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Synthesis and Aggregate Morphology of Protoporphyrin IX Derivative Having Phospholipid Residue

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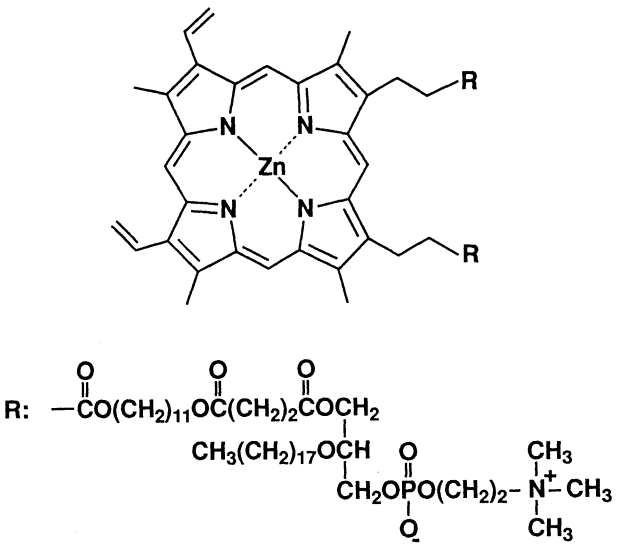
A protoporphyrin IX derivative having a phospholipid residue (lipid-porphyrin) was synthesized. The lipid-porphyrin itself formed an oval multilamellar vesicle in an aqueous medium. The spectral features of the lipid-porphyrin vesicle indicated a stacked configuration of the porphyrin moiety.

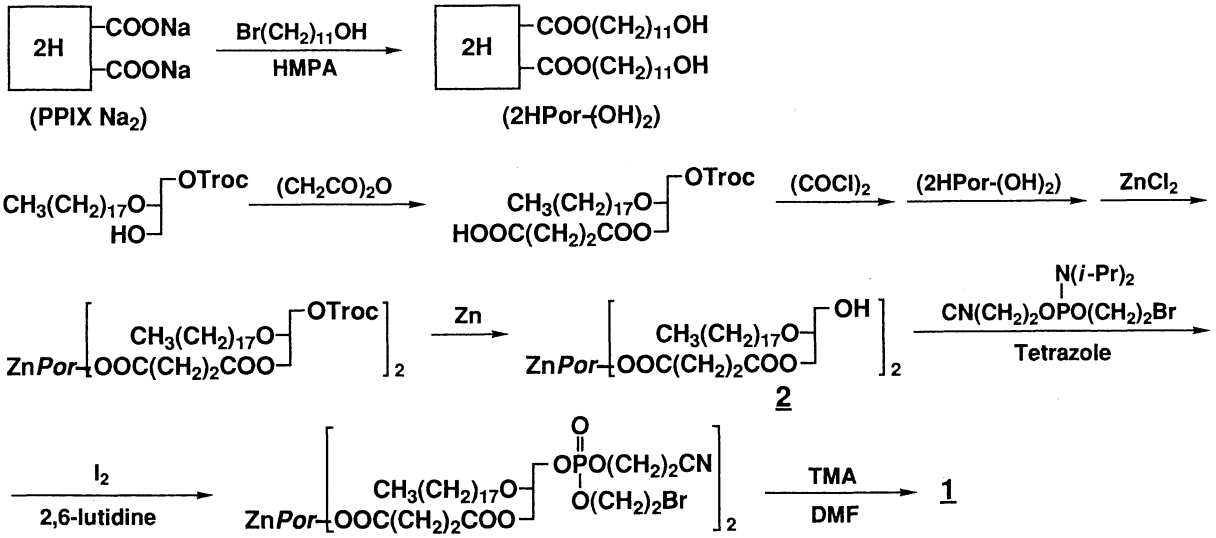
Molecular assembly containing an amphiphilic porphyrin is of current interest in the study of models for hemoproteins, photochemical systems, *etc.*[1-4] If a metalloporphyrin complex, which acts as an active center of various enzyme reactions, itself produces a highly organized aggregate in an aqueous medium, a super molecular assembly including an ordered reactive site with a high density is materialized. At present, however, few examples of self-assembled porphyrins have been reported. Fuhrhop et al. synthesized glycosamide derivatives of protoporphyrin IX (PPIX), which formed micellar fibers having a ribbon structure.[5] The porphyrinatozinc fibers are to be expected for multiple donors of electron in energy device. We also reported vesicles consisting of a tetraphenylporphin (TPP) derivative as a hemoglobin (Hb) model with O_2 binding ability under physiological conditions (pH 7.4, <40 °C).[6] The hydrophobic domain constructed by the assembled TPP derivatives enables the O_2 adduct to be stable against irreversible oxidation through a proton driven process. However, until now an Hb model made up of a molecular assembly consisting of PPIX,



Lipid-porphyrin (**1**)

which is the most important natural porphyrin, has not been reported.

We report herein the synthesis and aggregation morphology of a new PPIX derivative bearing covalently bound dialkylglycerophosphocholine groups: 2,18-bis(11-(3-(2-stearyloxy-3-(trimethylammonio-ethoxy)phosphonatoxy)propanoxycarbonylpropanoyloxy)undecanoxycarbonylethyl)-8,13-divinyl-3,7,12,17-tetramethylporphinatozinc(II), lipid-porphyrin (**1**).



The synthetic route for the lipid-porphyrin is as follows. PPIX disodium salt was suspended in hexamethylphosphoramide (HMPA) with 1-bromoundecanol at 50 °C for 20 h to give 2,18-bis(11-hydroxyundecan-oxycarbonylethyl)-8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine (2H*Por*-$(OH)_2$) (yield: 68%). 1-(2,2,2-Trichloroethoxycarbonyl)-2-stearyloxyglycerol was reacted with succinic anhydride in dry THF with 4-(N,N-dimethylamino)pyridine to afford the dialkylglyceroacid (yield: 51%).[4] Its acid chloride was allowed to couple with 2H*Por*-$(OH)_2$ in dry THF to yield a PPIX derivative bound the lipid moiety (yield: 52%). Insertion of zinc into this porphyrin was accomplished using $ZnCl_2$ in dry THF with 2,6-lutidine at 25 °C. The trichloroethoxycarbonyl (Troc-) group was selectively removed by activated zinc in acetic acid/THF to give the 3-hydroxyglycero derivative (**2**). If removal of the protective groups was carried out for the free-base porphyrin, ring decomposition occurred. Then the hydroxy groups were transformed into phosphocholine groups by an acid-catalyzed coupling with a dialkylphosphoroamidite followed by a one-step deprotection-substitution reaction.[7] An excess of tetrazole was added to a solution of **2** and 2-cyanoethyl-2-bromoethyl (N,N-diisopropylamino)phosphoramidite in dry THF/CH_3CN at 25 °C, and the solution was stirred for 1 h. The

intermediate phosphite triester was oxidized *in situ* by the addition of 2,6-lutidine and a solution of I_2 in aqueous THF. The neutral phosphate could easily be purified using silica-gel column chromatography ($CHCl_3/CH_3OH$:15/1, v/v)(yield: 89%). In the final step, simultaneous removal of the cyanoethyl group and displacement of the bromide were accomplished by heating in DMF solution of trimethylamine (TMA) in a pressure bottle at 60 °C for 14 h. The mixture was purified by gel chromatography on TOYOPEARL HW-40 (benzene/CH_3OH:4/1, v/v) to afford lipid-porphyrin (**1**) (Yield: 31%). **1** was characterized by IR, Vis, and COSY (^1H, ^{13}C) spectroscopy.[8)]

The lipid-porphyrin (**1**) was easily homogenized in deionized water by sonication (70W, 10min) ([**1**] = 5×10^{-6} mol dm^{-3}) to give a transparent red solution. The aggregate morphology was clearly elucidated by transmission electron microscopy (TEM). An aqueous solution of **1** was mixed with 1% uranyl acetate, and a mixed droplet was placed onto a carbon-coated copper grid. The grid was allowed to air-dry for 3 h and was observed in a JEOL JEM-100 CX at an acceleration of voltage 100 kV. The lipid-porphyrin formed an oval multilamellar vesicle with a major axis of 200-250 nm and a uniform thickness of each layer of *ca.* 7 nm (Fig. 1). Since the molecular length of **1** is *ca.* 4 nm, it can be presumed that the vesicle arises from bilayer aggregates of the porphyrin molecules. This is the first example of formation of a vesicle consisting of lipid-porphyrins.

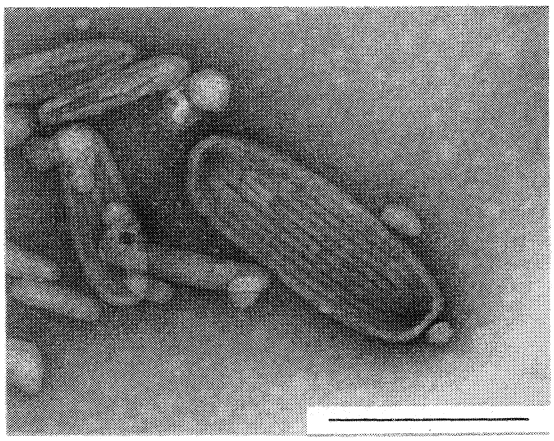


Fig. 1. TEM of multilamellar vesicle consisting of the lipid-porphyrin (**1**) in water (bar: 200 nm).

Visible absorption spectra of the aqueous dispersion of **1** showed a Soret-band (λ_{max}: 394 nm) which was shifted toward the blue region compared to that of a micellar solution containing **1** with Triton X-100 (λ_{max}: 418 nm) (Fig. 2) . A blue-shifted Soret band comes from transition dipole interactions of the stacked configuration of

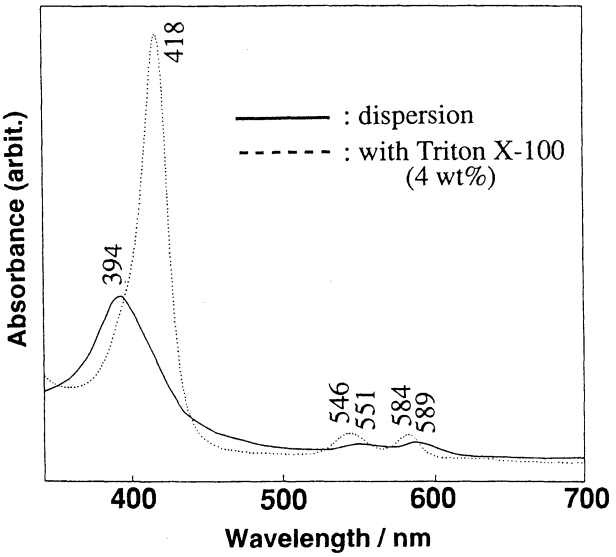


Fig. 2. Visible absorption spectra of the lipid-porphyrin (**1**) in water. [**1**]: 10×10^{-6} mol dm^{-3}.

the porphyrin moiety.[9] Fluorescence of the aqueous dispersion of **1** was completely quenched, also suggesting formation of π–π aggregation. These spectral features of the oval multilamellar vesicle remained essentially unchanged after 6 months and the aggregate did not seem to dissociate upon dilution or heating. Differential scanning calorimetry (DSC) of the **1** dispersion showed a broad endothermic peak at 50-60 °C.

The morphology of the **1** assembly was characterized by its oval form and a large particle size compared to a spherical phospholipid (*e.g.* 1,2-dipalmytoyl-*sn*-glycero-3-phosphocholine, DPPC) vesicle (ϕ; 40-50 nm) prepared by sonication. Because the porphyrin moiety of the lipid-porphyrin is a rigid and hydrophobic unit (0.8x0.8x0.4 nm^3; determined using CPK models), stacking of the porphyrin ring results in the small curvature of the vesicle.

In summary, a new protoporphyrin IX derivative having phospholipid residue was synthesized and its morphology in an aqueous medium was clarified. The lipid-porphyrin (**1**) itself formed an oval multilamellar vesicle with a stacked configuration of porphyrins. The lipid-porphyrin assembly would be useful as a new molecular structure for artificial proteins, photochemical devices, molecular wires, *etc.* Investigation of the O_2 binding ability of a lipid-porphyrinatoiron(II) vesicle is now in progress by the authors.

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8) IR (NaCl) 1734 (C=O), 1246 (P=O), 1090 (P-O-C) cm^{-1}; Vis ($CHCl_3$) $\lambda_{max}(\varepsilon_{relative})$/nm 584(0.92), 545(0.92), 417(10.0); ^1H NMR(400 MHz, $CDCl_3/CD_3OD$) δ 9.8-10.1(4H, m, meso H), 8.3(2H, m, vinyl -CH-), 6.1-6.3(4H, m, vinyl $=CH_2$), 3.9-4.3(24H, m, Por-CH_2-, glycero -CH_2-, -C(=O)OCH_2-, -CH_2OPO(=O)OCH_2-), 3.1-3.7(44H, m, glycero -CH(O)-, -CH_2O-, Por-CH_3, Por-CH_2CH_2C(=O)-, -$CH_2N(CH_3)_3$, 2.6(8H, m, $OOC(CH_2)_2COO$), 0.8-1.3(106H, m, -CH_2-, -CH_3).
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