CHEMISTRY LETTERS, pp. 1953-1956, 1994.

Synthesis of Protoporphyrin IX Derivatives Having Four Amphiphilic and/or Lipophilic Alkyl Chains and Their Dispersing Behavior in Phospholipid Bilayer

Teruyuki KOMATSU, Kazuhiro YAMADA, Satoru YANAGISAWA,
Hiroyuki NISHIDE, and Eishun TSUCHIDA*
Department of Polymer Chemistry, Waseda University, Shinjuku-ku, Tokyo 169

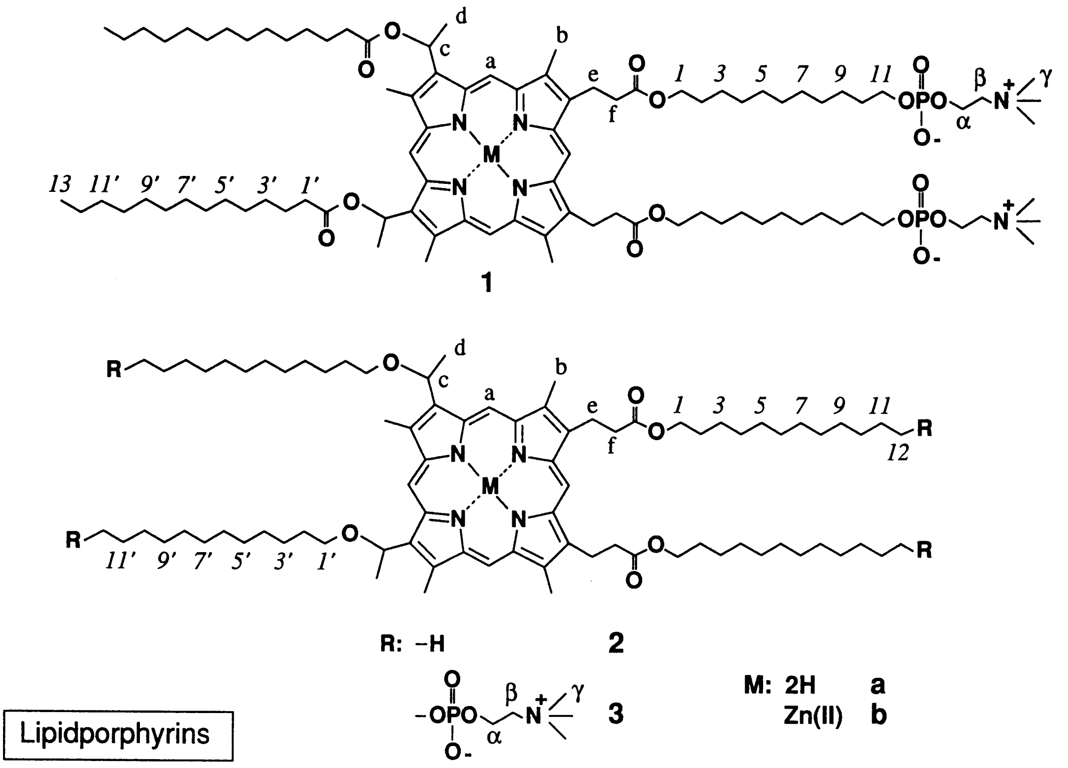
New protoporphyrin IX derivatives having four amphiphilic and/or lipophilic alkyl chains (lipidporphyrins) were synthesized. The lipidporphyrins were efficiently incorporated into the bilayer membrane of phospholipid vesicle without a stacked arrangement. Homogeneous dispersing property of the lipidporphyrins in the bilayer membrane was maintained after polymerization of the phospholipid molecules.

In hemoglobin (Hb) the globin protein protects the protoheme from irreversible oxidation by embedding it into the hydrophobic cavity (heme-pocket) and suppresses the proton-driven and μ-oxo forming oxidation of the O_2-coordinated heme. We have substituted the role of the heme-pocket with the hydrophobic region of the phospholipid bilayer and then designed a phospholipid vesicle embedded tetraphenylporphinatoiron(II) (TPP) derivative as a totally synthetic O_2-carrier under physiological conditions (pH 7.4, 37 °C).$^{1)}$ Besides O_2-transport, the phospholipid vesicle containing TPP derivative is of current interest in the study of models for biomimetic reactions (monooxygenation, energy transduction strategies of respiration *etc.*).$^{2-4)}$ However a remaining problem of this system as a hemoprotein model is using the modified TPP derivative which is non-natural porphyrin having different chemical reactivities, spectral features and bio-compatibility from the protoporphyrin IX (PPIX). Furthermore, the planar porphyrin (*e.g.* PPIX) often produce a π–π stacked aggregate which induces an unfavorable electron transfer leading to irreversible oxidation.

Recently, we have found that PPIX derivatives having four amphiphilic and/or lipophilic alkyl chains (lipidporphyrins) are homogeneously dispersed in the bilayer membrane of the phospholipid vesicle without a stacking. We describe herein the synthesis of new lipidporphyrinatozinc(II) complexes (**1b**, **2b**, **3b**) and their characteristic dispersing behaviors in the assembled phospholipid bilayer in aqueous solution.

The synthetic routes for the lipidporphyrins are shown in Scheme 1. The hematoporphyrin is the most labile of the natural porphyrins derived from PPIX.$^{5)}$ The hematoporphyrin disodium salt ($HPNa_2$) was reacted with 1-bromo-11-(2',2',2'-trichloroethoxycarbonyloxy)undecane in HMPA and then was allowed to couple with tetradecanoyl chloride in dry THF to yield four substituted porphyrin **4a**. Insertion of zinc into **4a** was accomplished using $Zn(AcO)_2$. The 2,2,2-trichloroethoxycarbonyl (Troc) protecting group of **4b** was selectively removed by zinc powder in acetic acid-THF to give **5b**. The hydroxy groups of **5b** were transformed into phosphocholine groups using previously reported procedure to afford **1b**.$^{6)}$

Lipophilic lipidporphyrin **2b** was synthesized as follows: protoporphyrin IX disodium salt ($PPNa_2$) was dissolved into 30 % HBr acetic acid solution and stirred at room temperature to give 8,13-bis(1-bromoethyl)-3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid (BrP). Then the carboxyl groups of the BrP

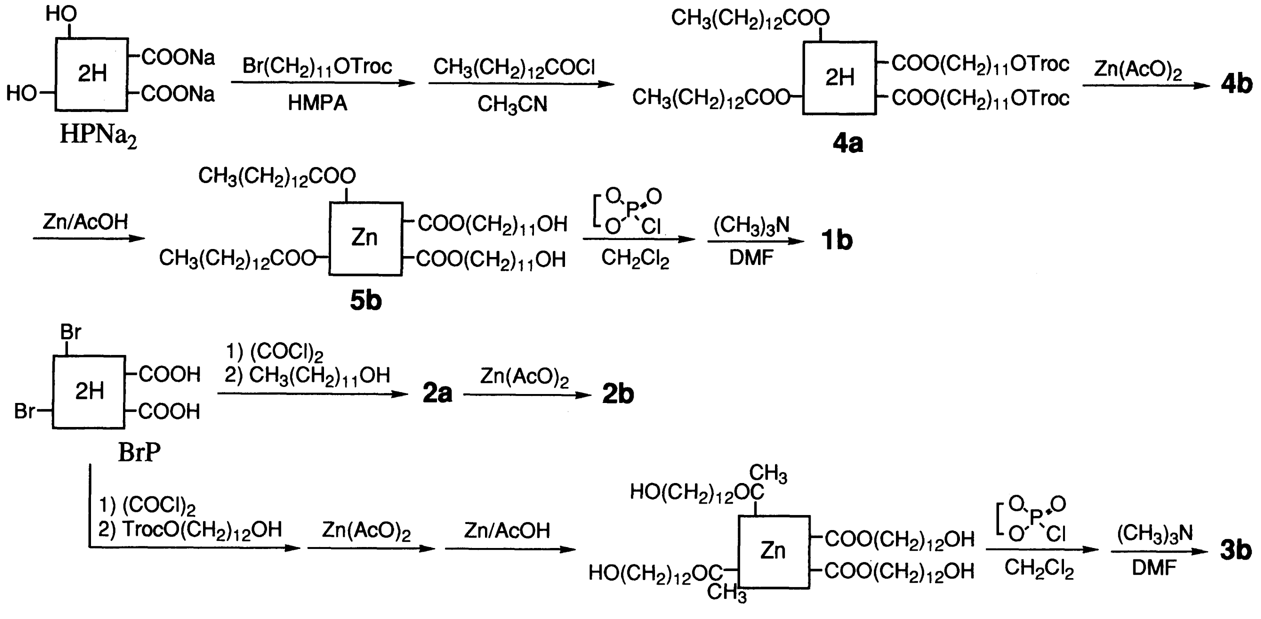


were converted to acid chlorides under argon and the dry CH_3CN solution of 1-dodecanol and 4-(N-dimethylamino)pyridine (DMAP) was added to the crude acid chloride, leading a simultaneous ester and ether formation to give **2a**. Zinc insertion into **2a** was carried out by $Zn(AcO)_2$.

Lipidporphyrin **3b** was synthesized according to the same manner for the preparation of **1b** and **2b**. All porphyrin derivatives were characterized by IR, UV-Vis and ^{1}H-NMR spectroscopy.[7)]

The lipidporphyrins were sonicated with a phospholipid molecule [1,2-bis(parmytoyl)-*sn*-glycero-3-phosphocholine (DPPC)] [lipidporphyrin:DPPC = 1:50 (molar ratio), [Por]= 1×10^{-5} mol dm^{-3}] in deionized water to give a phospholipid vesicle embedded lipidporphyrin solution. Incorporation of the lipidporphyrins in the vesicle was confirmed by gel-permeation chromatography using Sepharose CL-4B and monitored at 412 nm and 255 nm based on the porphyrin derivatives and phospholipid, respectively.[8)] The curves coincided with each other meaning that the lipidporphyrin is included in the phospholipid vesicle; more than 97% of **1b** or **2b** molecule was embedded into the bilayer membrane. From the TEM of these hybrid assemblies containing **1b** or **2b**, only the small unilamellar vesicles with a diameter of 40-50 nm were detected. However, the elusion curves of the phospholipid vesicle embedded **3b** were broad and a micelle fraction was also observed (embedding ratio of **3b** into the phospholipid bilayer: 68%). Lipidporphyrin **3b** is too hydrophilic to embed into the bilayer membrane efficiently and to produce a uniform phospholipid vesicle.

The orientation and stacking behaviors of the lipidporphyrin in the phospholipid assembly were estimated from its visible absorption spectral pattern. The visible absorption spectrum of the phospholipid vesicle embedded **1b** or **2b** appeared at 412 nm and almost same as that of the micellar dispersion with Triton X-100 (4 wt%) containing **1b** or **2b** at 25 °C (Fig. 1). On the other hand, the absorption spectrum of the DPPC vesicle embedded 2,18-bis(11-[(2-trimthylammonio)ethylphosphonatoxy]undecanoxycarbonyl)ethyl-8,13-divinyl-3,7,



Scheme 1.

12,17-tetramethyl-21H,23H-porphinatozinc(II) (lipidporphyrin having two alkylphosphocholine groups; **6b**[11]) showed low absorbance at λ_{max} (412 nm) and a broad shoulder at 390 nm. It is well known that the blue-shifted Soret band comes from transition dipole interaction of the face-to-face stacked porphyrin.[9,10] Although two substituted lipidporphyrin **6b** was incorporated into the DPPC vesicle,[11] its porphyrin moiety tend to be stacked in the bilayer membrane at 25 °C.

These results indicated that **1b** and **2b** were completely embedded into the bilayer membrane of the DPPC vesicle as a monomeric form. That is, introduction of four long alkyl chains to the 2,18- and 8,13-positions of the porphyrin ring and moderate lipophilic character are important factors for dispersing the lipidporphyrin molecule into the phospholipid bilayer membrane without stacking.

Polymerizable phospholipid (1,2-bis(octadecadienoyl)-*sn*-glycero-3-phosphocholine (DODPC)) vesicle embedded **1b** or **2b** [lipidporphyrin : DODPC = 1 : 50 (molar ratio), [Por] = 1×10^{-5} mol dm^{-3}] was polymerized by azobis(2-amidinopropane)dihydrochloride (AAPD) (5 mol% to DODPC) at 60 °C for 12 h under argon atmosphere.[12] The visible absorption spectrum of the poly-DODPC vesicle embedded **1b** or **2b** (polymerization conversion was 59%) was almost same as that of before polymerization (Fig. 1, absorption spectrum of the non-polymerized DODPC vesicle

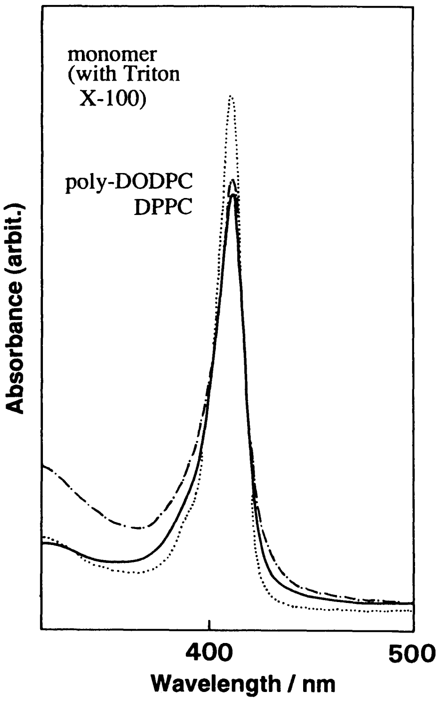


Fig. 1. Visible absorption spectra of the phospholipid vesicle embedded **1b**. [**1b**]/[phospholipid] =1/50 (molar ratio).

embedded **1b** was not shown). This indicated that a phase separation which induces the unfavorable stacking of the porphyrin ring was not occurred even in the polymerization process. The protoporphyrin IX derivatives (**1b** and **2b**) can be fixed independently in the hydrophobic environment of the non-polymerized and polymerized phospholipid vesicle. Study of the O_2-coordination to the lipidporphyrinatoiron(II) embedded into the phospholipid vesicle is now in progress by the authors.

This work was partially supported by a Grant-in-Aid for Scientific Research (No. 05403028, 05236103, and 053666) from the Ministry of Education, Science and Culture, Japan.

References

1) E. Tsuchida and H. Nishide, *Top. Curr. Chem.*, **132**, 64 (1986); E. Tsuchida, T. Komatsu, K. Arai, and H. Nishide, *J. Chem. Soc., Dalton Trans.*, **1993**, 2465.
2) J. T. Groves and R. Neumann, *J. Am. Chem. Soc.*, **111**, 2900 (1989).
3) M. Nango, A. Mizusawa, T. Miyake, and J. Yoshinaga, *J. Am. Chem. Soc.*, **112**, 1640 (1990).
4) N. Nishino, H. Mihara, K. Kobata, and T. Fujimoto, *J. Chem. Soc., Chem. Commun.*, **1993**, 162.
5) R. K. DiNello and C. K. Chang, "The Porphyrins I," ed by D. Dolphin, Academic Press Inc. (1976) p.289.
6) Y. Matsushita, E. Hasegawa, K. Eshima, and E. Tsuchida, *Chem. Lett.*, **1983**, 1387.
7) **1b**; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.8-1.3 (72H, m, *3-9, 3'-13'*), 1.5 (8H, m, *2, 10*), 1.7 (4H, m, *2'*), 2.3 (6H, m, d), 2.5 (4H, m, *1'*), 2.8-3.1 (18H, m, γ), 3.2 (4H, m, f), 3.4 (4H, m, β), 3.5-3.8 (16H, m, b, *11*), 4.0 (8H, m, *1*, α), 4.3 (4H, m, e), 7.5 (2H, m, c), 9.9-10.3 (4H, m, a). IR (NaCl): ν 1734 (C=O (ester)), 1240 (P=O), 1059 (PO) cm^{-1}. UV-Vis. ($CHCl_3/CH_3OH$, 1:1 v/v): λ_{max}= 576, 540, 500, 410 nm. **2b**; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.8-1.9 (92H, m, *2-12, 2'-12'*), 2.3 (6H, d, d), 3.3 (4H, t, f), 3.6-3.8 (12H, m, b), 3.9-4.1 (8H, m, *1,1'*), 4.3 (4H, t, e), 6.2 (2H, s, c), 10.0-10.7 (4H, m, a). FABMS: 1334 M^+. IR (NaCl): ν 1736 (C=O (ester)) cm^{-1}. UV-Vis. ($CHCl_3$): λ_{max}= 571, 534, 404 nm. **3b**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.8-1.8 (80H, m, *2-11, 2'-11'*), 2.3 (6H, d, d), 3.3 (4H, s, f), 3.5 (8H, s, β), 3.6 (12H, m, b), 3.7 (4H, t, *1'*), 3.8 (8H, m, *12, 12'*), 6.1 (2H, s, c), 10.0-10.6 (4H, m, a). IR (NaCl): ν 1732 (C=O (ester)), 1231 (P=O), 1090 (PO) cm^{-1}. UV-Vis. (CH_3OH): λ_{max}= 575, 539, 408 nm.
8) A small amount of phospholipid which contains unsaturated fatty acid residues, 1,2-bis(octadecadienoyl)-*sn*-glycero-3-phosphocholine (DODPC), was added as the UV probe.
9) J. H. van Esch, A.-M. H. Peters, and R. J. M. Nolte, *J. Chem. Soc., Chem. Commun.*, **1990**, 638.
10) B. C. Barber, R. A. Freitag-Beeston, and D. G. Whitten, *J. Phy. Chem.*, **95**, 4074 (1991).
11) E. Tsuchida, T. Komatsu, N. Toyano, S. Kumamoto, and H. Nishide, *J. Chem. Soc., Chem. Commun.*, **1993**, 728
12) H. Ohno, S. Takeoka, H. Iwai, and E. Tsuchida, *Macromolecules*, **22**, 61 (1989).

(Received July 18, 1994)