A New Synthetic Model for Myoglobin: "Tulip Garden" Porphyrin

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A new model compound "tulip garden" porphyrin, has a long half-lifetime, thus satisfying the demand for the synthetic analogue of myoglobin. At the same time, "tulip garden" porphyrin has high O_2 affinities as compared to other protected porphyrins; especially, its Co(II) complex shows almost the same affinity ($P_{1/2}O_2$) as coboglobins. The difference in oxygen affinities between "tulip garden" and "picket fence-type" porphyrins can be ascribed to the bulkiness of pendant groups. The strong "side" influence of the adamantyl group can be expected to bring about a high O_2 affinity.

Considerable efforts have recently been made to develop synthetic model compounds which bind O_2 reversibly in a manner analogous to that of hemoglobin (Hb) and myoglobin (Mb).[1] These model compounds satisfy the demands for the formation of more stable oxygen adducts at room temperature and show high O_2 affinities equal to those of natural respiratory hemoprotein. In Hb and Mb, an important function of the heme pocket is to prevent dimeric interactions between hemes. In accordance with this requirement, sterically protected porphyrins ("superstructure porphyrins"[2]) have been synthesized in recent years. Such superstructures have a *hydrophobic pocket* on the single face of the porphyrin plane and help to *inhibit irreversible autoxidation*.[3] At room temperature, however, only a limited number of the protected porphyrins, most notably the "picket fence,"[4] "pocket,"[5] "capped,"[6] "doubly bridged,"[7] "hanging imidazole,"[8] and "cofacial"[9] porphyrins, bind O_2 reversibly.

The O_2 affinities and half-lifetimes of oxygen complexes differ from compound to compound. This variation is attributable to the electronic nature of porphyrins,[10] local polarity at the binding site,[2,11] the effect of solvation on the binding site,[12] and the steric effects on coordination to the metal center.[13] As far as the steric effects are concerned, we may consider two types of model compounds. In "capped" porphyrin[13] and "cyclophane heme,"[14] a series of compounds has been synthesized by changing the cavity size between the porphyrin plane and the capping aromatic ring, and the influence of the steric hindrance upon O_2 binding has been studied. On the other hand, the oxygen-binding properties of the "picket fence" porphyrins containing different pendant groups have hardly been investigated at all. In order to investigate of the steric effect caused by the bulkiness of the pendant groups, we need to design new model compounds.

We wish to report here the synthesis of a new model compound of Mb, "tulip garden" porphyrin **5a** (Fig. 1), which is "modified picket fence porphyrin." A reversible oxygenation based on its Fe(II) and Co(II) complexes is also described. We further synthesized "picket fence-type" porphyrin **5b** (Fig. 1), whose pendant moiety is the *t*-butyl group. The bulkiness of the adamantyl group may lead to very strong steric effect on the porphyrin **5a**. In order to compare the local polarity effects of binding sites, we also furthermore synthesized "camphanoyl fence" porphyrin **5c** (see Scheme 1).

Experimental

The synthetic procedure[15] to **5a**, **5b**, and **5c** is shown in Scheme 1, while the metallation procedure to the Co(II) and Fe(II) complexes is shown in Scheme 2.

5,10,15,20-Tetrakis(2-nitro-4-t-butylphenyl)-21H,23H-porphyrin **2**. The porphyrin **2** was prepared by the Rothemund condensation of pyrrole with a nitrobenzaldehyde derivative **1**.[16] A 259-g portion of **1** (1.25 mol) was dissolved in 2 dm^3 of glacial acetic acid. The solution was then heated to its boiling point with continuous stirring. A 95-g portion of pyrrole (1.42 mol) was added, drop by drop, to the boiling solution. The solution was then allowed to reflux for 3 h. After the solution has cooled to room temperature, the resulting black solution was left overnight. The purple crystals were filtered by suction and purified by silica gel chromatography, using

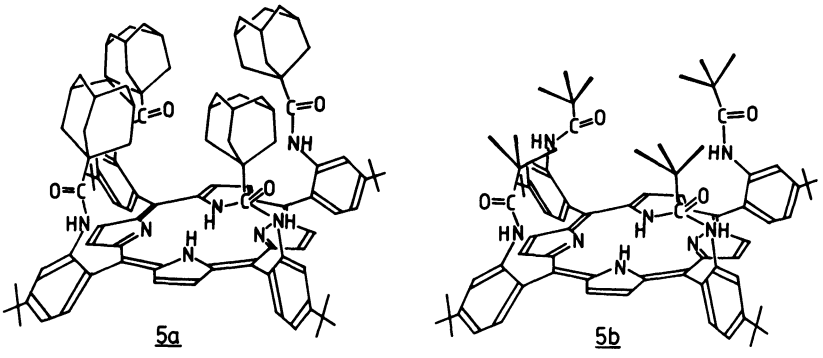
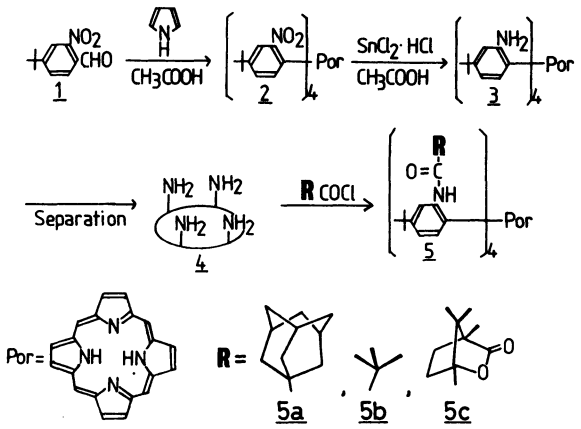


Fig. 1. "Tulip garden" porphyrin **5a** and "picket fence type" porphyrin **5b**.



Scheme 1. Synthetic procedure to **5a**, **5b**, and **5c**.

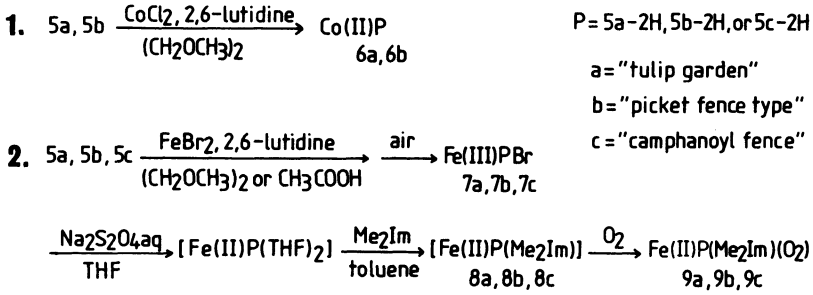
$CHCl_3$ as the eluent. A 46.0-g portion of the porphyrin **2** (0.0451 mol) was thus obtained; the overall yield was 14.4% based on the **1** consumed: Anal. Calcd for $C_{60}H_{58}N_8O_8$: C, 70.71; H, 5.74; N, 10.99. Found: C, 70.76; H, 5.70; N, 11.14. ^1H NMR ($CDCl_3$) δ=−2.6 (2H, s, internal pyrrole H), 1.7 (36H, s, *t*-butyl H), 8.0—8.5 (12H, m, phenyl H), 8.6 (8H, s,β-pyrrole H). UV-*vis*. ($CHCl_3$) λ/nm (ε/$M^{-1}cm^{-1}$)[17] 423 (3.3×10^5), 517 (2.0×10^4), 553 (7.2×10^3), 594 (6.0×10^3), 651 (2.6×10^3).

5,10,15,20-Tetrakis(2-amino-4-t-butylphenyl)-21H,23H-porphyrin **3**. A 4.0-g portion of the porphyrin **2** (3.9 mmol) was dissolved in a mixture of concentrated hydrochloric acid (500 cm^3) and glacial acetic acid (1 dm^3) at room temperature. The resulting green solution was heated to 65 °C, and then a solution of 14 g (62 mmol) of $SnCl_2\cdot2H_2O$ dissolved in 100 cm^3 of the mixed solvent (HCl:CH_3COOH=1:2) was slowly added during a 30-min period. After heating for 1.5 h, crushed ice and water were poured into this solution. The porphyrin **3** was extracted with $CHCl_3$ from the resulting solution. The product was purified by alumina-column chromatography ($CHCl_3$). The average yield was 3.2 g (3.6 mmol, 92%): Anal. Calcd for $C_{60}H_{66}N_8$: C, 80.14; H, 7.40; N, 12.46. Found: C, 79.92; H, 7.67; N, 12.71. ^1H NMR ($CDCl_3$) δ=−2.6 (2H, s, internal pyrrol H), 1.5 (36H, s, *t*-butyl H), 3.4 (8H, s, NH_2), 7.0 (8H, d, phenyl H), 7.7 (4H, d, phenyl H), 8.8 (8H, s,β-pyrrole H). UV-*vis*. ($CHCl_3$) λ/nm (ε/$M^{-1}cm^{-1}$): 422 (2.3×10^5), 517 (1.7×10^4), 553 (6.1×10^3), 591 (5.3×10^3), 647 (2.8×10^3).

Separation of $\alpha,\alpha,\alpha,\alpha$-Isomer. The resulting porphyrin **3** was a mixture of four atropisomers in statistical abundance. The most polar one, the $\alpha,\alpha,\alpha,\alpha$-isomer, could be easily separated from the other three atropisomers by thin-layer[18] and column chromatography. Before isomer separation, the preferential isomerization to the $\alpha,\alpha,\alpha,\alpha$-isomer was carried out by the a modification of literature methods.[19] Eight grams of the porphyrin **3** were dissolved in 1.5 dm^3 of toluene. This solution was refluxed under Ar, and a 40-g portion of alumina (Merck alumina 90) was added six times every three hours. After refluxing for 20 h, the solution was cooled to the ambient temperature. The isomerized product was then extracted from the alumina with a $CHCl_3$/acetone mixed solvent. After the solvent had been removed, the residue was redissolved in a minimum amount of benzene. The benzene solution was poured into a column of silica gel (Wako gel C-200) prepared as a slurry in benzene. All the material was loaded, and 2:1 benzene/diethyl ether was passed through the column until the eluate became very pale. The desired $\alpha,\alpha,\alpha,\alpha$-isomer was left in the column and eluted with 2:1 benzene/acetone. The solvent was removed using a rotary evaporator at 30 °C. Yield: 6.5 g (81%).

"Tulip Garden" Porphyrin **5a**. A 3.0-g portion (3.3 mmol) of the $\alpha,\alpha,\alpha,\alpha$-isomer **4** was dissolved in CH_2Cl_2 (200 cm^3) containing 10 cm^3 of pyridine. A 15-g portion (76 mmol) of 1-adamantanecarboxylic acid chloride was added, after which the solution was stirred for 3 h at the ambient temperature. A 200-cm^3 portion of 20% aqueous ammonia was then added, and the solution was stirred for an additional 30 min. The organic layer was separated and subsequently washed, first with dilute hydrochloric acid and then with aqueous ammonia. The solvent was evaporated using a rotary evaporator. The product was purified by chromatography on a silica-gel column (benzene), eluting with 3:1 benzene/ether. Yield 4.7 g (3.0 mmol, 91%): Anal. Calcd for $C_{104}H_{122}N_8O_4\cdot H_2O$: C, 79.95; H, 7.98; N, 7.15. Found: C, 79.52; H, 7.80; N, 7.24. ^1H NMR ($CDCl_3$) δ=−2.6 (2H, s, internal pyrrole H), 0.9—1.2 (60H, m, adamantyl H), 1.6 (36H, s, *t*-butyl H), 7.4—7.5 (12H, m, phenyl H), 8.9 (8H, s,β-pyrrole H), 8.9 (4H, d, NHCO). UV-*vis*. ($CHCl_3$) λ/nm (ε/$M^{-1}cm^{-1}$): 438 (3.5×10^4), 555 (8.3×10^3), 593 (6.5×10^3), 652 (2.5×10^3).

"Picket Fence-type" Porphyrin **5b** *and "Camphanoyl Fence" Porphyrin* **5c**. The synthetic procedure for **5b** and **5c** was similar to that used for **5a**. The porphyrin, **5b** or **5c**, was obtained by coupling the $\alpha,\alpha,\alpha,\alpha$-isomer **4** with pivaloyl chloride[20] or (−)-camphanoyl chloride respectively. The overall yield of **5b** was 95%, and that of **5c** was 60%: **5b** Anal. Calcd for $C_{80}H_{98}N_8O_4\cdot H_2O$: C, 76.65; H, 8.04; N, 8.94. Found: C, 76.55; H, 7.78; N, 8.77. ^1H NMR ($CDCl_3$) δ=−2.6 (2H, s, internal pyrrole H), 0.1 (36H, s, pivaloyl H), 1.6 (36H, s, *t*-butyl H), 7.2—7.8 (12H, m, phenyl H), 8.8 (8H, s,β-pyrrole H), 8.9 (4H, d, NHCO). UV-*vis*. ($CHCl_3$) λ/nm (ε/$M^{-1}cm^{-1}$): 423 (3.5×10^5), 515 (2.1×10^4), 549 (6.2×10^3), 589 (6.4×10^3), 646 (2.5×10^3). **5c** ^1H NMR ($CDCl_3$) δ=−2.6 (2H, s, internal pyrrole H), −0.1—1.3 (52H, m, camphanoyl H), 1.5 (36H, s, *t*-butyl H), 7.3—7.8 (12H, m, phenyl H), 8.4 (4H, d, NHCO), 8.8 (8H, s,β-pyrrole H). UV-*vis*.



Scheme 2. Metallation procedure.

($CHCl_3$) λ/nm: 422, 517, 551, 582, 653.

Cobalt Insertion. A 1.0-g portion (0.65 mmol) of **5a**, 0.65 g (5.0 mmol) of anhydrous $CoCl_2$, and 0.20 cm^3 of 2.6-lutidine were dissolved in 100 cm^3 of 1,2-dimethoxyethane under Ar. The resulting solution was stirred for 3 h at room temperature. The solution was then brought to dryness, and the residue was redissolved in $CHCl_3$. The product was purified by alumina-column chromatography, using $CHCl_3$ as the eluent. Yield: 0.90 g (87%). The porphyrin **6b** was prepared by a similar procedure. Yield: (50%): **6a** Anal. Calcd for $CoC_{104}H_{120}N_8O_4 \cdot H_2O$: C, 76.96; H, 7.58; N, 6.90. Found: C, 77.03; H, 7.84; N, 6.65. UV-*vis.* ($CHCl_3$) λ/nm (ε/M^{-1} cm^{-1}): 419 (2.3×10^5), 532 (1.7×10^4). **6b** UV-*vis.* ($CHCl_3$) λ/nm: 414, 526.

Iron Insertion. **5a** (0.8 g), 2.6-lutidine (2 cm^3), and anhydrous $FeBr_2$[21] were dissolved in 200 cm^3 of 1,2-dimethoxyethane under oxygen-free conditions. The resulting solution was stirred for 3 h at the ambient temperature. The solution was then brought to dryness, and the product was purified using an alumina column ($CHCl_3$). The eluate was stirred with 10% hydrobromic acid. The solvent was then evaporated by means of a rotary evaporator. Yield: 0.82 g (95%). The porphyrin **7b** was prepared by a similar procedure. Yield: (60%). The porphyrin **7c** was obtained by the reaction in a mixed solvent of 1:1 $CHCl_3$/acetic acid. Yield: (70%). **7a** Anal. Calcd for $FeC_{104}H_{120}N_8O_4Br$: C, 74.24; H, 7.19; N, 6.66. Found: C, 73.90; H, 7.21; N, 6.77. UV-*vis.* ($CHCl_3$) λ/nm: 425, 514, 578, 660, 694. **7b** Anal. Calcd for $FeC_{80}H_{96}N_8O_4Br$: C, 69.94; H, 7.20; N, 7.96. Found: C, 70.17; H, 7.07; N, 8.18. UV-*vis.* ($CHCl_3$) λ/nm: 422, 512, 586, 656, 685. **7c** UV-*vis.* ($CHCl_3$) λ/nm: 421, 508, 578, 656.

Dioxygen Adducts of Fe(II) Complexes. A 2.0-g portion (1.2 mmol) of Fe(III) porphyrin **7a** was dissolved in a mixed solvent of 50% THF/benzene (v/v), after which the solution was purged with Ar to remove any O_2. Then a 100-cm^3 portion of the deoxygenated aqueous 0.2 M $Na_2S_2O_4$ solution was added to this solution of **7a**. When the mixture was vigorously stirred for 30 min, the dark brown solution turned red-orange. The aqueous layer was discarded, and the organic layer was dried using anhydrous Na_2SO_4. After removing the solution from Na_2SO_4 by filtration under Ar, the crude iron(II) complex was redissolved in 30 cm^3 of toluene containing 1,2-Me_2Im[22] and then 100 cm^3 of heptane was added, drop by drop, for crystallization. The precipitate **8a** was separated by filtration and dried *in vacuo*. All the operations were carried out under Ar or N_2 atmosphere. After the exposure of the **8a** to oxygen at atmospheric pressure for 1 d, a pure "dioxygen" complex **9a** was obtained as deep-violet crystals. The "dioxygen" complex **9b** was prepared by a similar procedure. **9c** was immediately oxidized to the iron(III) state, and so we could not obtain the pure "dioxgen" complex. For that reason, we used a THF/benzene solution of the crude iron(II) complex for the spectrophotometric measurement: **9a** Anal. Calcd for $FeC_{109}H_{128}N_{10}O_6$: C, 75.67; H, 7.46; N, 8.10. Found: C, 75.47; H, 7.57; N, 8.06.

Oxygen-affinity Measurement. The oxygen equilibria constants were determined by means of spectrophotometric O_2 titration. A Hitachi 340 recording spectrophotometer was used in all the experiments. The measurements were done at *ca.* 20 °C in a toluene[23] solution containing the corresponding imidazole.[24] In general, metalloporphyrin concentration of 50—80 μM were used, and the spectra were recorded in the 600—350 nm range. Any spectral changes were recorded, in the absence of O_2 and at various increasing partial pressures of O_2. The oxygen partial pressures were determined by the injection of known volumes O_2 into the tonometer. After the measurements, the reversibility was checked by bubbling a sample solution with Ar in order to obtain a spectrum identical with initial one.

Results

$$M(P)(B) + O_2 \rightleftharpoons M(P)(B)(O_2) \quad M = Co, Fe$$

In solution, five-coordinate porphyrin complexes are suitable for the measurement for the oxygen equilibrium, an appropriate N-base should, however, be chosen to ensure that the five-coordinate complex is the dominant species. Sterically hindered 2-substituted imidazoles exclusively form the five-coordinate adducts with Fe(II) porphyrins.[25,26] These hindered imidazole adducts are considered to mimic the T-state (low affinity) hemoglobin.[26] In the Fe(II) porphyrin/unhindered imidazole system, which can be expected to be a model system for the R-state (high affinity) hemoglobin,[26] six-coordinate bis-ligated adducts are the dominant species in solution.[25] Our porphyrin **5a** exerts a strong steric effect on the sixth coordinating position, but cannot restrain the coordination of 1-MeIm[27] in the pocket. In Co(II) porphyrins, both unhindered and hindered imidazoles form five-coordinate adducts.[28] For that reason, we choose the Fe(II) porphyrin/1,2-Me_2Im system the for T-state model and Co(II) porphyrin/1-MeIm system for the R-state model.

The spectrophotometric oxygen titrations of Fe(II) and of the Co(II) complexes were carried out in a toluene solution. Isosbestic points were found in all titrations. Figure 2 shows the spectral changes which took place when a toluene solution containing 1,2-Me_2Im

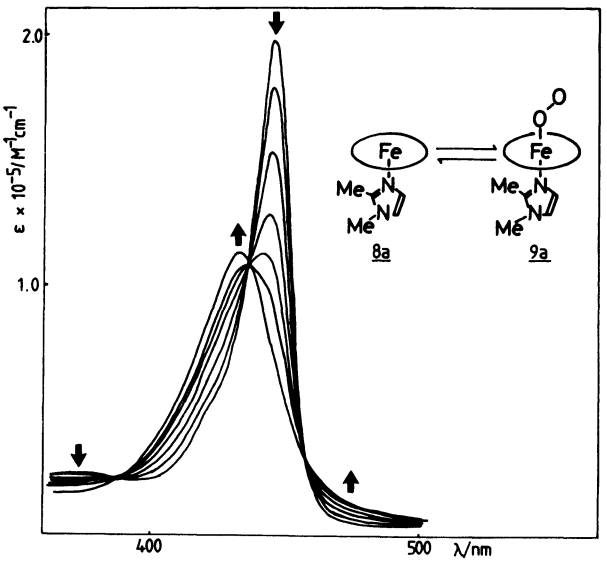


Fig. 2. Spectral changes of "tulip garden" iron(II) porphyrin under the various oxygen pressures. Arrows indicate the changes with increasing oxygen pressures.

TABLE 1. ELECTRON-ABSORPTION SPECTRA (TOLUENE)

Compound	λ/nm (ε/$M^{-1}cm^{-1}$)
6a(1-MeIm)	420(1.9×10^5), 537(1.1×10^4)
6a(1-MeIm)(O_2)	432(1.5×10^5), 554(1.3×10^4)
6b(1-MeIm)	416(*nd***), 536(*nd*)
6b(1-MeIm)(O_2)	423(*nd*), 549(*nd*)
8a	444(2.0×10^5), 540(7.3×10^3), 570(8.4×10^3), 613(4.3×10^3)
9a	432(1.1×10^5), 554(1.0×10^4), 592(5.3×10^3)
8b	432(*nd*), 535(*nd*), 565(*nd*)
9b	424(*nd*), 546(*nd*)

***nd*=not determined.

TABLE 2. HALF-SATURATION PRESSURES OF O_2 BINDING

Compound	$P_{1/2}O_2$ Torr
Co"tulip garden" (4.7×10^{-3} M 1-MeIm)	9.5
Co"picket fence type" (4.7×10^{-3} M 1-MeIm)	29
Fe"tulip garden" (9.9×10^{-2} M 1,2-Me_2Im)	26
Fe"picket fence type" (5.0×10^{-2} M 1,2-Me_2Im)	84

of Fe(II) porphyrin **8a** was exposed to various pressures of oxygen. However, the "camphanoyl fence" porphyrin Fe(II) complex was oxidized irreversibly, probably to the hydroxy iron(III) complex while the O_2 titration was being measured.[29] Both **9a** and **9b** showed full reversible oxygenation, and no spectrum of an oxidative species was observed within the period of the measurements. The electron-absorption data are listed in Table 1.

The Soret bands of the spectra were used for the spectrophotometric determination of oxygen-binding equilibria, because the most remarkable spectral changes throughout oxygenation took place at the Soret bands. The half-saturation pressures ($P_{1/2}O_2$) thus determined are listed in Table 2. The O_2 affinities of **8a** and **6a** (1-MeIm) were nearly three times those of **8b** and **6b** (1-MeIm). The half-lifetime for irreversible oxidation is >4d on **9a** and 36 h on **9b** at *ca.* 25 °C. In these ($P_{1/2}O_2$) and half-lifetime data, toluene was used as the solvent. The polarity effect of the solvent is negligible.

Discussion

(A) Half-lifetime. In synthetic analogues of Hb–Mb, it is desirable to form a stable oxygen adduct at room temperature. In this section, we will discuss the static stability of oxygen complexes. We consider that the half-lifetime of oxygen adducts at the ambient temperature indicates its static stability. The half-lifetimes of various oxygen complexes of Fe(II) porphyrins are given in Table 3. These data allow for comparison among a variety of synthetic Fe(II) porphyrins.

The protected porphyrins which have a nonprotic cavity on one side of the porphyrin plane, **9a**, **9b**, "FeTTTPP,"[2] "cofacial,"[9] "picket fence,"[4] "capped,"[48] "pocket,"[5] "doubly bridged,"[7] "hanging imidazole,"[8] and "cyclophane heme,"[31] showed long half-lifetimes (>5 h) at room temperature. In these models, the protected superstructures prevented bimolecular reactions between oxygen adducts and inhibited autoxidation. Other protected porphyrins, *e.g.*, "FeT(OMe)$_3$PP," "FeT(OEt)$_3$PP,"[32,33] and "strapped heme,"[34] whose superstructures could not prevent dimerization, binded O_2 partly reversibly at the ambient temperature. The subsequent autoxidation, however, took place immediately. Compared with these protected porphyrins, which oxygenated reversibly at room temperature, unprotected "chelated heme" had a short half-lifetime.[35] These data suggest that the protected superstructure is essential to stabi-

TABLE 3. HALF-LIFETIMES OF Fe(II) PORPHYRINS

Compound (base concentration)	$t_{1/2}$	Conditions	Ref.
FeTpivP (10^{-4} M 1,2-Me_2Im)	1 month	25 °C Toluene	3
Fepiv$_3$5CIm (tailed Im)	3 d		3
FePocpiv (0.1 M 1,2-Me_2Im)	1 d		5
(1.0 M 1-MeIm)	36 h		5
(0.1 M 1-MeIm)	7 h		5
FeMedPoc (0.1 M 1-MeIm)	2 d		5
Crown porphyrin (0.01 M 1-MeIm)	<3 min	24 °C DMA	44
(0.01 M 1-CPh$_3$Im)	>1 h	24 °C DMA	44
Fe-4-Cu	>12 h	25 °C Benzene	9
Fe-5-Cu	>12 h		9
FeTTTPP (1,2-Me_2Im)	>30 h	25 °C Toluene	2
(1,2-Me_2Im)	>2 h	60 °C Toluene	2
Chelated heme (tailed Im)	>10 s	25 °C CTAB	35
(tailed Im)	≈5 min	25 °C DMF	35
Fe(C_2-Cap) (0.64 M 1-MeIm)	5 h	25 °C Benzene	48
Basket handle (0.001 M 1-MeIm)	≈25 min	25 °C Toluene	45
(tailed Im)	≈30 min		45
(no axial base)	90 s		45
Hanging imidazole (tailed Im)	1 d	20 °C Toluene	8
Doubly bridged (tailed Im)	>2 d	20 °C DMF	7
9a (0.15 M 1,2-Me_2Im)	>4 d	20 °C Toluene	This work
9b (0.15 M 1,2-Me_2Im)	36 h		This work

lizing the oxygen complex at room temperature.

In the simple protoporphyrin/imidazole system, the oxygen adduct was less stable than "chelated heme." Although the protection was not enough to prevent dimerization, "hanging imidazole" porphyrin showed a long half-lifetime (1d).[8] In both "chelated heme" and "hanging imidazole" porphyrin, the imidazole arm was covalently linked to the porphyrin moiety, hence, the axial coordination was stabilized. Traylor[36] and Tsuchida[37] found that rigid and stable imidazole-heme coordination should result in a more stable oxygen adduct.

These protected porphyrins had various polarities at the ligand-binding site. "FeTTTPP" had a completely nonpolar pocket,[2] but other protected porphyrins were somewhat polar at the binding site. A series of "picket fence" and "pocket" porphyrins, whose polarities were quite similar, showed rather different values for their half-lifetimes (from 7 h to 1 month; see Table 3). Half-lifetime of "FeTTTPP" was over 30 h. Considering these variations of stability, it seems that the polarity is not strictly relevant to halflife time of oxygen adduct.

We will try to speculate tentatively on the relevance between the static stability and the polarity of a binding site. A related "picket fence" porphyrin complex, Fe(T*tos*PP)(N-Bu^{t}Im) (where *tos*=*p*-toluenesulfonamide), underwent irreversible oxidation.[4] Our **9c** was also autoxidized immediately at room temperature. Both these models contained an electron-withdrawing moiety. We attribute the rapid oxidation of these two models, in spite of the inhibition of dimerization by the "picket fence," to the strong polarity of the amide protons, which permits the protonation of coordinated dioxygen and consequent oxidation. These results suggest that the local polarity effect on the stability of the oxygen complex may be small unless the polarity is too strong to promote the protonation and subsequent autoxidation.

The half-lifetime of **9a**, which was more protected than **9b**, was longer than that of **9b**. This suggests that complete protection brings about good stability of the oxygen adduct. The relationship between the stability and the steric hindrance is vague. At present, though, we consider that the steric hindrance is not very relevant to the half-lifetime.

(B) Half-saturation Pressure of Oxygen. The dynamic stability of oxygen binding is estimated by means of the half-saturation pressures of oxygen ($P_{1/2}O_2$). Table 4 contains the half-saturation pressures for the oxygenation of unhindered-imidazole complexes of various Co(II) porphyrins. Table 5 contains similar data for hindered-imidazole complexes of Fe(II) porphyrins. The variations in these $P_{1/2}O_2$values, particularly the difference in oxygen affinities between **9a** and **9b**, lead to several speculations about O_2-binding behavior. We consider that the oxygen affinities of these model compounds depend on four factors. We will discuss these four factors in the following paragraphs.

Table 4. O_2 affinities of Co(II) porphyrins (1-MeIm)

Compound	$P_{1/2}O_2$	Conditions	Ref.
CoMb (sperm whale)	30	20°C Water	46,47
CoTpivP	140	25°C Toluene	26
Co(T(*p*-OCH_3)PP)	10000	15°C Toluene	28
Co(C_2-Cap)	140000		42
6a	29	20°C Toluene	This work
6b	84	20°C Toluene	This work

Table 5. O_2 affinities of Fe(II) porphyrins (1,2-Me_2Im)

Compound	$P_{1/2}O_2$	Conditions	Ref.
FeTTTPP	508	25°C Toluene	2
FeTpivP	38		3
FePocpiv	12.6		12
FeMedPoc	12.4		12
FeTalPoc	4		12
Fe(C_2-Cap)	4000		42
Fe(Np C_2-Cap)	613	0°C Toluene	42
9a	9.5	20°C Toluene	This work
9b	26		This work

Local Polarity of Ligand-binding Site. The polarity of the ligand-binding site considerably influences the oxygen affinities. It has previously been suggested that an increased polarity should increase the oxygen affinities.[38] This suggestion has been based on the measurements on "flat," unprotected porphyrins. In protected porphyrins, also, however, we consider a similar tendency to be found.

Recently, structural studies of oxyMb[39] and oxyHb[40] have established that the N^ε of His E7 forms a hydrogen bond with the bound oxygen. The N^ε atom stabilizes the coordinated molecular oxygen with this hydrogen bond. Structural studies[41] have also shown that, in the solid state, there is no interaction between the amide proton and the bound dioxygen in the "picket fence" porphyrins. Consequently, we are unable to expect the stabilization of oxygen binding by means of the hydrogen bond in these model systems (not even in **9a**). However, these amide groups can be expected to increase the polarity at the ligand-binding site.

Momenteau and Lavallete[11] observed O_2 binding in two similar "hanging base" porphyrins. Changing the mode of attachment from amide to ether linkages resulted in a difference, by a factor of *ca.* 10, in $P_{1/2}O_2$ values. They concluded that the presence of the amide groups strongly increased the stability of the oxygenated complexes. "FeTTTPP," which had a completely nonpolar pocket, showed a rather low O_2 affinity in comparison to the "picket fence," "pocket," and **9a**. This large difference between the oxygen affinities was attributed primarily to the loss of polarity in the binding pocket of "FeTTTPP." A number of protected porphyrins, **9a**, **9b**, "picket fence," and "pocket" porphyrins, which included four amide groups around the binding site, showed high O_2 affinities.

These results suggest that the local polarity is a

significantly important factor in determining the O_2 affinity in encumbered model systems. As has been mentioned in Section (A), if the local polarity is too strong, as, *e.g.*, in **9c** and "FeT*tos*PP," the protonation and autoxidation happen rapidly even if the systems show high O_2 affinities. For that reason, these porphyrins are unsuitable as "biomimetic models" of natural oxygen carriers.

Polarity effects are expected to be similar within **9a**, **9b**, "picket fence," and "pocket" porphyrins. Therefore, the affinity differences among these model compounds, especially that between **9a** and **9b**, can not be explained by the local polarity effect. We must consider other factors in order to explain the differences.

Electronic Nature of the Porphyrin. Electronic effects have been extensively studied. Recently, Traylor *et al.*[10] found that the electronic nature of the heme had a striking effect on the O_2 affinity. The electronic nature of the series of protected porphyrins seems to be similar. Especially in **9a** and **9b**, we consider that the electronic natures of these two porphyrins quite resemble one another. As Collman *et al.*[12] mentioned in his report, other factors must be primarily responsible for the high O_2 affinities of the protected porphyrins.

Solvation Effect. Collman *et al.*[12] have discussed the solvation effects on "flat" and "protected" porphyrins. They suggested that, in "flat" iron porphyrins, the unligated five-coordinate form might be subject to a stronger solvation stabilization than the "protected" porphyrins. This stabilization of the five-coordinated species could account for the lower gaseous-ligand affinities of these "flat" porphyrins relative to "protected" porphyrins. They concluded that the solvation effect was the dominant factor responsible for the lower affinities of the "flat" hemes as compared to the "protected" hemes.

In "capped" porhyrins,[42] "[6.6]cyclophane,"[14] and "cofacial" porphyrins,[9] the O_2 affinities of these models were lower those of "flat" hemes ("chelated heme," or TPP), although a solution stabilization did not take place (see Table 6). The steric hindrance restrained the coordination of oxygen. This is reason why "capped," "[6.6]-cyclophane," and "cofacial" porphyrins indicated rather low O_2 affinities. In **9a** and **9b**, the solvation effects were considered to be almost the same. Even if the solvation effects of these two models were slightly different, the influence on the O_2 affinity should not be large. We consider that the solvation effect is the dominant factor in the difference between the O_2 affinities of "flat" hemes and those of "protected" hemes, however, among the "protected" hemes, a little difference in the solvation effect has no significant meaning on the O_2 affinities.

Steric Effect. The steric hindrance extensively influences the O_2 affinities of the protected porphyrins. Table 6 shows the $P_{1/2}O_2$ values of various porphyrin complexes.

TABLE 6. O_2 AFFINITIES OF SELECTED Fe(II) PORPHYRINS

Compound (base)	$P_{1/2}O_2$	Conditions	Ref.
[7.7]Cyclophane (1,5-DCI)	1.4	20°C Benzene	10
[6.6]Cyclophane (1,5-DCI)	696		10
Fe-4-Cu (1-MeIm)	31		9
Fe-5-Cu (THP Im)	5		9
Fe(Np C_2-Cap) (1-MeIm)	2.3		42
Fe(C_2-Cap) (1-MeIm)	4.5	0°C Toluene	42
Fe(C_3-Cap) (1-MeIm)	120-180		42
FePocpiv (1,2-Me_2Im)	12.6	25°C Toluene	12
FeMedPoc (1,2-Me_2Im)	12.4		12
FeTalPoc (1,2-Me_2Im)	4		12
9a (1,2-Me_2Im)	9.5	20°C Toluene	This work
9b (1,2-Me_2Im)	26		This work

The steric hindrance caused by the capping aromatic ring was termed "central" and "peripheral" effects by Basolo.[13] We call this "top" hindrance. The "top" hindrance was investigated in several kinds of protected porphyrins; "capped,"[13] "cyclophane,"[14] "cofacial,"[9] and "pocket"[12] hemes. Clayden *et al.*[43] studied the cavity size of "C_2-Cap," "C_3-Cap," and "Np C_2-Cap" using the paramagnetic shift and relaxation effects observed in these Co(II) complexes. The cavity size was varied in this order; "Np C_2-Cap">"C_2-Cap">"C_3-Cap." The O_2 affinities of these porphyrins decreased in exactly the same order.[42] Traylor *et al.*[14] measured the O_2 affinity of "[6.6]cyclophane" and "[7.7]cyclophane." "[7.7]cyclophane," whose cavity was larger than that of "[6.6] cyclophane," indicated a higher O_2 affinity. Chang *et al.*[9] obtained a similar result using "cofacial" porphyrins, "Fe-4-Cu," and "Fe-5-Cu." These three model systems, "capped," "cyclophane," and "cofacial" hemes, revealed a large steric effect on O_2 binding. The cavity size of "C_3-Cap," "[6.6]cyclophane," and "Fe-4-Cu" was too small for the coordination of dioxygen, and these three models had lower O_2 affinities than those of "flat" porphyrins.

A similar tendency of "top" hindrance is observed in "pocket" porphyrins.[12] The cavity size was changed in this order: "FeTalPoc">"FeMedPoc">"FePocpiv," the O_2 affinity varying in the same order. "FeMedPoc" and "FePocpiv" porphyrins had almost the same values for resulted in a difference, by a factor of *ca.* 10, in $P_{1/2}O_2$. In both porphyrins, the covalently attached benzene rings are rigidly constrained above the porphyrin ring. In contrast to these two porphyrins, the benzene ring of "FeTalPoc" was "floppy", and the O_2 affinity of this porphyrin was three times as high as those of "FeMedPoc" and "FePocpiv."

In the "top" hindrance, if the cavity size becomes too far small, the oxygen affinity is considerably decreased.

In the "pocket" porphyrins, the effects of cavity size and of rigidity are not yet clear.

The adamantyl moiety, which was the pendant group of **5a**, was bulkier than the *t*-butyl group of **5b**. The steric congestion attributable to the bulkiness of the pendant groups is termed "*side*" influence. This "*side*" influence has been scarcely studied in model systems, and it is not clear that "*side*" influence causes either the inhibition or the promotion of O_2 binding. At the beginning of this work, we set up a hypothesis that a strong "side" influence may produce a high O_2 affinity. As was expected, $P_{1/2}O_2$ values of these two models (**8a** and **8b**, **6a** and **6b**) were different, by a factor of *ca.* 3 (see Tables 4 and 6). "Tulip garden" porphyrins, **8a** and **6a**, demonstrated high O_2 affinities. The electronic nature, local polarity, solvation effect, and "top" hindrance are expected to be quite similar in these two types of model compounds, "tulip garden" and "picket fence-type" porphyrins. Only the steric "*side*" influence is dissimilar. These results suggest that the O_2 affinity is clearly dependent upon the bulkiness of the pendant groups.

Camphanoyl group of **8c** had the almost same bulkiness as the adamantyl moiety. Unfortunately, **8c** was rapidly autoxidized to the iron(III) state. For that reason, we could not estimate the "*side*" influence of **8c**.

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