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Binding of Imidazoles and Pyridines to the Atropisomers of Zinc Tetra(*o*-pivalamidophenyl)porphyrin

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Complex formation of four atropisomers of zinc tetra(*o*-pivalamidophenyl)porphyrin with imidazoles (imidazole, 1-methylimidazole, 2-methylimidazole, and benzimidazole) and pyridines (pyridine, 3,5-dimethylpyridine, and 2,6-dimethylpyridine) was studied. The ratios of the absorbance of the β band to the α band in the adducts tended to be in the order of $\alpha\alpha\alpha\alpha > \alpha\alpha\beta\beta \geqq \alpha\alpha\alpha\beta > \alpha\beta\alpha\beta$. This order, which is the same as that of "electronic effect" on the physical properties of the four atropisomers of tetra(*o*-pivalamidophenyl)porphyrin, indicates the presence of "electronic effect" in the zinc derivatives of the porphyrin. Among the four isomers, the equilibrium constants of N-base binding tended to be in the order of $\alpha\beta\alpha\beta > \alpha\alpha\alpha\alpha \geqq \alpha\alpha\alpha\beta > \alpha\alpha\beta\beta$, suggesting the relatively greater importance of "steric effect" of the bulky pivalamido groups rather than "electronic effect" for the coordination of the N-bases. The differences in the equilibrium constants among the atropisomers are attributable to the contributions of both the steric effect and the electronic effect.

Keywords——atropisomer; picket-fence porphyrin; zinc tetra(*o*-pivalamidophenyl)porphyrin; imidazole; pyridine; axial coordination equilibrium; steric effect; electronic effect

Introduction

"Picket-fence" porphyrin, the $\alpha\alpha\alpha\alpha$-isomer of iron tetra(*o*-pivalamidophenyl)porphyrin (Fe*T*o*P*ivPP),[1] is a very good model for hemoglobin or myoglobin, being able to simulate the reversible O_2 binding in solution.[2] Two histidine molecules (proximal and distal) around the heme center are believed to play an important role in controlling the O_2 binding.[3] Thus, it is useful to study the binding properties of imidazoles to metalloporphyrins in order to understand the binding of histidines in heme proteins. There have been many reports on this subject[4] and "electronic effect" is reported to be important for the binding of N-bases to a metal center of the porphyrin.[4c,h,n] On the other hand, few reports have appeared on "steric effect" in the binding of N-bases to metalloporphyrins.[5]

There are four atropisomers in *ortho*-substituted tetraphenylporphyrin derivatives (T*o*XPP). In some cases (*e.g.* X=cyano[6] and X=amino[2]), those atropisomers are separable from each other by column chromatography. The structural differences among the atropisomers can be used to study the steric effect of the substituent X. We have already reported the separation and physico-chemical properties of the four atropisomers of tetra(*o*-pivalamidophenyl)porphyrin (H_2T*o*PivPP).[7] Zinc incorporation kinetics differed among the isomers of H_2T*o*PivPP and the differences were explained in terms of both electronic and steric factors.[8]

Here, we used four atropisomers of the zinc derivative of H_2T*o*PivPP to study the effect of the bulky pivalamido groups on the equilibrium of the coordination of N-bases such as imidazole and pyridine. Zinc was chosen as the central metal instead of iron, because many results are available for comparison, and only one axial ligand molecule is known to coordinate to zinc porphyrins, producing a 1:1 adduct.[4i-k] This property made the

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interpretation of the data easier than would have been the case in a system involving coordination of two axial ligands.

Experimental

Tetraphenylporphyrin (H_2TPP) and its zinc derivative, ZnTPP, were prepared by the reported methods.[9] Four atropisomers ($\alpha\beta\alpha\beta$, $\alpha\alpha\beta\beta$, $\alpha\alpha\alpha\beta$, $\alpha\alpha\alpha\alpha$)[10] of H_2T*o*PivPP were prepared as reported previously.[7] Zinc was incorporated into the $\alpha\beta\alpha\beta$-isomer as follows. The $\alpha\beta\alpha\beta$-isomer (100 mg) was dissolved in 20 ml of dimethylformamide (DMF), and then $Zn(OAc)_2 \cdot 2H_2O$ (*ca.* 800 mg) in 6 ml of H_2O/DMF (1 : 2) was added. The reaction vessel was incubated in a temperature-controlled bath (50 °C) for 32 h. The rotational isomerization was negligible under these conditions.[11] At the end of the reaction, the visible absorption spectrum was recorded to check the completion of the reaction. Excess H_2O was added to the reaction mixture and the resultant precipitate was collected and dried (about 90% yield). The dried powder was dissolved in chloroform and recrystallized by the addition of hexane and heptane. Zinc incorporation into the other isomers was done by the same method.

Stock solutions of the porphyrins were made by dissolving several mg of the porphyrins in volumetric flasks (25—100 ml) at 25 °C. Chloroform and benzene were distilled after being washed by the reported methods.[12] Ultra fine grade ethanol (Nakarai Chemicals, Ltd.) was used without further purification. Pyridine was distilled and kept on molecular sieves 4A. Imidazole and benzimidazole were recrystallized several times from benzene and hot water, respectively. The other chemicals were used as received.

Spectrophotometric measurements were made on a Hitachi 228 spectrophotometer equipped with a thermostated cell compartment attached to a circulating constant-temperature bath (Neslab, RTE-8). First, the spectrum of a porphyrin solution (3.0 ml) was recorded. To this solution in a cell, a few μl of a base stock solution (*ca.* 3×10^{-2} M) was added with a microsyringe and mixed quickly. The cuvette was equilibrated in the thermostated cell compartment for at least 4—5 min and then the visible spectrum was recorded. The same procedure was repeated several times up to a total of 20 μl addition of the base solution to obtain data at different concentrations of the base. Finally, non-diluted base (solid or liquid, 10—20 mg) was added to measure the spectrum of the completely ligated form of the porphyrin.

The stoichiometry of the reaction between zinc porphyrin and N-bases is known to be 1 : 1.[4i-k] Therefore, the coordination reaction can be described as,

$$P + L = PL \tag{1}$$

where P is the zinc porphyrin, L is the base, and PL is the porphyrin-base complex. According to this equation, the equilibrium constant (K_{eq}) can be written as,

$$\begin{aligned} K_{eq} &= [PL]/\{([P]_0 - [PL])([L]_0 - [PL])\} \\ &= X/\{(1-X)([L]_0 - X[P]_0)\} \end{aligned} \tag{2}$$

where $[P]_0$ and $[L]_0$ are the initial concentrations of the zinc porphyrin and the ligand, respectively, and X is the fraction of complexed porphyrin in total porphyrin ($X = [PL]/[P]_0$), which was determined from the absorbance at an appropriate wavelength.[4i] The K_{eq} of a given ligand concentration was calculated by using Eq. 2, and several values of K_{eq} at different ligand concentrations were averaged. In the 2,6-dimethylpyridine binding experiment, the equilibrium constant was too small to obtain the spectrum of completely complexed form. Therefore, the Ketelaar method[13] was used to determine the K_{eq} in this case.

Results and Discussion

Absorption maxima at the β band of atropisomers of ZnT*o*PivPP in several solvents are listed in Table I. The data for ZnTPP are also listed for comparison. A marked solvent effect of ethanol was seen with all the porphyrins examined, and the β bands of the porphyrins appeared at 8—10 nm longer wavelength than in chloroform or benzene. This effect is similar to the one reported by Nappa and Valentine.[14] Among the four isomers there is a tendency for the peak of the β band of $\alpha\beta\alpha\beta$-isomer to be red-shifted compared with those of the $\alpha\alpha\beta\beta$-isomer and $\alpha\alpha\alpha\alpha$-isomer in all the solvents.

Examples of spectral changes of $\alpha\beta\alpha\beta$-isomer and $\alpha\alpha\beta\beta$-isomer of ZnT*o*PivPP in chloroform arising from the coordination of imidazole are shown in Fig. 1. Good isosbestic points were observed in both cases. The spectra of imidazole complexes of the porphyrins were more red-shifted than those of non-coordinated $\alpha\beta\alpha\beta$- or $\alpha\alpha\beta\beta$-isomer of ZnT*o*PivPP.

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TABLE I. Spectral Data for the β Band of the Atropisomers of ZnT*o*PivPP and ZnTPP in Several Solvents

	Chloroform	Benzene	Ethanol
ZnT*o*PivPP ($\alpha\beta\alpha\beta$)	551.1[a]	549.2	559.2
	(1.84×10^4)[b]	(1.82×10^4)	(2.01×10^4)
ZnT*o*PivPP ($\alpha\alpha\beta\beta$)	547.5	548.4	557.8
	(2.13×10^4)	(2.03×10^4)	(2.11×10^4)
ZnT*o*PivPP ($\alpha\alpha\alpha\beta$)	549.0	549.2	557.6
	(2.09×10^4)	(2.13×10^4)	(2.17×10^4)
ZnT*o*PivPP ($\alpha\alpha\alpha\alpha$)	548.4	547.4	556.4
	(1.83×10^4)	(2.11×10^4)	(2.07×10^4)
ZnTPP	547.9	549.8	557.0
	(1.96×10^4)	(2.24×10^4)	(2.13×10^4)

a) Wavelength of the absorption maximum at the β band (nm). *b*) Extinction coefficient ($M^{-1}cm^{-1}$).

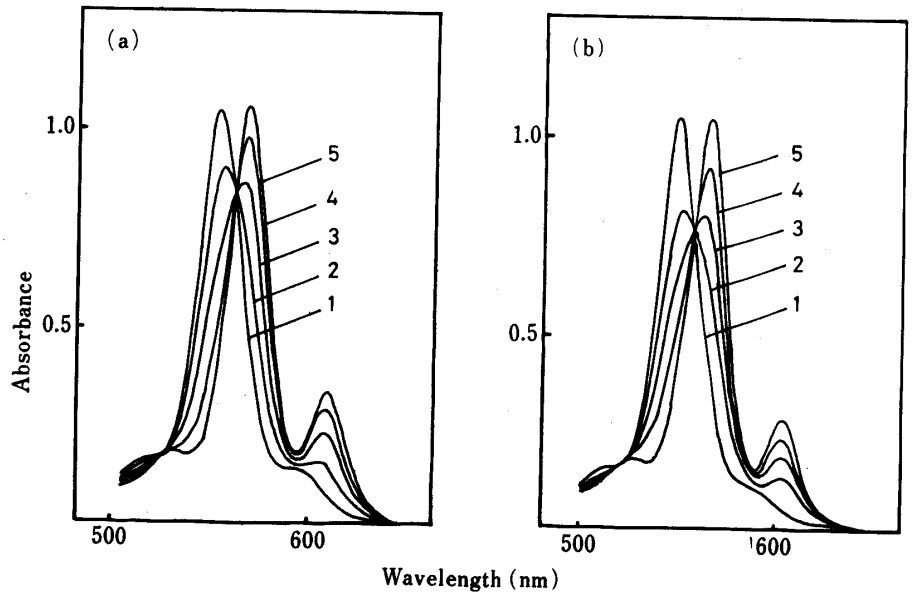

Fig. 1. Visible Spectral Changes Observed upon Addition of Imidazole to (a) the $\alpha\beta\alpha\beta$-Isomer, 6.16×10^{-5} M, and (b) the $\alpha\alpha\beta\beta$-Isomer, 4.95×10^{-5} M, of ZnT*o*PivPP in Chloroform Solution at 25 °C

Imidazole concentration (M): (a) 1) 0, 2) 2.37×10^{-5}, 3) 4.73×10^{-5}, 4) 9.47×10^{-5}, 5) *ca.* 5×10^{-2}; (b) 1) 0, 2) 4.73×10^{-5}, 3) 9.47×10^{-5}, 4) 1.89×10^{-4}, 5) *ca.* 5×10^{-2}.

Equilibrium constants of binding of various imidazoles and pyridines to the four atropisomers of ZnT*o*PivPP and to ZnTPP in chloroform at 25 °C are listed in Table II. The values of the ratio of the absorbance of β band to α band (β/α) are also listed in Table II. Although there are a few exceptions (BzIm, 2,6-dimethylpyridine (2,6-diMePy)), the magnitude of K_{eq} tended to be in the order of $\alpha\beta\alpha\beta > \alpha\alpha\alpha\alpha > \alpha\alpha\alpha\beta \geqq \alpha\alpha\beta\beta$. K_{eq} of the binding of 1-methylimidazole (1-MeIm) was the largest among the bases studied for all isomers, while that of 2,6-diMePy binding was the smallest. The order was 1-MeIm $\geqq$ 2-MeIm $\geqq$ BzIm $\geqq$ Im > 3,5-diMePy > Py $\gg$ 2,6-diMePy, showing that K_{eq} of the binding of imidazoles is larger than that of pyridines.

The values of β/α ratio among the isomers of N-base adducts of ZnT*o*PivPP were in the order of $\alpha\alpha\alpha\alpha > \alpha\alpha\beta\beta \geqq \alpha\alpha\alpha\beta > \alpha\beta\alpha\beta$. This is the same order as that found in some physicochemical properties of H_2T*o*PivPP atropisomers (visible spectrum, reduction potential, *etc.*).[7]

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TABLE II. Equilibrium Data for N-Bases Binding to the Atropisomers of ZnT*o*PivPP and to ZnTPP in Chloroform at 25 °C

Base[a]	Zinc porphyrins				
	$\alpha\beta\alpha\beta$	$\alpha\alpha\beta\beta$	$\alpha\alpha\alpha\beta$	$\alpha\alpha\alpha\alpha$	TPP
Im	15.1[b] ± 2.2[c] (3.12)[d]	2.79 ± 0.67 (3.60)	2.92 ± 0.45 (3.33)	4.55 ± 0.37 (4.16)	0.41 ± 0.04 (1.79)
1-MeIm	21.1 ± 3.8 (3.14)	5.69 ± 0.71 (3.68)	8.10 ± 0.95 (3.42)	12.2 ± 1.2 (4.43)	0.85 ± 0.07 (1.78)
2-MeIm	12.1 ± 3.6 (3.15)	5.92 ± 1.13 (3.50)	7.26 ± 1.81 (3.42)	8.69 ± 3.07 (4.49)	0.93 ± 0.08 (1.82)
BzIm	16.0 ± 0.5 (3.28)	2.45 ± 0.13 (3.54)	5.93 ± 0.74 (3.44)	4.36 ± 0.49 (3.26)	0.46 ± 0.02 (1.92)
Py	3.47 ± 0.52 (3.63)	0.89 ± 0.04 (4.46)	1.15 ± 0.05 (3.96)	1.50 ± 0.10 (5.45)	0.24 ± 0.01 (2.10)
3,5-diMePy	6.92 ± 0.91 (3.70)	0.95 ± 0.05 (4.30)	1.70 ± 0.09 (3.95)	2.39 ± 0.18 (5.18)	0.28 ± 0.04 (2.02)
2,6-diMePy	0.0017 (—)	0.0010 (—)	0.0013 (—)	0.0049 (—)	0.0004 (—)

a) See References and Notes for the abbreviations. *b*) $K_{eq} \times 10^{-4}$ (M^{-1}). *c*) Standard deviation. *d*) Ratio of absorbance of the β band to that of the α band (β/α) in the base complex.

These differences among H_2T*o*PivPP atropisomers were considered to stem from electronic effect, which may be caused by interactions between adjacent pivalamido groups.[7] This order of β/α ratio indicates that there are appreciable differences in electronic effect among atropisomers of ZnT*o*PivPP.

Electronic effects in axial ligand addition and spectroscopic trends of a series of symmetrical and unsymmetrical derivatives of ZnTPP were reported by McDermott and Walker.[4p] They used a series of (p-Cl)$_x$(p-NEt$_2$)$_y$TPPZn(II) as phenyl-substituted ZnTPP derivatives to change the Hammet σ constant (electronic effect). Their results were as follows. 1) The position of the α or β band in the visible absorption spectra of the ZnTPP derivatives is correlated to the value of $\log(\beta/\alpha)$ (large $\log(\beta/\alpha)$ corresponds to a blue shift of the α or β band). This type of correlation was also reported by Nappa and Valentine in various base adducts of ZnTPP.[14] 2) The position of the α or β band is also correlated to the σ constant of the porphyrins (large σ constant corresponds to blue shift of the α or β band). 3) There was a correlation between σ constant and $\log K_{eq}$ (larger σ constant corresponds to larger $\log K_{eq}$). These correlations mean that larger β/α, which is related to the electronic effect of the porphyrin, corresponds to larger K_{eq} in the series of ZnTPP derivatives. We will extend this relationship to the atropisomers of ZnT*o*PivPP. If the electronic effect were the only factor responsible for the difference in K_{eq} among the atropisomers of ZnT*o*PivPP, the order of K_{eq} should be $\alpha\alpha\alpha\alpha > \alpha\alpha\beta\beta \geqq \alpha\alpha\alpha\beta > \alpha\beta\alpha\beta$. However, the results shown in Table II are quite inconsistent with this expectation.

Now we focus on the difference in K_{eq} between the $\alpha\beta\alpha\beta$-isomer and $\alpha\alpha\beta\beta$-isomer instead of considering all the isomers, because these two isomers have symmetrical structures with respect to two sides of the porphyrin plane, which means that the observed K_{eq} is equal to the real equilibrium constant of the N-base binding. On the other hand, the K_{eq} of $\alpha\alpha\alpha\beta$ and $\alpha\alpha\alpha\alpha$ must reflect a mixture of two different equilibria because of the unsymmetrical distribution of the bulky pivalamido groups between the two sides of the porphyrin plane.

Figure 2 shows a schematic representation of the arrangement of the bulky pivalamido groups above and below the porphyrin plane and the expected orientation of the imidazole

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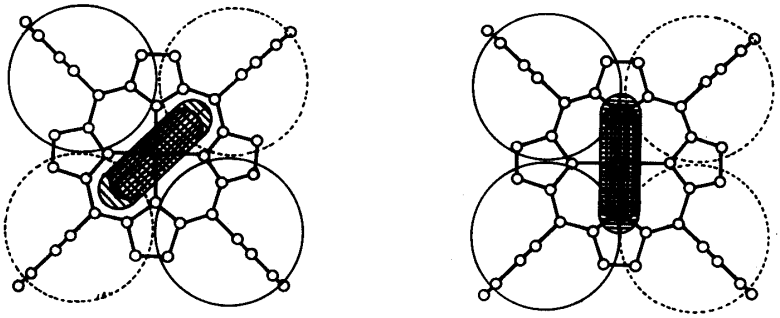

Fig. 2. Schematic Representation of Imidazole Binding to the $\alpha\beta\alpha\beta$-Isomer (a) and to the $\alpha\alpha\beta\beta$-Isomer (b) of ZnT*o*PivPP

Bulky pivalamido groups are shown as solid circles (α, above the plane) and dotted circles (β, below the plane). The size of the circles was calculated based on the van der Waals' radius and coordinates of each atom. Imidazole at about 2.6 Å above the plane is shown by hatching and imidazole at about 5.0 Å above the plane, by cross-hatching. The porphyrin plane was drawn based on the crystallographic data for ZnTPP.[15]

plane. This figure clearly indicates that there is little or no steric hindrance for the coordination of imidazole to the $\alpha\beta\alpha\beta$-isomer of ZnT*o*PivPP, while the coordination to the $\alpha\alpha\beta\beta$-isomer must be greatly hindered by the bulky pivalamido groups. Thus, the difference in the value of K_{eq} between the $\alpha\beta\alpha\beta$-isomer and $\alpha\alpha\beta\beta$-isomer ($\alpha\beta\alpha\beta > \alpha\alpha\beta\beta$) is mainly attributable to the steric effect of the bulky pivalamido groups existing at adjacent positions on the same side of the porphyrin plane.

A quantitative estimation of the steric effect is not easy, because the difference in K_{eq} contains the electronic effect in addition to the steric effect. We tried to separate these effects by estimating the electronic effect using the relationships cited above (1—3).[4p] Based on the difference (0.068) in $\log(\beta/\alpha)$ between the $\alpha\beta\alpha\beta$-isomer and $\alpha\alpha\beta\beta$-isomer, the difference in $\log K_{eq}$ between these isomers was estimated as about 0.17. This value means that if there were no steric effect, K_{eq} of $\alpha\beta\alpha\beta$ should be 2/3 of that of $\alpha\alpha\beta\beta$. However, the observed value of K_{eq} of $\alpha\beta\alpha\beta$ was 2 to 7 times larger than that of $\alpha\alpha\beta\beta$. Therefore, if there were no electronic effect, the steric effect would decrease the K_{eq} by a factor of 3 to 10. The adjacent location of the pivalamido groups on the same side of the porphyrin plane is essential for the group to disturb the binding of imidazole sterically.

This steric interference with the imidazole binding to ZnT*o*PivPP is remarkable compared to "bis-pocket" porphyrin(5,10,15,20-tetrakis(2,4,6-triphenylphenyl)porphyrin), where no steric effect of triphenyl groups on the binding of imidazole was found.[5] This difference may be explained by a rotation of the phenyl groups in the "bis-pocket" porphyrin. The rotation of the phenyl group can make enough space for imidazole to coordinate to the central metal atom without difficulty. On the other hand, the pivalamido groups existing at adjacent positions on the same side of the porphyrin plane are large enough to disturb the binding of imidazole on that side of the porphyrin plane. This may also be the case in the binding of other N-bases to the atropisomers of ZnT*o*PivPP, except in the case of 2,6-diMePy, where the interaction between the methyl groups at the 2,6 positions of pyridine and the porphyrin plane must be a major factor sterically controlling the equilibrium.

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References and Notes

1) Abbreviations: H_2T*o*PivPP, tetra(*o*-pivalamidophenyl)porphyrin; DMF, dimethylformamide; OAc, CH_3COO^-; K_{eq}, equilibrium constant; H_2TPP, tetraphenylporphyrin; Me, methyl; BzIm, benzimidazole; Im,

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