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Coaggregate of amphiphilic zinc chlorins with synthetic surfactants in an aqueous medium to an artificial supramolecular light-harvesting system

Tomohiro Miyatake,[a,*] Hitoshi Tamiaki,[b,*] Manabu Fujiwara[a] and Takayuki Matsushita[a]

[a]*Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan*
[b]*Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

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Abstract—Aqueous assemblies of zinc chlorins possessing a nonionic (oligo)oxyethylene, a cationic quaternary ammonium or an anionic sulfonate group were prepared in the presence of a synthetic surfactant. The nonionic zinc chlorin formed aggregates when admixed with a nonionic surfactant such as Triton X-100 to give a highly ordered oligomeric *J*-aggregate similarly as natural bacteriochlorophyll-*c* or *d* does in a chlorosome. In addition, the coassemblies of the cationic zinc chlorin with an anionic surfactant and of the anionic zinc chlorin with a cationic surfactant gave large oligomers of these chlorophyllous pigments. The structures of hydrophilic groups in both the zinc chlorin and surfactant molecules controlled their aqueous coassemblies.

1. Introduction

Light-harvesting antenna systems of photosynthetic organisms generally consist of pigment–protein complexes, in which chlorophylls are arranged for efficient energy transfer.[1] In a chlorosome of green photosynthetic bacteria, however, bacteriochlorophyll(BChl)-*c* and *d* molecules (Fig. 1) self-aggregate to produce a supramolecular light-harvesting system without any assistance of proteins.[2] A special C=O···HO···Mg bonding among the central metal, 13^1-oxo and 3^1-hydroxyl groups of the BChl molecule is necessary for building the self-aggregates.[3] Interestingly, the isolated BChl-*c* and *d* molecules self-organized to reproduce a chlorosomal aggregate in vitro.[4,5] Moreover, the synthetic zinc chlorin **1** possessing a 3^1-hydroxyl group (Fig. 1) similarly self-aggregated to give the chlorosome-type aggregate.[6,7] Efficient energy transfer from the aggregated zinc chlorin to an acceptor molecule was observed in the artificial systems.[7,8] Thus, the model pigments are available to produce a structural and functional model for natural antenna system.[6–12]

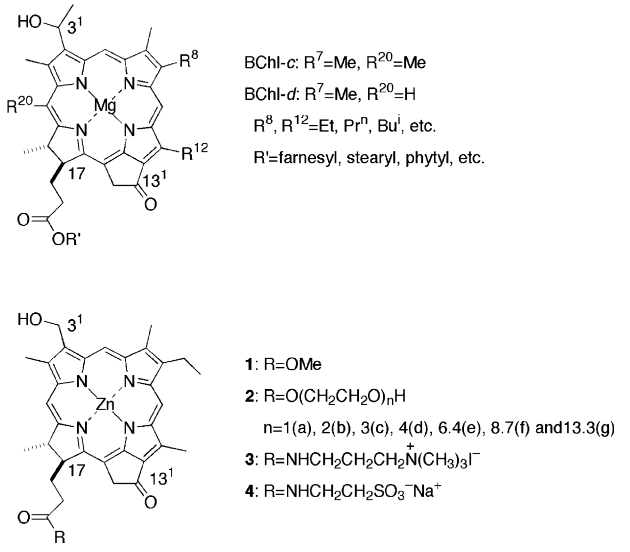

Figure 1. Molecular structures of chlorosomal chlorophylls (BChls-*c* and *d*) and synthetic zinc chlorins **1**–**4**.

In a natural chlorosome, self-aggregated BChl-*c* and *d* molecules are considered to be surrounded with a monolayer of galactolipids. Synthetic zinc chlorin **1** and isolated natural BChls-*c* and *d* hardly dissolve in water. Hence, some aqueous in vitro aggregates of the

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* Corresponding authors. Tel.: +81-77-543-5111; fax: +81-77-543-7483 (T.M.); tel.: +81-77-566-1111; fax: +81-77-561-2659 (H.T.); e-mail addresses: miyatake@rins.ryukoku.ac.jp; tamiaki@se.ritsumei.ac.jp

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chlorosomal chlorophylls and the synthetic analogues as in **1** were prepared in the presence of a lipid or surfactant. The BChl-*c*–lipid mixture in an organic medium was injected into water to reproduce a chlorosome-like pigment–lipid assemblies.[5] Similarly, self-aggregates of the synthetic zinc chlorins were surrounded with phospholipid or synthetic surfactant to form the artificial chlorosome in an aqueous medium.[7,10]

In general, amphiphilic molecules such as synthetic surfactants or natural lipids self-assemble to form supramolecular structures in an aqueous medium. The morphology of the aqueous assemblies is strongly dependent upon the structure of both hydrophobic and hydrophilic moieties in the amphiphilic molecule. In addition, varieties of aqueous assemblies were prepared by mixing any other amphiphilic molecules. In the previous study,[9] amphiphilic zinc chlorins possessing a nonionic (oligo)oxyethylene (**2**), cationic quaternary ammonium (**3**), and anionic sulfonate group (**4**) on the 17-position were prepared (Fig. 1). From the spectroscopic analyses, amphiphilic zinc chlorins **2**–**4** self-organized in an aqueous medium without any surfactants to form a small aggregate such as a dimer in an aqueous medium. In the same environments, other synthetic zinc chlorins possessing an alkyl ester as in **1** gave their large aggregates.[7,11] These results indicated that the hydrophilic groups of **2**–**4** prevented their further aggregation.

Here we report aqueous aggregates of amphiphilic zinc chlorins **2**–**4** in the presence of several synthetic surfactants shown in Figure 2. When **2**–**4** coassembled with specific surfactants, their chlorosome-type oligomers were formed in an aqueous medium. The formation of such a coassembly was dependent on both the molecular structures of amphiphilic zinc chlorin and synthetic surfactant used.

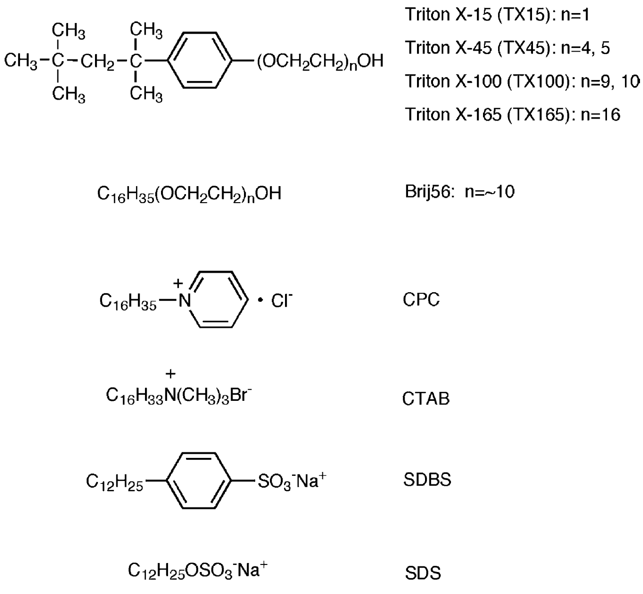

Figure 2. Molecular structures of the nonionic, cationic, and anionic surfactants used in this study.

2. Results and discussion

2.1. Coaggregation of nonionic zinc chlorin and Triton X-100 in an aqueous medium

Self-aggregates of nonionic zinc chlorins **2** in an aqueous medium containing no surfactant were already examined in the previous report.[9] The nonionic zinc chlorins possessing several numbers of oxyethylene group **2a** ($n = 1$), **2b** (2), **2c** (3), **2d** (4), **2e** (6.4), **2f** (8.7), and **2g** (13.3) were well soluble in methanol and gave a Qy absorption maximum at 652 nm showing that all **2a**–**g** were monomeric forms. When the methanol solution of zinc chlorins **2a**–**g** was diluted with a 99-fold volume of water, the amphiphilic zinc chlorins gave a red-shifted Qy band at 675 nm, indicating that **2a**–**g** formed a small aggregate in the aqueous medium.

In contrast, the addition of a nonionic surfactant induced further aggregation of the amphiphilic zinc chlorins to form a chlorosome-type large aggregate. When zinc chlorin **2b** was mixed with Triton X-100 (TX100) in methanol and injected into a 99-fold volume of water, a homogeneous green solution was obtained. The absorption spectrum of an aqueous solution of **2b** (10 μM) and TX100 (500 μM) showed a red-shifted Qy absorption band at 734 nm (the solid line of Fig. 3), indicating that **2b** formed a large aggregate as well as BChl-*c* and *d* does in a natural chlorosome. The zinc chlorin chromophore of **2b** formed strongly exciton coupled *J*-aggregate in a hydrophobic environment of a TX100 assembly. The dimension of pigment–surfactant aggregate was estimated about 60–120 nm by a dynamic light scattering (DLS) measurement, which was larger than that of a neat TX100 micelle, 7–10 nm.[13] The aqueous aggregate was stable, and the obtained absorption spectrum unchanged for several months.

The absorption spectrum of the zinc chlorin–TX100 assemblies was dependent upon the concentration of TX100 added. When 100 μM of TX100 was used, a minor Qy absorption band due to a large aggregate of **2b** was observed around 730 nm concomitant with a

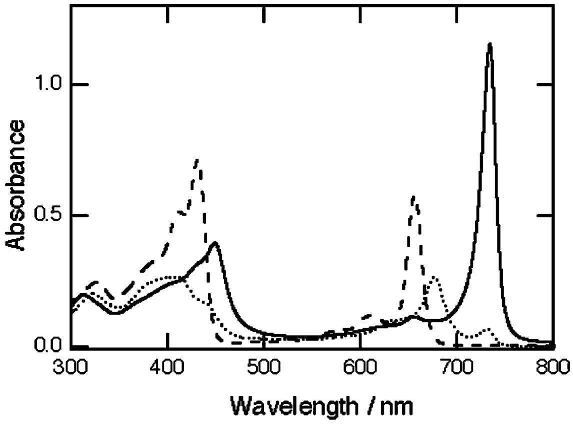

Figure 3. Visible absorption spectra of zinc chlorin **2b** (10 μM) in an aqueous medium with TX100; [TX100]=100 μM (the dotted line), 500 μM (the solid line), and 5000 μM (the dashed line).

major 675-nm band of a small aggregate described above (the dotted line of Fig. 3). The result showed that a small part of zinc chlorin **2b** formed chlorosome-type aggregate in a less TX100 concentration than its critical miceller concentration (CMC), 240 μM. Under the condition, **2b** partly coassembled with TX100 to give a similar large aggregate described above and almost **2b** dimerized in an aqueous methanol solution without lapping by TX100 reported previously. When an excess amount of TX100 (5000 μM) was used, the Qy absorption maximum of zinc chlorin **2b** appeared at 655 nm of the monomeric form. The excess TX100 completely disturbed the aggregation of zinc chlorins, suggesting that each zinc chlorin molecule might be surrounded with a TX100 assembly.

Zinc chlorin **1** (R = OMe) similarly formed a chlorosome-type aggregate in an aqueous TX100 solution. However, the spectroscopic properties of **1** aggregate were different from those of **2b** aggregate. The Qy absorption maxima of oligomeric **1** and **2b** were 743 and 735 nm, respectively (Fig. 4A). The spectral difference in the aggregated **1** and **2b** indicated that the hydrophilic dioxyethylene moiety of zinc chlorin **2b** affected the pigment–surfactant coassembly. The full widths at half maximum (fwhm) of the Qy absorption bands were 530 and 340 cm^{-1} for self-aggregates of **1** and **2b**, respectively. In addition, the CD spectrum of aggregated **2b** gave intense signals compared to that of **1** (Fig. 4B). These results suggested that coassembly of **2b** with TX100 afforded a highly ordered zinc chlorin aggregate. The hydrophilic dioxyethylene moiety of zinc chlorin **2b** might be oriented toward the bulk water, which assisted the arrangement of zinc chlorin moiety (Fig. 5).

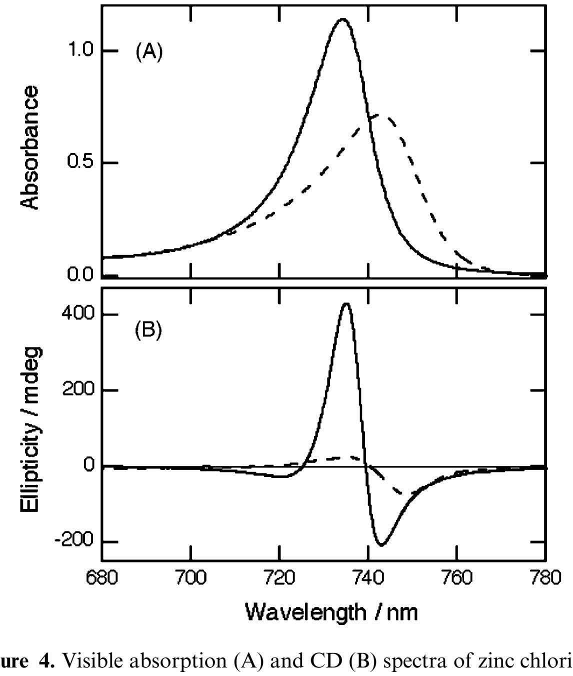

Figure 4. Visible absorption (A) and CD (B) spectra of zinc chlorins **1** (10 μM, the dashed line) and **2b** (10 μM, the solid line) in an aqueous medium with 500 μM of TX100.

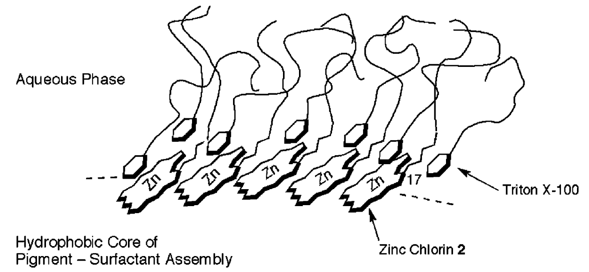

Figure 5. Schematic drawing of zinc chlorin **2**–TX100 coaggregate in an aqueous medium. The hydrophilic oligooxyethylene chain of both pigment and surfactant might be oriented toward the aqueous phase.

The length in the oligooxyethylene group on the 17-position of **2** affected the coassembly of zinc chlorin with TX100. Figure 6A showed visible absorption spectra of nonionic zinc chlorins **2a–g** in the presence of 500 μM TX100. The absorption spectra of coaggregates of **2a–d**

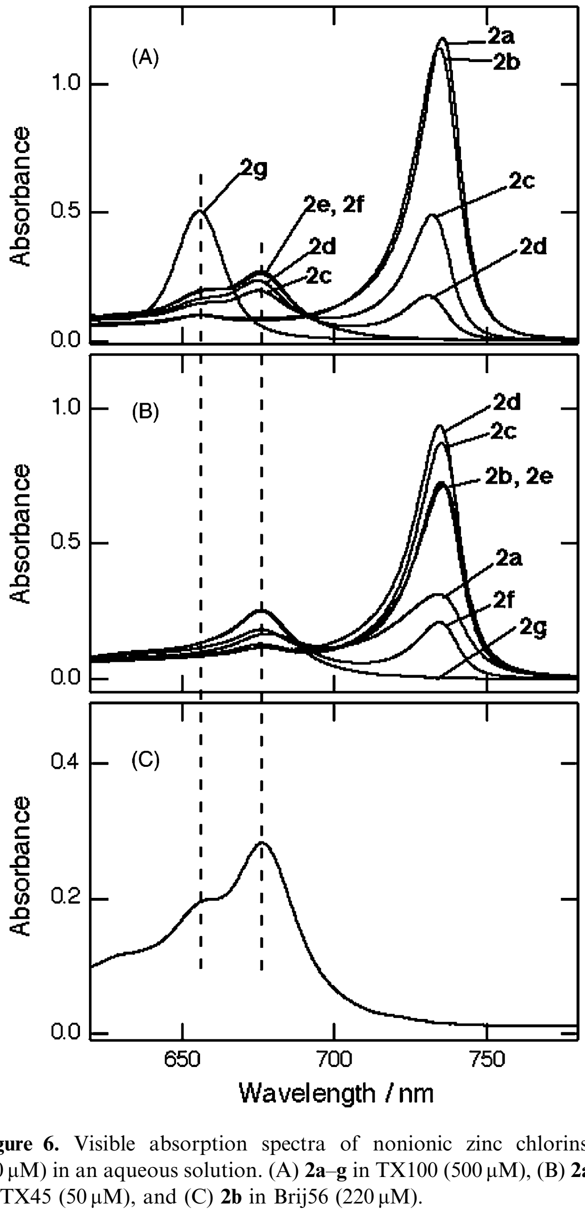

Figure 6. Visible absorption spectra of nonionic zinc chlorins **2** (10 μM) in an aqueous solution. (A) **2a–g** in TX100 (500 μM), (B) **2a–g** in TX45 (50 μM), and (C) **2b** in Brij56 (220 μM).

($n = 1$–4) with TX100 showed a Qy band at 731–735 nm, indicating that the zinc chlorins **2a–d** gave a large aggregate in the presence of TX100. Intensity of Qy band of the self-assembled zinc chlorin, however, apparently decreased (**2a** $>$ **2b** $>$ **2c** $>$ **2d**) with increasing the oligomerization number of oxyethylene group $(1 \rightarrow 4)$. Then, zinc chlorins **2e–g** with a longer oligooxyethylene chain ($n = 6.4$–13.3) did not show any absorption band around 730 nm of the large aggregate. A 675-nm band of the small aggregate was a major component in **2e** and **2f** coassemblies. Moreover, zinc chlorin **2g** showed a 655-nm band of monomeric zinc chlorin. Thus, the oligomerization number of oxyethylene group in **2** was larger than 6.4, a small aggregate or monomeric form of zinc chlorin was predominant in a TX100 solution. The long hydrophilic oligooxyethylene group of **2e–g** disturbed the oligomerization of zinc chlorin.

The CD spectra of the coassemblies of **2a–d** with TX100 gave an intense positive band around 730 nm accompanying with two weak negative bands around 740 and 720 nm (Fig. 7). Due to lacking absorption bands, zinc chlorins **2e–g** did not show any intense CD signals at 720–740 nm. In **2c** and **2d** assemblies, the additional weak CD signals appeared around 670 nm of a small aggregate. The CD spectral shapes of **2a–d** aggregates observed at 720–750 nm were similar, demonstrating that the basic arrangement of zinc chlorin chromophore in the coaggregate was independent of the length of oligooxyethylene moiety of **2**.

2.2. Coaggregation of nonionic zinc chlorins 2 with any other nonionic surfactants in an aqueous medium

The length of the oligooxyethylene group of the nonionic surfactant also affected the pigment–surfactant coassembly. Triton X-45 (TX45), possessing about half shorter oligooxyethylene group ($n = 4$, 5 in Fig. 2) than TX100, was mixed with nonionic zinc chlorins **2a–g** in an aqueous medium. Figure 6B showed visible absorption spectra of coaggregate of **2a–g** with TX45. Zinc chlorins **2a–f** showed a red-shifted Qy band at 733–735 nm, indicating that coassemblies of **2a–f** with TX45 gave chlorosome-type aggregate. In contrast to the coaggregate of **2** with TX100, the intensity of the Qy absorption band increased, as the oligomerization number of oxyethylene group of zinc chlorin **2** increased from 1 (**2a**) to 4 (**2d**). Then, the 734-nm band decreased with increasing the oligomerization number of oxyethylene chain from 4 (**2d**) to 13.3 (**2g**). Zinc chlorin **2d** possessing tetraoxyethylene group gave the largest Qy peak in an aqueous TX45 solution.

Coaggregate of **2** with other nonionic surfactants TX15 and TX165 ($n = 1$ and 16, respectively, in Fig. 2) were also prepared in an aqueous medium. Absorbance at 734 nm of chlorosome-type aggregate was plotted against the oligomerization number of oxyethylene chain in zinc chlorins **2** (Fig. 8). In the coassembly with TX15, an absorption band of large aggregate was found in zinc chlorins **2b–f** ($n = 2$–8.7). Zinc chlorin **2e** ($n = 6.4$) gave the most intense band around 734 nm. In the coassembly with TX45 and TX100, a 734-nm band appeared in zinc chlorins **2a–f** ($n = 1$–8.7) and **2a–d** ($n = 1$–4), respectively. Moreover, zinc chlorins **2a** ($n = 1$) and **2b** ($n = 2$) gave a large aggregate in an aqueous TX165 solution. Thus, when the length of oligooxyethylene chain of nonionic surfactants increased, the zinc chlorins with

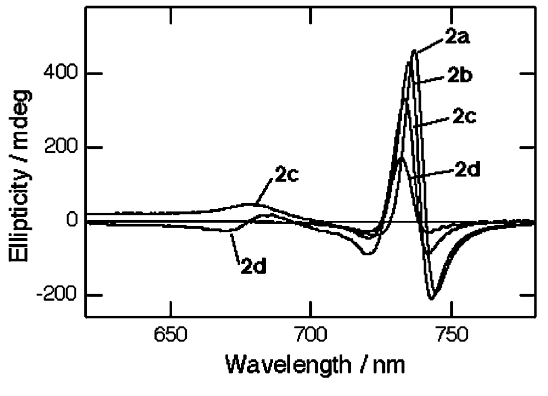

Figure 7. CD spectra of zinc chlorins **2a–d** (10 µM) in an aqueous TX100 (500 µM) solution.

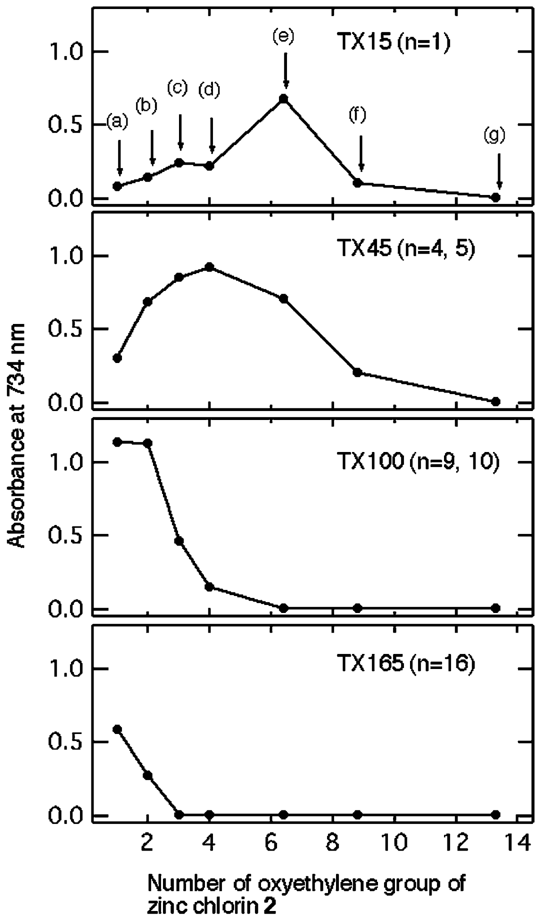

Figure 8. Dependency on the absorbance at 734 nm with oligomerization number of oxyethylene group of zinc chlorins **2** (10 µM) coaggregated with TX15 (40 µM), TX45 (50 µM), TX100 (500 µM), and TX165 (1 mM).

shorter (oligo)oxyethylene chain was favorable for formation of chlorosome-type large aggregate.

These studies indicated that the coassembly of amphiphilic zinc chlorin and nonionic surfactant was dependent on the length of the oligooxyethylene group of both zinc chlorin and surfactant. In the aqueous coassemblies, a hydrophobic zinc chlorin aggregate might be surrounded with the hydrophilic oligooxyethylene chains. If the appropriate amount of oligooxyethylene group was introduced for surrounding a zinc chlorin aggregate, a chlorosome-type aggregate preferentially formed in the pigment–surfactant coassembly.

The hydrophobic group of the nonionic surfactant also affected the coaggregation with amphiphilic zinc chlorins. An aqueous aggregate of amphiphilic zinc chlorin **2b** was prepared in the presence of Brij56 possessing a straight alkyl chain as the hydrophobic part (Fig. 2). The absorption spectrum of **2b** in an aqueous Brij56 solution gave a 676-nm absorption band of the small zinc chlorin aggregate concomitant with a 652-nm band of monomeric form (Fig. 6C). Although Brij56 has a similar hydrophilic lipophilic balance (HLB=12.9) to TX100 (HLB=13.5), the coaggregation of nonionic zinc chlorin **2b** with Brij56 was clearly different from that of **2b** with TX100. These results suggested that the coaggregation of the amphiphilic zinc chlorin with a nonionic surfactant was not only determined by HLB, but also a structure of the hydrophobic group of the nonionic surfactant. The aromatic ring of TX-surfactants could have π–π interaction with zinc chlorin moieties, which assisted the formation of the chlorosome-type aggregate.

2.3. Coaggregation of ionic zinc chlorins with ionic surfactants in an aqueous medium

The ionic zinc chlorins possessing a cationic quaternary ammonium group (**3**) and an anionic sulfonate group (**4**) were mixed with ionic surfactants in an aqueous medium. Figure 9A showed visible absorption spectra of the cationic zinc chlorin **3** with cationic CPC and with anionic SDBS. Both CPC and SDBS possess an aromatic ring in a molecule. In the presence of CPC, the absorption spectrum of **3** did not show any band around 700–750 nm (the dashed line of Fig. 9A). The 658- and 675-nm bands of monomer and small aggregate, respectively, of **3** appeared. In contrast, zinc chlorin **3** gave a 724-nm band in the presence of anionic SDBS concomitant with 658- and 675-nm bands (the solid line of Fig. 9A). The result suggested that a part of added cationic **3** coassembled with anionic SDBS to form a large aggregate in an aqueous medium. The ionic interaction between the cationic zinc chlorin and anionic surfactant assisted the formation of pigment–surfactant complexes.

While, the anionic zinc chlorin **4** showed large red-shifted bands at 700 and 730 nm of oligomeric zinc chlorin in the presence of cationic CPC (the dashed line

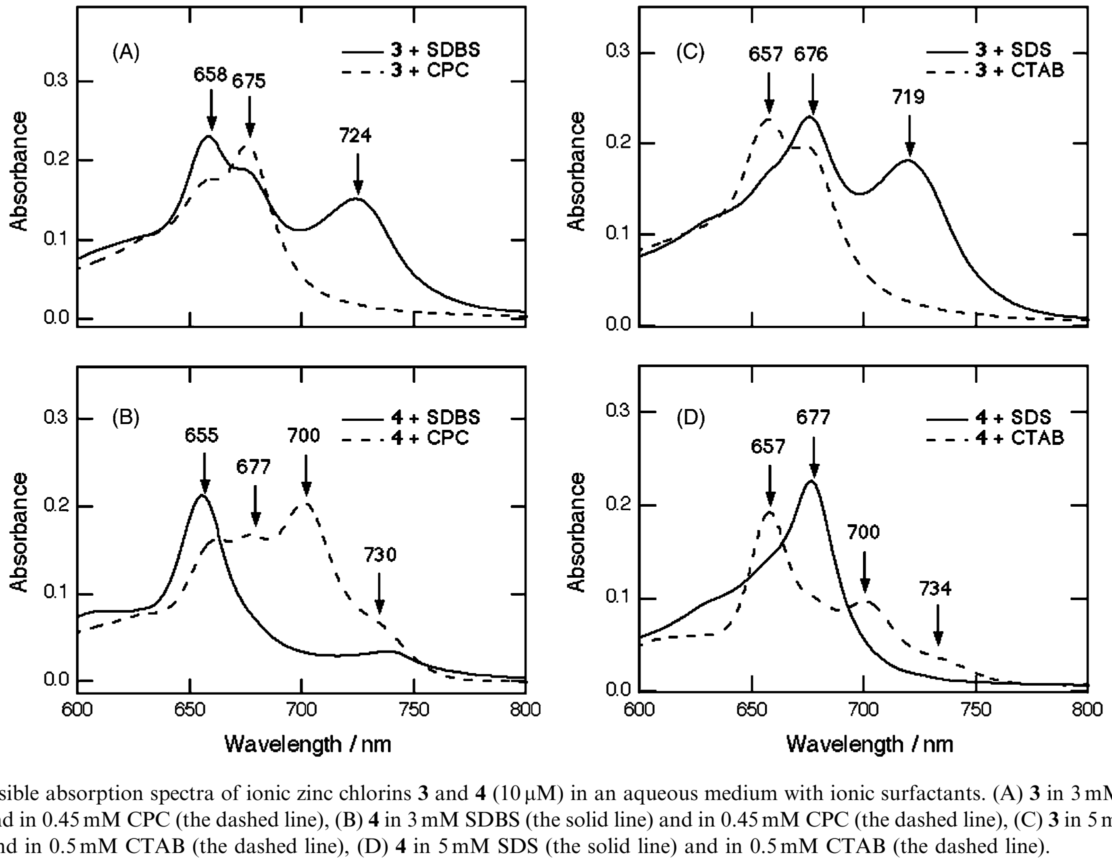

Figure 9. Visible absorption spectra of ionic zinc chlorins **3** and **4** (10 μM) in an aqueous medium with ionic surfactants. (A) **3** in 3 mM SDBS (the solid line) and in 0.45 mM CPC (the dashed line), (B) **4** in 3 mM SDBS (the solid line) and in 0.45 mM CPC (the dashed line), (C) **3** in 5 mM SDS (the solid line) and in 0.5 mM CTAB (the dashed line), (D) **4** in 5 mM SDS (the solid line) and in 0.5 mM CTAB (the dashed line).

of Fig. 9B). Mixing the anionic **4** and anionic SDBS did not afford an intense band around 700–730 nm (the solid line of Fig. 9B); a small 740-nm band would be ascribable to a slight formation of large aggregate **4**. These results showed that cationic or anionic zinc chlorins could coassemble with anionic or cationic surfactant, respectively, to form large aggregates in an aqueous medium.

The cationic CTAB and anionic SDS were used for coassembly with zinc chlorins **3** and **4**. The absorption spectra showed that large aggregates of ionic zinc chlorins were formed with the ionic surfactants possessing the opposite electric charges (Fig. 9C and D). The results indicated that the π–π interaction between the zinc chlorin and surfactant was not so important for the coassembly of ionic amphiphilic molecules. Moreover, the absorption band of the oligomeric zinc chlorin around 700–750 nm decreased by addition of 0.1 M NaCl, suggesting that the added Na^+ and Cl^- ions neutralized the ionic pair between the pigment and surfactant to de-aggregate the ionic coassembly.

3. Experimental

Synthetic zinc chlorins **1**–**4** (Fig. 1) were prepared according to the reported procedures.[7,9] Nonionic **2e**, **2f**, and **2g** were obtained as a mixture of zinc chlorins possessing a several length of oligooxyethylene chain and the average oligomerization number of oxyethylene group were 6.4, 8.7, and 13.3, respectively. Nonionic surfactants, Triton X-100 (TX100) and Triton X-45 (TX45), cationic surfactants, cetyl pyridinium chloride (CPC) and cetyl trimethylammonium bromide (CTAB), and anionic surfactants, sodium 4-dodecylbenzenesulfonate (SDBS) and sodium dodecylsulfate (SDS), were purchased from Nacalai Tesque, Japan, and Triton X-15 (TX15), Triton X-165 (TX165), and Brij 56 were purchased from Aldrich Chemical Co. Ltd. Visible absorption, circular dichloism spectra were measured with a Shimadzu UV-3100 spectrophotometer and a Jasco J-720W spectropolarimeter, respectively. DLS data were recorded with a Malvern Nano-NS particle size analyzer upon irradiation with a 4-mW He–Ne laser at a scattering angle of 173°.

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