Oxygen Binding to β-Alkylimidazolyl-*meso*-tetra(α,α,α,α-*o*-pivalamidophenyl)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-(α,α,α,α-*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.

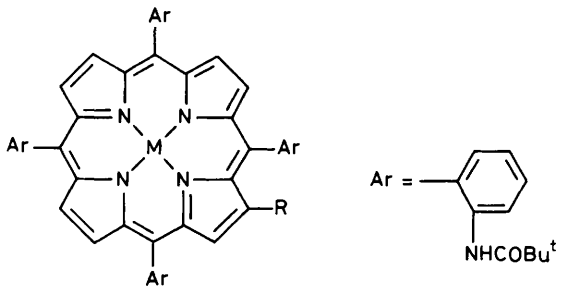
Recently we have found that the 5,10,15,20-tetra(α,α,α,α-*o*-pivalamidophenyl)porphinatoiron(II) complex (**2**) of mono(1-dodecyl-2-methylimidazole) embedded in a liposome of phosphatidylcholine binds molecular oxygen reversibly under physiological conditions (pH 7.0 aqueous medium, 37 °C) and the oxygen-binding 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.

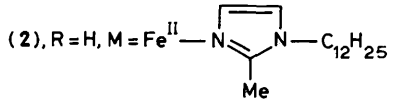
t-Butoxycarbonyl-L-aspartic acid β-benzyl ester was treated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide and with 1-(3-aminopropyl)-2-methylimidazole to give the ester (**3**). The ester (**3**) was debenzylated and then coupled with hexadecylamine to yield the amide (**4**); δ_H (100 MHz, $CDCl_3$, $SiMe_4$ standard) 0.88 (t, 3H, $[CH_2]_{15}Me$), 1.25 (s, 28H, $CH_2[CH_2]_{14}Me$), 1.48 (br., 2H, $CH_2[CH_2]_{14}Me$), 1.96 (q, 2H, $CONHCH_2CH_2CH_2$), 2.38 (s, 3H, imidazole-Me), 2.60 (d, 2H, $>CHCH_2CONH$), 3.20 (br. m, 4H, $>CHCONHCH_2CH_2CH_2$-imidazole, NH_2), 3.60 (t, 1H, >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(α,α,α,α-*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, $[CH_2]_{15}Me$), 1.25 (s, 28H, $CH_2[CH_2]_{14}Me$), 1.50 (br. s, 2H, $CH_2[CH_2]_{14}Me$), 2.00 (m, 2H, $CH_2CH_2CH_2$-imidazole), 2.40 (s, 3H, imidazole-Me), 2.60 (m, 2H, $>CHCH_2CONH$), 3.22 (m, 2H, $CH_2CH_2CH_2NHCOCH<$), 3.90 (t, 2H, $CH_2CH_2CH_2$-imidazole), 4.84 (m, 1H, >CH), 6.24 (t, 1H, CONH), 6.54 (d, *J* 16 Hz, 1H, *trans*-CH=CHCONH), 6.44 (t, 1H, CONH), 6.80 (s, 2H, imidazole 4- and 5-H), 6.90—9.20 (m, 28H, *trans*-CH=CHCONH, C_6H_4, porphyrin ring H, and CONH), and 8.36 (t, 1H, *trans*-CH=CHCONH). Compound (**1b**) was treated with $FeBr_2$ to give (**1a**); *m/z* 1594 (*M* + 1), $\lambda_{max.}$ (toluene) oxi: 424 and 578, deoxy: 442, 525, and 567, oxy: 427 and 558, CO adduct: 428 and 556 nm.

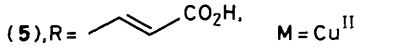
Compound (**1a**) and L-α-dimyristoylphosphatidylcholine (DMPC) were ultrasonicated to give a liposome dispersion as reported in ref. 1. Unilamellar vesicles of diameter 30—40 nm were observed by transmission electron microscopy. Compound (**1a**) was more efficiently incorporated into the DMPC liposome 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.

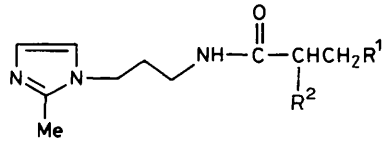
The spectrum of the liposome-embedded (**1a**) (Fe^{II}) showed u.v. and visible maxima (ε 8.23×10^4, 4.20×10^3, and 7.56×10^3 $dm^3\ mol^{-1}\ cm^{-1}$ at 443, 525, and 567 nm, respectively) which almost coincided with the spectrum of the five-coordinate deoxy porphinato iron complex. Furthermore, the 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 ($\lambda_{max.}$ 428 and 558 nm) on exposure to oxygen. The spectrum of the oxygen adduct changed to that of the CO adduct ($\lambda_{max.}$ 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

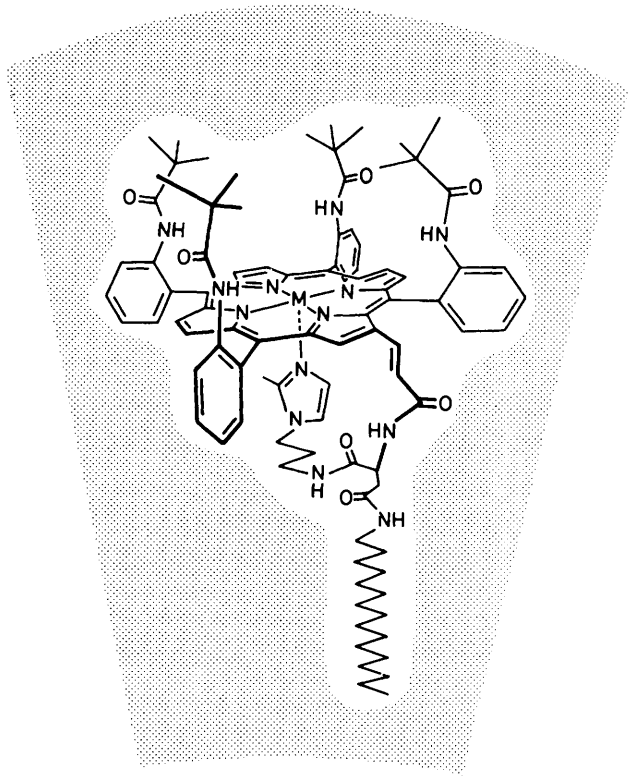








(**3**), $R^1 = CO_2CH_2Ph$, $R^2 = NHCO_2Bu^t$
(**4**), $R^1 = CONHC_{16}H_{33}$, $R^2 = NH_2$



(**1**) **a**; M = Fe^{II}
b; M = 2H

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

Heme	Solvent	Method[a]	k_{on}/dm^{3}mol^{-1}s^{-1}	k_{off}/s^{-1}	K/dm^3 mol^{-1}	$p_{1/2}$[b]/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_2I[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—2.0×10^7	10—30	0.67—1.0×10^6	0.50—1.0
Stripped hemoglobin[k]	pH 7.0—7.4	s.f., f.p.	3.3×10^7	12—13	2.5—2.7×10^6	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**)/Me_2I = 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-l-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 ($=k_{on}/k_{off}$) 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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