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Self-aggregates of a Synthetic Cadmium Chlorin in Solid Film as a Photosynthetic Antenna Model

Masaaki Amakawa and Hitoshi Tamiaki*

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

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Abstract—Self-aggregates of a synthetic cadmium chlorin possessing 3^1-hydroxyl and 13-carbonyl groups were prepared in dried thin film. The solid film was characterized by visible and infrared absorption spectroscopies at both transmission and reflection modes. The spectra given by the two different modes were essentially the same and resembled those in the extramembranous antennas of green photosynthetic bacteria. The circular dichroism and resonance Raman spectra also supported the similarity between the artificial self-aggregates and the natural systems. Electron diffraction of the aggregated film by transmission electron microscopy indicated the presence of an orderly structure with a 6.4-Å interval, which was estimated for the close Cd–Cd distance of the stacking in the self-aggregates. The in vitro self-assembly in the solid state is a good structural model for the in vivo antenna. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Green photosynthetic bacteria have extramembranous light-harvesting antennas which are called chlorosomes. The main parts of chlorosomes are self-aggregates of bacteriochlorophyll(=BChl)s-*c, d, e* (Fig. 1) and have no support of any proteins.[1] Numerous attempts have been made to determine their supramolecular structures, but the precise structures are still in debate.[1–4] Several synthetic models for naturally occurring BChls-*c, d* are available[4–8] and the in vitro self-assemblies showed the molecular requisites for self-aggregation: OH, keto-C=O groups, and metallated cyclic tetrapyrrole.[5–7] Magnesium and zinc complexes of synthetic chlorins possessing 3^1-OH and 13-C=O groups have been reported as good models (for example, **2** and **3** in Fig. 1).[5–10] Here, we report on the first synthesis of a cadmium complex of chlorin **4** and the preparation of stable self-aggregates in a solid film state[11] as a model for chlorosomal self-assembly.[12]

Results and Discussion

Methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (**1**)[5] was metallated by $Cd(OAc)_2{\cdot}2H_2O$ to give the corresponding cadmium complex (**4**). The simple and mild procedures of 2-h refluxing of the MeOH–CH_2Cl_2 (1:1) solution and recrystallization afforded the pure sample in a moderate yield (59%). The MeOH-CH_2Cl_2 (1:3) solution of **4** was dropped into excess cyclohexane, then the precipitate of **4** was separated from the colloidal solution, spontaneously or by centrifugation. The solid thin film formed was dried on several plates which were used for the following spectroscopic analyses. The dark green aggregate solid was stable and unchanged after prolonged storage even at room temperature, whereas the solution containing monomeric **4** changed rapidly to give metal free **1** and undetermined decomposed products.

The visible spectra (transmission mode) of **4** are shown in Figure 2(A). The solid line is the spectrum of the solid thin film of **4** on a quartz plate, and the broken line is that of MeOH-CH_2Cl_2 (1:3) solution of **4** in a quartz cuvette. In the polar organic solvents, **4** gave sharp Q_y (657 nm) and Soret bands (431 nm) which were characteristic of the monomer of chlorin compounds. In the solid film, the two peaks moved to 744 and 462 nm and the bands broadened. Similar behavior was observed in self-aggregates of the corresponding zinc complex **3**.[5] The Q_y band in the solid film of **4** (half-height bandwidth $(\Delta) = 1100\,cm^{-1}$) was broader than those of the in vivo chlorosomes and in vitro self-aggregated **2** and **3** in a solution ($\Delta = 600-700\,cm^{-1}$).[5] The 1.7-fold broadening was mainly ascribable to heterogeneity in the solid film prepared by spontaneous self-aggregation, because similar broadening was observed in a solid film of BChl-*c* aggregates ($\Delta \approx 1300\,cm^{-1}$).[11] The solid film **4** showed larger ellipticity than the solution (Fig. 2(B)) and especially the solid film gave large circular dichroism peaks in the red-shifted absorption regions. These results

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Key words: Natural products; mimetics; pigments; porphyrins and analogues.

*Corresponding author. Tel.: +81-77-561-2765; fax: +81-77-561-2659; e-mail: tamiaki@se.ritsumei.ac.jp

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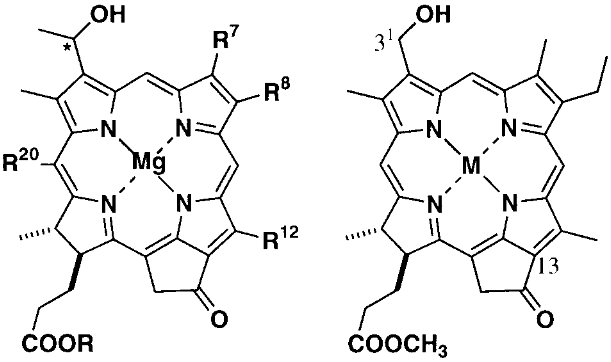
indicated that cadmium complex **4** self-aggregated in the solid state and exciton-coupling occurred among the molecules in the self-assembly. Furthermore, the reflection mode visible spectrum of the solid film ($\lambda_{max} \approx 740$ and 460 nm) was almost the same as the corresponding spectrum at transmission mode. The visible spectrum of cadmium chlorin **4** in the solid state is similar to those of in vivo self-aggregates of magnesium chlorins in natural chlorosomes, e.g. chlorosomal BChl-*c* aggregates of *Chlorobium tepidum* ($\lambda_{max} \approx 745$ and 460 nm).

The infrared spectrum (reflection mode) of **4** in the solid thin film on an aluminum coated glass plate is shown by the solid line of Figure 3. In a transmission mode, the same spectrum was observed in the solid thin film prepared on a KBr plate ($\nu = 3140$, 1736, 1647, 1589, 1532 and 1472 cm^{-1}). The peak around 3140 cm^{-1} was assigned to 3^1-OH stretching mode (Fig. 3(A)). When the solid film was exposed to pyridine vapor, the self-aggregate was dissolved by coordination of pyridine to the central cadmium and changed to the deaggregation forms[13] to give a $+250\,cm^{-1}$ shifted O–H peak (see the broken line of Fig. 3(A)). Such a shift indicates that the OH group is bonded with some other groups in the self-aggregate. The other region of the infrared spectra (Fig. 3(B)) showed that the ester C=O stretching at 1734 cm^{-1} and the C–C and C–N stretchings of the chlorin skeleton (1500~1600 cm^{-1}) were little changed by deaggregation and that the 13-keto-carbonyl peak moved from 1647 cm^{-1} to 1684 cm^{-1}. From these results, 3^1-OH was hydrogen-bonded with 13-C=O in the self-aggregates. Furthermore, the peak values of the O–H ($\approx 3140\,cm^{-1}$) and the C=O (1647 cm^{-1}) stretchings in the solid film are lower than those of normal hydrogen bonding. Compared with previous assignments,[5] the strong hydrogen-bonding is ascribed to special C=O···H–O···Cd bonding in the solid film.[14] The same intermolecular bonding was observed in natural chlorosomes.[1] Consequently, the in vitro self-aggregate of cadmium chlorin **4** in solid film is a good structural model for in vivo self-aggregates of BChls-*c,d*.



$R^7 = R^{20} = CH_3$: BChl-*c*
$R^7 = CH_3$, $R^{20} = H$: BChl-*d*
$R^7 = CHO$, $R^{20} = CH_3$: BChl-*e*
(R^8, R^{12} = alkyl groups,
R = long chains)

$M = H_2$: **1**
M = Mg : **2**
M = Zn : **3**
M = Cd : **4**

Figure 1. Chlorosomal bacteriochlorophylls (left) and synthetic chlorins (right).

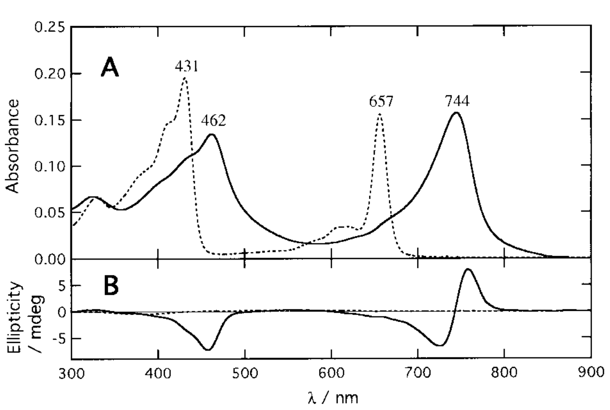


Figure 2. (A) Visible and (B) circular dichroism spectra (transmission mode) of cadmium chlorin **4** in the solid thin film (——) and in MeOH–CH_2Cl_2 (1:3) (- - - -).

The resonance Raman (RR) spectrum of cadmium chlorin **4** in solid film was measured upon excitation with a 457.9 nm line of an Ar^+ laser (Fig. 4). The peak at 1637 cm^{-1} was assigned to 13-C=O stretching mode, which was 10 cm^{-1} down-shifted to the corresponding IR peak described above. Similar shifts have been observed in self-aggregates of magnesium chlorin **2** ($1642\,cm^{-1} - 1650\,cm^{-1} = -8\,cm^{-1}$) and zinc chlorin **3** ($1649\,cm^{-1} - 1655\,cm^{-1} = -6\,cm^{-1}$).[5,9] The frequency was quite lower than those of monomeric chlorophylls (around 1700 cm^{-1}),[15] indicating the presence of the special C=O···H–O···Cd bonding in the solid film (vide

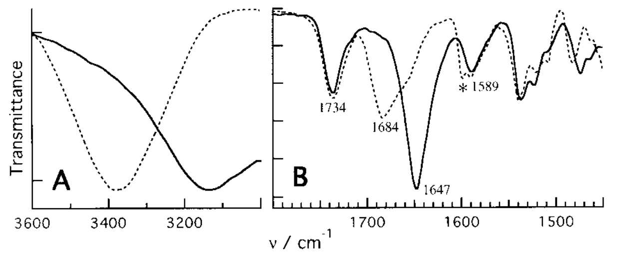


Figure 3. Infrared spectra (reflection mode) of cadmium chlorin **4** in the solid thin film (——) and after treatment of pyridine vapor (- - - -). *Due to pyridine.

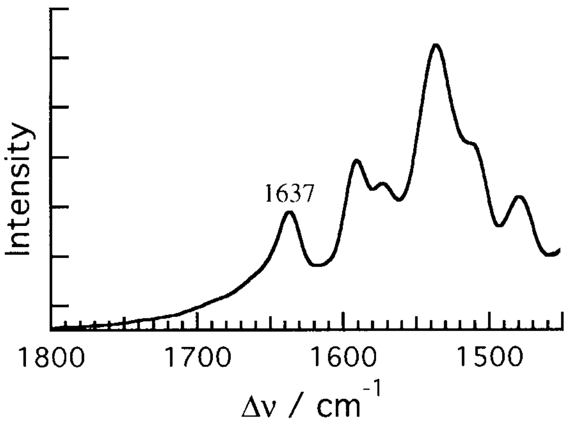


Figure 4. Resonance Raman spectra (excited at 457.9 nm) of cadmium chlorin **4** in the solid thin film.

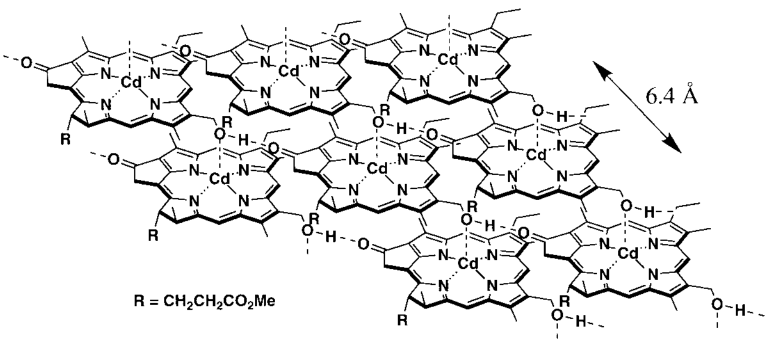


Figure 5. Proposed supramolecular structure of self-aggregated **4**.

supra). The RR peak positions at 1450∼1600 cm^{-1} were similar to the corresponding IR ones, while the intensities were different. The RR spectra also suggest the similarity of supramolecular structures for self-aggregates of synthetic **2–4** and natural BChls-*c, d.*

The electron diffraction of the solid thin film by transmission electron microscopy showed a clear ring pattern, indicating the presence of order in the self-aggregates. Analysis of the pattern gave an orderly structure with a 6.4-Å interval. This value is close to the Mg–Mg distance (6.8 ± 0.2 Å) in the stacking of methyl bacteriochlorophyllide-*d* estimated from molecular modeling.[2] Although the ion radius of Cd^{2+} is slightly larger than that of Mg^{2+}, the supramolecular structure of self-aggregates of cadmium complex **1** is almost the same as the naturally occurring self-assemblies of BChls-*c,d* (Fig. 5). Based on the proposed supramolecular structure, molecular modeling (MM+/PM3)[10] gave the close Cd–Cd distance of $6.8_5 \pm 0.1_5$ Å, which corresponded to the experimental value mentioned above.

Upon Soret-band excitation of the monomeric state in MeOH–CH$_2$Cl$_2$ (1:3), cadmium chlorin **4** gave a fivefold lower fluorescence emission (the solid line of Fig. 6(A)) than zinc chlorin **3** (the broken line of Fig. 6(A)), which is consistent with the reported data of the corresponding chlorophyll-*a* analogues.[16] On the other hand, the self-aggregates of **4** in non-polar organic solvents, MeOH–CH$_2$Cl$_2$-cyclohexane (1:3:396) at a low concentration ($\mathrm{Abs}_{751} \approx 0.13$)[17] were as fluorescent as those of zinc complex **3** (Fig. 6(B)). The self-aggregates of cadmium chlorin **4** are expected to function as efficient light-harvesting and energy-transfer media like the aggregates of **3**.[18]

In conclusion, the cadmium complex **4** was prepared by a simple procedure and easily self-aggregated to form stable oligomers which are slightly soluble in non-polar organic solvents. The self-aggregates in the solid thin film could serve for measurements of circular dichroism, resonance Raman spectra and X-ray scattering as well as visible and infrared spectra at both transmission and reflection modes. The results of these measurements indicate that the synthetic cadmium chlorin **4** self-aggregates to give a solid thin film as a good model for extramembranous light-harvesting antennas composed of BChls-*c,d,e* in the green photosynthetic bacteria.

Experimental

The transmission visible and circular dichroism spectra of aggregated film on a quartz plate were measured with a Hitachi U-3500 spectrophotometer and a Jasco J-720W spectropolarimeter, respectively The reflection visible spectra on an aluminum coated glass plate were recorded using a 60Φ integrating sphere attachment. FT-IR absorption spectra were measured with a Shimadzu FTIR-8600 spectrophotometer and an AIM-8000R microscope; the aggregated film on a KBr plate and on an aluminum coated glass plate were used for transmission and reflection modes, respectively. Resonance Raman spectra were measured with a Jasco NR-1800 laser Raman spectrophotometer. The aggregated film on a thin carbon film was used for morphological observation and electron diffraction experiments with a JEOL JEM-100C transmission electron micro-

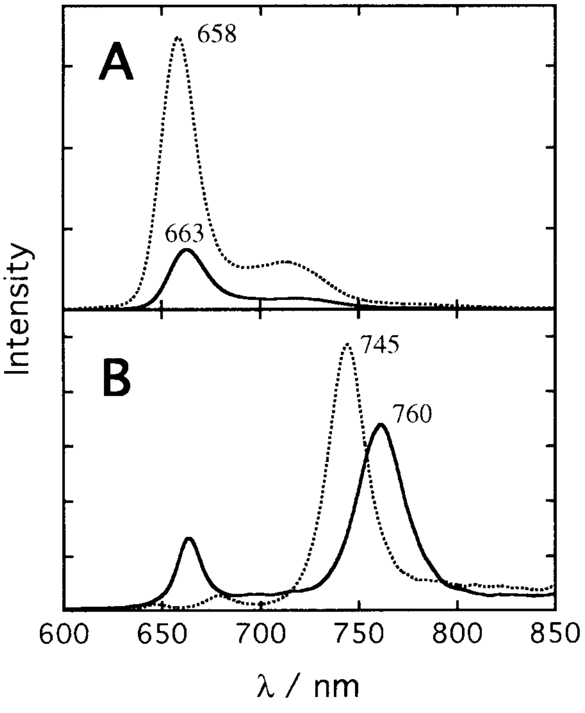


Figure 6. Fluorescence spectra (excited at the Soret peak) of zinc chlorin **3** (----, $\lambda_{\mathrm{Soret}} = 426$ (monomer) and 451 nm (aggregates)) and cadmium chlorin **4** (——, $\lambda_{\mathrm{Soret}} = 431$ (monomer) and 461 nm (aggregates)) in (A) MeOH–CH$_2$Cl$_2$ (1:3) ($\mathrm{Abs}_{\mathrm{Soret}} = 0.195$) and (B) MeOH–CH$_2Cl_2$-cyclohexane (1:3:396) ($\mathrm{Abs}_{\mathrm{Soret}} \approx 0.08$).

scope as described before.[19] Other equipment was described in our previous report.[20] Methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (**1**) was prepared according to the reported procedure.[5] Dry solvents for preparation of a solid thin film were prepared as follows. Dichloromethane was treated with H_2SO_4, separated, washed with aqueous saturated $NaHCO_3$ solution and water, dried over $CaCl_2$ and distilled. Cyclohexane was dried over molecular sieves 4A and distilled.

Synthesis of cadmium methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (4)

A solution of $Cd(OAc)_2 \cdot 2H_2O$ (100 mg) in MeOH (100 mL, freshly distilled) was added to a solution of metal-free chlorin **1**[4] (100 mg) in CH_2Cl_2 (100 mL, freshly distilled) and refluxed for ca. 2 h under Ar in the dark. The completion of the reaction was confirmed by visible spectroscopy (662→655 nm). After cooling to room temperature, the solution was poured into H_2O and washed with H_2O three times and the separated CH_2Cl_2 solution was dried in vacuo. The residue was recrystallized from MeOH–CH_2Cl_2 (1:4)/$CHCl_3$-hexane (1:10). To remove metal free chlorin **1** which was produced in the above work-up procedures, the solids were washed with CH_2Cl_2 and hexane until the filtrate was colorless to give pure titled compound **4** (71 mg) as dark blue crystals in 59% yield. The cadmium complex **4** was unstable in the monomeric state, easily demetallated in a polar organic solvent and must be stored in the pure crystal form (not the solution) in the freezer. Mp > 300 °C; VIS (THF) λ_{max} 650 (relative intensity, 0.71), 611 (0.12), 601 (0.11, sh), 577 (0.06), 531 (0.03), 495 (0.02), 427 (1.00), 405 (0.60, sh), 320 (0.22); ^1H NMR ($CDCl_3 + 0.6\%\ C_5D_5N$) δ 9.50 (1H, s, 10-H), 9.25 (1H, s, 5-H), 8.22 (1H, s, 20-H), 5.78 (2H, s, 3-CH_2), 5.25, 4.95 (1H + 1H, d, $J = 20$ Hz, 13^1-CH_2), 4.43 (1H, dq, $J = 2$, 7 Hz, 18-H), 4.11 (1H, dt, $J = 8$, 3 Hz, 17-H), 3.72 (2H, q, $J = 8$ Hz, 8-CH_2), 3.61, 3.28, 3.22 (6H + 3H + 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3), 2.61–2.77, 2.34–2.59, 2.14–2.31 (1H + 2H + 1H, m, 17-CH_2CH_2), 1.66 (3H, t, $J = 8$ Hz, 8^1-CH_3), 1.64 (3H, d, $J = 7$ Hz, 18-CH_3); MS (FAB) found: *m/z* 664. Calcd for $C_{33}H_{34}N_4O_4{}^{114}Cd$: M^+, 664.

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