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Changes of Aqueous Self-assemblies of Zinc Chlorophyll Derivatives Possessing a Hydrophilic Chain by Treatment with Organic Solvents

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Visible absorption and circular dichroism spectra of zinc chlorophyll derivatives possessing an oligo(oxyethylene) moiety at the 17-propionate in aqueous media were drastically changed by treatment with dichloromethane or chloroform, indicating that dimeric and monomeric species were converted into chlorosome-like large self-aggregates.

Structural changes of supramolecules by external stimuli have attracted much attention for the construction of smart nanomaterials and molecular machines.[1] In biological events, supramolecular structural changes including self-assembly and disassembly of biological molecules are important to sustain biological activities in living organisms.[2] Model systems mimicking structural changes of biological supramolecules are useful to elucidate such dynamic biological processes.

Photosynthetic light-harvesting complexes are exquisite biological supramolecules. In these complexes, chlorophyll (Chl) and bacteriochlorophyll (BChl) molecules are highly organized to capture sunlight energy and to transfer it to reaction centers efficiently. Formation and degradation dynamics of photosynthetic light-harvesting complexes still remains unclear, although X-ray crystallographic analyses clearly reveal some of their static three-dimensional structures.[3]

Green photosynthetic bacteria have unique light-harvesting complexes called chlorosomes. In chlorosomes, BChls *c*, *d*, and *e* (Figure 1A) self-aggregate without the help of proteins in hydrophobic environments surrounded by lipids.[4] Static chlorosomal self-aggregates by natural BChls and synthetic Chl derivatives have been extensively studied.[5,6] There are few reports, however, on the dynamic behavior of chlorosomal self-aggregates.[7–11] We report unique spectral changes of self-aggregates of amphiphilic zinc Chl derivatives **1**–**3** possessing a hydrophilic chain, poly(ethylene glycol) (PEG), at the 17-propionate (Figure 1B).

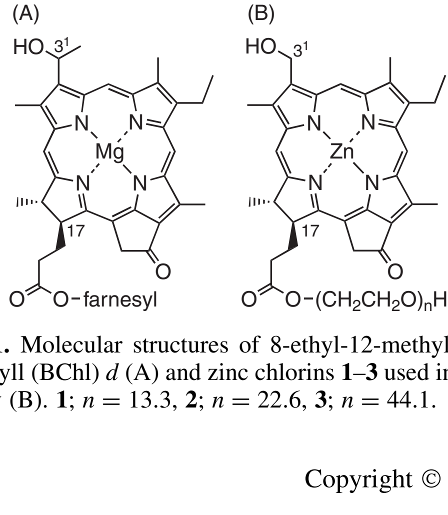

Figure 1. Molecular structures of 8-ethyl-12-methyl-bacteriochlorophyll (BChl) *d* (A) and zinc chlorins **1**–**3** used in the present study (B). **1**; $n = 13.3$, **2**; $n = 22.6$, **3**; $n = 44.1$.

Zinc chlorins **1**–**3**[12] were dissolved in methanol, followed by dispersion into 99-fold volume of distilled water (final concentration of zinc chlorins: ca. 10 μM). The aqueous solution was rigorously mixed with the same volume of organic solvents by a vortex mixer for 10 s, followed by standing in the dark. Visible absorption and circular dichroism (CD) spectra of zinc chlorins in the aqueous phase were measured before and after mixing with organic solvents with a Shimadzu UV-2450 spectrophotometer and a JASCO J-720 spectropolarimeter, respectively.

Spectral changes of zinc chlorin **1** in an aqueous 1% methanol solution by treatment with dichloromethane are depicted at the left in Figure 2. Before treatment, zinc chlorin **1** exhibited a Q_y peak at 675 nm. This would be due to their being dimeric species of zinc chlorins, in which the 3^1-hydroxy group of the two zinc chlorins is coordinated to the central zinc of the other.[12] After mixing the aqueous solution containing **1** with dichloromethane, the Q_y absorption band was shifted to 720 nm. At the same time, large reverse S-shaped CD signals appeared in this region. These indicate that the dimeric species of **1** in the aqueous phase was converted into chlorosome-like large self-aggregates[12,13] by treatment with dichloromethane. Zinc chlorin **2** exhibited the Q_y absorption band at 656 nm with a shoulder around

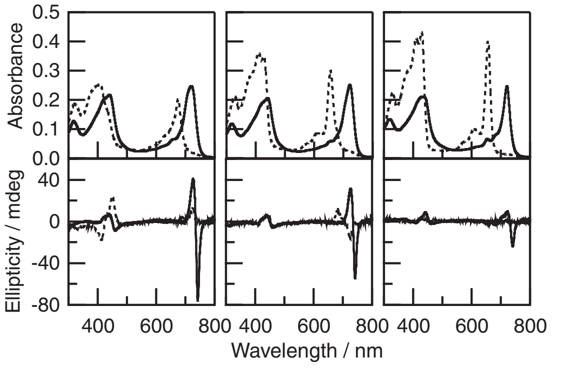

Figure 2. Visible absorption (top) and CD spectra (bottom) of **1** (left), **2** (center), and **3** (right) in 1% methanol/water before (broken curve) and after treatment with dichloromethane (solid curve). The concentration of zinc chlorins **1**–**3** was ca. 10 μM.

675 nm in aqueous media (see center of Figure 2). This spectral feature was ascribable to coexistence of monomeric and dimeric species. The Q_y band was also red-shifted to 720 nm after treatment of the aqueous solution of **2** with dichloromethane.

Zinc chlorin **3**, which possessed a longer PEG chain and was more hydrophilic than **1** and **2**, exhibited the Soret and Q_y absorption bands at 429 and 656 nm, respectively, in an aqueous solution (see right of Figure 2). This spectrum is characteristic of monomers. The monomeric zinc chlorin **3** was also converted to large self-aggregates with the Q_y peak position at 720 nm after mixing the aqueous phase with dichloromethane. Reverse S-shaped CD signals due to self-aggregates of **3** appeared. The CD intensities tended to decrease with increase of the length of PEG chains, implying that the organization of self-aggregates of zinc chlorins possessing a longer PEG chain might be somewhat moderate. When aqueous solutions containing **1**–**3** were treated with chloroform, essentially the same spectral changes could be observed in the aqueous phase (Figure S1).[14]

Diethyl ether induced different spectral changes of zinc chlorins **1**–**3** in aqueous media (Figure S2).[14] The 675-nm Q_y absorption band of zinc chlorin **1** was blue-shifted to 656 nm. In the case of **2**, the shoulder around 675 nm disappeared by treatment with diethyl ether. These revealed the conversion from dimeric species of **1** and **2** into monomeric forms by treatment with this solvent. The Q_y peak of zinc chlorin **3** was positioned at 656 nm both before and after treatment with diethyl ether, to give its monomeric species predominantly. It is noted that small amounts of **1**–**3** were extracted by dichloromethane, chloroform, and diethyl ether.

In contrast, nonpolar organic solvents such as hexane, cyclohexane, and tetrachloromethane hardly changed the Q_y peak position of **1**–**3** (Figures S3–S5).[14] CD spectroscopy also indicated little formation of large self-aggregates. These indicate that the three nonpolar organic solvents hardly changed the aqueous self-assemblies of zinc chlorins **1**–**3**.

Treatments of an aqueous methanol solution with organic solvents would decrease the methanol contents in the aqueous phase, which might affect the above spectral changes. Dialysis of aqueous solutions of zinc chlorins **1**–**3** overnight, however, gave no absorption band and CD signal around 720 nm ascribable to large self-aggregates. These indicate that the concentration change of methanol in aqueous solutions did not cause the present spectral changes.

Proposed supramolecular structural changes of amphiphilic zinc chlorins, which resulted in drastic spectral changes in this study, are illustrated in Figure 3. Small amounts of dichloromethane or chloroform could be dissolved in aqueous solutions[15] and their hydrophobic microdomains would be formed, where amphiphilic zinc chlorin molecules can assemble as chlorosome-like large self-aggregates. Since the solubilities of hexane, cyclohexane, and tetrachloromethane are much lower than those of dichloromethane and chloroform,[15] treatments with these nonpolar organic solvents would have little effect on assembly of amphiphilic zinc chlorins. In contrast, zinc chlorins would be deaggregated when diethyl ether, which is able to form a coordination bond with the central zinc and a hydrogen bond with the 3^1-hydroxy group, was dissolved in aqueous solutions. The present manipulation would be useful to construct model systems of formation and degradation of chlorosomal self-aggregates in green photosynthetic bacteria.

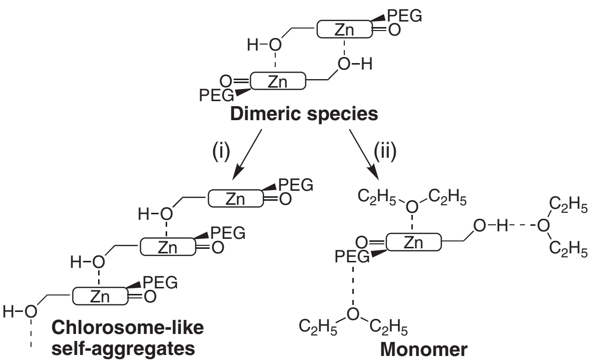

Figure 3. Proposed structural changes of self-aggregates of amphiphilic zinc chlorin **1** by treatments with dichloromethane or chloroform (i) and diethyl ether (ii).

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14 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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