O_2 and CO Binding Behavior of Double-Sided Porphyrinatoiron(II) Complexes Modified by Amide Residues

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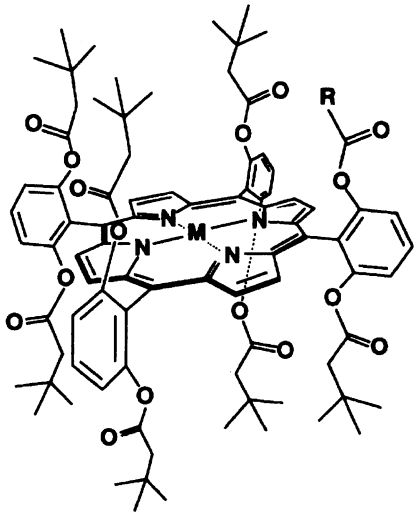
New double-sided porphyrinatoiron(II) complexes having a polar cavity which includes an amide moiety (5,10,15-tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[*N*-acetylvalyloxy]-6-(3,3-dimethylbutyryloxy)phenyl]-porphinatoiron(II) (**1b**) and 5,10,15-tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[3,5-bis(acetamido)benzoyloxy]-6-(3,3-dimethylbutyryloxy)phenyl]porphinatoiron(II) (**2b**)) were synthesized. ^{1}H NMR spectroscopy indicated that the amide residues are located on the porphyrin ring plane. The O_2 and CO binding affinities of **1b** and **2b** were higher than those of 5,10,15,20-tetrakis[2,6-bis(3,3-dimethylbutyryloxy)phenyl]porphinatoiron-(II) (**4b**), in response to the local polarity in the cavity. The polar amide residue resulted in a decreased O_2 dissociation rate. Thermodynamic parameters for the gaseous ligand bindings to the **2b** complex were also determined.

In hemoglobin (Hb) and myoglobin (Mb), heme-ligand binding properties are governed by the electric and steric effects of the microenvironment surrounding the coordination site of heme. X-Ray structural, neutron diffraction, and Raman studies of oxy-Hb and oxy-Mb have provided direct evidence that the terminal oxygen of the bound O_2 forms a hydrogen bond with the distal imidazole proton of histidine.[1—3] This electrostatic intermolecular interaction contributes to the stabilization of the O_2-heme binding.

5,10,15,20-Tetrakisphenylporphine (TPP) and their derivatives have been widely exploited in the development of bioinorganic systems,[4] and can provide an excellent starting point to fulfill many of synthetic requirements for mimicking the remarkable capabilities of hemoprotein. Many models of the natural O_2 carrier so far synthesized were also derived from TPP, having a steric cavity on the ring plane, in order to prevent μ-dioxo dimer formation leading to irreversible oxidation.[5—10] Prominent among these modified porphyrin complexes is 5,10,15,20-tetrakis(o-pivalamidophenyl)porphinatoiron(II) (picket-fence porphyrinatoiron; TpivPP).[5a] TpivPP forms a very stable O_2 adduct in toluene at 25 °C and has a long lifetime compared to those of the others. Therefore, the role of the pivalamido groups in the cavity of TpivPP regarding O_2 adduct formation has been discussed in comparison with the distal histidine in the heme pocket of Hb for a decade. Although the possibility of hydrogen bonding between the bound O_2 and pivalamido groups in TpivPP is denied by the IR and ^{17}O NMR spectra of the O_2 adduct complex, the amide dipole is known to contribute to the strong O_2 affinity.[7b,11]

In order to clarify the effect of the polar groups around the coordination site to the O_2 binding kinetics, Chang et al. synthesized model derivatives equipped with a range of polar groups situated at similar positions from the heme center.[12] They have demonstrated that dipolar forces and hydrogen bonding can play a significant role in regulating the O_2 affinities of the hemes. Furthermore, Kyuno et al. synthesized several modified tetraphenylporphinatocobalt(II)s which contain polar or nonpolar groups in their cavities, and discussed the relation between the O_2 affinity and the substituted groups.[13] Reed et al. recently synthesized picket-fence-type porphyrinatoirons having one of the four pivalamido groups replaced by a substituent ca-



R: $CH_3\overset{\overset{O}{\|}}{C}NCH(CH_3)_2-$ H — M: 2H 1a; Fe(III)Br 1b'; Fe(II) 1b

R: $CH_3\overset{\overset{O}{\|}}{C}N$ H, $CH_3\overset{\overset{O}{\|}}{C}N$ H — M: 2H 2a; Fe(III)Br 2b'; Fe(II) 2b

R: $(CH_3)_3CCH_2-$ — M: 2H 4a; Fe(III)Br 4b'; Fe(II) 4b

Chart 1.

pable of hydrogen bonding, and determined their O_2 binding affinities.[14] The effect of hydrogen bonding on the O_2 affinity was best illustrated by the 10-fold increase observed when one pivalamido group of TpivPP was replaced by a phenylurea substituent.

We synthesized highly symmetric "double-sided" porphyrinatoiron(II) complexes, having two cavities comprising four ester groups on both sides of the porphyrin plane, and reported on their O_2 and CO binding kinetics.[15] The "double-sided" enclosure showed a similar effect as that of the globin wrapping in Hb, and forms hydrophobic cavities around the axial coordination sites.[15d] However, the lower O_2 binding affinities of double-sided hemes, compared to that of the amide-residue-substituted derivatives (e.g. TpivPP), was ascribed to a decrease in a local polarity inside the cavity.

In the present study we designed and synthesized new double-sided porphyrinatoiron complexes having a polar cavity which includes an acetamido group in order to elucidate the influence of the ester and amide residues to the O_2 binding affinity: 5,10,15-tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(*N*-acetylvalyloxy)]-6-(3,3-dimethylbutyryloxy)phenyl]porphinatoiron(II) (**1b**) and 5,10,15-tris[2,6-bis-(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(3,5-bis(acetamido)benzoyloxy]-6-(3,3-dimethylbutyryloxy)phenyl]-porphinatoiron(II) (**2b**). We report here on the ligation property and kinetic behavior of the axial base, O_2, and CO to the hemes and discuss the effect of the ester and/or amide residues on O_2 and CO binding (Chart 1).

Experimental

General. Infrared spectra were recorded with a JASCO FT/IR-5300 spectrometer. ^{1}H NMR spectra were recorded on a JEOL GSX-400 instrument. The chemical shifts are expressed in ppm downfield from Me_4Si as an internal standard. FAB mass spectra were measured with a JEOL DX-303 spectrometer. Absorption spectra were recorded with Shimadzu UV-2100 spectrophotometer. 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.

Materials and Solvents. 1,2-Dimethylimidazole (1,2-Me_2Im) was purified before use by distillation in vacuo under reduced pressure. 3,3-Dimethylbutyryl chloride, 4-(dimethylamino)pyridine, *N*-acetyl-DL-valine, 3,5-diaminobenzoicacid dihydrochloride, 4-(1-pyrrolidinyl)pyridine, and dicyclohexylcarbodiimide (DCC) were commercially available as special grade and were used without further purification. Tetrahydrofuran (THF) and toluene were purified immediately before use by distillation from sodium and benzophenone. *N*,*N*-Dimethylformamide (DMF) was distilled in vacuo under argon and stored over 4A molecular sieves.

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-hydroxy-6-(3,3-dimethylbutyryloxy)phenyl]porphine (3). 5,10,15,20-Tetrakis(2,6-dihydroxyphenyl)porphine[15a] (3.0 g, 40.4 mmol) and 4-(dimethylamino)-pyridine (3.4 g, 28.0 mmol) were dissolved in dry THF (500 ml). To this ice-cooled solution 3,3-dimethylbutyryl chloride (3.9 cm^3, 28.0 mmol) was added dropwise with stirring under an argon atmosphere; the reaction mixture was further stirred for 4 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 was chromatographed on a silica-gel flash column using $CHCl_3$/diethyl ether (10/1 (v/v)) as the eluent. The second elution 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 a purple crystalline product (**3**) (0.59 g, 10.3%). R_f 0.59 ($CHCl_3$/diethyl ether (10/1 (v/v)). FAB MS $[M+1]^+$ 1429. IR (KBr) 3454 (ν_{OH}), 1762 cm^{-1} (ν_{CO}(ester)). ^{1}H NMR ($CDCl_3$) $\delta=-3.0$ (2H, s, inner H), −0.5—0.2 (63H, m, *t*-butyl), 1.2 (14H, m, $-CH_2-$), 7.0—7.9 (12H, m, phenyl H), 8.9 (8H, d, pyrrole-H). VIS. ($CHCl_3$)650, 579, 535, 507, and 413 nm.

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(*N*-acetylvalyloxy)]-6-(3,3-dimethylbutyryloxy)phenyl]porphine (1a). To a solution of *N*-acetyl-DL-valine (0.17 g, 1.1 mmol), **3** (0.03 g, 0.02 mmol), and 4-(1-pyrrolidinyl)pyridine (16 mg, 0.11 mmol) in dry THF (4 ml), DCC (0.22 g, 1.05 mmol) was added. The reaction mixture was stirred at 25 °C for 10 h. The precipitated *N*,*N*′-dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in benzene and insoluble DCU was removed by filtration. The filtrate was concentrated and the residue was chromatographed on a silica-gel flash column using $CHCl_3$/ethyl acetate (10/1 (v/v)) as the eluent. The eluent was collected and evaporated to dryness. The residue was then dried at room temperature for several hours in vacuo to afford a purple crystalline product (**1a**) (18 mg, 54.0%). R_f 0.35 ($CHCl_3$/ethyl acetate (5/1 (v/v))). FAB MS $[M]^+$ 1569. IR (KBr) 1763 (ν_{CO}(ester)), 1653 cm^{-1} (ν_{CO}(amide)). ^{1}H NMR ($CDCl_3$) $\delta=-3.1$ (2H, s, inner H), −1.8, −0.9 (6H, d, $-(CH_3)_2$), −0.5—0.4 (63H, m, *t*-butyl), 0.9 (3H, s, $-CH_3$ (acetamido), 1.2 (14H, m, $-CH_2-$), 3.5 (1H, m, $-C\underline{H}(CH_3)_2$), 4.8 (1H, d, NH (acetamido)), 7.4—7.9 (12H, m, phenyl H), 8.9 (8H, s, pyrrole-H). VIS. (toluene) 650, 583, 537, 507, and 414 nm.

3,5-Bis(acetamido)benzoic Acid (5). After 3,5-diaminobenzoic acid dihydrochloride (10.0 g, 44.4 mmol) was dissolved in 10 M–NaOH aqueous solution (40 ml), the mixture was stirred for 30 min in an ice–water bath. Acetyl chloride (63 ml, 88.8 mmol) was dropped into the solution at 5 °C for 3 h; stirring continued for an additional 2 h at room temperature. The reactant was filtered off and a white solid was washed with water and then dried in vacuo at 50 °C for 12 h to give **5** (75.1%). EI MS $[M]^+$ 236. IR (KBr) 3449 (ν_{OH}), 3272 (ν_{NH}(amide)), 1705 (ν_{CO}), 1655 cm^{-1} (ν_{CO}(amide)).

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(3,5-bis(acetamido)benzoyloxy)]-6-(3,3-dimethylbutyryloxy)phenyl]porphine (2a). To a solution of **5** (0.15 g, 0.63 mmol), **3** (45 mg, 0.03 mmol), and 4-(1-pyrrolidinyl)pyridine (9.3 mg, 0.06 mmol) in dry DMF (4 ml), DCC(0.13 g, 0.63 mmol) was added. The reaction mixture was stirred at 25 °C for 24 h. The DCU was filtered off and the filtrate was evaporated to dryness. After

the residue had been dissolved in benzene, insoluble DCU was removed by filtration. The filtrate was concentrated and the residue was chromatographed on a silica-gel flash column using $CHCl_3$/methanol (15/1 (v/v)) as the eluent. The elution was collected and evaporated to dryness. The residue was then dried at room temperature for several hours in vacuo to afford a purple crystalline product (**2a**) (19.7 mg, 38.0%). R_f 0.32 ($CHCl_3$/methanol (15/1 (v/v))). FAB MS $[M]^+$ 1646. IR (KBr) 3320 (ν_{NH}), 1757 (ν_{CO}(ester)), 1669 cm^{-1} (ν_{CO}(amide)). ^1H NMR ($CDCl_3$) $\delta=-3.1$ (2H, s, inner H), −1.2—0.4, (63H, m, *t*-butyl), 1.2 (14H, s, $-CH_2-$), 1.6 (6H, s, $-CH_3$(acetamido)), 6.8 (2H, s, NHCO), 7.2—8.0 (15H, m, phenyl H), 8.9 (8H, s, pyrrole-H). VIS. (toluene) 648, 583, 536, 508, and 414 nm.

Iron Insertion. After porphyrin derivatives and excess $FeBr_2$ had been dissolved in dry THF, the mixture was heated to reflux under argon for 12 h. The mixture was then brought to dryness on a rotary evaporator, extracted with $CHCl_3$; the resulting solution was chromatographed on a silica-gel flash column. The eluate was treated with concd HBr and dried at room temperature for several hours in vacuo to give corresponded dark-purple crystalline iron(III) complexes.

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(*N*-acetylvalyloxy)]-6-(3,3-dimethylbutyryloxy)phenyl]porphinatoiron(III)Bromide (1b′). R_f 0.64 ($CHCl_3$/ethylacetate (8/1 (v/v))). FAB MS $[M-Br]^+$ 1622. IR (KBr) 1763 (ν_{CO}(ester)), 1653 cm^{-1} (ν_{CO}(amide)). VIS. (toluene) 680, 648, 584, 509, and 412 nm.

5,10,15-Tris[2,6-bis(3,3-dimethylbutyryloxy)phenyl]-20-[2-[(3,5-bis(acetamido)benzoyloxy)]-6-(3,3-dimethylbutyryloxy)phenyl]porphinatoiron(III) Bromide (2b′). R_f 0.3 ($CHCl_3$/methanol (15/1 (v/v))). FAB MS $[M-Br]^+$ 1700. IR (KBr) 3320 (ν_{NH}), 1757 (ν_{CO}(ester)), 1669 cm^{-1} (ν_{CO}(amide)). VIS. (toluene) 680, 646, 584, 509, and 412 nm.

Iron(II) Complex. Reaction of the iron(III) complex to the iron(II) was carried out using $Na_2S_2O_4$ in a heterogeneous two-phase system under an argon atmosphere, as previously reported.[15]

1b (1,2-Me$_2$Im). Compound **1b** with 10^2—10^4 equiv of 1,2-dimethylimidazole produced **1b** (1,2-Me_2Im): VIS. (toluene) 557, 535, and 434 nm. +CO; 534 and 420 nm. +O_2; 546 and 421 nm.

2b (1,2-Me$_2$Im). Compound **2b** with 10^2—10^4 equiv of 1,2-dimethylimidazole produced **2b** (1,2-Me_2Im): VIS. (toluene) 558, 537, and 435 nm. +CO; 532 and 420 nm. +O_2; 548 and 421 nm.

Equilibrium Measurements. The equilbrium constants of the axial base were determined at 25 °C under argon by titration of the four-coordinated porphyrinato-iron(II)s with aliqouts of 1,2-dimethylimidazole in O_2 free toluene. For the bases there are two possible equilibria: Eqs. 1 and 2.

$$FeP + B \overset{K(B)}{\rightleftharpoons} FeP(B) \quad (1)$$

and

$$FeP(B) + B \overset{K(B)_2}{\rightleftharpoons} FeP(B)_2 \quad (2)$$

(FeP: Porphyrinatoiron(II), B: Imidazole derivative). Since 1,2-dimethylmidazole is a sterically hindered base, the titration shows isosbestic points (i.e. $K(B)_2=0$) and the data can be fitted to Drago's equation.[15b]

O_2 and CO binding to the porphyrinatoiron(II) complex can be expressed by

$$FeP(B) + L \overset{K(O)_2}{\rightleftharpoons} FeP(B)L \quad (3)$$

(L: Gaseous ligand (O_2 or CO)). The O_2 or CO binding affinity (gaseous pressure at half O_2 or CO binding for the porphyrinatoiron(II), ($P_{1/2}(L)=1/K(L)$) was determined as described previously.[15c] The heme concentration of 2×10^{-5} mol dm^{-3} was used for the equilibrium study, and the spectra were recorded within the range of 700—360 nm.

Kinetic Measurements. Kinetic measurements were performed by using a laser flash-photolysis technique. The laser flash-photolysis experiment and data analysis were carried out with a Unisoku (USP-500 series). Rhodamine 590 in anhydrous methanol was used as the dye. The heme concentration of 1×10^{-5} mol dm^{-3} was used, and most experiments were carried out at 25±0.2°C. After laser-flash photolysis of the O_2 and/or CO complexes, the five-coordinate adduct of the heme was momentarily obtained by using 1, 2-dimethylimidazole. Under a large excess of 1,2-dimethylimidazole, no species other than the five-coordinate complex Fe(B) formed following the flash photolysis; a recombination gave k_{obsd}, represented by

$$FeP(B)L \overset{h\nu}{\rightarrow} FeP(B) \underset{k_{off}(L)}{\overset{k_{on}(L)}{\rightleftharpoons}} FeP(B)L \quad (4)$$

where

$$k_{obsd} = k_{on}(L)[L] + k_{off}(L) \quad (5)$$

Since the gaseous ligand concentrations were always in large excess to the heme concentration, the pseudo-first-order approximation was applied throughout.

$K(O_2)$ was also determined using the competitive rebinding technique.[16,17] The photolysis of FeP(B)CO in the presence of an appropriate mixture of CO and O_2 first showed a rapid recombination to $FeP(B)O_2$, followed by a slow return to FeP(B)CO, since $K(CO)\gg K(O_2)$ and $k_{on}(CO)[CO]<k_{on}(O_2)[O_2]$ for a wide range of $[O_2]$ and [CO].

$$FeP(B)CO \overset{h\nu}{\rightarrow} FeP(B) \underset{k_{off}(O_2)}{\overset{k_{on}(O_2)}{\rightleftharpoons}} FeP(B)O_2 \quad (6)$$

(k_{obsd}(slow))

From Gibson's equation,

$$1/k_{obsd}(slow) = \frac{K(O_2)[O_2]}{k_{on}(CO)[CO]} + \frac{1}{k_{on}(CO)[CO]} + \frac{1}{k_{off}(O_2)} \quad (7)$$

a plot of $1/k_{obsd}$(slow) vs. $[O_2]/[CO]$ yields a straight line with a slope of the $K(O_2)/k_{on}(CO)$. The enthalpy and entropy changes (ΔH and ΔS) of the O_2 binding reaction were calculated in terms of the O_2 binding affinity at various temperatures using van't Hoff plots. The temperature of the solution was maintained to a precision of ±0.2 °C. Arrhenius activation parameters for ligand association were determined by measuring the ligand rebinding rates at 10—35 °C.

Results and Discussion

Double-sided porphyrinatoiron complexes (**1b** and **2b**) are equipped with amide residues around the ax-

ial coordination sites: *N*-acetylvaline, and the 3,5-bis(acetamido)phenyl ring. They include an acetamido substituent having an N–H group in their superstructure, which is potentially capable of hydrogen bonding to coordinated O_2. The 3,5-bis(acetamido)phenyl group was chosen as an amide functionality over the Fe–O_2 binding site. These compounds were synthesized by coupling seven substituted porphyrin with acetamido derivatives using DCC; **1b′** and **2b′** were soluble in common organic solvents. Identification and structural assignments of these complexes were based on some physicochemical analyses (see Experimental section).

In the ^{1}H NMR spectrum of **1a** the CH_3-(isopropyl) signals of *N*-acetylvaline appeared at −0.9, −1.8 ppm and shifted more up-field than those of free *N*-acetylvaline. A similar phenomenon was seen for the CH_3–(acetyl) protons (1.6 ppm) in **2a**. These results indicate that chemical shifts of the polar substituents of **1a** and **2a** are affected by the porphyrin ring current shifts, i.e. the acetamido groups are located on the porphyrin plane. Furthermore, the $(CH_3)_3$C-(3,3-dimethylbutyryloxy) signals of **2a** were split with 1 : 2 : 2 : 2 at 0.4, −0.1, −0.3, and −1.2 ppm (Fig. 1). This observation can be understood in terms of a ring-current shift of the bis(acetamido)phenyl groups, i.e. the plane of the phenyl ring is perpendicular to the adjacent two $(CH_3)_3$C– groups and the other $(CH_3)_3$C– group is nearly coplanar with the phenyl ring on the front side of the porphyrin for O_2 binding.

Read et al. explored the superstructure conformation of 5-[α-*o*-[3,5-bis(acetamido)benzamido]phenyl]-10α,15α,20α,-tris(*o*-pivalamidophenyl)porphine (**6**) by ^{1}H NMR spectroscopy. They suggested that the 3- and/or 5-acetamido groups are capable of intramolecular hydrogen bonding to the adjacent pivalamide residues. However, it was not possible to unambiguously assign a rigid conformation of **6**.[14]

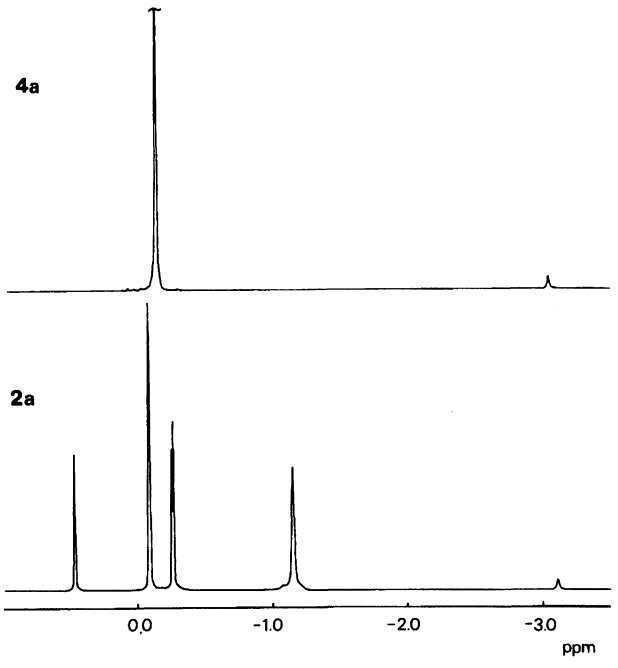


Fig. 1. ^{1}H NMR spectra of **4a** and **2a** in $CDCl_3$ at 25 °C.

Imidazole Binding Equilibrium. The binding for base to both-faces protected porphyrinatometal is usually depressed with an increase in the steric bulk of the substituents.[16] In a previous study we reported that the coordination of double-sided porphyrin to an axial base could be controlled by the nature and structure of the cavity.[15b] The *K*(B) for 1,2-dimethylimidazole to 5,10,15,20-tetrakis[2,6-bis(3,3-dimethylbutyryloxy)phenyl]porphinatoiron(II) (**4b**), having two cavities comprising 3,3-dimethylbutyryloxy groups on both sides of the ring plane, was slightly lower than that for single-face hindered porphyrin (e.g. TpivPP), indicated that the unfavorable steric repulsion on the rear side of **4b** is appreciably weakened.

The equilibrium constants for 1,2-dimethylimidazole to **1b** and **2b** were determined by the absorption spectral changes occurring upon titration of a toluene solution of the four-coordinate species with the imidazole derivatives under an argon atmosphere. The addition of 1,2-dimethylimidazole to **1b** or **2b** gave only five-coordinate high-spin species cleanly; well-defined isosbestic points were observed in the visible absorption spectra during titration. The *K*(B) for the binding of 1,2-dimethylimidazole to the double-sided heme complexes (**1b**, **2b** and **4b**), having other cavities on the porphyrin plane, are summarized in Table 1. The *K*(B) of **2b** and **4b** were nearly the same, suggesting that the 1,2-dimethylimidazole bound to the central iron of **2b** from the less-polar cavity side comprises by four 3,3-dimethylbutyryloxy groups. On the other hand, the *K*(B) of **1b** was slightly larger than those of the others. Since the *N*-acethylvaline residue is small and flexible compared to the *t*-butyl and the phenyl groups, only a few bases can bind to the central iron of **1b** from the polar-cavity side having *N*-acetylvaline.

O_2 and CO Binding Affinity. Compounds **1b** and **2b** with 1,2-dimethylimidazole gave stable and reversible O_2 adducts in toluene at 25 °C. Spectropho-

Table 1. Equilibrium Constants for the Binding of Axial Base to Fe(II) Porphyrin Complexes in Toluene at 25 °C[a)]

Porphyrin	Ligand	$10^{-3}K$(B) / dm^3 mol^{-1}	Ref.
1b	1,2-Me_2Im	2.4	This work
2b	1,2-Me_2Im	1.1	This work
4b	1,2-Me_2Im	1.3	15b
TpivPP	1,2-Me_2Im	37	5b
TPP	2-MeIm	24	b)

a) Estimated errors < 10% b) D. Brault and M. Rougee, *Biochem. Biophys. Res. Commun.*, **57**, 654 (1974).

Table 2. Parameters for Binding of O_2 and CO to Fe(II) Porphyrin (1,2-Me_2Im) Complexes in Toluene at 25 °C[a)]

Porphyrin	O_2			CO		Ref.
	$10^{-7}k_{on}$ / $dm^3\ mol^{-1}\ s^{-1}$	$10^{-4}k_{off}$ / s^{-1}	$P_{1/2}$ / Torr	$10^{-5}k_{on}$ / $dm^3\ mol^{-1}\ s^{-1}$	$10^3P_{1/2}$ / Torr	
1b	4.0	4.5	150	6.5	14	This work
2b	4.0	3.6	120	6.1	15	This work
4b	4.7	7.9	230	9.0	17	15c
TpivPP	11	4.6	38	14	9	15c

a) Estimated errors<10%.

Table 3. Thermodynamic Parameters for the Binding of O_2 and CO to Fe(II) Porphyrin (1,2-Me_2Im) Complexes in Toluene

Porphyrin	O_2					CO		
	ΔH / kJ mol^{-1}	ΔS / J K^{-1} mol^{-1}	E_a^{on} / kJ mol^{-1}	log A^{on} / —	$\Delta G^{\neq}$ / kJ mol^{-1}	E_a^{on} / kJ mol^{-1}	log A^{on} / —	$\Delta G^{\neq}$ / kJ mol^{-1}
2b	−59	−180	15	10	30	18	9	40
4b	−59	−188	20	11	30	33	12	39
TpivPP[a)]	−60	−176	13	10	27	—	—	—

a) Values calculated from $\Delta H^{\neq}$ and $k_{on}(O_2)$ in Ref. 18.

tomeric O_2 and CO titrations of **1b** (1,2-Me_2Im) and **2b** (1,2-Me_2Im) were carried out under conditions of excess base ([1,2-Me_2Im]=0.2 mol dm^{-3}). The isosbestic points were maintained in all of the titrations.

The values of $P_{1/2}(O_2)$ were determined from the spectral changes at various partial pressures of O_2 using an equation employed by Collman et al.,[18] since O_2 binding to **1b** and **2b** complexes at 25 °C were incomplete, even at 760 Torr (1 Torr=133.322 Pa) O_2. The $K(O_2)(=1/P_{1/2}(O_2))$ was also determined kinetically by the previously described Gibson's method.[17,18] This technique takes advantage of the approximately 10-fold faster rate of O_2 addition to heme compounds, compared to CO, and the much larger binding constant of CO to the hemes. Under all conditions, a plot of $1/k_{obsd}$(slow) vs. $[O_2]/[CO]$ gave $K(O_2)$. The $K(O_2)$ derived from the competition method was in accordance with that obtained directly under equilibrium conditions. The CO binding affinities of these complexes were large enough to apply Hill's plot.

The values of $P_{1/2}$ for O_2 and CO binding are summarized in Table 2. The O_2 binding affinities of the **1b** (1,2-Me_2Im) and **2b** (1,2-Me_2Im) complexes were significantly higher than that of **4b** (1,2-Me_2Im). Since the K(B) for the binding of 1,2-dimethylimidazole to **2b** was nearly the same as that for the **4b**, the difference in the O_2 affinities cannot be entirely attributed to an unfavorable steric repulsion between the axial imidazole and the 3,3-dimethylbutyryloxy groups. On the other hand the $P_{1/2}$(CO) values of these complexes were almost the same as that of the **4b** (1,2-Me_2Im) complex.

Several previous studies concerning O_2 carrier models have unmistakably demonstrated that O_2 binding to hemes is very sensitive to the solvent polarity and/or local polarity of the cavities on the ring plane. It is generally accepted that an increased solvent polarity enhances the O_2 binding due to a stabilization of the expected charge separation with Fe–O_2 bonding in unprotected hemes, and the dipole of the amide moiety contributes to the O_2 adduct in protected hemes.[7b,12—14,17—21] The O_2 binding affinity of 5, 10,15,20-tetrakis(2,4,6-triphenylphenyl)porphinatoiron having nonpolar and wide cavities on both sides of the porphyrin plane was very low compared with those of the TpivPP and **4b**.[19]

The 2-fold increase in the O_2 binding affinity of **2b**, compared to that of **4b**, suggests that the introduction of an acetamido group to the less polar cavity results in a slightly polar environment around central iron ion, which stabilizes the O_2 adduct. This corresponds to a $\Delta\Delta G$ of 1.7 kJ mol^{-1}, which is too small to contribute to stabilization of O_2 binding by hydrogen bonding. We suggest that the high O_2 binding affinity of **2b**, compared to that of **4b**, can be mainly attributed to a weak favorable dipole contribution of the amide moieties.

Kinetics of O_2 and CO Binding. In order to elucidate the influence of the amide residues to the O_2 and CO binding affinity, the dynamics of gaseous ligand binding were explored by laser-flash photolysis. When toluene solutions of the FeP(1,2-Me_2Im)CO ([heme]=1×10^{-5} mol dm^{-3}, [1,2-Me_2Im]=0.2 mol dm^{-3}) were photolyzed, linear decay plots of log ΔA vs. t were obtained. This indicates a clean monophasic rebinding. The value of k_{on} (CO) was estimated using Eq. 5. Under the same conditions, flash photolysis of the stable dioxygenated complex (FeP(1,2-Me_2Im)O_2) was carried

out over a range of O_2 concentrations. The $k_{on}(O_2)$ was obtained using Eq. 5 and $k_{off}(O_2)$ was calculated from $k_{on}(O_2)/K(O_2)$. Kinetic parameters for the gaseous ligation of double-sided porphyrinatoiron complexes are summarized in Table 2.

Traylor and Collman independently found several effects on O_2 and CO binding kinetics of synthetic porphyrinatoiron complexes.[17,18] These investigations suggested that the following factors affect the O_2 and CO binding kinetics of model hemes: (1) proximal base ligation as a fifth ligand of hemes, (2) a distal steric hindrance, and (3) an increased solvent polarity or electrostatic interactions.

In general, the $k_{on}(O_2)$ value has been reported to decrease only due to distal steric hindrance. The shapes of the cavities for O_2 and CO binding of our double-sided complexes are appreciably different from that of TpivPP, e.g. from the ^{1}H NMR spectra it is assumed that the acetamide groups of **1b** and/or **2b** are located on the porphyrin ring plane. This corresponds to slight difference in the $k_{on}(O_2)$ between the double-sided series and TpivPP. However, the higher O_2 binding affinities of **1a** and **2b** complexes, compared to **4b**, were mainly attributed to $k_{off}(O_2)$.

Momenteau et al. considered that the 10-fold increase in the O_2 binding affinity of the amide to the ether 'hanging-base' porphyrins resulted from a difference in the $k_{off}(O_2)$.[20] Traylor et al. suggested that the high O_2 binding affinity of the [5]heme[5](3,5)pyridinophane resulted from introducing two amide moieties and a polar pyridine ring in direct contact with the bound O_2.[21] Therefore, our result indicates that the introduction of the acetamido groups to the cavity increases a local polarity around the coordination site, and stabilizes the dioxygenated species derived from the decreased dissociation rate.

Our kinetic data concerning the CO complexes demonstrated that $k_{on}(CO)$ decreases in the order TpivPP>**4b**>**1b**,**2b**. The slightly decreased $k_{on}(CO)$ of **1b** and **2b** is mainly attributed to the distal steric hindrance of the polar cavity around the coordination site.

The enthalpy and entropy changes of the dioxygenation equilibrium of the **2b** (1,2-Me_2Im) complex were determined from van't Hoff plots (15—35 °C). The thermodynamic values are summarized in Table 3. Further, the activation energy for a gaseous ligand association was determined by Arrhenius plots. The values for the transition energy and the preexponential factor for the transition state are also summarized in Table 3. The thermodynamic values of the double-sided heme complexes are similar to those of TpivPP. No appreciable differences in the thermodynamics parameters were observed among them.

We studied the binding properties of double-sided porphyrinatoirons having an amide residue. The acetamido group of **1b** and/or **2b** was located on the porphyrin plane, and increased the local polarity around the coordination site, compared to that of **4b**, having less-polar cavities constructed by four 3,3-dimethylbutyryloxy groups. The polar cavity of the **1b** and **2b** complexes increased the O_2 binding affinity, as reflected by the decreased dissociation rate, compared to that of **4b**.

References

1) B. Shannan, *Nature*, **296**, 683 (1982); B. Shannan, *J. Mol. Biol.*, **171**, 31 (1983).

2) S. E. V. Philips and B. P. Schoenborn, *Nature*, **292**, 81 (1981).

3) T. Kitagawa, M. R. Ondris, D. L. Rousseau, M. Ikeda-Saito, and T. Yonetani, *Nature*, **298**, 869 (1982).

4) Y. Aoyama, M. Asakawa, Y. Matsui, and H. Ogoshi, *J. Am. Chem. Soc.*, **113**, 6233 (1991).

5) a) J. P. Collman, R. R Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975); b) J. P. Collman, J. I. Brauman, T. J. Collins, B. L. Iverson, G. Lang, R. B. Pettman, J. L. Sessler, and M. A. Walters, *J. Am. Chem. Soc.*, **105**, 3038 (1983); c) J. P. Collman, J. I. Brauman, J. P. Fitzgerald, J. W. Sparapany, and J. A. Ibers, *J. Am. Chem. Soc.*, **110**, 3480 (1988).

6) a) T. Hashimoto, R. L. Byer, M. J. Crossley, J. E. Baldwin, and F. Basolo, *J. Am. Chem. Soc.*, **104**, 2101 (1982); b) J. E. Baldwin, J. H. Cameron, M. J. Crossley, I. J. Dagley, S. R. Hall, and T. J. Klose, *J. Chem. Soc., Dalton Trans.*, **1984**, 1739.

7) a) M. Momenteau, J. Mispelter, B. Loock, and J-M. Lhoste, *J. Chem. Soc., Perkin Trans. 1*, **1985**, 221; 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.

8) E. Tsuchida, *Top. Curr. Chem.*, **132**, 64 (1986); E. Tsuchida, T. Komatsu, T. Babe, T. Nakata, H. Nishide, and H. Inoue, *Bull. Chem. Soc. Jpn.*, **63**, 2323 (1990).

9) K. Kim and J. A. Ibers, *J. Am. Chem. Soc.*, **113**, 6077 (1991).

10) Y. Uemori and E. Kyuno, *Inorg. Chem.*, **28**, 1690 (1989).

11) I. P. Gerothanasis, M. Momenteau, and B. Loock, *J. Am. Chem. Soc.*, **111**, 7006 (1989).

12) C. K. Chang, B. Ward, R. Young, and M. P. Kondylis, *J. Macromol. Sci. Chem.*, **25**, 1307 (1988).

13) H. Imai, S. Sekizawa, and E. Kyuno, *Inorg. Chim. Acta*, **125**, 151 (1986).

14) G. E. Wuenschell, G. Tetreau, D. Lavalette, and C. A. Reed, *J. Am. Chem. Soc.*, **114**, 3346 (1992).

15) a) T. Komatsu, E. Hasegawa, H. Nishide, and E. Tsuchida, *J. Chem. Soc., Chem. Commun.*, **1990**, 66; b) E. Tsuchida, E. Hasegawa, T. Komatsu, T. Nakata, K. Nakao, and H. Nishide, *Bull. Chem. Soc. Jpn.*, **64**, 888 (1991); c) T. Komatsu, S. Kumamoto, H. Nishide, and E. Tsuchida, *J. Chem. Soc., Dalton Trans.*, **1991**, 3281; d) E. Tsuchida, T. Komatsu, T. Nakata, H. Nishide, and H. Inoue, *J. Chem. Soc., Dalton Trans.*, **1991**, 3285; e) T. Komatsu, K. Arai, H. Nishide, and E. Tsuchida, *Chem. Lett.*, **1992**, 799.

16) C. M. Drain and B. B. Corden, *Inorg. Chem.*, **28**,

4374 (1989).
17) a) C. K. Chang and T. G. Traylor, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1166 (1975); b) D. K. White, J. B. Cannon, and T. G. Traylor, *J. Am. Chem. Soc.*, **101**, 2443 (1979); c) T. G. Traylor, S. Tsuchiya, D. Campbell, M. Mitchel, D. Stynes, and N. Koga, *J. Am. Chem. Soc.*, **107**, 604 (1985).
18) J. P. Collman, J. I. Brauman, B. L. Iverson, J. I. Sessler, R. M. Morris, and Q. H. Gibson, *J. Am. Chem. Soc.*, **105**, 3052 (1983).
19) K. S. Suslick and M. M. Fox, *J. Am. Chem. Soc.*, **105**, 3507 (1983).
20) M. Momenteau and D. Lavalette, *J. Chem. Soc., Chem. Commun.*, **1982**, 341.
21) T. G. Traylor, N. Koga, and L. A. Deardurff, *J. Am. Chem. Soc.*, **107**, 6504 (1985).