Cooperative Dioxygen Binding to the Bi-bridged Dimeric Porphyrinatocobalt(II) Complexes

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Abstract

Two types of bi-bridged dimeric porphyrinatocobalt(II) complexes, 'picket fence' type and 'strapped' type as a hemoglobin model, and the corresponding monomeric complexes were designed and synthesized. Their equilibrium constants for monodentate or bidentate axial ligands and their dioxygen affinities were measured in DMF. A strapped type dimer complex binds 1,2-bis(4-pyridyl)ethane to form a 'sandwich' structure and reveals the cooperative effect upon dioxygen binding. To explain such hemoglobin-like cooperativity, an intramolecular mechanism is postulated in keeping with the proposal of Perutz's stereochemical trigger mechanism.

Introduction

There have been many investigations on the cooperative dioxygen binding of hemoglobin (Hb) [1, 2]. On the molecular basis to the cooperative effect in Hb and its cobalt derivative (CoHb), Perutz suggests that the shift of the central iron atom toward the porphyrin plane upon O_2 binding could play a crucial role in this case [3]; *i.e.*, this shift would accompany the movement of proximal histidine and make some conformational changes leading to the increased affinity for other O_2 bindings.

Like the hemoglobin, both mechanisms of trigger and of informational conveyance must also coexist in the system of synthetic model molecules revealing the cooperativity. Based upon the idea mentioned above, we redesigned the dimeric model systems in which the shift of the first metal atom upon O_2 binding would be responsible for the regulation of dioxygen affinity on the second active site through a bridged ligand. Although a similar approach along this line was carried out for the series of mono-bridged dimeric complexes having suitable 'sandwich' structures, an appreciable effect was not found. However, the results suggest that to some extent the unfavorable strains might remain or be released even in the case of the mono-bridged dimeric complexes [4]. In the bi-bridged model complexes of the 'sandwich' structure with an appropriate bidentate ligand (see Fig. 1), the cobalt(II) atom will be moved into the porphyrin plane upon the first O_2 binding and this shift will accompany the movement of the bidentate ligand. Finally, it will certainly be transferred to another active site with some conformational changes in the bidentate ligand bond and/or displacement of the cobalt atom from the porphyrin plane. Therefore, if a difference in dioxygen affinities between the first and second O_2 bindings can be seen in our model system, an experimental background to the 'Perutz's stereochemical trigger' mechanism will be realized.

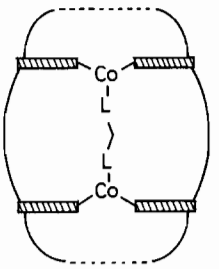

Fig. 1. A schematic representation of a 'sandwich' structure formed by a dimeric porphyrinatocobalt(II) complex and a bidentate ligand. L⌒L represents a bidentate ligand.

Experimental

Proton NMR spectra were recorded on a JEOL-FX-100 spectrometer. Mass spectra were obtained on a JEOL JMX-DX300 instrument. Electronic spectra were recorded on a HITACHI 340 spectrophotometer. Oxygenation or axial ligand equilibria were determined by spectrophotometric titration as reported earlier [4, 5]. $P_{1/2}$ values (half-saturation oxygen pressures of O_2 binding) were determined at several wavelengths and varied by less than 5% within a single run and from run to run.

All solvents were reagent grade. *N,N*-dimethylformamide (DMF) and pyridine were stored over 4

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Å molecular sieves for 3 days and were distilled at reduced pressure. 4,4'-Bipyridine (BIPY), 1,2-(4-pyridyl)ethane (BIPYEt) and 1,3-(4-pyridyl)propane (BIPYPr) were passed on alumina, then recrystallized.

5α,10β,15α,20β-Tetra(2-tert-butylcarbonylaminophenyl)porphyrin, **IIa**, and its cobalt(II) complex, **IIb**, were prepared according to a previously reported method [5].

Preparation of 5α,10β,15α,20β-Tetra(2-aminophenyl)-porphyrin, I

An atropmixture of *meso*-tetra(2-aminophenyl)-porphyrin [6] (10 g) was chromatographed on a silica-gel column (12 × 7 cm: $CHCl_3$); the elution with $CHCl_3$ gave a mixture of 5α,10β,15α,20β- and 5α,10α,15β,20β-tetra(2-aminophenyl)porphyrin and the solvent was evaporated to dryness. The resultant solid (3 g) was dissolved in $CHCl_3$ and loaded onto a 4 × 30 cm column of dry 230–400 mesh silica, and the elution with chloroform–ether (9:1 *v/v*) gave about 1 g of **I**.

Preparation of 5α,15α-Bis(2-aminophenyl)-10β-20β-(2-tert-butylcarbonylaminophenyl)porphyrin, III

To a solution of **I** (1 g) in CH_2Cl_2 (300 cm^3), a mixture of trimethylamine (0.46 cm^3) and triphenylmethylchloride (0.83 g) was added. After 1 h of stirring, methanol (30 cm^3) was added to the solution and the solvent was removed by a rotary evaporator. The resultant solid was dissolved in benzene and chromatographed on a silica-gel column (5 × 30 cm) using benzene–ether (24:1 *v/v*) as an eluent. The second from last band was collected and the solvent was removed by a rotary evaporator. The resultant solid was dissolved in CH_2Cl_2 (100 cm^3) and treated with 3 equivalents of pivaloyl chloride. After the solution was stirred for 1 h, concentrated HCl (100 cm^3) was added and stirred again at 35 °C for 30 min. Then, the HCl layer was neutralized with 10%-NH_4OH in an ice bath and was extracted with $CHCl_3$. After the solvent was stripped to dryness, the product was crystallized from benzene–hexane and gave 100 mg (8%) of **III**. *Anal.* Calc. for $C_{54}H_{50}N_8O_2$: C, 76.93; H, 5.98; N, 13.29. Found: C, 76.09; H, 5.84; N, 13.14%.

Preparation of 5α,15α-Bis(2-aminophenyl)-10β,20β-(2,2'-heptamethylene-1,9-dicarbonylaminophenyl)-porphyrin, IV, and 5α,15α:10β-20β-Bis(2,2'-heptamethylene-1,9-dicarbonylaminophenyl)porphyrin, Va

A dichloromethane solution (1 l) of precursor **I** (1 g) was treated with both pyridine (0.5 cm^3) and nonanedioyl dichloride (0.29 cm^3) at room temperature. The solution was stirred at room temperature for 2 h, then 10%-NH_4OH was added and the solution was stirred for 30 min. The organic layer was separated and stripped to dryness. The resultant solid was dissolved in $CHCl_3$ and chromatographed on a silica-gel column (3 × 30 cm). The elution with $CHCl_3$–ether (9:1 *v/v*) gave porphyrin **IV** and the product was recrystallized from benzene to give 425 mg of **IV**. The overall yield was 42% based on the compound **I** consumed. *Anal.* Calc. for $C_{53}H_{46}N_8O_2$: C, 76.97; H, 5.61; N, 13.55. Found: C, 76.95; H, 5.60; N, 13.49%. The elution with $CHCl_3$–acetone (4:1 *v/v*) gave porphyrin **Va** and the product was recrystallized from $CHCl_3$ to give 130 mg (11%) of **Va**. *Anal.* Calc. for $C_{62}H_{58}N_8O_4 \cdot 0.25CHCl_3$: C, 74.10; H, 5.82; N, 11.11. Found: C, 74.11; H, 5.81; N, 11.16%. Mass spectrum (FAB). Calc. for $C_{62}H_{58}N_8O_4 \cdot H^+$: 979. Found: 979.

Preparation of 5α,15α-Bis(2-pentylcarbonylaminophenyl)10β,20β-bis(2,2'-heptamethylene-1,9-dicarbonylaminophenyl)porphyrin, VIa

This was prepared from **IV** (500 mg), except valeryl chloride was used in place of noanedioyl dichloride. Average yields of 420 mg (69%) were obtained after chromatography and recrystallization from benzene. *Anal.* Calc. for $C_{63}H_{62}N_8O_4$: C, 76.03; H, 6.28; N, 11.26. Found: C, 75.64; H, 6.23; N, 11.28%.

Preparation of 'Strapped' Type Dimer, VIIa

A mixture of pyridine (0.2 cm^3) and glutaryl dichloride (0.1 cm^3) was added to a solution of **IV** (600 mg). The resulting solution was stirred at room temperature for 3 h, then 10%-NH_4OH was added and the solution was stirred again for 30 min. The organic layer was stripped to dryness and toluene (20 cm^3) was added to it. The solution was evaporated again to dryness. The resultant solid was dissolved in CH_2Cl_2 and the solution was chromatographed on a silica-gel (4 × 30 cm) using CH_2Cl_2–methanol (50:1 *v/v*) as an eluent. The third band was collected and stripped to dryness, and the product was recrystallized from $CHCl_3$ to give 200 mg (30%) of **VIIa**. *Anal.* Calc. for $C_{116}H_{100}N_{16}O_8 \cdot CHCl_3$: C, 71.50; H, 5.18; N, 11.40. Found: C, 71.70; H, 5.13; N, 11.35%.

Preparation of 'Picket Fence' Type Dimer, VIIIa

This was prepared from **III** (390 mg) by a procedure similar to that used for **VIIa**. Average yields of 55 mg (12%) were obtained after chromatography [1.5 × 20 cm column of 230–400 mesh silica-gel, $CHCl_3$–ether (2:1 *v/v*)] and recrystallization was carried out from benzene–hexane. *Anal.* Calc. for $C_{118}H_{108}N_{16}O_8$: C, 75.46; H, 5.80; N, 11.93. Found: C, 75.05; H, 5.91; N, 11.51%. Mass spectrum (FD). Calc. for $C_{118}H_{108}N_{16}O_8$: 1876. Found: 1876.

Cobalt(II) Insertion

(a) **VIIa** (250 mg) was dissolved in 300 cm^3 of acetic acid under N_2 atmosphere. The solution was

TABLE I. ^{1}H NMR Data[a]

	IIa	III	VIIIa	IV	VIa	Va	VIIa
Methyl protons[b]	0.25	0.25	0.13				
Pyrrole protons[c]	−2.49	−2.61	−3.04	−2.64	−2.59	−2.52	−3.04
Amino protons[d]		3.50		3.18			
Aliphatic protons[e]				0.94	[f]	0.94	1.10
				−0.60	−0.54	−0.48	−0.59
				−1.32	−1.25	−1.08	−1.36
				−2.61	−2.51	−2.28	−2.61

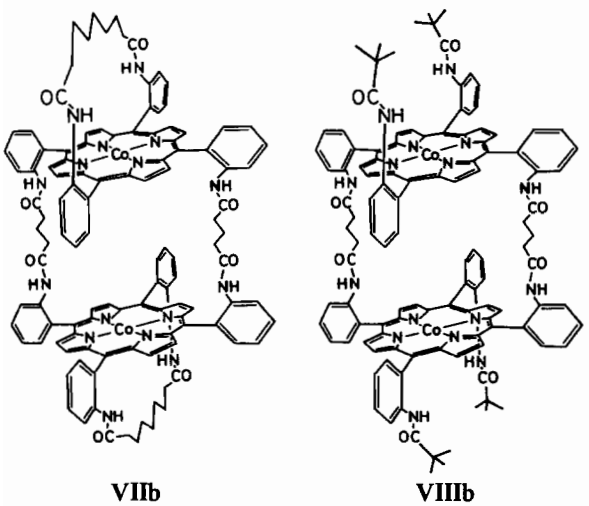
[a]Chemical shift (ppm) from TMS in $CDCl_3$. [b]Methyl protons in pivaloyl groups. [c]Internal pyrrole N–H protons. [d]Amino protons in phenylamino groups. [e]Protons in pentylcarbonyl groups. [f]Resonance was not assigned.

brought to reflux and excess of $Co(OAc)_2 \cdot 4H_2O$ (200 mg) was added. The solution was heated to reflux for 1 h, then the solvent was removed by trap-to-trap distillation. The residue was dissolved again and chromatographed on alumina. After elution with $CHCl_3$–DMF (10:1 *v/v*), the product was recrystallized from $CHCl_3$ as a **VIIb**. *Anal.* Calc. for $C_{116}H_{96}N_{16}O_8Co_2 \cdot 1.5CHCl_3$: C, 65.98; H, 4.59; N, 10.48. Found: C, 65.89; H, 4.59; N, 10.44%.

Vb was prepared from **Va** by the same procedure used for **VIIb**. *Anal.* Calc. for $C_{62}H_{56}N_8O_4Co \cdot 0.5CHCl_3$: C, 68.51; H, 5.20; N, 10.23. Found: C, 68.58; H, 5.41; N, 10.60%.

VIb was prepared from **VIa** in a similar manner used for **VIIb**, except heating was at 50 °C for 15 min instead of refluxing for an hour. *Anal.* Calc. for $C_{63}H_{60}N_8O_4Co$: C, 71.92; H, 5.75; N, 10.65. Found: C, 71.04; H, 5.76; N, 10.49%.

(b) **VIIIa** (80 mg) was treated with anhydrous $CoCl_2$ (75 mg) in THF (50 cm^3) and 2,6-dimethylpyridine (0.3 cm^3) at 50 °C for 45 min under N_2 atmosphere. Then, the solvent was removed by trap-to-trap distillation. The resultant solid was dissolved and chromatographed on alumina. After elution with benzene–ether (7:3 *v/v*), the product was recrystallized from CH_2Cl_2–hexane. *Anal.* Calc. for $C_{118}H_{104}N_{16}O_8Co_2$: C, 71.15; H, 5.26; N, 11.25. Found: C, 69.99; H, 5.03; N, 10.99%.



VIIb VIIIb

Fig. 2. Dimeric porphyrinatocobalt(II) complexes.

Results and Discussion

Synthesis and Characterization

Two types of dimeric porphyrinatocobalt(II) complexes were designed and synthesized, and are shown in Fig. 2. They have a cavity, non-coordination structural array in the vicinity of the metal ion site to stabilize the metal–O_2 bond, on each porphyrin plane outside of two porphyrin planes. The cavity introduced is the so called 'picket fence' type in **VIIIb** and 'strapped' type in **VIIb**, respectively. The distances between the two porphyrin planes in the dimers must have appropriate distance to accommodate bidentate ligands such as BIPY. From our previous study [4], it was shown that the dimeric porphyrinatocobalt(II) complex, prepared by coupling of two tetraphenylporphyrin derivatives with glutaryl dichloride, binds 1 mol of BIPY to form the stable five coordinate complex with a 'sandwich' structure. In this study, the similar bridging reagent was also employed.

Synthetic routes are illustrated in Fig. 3. Previous methods [5, 7] to obtain the precursor **I** were time-consuming and unsuitable for large scale preparations. Applying dry-column chromatography to the purification of the precursor, reduced the time and increased the effectiveness in separation. **VI** was obtained by treatment of the precursor **I** with 1 equivalent of nonanedioyl dichloride and pyridine in dry dichloromethane under a highly diluted condition, and **Va** was obtained as a byproduct. Dimerization of the porphyrins was also carried out by treating with glutaryl dichloride and pyridine in dry dichloromethane.

In the course of synthesis, each product was also characterized by ^{1}H NMR measurements. ^{1}H NMR data of the porphyrins prepared are listed in Table

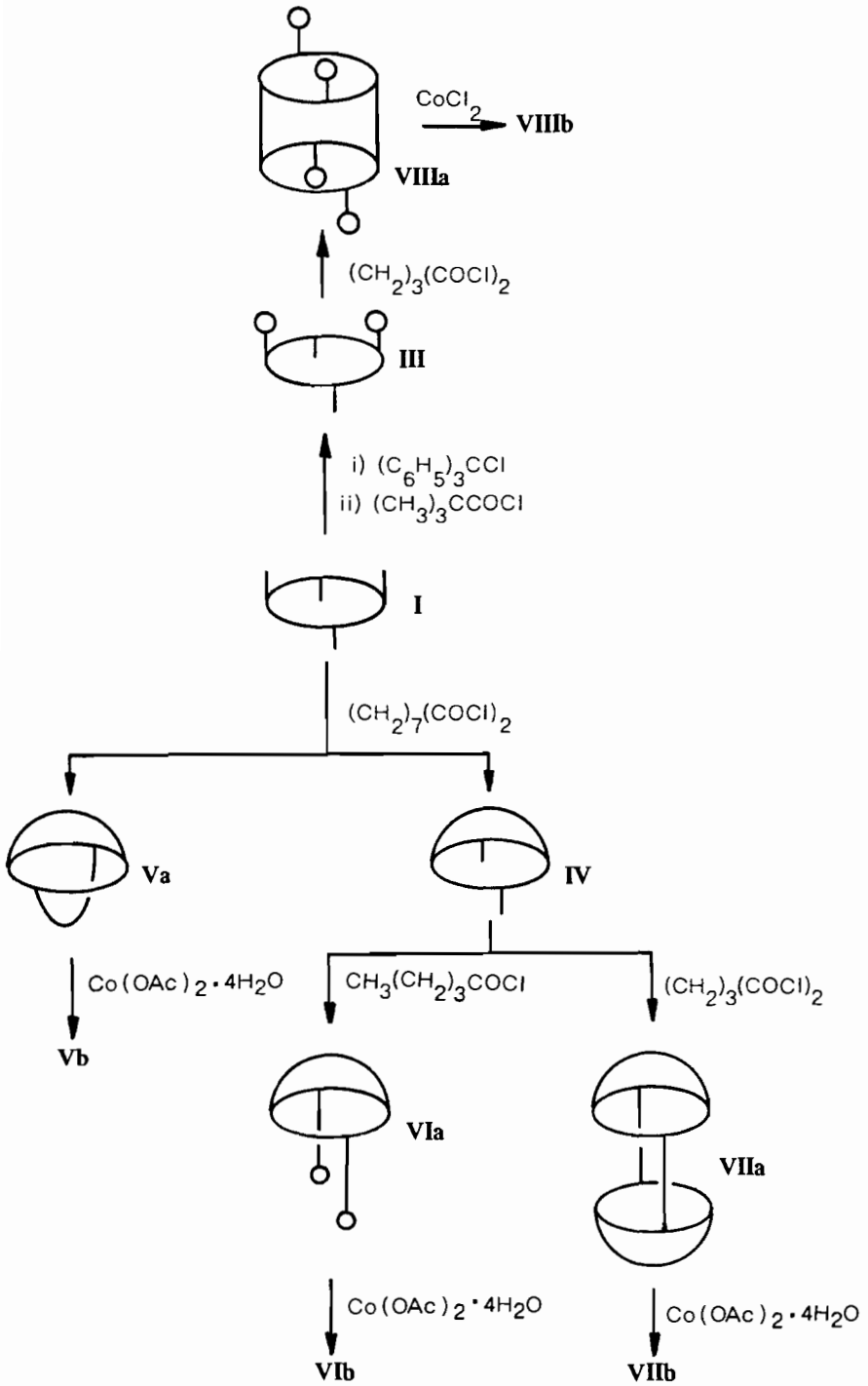


Fig. 3. Synthesis of porphyrins and their cobalt(II) complexes.

I. The chemical shifts of tert-butyl groups in **III** are consistent with those for **IIa**, thus confirming that two pivaloyl groups are introduced on two aminophenyl groups at 5 and 15-positions in the precursor **I**. In the case of porphyrin **IV**, the remarkable upfield shifts of aliphatic methylene protons were observed and these are characteristics of protons positioned above porphyrin planes [8, 9]. Therefore, the aliphatic methylene group in **IV** is confirmed as being bridged over two aminophenyl groups at 5 and 15-positions. Similar chemical shifts of aliphatic methylene protons were observed in **Va**, and the structure of **Va** is identified as having one methylene group bridged over two aminophenyl groups at 5

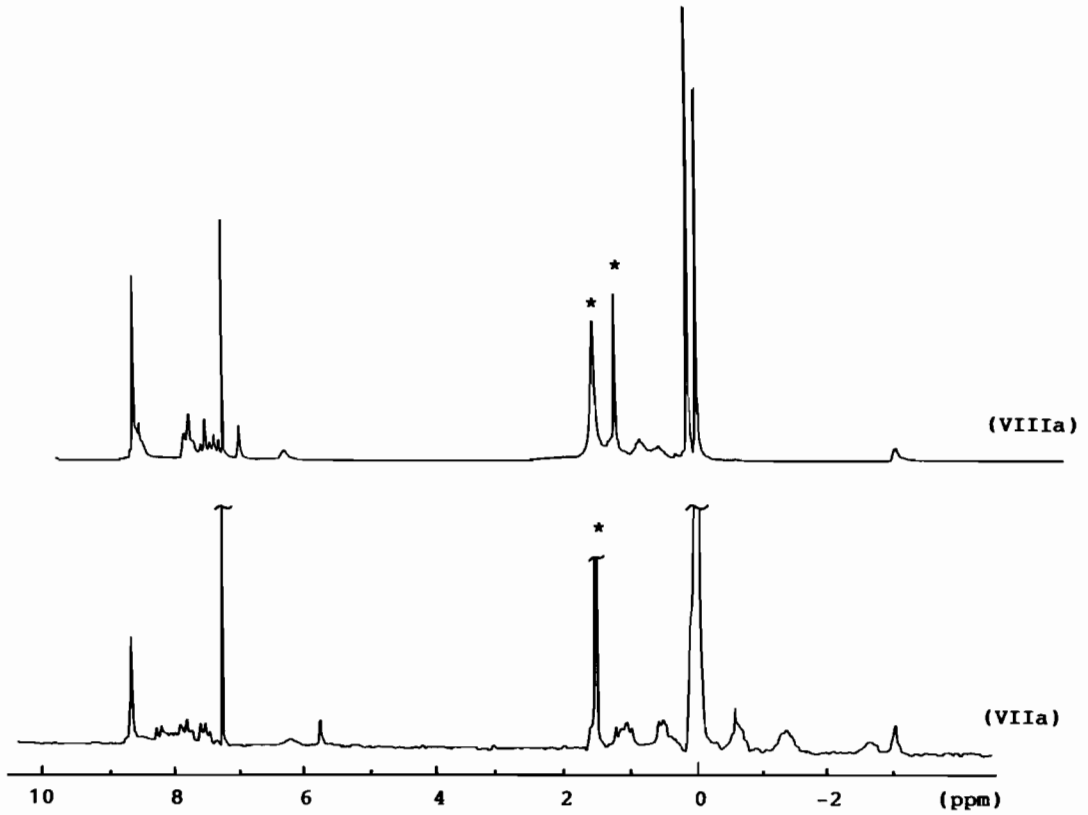


Fig. 4. ^{1}H NMR spectra in $CDCl_3$ of **VIIa** and **VIIIa.** *: Impurity.

and 15-positions and the other bridged over those at 10 and 20- positions.

Two porphyrin planes in the dimers, **VIIa** and **VIIIa** are located as 'face-to-face', because the internal pyrrole N–Hs are shifted to upfield [10] by *ca.* 0.4 ppm for both dimers in comparison with that of the monomeric porphyrin **III** (Fig. 4).

To avoid isomerization of the tert-butyl- or pentyl-carbonylaminophenyl groups, cobalt(II) insertions to **VIIIa** and to **VIa** were carried out under mild conditions. The lack of isomerization during cobalt(II) insertion was confirmed as follows. In the case of **VIIIb**, the ^{1}H NMR spectrum of the cobalt(III) complex prepared from **VIIIb** by oxidation with H_2O_2 in acidic conditions exhibited a single resonance (0.06 ppm) for t-butyl protons. On the other hand, the demetalated product of **VIIb**, which was obtained by treating with concentrated H_2SO_4 in a ice-salt bath, was confirmed as **VIIa** from TLC analysis. Therefore, it is concluded that any isomerization on tert-butyl- or pentyl-carbonylaminophenyl groups does not happen during cobalt insertion.

Cobalt(II) complexes prepared (except for **Vb**) bind O_2 reversibly in organic solvents (CH_2Cl_2 or toluene) containing 1-methylimidazole as a ligand at room temperature.

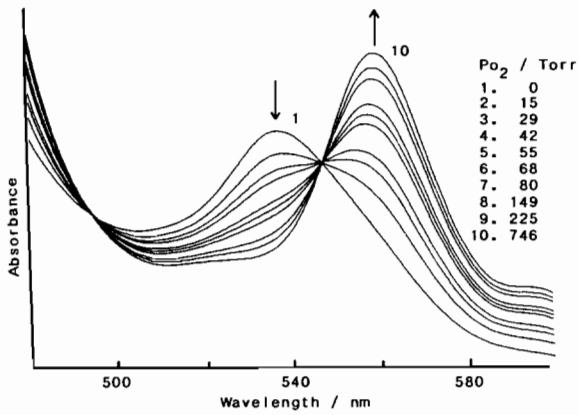


Fig. 5. Visible absorption spectra on oxygenation of **VIIb.** At −15.0 °C in DMF. Axial ligand: 1,2-bis(4-pyridyl)ethane.

Ligand Equilibria and Oxygenation

Because of low solubilities of complexes or ligands and of low dioxygen affinities in toluene, the measurements on oxygenation and the axial ligand equilibria, K_B were carried out in DMF at −15.0 ± 0.1 °C (Fig. 5). In the K_B measurements under above conditions, the formation constants for six coordinate adducts are known to be small [11] and these reactions are neglected in this study.

In our measurements, as the equilibrium constants are estimated from the spectral changes from four to five-coordinate porphyrinatocobalt(II) complexes, the obtained values are K_B on an axial ligand adduct formation reaction to a porphyrinatocobalt(II) unit.

(a) 'Picket fence' dimer

In this dimer system, **IIb** which has the same cavity as that of the dimer complex is regarded as a corresponding monomer complex. The reactions between monomeric four coordinate porphyrinatocobalt(II) complexes and axial ligands in solution are as follows:

$$[CoP] + L \rightleftharpoons [CoPL] \quad (1)$$

$$[CoP] + L\text{–}L \rightleftharpoons [CoP(L\text{–}L)] \quad (2)$$

$$2[CoP] + L\text{–}L \rightleftharpoons [CoP(L\text{–}L)CoP] \quad (3)$$

where CoP, L, and L–L represent porphyrinatocobalt(II) complexes, monodentate ligands and bidentate ligands, respectively. Spectral changes upon additions of bidentate ligands to the solution of **IIb** showed clear isosbestic points and resembled that of the monodentate ligand adduct. Experimentally, the equilibrium constants (K_B) for monodentate or bidentate ligand adduct formations were calculated according to eqns. (1) and (2), respectively. As seen in Table II, the $P_{1/2}$ and K_B values are virtually the same regardless of the axial bases employed, *i.e.*, monodentate or bidentate ligands. Therefore, it is concluded that the effect of these ligands on the dioxygen affinities is similar.

TABLE II Pyridines and Dioxygen Bindings to Model Complexes in DMF at −15.0 °C

		K_B[a] (M^{-1})	$P_{1/2}$[b] (Torr)
VIIIb	PY	1.4×10^3	8000[c]
	BIPY	1.0×10^5	490
	BIPYEt	4.8×10^3	7000[c]
IIb	PY	7.7×10^2	2600
	BIPY	1.2×10^3	2500
	BIPYEt	2.6×10^3	2400

[a]Binding constants to pyridines. [b]Half-saturation pressures for dioxygen binding. [c]Estimated errors <30%.

Compared with the monomer **IIb**, the equilibrium constant for the binding of pyridine to **VIIIb** is not changed as much; on the other hand the dioxygen affinity for the pyridine adduct to **VIIIb** is decreased drastically. In this case, the binding modes of pyridine to **VIIIb** are:

L–Co Co–L Co–L Co–L Co–L L–Co

A **B** **C**

where, Co Co represents a dimeric porphyrinatocobalt(II) complex. Their ratios (**A**:**B**:**C**) are ¼, ½ and ¼, respectively, based on statistical treatment. Therefore, the possibilities of binding of dioxygen toward picket fence sides in which the dioxygen affinity would be comparable to that of **IIb** should be a half. Furthermore, our previous result [4] suggests that aliphatic groups of straight-chain constructing a cavity in species **A** function less effectively than does picket fence as a cavity, and the dioxygen affinity of the species **A** might be reduced. Therefore, it will be responsible for the reduction of the dioxygen affinity for the pyridine adduct of **VIIIb**.

On the other hand, the equilibrium constant for the bindings of BIPY to **VIIIb** is 100 times larger than that to **IIb**, which can be explained by the chelating effects [12 13] on forming 'sandwich' structures as shown in Fig. 1.

Although the increment of dioxygen affinities is observed in the BIPY adduct of **VIIIb**, Hill's coefficient for the oxygenation is *ca.* 1.0, so that any cooperativity between the two active sites is not detected. Small values in the equilibrium constant and in $P_{1/2}$ for the BIPYEt adduct of **VIIIb** may be explained by the ligation on cavity sites as in the case of the PY adduct of **VIIIb**.

(b) 'Strapped' dimer

To prevent the bindings of axial ligands to one side of the porphyrinato complexes, an aliphatic chain was designed to bridge over each porphyrin plane. Ligand bindings (PY or BIPY) to the complex **Vb** were too small to evaluate ($K_B \ll 10$). Therefore, the two aliphatic chains bridged over each porphyrin plane construct suitable cavities around Co–O_2 and prevent the binding of a fifth ligand toward this side.

Both $P_{1/2}$ and K_B values of the monomeric complex **VIb** which has the same cavity as that of strapped type dimer **VIIb** are also the same regardless of the axial ligands employed (see Table III). It is

TABLE III. Pyridines and Dioxygen Bindings to Model Complexes in DMF at −15.0 °C

		K_B[a] (M^{-1})	$P_{1/2}$[b] (Torr)
VIIb	PY	1.4×10^3	320
	BIPY	2.3×10^5	450
	BIPYEt	9.2×10^4	49
	BIPYPr	6.0×10^4	160
VIb	PY	1.0×10^3	620
	BIPY	1.4×10^3	810
	BIPYEt	1.2×10^3	890

[a]Binding constants to pyridines. [b]Half-saturation pressures for dioxygen binding.

therefore concluded that the effect of axial ligands on the dioxygen affinities is similar.

In the case of a picket fence dimer, the equilibrium constants for the bindings of BIPY to **VIIb** are large compared to that of the monomeric complex, **VIb**. This tendency for larger equilibrium constants is also found for the bindings of BIPYEt or BIPYPr to **VIIb**. It is therefore concluded that all bidentate ligand adducts of **VIIb** have a 'sandwich' structure, even with BIPYPr which is a slightly larger molecule (see Fig. 6).

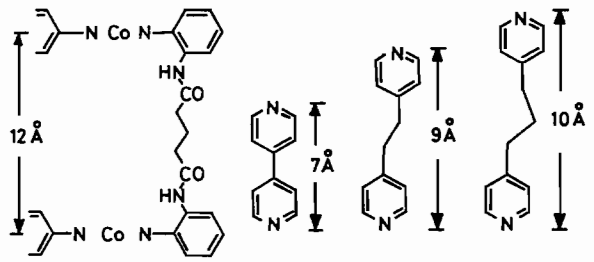


Fig. 6. Schematic representations of molecular sizes of dimeric porphyrinatocobalt(II) complexes (**VIIb** and **VIIIb**) and bidentate ligands.

The dioxygen affinity of the BIPY adduct of **VIIb** is increased compared with that of the monomer, and it is also comparable to that of the BIPY adduct of **VIIIb**. The dioxygen affinities on the bidentate ligand adducts of **VIIb** are increased in the order BIPY > BiPYPr > BIPYEt. The increment of dioxygen affinities will be attributed to the release of some unfavorable strains (e.g., displacement of a metal atom from a porphyrin plane) in the five coordinate deoxy complexes forming a 'sandwich' structure [4].

Most interesting evidence may be the cooperative O_2 binding to the complex of the 'sandwich' structure of **VIIb** with BIPYEt, where Hill's coefficient, n is 1.3 (Fig. 7). On the other hand, O_2 bindings to 'sandwich' complexes of **VIIb** with BIPY or BIPYPr are not cooperative (n = 1.0).

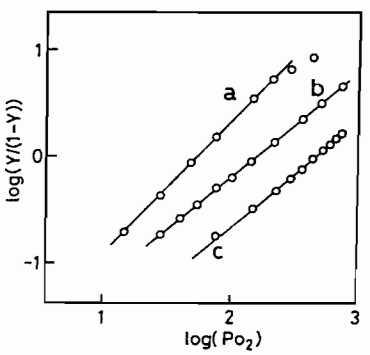


Fig. 7. Hill plots for O_2 binding to **VIIb** in DMF at −15.0 °C. (a) Axial ligand = BIPYEt, slope = 1.3; (b) axial ligand = BIPYPr, slope = 1.0; (c) axial ligand = BIPY, slope = 1.0.

In order to characterize further this cooperative O_2 binding, a dependence of ligand concentration on Hill's coefficient was determined. As a result, Hill's coefficient (n = 1.3) for O_2 binding to the BIPYEt adduct of **VIIb** is not changed within experimental error (±0.05) in the range of concentration of BIPYEt (2.5–10 mM), where the concentration of **VIIb** is 0.04 mM. In contrast to this observation, Tabushi's model system showed that Hill's coefficients on the O_2 bindings vary with the concentration of the base [13]. Therefore, he proposed an intermolecular mechanism for the cooperative dioxygen binding to his model system; upon the first O_2 binding to the complex, one of the ligand–metal bonds is broken and another existing ligand molecule comes in and binds to the complex. As a result, the dioxygen affinity of the second O_2 binding is increased. However, the mechanism mentioned above cannot be applied to cooperative dioxygen bindings on our model systems, because of lack of any dependence of Hill's coefficient on ligand concentration. Furthermore, the use of DMF as a solvent does not affect the cooperative dioxygen binding, because (i) all cobalt(II) complexes prepared have very small dioxygen affinities ($P_{1/2} \gg 2000$ Torr) in the absence of external axial ligand; (ii) the other model systems (*i.e.*, **VIIb** with BIPY or BIPYPr, **VIIIb** with bidentate ligands) do not show any cooperative dioxygen binding. An effect of electronic interaction between two cobalt atoms through the bidentate ligand (BIPYEt) can also be excluded, because the BIPY adduct of **VIIb** does not exhibit cooperativity in O_2 binding, where the BIPY adduct of **VIIb** is expected to have a stronger electronic interaction than the BIPYEt adduct in the sense of the separation between two cobalt atoms. However, it is clear that 'some information' (except for electronic changes) upon the O_2 binding to a first active site will be conveyed to the second one through the bridged BIPYEt molecule in an intramolecular manner. Subsequent to the computer simulation, $P_{1/2}$ values for first and second O_2 binding were estimated as 94 ± 13 and 23 ± 3 (Torr), respectively.

Instead, for the BIPYEt adduct of **VIIb** we propose a mechanism for the cooperative dioxygen binding based on structural aspects.

As seen in Fig. 6, the distance between two cobalt atoms in the model complex **VIIb** is about 12 Å, and the molecular sizes of BIPY, BIPYEt and BIPYPr are 7 Å, 9 Å and 10 Å, respectively, according to a DREIDING stereomodel. Assuming Co–N(pyridyl nitrogen) is about 2.4 Å [14], the distance between two cobalt atoms does not seem to be long enough to accommodate a BIPYEt molecule without constraint, therefore some steric restrictions might be imposed upon the bridged BIPYEt molecule bound to two cobalt atoms. Upon the first O_2 binding, the shift of a cobalt atom into a porphyrin plane

would release some restrictions in the BIPYEt molecule, so the second O_2 binding will be more favorable than the first. Since the BIPYPr molecule has a longer distance between two pyridyl nitrogens than BIPYEt, O_2 binding to the BIPYPr adduct of **VIIb** does not show cooperativity. This result may be explained by the difference in the molecular structures of the ethane or propane chain connecting the two pyridyl groups in the bidentate ligands; that is, such structural strains imposed on the BIPYEt molecule forming the 'sandwich' complex with **VIIb** will be smaller in the BIPYPr adduct of **VIIb,** because the propane chain in BIPYPr will be more flexible than the ethane chain in BIPYEt. Therefore, such restrictions might be not accumulated on the BIPY adduct of **VIIb**, because the BIPY molecule is rather small in size and could plunge between two cobalt atoms without constraint. Similarly, a negative cooperative effect on O_2 binding may be expected for the case of the BIPY adduct; however, it was not detected. This feature may be explained by suggesting that the bridging reagent is flexible enough to shrink the distance of the two porphyrin planes.

Our present work could give supporting evidence for the mechanism of cooperative effect on Hb by the following points; (i) the shift of a central metal atom upon O_2 binding can initiate structural changes as a trigger and (ii) these changes can be conveyed to other active sites in an intramolecular manner. Consequently, these mechanisms can regulate the dioxygen affinity with cooperativity.

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