Lipid–Porphyrin Vesicles: Morphology and O_2 Binding in Aqueous Medium

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Amphilphilic tetraphenylporphin derivatives having four dialkylglycerophosphocholine groups on the ring plane (lipid-porphyrins) form spherical unilamellar vesicles of diameter *ca.* 100 nm in water; the vesicles composed of lipid–porphyrinatoiron(II)–1-dodecylimidazole (Dlm), can bind dioxygen reversibly in aqueous medium.

Phospholipid vesicles containing porphyrin complexes have been prepared, as model systems for haemoproteins, and characterized in biomimetic reactions, *e.g.* O_2 transport by haemoglobin (Hb)[1] and regioselective oxidation by cytochrome P-450,[2] and electron transfer reaction in respiratory enzyme system.[3] We have shown that phospholipid vesicles embedded with porphyrinatoiron complexes have the same ability as Hb to reversibly bind dioxygen under physiological conditions (aqueous medium, pH 7.4, 37 °C).[4,5] We succeeded in utilizing the hydrophobic part of the phospholipid bilayer as

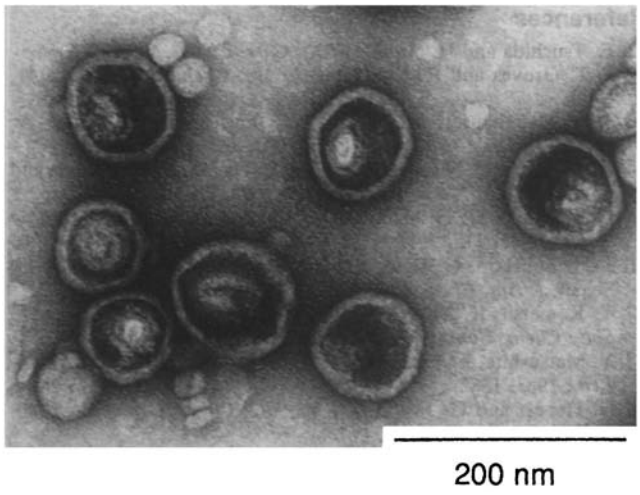
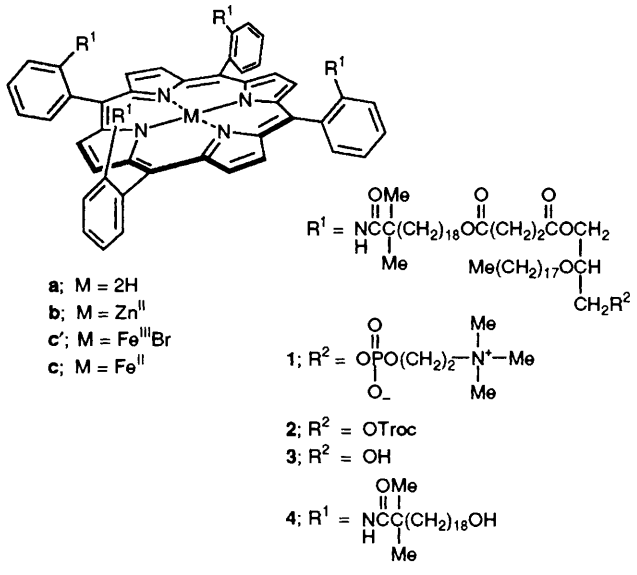


Fig. 1 Transmission electron micrograph of lipid–porphyrin **1b** vesicles in aqueous solution

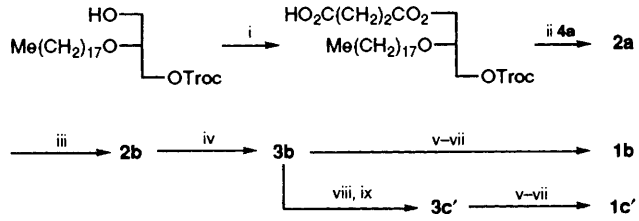


the efficient domain for the haem complex instead of the globin protein, therefore, its O_2 adduct was stable against irreversible oxidation through the proton driven process. However, a remaining disadvantage of this system was its indistinct structure, *e.g.* heterogeneous location of porphyrin molecules in the bilayer.

Self-organized porphyrin assemblies such as micelles or vesicles are topics of current interest. Fuhrhop *et al.* have found that protoporphyrin IX [3,17-bis(glycosamides)] formed long-lived micellar fibres having a ribbon structure.[6] We also elucidated that an eight-substituted tetraphenylporphin (octopus-porphyrin) produces fibrous aggregates in aqueous solution.[7] At present, however, few studies have been reported on self-organized porphyrinatometal assemblies that can mimic certain biological functions. If amphiphilic porphyrins themselves form a spherical vesicle with an intrinsic bilayer structure in aqueous medium, then more accurate and efficient biomimetic reactions would be realized.

More recently, we have found that amphiphilic tetraphenylporphin derivatives having four dialkylphosphocholine groups on one side of the ring plane (lipid–porphyrins) form spherical unilamellar vesicles in water. This paper describes the first example of 'porphyrin vesicle': we report the synthesis, morphology and O_2-binding of the porphyrin vesicle consisting of lipid–porphyrin complexes.

The synthetic route to the lipid-porphyrin derivatives is shown in Scheme 1. 1-(2′,2′,2′-Trichloroethoxycarbonyl)-2-



Scheme 1 *Reagents*: i, $(CH_2CO)_2O$, DMAP; ii, $SOCl_2$; iii, $ZnCl_2$, 2,6-lutidine; iv, Zn–$MeCO_2H$, THF; v, $NC(CH_2)_2P(Cl)(NPr^i_2)(CH_2)_2Br$, tetrazole; vi, I_2, 2,6-lutidine–H_2O, THF; vii, Me_3N; viii, dilute HCl; ix; $FeBr_2$, 2,6-lutidine

stearyloxyglycerol was treated with succinic anhydride in dry tetrahydrofuran (THF) with 4-(*N*,*N*-dimethylamino)pyridine (DMAP) to give the dialkylglyceroacid (yield: 51%). Its acid chloride was allowed to couple with 5,10,15,20-tetrakis-(α,α,α,α-*o*-2,2-dimethyl-20-hydroxyeicosanamido)phenylporphin **4a**[8] in dry THF to yield **2a** (yield: 56%). Insertion of zinc into **2a** was accomplished using $ZnCl_2$ in dry THF with 2,6-lutidine at 25 °C. Then the 2,2,2-trichloroethoxycarbonyl (Troc) protecting group of **2b** was selectively removed by activated zinc in acetic acid—THF at room temperature. The hydroxy groups of compound **3b** were transformed into phosphocholine groups **1b** by an acid-catalysed coupling with a dialkylphosphoroamidite, followed by a one-step deprotection–substitution reaction.[9] Iron porphyrin **1c′** was prepared from **3c′** in the same manner as previously described for **1b** from **3b**. All porphyrins were characterized by IR, VIS, ^1H, ^{13}C and COSY spectroscopy and elemental analysis.†

The lipid–porphyrins were easily dispersed in deionized water by a sonication method ([**1b**] = 1×10^{-5} mol dm^{-3}) to give a transparent red solution. The homogeneous dispersion did not change over six months. The aggregate morphology was clearly elucidated by electron microscopy. The lipid–porphyrins themselves formed a spherical unilamellar vesicle with a diameter of *ca.* 100 nmϕ in dilute aqueous solution (Fig. 1). This is the first successful example of formation of a porphyrin vesicle. The thickness of the membrane was *ca.* 7–8 nm. Since the molecular length of **1b** is *ca.* 3.5 nm, a bilayer structure was conceivable. The particle diameters agreed with the average sizes (94 ± 19 nm) estimated from a light scattering experiment.

Visible absorption spectra of the **1b** vesicles showed a Soret-band (λ_{max} 438 nm) which was shifted toward the red region compared with that of a micelle dispersion containing **1b** with Triton X-100 (λ_{max} 428 nm), whereas the Q-band absorption was not affected. The red-shifted Soret band was attributed to an in-line arrangement of one of the transition moments of the porphyrin molecules.[10,11] This spectroscopic behaviour of the lipid–porphyrin indicates that the porphyrins are oriented approximately in an edge-to-edge configuration. The fluorescence of an aqueous dispersion of **1b** showed a higher intensity compared with that of its micellar dispersion. No fluorescence quenching of the **1b** vesicle suggested that the formation of a 'face-to-face' aggregate did not occur. Differential scanning calorimetry of the **1b** dispersion showed a peak at 56 °C.

Porphyrin vesicles were obtained in a similar manner in a three-fold molar excess of dodecylimidazole (DIm) used as an axial base for the O_2 adduct. The porphyrinatoiron(III) vesicles (**1c′**–DIm, molar ratio: 1:3 in 1 mmol dm^{-3} phosphate buffer, pH 7.4) were reduced by the addition of a small

† Spectroscopic data for **1b**: δ_H (400 MHz, $CDCl_3$–CD_3OD = 2:1, $SiMe_4$) −0.3 (24H, m, CH_3), 0.7–1.3 (260H, alkyl chains CH_2, CH_2CH_3), 1.5–1.6 (16H, OCH_2CH_2, $(C{=}O)OCH_2CH_2$], 2.6 [16H, t, $(C{=}O)CH_2$], 3.2 [36H, s, $N(CH_3)_3$], 3.6–4.3 [52H, m, glycero, $(C{=}O)OCH_2$, OCH_2, $O(CH_2)_2N$], 7.5–8.7 (16H, m, phenyl-H), 8.8 (8H, s, pyrrole H); λ_{max} (benzene–MeOH = 2:1) 594, 557, 518, 486, and 425 nm. Satisfactory elemental analyses were obtained for **1b**.

excess amount of aqueous ascorbic acid under a nitrogen atmosphere. Excess ascorbic acid was removed by gel column separation with Sephadex G-25. The visible absorption spectrum of the iron(II) deoxy complex of lipid–haem vesicles (λ_{max} 434, 536 and 558 nm) changed to that of its O_2 adduct on exposure to O_2 (λ_{max} 432 and 540 nm). The spectrum changed reversibly in response to O_2 pressures. The O_2 adduct changed to the corresponding CO adduct upon bubbling CO gas through the solution (λ_{max} 431 and 544 nm). The O_2-binding affinity [$P_{\frac{1}{2}}(O_2)$; the O_2 partial pressure at half O_2-binding for the porphyrinatoiron(II)] of the lipid-haem vesicle, was estimated to be 43 Torr at 37 °C (1 Torr = 133.322 Pa).

In summary, lipid–porphyrins can themselves produce spherical unilamellar vesicles in aqueous medium and the vesicle **1c** can mimic the O_2-binding property of Hb. Furthermore, these porphyrin vesicles would also be useful as a new molecular structure in biomimetic reactions such as regioselective oxidation by utilizing their ordered bilayer.

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