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Self-aggregation of synthetic chlorophyll-*c* derivative and effect of C17-acrylate residue on bridging green gap in chlorosomal model

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ABSTRACT

A zinc bacteriochlorophyll-*d* analog possessing a fully π-conjugated porphyrin skeleton and an acrylate residue at the 17-position was prepared by chemical modification of an accessory photosynthetic pigment, chlorophyll-c_1 from a diatom *Chaetoceros* species. The synthetic zinc chlorophyll-c_1 derivative self-aggregated in a less polar organic solvent and in a solid film to give a red-shifted and broadened visible absorption spectrum, which is similar to that of the main light-harvesting antenna of a green photosynthetic bacterium, where magnesium chlorins with the 17-propionate residue including bacteriochlorophyll-*d* aggregate in a *J*-type fashion to form large oligomers. The resulting self-aggregates showed specific and intense absorption bands at around 500 and 600 nm due to the π-extension along the molecular x-axis, which could bridge the "green gap" observed in the above natural antenna system. Visible and infrared absorption spectral analyses revealed that the supramolecular structure of self-aggregates of the zinc chlorophyll-c_1 derivative are comparable to those of the natural antenna, in spite of double dehydrogenation at the 17,18- and 17^1,17^2-positions in the molecule.

1. Introduction

Green photosynthetic bacteria possess unique light-harvesting antennae called chlorosomes [1–6]. These apparatuses have attracted a lot of attention from various fields, such as biological and material sciences as well as supramolecular chemistry using natural or synthetic chlorophylls (Chls) and porphyrins [7–9]. Chlorosomes are constructed by supramolecular self-aggregates of specific chlorophyll molecules without supports of proteins [4–6,10–12]; their modelization and application are easier than those of any other antenna systems prepared through interaction of pigments with peptides. In addition, highly efficient absorption of sunlight and ultrafast long-range excited energy migration are possible in chlorosomes [13–16]. Most green bacteria biosynthesize bacteriochlorophyll(BChl)-*c* or *d* (Fig. 1 right), and their chlorosomes mainly absorb light at 700–800 nm in red to infrared and <500 nm in ultraviolet to visible regions [1–4,10,12,17]. The former and latter absorption bands are ascribable to Qy and Soret bands, respectively, of the composite chlorophyll pigments. Such natural chlorosomal bands were reconstructed by *in vitro* self-aggregation of the natural bacteriochlorophylls and their synthetic models [1,2,18–20]. These absorption regions are partly overlapped with solar radiation reaching at the surface of the earth, but the above chlorosomes less absorb visible lights of around 500–700 nm (green to orange/red) where the photon flux density of sunlight is high.

Oxygenic phototrophs produce Chl-*a* (Fig. 1 left) as a photosynthetically active pigment, which gives weak absorbance in the 500–600 nm (green to orange) region. To absorb sunlight in the region efficiently, Chl-*a* producing organisms utilize accessory pigments [21–23]: this issue is called green gap. The accessory pigments include bilins and carotenoids as well as other chlorophylls, *e.g.* Chl-*b* (Fig. 1 left) and Chl-*c* (Fig. 1 middle). The accessory chlorophyll molecules can absorb a green light more than Chl-*a*, due to extension of their π-conjugated systems along the molecular x-axis [24–27].

The 7-methyl group of BChl-*c* is substituted with the 7-formyl group to afford BChl-*e* (Fig. 1 right) for overcoming the "green gap" in chlorosomes; this is similar as the situation that Chl-*b* is utilized as the accessory pigment for Chl-*a* in oxygenic phototrophs [10,12,28,29]. On the other hand, Chl-*c* type pigments are not found in natural chlorosomes. Therefore, we propose that synthetic Chl-*c*

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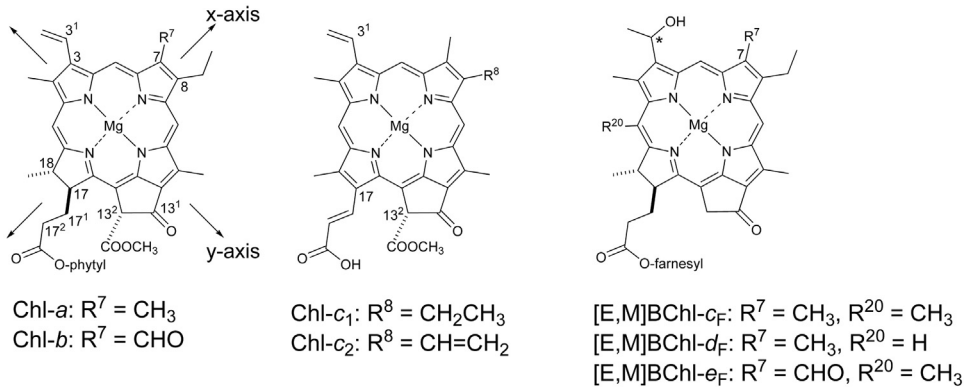

Fig. 1. Molecular structures of naturally occurring chlorophyll in most oxygenic phototrophs (left and middle) and chlorosomal chlorophylls in anoxygenic green bacteria (right).

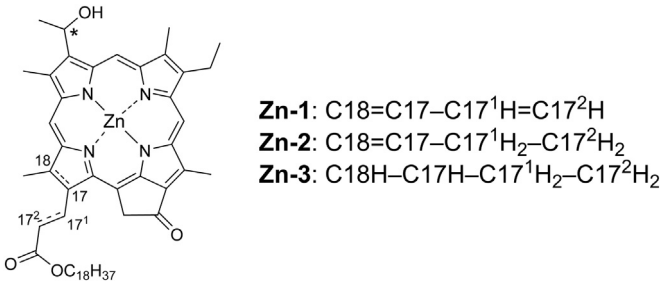

Fig. 2. Synthetic chlorosomal chlorophyll derivatives.

derivatives should be useful for creation of a chlorosomal model bridging the "green gap".

Here, we synthesized a chlorophyll-c_1 derivative possessing the 3^1-hydroxy group and lacking the 13^2-methoxycarbonyl group **Zn-1** (Fig. 2) as a novel model of the chlorosomal BChl-*d* molecule (Fig. 1 right) [30–33] and investigation of its self-aggregation by various spectroscopic analyses. Chl-*c* molecules have a porphyrin π-skeleton and an acrylate residue at the 17-position with more planar and less flexible conformation. Since almost all Chls-*c* are free carboxylic acids and lack a long hydrocarbon chain in the acrylate residue, their solubilities are low in ordinary organic solvents [34,35]. The less solubility is one of the reasons why Chl-*c* has been hardly used for photosynthesis researches. Thus, we esterified the 17-acrylate residue with a long alkyl (stearyl) chain to increase the solubility of the model compound. Additionally, to clarify the effect of the planarity, flexibility, and π-conjugation around the 17-position on the chlorosomal self-aggregation, zinc porphyrin/chlorin analogs possessing the 17-propionate residue **Zn-2/3** (Fig. 2) were prepared, and their self-aggregation was compared with that of **Zn-1**.

2. Experimental

2.1. General

Visible absorption spectra and circular dichroism (CD) spectra in a solution or solid film were measured by a Hitachi U3500 spectrophotometer and a Jasco J-720W spectropolarimeter, respectively, with a 10-mm quartz cell or on a quartz plate. FT-IR spectra were recorded on a Shimadzu IRAffinity-1 spectrometer with a Shimadzu AIM-8000R microscope for measurements of solid films on an aluminum coated glass (reflection mode). ^{1}H NMR spectra in chloroform-*d* or tetrahydrofuran(THF)-d_8 were recorded by a JEOL ECA-600 (600 MHz) spectrometer; tetramethylsilane (δ = 0.00 ppm) was used as an internal standard. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II spectrometer: atmospheric pressure chemical ionization (APCI) and positive mode in an acetonitrile solution.

All the reactions were performed in the dark under nitrogen or argon gas and monitored by thin layer chromatography (TLC) and visible absorption spectroscopy. TLC, preparative layer chromatography (PLC) or flash column chromatography (FCC) was performed with silica gel (Kieselgel 60 F_{254} or Kieselgel 60, 40–63 μm, 230–400 mesh; Merck).

Synthetic details of zinc porphyrins/chlorins possessing the 17-propionate residues **Zn-2/3** and **Zn-2V/3V** and ^{1}H NMR spectra of synthetic compounds **Zn-1/2/3** and **Zn-1V/2V/3V** (Figs. S1–S6) are shown in supplementary data. Before the optical measurements were made, chlorophyll derivatives were purified by reverse phase (RP) HPLC: a Shimadzu LC-10AD_{VP} pump, SPD-M10A_{VP} diode-array detector, SCL-10A_{VP} system controller and a packed ODS column (Inertsil ODS-P, 10 ϕ x 250 mm; GL Sciences) with an aqueous methanol eluent.

2.2. Synthesis of chlorophyll-c derivatives

2.2.1. Synthesis of stearyl pheophorbide-c_1/c_2 (**5**/**5′**)

Chls-c_1/c_2 were extracted from commercially available diatoms of *Chaetoceros gracilis* (5×10^{11} cells) and purified by FCC (3% MeOH $-CH_2Cl_2$), then dissolved in dry *N*,*N*-dimethylformamide (DMF, 200 mL), to which were added cesium fluoride (1.1 g, 7.1 mmol) and stearyl iodide (1.6 g, 4.1 mmol) [35]. After stirred for 1 day at room temperature, the reaction mixture was treated with an aqueous 4% HCl solution [34] to give demetalated pheophytins. The reaction mixture was extracted with chloroform, washed with an aqueous 4% $NaHCO_3$ solution and water, and dried over Na_2SO_4. After evaporated *in vacuo*, the resulting residue was purified by FCC (CH_2Cl_2) to give a mixture of **5**/**5′** (=2/5): VIS (CH_2Cl_2) λ_{max} = 650 (relative intensity, 0.01), 595 (0.07), 575 (0.08), 533 (0.07), 434 nm (1.00).

5: ^{1}H NMR ($CDCl_3$) δ = 9.58, 9.54, 9.03 (each 1H, s, 5-, 10-, 20-H), 8.41 (1H, d, *J* = 16 Hz, 17-CH), 8.00 (1H, dd, *J* = 11, 18 Hz, 3-CH), 6.52 (1H, d, *J* = 16 Hz, 17^1-CH), 6.24 (1H, d, *J* = 18 Hz, 3^1-CH *trans* to 3-C–H), 6.17 (1H, d, *J* = 11 Hz, 3^1-CH *cis* to 3-C–H), 6.12 (1H, s, 13^1-CH), 4.51 (2H, t, *J* = 7 Hz, 17^2-CO_2CH_2), 3.91 (2H, q, *J* = 8 Hz, 8-CH_2), 3.74, 3.71, 3.66, 3.47, 3.34 (each 3H, s, 2-, 7-, 12-, 18-CH_3, 13^2-CO_2CH_3), 1.98 (2H, quintet, *J* = 7 Hz, 17^2-CO_2CCH_2), 1.81 (3H, t, *J* = 8 Hz, 8^1-CH_3), 1.68–1.20 (30H, m, 17^2-$CO_2C_2(CH_2)_{15}$), 0.88 (3H, t, *J* = 7 Hz, 17^2-$CO_2C_{17}CH_3$), −4.31, −5.52 (each 1H, s, NH × 2); HRMS (APCI) found: *m*/*z* = 841.5264, calcd for $C_{53}H_{69}N_4O_5$: MH^+, 841.5262.