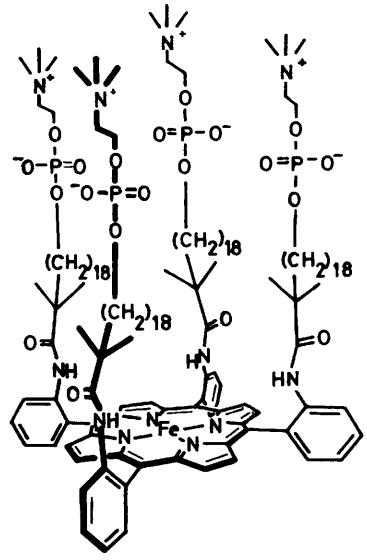
Iron–Ligand Bonding Properties of Synthetic Iron–Porphyrin Complexes with Oxygen Transporting Ability in Aqueous Media

Eishun Tsuchida,* Hiromi Maeda, Makoto Yuasa, and Hiroyuki Nishide
Department of Polymer Chemistry, Waseda University, Tokyo 160, Japan
Hidenari Inoue and Tsuneo Shirai
Department of Applied Chemistry, Keio University, Yokohama 223, Japan

Mössbauer spectra, i.r. spectra (Fe–CO stretching), and photodissociation quantum yields of the oxygen and CO adducts were measured for synthetic iron–porphyrin complexes with oxygen transporting ability in aqueous media: the 5,10,15,20-tetra{α-*o*-[2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido]phenyl}porphyrinatoiron(II) (lipid-heme) complex of 1-laurylimidazole (lauryl = dodecyl) or 1-lauryl-2-methylimidazole embedded in the bilayer of phospholipid liposome (abbreviated as liposome–lipid-heme) and the tetradecyl-substituted copper–iron(II)–diporphyrin (diheme) complex of 1-laurylimidazole solubilized in a surfactant micelle (micelle–diheme). The quadrupole splitting (ΔE_Q) for the CO adduct of the diheme indicated a large electric-field gradient at the iron nucleus, probably due to steric hindrance of the diheme structure. Its CO-stretching vibration (ν_{CO}) and photodissociation quantum yield (Φ), obtained by flash photolysis, were similar to those of carboxy hemoproteins and suggested the distorted ligation of CO to Fe. Mössbauer parameters for the oxygen adducts of liposome–lipid-heme and micelle–diheme in aqueous media agreed with those for the oxy hemoproteins.

Bonding properties of hemoglobin (hb) and myoglobin (mb) have been intensively studied because of the interest in their reversible oxygen transporting capacity. The bond between iron and oxygen or carbon monoxide (CO) has been the subject of considerable investigation by various physico-chemical methods. Attempts to synthesize protein-free iron–porphyrin complexes capable of reversible oxygenation have been developed, *i.e.* much effort has been made to mimic natural oxygen transporters like hb and mb by using modified synthetic iron–porphyrin complexes.[1–7] The bonding properties of these synthetic iron–porphyrin complexes have also been extensively studied by Mössbauer, i.r., Raman, and e.s.r. spectroscopy.[8–10] However the physico-chemical measurements were carried out on the complexes in organic solvents or the solid state, because these synthetic iron–porphyrin complexes could not form their reversible oxygen adducts in aqueous media.

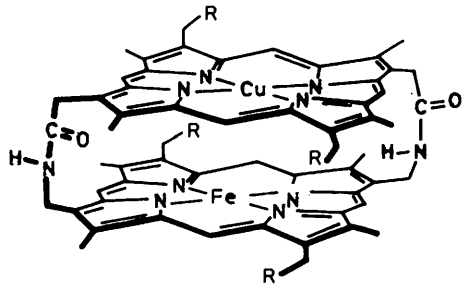
Recently, we found and reported that iron–porphyrin derivatives incorporated into phospholipid liposome and a surfactant micelle formed oxygen adducts reversibly even in aqueous media at room temperature, and transported oxygen effectively under physiological conditions.[11–14] In this paper, Mössbauer spectra were measured for the deoxy, CO, and oxygen adducts of the 5,10,15,20-tetra{α-*o*-[2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido]-phenyl}porphyrinatoiron(II) (lipid-heme, Scheme 1) complex of 1-laurylimidazole (li) (lauryl = dodecyl) or 1-lauryl-2-methyl-imidazole (lmi) embedded in the liposome of L-α-dimyristoyl-phosphatidylcholine (dmpc) (myristoyl = tetradecyl) (abbreviated as 'liposome–lipid-heme') and the tetradecyl-substituted copper–iron(II)–diporphyrin (diheme, Scheme 2) complex of li solubilized in the Triton X-100 micelle (abbreviated as 'micelle–diheme') in aqueous media. I.r. spectra of the CO adducts and photodissociation quantum yields of the CO and oxygen adducts were also measured. The iron–ligand bonding properties of the liposome–lipid-heme and the micelle–diheme are discussed in comparison with those previously reported for hb, mb, and synthetic iron–porphyrin complexes.



Scheme 1. Lipid-heme

Experimental

Materials.—The lipid-heme and the diheme were prepared as previously reported.[13,14] Iron-57-labelled derivatives of the lipid-heme and diheme, used for Mössbauer spectroscopic measurements, were also synthesized according to the above method, and as given below. The compound $^{57}FeCl_2$ was prepared from $^{57}Fe_2O_3$ according to the literature procedure.[15] The compounds 5,10,15,20-tetra[α-*o*-(20′-hydroxy-2′,2′-dimethyleicosanamido)phenyl]porphyrin[14] and $^{57}FeCl_2$ were dissolved in tetrahydrofuran–pyridine, and the mixture was refluxed under nitrogen for 6 h to give the iron-57-labelled derivative. [U.v.-visible spectrum(chloroform): before reaction, $\lambda_{max.}$ 416, 512, 544, 590, and 642 nm; after reaction $\lambda_{max.}$ 418, 503,



Scheme 2. Diheme; R = C_2H_5 or $C_{10}H_{21}$

574, 645, and 672 nm.] This iron-57-labelled derivative was allowed to react with 2-chloro-2-oxo-1,3,2λ^5-dioxaphospholane–triethylamine (1:1 w/w) and then anhydrous trimethylamine to yield the iron-57-labelled derivative of lipid-heme [u.v.–visible (methanol); $\lambda_{max.}$ 418, 504, 575, 645, and 673 nm].

In the synthesis of the iron-57-labelled derivative of diheme, the *N*,*N*-dimethylformamide solution of the parent dimeric porphyrin (copper–metal free diporphyrin complex)[14] and $^{57}FeCl_2$ was refluxed under nitrogen for 8 h. The resulting residue was chromatographed on neutral alumina with chloroform. Recrystallization from chloroform–hexane (1:1 v/v) gave the iron-57-labelled derivative of diheme. The u.v.–visible spectra characterized by absorptions at 383, 515, 544, and 576 nm of the parent dimeric porphyrin (copper-metal free diporphyrin complex) changed to those at 380, 524, and 564 nm of the iron-57-labelled derivative of diheme. The ligands li, lmi, and 1-tritylimidazole (ti) were prepared as reported in the literature.[11] 1-Methylimidazole (mim) and imidazole (im) were purchased from Tokyo Kasei (special grade). Dmpc and Triton X-100 were purchased from Sigma and Kanto Kagaku (special grade), respectively.

Preparation of the Liposome–Lipid-heme.—The liposome–lipid-heme was prepared according to the literature procedure.[14] The iron(III)–porphyrin bromide (lipid-hemin, 1 μmol) was reduced in the presence of li (3 μmol) or lmi (20 μmol) by mixing its methanol solution with Pd–C catalyst under hydrogen, and then carbon monoxide gas was bubbled through the mixture. The methanol solution was filtered, dried with molecular sieves, and added to a methanol solution of the phospholipid (50 μmol) saturated with carbon monoxide. By evaporating the solvent under reduced pressure, thin films were 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)–porphyrin complex (deoxy lipid-heme complex). Oxygen-free, phosphate buffer solution (pH 7.0, 20 cm^3) was added, and the mixture was shaken by a Vortex mixer. It was ultrasonicated and homogenized in an ice–water bath under nitrogen. The deoxy liposome–lipid-heme solution thus prepared was incubated at room temperature under a nitrogen atmosphere for 2 h. The red transparent solution of the deoxy liposome–lipid-hemes showed u.v.–visible absorption spectra with maxima at 426, 535, and 562 (sh) nm for the li complex, and 438, 535 (sh), and 562 nm for the lmi complex. The u.v.–visible absorption spectra of the deoxy complexes changed to those assigned to the oxygen adducts ($\lambda_{max.}$: 422 and 545 for the li complex, and 422 and 546 nm for the lmi complex) on exposure to oxygen. The spectra of the oxygen adducts changed to those of the CO adducts [$\lambda_{max.}$: 423, 540 (li) and 423, 540 nm (lmi)] on bubbling through with CO and returned to those of the deoxy complexes on bubbling with nitrogen. [For the lipid-heme complex of li in methanol,[4] $\lambda_{max.}$(nm): deoxy 427, 535, 562 (sh); CO 423, 540; and oxy 422, 546.]

Preparation of the Micelle–Diheme.—The micelle–diheme was prepared as reported.[13] The iron(III)–porphyrin complex (diheme, 1 μmol) was reduced in the presence of li (50 μmol) or mim (5 mmol) by mixing its benzene solution with aqueous sodium dithionite under nitrogen, and then carbon monoxide gas was bubbled through the mixture. The benzene phase was collected, dried with molecular sieves, and added to a benzene–chloroform (1:1 v/v) solution of a surfactant such as poly(ethylene oxide)–octylphenyl ether saturated with carbon monoxide. By evaporating the solvent under reduced pressure, thin films were 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)–porphyrin (deoxy diheme) complex. Oxygen-free, phosphate buffer solution (pH 7.0, 20 cm^3) was added, and the mixture was shaken by a Vortex mixer. The u.v.–visible absorption spectra of the aqueous micelle–diheme–li solution were as follows: $\lambda_{max.}$(nm); deoxy 395, 567; CO 398, 530, 568; and oxy 396, 534, 569. For diheme–mim in benzene:[16] $\lambda_{max.}$(nm); deoxy 390, 570; CO 396, 533, 572; and oxy 393, 536, 573.

Mössbauer Spectroscopic Measurements.—The Mössbauer spectrometer was of the constant-acceleration type. The source was used at room temperature and consisted of *ca.* 10 mCi (3.7×10^8 Bq) of ^{57}Co diffused in palladium foil. The absorbers, with a thickness of *ca.* 0.2 mg of iron per cm^2, were kept at 77 K. The Doppler velocity was calibrated with natural iron foil kept at room temperature, and zero velocity was taken as the centroid of its Mössbauer spectrum at room temperature. The spectra were fitted to Lorentzian line shapes by using a least-squares fitting program. Statistical uncertainties were 0.01 mm s^{-1} for all Mössbauer parameters.

I.r. Spectroscopic Measurements.—Solutions of the CO adducts of the diheme complex were prepared as mentioned above. By freeze-drying the solution, the CO adducts were isolated under a CO atmosphere. I.r. spectra were measured by the KBr tablet method with an i.r. spectrophotometer (Japan Spectroscopic, JASCO, IR-810).

Flash Photolysis Measurements.—Flash photolysis measurements were performed by the use of a pulse flash spectrophotometer (Unisoku, FP-2000).[17,18]

Results and Discussion

Mössbauer parameters for the liposome–lipid-heme, the micelle–diheme, and other hemes are summarized in Table 1. For the deoxy li-ligated heme complex of the liposome–lipid-heme, both isomer shift (δ) and quadrupole splitting (ΔE_Q) were small and nearly equal to those for the deoxy li-heme complex of the lipid-heme in methanol, which indicated that the iron was in the iron(II) low-spin state. The u.v.–visible absorption spectrum for the deoxy li-ligated lipid-heme complex differed from that for the deoxy lmi-ligated lipid-heme complex (see Experimental section); the latter is assigned to a five-co-ordinate, high-spin iron(II) heme complex. This result also supports an iron(II) low-spin state for the deoxy li-heme complex. In addition the large δ and ΔE_Q values for the deoxy lmi-heme complex of the liposome–lipid-heme showed the iron to be in the iron(II) high-spin state. This result agrees with the spectroscopic spin states of the heme irons reported for the 5,10,15,20-tetra(α-*o*-pivalamidophenyl)porphyrinatoiron(II), [Fe(tpapp)], complexes with mim and 1,2-dimethylimidazole (dmim) in toluene.[19]

For the diheme system, the large δ and ΔE_Q values of deoxy mim-heme complexes showed the iron to be in the iron(II) high-spin state. This suggested that the mono(mim)-ligated, or five-co-ordinate, diheme was formed during the deoxy state and that

Table 1. Mössbauer parameters of the liposome–lipid-heme and micelle–diheme complexes in aqueous media at 77 K

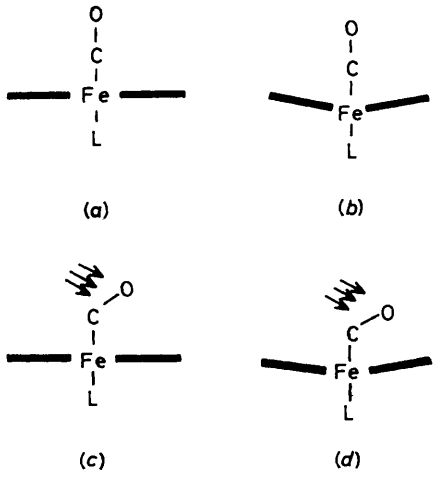
			Deoxy		CO		Oxy		
Heme	Ligand	Condition	δ/mm s^{-1}	ΔE_Q/mm s^{-1}	δ/mm s^{-1}	ΔE_Q/mm s^{-1}	δ/mm s^{-1}	ΔE_Q/mm s^{-1}	Ref.
Liposome–lipid-heme	li	H_2O	0.43	0.92	0.20	0.40	0.28	2.15	This work
	lmi	H_2O	0.98	2.09	0.25	0.39			This work
Lipid-heme	li	Methanol	0.49	0.85	0.22	0.44			This work
Micelle–diheme	li	H_2O	0.90	2.25	0.24	0.50	0.25	2.09	This work
Diheme	mim	Benzene	0.89	2.26	0.25	0.53	0.29	2.06	This work
Protoheme	im	Solid	0.44	0.98	0.23	0.33			20
	2Me-im	Solid	0.89	2.06	0.25	0.51			20
Chelated-heme	im	Solid	0.95	2.06	0.22	0.38			20
	2Me-im	Solid	0.93	2.05	0.26	0.49			20
[Fe(tpp)][b]	2Me-im	Solid	0.92	2.26					*a*
[Fe(tpapp)]	mim	Solid	0.44	1.02	0.27[c]	0.27[c]	0.27	2.04	27
Hemoglobin		Solid	0.92[c]	2.37	0.26[c]	0.26[c]	0.26	2.19	25,26
Myoglobin		Solid	0.90	2.20	0.27[c]	0.36[c]	0.22	2.27	25,26

[a] J. P. Collman and C. A. Reed, *J. Am. Chem. Soc.*, 1973, **95**, 2048; J. L. Hoard and W. R. Scheidt, *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 3919. [b] tpp = 5,10,15,20-Tetraphenylporphyrinate. [c] At 4.2 K.

Table 2. CO stretching vibration data for the diheme complexes at room temperature

Heme	Ligand	Condition	ν_{CO}/cm^{-1}	Ref.
Diheme	ti	KBr tablet	1 940	This work
	im	KBr tablet	1 945	This work
Heme[a]	mim	KBr tablet	1 955	This work
Protoheme[b]	ti	KBr tablet	1 954	This work
	im	KBr tablet	1 960	This work
Hemoglobin		H_2O	1 951	*c,d*
Myoglobin		H_2O	1 945	*c,e*

[a] The iron(II) complex of the dimethyl ester of 7,17-diethyl-3,8,13,18-tetramethyl-porphyrin-2,12-diacetic acid. [b] Iron(II)–protoporphyrin IX dimethyl ester. [c] W. S. Caughey, *Ann. N.Y. Acad. Sci.*, 1970, **174**, 148. [d] S. R. Anderson and E. Antonini, *J. Biol. Chem.*, 1968, **243**, 2918. [e] E. Antonini and M. Brunori, 'Hemoglobin and Myoglobin in Their Reaction with Ligands,' North-Holland, Amsterdam, 1971.



Scheme 3. L = ligand

the mim can co-ordinate to the iron from only one side of the porphyrin plane, due to steric hindrance of the faced copper–porphyrin cap.[16]

For the CO adducts of the lipid-heme system, the Mössbauer parameters indicated that iron was in the iron(II) low-spin state. Both isomer shifts and quadrupole splittings were smaller than those of the deoxy li-heme complex of the lipid-heme, in the iron(II) low-spin state (Table 1), owing to π back donation to the co-ordinated CO. The δ value for the lmi complex was larger than that of the li complex. This means that σ donation of the co-ordinated CO is reduced as the iron is pulled out from the porphyrin plane by the 2-methyl group of lmi.

The ΔE_Q values for the CO adducts of the diheme system were much larger than those of the other iron–porphyrin complexes [except for the 2-methylimidazole (2Me-im) complexes], hb, and mb, probably because of the steric structure of the diheme. The iron–porphyrin plane is slightly distorted due to the covalently di-bridged and faced copper–porphyrin (see Scheme 2), which probably causes a large electric field gradient at the iron nucleus and brings about the large ΔE_Q values. Similarly, large ΔE_Q values were observed for the 2Me-im-ligated protoporphyrinatoiron (protoheme) (protoporphyrin IX = 3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionic acid) complex and the 2Me-im-chelated heme as shown in Table 1. The latter values have been explained by the steric hindrance between the 2-methyl group and the porphyrin plane[20] or by the distortion of the porphyrin plane, as shown in (*b*) of Scheme 3, such as doming or ruffling in the porphyrin plane.[6,21] A distorted bonding structure is also assumed for the diheme complex based upon the ΔE_Q value mentioned above.

For the CO adduct of the diheme system, the CO stretching vibration (ν_{CO}) was measured and is shown in Table 2. The ν_{CO} values of the diheme system were shifted to lower wavenumber in comparison with the values of the corresponding protoheme complexes. This means that ν_{CO} of the diheme system is influenced by the steric structure of the diheme; a distorted structure for the diheme is also supported. From the ΔE_Q and ν_{CO} values, the schematic bonding structure proposed for the CO adduct of the diheme is as shown in (*d*) of Scheme 3.

The quantum yields (Φ) for the photodissociation of the CO and oxygen adducts were determined by flash photolysis measurements. The relationship between relative flash intensities and percentage photodissociation of the CO and oxygen adducts was measured by varying the intensities of the flash with neutral density filters. The resulting curves showed a hyperbolic relationship and high efficiency in the photodissociation. The reciprocal of the flash intensity at 50% photodissociation, $(I_{\frac{1}{2}})^{-1}$, is a parameter of the photosensitivity.[17,18] The parameter $(I_{\frac{1}{2}})^{-1}$ is represented by equation (1), where ε_{mM} is

$$(I_{\frac{1}{2}})^{-1} = C \cdot \varepsilon_{mM} \cdot \Phi \qquad (1)$$

the absorption coefficient of the CO and oxygen adducts at the

Table 3. Quantum yields for the photodissociation of the CO and O_2 adducts of the diheme complex

Heme	Ligand	Solvent	Φ		Ref.
			CO	O_2	
Diheme	mim	Benzene	0.39	0.035	This work
Protoheme	mim	Benzene	0.20		This work
Chelated-heme	im	Benzene	0.28		This work
Hemoglobin		H_2O	0.40	0.008	*a,b*
Myoglobin		H_2O	1 [c]	0.030	*b*

[a] R. W. Noble, M. Brunori, J. Wyman, and E. Antonini, *Biochemistry*, 1968, **6**, 1216. [b] See footnote *e*, Table 2. [c] Defined as 1.

Soret band, Φ is the quantum yield of each complex, and *C* is a constant originated from the apparatus. Quantum yields for the photodissociaton of the CO and oxygen adducts were calculated from the above equation. The value of Φ for the photodissociation of the CO adduct of the diheme system was larger than those of the protoheme and the chelated-heme systems, and similar to those of hb and mb. It has been reported that there is a correlation between Φ and ν_{CO} for the CO adducts of hemoproteins.[17,22] Correlation between Φ and ν_{CO} was noticed in Tables 2 and 3. The relationship is associated with the bonding property of FeCO. Namely, a decrease in ν_{CO} or an increase in the π-bonding character in FeCO is more favourable for the photodissociation.[17,22] The fact that the value of Φ for the carboxy–diheme complex was similar to that for carboxy–hb and carboxy–mb means a bent character [as shown in (*c*) of Scheme 3[23,24]] in the FeCO bonding of the diheme in addition to the distorted structure of the porphyrin plane, as shown in Scheme 3(*d*).

For the oxygen adducts of the liposome–lipid-heme and the micelle–diheme in aqueous media, the Mössbauer parameters were consistent with those of oxy hb and oxy mb. The u.v.–visible absorption spectra of the lipid-heme and diheme complexes under the same experimental conditions clearly showed the oxygen adduct formation with the maxima at 545 nm (oxy lipid-heme) and 534, 569 nm (oxy diheme), respectively. The small δ value and the large ΔE_Q value for the oxygen adducts means that the iron ions are in an iron(III) low-spin state based on the charge separated structure Fe^{III}–O_2^- which has been reported for oxy hb,[25,26] oxy mb,[25,26] and oxy [Fe(tpapp)].[27]

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