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Kinetics of Zinc Incorporation into the Atropisomers of Tetra(*o*-pivalamidophenyl)porphyrin

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The rates of zinc incorporation into four atropisomers of tetra(*o*-pivalamidophenyl)-porphyrin in dimethylformamide have been measured spectrophotometrically under pseudo-first-order conditions. The anion species of zinc salts used have substantial effects on the kinetics of the porphyrin metallation. When $Zn(OAc)_2$ was used as a metal salt, the values of the first-order rate constant were in the order $\alpha\beta\alpha\beta > \alpha\alpha\alpha\beta \simeq \alpha\alpha\beta\beta > \alpha\alpha\alpha\alpha$, while when $Zn(NO_3)_2$ was used, the order was $\alpha\alpha\alpha\alpha > \alpha\alpha\alpha\beta > \alpha\beta\alpha\beta \simeq \alpha\alpha\beta\beta$. Two factors, an "electronic factor" and a "steric factor," were considered to affect the reaction. The electronic factor is dominant in the former case, while the steric factor is dominant in the latter case. On the basis of the zinc concentration dependence and temperature dependence of the rate constants, the difference in order among the atropisomers could be explained by a hypothetical reaction mechanism involving two alternative rate-determining steps.

Keywords——kinetics; zinc incorporation; atropisomer; picket fence porphyrin; tetra(*o*-pivalamidophenyl)porphyrin; steric effect; electronic effect

Hemoglobin or myoglobin can reversibly take up oxygen. A typical model compound for this property is the iron complex of the picket fence porphyrin.[2] It is thus important to investigate how and why the iron complex of the picket fence porphyrin can form the dioxygen adduct reversibly.

Four atropisomers exist in *ortho*-substituted tetraarylporphyrins.[2,3] They are distinguished by the distribution of the substituents between the two sides of the porphyrin plane. The picket fence porphyrin is one of the atropisomers of tetra(*o*-pivalamidophenyl)porphyrin (ToPivPP). We have been interested in the effect of subtle differences in the stereochemical structure around the porphyrin periphery on the nature of the porphyrin in relation to the role of apoprotein in hemoproteins.

In a previous paper,[3e] we reported that the physicochemical properties, such as visible spectra, nuclear magnetic resonance (NMR) spectra, and reduction potentials, differ among the four possible atropisomers of ToPivPP. In addition to such static properties, we are also interested in the dynamic properties of the atropisomers of ToPivPP. Here, we report the kinetics of zinc incorporation into the atropisomers of ToPivPP. Turay and Hambright have studied the copper incorporation into the atropisomers of ToPivPP.[4] We studied the reaction kinetics using zinc in order to compare this reaction with the copper incorporation reaction.

Another important aim of this work was to estimate the steric effect of the bulky pivalamido group on the reaction. We found that among the atropisomers the order of the values of the reaction rate depends on the kind of zinc salt used. The results are discussed in detail in relation to a general mechanism of porphyrin metallation.

Experimental

Four atropisomers of ToPivPP and tetraphenylporphyrin (TPP) were prepared as described previously.[3e] Reagent-grade $Zn(NO_3)_2 \cdot 6H_2O$, $Zn(OAc)_2 \cdot 2H_2O$, $Zn(ClO_4)_2 \cdot 6H_2O$, and $ZnCl_2$ were used without further purification. Dimethylformamide (DMF) was distilled and dried on molecular seive 4A.

The kinetic runs were conducted under pseudo-first-order conditions with zinc ion in large excess. The incorporation reaction was started by mixing of 0.5 ml of porphyrin stock solution with 2.5 ml of zinc stock solution in a ultraviolet (UV) cell. The reactions were followed spectrophotometrically with a Shimadzu UV200 or a Hitachi 228 spectrophotometer. The reaction temperature was kept constant during the reaction by circulating temperature-controlled water through the cell holder. The reaction temperature was measured occasionally with a thermometer inserted directly into the cell.

Results

The kinetics of zinc incorporation into ToPivPP atropisomers are first-order with respect to porphyrin concentration. Figure 1 shows spectral changes during the reaction of $Zn(OAc)_2$ with $\alpha\beta\alpha\beta$-isomer of ToPivPP. There are isosbestic points at 453, 541, 587, 599, and 630 nm in this case. The positions of the isosbestic points vary somewhat among the isomers, because there are distinct differences in the absorption spectra of the isomers.[3e]

The rate law at constant zinc concentration can be written as

$$\text{rate} = -\mathrm{d}[H_2P]/\mathrm{d}t = k_{\text{obs}}[H_2P]\,,$$

where k_{obs} is the first-order rate constant (involving the zinc concentration). The observed rate

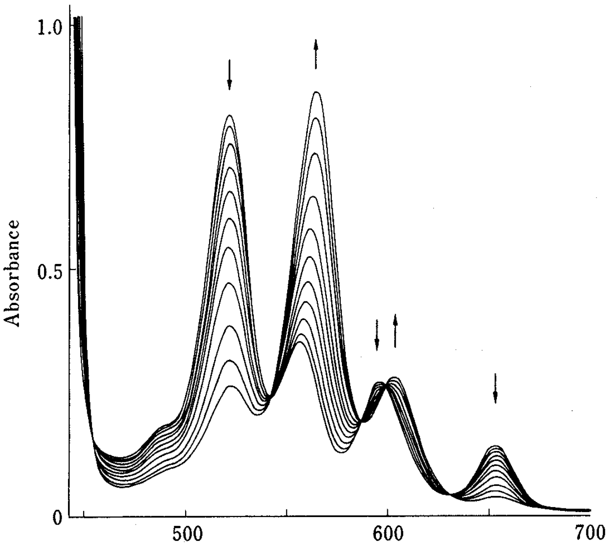

Fig. 1. Spectral Changes during the Reaction of $Zn(OAc)_2$ with $\alpha\beta\alpha\beta$-Isomer of ToPivPP

The change was followed for about 4 h, and the direction of the change is indicated by arrows. The concentrations of the porphyrin and zinc were 5.38×10^{-5} and 1.07×10^{-3} M, respectively. The reaction temperature was 48 °C.

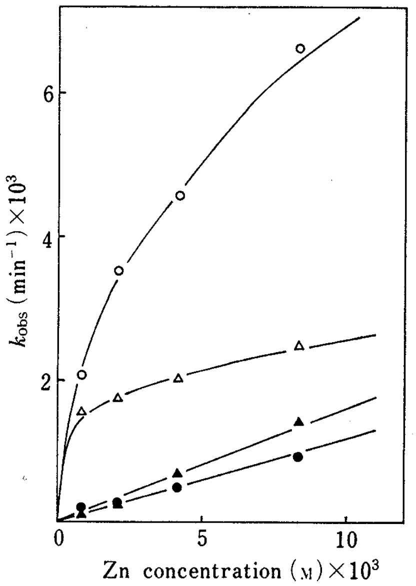

Fig. 2. Dependence of k_{obs} on the Concentration of Zinc

○, $\alpha\beta\alpha\beta$-isomer + $Zn(OAc)_2$; ●, $\alpha\beta\alpha\beta$-isomer + $Zn(NO_3)_2$; △, $\alpha\alpha\alpha\alpha$-isomer + $Zn(OAc)_2$; ▲, $\alpha\alpha\alpha\alpha$-isomer + $Zn(NO_3)_2$.

constants, k_{obs}, for the four atropisomers of ToPivPP using $Zn(NO_3)_2$ and $Zn(OAc)_2$ as zinc salts are listed in Table I. The rate constants for TPP are also listed for reference. The values for ToPivPP are much smaller than those for TPP. This is consistent with the result in the case of copper incorporation.[4] Among the atropisomers, the order of the values of the rate constants differs in the cases of $Zn(OAc)_2$ and $Zn(NO_3)_2$. When $Zn(OAc)_2$ is used, the rate constant is largest for $\alpha\beta\alpha\beta$-isomer and smallest for $\alpha\alpha\alpha\alpha$-isomer. The order of the rate constants is $\alpha\beta\alpha\beta > \alpha\alpha\alpha\beta \simeq \alpha\alpha\beta\beta > \alpha\alpha\alpha\alpha$. On the other hand, when $Zn(NO_3)_2$ is used, the order is $\alpha\alpha\alpha\alpha > \alpha\alpha\alpha\beta > \alpha\beta\alpha\beta \simeq \alpha\alpha\beta\beta$. This is the same order as that observed in copper incorporation by Turay and Hambright.[4] $ZnCl_2$ and $Zn(ClO_4)_2$ were also used as metal salts. However, the $ZnCl_2$ incorporation rate was very slow and $Zn(ClO_4)_2$ incorporation was complex at the initial phase instead of giving a good first-order reaction. Therefore, detailed experiments were not performed using these salts.

Figure 2 shows the zinc concentration dependence of the rate constants for $\alpha\beta\alpha\beta$-isomer and $\alpha\alpha\alpha\alpha$-isomer. The rate constants increase with increase of the concentration of $Zn(OAc)_2$, but the reaction order is less than 1 and the dependence tends to saturate at high concentrations of zinc for both isomers. On the other hand, the rate constants depend linearly on the concentration of $Zn(NO_3)_2$ with a reaction order of about 1.

The temperature dependence of the rate constant was investigated and the results are listed in Table II. The activation free energy was calculated by use of the Eyring equation,

$$k = \kappa(k_B T/h) \exp(-\Delta G^*/kT),$$

in which κ is assumed to be 1. ΔH^* and ΔS^* were calculated by the use of the relationships, $\Delta H^* = E_a + RT$ and $\Delta G^* = \Delta H^* - T\Delta S^*$, where E_a is an apparent activation energy. When $Zn(OAc)_2$ is used, ΔH^* for the $\alpha\alpha\alpha\alpha$-isomer is larger than that for $\alpha\beta\alpha\beta$-isomer. On the other

TABLE I. The Rate Constants of Zinc Incorporation.[a]

Salt	k_{obs} (min^{-1}) × 10^3				
	$\alpha\beta\alpha\beta$	$\alpha\alpha\beta\beta$	$\alpha\alpha\alpha\beta$	$\alpha\alpha\alpha\alpha$	TPP
OAc	2.36	1.21	1.42	0.79	4.52
	(2.99)[b]	(1.53)	(1.80)	(1.00)	(5.72)
NO_3	0.73	0.64	0.95	1.42	10.6
	(0.51)	(0.45)	(0.67)	(1.00)	(7.46)

a) Reaction conditions: 48 °C, [Zn] = 8.33×10^{-3} M, in DMF.
b) Relative value with respect to $\alpha\alpha\alpha\alpha$-isomer.

TABLE II. Activation Parameters for Zn Incorporation Reaction in DMF[a]

Salt	Atropisomer	E_a[b]	ΔG^*[b]	ΔH^*[b]	ΔS^*[c]
OAc	$\alpha\beta\alpha\beta$	20.6	24.4	20.0	−13.7
	$\alpha\alpha\beta\beta$	21.4	25.0	20.8	−13.4
	$\alpha\alpha\alpha\beta$	20.2	25.0	19.5	−16.9
	$\alpha\alpha\alpha\alpha$	23.1	25.5	22.5	−9.3
NO_3	$\alpha\beta\alpha\beta$	20.2	25.1	19.6	−17.0
	$\alpha\alpha\beta\beta$	—	—	—	—
	$\alpha\alpha\alpha\beta$	—	—	—	—
	$\alpha\alpha\alpha\alpha$	17.2	24.7	16.6	−25.1

a) The concentration of the zinc salts is 2.6×10^{-2} M.
b) kcal/mol. *c*) e.u.

hand, when $Zn(NO_3)_2$ is used, ΔH^* for the $\alpha\beta\alpha\beta$-isomer is larger than that for the $\alpha\alpha\alpha\alpha$-isomer. In both cases, ΔH^* for the $\alpha\beta\alpha\beta$-isomer takes a similar value. ΔS^* takes negative values in all cases, and is more negative with $Zn(NO_3)_2$ than with $Zn(OAc)_2$.

Discussion

Taking account of the role of the picket, two factors may affect the zinc incorporation reaction rate: the steric effects of bulky pivalamido groups and the conformational effect on the electronic nature of the porphyrin ring, especially on the central pyrrole proton dissociation. We call these factors the "steric factor" and the "electronic factor," respectively.

Hambright *et al.*[5)] have demonstrated that the rate constants of acid solvolysis of zinc porphyrins and metal-exchange reaction of cadmium porphyrins with zinc increase as the reduction potential becomes more negative. This correlation can be extended to the zinc incorporation reaction. In a previous paper,[3e)] we have reported that the reduction potential is most negative for $\alpha\beta\alpha\beta$-isomer and least negative for $\alpha\alpha\alpha\alpha$-isomer among the atropisomers of ToPivPP. The order of negativity is $\alpha\beta\alpha\beta > \alpha\alpha\alpha\beta \simeq \alpha\alpha\beta\beta > \alpha\alpha\alpha\alpha$. This is parallel to the order of k_{obs} among the isomers observed when $Zn(OAc)_2$ was used. Thus, the order of k_{obs} among the atropisomers with $Zn(OAc)_2$ appears to be related to the electronic factor.

On the other hand, we can explain the order of k_{obs} obtained with $Zn(NO_3)_2$ in terms of the steric factor as follows. Since the two sides of the porphyrin plane of the $\alpha\alpha\alpha\beta$-isomer and $\alpha\alpha\alpha\alpha$-isomer are distinguishable, the rate constant, k_{obs}, for both isomers can be expressed as

$$k_{obs} = k^u + k^b \qquad (k^u \leqq k^b),$$

where k^u is the rate constant of the reaction from the upside (pivalamido group-rich side) of the prophyrin plane, and k^b is that from the bottom side of the porphyrin plane. Since the two sides of the porphyrin plane are indistinguishable for $\alpha\beta\alpha\beta$- and $\alpha\alpha\beta\beta$-isomer, the equation can be written for these isomers as

$$k_{obs} = 2k^u = 2k^b.$$

We introduce the steric retarding factor due to the bulky pivalamido group for the reaction by assuming that each individual group independently retards the reaction. A value of 1/2 is tentatively taken as the retarding factor of one pivalamido group, leading to the following relative rates for the reactions of the respective isomers with $Zn(NO_3)_2$: $2 \times (1/2)^2$ for $\alpha\beta\alpha\beta$- and $\alpha\alpha\beta\beta$-isomer, $1/2 + (1/2)^3$ for $\alpha\alpha\alpha\beta$-isomer, and $1 + (1/2)^4$ for $\alpha\alpha\alpha\alpha$-isomer. The calculated ratio of k_{obs} relative to k_{obs} of the $\alpha\alpha\alpha\alpha$-isomer is 0.47 : 0.47 : 0.59 : 1 in the order mentioned above. This is in excellent agreement with the experimental results shown in Table I.

Prior to discussing the incorporation reaction mechanism, the following factors should be considered: the state of the metal salt in the solution and the state of porphyrin in the solution. There are no definite data on the state of zinc salts in DMF, although several reports have suggested the existence of complex forms of metal salts in solution.[6)] Thus, we can only assume that the concentration of the active zinc species is proportional to the overall concentration of the zinc salts, regardless of what is really the active species. The structure of the porphyrin in DMF must be monomer since the intensity of the visible absorption spectra depends linearly on the concentration of the porphyrin. The reaction species of porphyrin has been claimed to be neutral monomer (H_2P) for this kind of porphyrin in DMF.[7)]

Now we will consider the reaction mechanism of zinc incorporation in an attempt to explain the difference in the order of k_{obs} among the atropisomers of ToPivPP depending on the zinc salts used. A general mechanism of porphyrin metallation has been proposed in several papers.[8)] Although some differences exist among the mechanistic schemes, several common features appear and seem to be generally accepted, as pointed out by Pasternack *et* *al.*[8e)]

The metallation reaction involves several steps, as follows: (1) interaction of metal with porphyrin to form an outer-sphere complex, (2) deformation of the porphyrin, (3) loss of coordinated ligands (including solvent molecules) from the metal, (4) formation of a covalent bond between the metal and the porphyrin, (5) incorporation of the metal into the hole of the porphyrin ring, (6) loss of central ring protons from the porphyrin. The above order represents a possible reaction sequence. It is difficult to establish individual steps based on the experimental results. Therefore, we simplify these steps to two primary steps: (A) interaction between the metal and the porphyrin to form an intermediate complex, probably involving steps (1) and (2), and (B) formation of a stable metalloporphyrin, probably involving steps (3)—(6). The simplified mechanism can be written as

$$\mathrm{H_2P + M^{2+}} \underset{k_{-\mathrm{A}}}{\overset{\overset{\text{step A}}{k_\mathrm{A}}}{\rightleftarrows}} \mathrm{H_2P\text{---}M^{2+}} \overset{\overset{\text{step B}}{k_\mathrm{B}}}{\longrightarrow} \mathrm{MP + 2H^+}$$

In this mechanism it is possible for either step A or B to be rate-limiting. This mechanism is similar but not identical with the mechanism proposed by Pasternack *et al.*[8e)] In their scheme step A is considered as a preequilibrium step and B as the rate-limiting step.

If step A is rate-limiting, the reaction rate should simply be first-order in metal concentration, while if step B is rate-limiting, the reaction rate should depend on the concentration of the intermediate complex, H_2P---M^{2+}, and, therefore, should not be as simple as in the first case. When $Zn(OAc)_2$ is used as the metal ion source k_{obs} for both $\alpha\beta\alpha\beta$- and $\alpha\alpha\alpha\alpha$-isomer does not show a first-order dependence on zinc concentration. When $Zn(NO_3)_2$ is used, k_{obs} shows a good first-order dependence on zinc concentration. These results indicate, as predicted above, that step B is important in determining the overall rate ($k_A > k_B$) when $Zn(OAc)_2$ is used, while step A is suggested to be rate-determining ($k_B > k_A$) when $Zn(NO_3)_2$ is used.

The temperature dependence of k_{obs} is different depending on the zinc salts used as the metal ion source. The activation entropy is smaller when $Zn(OAc)_2$ is used than when $Zn(NO_3)_2$ is used. The entropy change will be smaller when step B determines the overall rate rather than step A, because step A is an intermolecular reaction, while step B is a quasi-intramolecular reaction. This result also suggests that step B is important when $Zn(OAc)_2$ is used, while step A is important when $Zn(NO_3)_2$ is used.

When $Zn(OAc)_2$ is used, the order of k_{obs} among the isomers is related to the electronic factor, and when $Zn(NO_3)_2$ is used, the order is related to the steric factor. Therefore, by combining this phenomenon with the results on zinc concentration dependence and temperature dependence mentioned above, we can say that the steric factor affects step A and the electronic factor affects step B.

From presently available kinetic data, we cannot elucidate the structure and properties of the intermediate which might explain why $k_A > k_B$ with $Zn(OAc)_2$ and why $k_B > k_A$ with $Zn(NO_3)_2$ in DMF. However, the controversy regarding the porphyrin metallation mechanism and the contradictory experimental results concerning the metallation reaction so far reported[9)] may be accommodated by the idea that the relationship of the rate constants for the two sequential steps alternates between $k_A > k_B$ and $k_B > k_A$ depending on the reaction conditions. The cases where the rate linearly depends on the concentrations of metal and porphyrin[6a,7,8b,c)] fall under the category of $k_B > k_A$. On the other hand, the cases where no linear dependence of the rate on metal concentration is observed[6a,e,8e,10)] can be classified into the category of $k_A > k_B$. This classification of porphyrin metallation may be a useful first step in elucidating the general mechanism including the nature of the intermediate(s).

References and Notes

1) Abbreviations: ToPivPP = tetra(*o*-pivalamidophenyl)porphyrin; TPP = tetraphenylporphyrin; OAc = CH_3COO; DMF = dimethylformamide; H_2P = free base porphyrin; M^{2+} = metal dication; MP = metalloporphyrin.
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