Oxygen- and Carbon Monoxide-binding to a Lipophilic Diporphyrinatocopperiron Complex Solubilized in an Aqueous Medium with a Micelle†

Hiroyuki Nishide, Yukio Hashimoto, Hiromi Maeda, and Eishun Tsuchida *
Department of Polymer Chemistry, Waseda University, Tokyo 160, Japan

A tetradecyl-substituted diporphyrinatocopperiron complex was synthesized and solubilized in an aqueous medium with a surfactant. Both the oxygen- and carbon monoxide-binding rate constants decreased due to the steric hindrance of the porphyrinatocopper cap, which brought about a reduced gas-binding affinity in comparison with other synthetic porphyrinatoiron complexes.

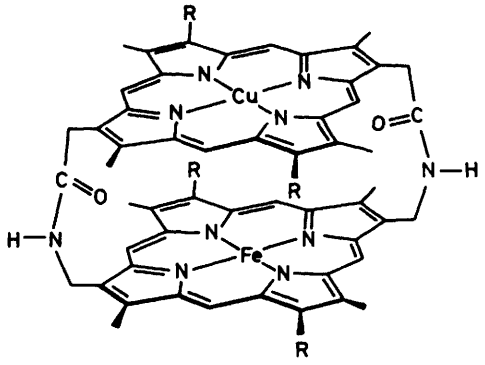
The synthesis of a protoporphyrinatoiron complex with oxygen-binding ability in an aqueous medium is of great interest. Chang and co-workers[1] have reported that the diporphyrinatocopperiron (**1b**)–imidazole complex reversibly forms its oxygen adduct and its half-life is *ca.* 1 week in dry benzene at room temperature.[1] Molecular oxygen binds to the porphyrinatoiron moiety through an opening in the face-to-face structure of the diporphyrin. They proposed that the inert porphyrinatocopper tightly linked to the porphyrinatoiron protects the oxygen adduct. The carbon monoxide-binding affinity of (**1b**) was much reduced and similar to that of hemoglobin in comparison with those of other synthetic porphyrinatoiron(II) complexes, also due to the steric hindering effect of the porphyrinatocopper cap effectively covering the gaseous molecule binding site.[1] We have reported the reversible oxygen-binding and reduced carbon monoxide-binding affinity for the diporphyrinatocopperiron (**1c**)–imidazole complex embedded in polymer films.[2]

We have recently found that a modified and lipophilic 5,10,15,20-tetraphenylporphyrinatoiron(II) complex of 1-dodecylimidazole solubilized with a phospholipid or a surfactant binds molecular oxygen reversibly under physiological conditions (at pH 7 in aqueous medium, 37 °C).[3–7] It was considered that the porphyrinatoiron complex was embedded in a bilayer of the phospholipid or incorporated in a micelle of the surfactant and that the hydrophobic environment of the inner region of the bilayer or the micelle protected the oxygen adduct from its proton-driven irreversible oxidation.

The use of a protoporphyrinatoiron complex having *meso* hydrogens instead of 5,10,15,20-tetraphenylporphyrinatoiron is important for a more accurate model of hemoglobin or red blood cells. The diporphyrinatocopperiron complexes are characterized by having eight *meso* hydrogens, which leads to visible spectra and a porphyrin cleavage reaction which are very similar to those of natural protoporphyrinatoiron IX [3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionatoiron(II)] isolated from hemoglobin. We have also preliminarily reported that a tetradecyl-substituted diporphyrinatocopperiron complex, which increases the compatibility of (**1**) with the hydrophobic region of the micelle and is solubilized in an aqueous medium with a surfactant, forms its oxygen adduct reversibly.[8] This paper describes the synthesis of this lipophilic derivative of the diporphyrinatocopperiron complex (**1a**) and small molecule-binding profiles to (**1a**) solubilized in an aqueous medium in comparison with those of other porphyrinatoiron complexes.

Experimental

The porphyrin complex (**1a**) was synthesized from the dimethyl ester of 3,8,13,18-tetramethyl-7,17-didecylporphyrin-2,12-diacetic acid, (**2**) (Scheme 1). The dimethyl ester (**2**) was synthesized from 4-decyl-5-formyl-3-methylpyrrole-2-carboxylic acid (**3**) and 4-methoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylic acid (**4**). The synthesis of (**3**) was as follows (Scheme 2). Benzyl 3,5-dimethylpyrrole-2-carboxylate (**5**) as the precursor of (**3**) was obtained in the presence of zinc powder (294 g) and sodium acetate (300 g) by the reaction of acetylacetone (134 g) with diethyl hydroxyiminomalonate prepared from diethyl malonate (204 cm^3) and sodium nitrite, followed by benzylation of the 2-ethyl ester. Yield: 73.7 g (0.42 mol), m.p. 106.2—107.1 °C; ^{1}H n.m.r. ($CDCl_3$): δ 2.23 (3 H, s, 5-CH_3), 2.38 (3 H, s, 3-CH_3), 5.38 (2 H, s, –CH_2Ph), 5.92 (1 H, s, 4-H), 7.61 (5 H, s, Ph), and 9.91 p.p.m. (1 H, s, NH); i.r. (KBr): 3 310 (ν_{N-H}), 1 665 ($\nu_{C=O}$), and 1 500 ($\nu_{C=O}$) cm^{-1}.



(**1a**), R = $C_{10}H_{21}$

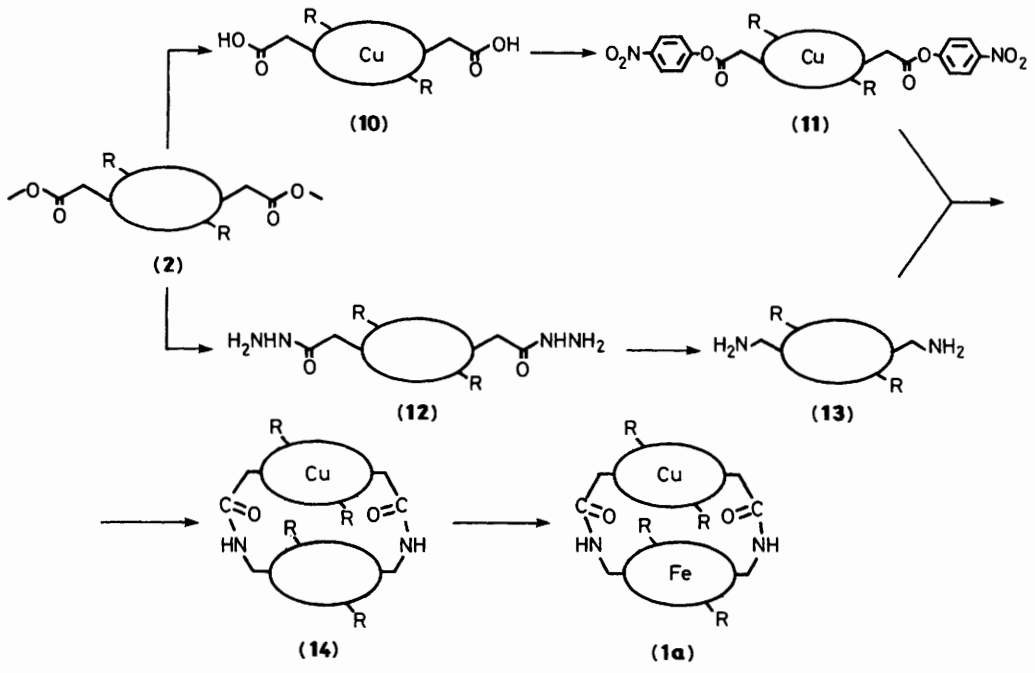
(**1b**), R = C_5H_{11}

(**1c**), R = C_2H_5

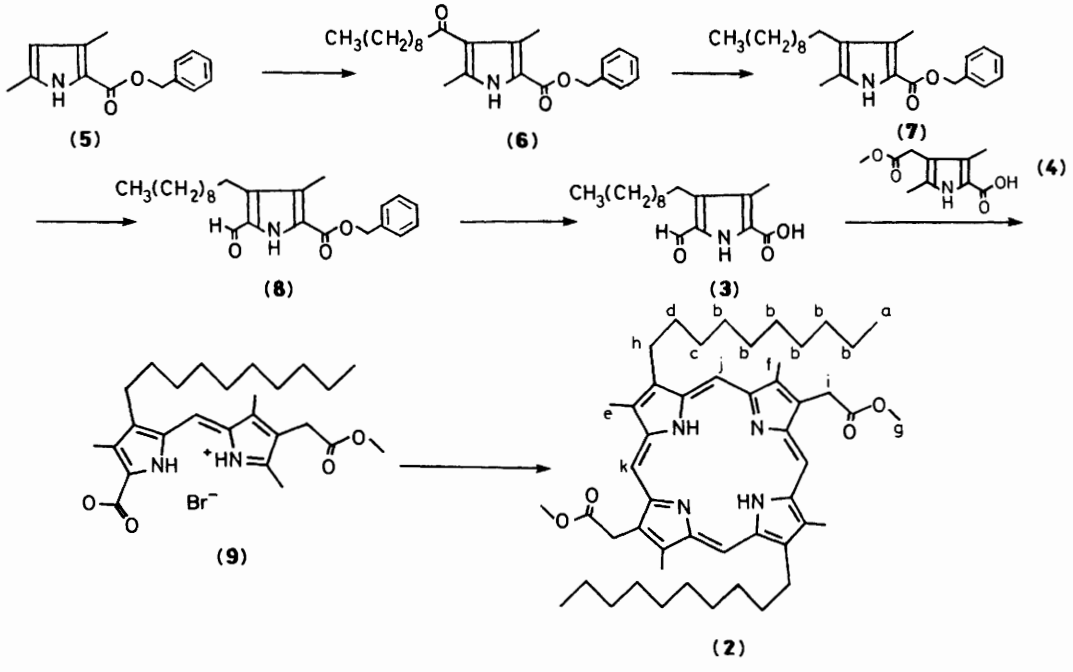
The decyl group was introduced by acylation of (**5**) (70.5 g) with decanoyl chloride (63 g) in dichloromethane–nitromethane (1:1, 600 cm^3) in the presence of anhydrous tin(IV) chloride (50 cm^3), giving (**6**). Yield: 70.1 g (0.182 mol), m.p. 48.1—49.2 °C; ^{1}H n.m.r. ($CDCl_3$): δ 0.88 (2 H, t, –CH_2CH_3), 1.08—2.55 (14 H, m, –CH_2–), 2.61 (3 H, s, 5-CH_3), 2.70 (3 H, s, 3-CH_3), 2.85 (2 H, m, –CH_2CO), 5.38 (2 H, s, –CH_2Ph), 7.61 (5 H, s, Ph), and 9.91 p.p.m. (1 H, s, NH); i.r. (KBr): 3 310 (ν_{N-H}), 2 930, 2 860 (ν_{C-H}), 1 670 ($\nu_{C=O}$, ester), and 1 640 ($\nu_{C=O}$, ketone) cm^{-1}.

Compound (**6**) (70.1 g) was reduced with sodium tetrahydroborate (10 g), giving (**7**). Yield: 65.9 g (0.164 mol), m.p. 59.3—60.7 °C; ^{1}H n.m.r. ($CDCl_3$): δ 0.89 (2 H, t, –CH_2CH_3), 1.09—1.65 (16 H, m, –CH_2–), 2.21 (3 H, s, 5-CH_3), 2.35 (3 H, s, 3-CH_3), 2.37 (2 H, m, 4-CH_2), 5.48 (2 H, s, –CH_2Ph), 7.67 (5 H, s, Ph), and 9.35 p.p.m. (1 H, s, NH); i.r. (KBr): 3 330 (ν_{N-H}), 2 940, 2 860 (ν_{C-H}), 1 670 ($\nu_{C=O}$), and 1 500 ($\nu_{C=O}$) cm^{-1}.

† *Non-S.I. unit employed:* mmHg ≈ 13.6 × 9.8 Pa.



Scheme 1.



Scheme 2.

The 5-methyl group of (**7**) was dichlorinated with sulphonyl chloride (20 cm^3) at high dilution, and the mixture was stirred with water overnight to afford the pyrrole aldehyde (**8**). Yield: 27.3 g (0.071 mol), m.p. 42.5—43.0 °C; ^1H n.m.r. ($CDCl_3$): δ 0.89 (3 H, t, CH_2CH_3), 1.08—1.64 (16 H, m, $-CH_2-$), 2.33 (3 H, s, 3-CH_3), 2.59 (2 H, t, 4-CH_2), 5.48 (2 H, s, $-CH_2Ph$), 7.66 (5 H, s, Ph), 9.53 (1 H, s, NH), and 9.95 p.p.m. (1 H, s, CHO); i.r. (KBr): 3 320 (ν_{N-H}), 1 750 ($\nu_{C=O}$, aldehyde), and 1 670 ($\nu_{C=O}$, ester) cm^{-1}; R_f = 0.8 [$CHCl_3$–CH_3OH (20:1)].

After catalytic debenzylation of (**8**) (8.9 g) with hydrogen in the presence of Pd–C, the free acid (**3**) was mixed with equimolar pyrrole carboxylic acid (**4**) (5.0 g), which was synthesized by the previous method in acetonitrile–methanol (1:1, 150 cm^3). The mixture was treated with 48% hydrobromic acid–acetic acid solution (25 cm^3) at refluxing temperature to afford the dipyrromethene (**9**). After the solvent was removed, the crude dipyrromethene was self-condensed in hot formic acid (25 cm^3) with excess bromine. After re-esterification with

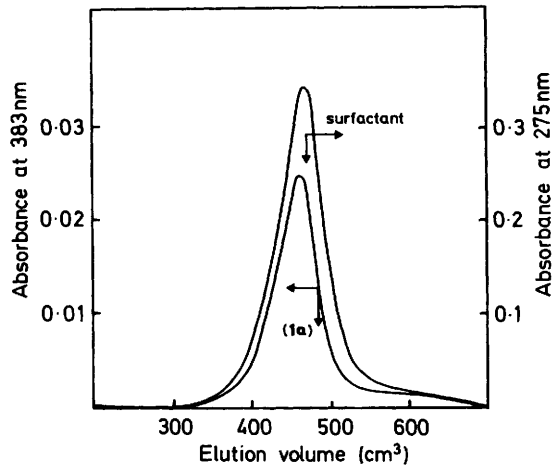


Figure 1. Elution curves of the (**1a**) micellar solution by gel-permeation chromatography detected at 275 nm based on the surfactant and at 383 nm based on (**1a**)

methanol (200 cm^3) and concentrated H_2SO_4 (2 cm^3), the solvent was removed and the residue washed with methanol. The crude product was purified by column chromatography [silica gel, chloroform–methanol (20:1)], followed by recrystallization from chloroform–methanol to give (**2**). Yield: 0.52 g (0.66 mmol); ^1H n.m.r. ($CDCl_3$): δ −3.99 (2 H, s, NH), 0.81 (6 H, t, H^a), 1.2 (24 H, m, H^b), 1.45 (4 H, m, H^c), 2.28 (4 H, m, H^d), 3.59 (6 H, s, H^e), 3.63 (6 H, s, H^f), 3.69 (6 H, s, H^g), 4.06 (4 H, t, H^h), 5.01 (4 H, s, H^i), 10.01 (2 H, s, H^j), and 10.12 (2 H, s, H^k); m/e = 790.

Under an inert atmosphere (**2**) (200 mg) was treated with copper(II) chloride (250 mg) in dimethylformamide at 100 °C, and after washing with water recrystallization from chloroform–methanol gave the copper complex of (**2**) (206 mg). This was hydrolyzed with 2 N aqueous potassium hydroxide (15 cm^3) in a mixture of pyridine and isopropyl alcohol to yield the copper diacid derivative of (**2**). After removing the solvent the residue was dissolved in water, precipitated in concentrated hydrochloric acid, and washed with sufficient water, yielding pure (**10**) (198 mg). Complex (**10**) (198 mg) was then dissolved in dry pyridine and treated with *p*-nitrophenyl trifluoroacetate (1.5 g) at room temperature in the dark. The desired copper active diester (**11**) was obtained after washing with hexane until free from pyridine. Yield: 117 mg (0.102 mmol); i.r. (KBr): 1 760 ($\nu_{C=O}$), 1 520, 1 340 (ν_{NO_2}), and 1 200 (ν_{C-O}) cm^{-1}, R_f = 0.9 [$CHCl_3$–CH_3OH (20:1)].

On the other hand, the diamino derivative of (**2**) was prepared as follows. Compound (**2**) (400 mg) was treated with anhydrous hydrazine (15 cm^3) in refluxing dry pyridine (100 cm^3) in the dark for 40 h. After cooling, the porphyrin dihydrazine derivative (**12**) was filtered off and washed with methanol (380 mg). Compound (**12**) was dissolved in cool acetic acid (200 cm^3), 3 N hydrochloric acid (20 cm^3) was added, and then sodium nitrite (5 cm^3) added dropwise. After adding sodium acetate (80 cm^3), the porphyrin diazide derivative was refluxed in toluene (200 cm^3) to yield the isocyanate derivative. After washing with hydrochloric acid, the resulting mixture was vigorously stirred at reflux to hydrolyze the isocyanate groups. The crude diamino porphyrin (**13**) was obtained from the aqueous layer, redissolved in methanol, and precipitated in ammonium hydroxide to yield pure (**13**) (83 mg) (0.08 mmol). Compound (**13**) (41 mg, 0.023 mmol) was treated with (**11**) (55 mg, 0.023 mmol) in vigorously stirred pyridine (200 cm^3) in the dark. The reaction residue was chromatographed on silica gel with $CHCl_3$–CH_3OH (20:1). The resulting copper(II) diporphyrin (**14**) was recrystallized from toluene. Yield: 48 mg (0.036 mmol); $\lambda_{max.}$ ($CHCl_3$): 383, 515, 544, and 576 nm.

Compound (**14**) (47 mg) was heated at 80—90 °C with iron(II) bromide in dimethylformamide, the resulting residue being chromatographed on neutral almina with $CHCl_3$. Recrystallization from chloroform–hexane gave the iron(II) complex of (**1a**). Yield: 19 mg (0.0074 mmol) (Found: C, 69.6; H, 8.0; Br, 6.6; Cu, 4.2; Fe, 3.5; N, 8.1. Calc. for $C_{94}H_{126}BrCuFeN_{10}O_2$: C, 69.3; H, 7.7; Br, 6.9; Cu, 4.2; Fe, 3.4; N, 8.1%); $\lambda_{max.}$ ($CHCl_3$): 383, 534, and 564 nm.

The iron(II) complex of (**1a**) was prepared as follows. The iron(III) of complex (**1a**) (1.0 μmol) was reduced in the presence of 1-dodecylimidazole (10 μmol) under hydrogen by mixing the benzene solution with Pd–C, and then carbon monoxide gas was bubbled through the mixture. The benzene solution was filtered and the benzene solution of a surfactant such as poly(ethylene oxide) octylphenyl ether, p-$(C_8H_{17})C_6H_4O(CH_2CH_2O)_nH$, (100 mg) saturated with carbon monoxide was added. By evaporating the solvent under reduced pressure, a thin film was prepared on the glass wall of a large round flask. This was dried *in vacuo* for *ca.* 1 h at 90 °C to remove carbon monoxide, giving the iron(II) complex of (**1a**). Oxygen-free phosphate buffer (pH 7.4, 12.5 cm^3) was added, and the mixture was then shaken by a Vortex mixer.

Measurements.—Electronic absorption spectra were measured with a Hitachi UV-320 spectrophotometer. Oxygen- and carbon monoxide-binding affinities [$p_{\frac{1}{2}}$: pressure required for 50% oxygen or carbon monoxide binding for the porphyrinato-iron complex] of (**1a**) solubilized in the phosphate buffer (pH 7.4) solution were determined by spectroscopic measurement of the binding- and dissociation-equilibrium curves as in the previous paper.[8] The binding affinity of n-butyl isocyanide was measured spectroscopically by titrating (**1a**)–1-methylimidazole in benzene solution with the isocyanide solution.

The oxygen- and carbon monoxide-binding reactions were also measured using a pulse flash spectrophotometer (Union Giken RA-401) equipped with a kinetic data processor. Rate constants for the oxygen- and carbon monoxide-binding and dissociation were determined under pseudo-first-order kinetic conditions.

Results and Discussion

The R_f value for (**1a**) was 0.75 [$CHCl_3$–CH_3OH (20:1) on silica gel] and larger than that (0.30) of (**1c**); this demonstrates that the lipophilicity of (**1a**) increases in comparison with (**1c**) as a result of the introduction of the decyl substituents to the porphyrin plane.

The incorporation of (**1a**) in the micelle was confirmed by gel-permeation chromatography (Sepharose 4B column) monitored by the absorptions at 275 and 383 nm based on the surfactant and (**1a**) respectively (Figure 1). The elution curves coincide with each other, indicating that (**1a**) is incorporated in the micelle.

The deoxy (**1a**) solution turned brilliant red on exposure to oxygen. The single peak in the visible absorption spectrum for the deoxy complex ($\lambda_{max.}$ 567 nm) was replaced by two peaks ($\lambda_{max.}$ 534 and 569 nm) assigned to the oxygen adduct, with isosbestic points at 458 and 581 nm (Figure 2). The spectrum of the oxygen adduct changed to that of the carbon monoxide adduct ($\lambda_{max.}$ 530 and 568 nm) on bubbling through carbon monoxide (isosbestic points 455 and 479 nm) and the spectrum of the oxygen adduct returned to that of the deoxy complex on bubbling through nitrogen. These spectra agreed with those previously reported[1] for complex (**1b**) in benzene. The oxy–deoxy cycle could be repeated a hundred times at 37 °C. This is

the first example of a synthetic and non-tetraphenyl-type porphyrinatoiron complex which has been shown reversibly to bind oxygen under semiphysiological conditions (in pH 7.4 aqueous medium at 37 °C).

The same measurements were carried out for (**1a**)–1-methylimidazole {molar ratio [imidazole]/[(**1a**)] = 10:1}. The absorption spectrum of (**1a**) was broadened and irreversibly oxidized on exposure to oxygen, and a large excess (molar ratio > 1000) of 1-methylimidazole was necessary to complete the complexation of the micellar (**1a**) with the imidiazole and to form the oxygen adduct. This is probably because lipophilic 1-methylimidazole is less effectively incorporated and concentrated in the micelle containing (**1a**) in comparison with 1-dodecylimidazole, for example.

The oxygen-binding equilibrium curve (Figure 3) shows that (**1a**) solubilized in an aqueous medium binds molecular oxygen in response to the partial oxygen pressure. The oxygen-binding affinity [$p_{\frac{1}{2}}(O_2)$] of (**1a**) was determined from the equilibrium curve and listed in Table 1 together with reference data. The value of $p_{\frac{1}{2}}(O_2)$ agreed with that previously reported [1] for (**1b**) in benzene and was lower than those of other synthetic porphyrinatoiron complexes (except a capped one). It is significant that (**1a**) in an aqueous medium has a very similar oxygen binding affinity to (**1b**) in a non-polar medium. Normally the

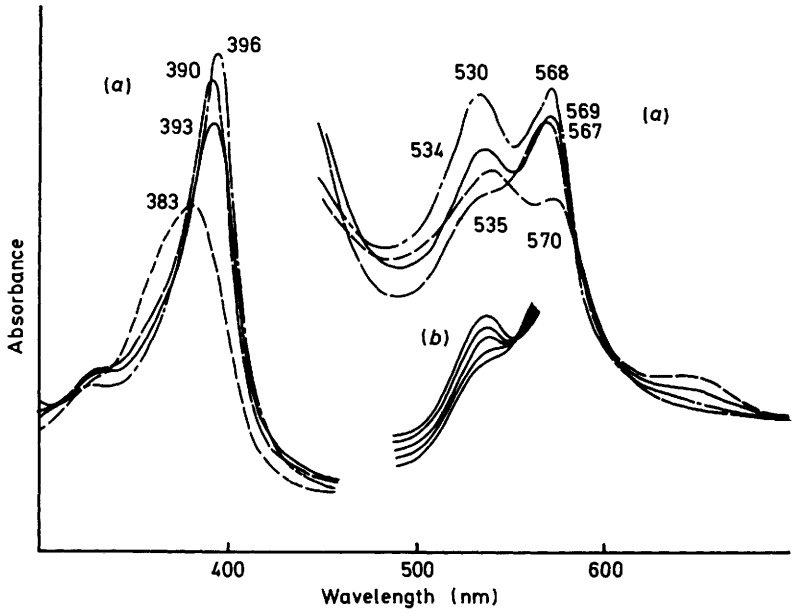


Figure 2. U.v.–visible spectra of the (**1a**) solution: (*a*) oxygen adduct (——), deoxy (– – –), carbon monoxide adduct (— – —), and oxidized complex (------) of (**1a**); (*b*) the spectral change from the deoxy to the oxygen adduct

Table 1. Rate constants and binding affinities with oxygen and carbon monoxide

		O_2				CO					
Porphyrinatoiron	Solvent (pH)	k_{on}/dm^3 mol^{-1} s^{-1}	k_{off}/s^{-1}	K/dm^3 mol^{-1}	$p_{\frac{1}{2}}$/mmHg	k_{on}/dm^3 mol^{-1} s^{-1}	k_{off}/s^{-1}	K/dm^3 mol^{-1}	$p_{\frac{1}{2}}$/mmHg	M	Ref.
(**1a**)	Water (7.4)	4.5×10^5	140	3.2×10^3	36	2.5×10^4	0.05	5.0×10^5	0.14	260	This work
(**1b**)	Benzene	2.5×10^5	160	1.6×10^3	31	2.0×10^4	0.02	1.0×10^6	0.1	310	1
Chelated heme[a]	Water (7.3)	2.6×10^7	47	5.5×10^5	1.0	4.0×10^6	0.009	4.0×10^8	0.002	500	*b*
Tailed picket fence heme	Toluene	4.3×10^8	2 900	1.5×10^5	0.58	3.6×10^7	0.0078	4.6×10^9	0.000 022	26 600	*c*, *d*
Hanging base heme	Toluene	3.1×10^8	620	5.0×10^5	0.29	4.0×10^7	0.0067	6.0×10^9	0.000 017	17 000	*e*
Red blood cell	Water (7.4)	4.2×10^4	0.62	6.8×10^4	27	3.7×10^4	0.1	3.7×10^5	0.1	270	This work
Hemoglobin	Water (7.4)	3.3×10^7	13	2.5×10^6	0.15	5.1×10^6	0.009	5.1×10^8	0.001—0.004		*f*, *g*

[a] Measured in an aqueous medium protected with carbon monoxide. [b] T. G. Traylor and A. P. Berzinis, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3171. [c] J. P. Collman, J. I. Brauman, B. L. Iverson, J. L. Sessler, R. M. Morris, and Q. H. Gibson, *J. Am. Chem. Soc.*, 1983, **105**, 3052. [d] G. B. James, F. S. Molinaro, J. A. Ibers, J. P. Collman, J. I. Brauman, E. Rose, and K. S. Suslick, *J. Am. Chem. Soc.*, 1980, **102**, 3324. [e] M. Momenteau, B. Loock, and D. Lavalette, *J. Chem. Soc., Chem. Commun.*, 1983, 962. [f] P. I. Reisberg and J. S. Olson, *J. Biol. Chem.*, 1980, **255**, 4159. [g] V. S. Sharma, M. R. Schmit, and H. M. Ranney, *J. Biol. Chem.*, 1976, **251**, 4267.

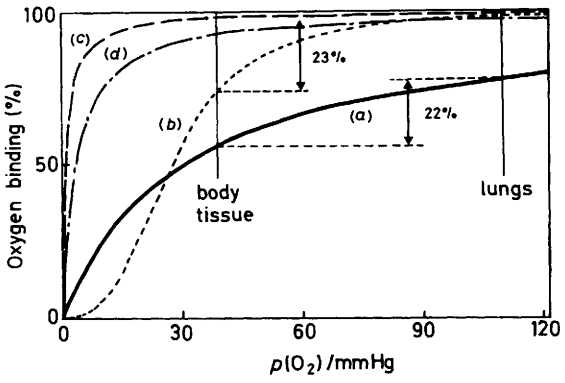


Figure 3. Oxygen-binding equilibrium curve of (**1a**) solubilized in an aqueous medium: (*a*) (**1a**), (*b*) hemoglobin in blood, (*c*) myoglobin, and (*d*) chelated heme

polarity of the medium has a marked effect on $p_{\frac{1}{2}}$, emphasising the charge separation on oxygen binding.[9] For the micellar (**1a**) complex in aqueous medium, it is considered that the hydrophobic property of the inside part of the micelle and/or non-polar cavity around the oxygen binding site of (**1a**) cancel out the polar effect of the aqueous medium. The $p_{\frac{1}{2}}(O_2)$ value (36 mmHg) of (**1a**) is close to that of hemoglobin in blood, but fairly removed from that of myoglobin. This suggests that (**1a**) has the potential to act as an oxygen carrier under physiological conditions which transports oxygen from the lungs [$p(O_2)$ *ca.* 110 mmHg] to myoglobin in body tissue [$p(O_2)$ 40 mmHg].

The carbon monoxide-binding affinity of (**1a**) solubilized in an aqueous medium was determined from the carbon monoxide-binding equilibrium curve and given in Table 1 together with reference data. The $p_{\frac{1}{2}}(CO)$ value agreed with that previously reported for (**1b**) in benzene and was much lower than those of other synthetic porphyrinatoiron complexes. The value of M [$p_{\frac{1}{2}}(O_2)/p_{\frac{1}{2}}(CO)$] for (**1a**) was also reduced compared to other synthetic porphyrinatoiron complexes, which indicates a resistance of (**1a**) to carbon monoxide poisoning.

As mentioned above, (**1**) shows a reduced affinity for gaseous molecules. This is probably caused by its cofacial structure (small gap between porphyrins acts as a steric hindrance for the binding) or a doming effect[10] caused by its doubly bridged diporphyrin nature. The oxygen- and carbon monoxide-binding rate constants [equation (1)] were measured using a flash

$$\text{Fe} + \text{O}_2 \text{ (or CO)} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Fe–O}_2 \text{ (or –CO)} \qquad (1)$$

photolysis method and are given in Table 1. The rate constants for (**1a**) and (**1b**) were similar in spite of different media, which means that the solvent and the micellar surroundings do not affect the kinetic process of the binding reaction. In comparison with other synthetic porphyrinatoiron complexes, both oxygen- and carbon monoxide-binding rate constants (k_{on}) decreased for (**1a**) probably due to the steric hindrance of the porphyrinatocopper cap which brings about a lower gas-binding affinity. The dissociation rate constants (k_{off}) of oxygen for (**1**) are comparable to those of other synthetic porphyrinatoiron complexes but the k_{off} values of carbon monoxide for (**1**) are higher than those of the other complexes. A previous paper[11] on iron–ligand bonding properties including (**1**) indicated that the steric hindrance of the cofacial structure causes a bent or distorted ligation of CO to Fe. This steric effect of (**1**) on the CO ligation probably brings about the higher k_{off} values of CO and also the lower M values for (**1**).

Table 2. Isocyanide binding affinities

Porphyrinatoiron	Solvent (pH)	K/dm^3 mol^{-1} n-Butyl isocyanide	K/dm^3 mol^{-1} t-Butyl isocyanide	Ref.
(**1a**)	Benzene	1.4×10^4	$<1.0 \times 10^{-3}$	This work
Chelated heme	Benzene	4.4×10^8	2.3×10^8	*a*
Hemoglobin	Water (7.0)	1.1×10^5	100	*b*, *c*

[a] T. G. Traylor, C. K. Chang, J. Geibel, A. Berzinis, T. Mincey, and J. Cannon, *J. Am. Chem. Soc.*, 1979, **101**, 2443. [b] See footnote *f* of Table 1. [c] M. P. Mims, A. G. Porras, J. S. Olson, R. W. Noble, and J. A. Peterson, *J. Biol. Chem.*, 1938, **258**, 14219.

To evaluate the steric hindrance of the porphyrinatocopper cap, (**1a**) was ligated with isocyanides. The visible absorption spectrum of (**1a**) changed to that of the (**1a**)–isocyanide complex with $\lambda_{max.}$ at 398 nm through an isosbestic point at 393 nm. The binding equilibrium constants K ($= p_{\frac{1}{2}}^{-1}$) are given in Table 2. The K values for (**1a**) were much smaller than those for other synthetic porphyrinatoiron complexes. Bulky isocyanide, especially t-butyl isocyanide, is inhibited to co-ordinate to the porphyrinatoiron moiety of (**1a**), which also supports the steric hindrance of the porphyrinatocopper cap covering the small molecule binding site. The K values for hemoglobin are also given in Table 2. Changing from n-butyl to t-butyl isocyanide results in a lowering of the K value for both (**1a**) and hemoglobin, although not to the same extent. This suggests that the steric effect around the porphyrinatoiron reduces the binding affinity of bulky ligands to a different degree in (**1a**) and hemoglobin.

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