

Oxygen Binding to β -Alkylimidazolyl-meso-tetra($\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamido-phenyl)porphinatoiron(II) Embedded in a Phospholipid Bilayer under Physiological Conditions

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2-Mono(1- $\{N$ -[3-(2-methylimidazol-1-yl)propyl]carbamoyl}-2-(N -hexadecylcarbamoyl)ethyl-*trans*-acrylamido)-5,10,15,20-tetra-($\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)porphinatoiron(II) (**1a**) was synthesized and solubilized in a physiological solution with phospholipid; the oxygen-binding rate constant and affinity were similar to those of erythrocytes in suspension.

Recently we have found that the 5,10,15,20-tetra($\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)porphinatoiron(II) complex (**2**) of mono(1-dodecyl-2-methylimidazole) embedded in a liposome of phosphatidylcholine binds molecular oxygen reversibly under physiological conditions (pH 7.0 aqueous medium, 37 °C) and the oxygen-binding affinity and rate parameters were similar to those of hemoglobin in blood.^{1,2} It was considered that the porphinato iron complex was embedded in a bilayer of the liposome and that the hydrophobic environment of the inner region of liposome protected the oxygen adduct from its irreversible oxidation. In the present communication, we

synthesized a new and lipophilic porphinato iron complex (**1a**), to corroborate the role of the liposome-embedded (**2**) as an oxygen carrier under physiological conditions. The coordination equilibrium of the porphinato iron with imidazole is less important in (**1a**) since the lipophilic part of the molecule is covalently attached to the porphyrin. Compatibility of the porphyrin with phospholipid and oxygen-binding ability are also expected to be much improved. The oxygen-binding affinity and rate parameters of the liposome-embedded (**1a**) were measured and discussed in comparison with those for erythrocytes.

t-Butoxycarbonyl-L-aspartic acid β -benzyl ester was treated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide and with 1-(3-aminopropyl)-2-methylimidazole to give the ester (3). The ester (3) was debenzylated and then coupled with hexadecylamine to yield the amide (4); δ_{H} (100 MHz, CDCl_3 , SiMe_4 standard) 0.88 (t, 3H, $[\text{CH}_2]_{15}\text{Me}$), 1.25 (s, 28H, $\text{CH}_2[\text{CH}_2]_{14}\text{Me}$), 1.48 (br., 2H, $\text{CH}_2[\text{CH}_2]_{14}\text{Me}$), 1.96 (q, 2H, $\text{CONHCH}_2\text{CH}_2\text{CH}_2$), 2.38 (s, 3H, imidazole-Me), 2.60 (d, 2H, $>\text{CHCH}_2\text{CONH}$), 3.20 (br. m, 4H, $>\text{CHCONHCH}_2\text{CH}_2\text{CH}_2$ -imidazole, NH_2), 3.60 (t, 1H, $>\text{CH}$), 3.90 (t, 2H, CH_2 -imidazole), 6.07 (t, 1H, CONH), 6.85 (d, 1H, imidazole-4- or 5-H), 6.89 (d, 1H, imidazole-5- or 4-H), and 7.65 (t, 1H, CONH); m/z 477 (*M*). 5,10,15,20-Tetra($\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)-porphinatocopper(II) was formylated,^{3,4} and then treated with malonic acid in the presence of piperidine (Knoevenagel condensation) to give compound (5). Compound (5) was demetallated to give the corresponding free base, which was treated with (4) to give (1b); δ_{H} (100 MHz, CDCl_3 , SiMe_4 standard) -2.48 (s, 2H, pyrrole-NH), -0.02—0.29 (m, 36H, Bu^t), 0.86 (t, 3H, $[\text{CH}_2]_{15}\text{Me}$), 1.25 (s, 28H, $\text{CH}_2[\text{CH}_2]_{14}\text{Me}$), 1.50 (br. s, 2H, $\text{CH}_2[\text{CH}_2]_{14}\text{Me}$), 2.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$ -imidazole), 2.40 (s, 3H, imidazole-Me), 2.60 (m, 2H, $>\text{CHCH}_2\text{CONH}$), 3.22 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCOCH}$), 3.90 (t, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$ -imidazole), 4.84 (m, 1H, $>\text{CH}$), 6.24 (t, 1H, CONH), 6.54 (d, *J* 16 Hz, 1H, *trans*- $\text{CH}=\text{CHCONH}$), 6.44 (t, 1H, CONH), 6.80 (s, 2H, imidazole 4- and 5-H), 6.90—9.20 (m, 28H, *trans*- $\text{CH}=\text{CHCONH}$, C_6H_4 , porphyrin ring H, and CONH), and 8.36 (t, 1H, *trans*- $\text{CH}=\text{CHCONH}$). Compound (1b) was treated with FeBr_2 to give (1a); m/z 1594 (*M* + 1), λ_{max} (toluene) oxi: 424 and 578, deoxy: 442, 525, and 567, oxy: 427 and 558, CO adduct: 428 and 556 nm.

Compound (1a) and L- α -dimyristoylphosphatidylcholine (DMPC) were ultrasonicated to give a liposome dispersion as

reported in ref. 1. Unilamellar vesicles of diameter 30—40 nm were observed by transmission electron microscopy. Compound (1a) was more efficiently incorporated into the DMPC liposome than (2) was; 50 mol of DMPC were sufficient to solubilize 1 mol of (1a) completely in water, while more than 200 mol were necessary for (2). This could be explained by the high compatibility or affinity of (1a) for the lipid bilayer which is caused by the introduction of the hexadecyl group into the porphinato iron.

The spectrum of the liposome-embedded (1a) (Fe^{II}) showed u.v. and visible maxima (ϵ 8.23×10^4 , 4.20×10^3 , and $7.56 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 443, 525, and 567 nm, respectively) which almost coincided with the spectrum of the five-coordinate deoxy porphinato iron complex. Furthermore, the absorption spectrum was not influenced by the concentration of (1a) and DMPC. These results suggest that the coordination equilibrium between the porphinato iron and the 2-methylimidazole was completed for (1a) intramolecularly and was essentially unaffected by the presence of the lipid.

The spectrum of the deoxy (1a) changed to that assigned to the oxygen adduct (λ_{max} 428 and 558 nm) on exposure to oxygen. The spectrum of the oxygen adduct changed to that of the CO adduct (λ_{max} 429 and 556 nm) when carbon monoxide was bubbled through the solution and returned to that of deoxy-heme on bubbling nitrogen. The oxy-deoxy cycle could be repeated dozens of times under physiological conditions: degradation of the oxygen adduct of the liposome-embedded (1a) could be neglected during the following experiments. The oxygen-binding affinity ($p_{1/2}$: oxygen pressure at half oxygen-binding for the porphinato iron) of the liposome-embedded (1a) was determined by oxygen-binding equilibrium curve measurement (Table 1). The $p_{1/2}$ value of the liposome-embedded (1a) is 41 mmHg at 37 °C and close to that of hemoglobin in blood,^{6,10} but considerably different from that

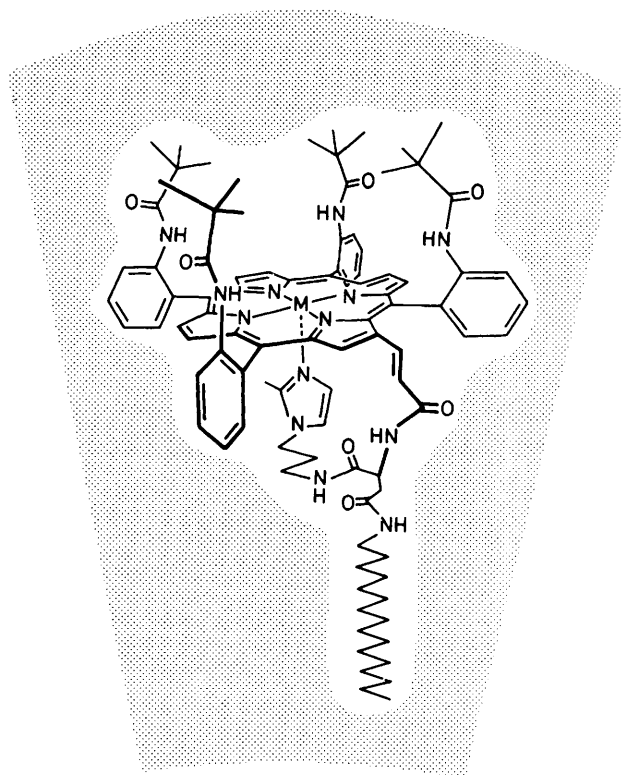
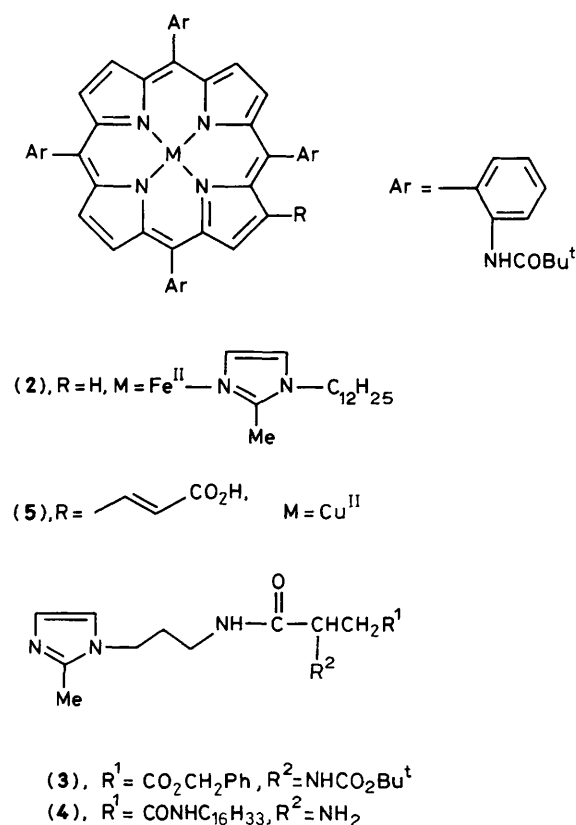


Table 1. Oxygen-binding rate parameters at 25 °C and affinity at 37 °C of liposome-embedded (**1a**).

Heme	Solvent	Method ^a	$k_{\text{on}}/\text{dm}^3\text{mol}^{-1}\text{s}^{-1}$	$k_{\text{off}}/\text{s}^{-1}$	$K/\text{dm}^3\text{mol}^{-1}$	$p_{1/2}^b/\text{mmHg}$
Liposome-embedded (1a) ^c	pH 7.0	s.f.	7.4×10^3	0.21	3.5×10^4	41
Liposome-embedded (2) ^{c,d}	pH 7.0	s.f.	7.9×10^3	0.32	2.5×10^4	49
Erythrocyte suspension ^e	pH 7.4	s.f.	1.1×10^4	0.16	6.8×10^4	27 ^{e,f}
(1a)	Toluene	f.p.	7.8×10^7	—	—	—
(2)/Me ₂ I ^g	Toluene	f.p.	1.1×10^8	46000	2.3×10^3	38 ^h
Chelated heme ⁱ	pH 7.3	f.p.	2.6×10^7	47	5.5×10^5	—
Myoglobin ^j	pH 7.0–7.4	s.f., f.p.	$1.0\text{--}2.0 \times 10^7$	10–30	$0.67\text{--}1.0 \times 10^6$	0.50–1.0
Stripped hemoglobin ^k	pH 7.0–7.4	s.f., f.p.	3.3×10^7	12–13	$2.5\text{--}2.7 \times 10^6$	0.22–0.36

^a s.f. = stopped flow; f.p. = flash photolysis ^b $p_{1/2}$ Values determined from the oxygen-binding equilibrium curve measurements.

^c Liposome-embedded heme composed of dimyristoylphosphatidylcholine. ^d From refs. 2 and 5. ^e From ref. 2. ^f From ref. 6. ^g From refs. 7 and 8. (**2**)/Me₂I = 5,10,15,20-tetra(α,α,α,α-o-pivalamidophenyl)porphinatoiron(II) complex of mono(1,2-dimethylimidazole). ^h At 25 °C. ⁱ From ref. 9, chelated-heme in pH 7.3 = protoheme N-(3-imidazol-ylpropyl)amide methyl ester solubilized by myristoyltrimethylammonium bromide at 20 °C. ^j From ref. 10, at 20 °C. ^k From refs. 10 and 11 at 20 °C.

of myoglobin.¹⁰ This means that the liposome-embedded (**1a**) has the potential to act as an oxygen carrier under physiological conditions.

The kinetics of the oxygen-binding reaction were studied using a stopped-flow spectrophotometer. Binding and dissociation rate constants (k_{on} and k_{off}) are summarized in Table 1. The $p_{1/2}$ value calculated from the $K (=k_{\text{on}}/k_{\text{off}})$ value is consistent with that determined from the oxygen-binding equilibrium curve. These results support the validity of the measurement. The k_{on} value of the liposome-embedded (**1a**) is similar to that of the erythrocyte suspension. The oxygen-binding reactions for the liposome-embedded (**1a**) and the erythrocyte suspension show the same features. The oxygen-binding rate parameters of (**1a**) in toluene and those given in the literature for homogeneous systems are also listed in Table 1; they are about 10^3 times larger than those of the liposome-embedded (**1a**) and the erythrocyte suspension. Our results show that liposome-embedded (**1a**) could act as a good model under physiological conditions for the oxygen-carrier function of erythrocytes.

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