Amphiphilic Porphinatoirons Having Steroid Groups and Their Oxygen-Adduct Formation in an Aqueous Medium

Eishun TSUCHIDA,* Teruyuki KOMATSU, Takeshi BABE, Taisaku NAKATA, Hiroyuki NISHIDE, and Hidenari INOUE†

Department of Polymer Chemistry, Waseda University, Tokyo 169
†Department of Applied Chemistry, Keio University, Yokohama 223

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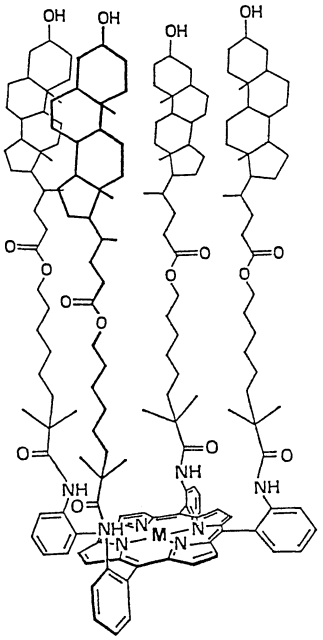
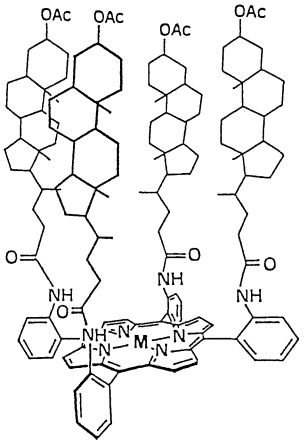
Amphiphilic heme derivatives having four steroid groups (steroid-heme), 5,10,15,20-*tetrakis*[α,α,α,α-*o*-[2,2-dimethyl-8-(3α-hydroxycholan-24-oyloxy)octanamido]phenyl]porphinatoiron (**1b**) and 5,10,15,20-*tetrakis*-[α,α,α,α-*o*-(3-acetoxycholan-24-oylamino)phenyl]porphinatoiron (**2b**), were synthesized as a model of an oxygen carrier. They were efficiently embedded in the bilayer of a phospholipid liposome based on their high compatibility with the lipid. The oxygen-binding ability of **1b** was compared with those of hemoglobin (Hb) and myoglobin (Mb). The dioxygen adduct of **1b** was also characterized by Mössbauer and infrared spectroscopy.

We have reported that phospholipid derivatives of tetraphenylporphinatoiron, such as 5,10,15,20-*tetrakis*-[α,α,α,α-*o*-[2,2-dimethyl-20-[2-(trimethylammonioethoxy)phosphinatoxy]eicosanamido]phenyl]porphinatoiron (II) (abbreviated as lipid-heme), are efficiently embedded in the bilayer of a phospholipid liposome and transport oxygen reversibly under physiological conditions (pH 7.0 aqueous medium, 37 °C), just as Hb does.[1-3] The good compatibility of the porphinatoiron with a phospholipid molecule enhances not only the trapping efficiency of the porphinatoiron in the liposome, but also the oxygen-binding ability of the porphinatoiron in an aqueous medium.



M; 2H (**1a**)
Fe (**1b**)



M; 2H (**2a**)
Fe (**2b**)

It is well-known that a sterol molecule, such as cholesterol, is a good packing reagent for a phospholipid bilayer assembly and stabilizes the phospholipid bilayer structure in biological membranes.[4] Recently, Groves et al. synthesized a steroid derivative of porphinatoiron, 5,10,15,20-*tetrakis*[α,β,α,β-*o*-[(3-hydroxy-5-cholan-24-oyl)amino]phenyl]porphinatoiron, as a model compound of oxygenase P-450 and reported that it has a high compatibility with a phospholipid bilayer after performing ^{31}P NMR and ESR spectroscopy.[5] Though it has two steroid moieties appended from either side of the porphyrin plane, the two fences on both sides of the macrocycle, respectively, could not construct a sufficient hydrophobic pocket around the metal sufficiently to form a stable oxygen adduct under physiological conditions. In the present study, we synthesized steroid derivatives of porphinatoiron (steroid-heme), 5,10,15,20-*tetrakis*[α,α,α,α-*o*-[2,2-dimethyl-8-(3α-hydroxycholan-24-oyloxy)octanamido]phenyl]-porphinatoiron (**1b**) and 5,10,15,20-*tetrakis*[α,α,α,α-*o*-(3α-acetoxycholan-24-oylamino)phenyl]porphinatoiron (**2b**), having four steroid groups on one side of the porphyrin plane. Their high compatibility with a phospholipid or the stabilization effect of the substituted steroid groups on the formed liposome was estimated by the trapping efficiency of **1b** and **2b** in the phospholipid bilayer and their liposome structure. The effect of steroid groups substituted on the porphyrin plane was also analyzed through an oxygen-binding reaction of the liposome embedded steroid-heme (**1b**) under physiological conditions and through a characterization of the oxygen adduct by both Mössbauer and infrared spectroscopy, in comparison with those of Hb.

Experimental

5,10,15,20-*tetrakis*[α,α,α,α-*o*-[2,2-dimethyl-8-(3α-hydroxycholan-24-oyloxy)octanamido]phenyl]porphinatoiron (1b). 3α-(formyloxy)cholan-24-oyl chloride (4.0 g, 9.76 mmol) was allowed to react with 5,10,15,20-*tetrakis*[α,α,α,α-*o*-(2,2-dimethyl-8-hydroxyoctanamido)phenyl]porphine[6] (1.88 g, 1.39 mmol) in dry THF (300 cm^3) containing pyridine (0.77 g, 9.76 mmol); the mixture was heated to reflux under nitrogen and continuous stirring for 10 hr. The

solution was then dried on a rotary evaporator and extracted with CH_2Cl_2. The organic layer was washed, first with dilute HCl and then with aqueous ammonia. The resulting solution was evaporated and the residue chromatographed on a dry silica-gel column using 10 : 1 (v/v) $CHCl_3$-diethyl ether as the eluent. The elution was reduced to a small volume on a rotary evaporator and the residue dried at room temperature for several hours in vacuo, to give a purple crystalline product, 5,10,15,20-*tetrakis*[$\alpha,\alpha,\alpha,\alpha$-*o*-[3$\alpha$-(formyl-oxycholan-24-oyloxy)octanamido]phenyl]porphine (3.57 g, 1.23 mmol). This porphyrin (3.57 g, 1.23 mmol) was deformylated by potassium hydrogencarbonate (0.74 g, 7.4 mmol) in CH_2Cl_2-CH_3OH-water (100 cm^3). The mixture was continuously stirred at 40—50 °C for 2 h, and then extracted with CH_2Cl_2. The extract was concentrated and separated by a dry silica-gel column using 20 : 1 (v/v) $CHCl_3$-CH_3OH as the eluent to give 5,10,15,20-*tetrakis*[$\alpha,\alpha,\alpha,\alpha$-*o*-[2,2-dimethyl(3$\alpha$-hydroxycholan-24-oyloxy)octanamido]-phenyl]porphine (**1a**) (2.13 g, 54.7%). Anal. Calcd for $C_{180}H_{258}N_8O_{16}$: C, 78.1; H, 8.6; N, 5.0. Found: C, 77.9; H, 8.6; N, 4.9. IR (KBr); 3350 cm^{-1} (ν_{OH}), 1740 cm^{-1} (ν_{CO} (ester)), 1685 cm^{-1} (ν_{CO} (amide)). ^1H NMR ($CDCl_3$) δ=−2.4 (2H, s, inner H), −0.2 (24H, s, 2,2-dimethyl H), 0.2—2.0 (40H, m, alkyl chain H), 0.2—2.3 (104H, m, steroid), 0.6 (12H, s, C_{21} steroid), 0.9 (24H, m, C_{18}, C_{19} steroid), 2.2 (8H, s, C_{23} steroid), 3.5 (4H, s, C_3 steroid), 4.0 (8H, t, alkyl chain H(CH_2OCO-)), 7.1—7.9 (24H, m, *meso*-phenyl H), 8.7 (4H, s, amido H), 8.8 (8H, s, β-H of porphyrin ring). VIS. ($CHCl_3$) 641, 588, 545, 511, and 418 nm. Iron insertion of the porphyrin was carried out as follows: **1a** (1 g, 0.36 mmol) and anhydrous iron (II) bromide (7.98 g, 27 mmol) were dissolved in dry THF (200 ml); the mixture was then heated to reflux under nitrogen. The reaction was came to completion after 6 h. The mixture was then dried on a rotary evaporator and extracted with CH_2Cl_2. The resulting solution was chromatographed on a dry silica-gel column using 20 : 1 (v/v) $CHCl_3$-CH_3OH as the eluent. The elution was treated with concentrated HBr (0.2 ml) and dried at room temperature for several hours in vacuo, to give a dark-purple crystalline product (**1b**) (1.0 g, 94.4%). Anal. Calcd for $C_{180}H_{256}N_8O_{16}FeBr$: C, 73.9; H, 8.8; N, 3.8; Fe, 1.9; Br, 2.7%. Found: C, 74.0; H, 9.0; N, 3.9; Fe, 1.8; Br, 2.6%. IR (KBr) 3350 cm^{-1} (ν_{OH}), 1740 cm^{-1} (ν_{CO}(ester)), 1685 cm^{-1} (ν_{CO}(amide)). VIS. ($CHCl_3$), 678, 646, 575, 506, and 419 nm. An iron 57-labeled derivative of **1b**, used for Mössbauer spectroscopic measurements, was also synthesized according to the above method.

5,10,15,20-*tetrakis*[$\alpha,\alpha,\alpha,\alpha$-*o*-(3$\alpha$-acetoxycholan-24-oylamino)phenyl]porphinatoiron (2b). 3α-Acetoxycholan-24-oyl chloride (4.05 g, 9.56 mmol) was allowed to react with 5,10,15,20-*tetrakis*($\alpha,\alpha,\alpha,\alpha$-*o*-aminophenyl)porphine (0.92 g, 1.37 mmol) in dry THF (300 cm^3) containing pyridine (1.08 g, 13.7 mmol); the mixture was continuously stirred for 10 h. at 25 °C under nitrogen. The solution was then dried on a rotary evaporator and extracted with CH_2Cl_2. The organic layer was washed, first with dilute HCl and then with aqueous ammonia. The resulting solution was evaporated and the residue chromatographed on a dry silica-gel column using 10 : 1 (v/v) $CHCl_3$-diethyl ether as the eluent. The elution was reduced to a small volume on a rotary evaporator and residue dried at room temperature for several hours in vacuo, to give a purple crystalline product, 5,10,15,20-*tetrakis*[$\alpha,\alpha,\alpha,\alpha$-*o*-(3$\alpha$-acetoxycholan-24-oyl-amino)phenyl]porphine (**2a**) (2.90 g, 92.9 %). Anal. Calcd for $C_{148}H_{194}N_8O_{12}$: C, 78.1; H, 8.6; N, 4.9. Found C, 78.4; H, 8.9; N, 4.8. IR (KBr) 1725 cm^{-1} (ν_{CO}(ester)), 1685 cm^{-1} (ν_{CO}(amide)); ^1H NMR ($CDCl_3$) δ=−2.4 (2H, s, inner H), 0.2—2.3 (104H, m, steroid), 0.6 (12H, s, C_{21} steroid), 0.9 (24H, m, C_{18}, C_{19} steroid), 2.0 (12H, s, C_3-OCO-$\underline{CH_3}$), 4.0 (4H, s, C_3 steroid), 7.1—7.9 (24H, m, *meso*-phenyl H), 8.7 (4H, s, amido H), 8.8 (8H, s, β-H of porphyrin ring). VIS. ($CHCl_3$) 641, 588, 545, 511, and 418 nm. Iron insertion was carried out as described above. (**2b**); Anal. Calcd for $C_{148}H_{192}N_8O_{12}FeBr$: C, 73.4; H, 8.0; N, 4.7; Fe, 2.3; Br, 3.3%. Found C, 73.0; H, 7.9; N, 4.6; Fe, 2.1; Br, 3.4%. IR (KBr) 1725 cm^{-1} (ν_{CO}(ester)), 1685 cm^{-1} (ν_{CO}(amide)) VIS. ($CHCl_3$) 680, 651, 582, 511, and 419 nm.

Other Materials. Potassium hydrogencarbonate was commercially available as a special grade and used without further purification. Tetrahydrofuran (THF) and benzene were purified immediately before use by distillation from sodium and benzophenone under nitrogen. Dichloromethane (CH_2Cl_2) and pyridine were purified before use by distillation from calcium hydride under nitrogen. 1-Dodecylimidazole (dim) and 1-dodecyl-2-methylimidazole (dmi) were synthesized as reported in previous papers.[7] Ditetradecylphosphatidylcholine (DMPC) and egg york lecithin (EYL) were purchased from Sigma (special grade).

Preparation of the Liposomal Steroid-heme. The liposome-embedded iron(II) porphyrin (deoxy type) was prepared as described in a previous paper.[3] A suspension including the steroid-heme, the imidazole derivative and the phospholipid (molar ratio 1 : 3—40 : 50—200) was ultrasonicated and homogenized in a 0.1 mol dm^{-3} phosphate buffer solution (pH 7.0) to give red and transparent solutions of the liposome-embedded steroid-heme.

Spectroscopic Measurements. The spectra were recorded on a Shimazu UV-2100 high-sensitive spectrophotometer equipped with a thermostated cell holder and a bath. Infrared (IR) spectra were taken with JASCO IR-810 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on JEOL GSX-400 instrument. Chemical shifts are expressed in parts per million downfield from Me_4Si as an internal standard.

Oxygen-Binding to the Liposomal Steroid-heme. The oxygen-binding affinity (P_{50}: oxygen pressure at half oxygen-binding for the metalloporphyrin.) was determined by the spectral changes at various partial pressures of oxygen. The enthalpy and entropy change of oxygenation (ΔH, ΔS) were obtained from van't Hoff plots of P_{50} vs. $1/T$ (T=20—37 °C).

Kinetic Measurement. The kinetics of the oxygen-binding was monitored by flash-photolysis spectroscopy, using a Unisoku USP-500. The oxygen-binding rate constants (k_{on}, k_{off}) were determined by pseudo-first-order kinetics at various partial pressures of oxygen.

Infrared Spectroscopy. The infrared differential spectra were measured under $^{16}O_2$ vs. $^{18}O_2$ and $^{12}C^{16}O$ vs. nitrogen atmospheres. The heme concentration was 10 mmol dm^{-3}, and the cells used were precisely matched in terms of path length (0.1 mm) and NaCl window thickness. The spectrum was recorded with double-beam type IR spectrometer in the absorbance mode.

Mössbauer Spectroscopy. The Mössbauer spectra were measured under argon, CO, or O_2, respectively, at 77 K. The Mössbauer spectrometer was a constant-acceleration

type. The source was used at 25 °C and consisted of ca. 10 mCi (3.7×10^8 Bq) ^{57}Co diffused palladium foil. The absorbers were maintained at 77 K. The Doppler velocity was calibrated with natural iron foil kept at 25 °C; the zero velocity was taken as the centroid of its Mössbauer spectrum at 25 °C. The spectra were fitted to Lorentzian line shapes by using a least-squares fitting program.

Other Measurements. The gel permeation chromatography (GPC) elution curve was measured with a Sepharose 4B column (Pharmacia Fine Chemical, 2.5×50 cm. A small amount of the phospholipid, which contains an unsaturated fatty acid residue, 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phospocholine, was added for the probe. Transmission electron microscopy (TEM) (Hitachi H-500) of the liposomal steroid-heme was carried out by a negative staining method using uranyl acetate. The particle size of the liposomal steroid-heme was also measured by a submicron particle analyzer (Coulter Electronics N4 SD).

Results and Discussion

The incorporation of **1b** or **2b** in the lipid bilayer of liposome was confirmed by gel permeation chromatography monitored by absorption at 418 and 255 nm, based on the heme derivative and the phospholipid, respectively. The curves coincided with each other, meaning that **1b** or **2b** are included in the liposome. The trapping efficiency of **1b** or **2b** in the liposome was also measured by the GPC. The efficiency was more than 80%, which was superior to those of 5,10,15,20-tetraphenylporphinatoiron. This indicates that the steroid substituent groups improve the compatibility of the porphyrin with the phospholipid bilayer.

From TEM the liposomal steroid-heme looked like a unilamellar or single-walled liposome with diameters of ca. 30—40 nm. It shows that steroid-heme was incorporated in the phospholipid assembly without disordering their bilayer structure, owing to its high compatibility to the liposome. The average particle size of the liposomal steroid-heme was also measured by the particle analyzer; the diameter of the particle sizes was distributed in the range 25—35 nm.

The metal-free porphyrin derivatives, **1a** and **2a**, are fluorescent active and are used as a fluorescent probe to estimate the position of the porphyrin in the bilayer membrane. The fluorescence spectrum of the liposome embedded **1a** with maxima at 585 and 650 nm agreed with those in aprotic and organic solvents. Furthermore, its fluorescence intensity was intermediate between those in benzene and dichloromethane. These results indicate that the porphyrin is molecularly dispersed in the bilayer and surrounded by an environment similar to that in aprotic and organic solvents.

The visible spectrum of the deoxy-liposome embedded steroid-heme **1b**/dmi complex (λ_{max} 439, 533, and 561 nm) changed to that assigned to the oxygen adduct (λ_{max} 424 and 546 nm) upon exposure to oxygen. The spectrum of the oxygen adduct changed to that of the CO adduct (λ_{max} 426 and 544 nm) when CO was bubbled through the solution; it returned to that of deoxy-heme upon bubbling nitrogen. The deoxy-oxy cycle could be repeated more than one hundred times under physiological conditions. On the other hand, the spectrum of the deoxy-liposome embedded **2b** (λ_{max} 437, 533, and 561 nm) changed upon exposure to oxygen to that assigned to the irreversibly oxidized Fe(III) form of the porphinatoiron. This result suggests that the two methyl groups on the octanamido side chain of tetraphenylporphinatoiron play a crucial role to form an oxygen adduct and that steric substituents on the porphyrin plane efficiently suppress the irreversible oxidation of the oxygen adduct.

The oxygen-binding affinity was determined by an oxygen-binding equilibrium curve measurement in the aqueous medium (Table 1). The P_{50} value of the embedded liposome (**1b**) is 55 mmHg at 37 °C, close to that of Hb in blood[8] and our previously reported lipid-heme.[1,3,7] This indicates that the liposome-embedded steroid-heme (**1b**) acts as an effective oxygen

Table 1. Rate Constants, Affinity, and Thermodynamic Parameters for the Oxygen-Binding of the Liposomal Steroid-heme in pH 7.0 Aqueous Solution at 37 °C

Heme	$10^{-4}k_{on}$	k_{off}	p_{50}	ΔH	ΔS
	$dm^3\,mol^{-1}\,s^{-1}$	s^{-1}	mmHg[a]	kcal mol^{-1}[b]	cal $K^{-1}\,mol^{-1}$
Liposomal steroid-heme(**1b**)					
EYL[c]	3700	2900	55	−12	−35
EMPC[c]	3600	2900	58	−12	−36
Liposomal lipid-heme[d,e]	9800	8200	53	−15	−40
Liposomal picket fence-heme[d,f]	0.79	0.32	51	−16	−46
Red blood cell[g] suspension	1.1—4.2	0.16	27	−14	−42
Stripped Hb[h]	3300	12—13	0.22—0.36	−14—−15	ca. −40
Mb[h]	1000—2000	10—30	0.37—1.0	−14—−21	ca. −40

a) mmHg=133.322 Pa. b) cal=4.184 J. c) Liposome composed of EYL or DMPC. Ligand is dmi. d) Liposome composed of DMPC. Ligand is dim. e) From Ref. 11. f) From Ref. 3. g) From Ref. 8 and K. Imai, H. Morimoto, M. Kotani, H. Watari, and M. Kuroda, *Biochim. Biophys. Acta*, **200**, 189 (1970). h) From Ref. 10, 12, and M. R. Wang, B. M. Hoffman, S. J. Shire, and F. R. N. Gurd, *J. Am. Chem. Soc.*, **101**, 7394(1979), at 20 °C.

carrier under physiological conditions.

A kinetic profile of the oxygen-binding reaction was studied using flash photolysis. The oxygen-binding association and dissociation rate constants (k_{on}, k_{off}) are summarized in Table 1. The k_{on} value for the liposome-embedded 5,10,15,20-*tetrakis*(α,α,α,α-*o*-pivalamidophenyl)porphinatoiron (abbreviated as picket fence-heme) was similar to that of the red blood cell suspension.[9] The oxygen-binding rate constants for the homogenous system, such as Mb and Hb, were ca. 1000-times larger than those of the liposome-embedded picket fence-heme and the red blood cell suspension.[10] The oxygen-binding reaction is assumed to be largely retarded by a diffusion process of oxygen in and through the phospholipid membrane. The k_{on} values for the liposome-embedded steroid-heme and liposome-embedded lipid-heme were comparable with those of Hb and Mb.[11] These results suggest that the steric alkyl or steroidal groups above the porphyrin plane form an oxygen-binding pathway from the outside water phase to the oxygen-binding site of the heme and accelerate diffusion of oxygen in the bilayer membrane.

The thermodynamic parameters for the oxygen binding are also shown in Table 1. The enthalpy change (ΔH) and entropy change (ΔS) for the oxygen binding of the embedded liposome (**1b**) were estimated to be -12 kcal mol^{-1} and -35 cal K^{-1} mol^{-1}, respectively. These values are comparable to those of Hb[10,12] and the liposome-embedded lipid-heme.[1] These results indicate that the oxygen binding of the embedded liposome embeded (**1b**) proceeds in the same way as the binding of Hb and the liposome-embedded lipid-heme.

Mössbauer spectra were measured for the deoxy complex and the CO and oxygen adducts of **1b** at 77 K. The Mössbauer parameters for **1b** and other porphinatoiron are summarized in Table 2. For the deoxy-dim ligated porphinatoiron complex of **1b**, both the isomer shifts(δ) and the quadrupole splitting(ΔE_Q) were nearly equal to those of the deoxy complex of the picket fence-heme, which indicates that the iron ion is in the iron(II) low-spin state.[13] For the CO adduct of **1b**, both δ and ΔE_Q were smaller than those of the deoxy complexes of **1b**, in the iron(II) low-spin state, owing to a π-back donation effect at the coordinated CO. For the oxygen adduct of **1b**, the Mössbauer parameters were consistent with those of oxy-Hb and oxy-Mb. The visible absorption spectra of the **1b** complex under the same experimental conditions supported the oxygen adduct formation. The small δ value and the larger ΔE_Q value for the oxygen adduct mean that the iron ion is in an iron(III) low-spin state. This suggests a charge-separated structure Fe(III)-O_2^- which has been reported for oxy-Hb,[14,15] oxy-Mb and the oxygen adduct of the picket fence-heme.

Table 2. Mössbauer Parameters of the Steroid-heme Complexes

Heme	Ligand	Solvent	Deoxy		CO		Oxy	
			δ	ΔE_Q	δ	ΔE_Q	δ	ΔE_Q
			mm s^{-1}	mm s^{-1}	mm s^{-1}	mm s^{-1}	mm s^{-1}	mm s^{-1}
Steroid-heme(**1b**)	dim	Toluene	0.44	0.99	0.25	0.33	0.25	1.98
Picket fence-heme[a]	mim	Toluene	0.44	0.99	0.27[b]	0.27[b]	0.27	2.04
Lipid-heme[c]	dim	Methanol	0.49	0.85	0.22	0.44	0.28	2.15
Chelated-heme[d]	im	—	0.95	2.06	0.22	0.38	—	—
Hb[e]	—	—	0.92[b]	2.42[b]	0.26[b]	0.36[b]	0.26	2.19
Mb[e]	—	—	0.90	2.21	0.27[b]	0.36[b]	0.22	2.27

a) From Ref. 13. b) At 4.2 K. c) From Ref. 19. d) From E. Tsuchida, H. Nishide, H. Yokoyama, H. Inoue, and T. Shirai, *Polym. J.*, **16**, 325 (1984). e) From Refs. 15 and 19.

Table 3. Infrared Spectra Data of the Steroid-heme

Heme	Oxy		CO	Intensity ratio A(O_2)/A(CO)
	$\nu_{^{16}O-^{16}O}(\nu_{1/2})$	$\nu_{^{18}O-^{18}O}$	$\nu_{CO}(\nu_{1/2})$	
	cm^{-1}	cm^{-1}	cm^{-1}	
Steroid-heme(**1b**)[a]	1162(13)	1078	1970	0.14
Picket fence-heme[b]	1159(—)	1075	1968	—
Hb[c]	1107(9±1)	1065	1951(8)	0.19
Mb[d]	1103(8±1)	1065	1944(12)	0.10
O_2 or CO gas[e]	1556	—	2143	—

a) Ligand is dim. Benzene solution. b) Ligand is mim. Nujol mulls. From Ref. 15. c) From C. H. Barlow, J. C. Maxwell, W. J. Wallace, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **55**, 91 (1973). d) J. G. Maxwell, J. A. Volpe, C. H. Barlow, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **58**, 166 (1974). e) $^{16}O_2^-$=1145 cm^{-1}. From Ref. 17.

Data concerning the infrared absorption spectra are summarized in Table 3. The C-O stretching frequency (ν_{CO} of **1b**-CO) was 1970 cm^{-1}, similar to that of the picket fence-heme-CO complex of 1-methylimidazole (mim) in Nujol mulls (ν_{CO}=1969 cm^{-1}).[13] Similarly, the difference spectrum of the CO vs. the $^{16}O_2$ adducts of **1b** showed an intense band at 1970 cm^{-1}. The difference spectrum of the $^{16}O_2$ adduct vs. the CO adduct of **1b** showed an intense band at 1162 cm^{-1} with a band-width of 13 cm^{-1} at 1/2 height, a value which agreed with those of the picket fence-heme complex in Nujol mulls.[16] The intensity relative to the CO band of **1b** was similar to those of Mb and Hb. For the difference spectra of the $^{16}O_2$ vs. the $^{18}O_2$ adducts of **1b**, the $^{16}O_2$ adduct showed an intense band at 1162 cm^{-1} while the $^{18}O_2$ adduct showed prominent bands at 1122 and 1078 cm^{-1} based on the frequency of ^{16}O-^{18}O and ^{18}O-^{18}O, respectively. The O-O stretching frequency of **1b** differs from that of the gaseous molecular oxygen (ν_{O-O}=1556 cm^{-1}),[17] but is similar to that of superoxide (O_2^-, 1145 cm^{-1}).[17] It is concluded that the coordination structure of the oxygen adduct of **1b** at room temperature is a bent end-on type similar to oxy-Mb, oxy-Hb, and the oxygen adduct of the picket fence-heme.[18]

Our results show that the liposome embedded steroid heme (**1b**) acts as an effective Hb and Mb model under physiological conditions.

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