Octopus-porphyrins: their Assembly and Oxygen-binding in Aqueous Medium

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Amphiphilic tetraphenylporphyrins having four alkyl phosphocholine groups on each side of the ring plane (octopus-porphyrins) form fibrous aggregates in dilute aqueous solution; the vesicles, constituted by iron(II) derivative–1-dodecyl-2-methylimidazole (DMIm), have the ability to bind dioxygen reversibly in aqueous medium.

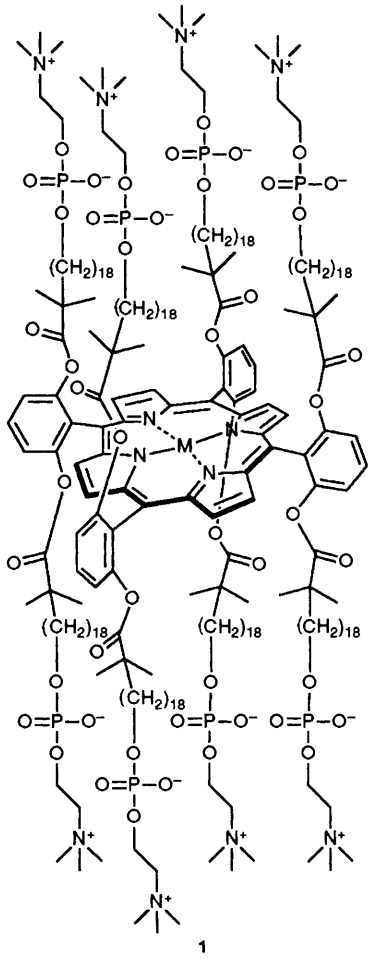
Various metalloporphyrins have been synthesized as models for haemoglobin (Hb) and myoglobin (Mb) and their O_2-binding behaviour studied in detail in aprotic organic solvents.[1–7] In particular, porphyrinatoiron complexes with both faces hindered are considered to be more effective than those with a single hindered face, since the bulky substitutes on each side of the macrocycle impede the formation of an intermolecular μ-oxo dimer leading to irreversible oxidation.[8–11]

We have also found that phospholipid vesicles incorporating the superstructured haems can reversibly bind dioxygen under physiological conditions (aqueous medium, pH 7.4, ≤ 40 °C).[12] The O_2-binding site of the haem derivatives is completely surrounded by the hydrophobic region of the phospholipid bilayer, which stabilizes the O_2 adduct species against irreversible oxidation through a proton-driven process.

However, less attention has been paid to molecular assemblies consisting of amphiphilic metalloporphyrins, *i.e.* porphyrin micelles or vesicles, as synthetic O_2-carriers in aqueous solution. Some amphiphilic porphyrins themselves form stable micellar fibres,[13] however, no study has been reported of the highly organized metalloporphyrin assemblies which can mimic certain biological functions. We have recently found that amphiphilic tetraphenylporphyrins having four alkyl phosphocholine groups on each side of the ring plane (octopus-porphyrins) and their iron derivatives form some self-organized aggregates in aqueous medium as elucidated by electron microscopy. This paper describes the synthesis, aggregate morphology and O_2-binding property of molecular assemblies made of octopus-haems.

The synthetic route for the two octopus-haems 5,10,15,20-tetrakis[2,6-bis{2,2-dimethyl-20-[{(2-trimethylammonio)ethoxy}phosphonatoxy]icosanoyloxy}phenyl]porphyrinatoiron(III) bromide **1b′** and 5,10,15,20-tetrakis{2,6-bis[3,3-dimethyl-4-{1-[{(2-trimethylammonio)ethoxy}phosphonatoxy]dodecanoxycarbonyl}butyroyloxy]phenyl}porphyrinatoiron(III) bromide **2b′** are shown in Scheme 1. 5,10,15,20-Tetrakis[2,6-bis(2,2-dimethyl-20-hydroxyeicosanoyloxy)phenyl]porphyrin **3a** or its iron(III) derivatives **3b′**[14] were phosphorylated and then the resultant phosphate triesters were cleaved by anhydrous trimethylamine in dry dimethylformamide (DMF), to afford **1a** or **1b′** (yield: 65%).[15]

Compound **2a** and its iron(III) derivatives **2b′** were synthesized as follows: 1,12-dodecanediol was monotritylated with trityl chloride and then treated with 3,3-dimethylglutaric acid anhydride, to give the tritylated alkyl acid **4** (yield: 24%). Compound **4** was allowed to couple with 5,10,15,20-tetrakis[2,6-bis(hydroxy)phenyl]porphyrin using *N,N*-dicyclo-



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a; M = 2H

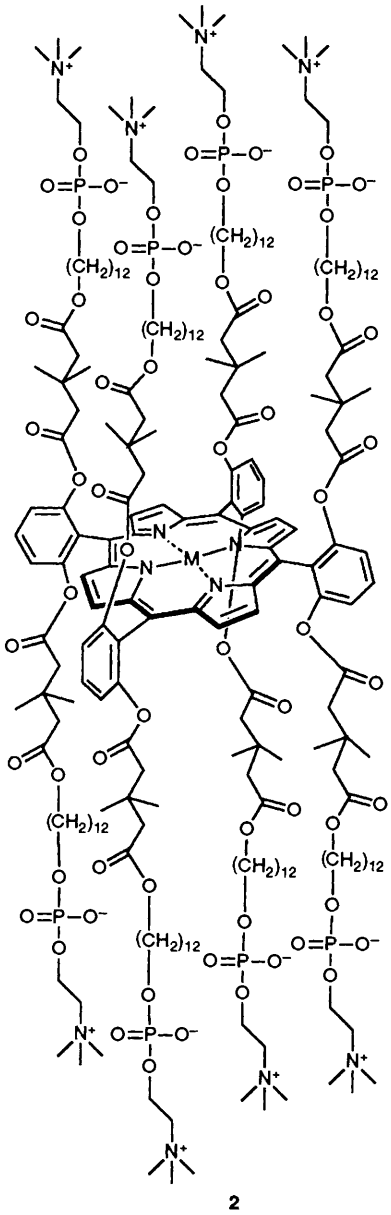
b′; M = $Fe^{III}Br$

b; M = Fe^{II}

Table 1 O_2- and CO-binding parameters of octopus-haem assemblies at 25 °C[a]

System	Solvent	$10^{-7}k_{on}(O_2)$/ $dm^3\,mol^{-1}\,s^{-1}$	$10^{-3}k_{off}(O_2)$/ s^{-1}	$P_{1/2}(O_2)$/ Torr	$10^{-5}k_{on}(CO)$/ $dm^3\,mol^{-1}\,s^{-1}$	$10^2 P_{1/2}(CO)$/ Torr
2b–DMIm vesicle[b]	p.b.[c]	4.4	12	160	13	4.4
2b–DMIm–DPPC vesicle[d]	p.b.[c]	4.1	7.5	110	20	3.3
2b–DMIm	DMF (10% MeOH)	2.6	22	140	9.5	—

[a] Estimated errors <10%. [b] **2b**–DMIm: 1:20 (mol ratio). [c] p.b.; 1 × 10^{-6} dm^3 mol^{-1} phosphate buffer (pH 7.4). [d] **2b**–DMIm–DPPC: 1:20:5 (mol ratio).



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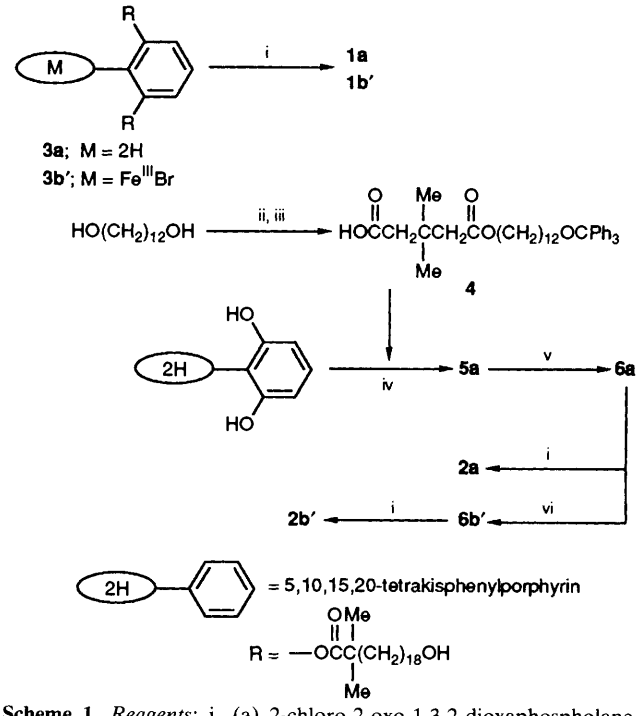
a; M = 2H
b′; M = $Fe^{III}Br$
b; M = Fe^{II}

hexylcarbodiimide (DCC) and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) in dry tetrahydrofuran (THF) to yield **5a** (yield: 56%). Compound **5a** was detritylated by BF_3MeOH in CH_2Cl_2 to give **6a** (yield: 75%). Insertion of iron into the **6a** was accomplished using the $FeBr_2$ method and refluxing in dry THF. Final products (**2a** and **2b′**) were obtained from **6a** or **6b′** in the same manner as previously described for **1a** from **3a**. All



Scheme 1. *Reagents*: i, (a) 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N, (b) Me_3N; ii, Ph_3CCl, pyridine; iii, 3,3-dimethylglutaric acid anhydride, DMAP; iv, DCC, DMAP; v, BBr_3CH_3OH; vi, $FeBr_2$, 2,6-lutidine

porphyrins were characterized by IR, UV–VIS, ^1H, ^{13}C NMR spectroscopy and elemental analysis.†

Octopus-porphyrins were easily dispersed in deionized water by vortex mixing ([porphyrin] = 1 × 10^{-4} mol dm^{-3}) to give a transparent, red solution. The homogeneous dispersion did not change for several months or longer. The critical micelle concentration (cmc) of octopus-porphyrins was *ca.* 1 × 10^{-6} mol dm^{-3} as estimated using the Wilhelmy method. Above this concentration, the aggregate morphology was clearly elucidated by electron microscopy.[13,16,17] Compound **1a** in dilute aqueous solution formed fibrous aggregates with uniform widths of *ca.* 6 nm (Fig. 1). Since the molecular length of **1a** is *ca.* 5 nm, it can be presumed that the fibres arise from the edge-to-edge aggregates of individual porphyrin molecules.

The VIS absorption spectra of the aqueous suspension of **1a** showed a Soret band (λ_{max}: 419 nm), which was shifted toward the red region compared with that of the micelle dispersion containing **1a** with Triton X-100 (λ_{max}: 413 nm). In contrast, the Q-band spectral feature did not change. The red-shifted Soret band was attributed to an in-line arrangement of one of the transition moments of the porphyrin molecules.[13,18,19] This spectroscopic behaviour also suggested edge-to-edge arrays of the octopus-porphyrins. The fluorescence of an

† Satisfactory elemental analyses were obtained for **1a**, **1b′**, **2a** and **2b′**.

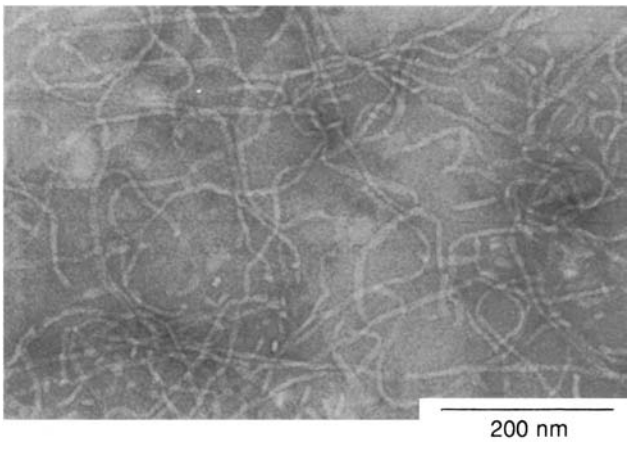


Fig. 1 Transmission electron micrograph of octopus-porphyrin **1a** aggregates in aqueous solution

aqueous dispersion of **1a** was present at the same intensity as that of its micellar dispersion. No quenching for fluorescence of the octopus-porphyrin assemblies showed that four alkyl amphiphilic chains on each side of the porphyrin plane impede the formation of face-to-face dimers.

The morphology of the octopus-porphyrin assembly was transformed into a totally different structure by adding alkyl imidazole and/or phospholipid derivatives. Aggregates of **2b′** were dispersed with a 20-fold molar excess of 1-dodecyl-2-methylimidazole (DMIm) by vortex mixing in 1×10^{-3} mol dm^{-3} phosphate buffer (pH 7.4), to give spherical unilamellar vesicles with a diameter of *ca.* 100 mm φ. The aggregate morphology of **2b′**–DMIm–1,2-bis(hexadecanoyl)-*sn*-glycero-3-phosphocholine (DPPC) (mol ratio: 1:20:5) prepared by sonication method in 1×10^{-3} mol dm^{-3} phosphate buffer also consisted of a spherical unilamellar vesicle (*ca.* 100 nm). These particle diameters agreed with the average sizes estimated from a light-scattering experiment.

Porphyrinatoiron(III) in the assembly was reduced by the addition of a small excess of aqueous ascorbic acid under a nitrogen atmosphere. The VIS absorption spectrum of the iron(II) deoxy complex of the octopus-haem vesicle (**2b**–DMIm, mol ratio: 1:20)(λ_{max}: 436, 535 and 558 nm) changed to an O_2-adduct upon exposure to O_2 (λ_{max}: 421 and 545 nm). The spectrum changed reversibly in response to O_2 pressures. The O_2-adduct changed to the corresponding CO-adduct on bubbling CO gas through the solution (λ_{max}: 422 and 535 nm). The O_2- and CO-binding parameters of the **2b** aggregates were determined using laser flash photolysis (Table 1).[3b,4,11a] The O_2 binding affinity [$P_{1/2}(O_2)$, the O_2 partial pressure at half O_2-binding for the porphyrinatoiron(II)] of the octopus-haem vesicle was estimated to be 160 Torr at 25 °C (1 Torr = 133.322 Pa), which is almost the same as that of **2b**–DMIm complex in DMF solution [$(P_{1/2}(O_2)$: 140 Torr]. Our results show that the molecular assemblies composed of octopus-haem complexes can mimic O_2-binding properties of Hb. This is to be linked to a new molecular architecture in biomimetic system.

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