*R*** (1140 cm^{-1}) is similar to those of Zn–**1*R*/*S*** (1020 cm^{-1}) and Zn–**2*R*/*S*** (1200 cm^{-1}), while Zn–**3*S*** gave a larger red-shift (1940 cm^{-1}) and moved to 744 nm leaving a shoulder of small aggregate species at 668 nm. This large red-shift is comparable to those of the 3^1-demethylated derivatives of Zn–**1**/**2**, 1650/1730 cm^{-1},[3] respectively. Change of the 3-(1-hydroxyethyl) to 3-hydroxymethyl group induces a less steric hindrance around the hydroxy group at the 3^1-position as one of binding components for self-aggregation by C=O···H–O···Zn, and tight packing of composite chlorin rings in a supramolecule gave larger red-shifts in the 3-CH_2OH than in the 3-CH(OH)Me.[10b] Therefore, Zn–**3*S*** would self-aggregate in 6% THF–water more tightly than Zn–**3*R***. The strong positive CD peak was observed at the region of Q_y band in self-aggregates of Zn–**3*S*** (solid line of Fig. 2b), suggesting also the formation of ordered large aggregates of Zn–**3*S***.

In a non-polar organic medium (1% (v/v) CH_2Cl_2–cyclohexane), Zn–**3*R*/*S*** gave the same Q_y peaks at 707 nm, and no diastereomeric control on their self-aggregation was observed. The large difference between self-aggregates of Zn–**3*R*/*S*** in 6% THF–water is ascribable to their environmental conditions. Water and/or THF molecules would associate self-aggregates of Zn–**3*S***[11] to form such large aggregates in the aqueous organic solvent.

When excited in the Soret region, the self-aggregate of Zn–**3*S*** in 6% THF–water showed an intense fluorescence emission at a peak maximum of 749 nm, as indicated by the dotted line of Fig. 2b. Although BChl-*e* mainly absorbs longer wavelength light around 500 nm than BChls-*c*/*d* does, its emission peaks are located at a shorter wavelength region (~730 nm) due to its blue-shifted Q_y peaks.[8c,12] Therefore, the self-aggregate of Zn–**3*S*** in the aqueous medium is quite interesting in view of both its longer wavelength Soret absorption (up to ca. 530 nm) and emission bands (~750 nm), compared with those of BChls-*c*/*d*/*e*. Since the self-aggregate can effectively absorb the most intense region of the solar spectrum and the excited singlet energy can closely fit that of energy-acceptor in natural chlorosomes, baseplate (795-nm maximum),[12] Zn–**3*S*** self-aggregates are promising for construction of an efficient artificial antenna system.

Experimental

General. ^{1}H NMR spectra in $CDCl_3$ were recorded on a Bruker AC-300 spectrometer. All chemical shifts are reported relative to the residual non-deuterated solvent peak: $\delta = 7.26$ ($CHCl_3$). Visible absorption and CD spectra were measured on a Hitachi U-3500 spectrophotometer and a JASCO J-720W spectropolarimeter, respectively. FAB-MS spectra were recorded on a JEOL HX-100 spectrophotometer. HPLC was carried out with a packed ODS column (Cosmosil 5C18-ARII, 10 mmϕ × 250 mm). Methyl 8-vinyl-12-methyl-bacteriopheophorbide-*d* (**4**) was prepared according to the reported procedure.[4b] Zinc-metallation of free base chlorin was done similar to the reported procedure.[5b,6,7] 3^1-Epimerically pure zinc complexes Zn–**1*R*/*S***[5] were prepared as previously reported. Other reagents were employed as purchased without further purification. All synthetic procedures were done in the dark.

Methyl 8-Formyl-12-methyl-bacteriopheophorbide-*d* (3). To a solution of **4** (23 mg, 0.041 mmol; $3^1R/S = 1/1$) in 5 mL of THF was added OsO_4 (20 mg, 0.079 mmol), and the mixture was stirred for 5 min at 0 °C. Then a solution of $NaIO_4$ (47 mg, 0.23 mmol) in 0.1 mL of AcOH and 1 mL of water was added, and the reaction mixture was stirred for 12 h at room temperature. The mixture was diluted with water and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (Et_2O–CH_2Cl_2, 1:4) to give 8-formylchlorin **3** (14 mg, 61%; $3^1R/S = 1/1$) as a black solid; Vis (CH_2Cl_2) λ_{max} 660 (relative intensity, 23%), 607 (5), 572 (5), 521 (8), 435 (100) nm; ^1H NMR ($CDCl_3$) δ 10.80/10.78 (1H, s, CHO), 9.75/9.72, 9.64/9.63, 8.57/8.55 (each 1H, s, 5-, 10-, 20-H), 6.39 (1H, m, 3-CH), 5.12, 4.97 (each 1H, d, $J = 20$ Hz, 13^1-CH_2), 4.46–4.49 (1H, m, 18-H), 4.17–4.26 (1H, m, 17-H), 3.69/3.65, 3.44, 3.37, 3.34 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.55–2.69, 2.17–2.35 (each 2H, m, 17-CH_2CH_2), 2.12 (3H, d, $J = 7$ Hz, 3^1-CH_3), 1.94 (3H, d, $J = 7$ Hz, 18-CH_3), −0.94, −2.48 (each 1H, s, NH); MS (FAB) m/z 566 (M^+).

Zinc Methyl 8-Formyl-12-methyl-bacteriopheophorbide-*d* (Zn–3*R*/*S*). 8-Formylchlorin **3** was zinc-metallated by refluxing a CH_2Cl_2 solution of **3** with excess $Zn(OAc)_2 \cdot 2H_2O$ dissolved in MeOH, and the crude product was purified by chromatography on silica gel (MeOH–CH_2Cl_2, 3:97) followed by HPLC (MeOH; 2.0 mL/min) to give zinc chlorins Zn-**3** as an epimeric mixture ($t_R = 7$ min). Each 3^1-epimer was further separated by HPLC (MeOH–H_2O, 85:15; 2.0 mL/min). Zn–**3*R***: $t_R = 19$ min; mp > 300 °C; Vis (THF) λ_{max} 650 (rel. 33%), 448 (100), 423 (29) nm; ^1H NMR (1% pyridine-d_5–$CDCl_3$) δ 11.26 (1H, s, CHO), 10.38, 9.71, 8.30 (each 1H, s, 5-, 10-, 20-H), 6.36 (1H, q, $J = 7$ Hz, 3-CH), 5.12, 5.02 (each 1H, d, $J = 20$ Hz, 13^1-CH_2), 4.37 (1H, m, 18-H), 4.15 (1H, m, 17-H), 3.67, 3.62, 3.56, 3.31 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.53, 2.3 (each 2H, m, 17-CH_2CH_2), 2.12 (3H, d, $J = 7$ Hz, 3^1-CH_3), 1.72 (3H, d, $J = 7$ Hz, 18-CH_3); HRMS (FAB) found m/z 628.1691, calcd for M^+, $C_{33}H_{32}N_4O_5Zn$ 628.1664. Zn–**3*S***: $t_R = 21$ min; mp > 300 °C; Vis (THF) λ_{max} 650 (rel. 34%), 447 (100), 421 (34) nm; ^1H NMR (1% pyridine-d_5–$CDCl_3$) δ 11.26 (1H, s, CHO), 10.38, 9.77, 8.30 (each 1H, s, 5-, 10-, 20-H), 6.33 (1H, q, $J = 7$ Hz, 3-CH), 5.12, 4.99 (each 1H, d, $J = 20$ Hz, 13^1-CH_2), 4.37 (1H, m, 18-H), 4.16 (1H, m, 17-H), 3.67, 3.63, 3.57, 3.29 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.55, 2.3 (each 2H, m, 17-CH_2CH_2), 2.12 (3H, d, $J = 7$ Hz, 3^1-CH_3), 1.70 (3H, d, $J = 7$ Hz, 18-CH_3); HRMS (FAB) found m/z 628.1667, calcd for M^+, $C_{33}H_{32}N_4O_5Zn$ 628.1664.

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