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Incorporation of a photosynthetic supramolecular complex by using imidazolyl Zn porphyrin dimers in bilayer lipid membrane

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Abstract—Incorporation of an artificial photosynthetic complex in bilayer lipid membrane by using seven porphyrin units through a supramolecular approach.

Crystallographies of photosynthetic systems disclosed the core structural arrangement of important chromophores involved in the reaction center and light-harvesting systems.[1–5] Then, how to construct artificial systems from appropriate components in a simple and efficient way became our target of challenge. Various designs towards accomplishment of artificial photosynthesis have been reported based on not only covalent linking,[6] but also supramolecular organization of porphyrins and other pigments.[7] Photophysical properties such as excitation energy transfer and charge separation have been examined mostly in homogeneous solutions. Although the photosynthesis is operated in the membrane phase in nature, limited examples of artificial photosynthetic systems were examined in the membrane hitherto,[8] and supramolecular systems developed by us were attempted here to be incorporated in the bilayer lipid membrane.

Inspired by the crystallography of bacterial photosynthetic reaction center,[5] we have introduced an idea of slipped cofacial dimer formation of imidazolyl (Im) Zn porphyrin by complementary coordination of Im→Zn as the simplest expression of the special pair by appending Im to porphyrin itself instead of coordination from membrane penetrating polypeptide helices.[9,10] This dimer arrangement was found by later crystallographies to be also a unit constituting light-harvesting complexes LH1 and LH2 from bacteria.[1–4] In the present approach, three dimer units were attached to free base (Fb) porphyrin as an energy acceptor, to afford a supramolecular heptaporphyrin system. Dimers in this approach therefore may be regarded as not only special pair but also antenna units to express a photosynthetic system even partly. The whole arrangement was then tried to be incorporated in liposomal membrane.

Scheme 1 illustrates the synthetic route leading to compound **4**. Covalently linked porphyrin nucleus **3** was synthesized by condensation of tris(aminophenyl)-porphyrin **1** with *N*-methylimidazolylporphyrinatozinc(II) having a carboxylic acid **2** using $(EtO)_2P(O)CN$ as the condensing agent.[11–13] Since imidazolyl and Zn (II) in the porphyrin moiety are components of complementary coordination, **3** exists as polymeric mixtures in non-polar media. In order to obtain heptaporphyrin complex **4**, 1 equiv. of **3** and 50 equiv. of **2**, existing as a dimer too, were mixed in pyridine, which dissociated all the complementary coordination structure of Im→Zn by competitive coordination. Then, pyridine was removed under reduced pressure to organize **3** into **4** in the presence of excess **2**. Dissolution of the residue in $CHCl_3$ afforded heptaporphyrin **4** and dimer of porphyrin **2**. Pure **4** was isolated as a single peak by gel permeation chromatography (GPC) using a $CHCl_3$ eluent.[14]

Absorption spectrum of **4** in $CHCl_3$ was expressed reasonably by the sum of one unit of **1** and three units of **2** as shown in Figure 1. In contrast, the fluorescence emission spectrum was completely different from the sum of the components because of quenching processes. When Zn porphyrin was irradiated at 438 nm, 95% contributing to the excitation of **2** and 5% to that of **1**, the fluorescence intensities from Zn porphyrin (at 628

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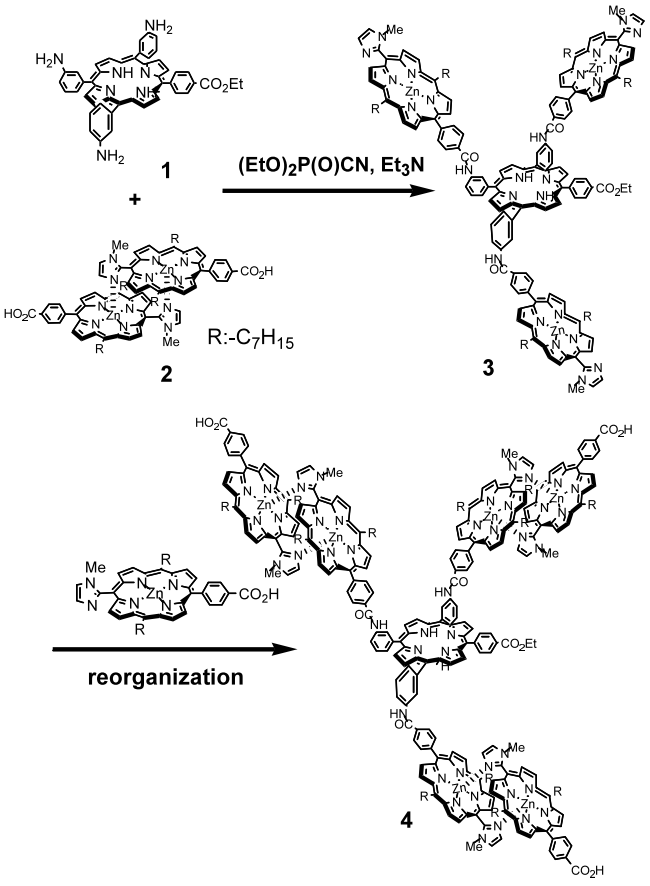

Scheme 1. Synthesis and supramolecular organization leading to heptaporphyrin **4**. Imidazolyl Zn porphyrin **2** is of dimeric structure in $CHCl_3$ and monomeric in pyridine.[9]

and 684 nm) were decreased to one-fifth and those from Fb porphyrin (at 652 and 715 nm) were increased by three times as shown in Figure 2. In other words, 80% of the excitation energy of Zn porphyrin was lost by the presence of Fb porphyrin and other factors. If all the energy from Zn porphyrin is utilized for the excitation of Fb porphyrin, the fluorescence intensity from Fb porphyrin must increase by a factor of 16.[15] Since the

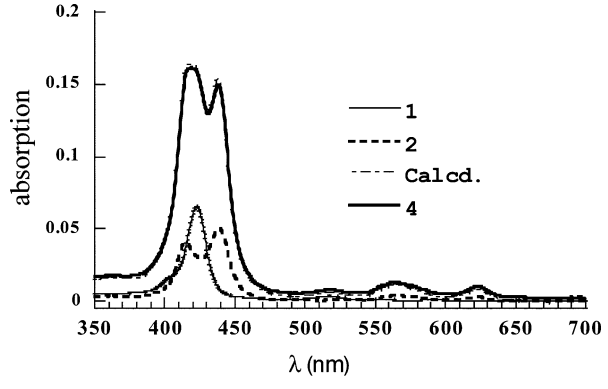

Figure 1. UV–vis spectra of **4** and components **1** and **2** in $CHCl_3$ at room temperature. $[\mathbf{4}]=[\mathbf{1}]=[\mathbf{2}]=1.55\times10^{-7}$ M. The calculated spectrum is a sum of the spectra of one and three units of **1** and **2**, respectively.

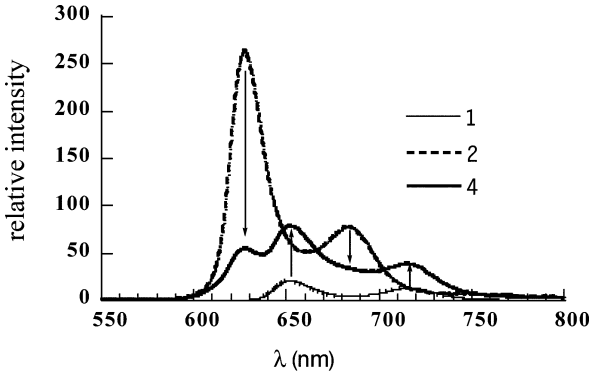

Figure 2. Fluorescence spectra of compound **4** and its components **1** and **2** in $CHCl_3$. Excitation at 438 nm. In all measurements of **4**, **1**, and **2**, the absorption intensities at 438 nm were 0.1.

intensity was increased by only three times, the energy transfer efficiency was estimated as $80\times(2/15)=11\%$. Then the remaining excitation energy of Fb porphyrin, 69% was lost probably by other quenching processes, such as self-quenching among three Zn porphyrin dimer units in **4** because each dimer units may be close to another dimer.

Next, large unilamellar vesicle (LUV) was prepared by injecting an ether solution of soybean lecithin and **4** (2000:1) slowly into a buffer solution of pH 7.0 (HEPES-Tris, 5 mM) at 60°C. Incorporation of **4** in the vesicle was performed at a very low concentration (4.3×10^{-8} M) to prevent fluorescence quenching by intermolecular energy transfer, and the ether injection method was applied because the complementary coordination of Im→Zn in **4** was decomposed by ultrasonication method. A turbid solution obtained was passed through a Sephadex G-50 column. No porphyrin was retained at the top of the column upon elution with a buffer solution of pH 7.0 (HEPES-Tris, 5 mM), indicating that porphyrins were not separated from lipid, but incorporated successfully into vesicle because porphyrins were totally insoluble in a buffer solution. Furthermore, an almost clear solution obtained was filtered through a 0.45 μm filter. Dynamic light scattering (DLS) measurement suggested the formation of LUV whose diameter was estimated as 150±42 nm. It was difficult to confirm the incorporation of **4** with a UV–vis spectrum because of the too low concentration and large scattering from lipids (Fig. 3, inset). However, the excitation spectrum (Fig. 3) monitored at 620 nm, where only Zn porphyrin emits, clearly showed the existence of a porphyrin component in the solution. Moreover, the Soret bands of porphyrins in the spectrum clearly split into twin peaks at 419 and 438 nm to show specifically the Zn porphyrin dimer in **4**.[9] Therefore, the complementary coordination structure of Im to Zn of the imidazolyl-substituted Zn porphyrins is also maintained in the environment of liposomal membrane. It is noteworthy that the shape of emission spectrum excited at 438 nm in the liposome was almost identical to that observed in a $CHCl_3$ solution and that

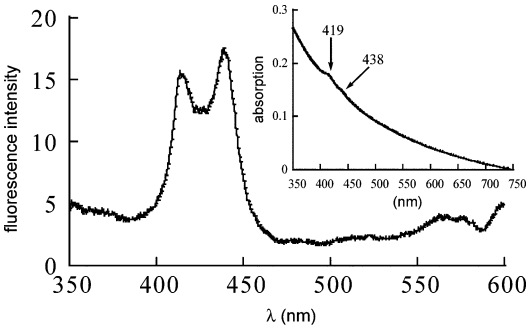

Figure 3. Excitation spectrum of **4** incorporated into liposomal membrane. Observation at 620 nm. Inset: absorption spectrum of **4** in liposome.

the quenching energy of emission from Zn porphyrin was maintained similarly to the case in a $CHCl_3$ solution (Fig. 4).

In the natural system, highly efficient energy and electron transfer in the photosynthetic chromophores is accomplished among complexes structured with proteins in a bilayer lipid membrane. In the present model, **4** was designed so that the structure might be formed itself in the membrane without the assistance of protein matrices. For this purpose, a carboxyl group was introduced at each terminal of three Zn porphyrin parts around Fb porphyrin in order to place the dissociated carboxylates near the membrane surface. This arrangement may drive the Fb part at the central part of the membrane in either a membrane-expanded or a tripod form for major (statistically 75%) and minor (25%) atropisomers, respectively. Incorporation of such a big supramolecular assembly seems successfully incorporated with keeping the complementary coordinated structure even located near the membrane surface.

It is concluded that the structure of strong imidazolyl-to-Zn complementary coordination is maintained in a similar manner as a photosynthetic supramolecular complex in the membrane. The emission and excitation spectra of **4** in the liposomal membrane suggested the quenching of energy emitted from Zn porphyrin similar to the case in a $CHCl_3$ solution. In this context, the model represents a special pair-pheophytin dyad in the photosynthetic reaction center in combination with a light-harvesting system. Investigation on transient absorption measurement is now under active investigation in this laboratory. Furthermore, attachment of another electron acceptor such as quinone to the ester part of Fb porphyrin will provide a complete set of an antenna–special pair–pheophytin–quinone model.

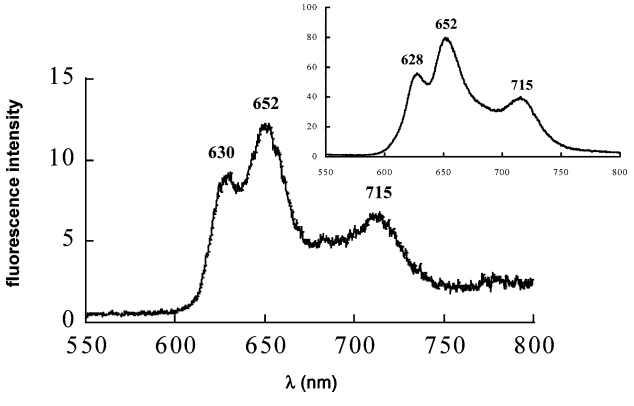

Figure 4. Emission spectrum of **4** incorporated into liposomal membrane. Excitation at 438 nm. Inset: emission spectrum of **4** in $CHCl_3$ solution.

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10. The binding constant of **2** was estimated as $K=5.6\times10^9$ M^{-1} in $CHCl_3$ by competitive equilibrium experiments with pyridine.
11. Synthesis of compound **1**. (a) Synthesis of 5,10,15-tris(3-nitrophenyl)-20-(4-ethoxycarbonylphenyl)porphine (**S1**): Terephthalaldehydic acid ethylester (0.828 mmol), *m*-

nitrobenzaldehyde (2.48 mmol), and pyrrole (3.31 mmol) were dissolved in 15 mL of propionic acid. After refluxing for 0.5 h, the reaction solution was evaporated. The crude product was purified by silica gel column chromatography (eluent: chloroform) to yield 0.046 mmol (5.5%) of **S1**. MS (MAL-DI-TOF) found for $[M+H]^+$, 822.2, calcd 821.2. (b) Synthesis of 5,10,15-tris-(3-aminophenyl)-20-(4-ethoxycarbonylphenyl)porphine (**1**): **S1** (0.0139 mmol) was dissolved in 5.5 mL of conc. HCl, and $SnCl_2$ (0.175 mmol) was added. After stirring for 1 h at 60°C, aq. NH_3 was added (pH 10). The reaction solution was extracted with $CHCl_3$. Organic layer was washed with aq. NH_3 (pH 10) and water. The solvent was removed by evaporation. The crude product was purified by silica gel column chromatography (eluent: chloroform/methanol = 8:1) to yield 0.072 mmol (52%) of **1**. MS (MALDI-TOF) found for $[M+H]^+$, 732.1, calcd 731.3; λ_{abs} (chloroform) 422 (100), 516 (5.13), 551 (1.96), 589 (1.61), 646 (1.04) nm; λ_{em} (chloroform) 651 (100), 717 (63.7) nm; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 8.95$ (2H, d, $J = 4.86$ Hz, pyrroleβH), 8.93 (3H, s, aminophenyl 2-H), 8.76 (2H, d, $J = 4.86$ Hz, pyrroleβH), 8.44 (2H, d, $J = 8.10$ Hz, pyrroleβH), 8.39 (2H, d, $J = 8.10$ Hz, pyrroleβH), 8.76–7.47 (10H, aminophenyl 4, 5-H and carbonylphenyl 2, 3, 5, 6-H), 7.10 (3H, d, $J = 8.10$, aminophenyl 6 position-H), 4.58 (2H, q, $J = 7.01$, -CH_2-), −2.81 (2H, d, pyrroleNH).

12. Synthesis of compound **2**. (a) Synthesis of 5,15-bis(*n*-heptyl)-10-(1-methylimidazol-2-yl)-20-(4-ethoxycarbonylphenyl)porphine (**S2**): Under an argon atmosphere, 1-methylimidazole-2-carboxyaldehyde (2.05 mmol), terephthalaldehydic acid ethylester (2.05 mmol), and meso-(*n*-heptyl)dipyrromethane (4.10 mmol) were dissolved in 410 mL of chloroform, and then trifluoroacetic acid (4.10 mmol) was added. After stirring for 6 h at rt, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (6.15 mmol) was added and the reaction mixture was stirred for further 1 h. The reaction solution was washed with aqueous sodium bicarbonate, and then organic layer was evaporated. The crude product was purified by silica gel column chromatography (eluent: chloroform/methanol = 20:1) to yield 0.143 mmol (7.0%) of **S2**. MS (MALDI-TOF) found for $[M+H]^+$, 735.42, calcd 734.43; λ_{abs} (chloroform) 418 (100), 517 (5.40), 552 (2.67), 593 (1.87), 649 (1.71) nm; λ_{em} (chloroform) 656 (100), 723 (44.0) nm; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 9.46$ (2H, d, $J = 4.86$ Hz, pyrroleβH), 9.41 (2H, d, $J = 4.86$ Hz, pyrroleβH), 8.81 (2H, d, $J = 3.24$ Hz, pyrroleβH), 8.78 (2H, d, $J = 3.24$ Hz, pyrroleβH), 8.44 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.41 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.33 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.20 (1H, d, $J = 7.83$ Hz, phenyl-H), 7.68 (1H, s, imidazol-H), 7.48 (1H, s, imidazol-H), 4.95 (4H, m, αCH_2), 4.60 (2H, q, $J = 7.29$ Hz, CO_2CH_2), 3.41 (3H, s, NCH_3), 2.50 (4H, m, βCH_2), 1.32 (3H, t, $J = 7.29$ Hz, CO_2CH_2-CH_3), 0.88 (6H, t, $J = 7.29$ Hz, alkyl CH_3), −2.67 (2H, d, pyrroleNH). (b) Synthesis of 5,15-bis(*n*-heptyl)-10-(1-methylimidazol-2-yl)-20-(4-ethoxycarbonylphenyl)porphinatozinc (II) (**S3**): To a solution of free base porphyrin **S2** (18.9 μmol) in 2 mL of chloroform was added 0.6 mL of saturated solution of zinc acetate dihydrate in methanol. After stirring for 3 h at room temperature, the solution was washed with water, and then organic layer was evaporated to yield 18.7 μmol (99%) of **S3**. MS (MALDI-TOF) found for $[M+H]^+$, 797.43, calcd 796.34; λ_{abs} (chloroform) 414 (82.3), 438 (100), 565 (8.23), 622 (6.63) nm; λ_{em} (chloroform) 627 (100), 684 (90.4) nm. (c) Synthesis of 5,15-bis(heptyl)-10-(1-methylimidazol-2-yl)-20-(4-carboxylphenyl)-porphinatozinc (II) (**2**): To a solution of **S3** (18.7 μmol) in 2.1 mL of methanol/THF (1:2) was added 0.5 mL of 8N NaOH. After stirring for 2 h at room temperature, the reaction solution was evaporated. To the solution was added conc. HCl (pH 2), and extracted with $CHCl_3$. Organic layer was washed with water. The solvent was removed by evaporation to yield 17.0 μmol (91%) of **2**. MS (MALDI-TOF) found for $[M+H]^+$, 769.91, calcd 768.31; λ_{abs} (chloroform) 414 (82.4), 438 (100), 565 (8.36), 622 (7.09) nm; λ_{em} (chloroform) 628 (100), 685 (29.0) nm; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 9.59$ (2H, d, $J = 4.86$ Hz, pyrroleβH), 9.00 (2H, d, $J = 3.24$ Hz, pyrroleβH), 8.91 (2H, d, $J = 4.86$ Hz, pyrroleβH), 8.78 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.63 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.47 (1H, d, $J = 7.83$ Hz, phenyl-H), 8.24 (1H, d, $J = 7.83$ Hz, phenyl-H), 5.56 (1H, s, imidazol-H), 5.43 (2H, d, $J = 3.24$ Hz, pyrroleβH), 4.28 (4H, m, αCH_2), 2.94 (4H, m, βCH_2), 2.13 (1H, s, imidazol-H), 2.05 (4H, m, γCH_2), 1.69 (3H, s, NCH_3), 1.01 (6H, t, $J = 7.29$ Hz, alkyl CH_3).

13. Synthesis of 5,10,15-tris-(3-{4-5,15-bis-(*n*-heptyl)-10-(1-methylimidazol-2-yl)-20-zinc(II) porphinyl-phenylamino-carbonyl}-20-(4-ethoxycarbonylphenyl)porphine (**3**): Under an argon atmosphere, **1** (0.48 mmol), **2** (2.78 mmol), and diethyl phosphorocyanidate (DEPC) (0.41 mmol) were dissolved in 20 mL of chloroform, and triethylamine (4.08 mmol) was added at 0°C. After stirring for 5 h at 0°C and 12 h at room temperature, the reaction solution was evaporated. The crude product was purified by gel permeation chromatography (eluent: pyridine) to yield 0.10 mmol (21%) of **3**. MS (MALDI-TOF) found for $[M+H]^+$, 2983.5, calcd 2982.2; λ_{abs} (pyridine) 431 (100), 516 (2.13), 561 (4.46), 584 (2.35), 643 (0.78) nm; λ_{em} (pyridine) 624 (25.4), 654 (100), 719 (51.2) nm.

14. Synthesis of **4**: **3** (5.5 μmol) and **2** (275 μmol) were dissolved in 3 mL of pyridine. Then, pyridine was removed under reduced pressure to organize **3** into **4** in the presence of excess **2**. Dissolution of the residue in $CHCl_3$ afforded heptaporphyrin **4** and dimer of porphyrin **2**, and gave two peaks by gel permeation chromatography (GPC) using $CHCl_3$. First peak was isolated by GPC using a $CHCl_3$ eluent to yield 0.57 μmol (10%) of **4**. Absorption spectrum of **4** in $CHCl_3$ was expressed reasonably by the sum of one unit of **1** and three units of **2** (Fig. 1). λ_{abs} (pyridine) 419 (100), 438 (93.8), 518 (3.84), 565 (7.31), 622 (5.24), 649 (0.73) nm; λ_{em} (pyridine) 628 (68.9), 652 (100), 715 (49.6) nm.

15. Consideration about the Fluorescence Intensity of the Fb porphyrin in Fig. 2: When no pathway of fluorescence quenching exists, the fluorescence intensities of Zn and Fb porphyrins are expressed by Eq. (1).

$$F_{Zn} = Ir\varphi_{Zn},\ F_{Fb} = I(1-r)\varphi_{Fb} \quad (1)$$

The fluorescence intensity of Zn porphyrin is decreased to F'_{Zn} by the presence of quenching pathway.

$$F'_{Zn} = Ir(1-\alpha)\varphi_{Zn} \quad (2)$$

Their ratio, $f_{Zn}=F'_{Zn}/F_{Zn}$ is 0.20 to give $\alpha=0.80$. If the quenching energy from Zn porphyrin is utilized solely for the excitation of Fb porphyrin, the fluorescence intensity of Fb porphyrin F'_{Fb} is calculated by Eq. (3).

$$F'_{Fb}=I(1-r)\varphi_{Fb}+Ir\alpha\varphi_{Fb} \quad (3)$$

Introduction of $r=0.95$ produces the ratio $f_{Fb}=F'_{Fb}/F_{Fb}=16$.

I: Absorption of **4** at 438 nm, r: fractional absorption of Zn porphyrin at 438 nm = 0.95, α: fluorescence efficiency of Zn porphyrin, φ_{Zn}: fluorescence quantum yield of Zn porphyrin, φ_{Fb}: fluorescence quantum yield of Fb porphyrin.