Monolayered Octopus-porphyrin Vesicle: Microstructure and Oxygen-binding in Aqueous Medium

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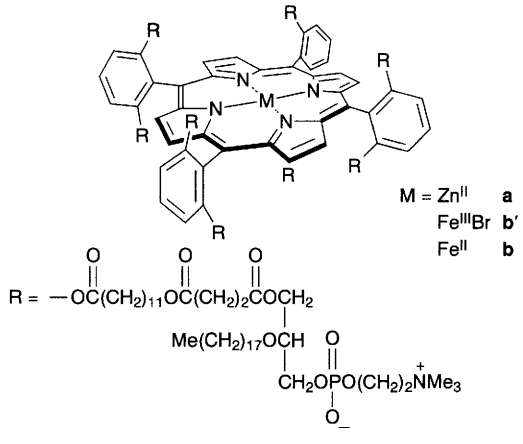
The amphiphilic tetraphenylporphin derivative having four dialkylglycerophosphocholine groups on both sides of the ring plane (octopus-porphyrin) forms spherical monolayered vesicle membranes in water with diameters of *ca.* 100 nm; the vesicle constituted by octopus-porphyrinatoiron(II)/1-dodecyl-2-methylimidazole (DMIm) can reversibly bind dioxygen at 25 °C.

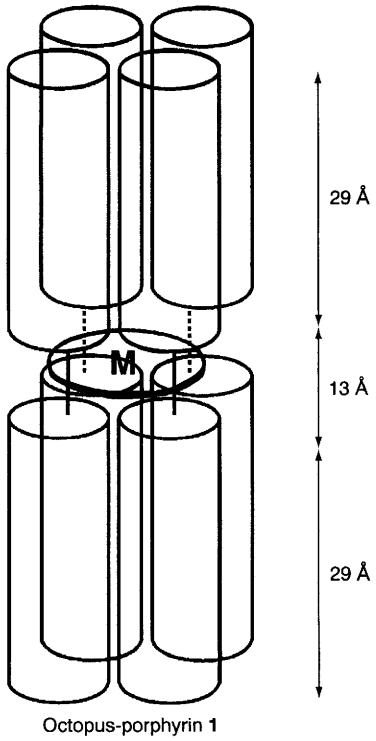
Self-organized porphyrin assemblies in aqueous media are topics of current interest in the chemistry of haemoprotein models, artificial photosynthetic systems and nanoscale molecular devices. We have reported that several amphiphilic porphyrins (lipidporphyrins) form long-lived micellar fibres and uni- or multi-lamellar vesicles;[1,2] aminoethylporphyrin fibre produces multiple charge separation species after laser flash irradiation[3] and lipidporphyrinatoiron(II) bilayer vesicles reversibly form stable dioxygen adducts under physiological conditions (aqueous medium, pH 7.4, 37 °C).[4] The elucidation of the microstructure of these highly ordered porphyrin aggregates is the key to their precise design and regulation of function.

More recently, we have found that tetraphenylporphyrin derivatives having four dialkylglycerophosphocholine groups on both sides of the ring plane (octopus-porphyrins) produce spherical monolayered vesicles in water and the porphyrin moiety in the centre of the membrane is clearly observed by cryoelectron microscopy. This paper describes for the first time the precise microstructure and O_2-binding property of the 'monolayered octopus-porphyrin vesicle'.

The synthetic route for the octopus-porphyrin is as follows. 12-Trityloxydodecanoic acid was coupled with 5,10,15,20-tetrakis[2,6-bis(hydroxy)phenyl]porphine using DCC and DMAP in dry THF to afford 5,10,15,20-tetrakis[2,6-bis(12-trityloxydodecanoyloxy)phenyl]porphine (yield: 58%). This compound was detritylated by BF_3MeOH in CH_2Cl_2, giving the octa[2,6-bis(hydroxydodecanoyloxy)] derivative (yield: 64%). The introduction of monoalkylglycerophosphocholine groups was carried out by our previously reported procedure.[4] Final products (**1a** and **1b′**) were obtained as red–purple and dark-purple solids, respectively. All porphyrins were characterized by IR, UV–VIS, ^{1}H, ^{13}C, COSY spectroscopy and elemental analysis.†

The octopus-porphyrin was easily dispersed in deionized water by sonication ([**1a**] = 1×10^{-5} mol dm^{-3}) to give a clear, red solution. The homogeneous dispersion did not change for several months. The aggregate morphology was elucidated by TEM equipped with a cryosystem. The vitrified specimen was prepared and observed as described elsewhere.[4b] The octopus-porphyrins themselves produced a spherical unilamellar vesicle with a uniform diameter of *ca.* 100 mm [Fig. 1(*a*)]. The particle diameters agreed with the average sizes (88 ± 24 nm) determined from a light-scattering experiment. The thickness of the membrane was estimated to be 70 ± 5 Å [Fig. 1(*b*)], which corresponds to the length of the side chains of **1a** (71 Å). Therefore, this membrane is regarded as a monolayered structure. Surprisingly, a white line with a diameter of *ca.* 15 Å was observed in the middle of the membrane [Fig. 1(*b*)]. This suggested that the electron density of the centre of the membrane was lower than those of the edge areas (29 Å width). Since this vesicle was made of monolayered porphyrins, it indicated that the density of the molecular packing in the centre of the membrane is poor in comparison with those of the oriented alkyl chain moieties. Thus, the white line with the width of *ca.* 15 Å is assumed to be a porphyrin layer with low molecular packing. It is known that tetraphenylporphyrin crystals produce large voids in crystals[5] and that cryoelectron micrographs record density differences in thin layered materials with great sensitivity.[6] We have observed for the first time the location of the porphyrin moiety in the centre of the membrane by cryo TEM.





Octopus-porphyrin **1**

Visible absorption maxima of the Soret band of the **1a** vesicle showed λ_{max} = 424 nm (comparable with that of the benzene/MeOH dispersion of **1a**, λ_{max} = 423 nm). Fluorescence of an aqueous dispersion of **1a** was present with a higher intensity compared with that of its benzene/MeOH non-aggregated solution. These results suggested that the porphyrin moieties of the vesicle formed 'edge-to-edge' aggregates.

The octopus-haem vesicle was obtained in a similar manner, such that the excess molar coexistence of 1-dodecyl-2-methylimidazole (DMIm) was used as an axial base for the O_2-adduct. The **1b′** vesicle (**1b′**/DMIm, molar ratio: 1/20 in 1 mmol dm^{-3} phosphate buffer, pH 7.4) was reduced by the addition of a small excess amount of aqueous $Na_2S_2O_4$ under an Ar atmosphere. The VIS spectrum of the iron(II) deoxy complex of the octopus-haem vesicle (λ_{max}: 437, 531, and 558 nm) showed formation of 5-*N*-coordinated deoxy species and changed to that of its O_2-adduct upon exposure to O_2 (λ_{max}: 432 and 545 nm). The spectrum changed reversibly in response to O_2-pressures. The O_2-adduct changed to the corresponding CO-adduct immediately upon bubbling CO gas through the solution (λ_{max}: 431 and 542 nm). The O_2-binding affinity [$P_{1/2}(O_2)$: the O_2-partial pressure at half O_2-binding for the haem] of the **1b** vesicle was estimated to be 72 Torr at 25 °C. The halflife of the dioxygenated **1b** vesicle was 20 min at 25 °C. Most importantly, it was shown that the octopus-haem lacking dimethyl groups at the bottom of the alkyl chains can form a stable O_2-adduct in an aqueous medium. Until now, rigid groups on the porphyrin plane have been considered necessary for O_2-adduct formation against irreversible oxidation, *e.g.* the dimethyl group on the *ortho*-position of the tetraphenyl rings,[7] phenyl-capped[8,9] and strapped-alkyl chain.[10] However, for **1b**, porphyrin moieties were precisely fixed in the centre of the hydrophobic area of the membrane at 29 Å from the surface, therefore preventing irreversible oxidation through a proton-driven process or μ-oxo dimer formation, even in water. This is the first example of O_2-adduct formation of the tetraphenylhaem with no sterically hindered groups near the O_2-binding site in aqueous solution.

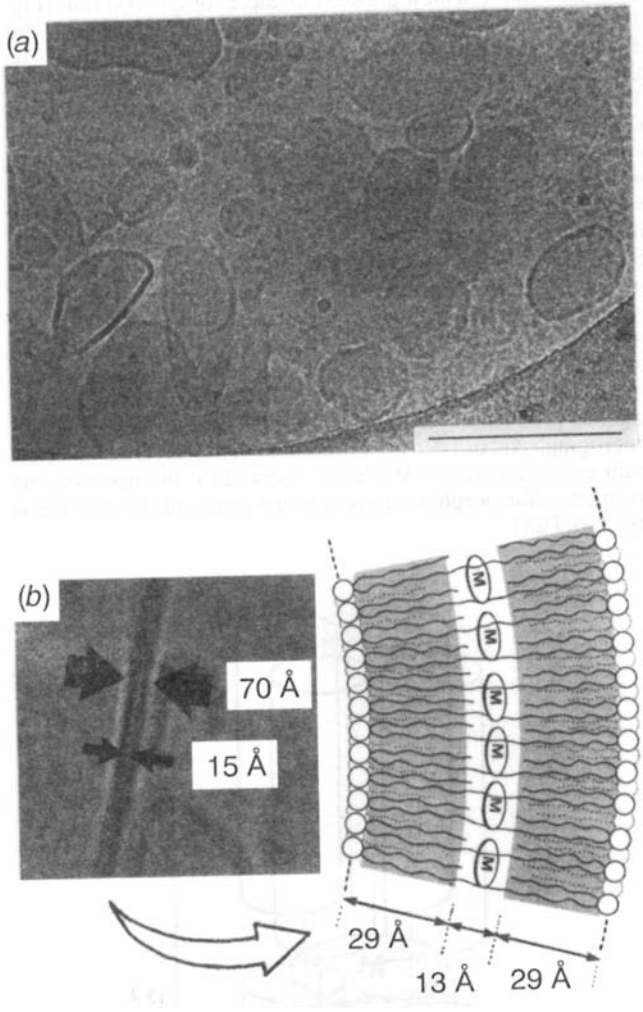


Fig. 1 Cryo TEMs of octopus-porphyrin **1a** vesicle in aqueous solution. [bar: 200 nm]

Table 1 O_2 and CO binding parameters of octopus-haem vesicle in phosphate buffer (pH 7.4, 10^{-6} mol dm^{-3}) at 25 °C[a]

System	$k_{on}(O_2)$/ 10^7 dm^3 mol^{-1} s^{-1}	$k_{off}(O_2)$/ 10^4 s^{-1}	$P_{1/2}(O_2)$/ Torr	$k_{on}(CO)$/ 10^6 dm^3 mol^{-1} s^{-1}
1b/DMIm vesicle[b]	73	8.7	72	4.6
2b/DMIm vesicle[c]	4.4	1.2	160	1.3

[a] Estimated errors < 10%. [b] **1b**/DMIm: 1/20 (molar ratio). [c] **2b**/DMIm: 1/20 (molar ratio). From ref. 2a.

The kinetic parameters for the O_2- and CO-binding to the octopus-haem vesicle, which were explored using laser flash photolysis,[4b,7b] are summarized in Table 1. The O_2-binding affinity of the **1b** vesicle is higher than that of our previously reported octopus-type porphyrinatoiron(II) having dimethyl groups at the bottom of the alkyl chains, 5,10,15,20-tetrakis-{2,6-bis[3,3-dimethyl-4-{1-[{(2-trimethylammonio)ethoxy}-phosphonatoxy]dodecanoxycarbonyl{butyroyloxy]phenyl}-{porphyrinatoiron(II) (**2b**).[2a] This change was kinetically attributed to the large k_{on} value of **1b**. This result agrees quite well the reduction of the steric hindrance around the O_2-binding site that leads to an increase in the k_{on} value in other porphyrinatoiron(II) models.[7a,10]

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† Satisfactory elemental analyses were obtained for **1a** and **1b′**.

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