Synthesis and Dioxygen-binding Properties of Double-sided Porphyrinatoiron(II) Complexes bearing Covalently Bound Axial Imidazole†

Eishun Tsuchida,* Teruyuki Komatsu, Kenji Arai and Hiroyuki Nishide
Department of Polymer Chemistry, Waseda University, Tokyo 169, Japan

Double-sided porphyrinatoiron(II) complexes bearing covalently bound axial imidazole, 5-[2-(5-imidazolylvaleryloxy)-6-(pivaloyloxy)phenyl]-10,15,20-tris[2,6-bis(pivaloyloxy)phenyl]porphyrinatoiron(II) and 5-[2-(3,3-dimethylbutyryloxy)-6-(5-imidazolylvaleryloxy)phenyl]-10,15,20-tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]porphyrinatoiron(II), have been synthesized. On the basis of their absorption and ^{1}H NMR spectra, the axial imidazole group is co-ordinated. The complexes reversibly form stable dioxygen adducts in toluene at 25 °C, and the kinetics of binding of O_2 and CO has been investigated. When embedded in phospholipid unilamellar vesicles, the complexes possess the ability to transport dioxygen in an aqueous medium. The binding affinity of the pivaloyloxy derivative [$P_{\frac{1}{2}}(O_2)$ = 27 Torr] is equal to that of a red blood cell suspension and the half-life of the dioxygen adduct formed was 1.5 d under physiological conditions (pH 7.4, 37 °C).

The haem prosthetic group of haemoglobin (hb) is enveloped within a highly functional architecture consisting of a globin chain, which enables the formation of a dioxygen adduct stable against irreversible oxidation through a proton-driven process under physiological conditions (aqueous medium, pH 7.4, 37 °C). Furthermore, the binding of O_2 and CO by the haems are controlled by electronic and steric effects of the enclosure surrounding the co-ordination sites.

As a model compound for hb, many synthetic haems have been prepared and their characteristic binding properties for O_2 discussed.[1-13] Much effort has been made to construct a pseudo-haem pocket on the haem plane by modifying porphyrin molecules. Typical of prominent models are 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatoiron [Fe(tpvp)] (picket-fence haem)[1] and 5,10,15,20-tetraphenylporphyrin capped at the *o*-position of each of the phenyl rings by $C_6H_2[C(O)O(CH_2)_2O]_4$-1,2,4,5 (capped haem).[2,3] The complex [Fe(tpvp)] has a pivalamide cavity on one side of the ring plane and can form a stable dioxygen adduct in toluene at 25 °C. Interestingly, the pivalamide groups are believed to provide a distal moiety exhibiting a favourable electrostatic interaction with a co-ordinated dioxygen and contributing to increased affinity for O_2, like the distal histidine (E8-His) in the haem pocket of hb.

Some previous reports showed that the amide groups are not crucial for the formation of a stable dioxygen adduct, however, the affinity of non-amide-substituted haems for O_2 was slightly lower than that of amide-substituted ones, *e.g.* [Fe(tpvp)].[2-5,8,12]

Recently, we have found that the porphyrinoctaesters **2** and **4** containing covalently bound axial imidazole forms stable dioxygen adducts in toluene at 25 °C.[12c] These new porphyrinatoiron molecules having a pseudo-globin wrapping containing an axial base on the ring plane, reproduce the haem pocket of hb. This paper reports their synthesis and kinetics of binding to O_2 and CO.

In aqueous media, the only example of reversible dioxygen binding found so far is with our phospholipid vesicle-embedded amphiphilic haem (lipid haem).[9] The haem complex is considered to be embedded in the bilayer of the phospholipid vesicle and the hydrophobic environment of the vesicle protects the adduct from irreversible oxidation. However, a phospholipid:haem molar ratio of 200:1 was required to prepare even a multilamellar vesicle (diameter *ca.* 80 nm), due to the presence of the alkylimidazole derivative (*e.g.* 1-dodecyl-2-methyl imidazole) used as axial base.[9c] In the case of the double-sided porphyrinatoiron(II) complexes bearing a covalently bound axial base it is not necessary to add an external lipophilic imidazole in the bilayer. Herein we also describe the morphology and dioxygen-binding ability of phosphilipid vesicle-embedded **2** or **4** compared to that of red blood cells under physiological conditions.

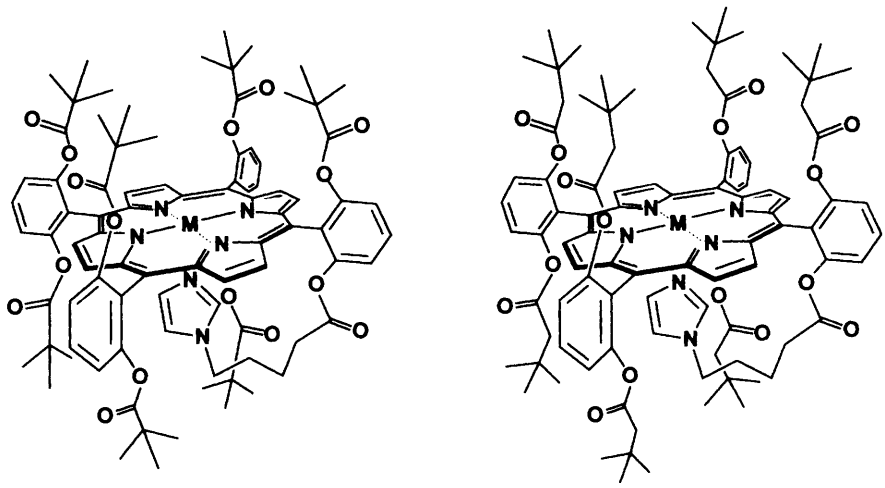
† *Non-SI unit employed:* Torr = 133.322 Pa.

Experimental

General.—Infrared spectra were recorded with a JASCO FT/IR-5300 spectrometer, ^{1}H NMR spectra on a JEOL GSX-400 instrument. Chemical shifts are expressed in ppm downfield from $SiMe_4$ as an internal standard. Fast atom bombardment (FAB) mass spectra were measured with a JEOL DX-303 spectrometer, absorption spectra with a Shimadzu MPS-2000 spectrophotometer. Elemental analyses were performed on a Yanagimoto MT3 CHN corder. Thin-layer chromatography (TLC) was carried out on 0.2 mm precoated plates of silica gel 60 F-254 (Merck). Purification was performed by silica gel 60 (Merck) flash-column chromatography. Transmission electron microscopy (TEM) was carried out with a JEOL JEM-100CX instrument by a negative staining method with uranyl acetate. The particle size of the phospholipid vesicle-embedded haem was measured by a submicron particle analyser (Coulter Electronics N4-SD).

Materials and Solvents.—Pivaloyl chloride, 3,3-dimethylbutyryl chloride, oxalyl chloride, lithium hydride, iron pentacarbonyl, and iodine were all special grade from Kanto Chem. Co. and used without further purification. 4-(Dimethylamino)pyridine (Tokyo Kasei Co.) was used without further purification. 1,2-Bis(myristoyl)-*sn*-glycerophosphocholine (dmpc) was from Nippon Oil & Fats Co. Tetrahydrofuran (thf), toluene and triethylamine were purified immediately before use by distillation from sodium. Acetonitrile was purified before use by distillation from diphosphorus pentaoxide. Imidazole was purified by repeated recrystallization from benzene.

5-(6-*Hydroxy*-2-*pivaloyloxyphenyl*)-10,15,20-*tris*[2,6-*bis*-(*pivaloyloxy*)*phenyl*]*porphyrin* H_2L^3. 5,10,15,20-Tetrakis(2,6-



H_2L^1	M	H_2L^2
1	2H	3
2	$Fe^{III}Br$	4
	Fe^{II}	

dihydroxyphenyl)porphyrin (1.5 g, 2.0 mmol) and 4-(dimethylamino)pyridine (1.8 g, 15 mmol) were dissolved in dry thf (500 cm^3). A tetrahydrofuran solution (100 cm^3) of pivaloyl chloride (1.78 cm^3, 14.4 mmol) was added dropwise with stirring for 4 h; the mixture was then stirred for 6 h at room temperature. The solution was brought to dryness on a rotary evaporator and extracted with $CHCl_3$. The organic layer was washed, first with dilute hydrochloric acid and then with aqueous $NaHCO_3$. The organic phase dried over anhydrous Na_2SO_4 was concentrated and the residue chromatographed on a silica gel flash-column using $CHCl_3$–diethyl ether (20:1 v/v) as the eluent. The second band eluted was collected and reduced to a small volume on a rotary evaporator. The residue was then dried at room temperature for several hours *in vacuo* to give purple crystalline H_2L^3 (0.46 g, 17.3%), R_f 0.18 [$CHCl_3$–diethyl ether (20:1 v/v)]. FAB mass spectrum: *m/z* 1331, M^+ (Found: C, 71.45; H, 6.75; N, 4.05. $C_{79}H_{86}N_4O_{15}$ requires C, 71.25; H, 6.50; N, 4.20%). IR (KBr): 3453 (ν_{OH}), 1757 cm^{-1} [ν_{CO}(ester)]; $\delta_H(CDCl_3)$ −3.0 (2 H, s, inner H), −0.5 to −0.1 (63 H, m, Bu^t), 7.4–7.9 (12 H, m, phenyl H) and 8.8 (8 H, d, pyrrole H); $\lambda_{max}(CHCl_3)$ 653, 583, 535, 506 and 412 nm.

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-(3,3-dimethylbutyryloxy)-6-hydroxyphenyl]porphyrin H_2L^4 was prepared according to our previous report.[12d]

5-(*Imidazolyl*)*valeric acid hydrochloride*. Diphenylmethyl 5-bromovalerate was synthesised according to Collman *et al.*[13] Lithium hydride (0.78 g, 0.1 mol) was added to a thf solution (200 cm^3) of imidazole (7.4 g, 0.1 mol) under argon and the mixture was refluxed for 12 h, changing to a pale yellow homogeneous solution. Then a thf solution (200 cm^3) of the above valerate (17.0 g, 49 mmol) was added and reflux continued for 12 h. The solvent was evaporated and $CHCl_3$ (300 cm^3) was added to the residue. The solution was washed with water and dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the remaining yellow oil purified by a silica gel column chromatography using $CHCl_3$–MeOH (40:1 v/v) as eluent to give a pale yellow oil (5.6 g, 33.5%), R_f 0.56 [$CHCl_3$–MeOH (40:1 v/v)].

The oil (5.6 g, 16.7 mmol) was dissolved in glacial acetic acid. Dry HCl was passed through the solution with vigorous stirring for 1.0 h followed by stirring for 2 h at room temperature. Acetic acid and HCl were removed under reduced pressure. The residue was dissolved in water and washed with diethyl ether and acetone to produce a pale yellow-white solid. Drying *in vacuo* afforded a white solid (2.7 g, 79.4%); $\delta_H(CD_3OD)$ 1.5–2.1 (4 H, m, CH_2), 2.4 (2 H, t, CH_2CO_2H), 4.3 (2 H, t, NCH_2), 7.6, 7.7 and 9.0 (3 H, imidazole).

5-[2-(5-*Imidazolylvaleryloxy*)-6-(*pivalolyloxy*)*phenyl*]-10,15,20-*tris*[2,6-*bis*(*pivaloyloxy*)*phenyl*]*porphyrin* H_2L^1. Synthesis and purification of the imidazole-bound porphyrins were carried out in a darkroom illuminated by a red light. Oxalyl chloride (4.6 cm^3, 54.2 mmol) was added to a dry MeCN solution of 5-(imidazolyl)valeric acid hydrochloride (2.1 g, 12.6 mmol) under argon and the mixture was refluxed for 1 h. The excess of oxalyl chloride and MeCN were removed *in vacuo* to yield a yellow solid. A dry MeCN (30 cm^3) solution of H_2L^3 (0.27 g, 0.2 mmol) and NEt_3 (4.0 g, 20 mmol) was added dropwise to the crude acid chloride at 25 °C. The mixture was refluxed for 6 h and then brought to dryness on a rotary evaporator. The residue was extracted with $CHCl_3$ and the organic layer washed with water and aqueous $NaHCO_3$. After drying over Na_2SO_4, the organic layer was evaporated to dryness and the residue chromatographed on a silica gel flash-column using $CHCl_3$–MeOH (20:1 v/v) as eluent. The major band was collected and reduced to a small volume on a rotary evaporator. The residue was then dried at room temperature for several hours *in vacuo* to give the purple crystalline product H_2L^1 (0.2 g, 70.3%), R_f 0.32 [$CHCl_3$–MeOH (20:1 v/v)]. FAB mass spectrum: *m/z* 1481, M^+ (Found: C, 70.60; H, 6.50; N, 5.60. $C_{87}H_{96}N_6O_{16}$ requires C, 70.50; H, 6.55; N, 5.65%). IR (KBr): 1757 cm^{-1} [ν_{CO}(ester)]; $\delta_H(CDCl_3)$ −3.0 (2 H, s, inner H), −0.6 to −0.1 (63 H, m, Bu^t), 3.6 [2 H, t, OC(=O)CH_2], 6.6, 6.9, 7.2 (3 H, imidazole), 7.3–7.9 (12 H, m, phenyl H) and 8.8 (8 H, d, pyrrole H); $\lambda_{max}(CHCl_3)$ 654, 582, 535, 506 and 412 nm.

5-[2-(3,3-*Dimethylbutyryloxy*)-6-(5-*imidazolylvaleryloxy*)-*phenyl*]-10,15,20-*tris*[2,6-*bis*-(3,3-*dimethylbutyryloxy*)*phenyl*]-*porphyrin* H_2L^2. This was prepared as described above for H_2L^1. A purple crystalline product was obtained (54.9%), R_f 0.24 [$CHCl_3$–MeOH (20:1 v/v)]. FAB mass spectrum: *m/z* 1578, M^+ (Found: C, 71.35; H, 7.15; N, 5.25. $C_{94}H_{110}N_6O_{16}$ requires C, 71.45; H, 7.00: N, 5.30%). IR (KBr): 1761 cm^{-1} [ν_{CO}(ester)]; $\delta_H(CDCl_3)$ −3.0 (2 H, s, inner H), −0.5 to 0.4 (63 H, m, Bu^t), 1.2 (14 H, m, CH_2), 2.8 [2 H, t, OC(=O)CH_2], 6.1, 6.7, 6.8 (3 H, imidazole), 7.3–7.8 (12 H, m, phenyl H) and 8.8 (8 H, d, pyrrole H); $\lambda_{max}(CHCl_3)$ 654, 583, 536, 507 and 415 nm.

Iron insertion. The ligand H_2L^2 (0.2 g) and an excess of $FeBr_2$ (0.3 g) were dissolved in dry thf (50 cm^3) and the mixture was heated to reflux under argon for 12 h. It was then brought to dryness on a rotary evaporator and extracted with $CHCl_3$.

After drying over Na_2SO_4, the $CHCl_3$ layer was evaporated to dryness and the residue was chromatographed on a silica gel flash-column. The eluate was treated with concentrated HBr and dried at room temperature for several hours *in vacuo*, to give dark purple crystalline [FeBr(L^2)] **3** (61.5%), R_f 0.25 [$CHCl_3$–MeOH (20:1 v/v)]. FAB mass spectrum: *m/z* 1713, $[M + 1]^+$ (Found: C, 66.2; H, 6.45; N, 4.80. $C_{94}H_{108}BrFeN_6O_{16}$ requires C, 65.90; H, 6.35; N, 4.90%). IR (KBr): 1762 cm^{-1} [ν_{CO}(ester)]; λ_{max}($CHCl_3$) 680, 648, 582, 510 and 413 nm.

On the other hand insertion into ligand H_2L^3 cannot be accomplished by the usual methods using $FeBr_2$ but can be achieved as follows. The compound [$Fe(CO)_5$] (1.57 cm^3) and I_2 (0.1 g, 0.4 mmol) were added to a dry toluene (50 cm^3) solution of H_2L^3 (0.26 g, 0.2 mmol) under an argon atmosphere. The mixture was heated at 100 °C for 12 h, aqueous NaCl solution added at room temperature and then stirred for 2 h. The mixture was extracted into benzene and washed with aqueous NaCl solution. After drying over Na_2SO_4, the organic layer was evaporated to dryness and the residue chromatographed on a silica gel flash-column using $CHCl_3$–MeOH (100:1 v/v) as eluent. The major band was collected and reduced to a small volume on a rotary evaporator. It was treated with concentrated HBr and then dried at room temperature for several hours *in vacuo* to give purple crystalline [FeBr(L^3)] **5** (0.21 g, 72.2%), R_f 0.14 [$CHCl_3$–MeOH (100:1 v/v)]. FAB mass spectrum: *m/z* 1466, M^+, IR(KBr) 3454 (ν_{OH}), 1756 cm^{-1} [ν_{CO}(ester)]. λ_{max}($CHCl_3$) 680, 649, 586, 507 and 411 nm.

The complex [FeBr(L^1)] was prepared from **5** by the same procedure as described for H_2L^1. A purple crystalline product was obtained (63.6%), R_f 0.33 [$CHCl_3$–MeOH (50:1 v/v)]. FAB mass spectrum: *m/z* 1536, $[M - Br + 1]^+$ (Found: C, 64.40; H, 6.00; N, 4.90. $C_{87}H_{94}BrFeN_6O_{16}$ requires C, 64.70; H, 5.85; N, 5.20%). IR (KBr): 1757 cm^{-1} [ν_{CO}(ester)]; λ_{max}($CHCl_3$) 670, 642, 576, 507 and 414 nm.

Spectroscopy of Iron(II) *Complexes*.—Reduction to the iron(II) complex was carried out by using toluene–aqueous $Na_2S_2O_4$ in a heterogeneous two-phase system under anaerobic conditions as previously reported.[12] After separation of the two phases, the organic layer containing the reduced compound was transferred under argon gas into the optical cell or NMR tube (diameter 5 mm). For visible absorption spectroscopy, haem concentrations of 2×10^{-5} mol dm^{-3} were used, and the spectra were recorded within the range 700–360 nm. The differential IR spectra were measured for the CO adduct *vs.* the deoxy form. The concentration of porphyrinatoiron was 5 mmol dm^{-3} in benzene and the cells used were precisely matched in terms of path length (25 μm) and CaF_2 window thickness.

Preparation of Phospholipid Vesicle-embedded Haems.—A $CHCl_3$ solution of the mixture of the porphyrinatoiron(III) and dmpc (haem:dmpc molar ratio = 1:100) was evaporated to give a thin film on the glass wall of a round flask. Phosphate buffer (3×10^{-2} mol dm^{-3}, pH 7.4, 10 cm^3) was added and the mixture was ultrasonicated (60 W, 10 min) and homogenized under argon in an ice–water bath. The resulting dispersion was incubated at room temperature for a few hours. Porphyrinatoiron(III) was reduced as previously described.[14]

Kinetic Measurements.—Kinetic measurements were performed by using laser flash-photolysis techniques. The experiments and data analysis were carried out with a Unisoku USP-500. Rhodamine 590 in absolute anhydrous methanol was used as the dye. Haem concentrations of 1.0×10^{-5} mol dm^{-3} were used and most experiments were carried out at 25 ± 0.2 °C. When no species other than the five-co-ordinate iron(II) complex Fe(por)B (por = porphyrinate, B = axial imidazole group) is formed [equation (1)] following flash photolysis, the gaseous ligand L (O_2 or CO) recombines with rate constant k_{obs} given by equation (2). The gaseous ligand concentrations were always in large excess compared to the haem concentration

$$\text{Fe(por)B(L)} \xrightarrow{h\nu} \text{Fe(por)B} \underset{k_{off}(L)}{\overset{k_{on}(L)}{\rightleftharpoons}} \text{Fe(por)B(L)} \quad (1)$$

$$k_{obs} = k_{on}(L)[L] + k_{off}(L) \quad (2)$$

so that the pseudo-first-order approximation could be applied throughout. The value of $K(O_2) = k_{on}(O_2)/k_{off}(O_2)$ was determined using the competitive rebinding technique.[1b,6] From Gibson's equation,[1b] a plot of $1/k_{obs}$(slow) *vs.* [O_2]/[CO] yielded a straight line with slope $K(O_2)/k_{on}$(CO). Values of M [K(CO)/$K(O_2)$] for the competitive binding of CO in the presence of O_2 were determined using the flow method by mixing pure O_2 with premixed (9.43 or 19 ppm) CO in N_2.[1b]

The thermodynamic parameters (ΔH and ΔS) of dioxygen binding were calculated from the binding affinity at various temperatures, using van't Hoff plots. The temperature of the solution was maintained to a precision of ±0.2 °C.

Results and Discussion

Synthesis and Structure.—One of the advantages of double-sided porphyrins is a reduction in the complexity of the diastereoisomeric properties during preparation. *meso*-Tetrakis(2,6-dihydroxyphenyl)porphyrin was treated with 7.2 equivalents of alkanoyl chloride, affording '7-substituted' porphyrins H_2L^3 and H_2L^4 after flash chromatography: the second band eluted was identified on the basis of its ^1H NMR and FAB mass spectra and elemental analysis (10–17% yield for H_2L^3 or H_2L^4).

Introduction of an axial base into the octaester porphyrins (H_2L^3 or H_2L^4) was achieved using 5-(imidazolyl)valeryl chloride, leading to the corresponding free-base porphyrins (H_2L^1 and H_2L^2). The length of the alkyl spacer unit required to achieve imidazole binding to the central iron(II) was confirmed by using Carey–Pauling–Koltun (CPK) models. These compounds were characterized by physicochemical analysis (see Experimental section).

Iron insertion into H_2L^3 cannot be accomplished by the usual methods using $FeBr_2$ due to its bulky pivaloyloxy groups around the porphyrin core, but can be achieved with [$Fe(CO)_5$] and I_2 in toluene at 100 °C.

The reduction of porphyrinatoiron(III) was carried out using aqueous $Na_2S_2O_4$ in a two-phase system (toluene–water) under anaerobic conditions. The reduced porphyrinatoiron(II) complexes in toluene are assigned as high-spin, five-co-ordinated species on the basis of their absorption [λ_{max}: **2**, 605 (sh), 559 (sh), 538 and 435; **4**, 605 (sh), 555 (sh), 535 and 432 nm] and ^1H NMR spectra. Five-co-ordinated tetraphenylporphyrinatoiron complexes with a single axial nitrogen base exhibit a sharp band in the Soret region and three distinct absorptions near 600, 560 and 535 nm.[13] The visible spectral patterns of the present five-co-ordinated species did not change over the range −20 to 50 °C.

The ^1H NMR spectra of complexes **2** and **4** in $CDCl_3$ were obtained at 22 °C. In general, the large isotropic shifts of the pyrrole β-protons are very characteristic of the oxidation and spin states of the central metal.[13,15] The β-protons of the double-sided haems gave rise to four singlets (δ 52.1–59.5 for **2**, 47.6–59.1 for **4**) indicating that the complex is five-co-ordinate and high spin ($S = 2$). When the temperature is lowered, the Curie effect caused the peaks to shift to low field. The peaks of the β-proton due to the square-planar porphyrinato iron(II) complex ($S = 1$) were not observed (δ −10 to 0) until −10 °C. The NMR spectra of complexes **2** and **4** reveal no trace of dimerization (mixture of six- and four-co-ordinate species) in the range of −10 to 40 °C.

Binding Affinity for O_2 *and* CO.—The addition of O_2 and CO to a toluene solution of complex **2** or **4** gave O_2 and CO adducts, respectively, on the basis of their absorption spectra [λ_{max}(toluene): **2**·O_2, 545 and 425; **2**·CO, 541 and 424; **4**·O_2, 544 and 420; **4**·CO, 540 and 421]. The half-life of the dioxygen

Table 1 Parameters for binding of O_2 and CO to iron(II) porphyrins in toluene at 25 °C[a]

Complex	$10^{-7}k_{on}(O_2)$/ dm^3 mol^{-1} s^{-1}	$k_{off}(O_2)$/s^{-1}	$P_{\frac{1}{2}}(O_2)$/ Torr	$10^{-6}k_{on}(CO)$/ dm^3 mol^{-1} s^{-1}	$10^4P_{\frac{1}{2}}(CO)$/ Torr	Ref.
2	2.4	2.4×10^3	13	1.8	4.9	This work
4	6.3	1.2×10^3	2.5	13	0.72	This work
6	3.0	27	0.13	1.8	1.2	5*b*
7	1.7	71	0.36	1.5	6.5	1*b*
Hb(R state) α[b]	3.3	13.2	0.22	4.6	14	16, 17
mb[b]	1–2	10–30	0.37–1	0.3–0.5	140–250	1*b*, 18

[a] Estimated errors < 10%. [b] pH 7.0–7.4, at 20 °C. [c] R state = relaxed state.

Table 2 Stretching vibration of carbonylated iron(II) porphyrin complexes at 25 °C

Complex	Solvent	ν(C=O) ($\nu_{\frac{1}{2}}$)/cm^{-1}	Ref.
2	Benzene	1977(11)	This work
4	Benzene	1962(11)	This work
8[a]	Benzene	1979(12)	12*b*
9[b]	Benzene	1964(12)	12*b*
[Fe(tpvp)]·hxim	Benzene	1968(12)	12*b*
[Fe(tpvp)]·mim	Nujol mull	1969	21
hb	Water	1951(12)	18, 22
mb	Water	1945(8)	18, 22

[a] 5,10,15,20-Tetrakis[2,6-bis(pivaloyloxy)phenyl]porphyrinatoiron(II)–1-hexylimidazole. [b] 5,10,15,20-Tetrakis[2,6-bis(3,3-dimethylbutyryloxy)phenyl]porphyrinatoiron(II)–1-hexylimidazole. [c] hxim = 1-Hexylimidazole. [d] mim = 1-Methylimidazole.

adduct with respect to irreversible oxidation to Fe^{III} was > 2 d in toluene at 25 °C. Dioxygen-binding affinities [$P_{\frac{1}{2}}(O_2)$] were kinetically determined.[1b,6] Carbon monoxide-binding affinities [$P_{\frac{1}{2}}(CO)$] were derived from $P_{\frac{1}{2}}(O_2)/M$ (Table 1).

The dioxygen-binding affinities of complexes **2** and **4** were 7–100-fold lower than that of α-5,15-[2,2-(decanamido)-diphenyl]-α,α-10,20-bis(*o*-pivalimidophenyl)porphinatoiron-(II)(1-methylimidazole) **6**[5b] and the 5,10,15-[1,3,5-tris(benzene-tripropionyl)tris(α,α,α-*o*-aminophenyl)-20-(α-*o*-pivalamido-phenyl)porphyrinatoiron(II)–1-methylimidazole **7**[1b] having four amide groups around the binding site.

It is well known that amide residues in the porphyrin-ring plane contribute to high dioxygen-binding affinity.[5,6c,8,11b,19] In recent work by Reed and co-workers[20] the [Fe(tpvp)] derivative having one of the four pivalamide groups replaced by a substituent capable of hydrogen bonding has been synthesised and its dioxygen-binding affinity determined. The effect of hydrogen bonding on dioxygen-binding affinity was best illustrated by the 10-fold increase observed when one pivalamide group is replaced by a phenylurea substituent. Although an amide group is not crucial for formation of a stable dioxygen adduct of synthetic haems,[2–4,8,12,19] there remains an unsolvable problem that the binding affinities of non-amide type haems are lower than those of hb and myoglobin (mb).

The dioxygen-binding affinities of complexes **2** and **4** are sufficiently high to be taken as a model for hb and mb. These results show that the pseudo-globin wrapping constructed by the eight ester substituents in the ring plane enables the formation of a stable dioxygen adduct of the tetraphenylporphyrinatoiron. This is the first successful case of an efficient dioxygen carrier molecule comprised of eight ester functions.

The binding affinities of complex **4** for O_2 and CO were significantly higher than those of **2**. Our previous work on double-sided porphyrinatoiron complexes showed that $P_{\frac{1}{2}}(O_2)$ was controlled by the size of the space at the rear side of the ring plane available for binding of an axial base.[12a,b] Thus, the lower $P_{\frac{1}{2}}$ values of **2** compared with **4** are also attributed to unfavourable steric repulsion between the rear cavity and the attached imidazole.

Kinetics of Binding of O_2 *and* CO.—In order to elucidate the O_2- and CO-binding properties of the double-sided haem bearing a covalently bound axial base the dynamics of binding were explored by laser flash photolysis. When a toluene solution of a carbon monoxide adduct ([haem] = 1×10^{-5} mol dm^{-3}) was photolysed linear decay plots of log ΔA *vs.* *t* were obtained. This indicates clean one-step rebinding. The value of $k_{on}(CO)$ was obtained using equation (2). Under the same conditions, flash photolysis of stable dioxygen adducts was carried out over a range of dioxygen concentrations. The value of $k_{on}(O_2)$ was also obtained using equation (2) and $k_{off}(O_2)$ was calculated from $k_{on}(O_2)/K(O_2)$. Kinetic parameters for the binding of O_2 and CO to the double-sided porphyrinatoiron complexes are summarized in Table 1.

The lower dioxygen-binding affinities of complexes **2** and **4** (ester type) compared with those of **6** and **7** (amide type) arise mainly from the increase in the dioxygen dissociation rate constants. Several investigations have suggested that the following factors control $k_{off}(O_2)$ of synthetic haems: (1) proximal base ligation as a fifth ligand, (2) distal steric hindrance, and (3) local polarity around the binding site (solvent effect, electrostatic interactions, *etc.*).[1b,5a,6,11b] It is assumed that changing the attachment of the groups in the distal moiety from amide to ester causes a decrease in the local polarity of the cavity and weakens the dioxygen adduct, leading to a higher value of $k_{off}(O_2)$.

Infrared Spectral Measurements.—The ν(CO) value of the adduct **4**·CO (1962 cm^{-1}) is lower than that of **2**·CO (1977 cm^{-1}, Table 2). This difference reflects the electron-donating ability of the imidazole ligand.[12b] Since the imidazole binding to the iron of **2** is sterically restrained by the bulky pivaloyloxy groups, the electron donation from the p_π orbital of the axial base to the d_π orbital of the iron might be decreased compared to that of **4**, where the imidazole residue is intramolecular bound to the central iron(II) without the unfavourable steric repulsion on the rear side.

Dioxygen Binding under Physiological Conditions.—As an hb model in aqueous media, we have developed a hybrid system of phospholipid vesicle-embedded haem.[9] According to TEM, the phospholipid vesicle-embedded complex **2** or **4** (phospholipid: haem molar ratio = 100:1) looked like a unilamellar and single-walled vesicle with diameters of *ca.* 40–50 nm without disorder of the bilayer structure (Fig. 1). The average particle size was also measured by the particle analyser; the diameter was 42 ± 10 nm. The homogeneous dispersion did not change for several months and there was no precipitation.

The incorporation of the double-sided porphyrinatoiron in the lipid bilayer of the vesicle was confirmed by gel permeation chromatography monitored by the absorptions of the haem derivative and the phospholipid at 420 and 255 nm respectively.* The elution curves coincided with each other, *i.e.* complexes **2** and **4** are included in the phospholipid vesicle.

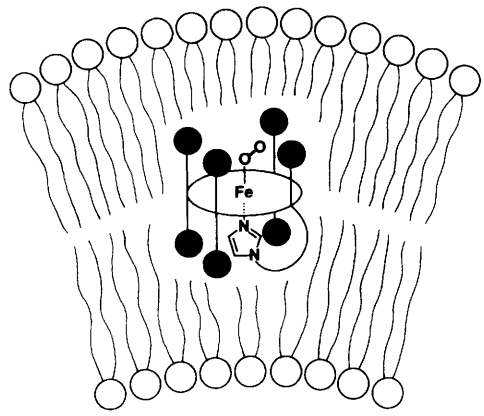


Fig. 1 A phospholipid vesicle-embedded dioxygenated double-sided porphyrinatoiron complex bearing a covalently bound axial imidazole

Table 3 Dioxygen-binding affinities in aqueous media (pH 7.4, 37 °C) and associated thermodynamic parameters

Complex	$P_{\frac{1}{2}}(O_2)$/ Torr	ΔH/ kJ mol^{-1}	ΔS/ J K^{-1} mol^{-1}	Ref.
2–dmpc vesicle[a]	27	−59.4	−163	This work
Lipid haem–eyl vesicle[b]	53	−62.8	−167	23
hb[c]	0.22[c]	−56.9 to −64.9	−116 to −133	24
mb[c]	0.37–1[c]	−64.0 to −87.9	−159 to −235	25
Red blood cell	27			18

[a] 3×10^{-2} mol dm^{-3} phosphate buffer. [b] Lipid haem–1-dodecyl-2-methylimidazole complex embedded in phospholipid vesicle comprised egg-yolk lechitin (eyl); 3×10^{-2} mol dm^{-3} phosphate buffer, pH 7.0. [c] pH 7.0–7.4 at 20 °C. See Table 1.

The visible absorption spectrum of the phospholipid vesicle-embedded complex **2** revealed a five-co-ordinated species [λ_{max} 605 (sh), 565 (sh), 536 and 435 nm] even in aqueous media. The spectrum changed to that of the dioxygen adduct (λ_{max} 546 and 422 nm) upon exposure to dioxygen. This spectrum in turn changed to that of the carbon monoxide adduct (λ_{max} 543 and 424 nm) when CO was bubbled through the solution. The formation of the dioxygen adduct was reversible under physiological conditions.

The $P_{\frac{1}{2}}(O_2)$ value for the phospholipid vesicle-embedded complex **2** is 27 Torr at 37 °C, similar to that of a red blood cell suspension (Table 3). The half-life of the dioxygen adduct of **2** with respect to irreversible oxidation in aqueous medium was > 1.5 d. These results suggest that the phospholipid vesicle-embedded **2** acts as an effective dioxygen carrier under physiological conditions, like a red blood cell substitute.

The thermodynamic parameters for the dioxygen-binding are summarized in Table 3. The enthalpy (ΔH) and entropy (ΔS) changes for complex **2** embedded in the phospholipid vesicle were estimated to be −59.4 kJ mol^{-1} and −163 J K^{-1} mol^{-1}, respectively, comparable to those of hb.

In this paper, we have demonstrated the O_2- and CO-binding properties of double-sided porphyrinatoiron complexes bearing covalently bound axial imidazole in toluene and in an aqueous medium. Specifically, phospholipid vesicle-embedded complex **2** formed a stable unilamella without disorder of the bilayer structure (diameter *ca.* 40–50 nm) and underwent reversible dioxygen adduct formation with the same $P_{\frac{1}{2}}(O_2)$ as that of red blood cells under physiological conditions. These results indicate that **2** is an efficient dioxygen carrier molecule and represents a totally synthetic red blood cell substitute.

* A small amount of phospholipid, which contains unsaturated fatty acid residues, 1,2-bis(octadeca-2,4-dienoyl)-*sn*-glycerophosphocholine, was added as the UV probe.

References

1 (*a*) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang and W. T. Robinson, *J. Am. Chem. Soc.*, 1975, **97**, 1427; (*b*) J. P. Collman, J. I. Brauman, B. L. Iverson, J. L. Sessler, R. M. Morris and G. H. Gibson, *J. Am. Chem. Soc.*, 1983, **105**, 3052.
2 J. Almog, J. E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, 1975, **97**, 227.
3 J. E. Linard, P. E. Ellis, jun., J. R. Budge, R. D. Jones and F. Basolo, *J. Am. Chem. Soc.*, 1980, **102**, 1896; T. Hashimoto, R. L. Dyer, M. J. Crossley, J. E. Baldwin and F. Basolo, *J. Am. Chem. Soc.*, 1982, **104**, 2101.
4 J. E. Baldwin, J. H. Cameron, M. J. Crossley, I. J. Dagley, S. R. Hall and T. Klose, *J. Chem. Soc., Dalton Trans.*, 1984, 1739.
5 (*a*) M. Momenteau, *Pure Appl. Chem.*, 1986, **58**, 1493; (*b*) M. Momenteau, B. Loock, C. Tetreau, D. Lavalette, A. Croisy, C. Schaeffer, C. Huel and J.-M. Lhoste, *J. Chem. Soc., Perkin Trans. 2*, 1987, 249; (*c*) I. P. Gerothanassis, M. Momenteau and B. Loock, *J. Am. Chem. Soc.*, 1989, **111**, 7006.
6 (*a*) C. K. Chang and T. G. Traylor, *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 1166; (*b*) D. K. White, J. B. Cannon and T. G. Traylor, *J. Am. Chem. Soc.*, 1979, **101**, 2443; (*c*) T. G. Traylor, S. Tsuchiya, D. Campbell, M. Mitchell, D. Stynes and N. Koga, *J. Am. Chem. Soc.*, 1985, **107**, 604.
7 A. R. Battersby, A. S. J. Bartholomew and T. Nitta, *J. Chem. Soc., Chem. Commun.*, 1983, 1291.
8 K. S. Suslick and M. M. Fox, *J. Am. Chem. Soc.*, 1984, **106**, 4522.
9 (*a*) E. Tsuchida, *Top. Curr. Chem.*, 1986, **132**, 64; (*b*) M. Yuasa, H. Nishide and E. Tsuchida, *J. Chem. Soc., Dalton Trans.*, 1987, 2493; (*c*) E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa and Y. Matsushita, *J. Chem. Soc., Dalton Trans.*, 1984, 1147.
10 Y. Uemori and E. Kyuno, *Inorg. Chem.*, 1989, **28**, 1690.
11 (*a*) B. Ward, C. B. Wang and C. K. Chang, *J. Am. Chem. Soc.*, 1981, **103**, 5236; (*b*) C. K. Chang, B. Ward, R. Young and M. P. Kondylis, *J. Macromol. Sci., Chem.*, 1988, **25**, 1307.
12 (*a*) T. Komatsu, E. Hasegawa, S. Kumamoto, H. Nishide and E. Tsuchida, *J. Chem. Soc., Dalton Trans.*, 1991, 3281; (*b*) E. Tsuchida, T. Komatsu, T. Nakata, E. Hasegawa, H. Nishide and H. Inoue, *J. Chem. Soc., Dalton Trans.*, 1991, 3285; (*c*) T. Komatsu, K. Arai, H. Nishide and E. Tsuchida, *Chem. Lett.*, 1992, 799; (*d*) T. Komatsu, S. Kumamoto, H. Nishide and E. Tsuchida, *Bull. Chem. Soc., Jpn.*, 1993, **66**, 1640.
13 J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, E. Bunnenberg, E. Linder, G. N. LaMar, J. D. Gaudio, G. Lang and K. Spartalian, *J. Am. Chem. Soc.*, 1980, **102**, 4182.
14 E. Tsuchida, H. Nishide, M. Yuasa and M. Sekine, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 776.
15 H. Goff and G. N. LaMar, *J. Am. Chem. Soc.*, 1977, **99**, 6599.
16 Q. H. Gibson, *J. Biol. Chem.*, 1970, **245**, 3285; J. S. Olson, M. E. Anderson and Q. H. Gibson, *J. Biol. Chem.*, 1971, **246**, 5919.
17 V. S. Sharma, M. R. Schmidt and H. M. Ranney, *J. Biol. Chem.*, 1976, **251**, 4267.
18 F. Antonini and M. Brunori, *Hemoglobin and Myoglobin and Their Reactions with Ligands*, North Holland, Amsterdam, 1971; M. Brunori and T. M. Schuster, *J. Biol. Chem.*, 1969, **244**, 4046.
19 M. Momenteau and D. Lavalette, *J. Chem. Soc., Chem. Commun.*, 1982, 341.
20 G. E. Wuenschell, C. Tetreau, D. Lavalette and C. A. Reed, *J. Am. Chem. Soc.*, 1992, **114**, 3346.
21 J. P. Collman, J. I. Brauman, T. R. Halbert and K. S. Suslick, *Proc. Natl. Acad. Sci., USA*, 1976, **73**, 3333.
22 W. S. Caughey, *Ann. N. Y. Acad. Sci.*, 1970, **174**, 148.
23 E. Tsuchida, H. Nishide, M. Yuasa, E. Hasegawa, Y. Matsushita and K. Eshima, *J. Chem. Soc., Dalton Trans.*, 1985, 275.
24 K. Imai and T. Yonetani, *J. Biol. Chem.*, 1975, **250**, 7093.
25 M. Wang, B. M. Hoffman, S. J. Shire and F. R. N. Gurd, *J. Am. Chem. Soc.*, 1979, **101**, 7394.

Received 18*th January* 1993; *Paper* 3/00276D