Lipid–Porphyrin Fibres: Morphology and Incorporation into Phospholipid Vesicle

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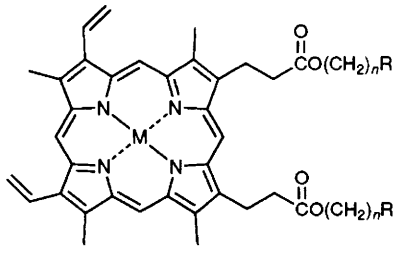
Protoporphyrin IX derivatives having two alkylphosphocholine groups (lipid–porphyrins) forms stable fibrous aggregates in aqueous medium; fibres have been spontaneously incorporated into the bilayer of the phospholipid vesicle.

Self-organized porphyrin aggregate in aqueous medium is a subject of current interest in the study of the artificial protein and redox system for photochemical reactions.[1,2] The advantages of the porphyrin assembly are its precise structure and ordered reactive sites with high density. We previously reported that the phospholipid derivatives of the tetraphenylporphyrin (tpp) complex formed fibres or spherical unilamellar vesicles in deionized water.[1] In particular, tetraphenylporphinatoiron(II) vesicles have the ability to bind reversibly dioxygen under physiological conditions (pH 7.4, 37 °C) as does haemoglobin (Hb). However, a remaining problem of this system, as an Hb model, is utilizing the superstructured tpp derivative, which is not easily prepared and is another non-natural porphyrin. Therefore, the aim of our present investigation is to develop a reversible O_2-binding system for molecular assembly containing the protoporphyrin IX (ppIX) derivative, which is the most important natural porphyrin.

We have recently found that ppIX derivative having two alkylphosphocholine groups (lipid–porphyrin) itself formed a highly organized fibrous aggregate in aqueous medium. This communication describes the synthesis, morphology and dynamic behaviour of lipid–porphyrin fibre. Moreover, the O_2-binding parameter of the phospholipid vesicle embedded lipid–porphyrinatoiron(II) is also reported.

The synthetic route for the lipid–porphyrin derivative is as follows. The protoporphyrin IX disodium salt was suspended in hexamethylphosphoramide with 1-bromohexanol at 50 °C for 20 h to give 2,18-bis(6′-hydroxyhexanoxycarbonylethyl)-8,13-divinyl-3,7,12,17-tetramethyl-21*H*,23*H*-porphine **3a** (yield: 68%). Compound **3a** was phospholylated using 2-chloro-2-oxo-1,3,2-dioxaphospholane and then the resultant phosphate triesters were cleaved by anhydrous trimethylamine in dry dimethylformamide (DMF) to afford **1a** (yield: 88%).[3] Compound **2a** having long alkylchains was prepared from **4a** in the same manner as **1a**. Zinc insertion into the lipid–porphyrin (**1a**, **2a**) was accomplished using Zn(MeCOO)$_2$ in methanol at 60 °C to give the Zn^{II} complex (**1b**, **2b**). Insertion of iron into **3a** and/or **4a** was carried out using the $FeCl_2$ method in dry DMF at 60 °C to afford the iron(III) complex (**3c′**, **4c′**). Lipid-haemin derivatives (**1c′**, **2c′**) were obtained from **3c′** or **4c′** as previously described for **1a** from **3a**. All porphyrin derivatives were characterized by IR, VIS, and COSY (^1H, ^{13}C) spectroscopy.†

The lipid–porphyrins were easily dispersed in deionized water (1×10^{-5} mol dm^{-3}) to give a transparent red solution. The homogeneous dispersion did not change even after storage for one year. The aggregate morphology was elucidated by transmission electron microscopy (TEM). Com-



1; n = 6, R = OPO(CH$_2$)$_2$—N$^+$(Me)$_2$—Me, O$^-$
2; n = 11
3; n = 6, R = OH
4; n = 11

a; M = 2H
b; M = Zn^{II}
c′; M = $Fe^{III}Cl$
c; M = Fe^{II}

† Satisfactory spectroscopic data were obtained for all compounds described.

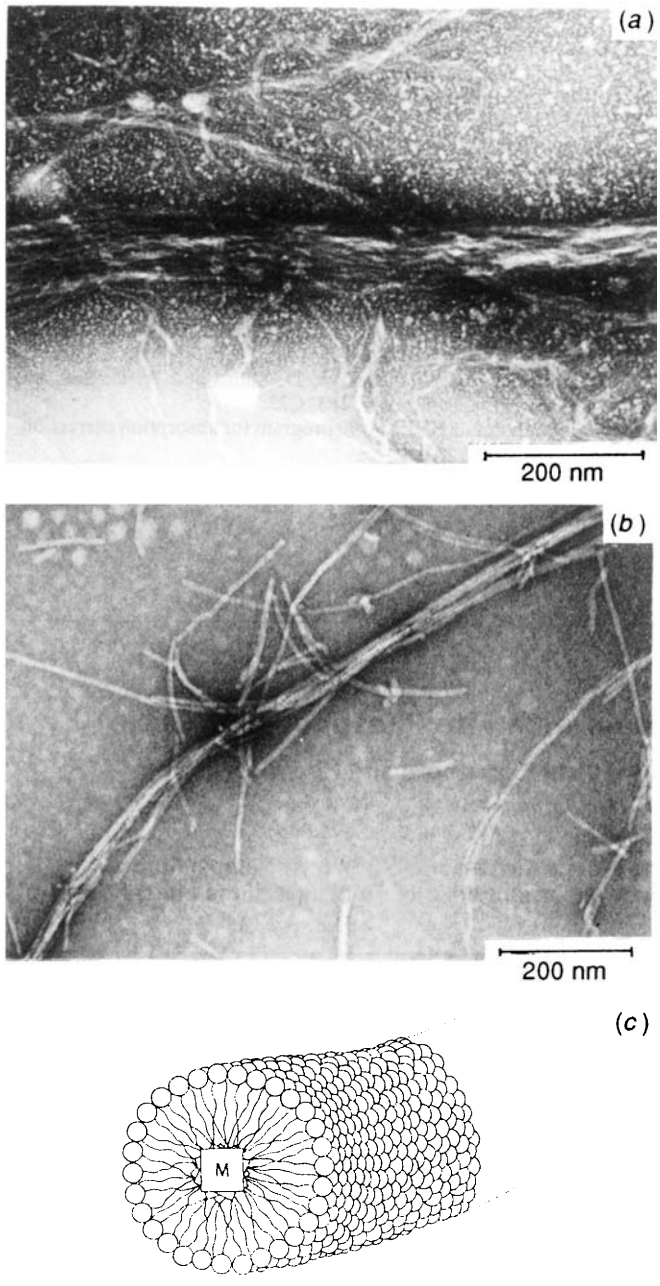


Fig. 1 Transmission electron micrographs of lipid–porphyrinatozinc(II) fibres (*a*) **1b**, (*b*) **2b** in aqueous solution and (*c*) proposed structure of fibres of **2b**

pound **2b** itself produced highly organized rod-like fibres with *ca.* 7 nm widths in dilute aqueous solution [Fig. 1(*b*)]. The length of the fibres varied from 0.2 to 1.5 μm. Since the molecular length of **2b** is *ca.* 3.5 nm, it might be presumed that the fibres arise from the stacked lipid–porphyrinatozinc(II) aggregate. On the other hand, **1b** formed ribbon-like fibres. A few thicker and longer aggregates of many such fibres were also present [Fig. 1(*a*)]. The morphology of the **1b** fibres resembles that of the micellar fibres consisting of protoporphyrin IX [3,17-bis(glycosamides)] previously reported by Fuhrhop *et al.*[2]

VIS absorption spectra of the lipid-porphyrinatozinc(II) fibres showed Soret bands at λ_{max} = 386 nm for **1b** and 389 nm for **2b**, which were shifted toward the blue region compared with that of the micellar dispersion containing monomeric

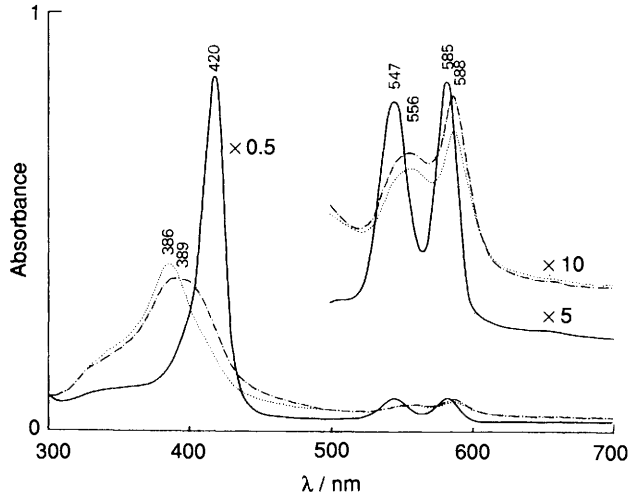


Fig. 2 VIS absorption spectra of lipid–porphyrinatozinc(II) fibres **1b** (. . . . .), **2b** (— · —) and micellar dispersion of **2b** with Triton X-100 (2% m/m) (——) in aqueous medium; [porphyrinatozinc]: 1 × 10^{-5} mol dm^{-3}. Spectra between 500–700 nm are magnified.

form of **1b** or **2b** with Triton X-100 (λ_{max} = 420 nm) (Fig. 2).‡ In general, the blue-shifted Soret band comes from transition dipole interactions of the face-to-face stacked porphyrin configuration.[4,5] Fluorescence of the aqueous dispersion of the lipid-porphyrinatozinc(II) was not present, which also suggested formation of a π-π aggregation. So it can be assumed that the **1b** fibre consists of the connected face-to-face porphyrinatozinc dimers and **2b** having two long alkylphosphocholine groups formed highly organized rod-like fibres in which the porphyrin units might be piled up [Fig. 1(*c*)]. However, considering the relationship between the thickness of the porphyrin plane and the number of the alkylphosphocholine groups, the observed stacking structure of lipid–porphyrin might be twisted as a whole. Analysis on the fine structure of the lipid–porphyrin assembly has been studied continuously through the model calculation and graphics.

The spectral features of the lipid–porphyrinatozinc(II) fibres remained essentially unchanged after a year and were not influenced by the addition of NaCl (*ca.* 0.15 mol dm^{-3}). The fibrous aggregate seems not to dissociate upon dilution or heating. The blue-shifted Soret band changed to a single 420 nm band upon the addition of methanol; the 420 nm Soret band became prominent in water–MeOH [1/1 (v/v)]. These results showed that long-lived lipid-porphyrin fibres did not change their structure even under physiological conditions.

In Hb, the protohaem group is included in the 'haem-pocket' constructed from the globin chain. The O_2-binding site is completely surrounded by the hydrophobic environment of the molecular assembly, *i.e.* apoprotein, which enables the formation of the O_2-adduct, which is stable against irreversible oxidation, through a proton driven process and/or formation of the μ-oxo dimer. Therefore, for the reversible O_2-binding of the protohaem under physiological conditions, the porphyrin needs to be positioned in the hydrophobic domain constructed by the assembled molecules.

To our surprise, the lipid–porphyrin fibres were spontaneously incorporated into the bilayer of the phospholipid [1,2-bis(palmitoyl-*sn*-glycero-3-phosphocholine (DPPC)] vesicle (40 ~ 50 nm). The absorption spectrum of the mixed solution of the **1b** fibre (4 × 10^{-5} mol dm^{-3}) and DPPC vesicle (2 × 10^{-3} mol dm^{-3}) changed from 386 nm to 420 nm within 30 min at 25 °C. From a TEM of the mixed solution of the **1b** fibre

‡ **2b** was dispersed with Triton X-100 (2% m/m) by vortex mixing in deionized water.

and DPPC vesicle, only small unilamellar vesicles with diameters of *ca.* 50 nm were detected. These results indicate that the lipid–porphyrin fibres **1b** were incorporated into the vesicle and the porphyrin molecules were embedded in the bilayer of the membrane. On the other hand, the incorporation of the **2b** fibre into the DPPC vesicle was slower than that of **1b** at 25 °C.

The lipid-porphyrinatoiron(III) (**1c'** or **2c'**) was dispersed with 1-dodecyl-2-methylimidazole (DMIm) and DPPC [**1c'**–DMIm–DPPC (mol ratio) = 1:20:100] by a sonication method in 3×10^{-2} mol dm^{-3} phosphate buffer (pH 7.4) to give a spherical unilamellar vesicle with a diameter of *ca.* 50 nm. Porphyrinatoiron(III) in the DPPC vesicle was reduced by the addition of a small excess of aqueous ascorbic acid under an argon atmosphere. The VIS absorption spectrum of the **1c**–DMIm deoxy complex of the DPPC vesicle embedded **1c** (λ_{max}: 433 and 555 nm) changed to a CO adduct upon exposure to CO (λ_{max}: 419, 539 and 564 nm) The O_2-binding affinity [$P_{1/2}(O_2)$, the O_2 partial pressure at half O_2-binding for the porphyrinato-iron(II)] of the DPPC vesicle embedded lipid-porphyrinatoiron(II), was estimated using laser flash photolysis;[6,7] the $P_{1/2}(O_2)$ was 170 Torr (1 Torr = 133.3 Pa) for **1c** and 140 Torr for **2c** at 25 °C.

In summary, the lipid–porphyrins having only two alkylphosphocholine groups themselves formed highly organized porphyrin fibres in aqueous medium. Interestingly, these porphyrin fibres were spontaneously incorporated into the DPPC vesicle and then the porphyrin molecules were homogeneously embedded into the bilayer of the membrane. Furthermore the $P_{1/2}(O_2)$ of the DPPC vesicle embedded lipid–porphyrinatoiron(II) was 140–170 Torr at 25 °C.

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References

1 T. Komatsu, K. Nakao, H. Nishide and E. Tsuchida, *J. Chem. Soc., Chem. Commun.*, 1993, 728; E. Tsuchida, T. Komatsu, K. Arai and H. Nishide, *J. Chem. Soc., Chem. Commun.*, 1993, 730.
2 J.-H. Fuhrhop, C. Demoulin, C. Boettcher, J. Koning and U. Siggel, *J. Am. Chem. Soc.*, 1992, **114**, 4159.
3 Y. Matsushita, E. Hasegawa, K. Eshima and E. Tsuchida, *Chem. Lett.*, 1983, 1387.
4 C. K. Chang, *J. Heterocycl. Chem.*, 1977, **14**, 1285.
5 C. A. Hunter, J. K. M. Sanders and A. J. Stone, *Chem. Phys.*, 1989, **133**, 395.
6 J. P. Collman, J. I. Brauman, B. L. Iverson, J. L. Sessler, R. M. Morris and G. H. Gibson, *J. Am. Chem. Soc.*, 1983, **105**, 3052.
7 T. G. Traylor, S. Tsuchiya, D. Campbell, M. Mitchell, D. Stynes and N. Koga, *J. Am. Chem. Soc.*, 1985, **107**, 604.