Discrimination Reaction of O_2 and CO to 'Cross-strapped' Type Iron(II) Porphyrins

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Abstract

The syntheses and characterization of cross-strapped type iron(II) porphyrins with sterically protected cavities on one face are described. The porphyrins, in which one set of two amino groups in the *trans* position was bridged by heptamethylenedicarbonyl and the other by tetradecamethylenedicarbonyl or octadecamethylenedicarbonyl, were derived from the $\alpha,\alpha,\alpha,\alpha$-isomer of *meso*-tetra(*o*-aminophenyl)porphyrin. The heptamethylene chain was found to be crushed by the steric interaction with the tetradecamethylene chain bridged over the heptamethylene chain. The iron(II) complexes bind O_2 and CO reversibly in toluene containing 1,2-dimethylimidazole at 20 °C, and their O_2 and CO affinities have been determined. The O_2 and CO adducts of the iron(II) complexes have been characterized by ^1H NMR and IR spectra. The changes of the O_2 and CO affinities of the iron(II) complexes are discussed in terms of the polar effect of amide groups, the H-bonding of amide groups with O_2, and the steric effects of cavities.

Introduction

The proteins in hemoglobins and myoglobins play important roles in the discrimination reaction against the binding of CO relative to that of O_2. The distal histidine (E7) is thought to be responsible for the reduced CO affinities, since the isolated β chain in HbZu** (β_{63}His → Arg) has a high association rate for CO binding and a high CO affinity [1]. The histidine can reduce CO affinity by steric and electronic effects. However, it is still unresolved which of the two effects is more important in lowering the CO affinity [2–9]. Furthermore, the studies have been designed and synthesized to fulfil the purpose of bridging the gap between the basic research conducted in the field of native hemoprotein and the simple model system thereof. From model complexes, many conflicting reports regarding the relative importance of these two effects on the discrimination have been given [10–14].

In order to address the steric role on the discrimination reaction in hemoproteins, a series of model porphyrins were designed and synthesized as shown in Fig. 1. We refer to the model porphyrins as 'cross-strapped' type porphyrins because one aliphatic chain overpasses another aliphatic chain bridging the same side of a porphyrin plane in these porphyrins. Here, each porphyrin has a cavity constructed by a so-called 'basket handle' and the size of cavity

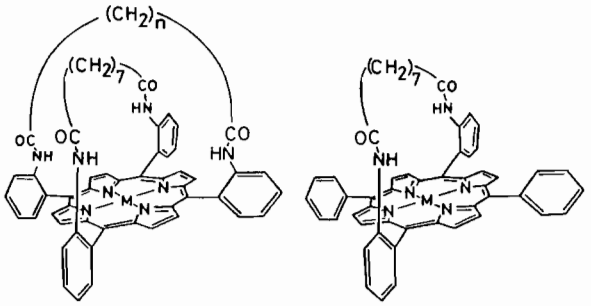

Fig. 1. 'Cross-strapped' and 'strapped' porphyrins.

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**Abbreviations used: HbA, human adult hemoglobin; HbZu, hemoglobin Zurich; 1,2-Me_2Im, 1,2-dimethylimidazole; 1-MeIm, 1-methylimidazole; 1,5-DCIm, 1,5-dicyclohexylimidazole; H_2-Azam$\alpha\alpha$, dianion of 5α,15α-bis(2-aminophenyl)-10α,20α-(nonanediamidodi-*o*-phenylene)porphyrin; H_2-Azam$\beta\beta$, dianion of 5β,15β-bis(2-amiophenyl)-10α,20α-(nonanediamidodi-*o*-phenylene)porphyrin; H_2-Azampiv$\alpha\alpha$, dianion of 5α,15α-bis[2-(2,2-dimethylpropanamido)phenyl]-10α,20α-(nonanediamidodi-*o*-phenylene)porphyrin; [Fe(piv$_2$-C$_8$)], iron(II) complex of 5α,15α-bis[2-(2,2-dimethylpropanamido)phenyl]-10α,20α-(octanediamidodi-*o*-phenylene)porphyrin; [Fe(PPXIDMe)], iron(II) complex of protoporphyrin IX dimethyl ether; [Fe(PocPiv)], 5,10,15-(1,3,5-benzenetriyltriacetyl)-20-α-*o*-pivalamidophenyl)porphyrinato iron(II); [Fe(MedPoc)], 5,10,15-(1,3,5-benzenetripropionyl)-20-α-*o*-pivalamidophenyl)porphyrinato iron(II); [Fe(piv$_2$C$_9$)], iron(II) complex of 5α,15α-bis[2-(2,2-dimethylpropanamido)phenyl]-10α,20α-(nonanediamidodi-*o*-phenylene)porphyrin; $P_{1/2}$ (X), partial pressure of gaseous ligand (O_2, CO) at half-saturation. $M = P_{1/2}(O_2)/P_{1/2}(CO)$; 'ruffling' refers to a distortion of the porphyrin ring toward D_{2d} geometry (see ref. 26).

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can be varied by the changes in the length of aliphatic chain overpassing the basket handle. In this paper, we report the syntheses of the porphyrins, the O_2 and CO affinities of their Fe(II) complexes, and their IR and ^1H NMR data. The O_2 and CO binding to the porphyrins is discussed in terms of the polar effect of amide groups, the H-bonding of amide groups with O_2, and the steric effects of cavities.

Experimental

General Information

Electronic spectra were recorded on a Hitachi 340 spectrophotometer. O_2 and CO affinities were determined by spectrophotometric titration using the flow method as described earlier [15]. The Fe(II) porphyrins were prepared by the mixing of Fe(III) porphyrins in toluene with aqueous sodium dithionite under Ar [13, 16]. Temperatures of the solutions were maintained at 25 ± 0.1 °C by the use of a constant-temperature circulation pump (Neslab Model RTE-8) and a variable-temperature cell holder (Hitachi). Various partial pressures of O_2 or CO were obtained by a gas mixture instrument (Kofloc Model GM-3A) constructed with mass flow controllers and flow meters. Concentrations of 1,2-Me_2Im and Fe(II) porphyrins were 0.07 M and *c.* 1 × 10^{-5} M, respectively. The spectra were recorded in the 500–350 nm range. $P_{1/2}$ values (half-saturation gas pressures for O_2 or CO binding) were calculated by the method of Beugelsdijk and Drago [17]. Reversibility was checked after the last CO or O_2 addition by purging with N_2 gas (7 ml/min) for 30 min; more than 90% reversibility was achieved after 2 h of carbonylation or oxygenation.

Proton NMR spectra were recorded on a JEOL GSX-400 spectrometer. Preparation of NMR samples were as follows. The Fe(III) porphyrin in CH_2Cl_2 was reduced with aqueous sodium dithionite under Ar. After separation of the two phases, the dichloromethane layer was washed with degassed H_2O and the solvent stripped off by passing Ar. The reduced product was then dissolved in degassed toluene-d^8 containing 1,2-Me_2Im and transferred to 5 mm NMR tube via a stainless steel tube. The solution was then exposed to an atomosphere of O_2 or CO at room temperature. The spectra for the O_2 and CO adducts were obtained at −20 and 24 °C, respectively. The concentrations of the Fe(II) porphyrins and 1,2-Me_2Im were *c.* 5 × 10^{-3} M and 0.1 M, respectively.

Infrared spectra were obtained in benzene-d^6 with a CsI cell, using a JASCO DS-701G spectrometer. Samples were prepared in the same manner used for the preparations of ^1H NMR samples of CO adducts and recorded at room temperature. The concentrations of Fe(II) porphyrins and 1,2-Me_2Im were *c.* 5 × 10^{-3} M and *c.* 0.1 M, respectively.

Mass spectra were obtained on a JEOL JMS-DX300 instrument.

Materials

All solvents were of reagent grade, and used without further purification, except as noted below. Toluene was stirred with concentrated H_2SO_4 and then washed with 5% NaOH and H_2O, dried over $CaCl_2$, and distilled. 1-MeIm and 1,2-Me_2Im were vacuum distilled from KOH. Silica gel (Wakogel C-200) was used for column chromatography. The 8.97% O_2 in N_2 mixture and the 995 ppm CO in N_2 mixture were commercially purchased.

Synthesis

5,15-diphenyl-10α,20α-bis(nonanediamidodi-*o*-phenylene)porphyrin (H_2-AzP) and its iron(III) complexes were prepared by the method described before [15, 18]. The acid chlorides were prepared by treating the appropriate acids with thionyl chloride [11c]. 1,5-DCIm was prepared according to the literature [14d].

5α,15α-bis(nonanediamidodi-o-phenylene)-10α,20α-bis(eicosanediamidodi-o-phenylene)-porphyrin (H_2-AzC18α)

A CH_2Cl_2 solution (400 ml) of H_2-Azamαα [15] (300 mg, 0.363 mmol) was treated with pyridine (0.5 ml) and eicosanedioyl dichloride (0.3 g, 0.8 mmol) at room temperature. The solution was stirred for 1 h, then 10% aqueous ammononia (100 ml) was added, and the solution was stirred for 0.5 h. The organic layer was separated and evaporated to dryness. The resultant solid was dissolved in $CHCl_3$ and chromatographed on a silica gel column ($CHCl_3$, 4 × 30 cm). The column was eluted with $CHCl_3$/ether (9:1). The product was recrystallized from benzene hexane, yielding 210 mg (52%). *Anal.* Calc. for $C_{73}H_{80}N_8O_4$: C, 77.35; H, 7.11; N, 9.89. Found: C, 77.21; H, 6.96; N, 9.61%. FAB-MS: *m/e* 1133 (M^+ + 1). ^1H NMR data are shown in Table 1.

5α,15α-bis(nonanediamidodi-o-phenylene)-10α,20α-bis(hexadecanediamidodi-o-phenylene)-porphyrin (H_2-AzC14α)

This was prepared from H_2-Azamαα (200 mg, 0.242 mmol) in the same manner as H_2-AzC18α, except hexadecanedioyl dichloride (0.3 g, 0.9 mmol) in place of eicosanedioyl dichloride was used, yielding 160 mg (61%). *Anal.* Calc. for $C_{69}H_{72}N_8O_4$: C, 76.92; H, 6.74; N, 10.40. Found: C, 76.53; H, 6.64; N, 9.79%. FAB-MS: *m/e* 1077 (M^+ + 1). ^1H NMR data are shown in Table 1.

TABLE 1. ^{1}H NMR data

	Protons of the C7-chain[a]				Amide	Protons	Remarks
	α	β	γ	δ			
H_2-AzP	+1.16	−1.22	−0.50	−2.50		+6.00	b, h
[Zn(AzP)]	+1.17	−1.36	−0.50	−2.65		+6.05	b
[Zn(AzP)(1,2-Me_2Im)]	+1.11	−1.29	−0.55	−2.63		+6.06	c
[Fe(AzP)(1,2-Me_2Im)(CO)]	+0.57	+0.57	+0.11	−1.02		+5.83	d, h
[Fe(AzP)(1,2-Me_2Im)(O_2)]	+1.28	+0.95	+0.17	−0.98	+7.96	+4.56	e, h
H_2-AzC14α	+1.21	−1.67	−1.50	−3.79	+6.72,	+6.42	b
[Zn(AzC14α)]	+1.11	−1.84	−1.46	−3.84	+6.65	+6.39	b
[Zn(AzC14α)(1,2-Me_2Im)]	+1.21	−1.87	−1.39	−4.12	+6.75	+6.46	c
[Fe(AzC14α)(1,2-Me_2Im)(CO)]	+1.20	−0.44	−0.02	−1.38	+6.90	+6.29	d
[Fe(AzC14α)(1,2-Me_2Im)IO_2)]	+1.09	−0.67	−0.16	−1.39	+8.25	+5.67, +4.59	e
H_2-AzC18β	+1.17	−1.32	−0.55	−2.60	+6.71	+6.00	b
H_2-AzC18α	+1.21	−1.47	−1.17	−3.11	+7.12	+6.34	b
[Zn(AzC18α)]	+1.14	−1.65	−1.16	−3.27	+6.98	+6.30	b
[Zn(AzC18α)(1,2-Me_2Im)]	+1.21	−1.72	−1.17	−3.54	+7.01	+6.37	c
[Fe(AzC18α)(1,2-Me_2Im)(CO)]	+0.86	+0.58	+0.34	−0.50	+7.01	+5.84	d
[Fe(AzC18α)(1,2-Me_2Im)(O_2)]	f	f	+0.39	−0.42	+7.73	g, +4.62	e

[a]The letters (α–δ) refer to $-NHCO-\underset{\alpha}{CH_2}\underset{\beta}{CH_2}\underset{\gamma}{CH_2}\underset{\delta}{CH_2}\underset{\gamma}{CH_2}\underset{\beta}{CH_2}\underset{\alpha}{CH_2}-CONH-$. [b]Solvent = $CDCl_3$. [c]Solvent = $CDCl_3$ containing 1,2-dimethylimidazole (0.1 M). [d]Solvent = toluene-d^8 containing 1,2-dimethylimidazole (0.1 M). [e]Solvent was the same in d, measured at −20 °C. [f]Resonances were obscured by the signals of the C18-chain (+0.82 to 1.61 ppm). [g]Resonance was obscured by either the signals of 1,2-dimethylimidazole (6.25–6.4 and 7.1–7.25 ppm) or those of undeuterated toluene (6.9–7.1 and 7.1–7.2 ppm). [h]Ref. 18.

5α,15α-bis(nonanediamidodi-o-phenylene)-10β,20β-bis(eicosanediamidodi-o-phenylene)-porphyrin (H_2-AzC18β)

This was prepared from H_2-Azamββ [19] (150 mg, 0.182 mmol) in the same manner as H_2-AzC18α, yielding 100 mg (49%). *Anal.* Calc. for $C_{73}H_{80}N_8O_4$; C, 77.35; H, 7.11; N, 9.89. Found: C, 76.74; H, 7.15; N, 9.81%. FAB-MS: *m/e* 1133 (M^+ + 1). ^{1}H NMR data are shown in Table 1.

Fe(III) insertion

Iron(III) complexes were prepared by heating the porphyrins with $FeBr_2$ in acetic acid containing 2% sodium acetate (wt./wt.) at 70 °C [20]. Purification was carried out on a silica gel column using $CHCl_3$/CH_3OH (95:5) as the eluent. The product was evaporated to dryness and then treated with concentrated HBr in $CHCl_3$. After being dried over Na_2SO_4, the solvent was reduced in volume on a rotary evaporator and the product was precipitated by adding hexane.

[Fe(AzC18α)]Br. Anal. Calc. for $C_{73}H_{78}N_8O_4$-FeBr·$CHCl_3$: C, 64.10; H, 5.74; N, 8.08. Found: C, 64.38; H, 6.04; N, 8.03%. UV–Vis λ_{max} ($CHCl_3$): 420 nm (log ε 4.93), 515 (4.13), 584 (3.51), 664 (3.45), 690 (3.48).

[Fe(AzC14α]Br. Anal. Calc. for $C_{69}H_{70}N_8O_4$-FeBr·$CHCl_3$: C, 63.19; H, 5.38; N, 8.42. Found: C, 63.30; H, 5.47; N, 8.74%. UV–Vis λ_{max} ($CHCl_3$): 419 nm (log ε 4.95), 510 (4.16), 580 (3.56), 652 (3.53), 680 (3.47).

Zn(II) insertion

Zn(II) complexes were prepared by heating the porphyrins with $Zn(CH_3COO)_2\cdot 2H_2O$ in acetic acid at 70 °C. The reaction mixture was then evaporated to dryness. The residue was dissolved in $CHCl_3$ and washed with 10% NaOH/H_2O. After being dried over Na_2SO_4, the solution was evaporated to dryness and the product was crystallized from hot methanol.

[Zn(AzP)]. Anal. Calc. for $C_{53}H_{42}N_6O_2Zn\cdot CH_3OH\cdot \frac{1}{2}CHCl_3$: C, 68.76; H, 4.92; N, 8.83. Found: C, 68.62; H, 4.70; N, 8.73%.

[Zn(AzC18α)]. Anal. Calc. for $C_{73}H_{78}N_8O_4Zn\cdot H_2O$: C, 72.17; H, 6.64; N, 9.22. Found: C, 72.00; H, 6.75; N, 9.10%.

[Zn(AzC14α)]. Anal. Calc. for $C_{69}H_{70}N_8O_4Zn$: C, 72.65; H, 6.19; N, 9.82. Found: C, 72.09; H, 6.24; N, 9.74%.

Results

Synthesis

The treatment of H_2-Azamαα with approximately two molar equivalents of $ClCO(CH_2)_nCOCl$ (n = 14

and 18) gave H_2-AzC14α and H_2-AzC18α, respectively. For the aid of characterization, the atropisomer of H_2-AzC18α was prepared from the reaction of H_2-Azamββ with $ClCO(CH_2)_{18}COCl$. The yields of H_2-AzC14α and H_2-AzC18α were 61% and 52%, respectively. The porphyrins were characterized by ^1H NMR, mass spectroscopy and elemental analysis.

Because of C_{2v} symmetry for both H_2-AzC14α and H_2-AzC18α, and D_{2h} symmetry for H_2-AzC18β, these porphyrins show four separated signals for the protons of the C7-chains in the ^1H NMR spectra. Each signal could be assigned on the basis of spin-decoupling experiments and their relative intensities (Table 1). The β proton signals appeared at higher magnetic fields than the γ proton signals. The δ proton signal of each H_2-AzC14α and H_2-AzC18α was shifted to higher magnetic field by 1.29 and 0.61 ppm, respectively, compared with that of H_2-AzP. The γ and β proton signals of H_2-AzC14α also appeared at higher fields than those of H_2-AzC18β and H_2-AzP, while the α proton signals appeared to be nearly constant among the porphyrins prepared. The signals of the C14-chain and the C18-chain overlapped, and therefore could not be assigned well.

The iron(III) complexes were prepared from the porphyrins by the acetate method [20]. The reaction temperatures were kept below 70 °C to prevent the atropisomerization of phenyl rings. Such isomerization was not observed for the case of H_2-AzC14α even in refluxing toluene for 3 h, while 10% of H_2-AzC18α was found to isomerize into H_2-AzC18β under these conditions. The lack of isomerization during iron insertion was confirmed by the ^1H NMR spectra of the CO adducts.

O_2 and CO Affinities

The O_2 and CO affinities for the iron(II) porphyrins were determined spectrophotometrically in toluene containing 1,2-Me_2Im as the axial base. The spectral changes upon O_2 and CO titrations are shown in Figs. 2 and 3, respectively. The $P_{1/2}(O_2)$ value for [Fe(AzC14α)(1,2-Me_2Im)] was extrapolated from the van't Hoff plots. The following data used in the plots, represent the pairs of temperature (°C) and $P_{1/2}(O_2)$ (torr): (11, 893), (5.4, 667), (0, 283), (−9.1, 127), (−15.7, 58), and (−22.4, 28). Table 2 lists the data of O_2 and CO affinities, together with that of related complexes. The solvent dependence of O_2 affinities for [Fe(AzC14α)] was also examined at 0 °C with 1,2-Me_2Im as an axial base. The $P_{1/2}(O_2)$ values for [Fe(AzC14α)] in mesitylene, toluene and *o*-dichlorobenzene were 260, 283 and 362 torr, respectively.

^1H NMR Spectra for the O_2 and CO Adducts

To determine the structural details of the O_2 and CO adducts, ^1H NMR spectra were measured in toluene-d^8 solution containing Fe(II) porphyrins (*c.* 5 mM) and 1,2-Me_2Im (*c.* 0.1 M), and are shown in Figs. 4 and 5, and Table 1. The spectra were recorded at −20 °C for the O_2 adducts to minimize the influence of five coordinate species. The ^1H NMR spectra of both Zn(II) complexes and their 1,2-Me_2Im adducts were virtually identical with those of the corresponding free base porphyrins. Contrary to this, the chemical shifts of C7-chain protons in the O_2 and CO adducts were significantly

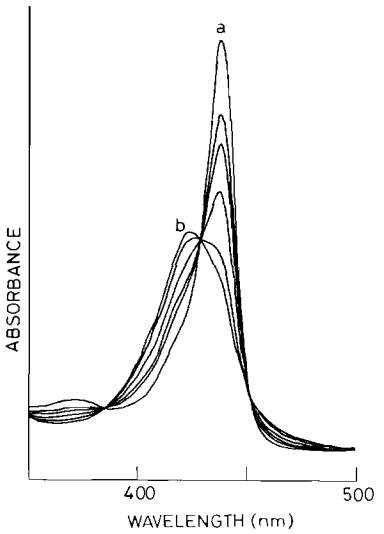

Fig. 2. Spectral changes upon addition of O_2 to [Fe(AzC14-α)], *c.* 1 × 10^{-5} M in toluene, 0.07 M in 1,2-Me_2Im, −9.1 °C: curve (a) under N_2; curve (b) under 760 Torr of O_2. The following partial pressures of O_2 were used: 43, 76, 152 and 380 Torr.

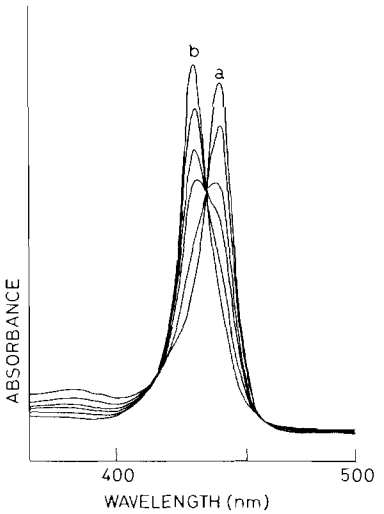

Fig. 3. Spectral changes upon addition of CO to [Fe(Az-C14α)], *c.* 1 × 10^{-5} M in toluene, 0.07 M in 1,2-Me_2Im, 20 °C: curve (a) under N_2; curve (b) under 393 Torr of CO. The following partial pressures of CO were used: 16, 46, 81 and 160 Torr.

TABLE 2. O_2 and CO binding to iron(II) porphyrins

Complexes	$P_{1/2}(O_2)$ (Torr)	$P_{1/2}(CO)$ (Torr)	M[a]	Conditions	References
[Fe(AzP)]	18	0.05	360	[b]	this work
[Fe(AzC14α)]	2150	62	35	[b]	this work
[Fe(AzC14α)]	18	0.31	58	[c]	this work
[Fe(AzC18α)]	15	0.09	170	[b]	this work
[Fe(PocPiv)]	12.6	0.067	216	[d]	11d
[Fe(MedPoc)]	12.4	0.026	480	[d]	11d
[Fe(piv_2C8)]	0.1	0.011	7	[e]	13
[Fe(piv_2C9)]	0.033	0.00028	89	[e]	13
5,5-Pyridine Cyclophaneheme	540	37	14	[f]	14e

[a]$M = P_{1/2}(O_2)/P_{1/2}(CO)$. [b]At 20 °C in toluene, 0.07 M in [1,2-dimethylimidazole (1,2-Me_2Im)]. [c]At 20 °C in toluene, 0.07 M in [1,5-dicyclohexylimidazole (1,5-DCIm)]. [d]At 25 °C in toluene, 0.1 M in [1,2-Me_2Im]. [d]At 20 °C in toluene, 0.01 M in [1-methylimidazole]. [f]At 20 °C in toluene, 0.7 M in [1,5-DCIm].

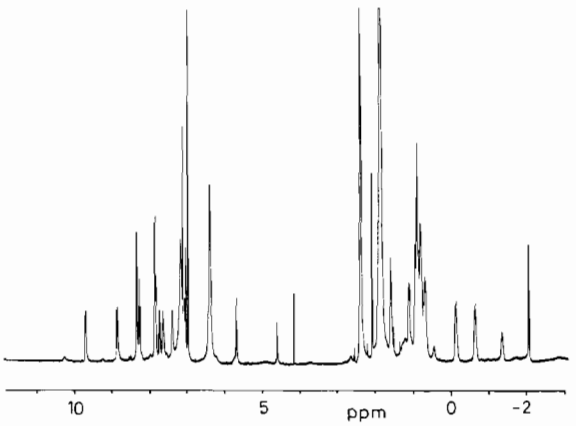

Fig. 4. ^{1}H HNR spectrum of the O_2 adducts of [Fe(AzC14α)], *c.* 5 × 10^{-3} M in toluene-d^8 containing 1,2-Me_2Im (0.1 M) at −20 °C.

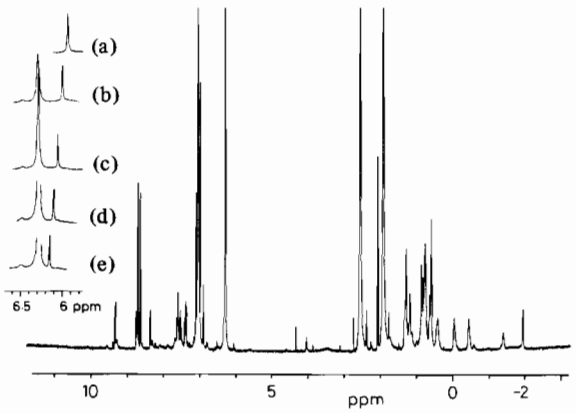

Fig. 5. ^{1}H NMR spectrum of the CO adducts of [Fe(AzC14α)], *c.* 5 × 10^{-3} M in toluene-d^8 containing 1,2-Me_2Im (0.1 M) at 24 °C. Insert: The shifts of the amide proton signals upon addition of 1,2-dimethylimidazole (1,2-Me_2Im); [Fe(AzC14α)] = 4.5 mM. Concentration of 1,2-Me_2Im are (a), 9; (b), 26; (c), 43; (d), 61; (e), 78 mM. The peak at 6.3 ppm is due to excess 1,2-Me_2Im.

TABLE 3. CO stretching frequencies

	ν(CO) (cm^{-1})	References
[Fe(AzP)]	1960[a], 1955[b]	this work
[Fe(AzC14α)]	1948[a], 1945[b]	this work
[Fe(AzC18α)]	1951[a], 1948[b]	this work
HbA	1951[c]	21
[Fe(PPIXDMe)]	1980[d], 1969[e], 1959[f]	21

[a]Solvents are benzene-d^6, 0.1 M in [1-methylimidazole(1-MeIm)]. [b]Solvents are benzene-d^6, 0.1 M in [1,2-dimethylimidazole]. [c]Aqueous media. [d]Solvents are 1-MeIm in CCl_4. [e]Solvents are 1-MeIm in $CHCl_3$. [f]Solvents are 1-MeIm in $ClCH_2$–CH_2Cl.

different from those of the corresponding free base porphyrins.

IR Spectra for the CO Adducs

Table 3 lists CO stretching frequencies. In [Fe(AzC18α)(1,2-Me_2Im)(CO)], the CO stretching frequency was independent of 1,2-Me_2Im concentration when between approximately 2 and 400 molar equivalents of the base were used. The ν(CO) value of [Fe(AzC14α)(1,2-Me_2Im)(CO)] was found to increase by 2 cm^{-1}, while that of [Fe(AzP)(1,2-Me_2Im)(CO)] was found to decrease by 2 cm^{-1} upon addition of 1,2-Me_2Im.

Discussion

Synthesis

Considering their structures, the tetradecamethylene (C14-chain) or octadecamethylene (C18-chain) chain must bridge 'over' the heptamethylene chain (C7-chain) in the α isomers, it is interesting that the yield of these porphyrins is comparable to that of H_2-AzC18β.

Fig. 6. Schematic representation of the strapping chains. The ovals represent porphyrin planes and the loops represent the strapping chains.

The influence of ring current of a porphyrin increases in strength both approaching the porphyrin plane and the ligand axis through the metal [22]. The high field shifts of resonances for the C7-chain protons in H_2-AzC18α are mainly due to the prevention of lateral movement of the C7-chain, because these values are comparable to those of H_2-Azpivαα [13, 15] in which the C7-chain is considered to lie just above the porphyrin center by the steric interaction with two pivalamido groups [23]. According to CPK models, the C7-chain in H_2-AzC18α lies approximately 2.8 Å below the C18-chain, while that of H_2-AzC14α lies in contact with a C14-chain. In addition to the shift of the δ protons to the higher magnetic field, the chemical shift value of the γ protons in H_2-AzC14α approaches the values of the β protons. This means that both the δ and γ protons are pushed down to the porphyrin plane. Thus, it is reasonable to expect that the C7-chain is crushed by the steric repulsion with the C14-chain as shown in Fig. 6. Therefore, the shifts of the C7-chain proton signals in [Fe(AzC14α)(1,2-Me_2Im)(CO)] are concluded to be the result of the crushing of the C7-chain.

^1H NMR Spectra for the O_2 and CO Adducts

The δ proton signals of [Fe(AzP)(1,2-Me_2Im)-(CO)] appear at higher magnetic field than those of [Fe(AzC18α)(1,2-Me_2Im)(CO)]. This may result from the lateral movement of the C7-chain in [Fe-(AzP)(1,2-Me_2Im)(CO)]. By this movement, the δ protons suffer less shielding of the ring current by the bound CO molecule than the others. In addition to the case of the free base porphyrins, the factors affecting the chemical shifts of the C7-chain protons in the CO adducts are considered to be both the shielding of the porphyrin ring current by CO and magnetic anisotropy of CO. Furthermore, it is difficult to evaluate the two effects separately in the present study. Thus, further discussion on the interaction of the C7-chain with CO using ^1H NMR data will be impossible. Increasing the concentration of 1,2-Me_2Im, the amide proton signals of the C7-chain in [Fe(AzC14α)(1,2-Me_2Im)(CO)] shift from 5.95 to 6.25 ppm, as shown in Fig. 5. Such shift of the amide proton signals is not observed for the other two complexes. Therefore, these observations imply that the amide protons of the C7-chain in [Fe(Az-C14α)(1,2-Me_2Im)(CO)] are directed to the position where the amide protons receive the interaction with the excess 1,2-Me_2Im added.

The C7-chain resonances for the O_2 adducts also differ from each other, as in the case of the CO adducts. The amide proton signals in [Fe(AzC14α)-(1,2-Me_2Im)(O_2)] appear at 8.25, 5.67 and 4.59 ppm (relative intensity was 1:2:1). The split of the amide proton signals at 8.25 and 4.59 ppm is due to H-bonding with O_2 [24, 18]. On the other hand, the signals of the amide protons forming the H-bonding with O_2 in [Fe(AzC18α)(1,2-Me_2Im)(O_2)] appear at 7.73 and 4.65 ppm, but any other amide proton signal was obscured by the resonances due to 1,2-Me_2Im or undeuterated toluene. The amide proton signals of the C14-chain and the C18-chain appear at similar positions in the CO adducts and the Zn(II) complexes. Thus, the signal at 5.67 ppm in the spectrum for [Fe(AzC14α)(1,2-Me_2Im)(O_2)] is assigned to the amide protons of the C7-chain: if that signal is due to the amide protons of the C14-chain, the amide proton signal of the C18-chain in [Fe(Az-C18α)(1,2-Me_2Im)(O_2)] must appear near 5.6 ppm, however, such a signal was not observed near 5.6 ppm. Therefore, it is concluded that the amide groups forming H-bonding with the bound O_2 molecules are not of the C7-chain, but of the C14-chain. The conformational changes in the amide groups might be due to the influence of the crushing in the C7-chain. On the other hand, the amide protons forming the H-bonding in [Fe(AzC18α)(1,2-Me_2Im)(O_2)] are the same as in the case of [Fe(AzP)(1,2-Me_2Im)(O_2)] [18]. The β and γ proton signals of the C7-chain in [Fe(AzC14α)(1,2-Me_2Im)(O_2)] are shifted to higher magnetic fields compared with the corresponding CO adducts. Such shifts are not observed in the other two complexes; therefore, these may be responsible for the differences in the interactions of O_2 or CO with the C7-chain.

IR Spectra for the CO Adducts

The solvent dependences of ν(CO) for the CO adducts are small compared to those observed by Maxwell and Caughy [21] (*c.* 20 cm^{-1}); the reduction of ν(CO) for [Fe(AzP)(1,2-Me_2Im)(CO)] results from the changes in solvent polarity. Thus, this means that the Fe–CO bond in [Fe(AzC18α)(1,2-Me_2-Im)-(CO)] is shielded from the solvent polarity as observed in the pocket porphyrin complexes [11c]. Contrary to this, the increase of ν(CO) for [Fe-AzC14α)(1,2-Me_2Im)(CO)] suggests the unique conformation of the amide groups as revealed by the ^1H NMR data. Comparing the ν(CO) value of [Fe(AzP)-(1,2-Me_2Im)(CO)], the small ν(CO) values of both [Fe(AzC14α)(1,2-Me_2Im)(CO)] and [Fe(AzC18α)-(1,2-Me_2Im)(CO)] can be ascribed to four amide groups in the cavities, since the amide groups can

play the role of a primary solvation shell [25]. Although the change in ν(CO) between [Fe(AzC14α)(1,2-Me_2Im)(CO)] and [Fe(AzC18α)(1,2-Me_2Im)(CO)] was small, the change may be due to the steric repulsion on the bound CO molecule in [Fe(AzC14α)(1,2-Me_2Im)(CO)], since these two complexes have identical numbers of amide groups.

Effect of the Ruffling [26] of the Porphyrin Plane on O_2 or CO Binding

The porphyrin rings in 'strapped' porphyrins are considered to be ruffled by the strapping chains [27, 28]. Using 1,2-Me_2Im as an axial base, the O_2 and CO affinities of iron(II) porphyrins decrease to approximately 1/40–1/50, compared with the case using 1-MeIm or 1,5-DCIm as an axial base [11d]. This is explained by the steric repulsion between the 2-methyl group and the porphyrin ring preventing movement of iron(II) toward the prophyrin plane upon O_2 or CO binding. Changing the axial base from 1,2-Me_2Im to 1,5-DCIm, the O_2 and CO affinities of [Fe(AzC14α)] decrease to approximately 1/200 and 1/120, respectively, as shown in Table 2. Thus, the larger changes in both O_2 and CO affinities suggest that the ruffling of the porphyrin ring in [Fe(AzC14α)] is more severe than in flat porphyrins.

Solvent Effect on O_2 Binding

The solvent dependence of O_2 affinities for [Fe(AzC14α)] was also examined at 0 °C with 1,2-Me_2Im as an axial base. The $P_{1/2}(O_2)$ values for [Fe(AzC14α)] in mesitylene, toluene and *o*-dichlorobenzene were 260, 283 and 362 torr, respectively. The O_2 affinities for iron(II) porphyrins are known to increase with the increase of solvent polarity [11b, 14a, 14e]. Thus, the independence of the solvent polarity to the O_2 affinity suggests that the cavity in [Fe(AzC14α)] is well shielded from solvent polarity.

Effect of Cavity Structure on O_2 or CO Binding

As seen in Table 2, the *M* value for [Fe(AzP)(1,2-Me_2Im)] is smaller than that for [Fe(TpivPP)(1,2-Me_2Im)] [5b] and is comparable to that for [Fe(piv_2C9)(1,2-Me_2Im)] [12]. The strapping group in [Fe(AzP)] is the same as that in [Fe(piv_2C9)]; therefore, it is reasonable to expect that the C7-chain in [Fe(AzP)] may cause certain effects such as a steric effect on O_2 or CO binding.

The O_2 affinity of [Fe(AzC18α)] increases slightly, but the CO affinity decreases to 1/2, compared with the corresponding O_2 and CO affinities of [Fe(AzP)]. The increased O_2 affinity for [Fe(AzC18α)(1,2-Me_2Im)] will be responsible for the presence of four amide groups which play the role of primary solvation shell [24a]. This suggestion is supported by the finding that ν(CO) of [Fe(AzC18α)(1,2-Me_2Im)(CO)] is smaller than that of [Fe(AzP)(1,2-Me_2Im)(CO)]. It is concluded that the amide groups of the C18-chain stabilize the Fe–O_2 bond not by H-bonding, since the amide H protons of the C7-chain form H-bonding in [Fe(AzC18α)(1,2-Me_2Im)(O_2)] as discussed in the ^{1}H NMR results. On the other hand, the reduced CO affinity of [Fe(AzC18α)(1,2-Me_2Im)] is partly responsible for the polar contribution of the amide groups. Nevertheless, it is difficult to explain the larger change in CO affinity than in O_2 affinity, because the effects of solvent polarity on the CO affinities are to no greater extent than in the O_2 affinities [12, 14e]. Thus, the reduced CO affinity must be mainly due to the steric effect of the C7-chain. Such a steric effect will be more evident in [Fe(AzC14α)(1,2-Me_2Im)], in which the C7-chain is crushed by the steric interaction with the C14-chain as discussed in the ^{1}H NMR spectra.

The O_2 and CO affinities for [Fe(AzC14α)(1,2-Me_2Im)] decrease to approximately 1/140 and 1/660 compared with those of [Fe(AzC18α)(1,2-Me_2Im)]. Since both [Fe(AzC18α)(1,2-Me_2Im)] and [Fe(AzC14α)(1,2-Me_2Im)] have the four amide groups in their cavities, it is unreasonable to expect that the changes in both O_2 and CO affinities between two complexes are due to the polar effect of the amide groups in the cavities. Contrary to this, the difference between O_2 and CO affinities is well explained by the structural changes between the Fe–O_2 and Fe–CO moieties. The CO molecule binds linearly to the iron center [29], while O_2 molecule binds to the iron center in a bent fashion [30]. Thus, the Fe–CO bond has greater steric interaction with the strapped C7-chain than the Fe–O_2 bond, therefore a greater change in CO affinity than in O_2 is expected. In agreement with this, the change in the CO affinity for [Fe(AzC14α)] is greater than in the O_2 affinity, compared with the CO and O_2 affinities for [Fe(AzC18α)]. As a result, we postulate that the steric effect plays a major role in the discrimination between O_2 and CO bindings to [Fe(AzC14α)]. There are no X-ray structural data at present and a recent report on a 'pocket' porphyrinatoiron(II) complex [31] also suggests that further work such as X-ray crystallographic study should be necessary to confirm our postulation.

Since the *M* value is equal to the ratio of the CO affinity to the O_2 affinity, the *M* value varies with both O_2 and CO affinities. Traylor *et al.* [14e] found a small *M* value (M = 14) for 5,5-pyridine cyclophaneheme and they concluded that the small *M* value is mainly responsible for the increase in O_2 affinity by a polar effect. In contrast to this, we propose that the discrimination reaction between O_2 and CO to [Fe(AzC14α)] is mainly responsible for the decrease in the CO affinity by a steric effect, as reported by Collman *et al.* [11d]. Comparing our study with the geometry of the active sites of de-

oxymyoglobin [32], oxymyoglobin [32] and carbonylmyoglobin [8], determined by X-ray analyses, there is reason to believe that the presence of imidazole in distal histidine increases the O_2 affinity by a polar effect, while on the other hand, it decreases the CO affinity by a steric effect. Therefore, our results may serve as useful information for the O_2 and CO discrimination reaction of hemoproteins.

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