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Sitting-atop complex formation of 2,3,7,8,12,13,17,18octaethylporphyrin with copper(II) ion in acetonitrile

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Abstract

The reaction of 2,3,7,8,12,13,17,18-octaethylporphyrin (H₂OEP) with copper(II) triflate and copper(II) perchlorate in acetonitrile was studied using spectrophotometry. The reaction product is the so-called sitting-atop complex where two pyrrolenine nitrogen atoms of the porphyrin coordinate to the incoming metal ion and two protons on the pyrrole nitrogen atoms still remain. The composition of the sitting-atop complex was determined by the mole ratio method, and it was found that the H₂OEP molecule binds two copper(II) ions in the product. The mechanism of the reaction was confirmed to be a series of second-order reactions with the first and second step of the reactions being the outer sphere complex formation between the H₂OEP molecule and copper(II) ion and the rate determining sitting-atop complex formation reaction, respectively, based on the mole ratio method. The reaction is relatively fast, and the second-order rate constants for the reaction of H₂OEP with copper(II) ion was determined to be $k = (3.2 \pm 0.3) \times 10^6$ M⁻¹ s⁻¹ (T = 25.0 °C) for the copper(II) triflate and $k = (3.0 \pm 0.2) \times 10^6$ M⁻¹ s⁻¹ (T = 25.0 °C) for the copper(II) perchlorate under the second-order conditions. The pK_a values of the mono- and diprotonated forms of the conjugate acid of several porphyrins including H₂OEP were determined by spectrophotometric titration in acetonitrile. The higher reactivity of H₂OEP toward copper(II) ion as compared with other porphyrins such as 5,10,15,20-tetraphenylporphyrin was attributed to its higher basicity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Porphyrin metallation; Copper(II) complexes; Reaction intermediate; Sitting-atop complexes; pKa of porphyrin in acetonitrile

1. Introduction

Metalloporphyrins and related compounds play important roles in biological systems. One of the most significant processes is the metalloporphyrin formation reaction with special reference to the heme biosynthesis [1-6]. The terminal step in the heme biosynthesis, the insertion of ferrous iron into protoporphyrin IX to form protoheme, is catalyzed by the enzyme ferrochelatase. Although the crystal structures of the ferrochelatases along with years of biophysical and kinetic studies have led to a better understanding of the catalytic mechanism of the ferrochelatase, the mechanism of the complex formation reaction of porphyrins has not been fully established not only in biological systems but in solution. The overall metalloporphyrin formation reaction

consists of the coordination of pyrrole nitrogen atoms to metal ion and the release of two pyrrole protons. Concerning the pyrrole protons, a typical reaction intermediate was proposed by Fleischer and Wang in 1960 [7]. They studied the reaction of protoporphyrin IX derivatives with several metal ions in chloroform and observed the formation of the reaction intermediate. This intermediate is called a sitting-atop complex, in which two pyrrolenine nitrogen atoms of the porphyrin coordinate to the metal ion and two pyrrole protons still remain. Several sitting-atop complexes of naturally occurring and synthetic porphyrins including such ions as platinum(II), copper(II), and rhodium(I) were then reported [8-10]. Recently, more clear evidence for the sitting-atop complex was spectrophotometrically obtained during the reaction of 5,10,15,20-tetraphenylporphyrin (H₂TPP) with copper(II) ion in acetonitrile [11]. The Brønsted basicity of acetonitrile is very weak [12,13], and, therefore, the pyrrole protons of H₂TPP

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are not easily released even after forming the intermediate with the copper(II) ion without additional proton acceptors. This acid-base property of the solvent is responsible for the stability of the sitting-atop intermediate. The rate of the sitting-atop complex formation reaction of H₂TPP with several 3d-block metal(II) cations in acetonitrile has also been determined, and the general mechanism of the reaction has been proposed on the basis of the parallel correlation between these values and the rate of the solvent exchange reaction of the corresponding cations [14]. The kinetics of the sitting-atop complex formation reaction have also been investigated for other *meso*-substituted porphyrins, 5,10,15,20-tetrakis(4-chlorophenyl)porphyrin i.e. $(H_2T(4-CIP)P)$, and 5,10,15,20-tetramesitylporphyrin (H₂TMP), and the mechanism of the reaction was discussed in terms of the electronic and steric effects of the substituents of the porphyrin peripherals on the reaction rate [15]. By using acetonitrile, having a weak basicity and a coordination ability to metal ions, as a solvent [16], it was possible to characterize the sittingatop complex of H₂TPP and other meso-substituted porphyrins.

In a previous paper, we also studied the sitting-atop complex formation of 2,3,7,8,12,13,17,18-octaethylporphyrin (H₂OEP) with copper(II) ion in acetonitrile and attempted to determine the rate constant of the reaction [15]. However, the rate is too fast to be followed under pseudo-first-order conditions where the copper(II) ion exists in large excess over H₂OEP. The higher reactivity of H₂OEP toward the complex formation reactions may be ascribed to its higher basicity and/or higher flexibility compared with other meso-substituted porphyrins. In the present study, we investigated the sitting-atop complex formation reaction of H₂OEP with copper(II) ion in acetonitrile and successfully determined the rate constant under the second-order conditions. We also determined the pK_a values of the porphyrins in acetonitrile using spectrophotometric titration in order to discuss the reactivity of the porphyrins towards metal ions in terms of the basicity of the porphyrins as well as the molecular structure and substituent effects of the porphyrins.

2. Experimental

2.1. Chemicals

Acetonitrile (Romil, >99.9%, Super Purity Solvent (far UV), water content <0.005%) was the same as used in a previous study [17]. Acetonitrile (Wako Pure Chemical Industries, Japan) was dried over activated 3 Å molecular sieves for several days and distilled under nitrogen atmosphere. H₂OEP (Tokyo Chemical Industry) was used without further purification. Pyridine (Fluka, >99.8%), 2,6-dimethoxypyridine (Aldrich, 98%), 2-chloropyridine and 3-chloropyridine (both TCI, >98%) were also used without additional purification. 8-Aminoquinaldine (REAKHIM, 'pure') was recrystallized from ethyl ether. 2-Methoxypyridine (Aldrich, 98%) was fractionally distilled from MgSO₄ under reduced pressure. Solutions of trifluoromethanesulfonic acid (Aldrich, 99+%) and triethylamine (Aldrich, 99%) were used as the acidic and basic titrants, respectively. Copper(II) perchlorate, Cu(ClO₄)₂, was prepared by treating copper(II) oxide (Wako Pure Chemicals, 99.9%) with perchloric acid (Wako Pure Chemicals, Super Special Grade), recrystallized from distilled water, and vacuum dried. Copper(II) triflate, Cu(CF₃SO₃)₂, was prepared according to the literature procedure [11].

2.2. Measurements

The UV–Vis absorption spectra were measured using a Hitachi U-3000 spectrophotometer. The spectral change for the reaction of the porphyrin with the copper(II) ion in acetonitrile was measured by a stopped-flow apparatus equipped with a rapid-scanning spectrophotometer (RSP-801, Unisoku, Japan). The temperature of all the measurements was controlled within ± 0.1 °C using a circulating thermostated water bath. The water content of the solutions was determined by Karl Fischer titration to be between 1.3 and 3 mM with the one exception of 20 mM for the spectrophtometric titration study and less than 15 mM for the kinetic study.

2.3. Spectrophotometric titration

The spectrophotometric titration method in a glove box was very similar to the one used in a previous study [17]. The main difference from the previous study was that the saturated solutions of the porphyrins were made and these solutions were diluted for titration experiments due to the poor solubility of the porphyrins in acetonitrile. The dilution factor was from 1.1 to 5, depending on the porphyrin and the reference compound. The concentrations of the porphyrins in the titration solutions were from 0.8 to 2×10^{-6} M. The concentrations of the other bases were usually in the 10^{-5} M range and the total concentration of the bases in our experiments never exceeded 1.8×10^{-4} M.

2.4. Calculation methods

The basicity of a polybasic base, $H_m B^{m+}$, in solvent S is defined using Eq. (1) and is expressed as the dissociation constant, K_a , of the conjugate acid, $H_{m+1}B^{(m+1)+}$, of the base, $H_m B^{m+}$, or more commonly its negative logarithm, pK_a .

$$H_{m+1}B^{(m+1)+} + S \stackrel{K_a}{\rightleftharpoons} H_m B^{m+} + HS^+$$
(1)

$$K_{\rm a} = \frac{a({\rm H}_m {\rm B}^{m+})a({\rm HS}^+)}{a({\rm H}_{m+1} {\rm B}^{(m+1)+})}$$
(2)

$$pK_{a} = -\log\left(\frac{a(H_{m}B^{m+})a(HS^{+})}{a(H_{m+1}B^{(m+1)+})}\right)$$
(3)

If m = 0, then $H_{m+1}B^{(m+1)+}$ is the conjugate cationic acid of the neutral base B and if m = +1, then $H_{m+1}B^{(m+1)+}$ is the conjugate dicationic acid of cationic base HB⁺.

To exclude the necessity for measuring the hydrogen ion activity (see Eq. (3)), we studied the equilibrium between two bases, $H_n B_1^{n+}$ and $H_m B_2^{m+}$.

$$H_{m}B_{2}^{m+} + H_{n+1}B_{1}^{(n+1)+} \rightleftharpoons H_{m+1}B_{2}^{(m+1)+} + H_{n}B_{1}^{n+}$$
(4)

The relative basicity, ΔpK_a , of bases $H_nB_1^{n+}$ and $H_mB_2^{m+}$ is expressed as:

$$\Delta p K_{a} = p K_{a} (H_{m+1} B_{2}^{(m+1)+}) - p K_{a} (H_{n+1} B_{1}^{(n+1)+})$$
$$= \log \left(\frac{a (H_{m+1} B_{2}^{(m+1)+}) a (H_{n} B_{1}^{n+})}{a (H_{m} B_{2}^{m+}) a (H_{n+1} B_{1}^{(n+1)+})} \right)$$
(5)

The mixture of bases as well as both bases separately were titrated with an optically transparent acid or base and the data for the calculations was obtained from the UV–Vis spectra. From each titration experiment, the $\Delta p K_a$ was determined as the mean of 5–20 values using mostly the same calculation methods as previously described [17–19]. For the complicated cases appearing in this study, where $p K_a (HB^+)$ and $p K_a (H_2B^{2+})$ of the two polybasic porphyrins are too close (less than 3 $p K_a$ units) to observe the pure spectrum of the monoprotonated form (HB⁺), a different approach was used. At wavelength λ , the absorbance of a solution (with a 1 cm path length) containing all forms of the dibasic compound B is expressed as follows:

$$A^{\lambda} = [\mathbf{B}]\varepsilon_{\mathbf{B}}^{\lambda} + [\mathbf{H}\mathbf{B}^{+}]\varepsilon_{\mathbf{H}\mathbf{B}^{+}}^{\lambda} + [\mathbf{H}_{2}\mathbf{B}^{2+}]\varepsilon_{\mathbf{H}_{2}\mathbf{B}^{2+}}^{\lambda}$$
(6)

The analytical concentration of the base expresses:

$$C = [\mathbf{B}] + [\mathbf{H}\mathbf{B}^+] + [\mathbf{H}_2\mathbf{B}^{2+}]$$
(7)

To use the least-squares of the linear combinations method (see [18]), the spectra of B, HB⁺ and H₂B²⁺ are needed. The absorbances of the solutions containing only B (Eq. (8)) or H₂B²⁺ (Eq. (10)) are experimentally obtained and the absorbance of HB⁺ (Eq. (9)) is calculated as described below.

$$A_{\rm B}^{\lambda} = C \varepsilon_{\rm B}^{\lambda} \tag{8}$$

$$A_{\rm HB^+}^{\lambda} = C \varepsilon_{\rm HB^+}^{\lambda} \tag{9}$$

$$A^{\lambda}_{\rm H_2B^{2+}} = C\varepsilon^{\lambda}_{\rm H_2B^{2+}} \tag{10}$$

In the solutions of low acidity, the concentration of H_2B^{2+} is very low and the third member in Eqs. (6) and (7) can be ignored. Expressing the ε -s from Eqs. (8) and

(9) and HB^+ from Eq. (7) and substituting these into Eq. (6) gives:

$$A^{\lambda} = \frac{[\mathbf{B}]}{C} A^{\lambda}_{\mathbf{B}} + \frac{C - [\mathbf{B}]}{C} A^{\lambda}_{\mathbf{HB}^{+}}$$
(11)

After rearrangement and substituting x = [B]/C and y = 1/(1-[B]/C), we get for *i*-th spectrum.

$$A_{\mathrm{HB}^{+}(i)}^{\lambda} = y_{i}A^{\lambda} - x_{i}y_{i}A_{\mathrm{B}}^{\lambda}$$
(12)

On the other hand, in the solutions of high acidity, the concentration of B approaches zero and in a similar way, we can express the absorbance of HB^+ :

$$A_{HB^{+}(i)}^{\lambda} = y_{i}A^{\lambda} - x_{i}y_{i}A_{H_{2}B^{2+}}^{\lambda}$$
(13)

If we have a set spectra for which either Eq. (12) or Eq. (13) can be applied, we then get the spectrum of HB⁺ using the least-squares minimization. The minimized quantities are the sums *S* (Eq. (15)) of dispersions σ_{λ}^{2} (Eq. (14)) of $A_{\text{HB}+(i)}^{\lambda}$ over the range of wavelengths $\lambda_{\text{start}} - \lambda_{\text{end}}$. This range of wavelengths was chosen so that it would incorporate the most characteristic absorption bands of the three forms of base B.

$$\sigma_{\lambda}^{2} = \frac{\sum_{i=1}^{n} (\bar{A}_{HB^{+}}^{\lambda} - A_{HB^{+}(i)}^{\lambda})^{2}}{n-1}$$
(14)

$$S = \sum_{\lambda = \lambda_{\text{start}}}^{\lambda_{\text{end}}} \sigma_{\lambda}^2 \tag{15}$$

 $\bar{A}^{\lambda}_{\mathrm{HB}^+}$ is the average of the $A^{\lambda}_{\mathrm{HB}^+(i)}$ values at wavelength λ . The adjusted quantities are x_i and y_i . In addition, if the isosbestic points were present in the wavelength range $\lambda_{\text{start}} - \lambda_{\text{end}}$, then the spectra were 'pushed' through the isosbestic points by setting higher weights (usually 100 times) to the dispersions $\sigma_{\lambda_{isobestic}}^2$ in Eq. (15). The spectrum of HB⁺ obtained this way is used in the ΔpK_a calculation procedure to calculate the difference between $pK_a(HB^+)$ and $pK_a(H_2B^{2+})$. If in Eq. (5), m =n = 0, then we assumed that the ratio of the activity coefficients does not change during titration experiments. If m = +1 and n = 0, then we calculated the activity coefficients using the Debye-Hückel equation as described in [20]. It appears that in very diluted solutions ($< 10^{-4}$ M), the corrections arising from the accounting activity coefficients are very small (< 0.04 pK_a units).

3. Results

3.1. Basicity of porphyrins

The relative basicity measurements between ten compounds were carried out and 26 relative acid-base

Table 1

Directly measured relative basicity values for neutral and cationic bases and estimated absolute pK_a values for their conjugate acids in acetonitrile **Directly measured** ΔpK_a **Base**



equilibrium constants were found. These values produce the self-consistent and continuous basicity scale presented in Table 1. Multiple overlapping measurements make the results more reliable and help to estimate their self-consistency. The entire basicity range involves at least two independent pathways of measurements and the relative basicity of any two bases can be obtained by combining at least two independent sets of measurements. The reversibility of the protonation/deprotonation process of all bases was checked. All equilibria were reached in minutes and were stable.

The method used in this study has the disadvantage that only the relative basicity can be determined. To assign the absolute pK_a values for the conjugate acids of the bases, the scale has to be anchored to a reference compound or compounds for which the pK_a value(s) are known. The absolute pK_a values of the bases were calculated in a way similar to the previous studies [17,19]. The scale was anchored to the pK_a value (12.33) of pyridine [21]. The precision *s* of the measurements was calculated as in [19]; $n_m = 26$, $n_c = 14-1 = 13$. For our results, s = 0.09 p K_a units.

3.2. Formation of the sitting-atop complex in acetonitrile

Fig. 1 shows the spectra of H₂OEP and its copper(II) complexes in acetonitrile. Upon the addition of copper(II) ion to the porphyrin solution, the spectrum rapidly changes to spectrum **B** in Fig. 1 with broader and weaker Soret band. The characteristic broader Soret band of the product indicates that the porphyrin ring should be distorted from the planar structure. The addition of nitrogen bases such as pyridine to the obtained solution causes a spectral change to that of the copper(II)-OEP complex, [Cu(OEP)]. These findings indicate that the intermediate species still binds the pyrrole protons and that the added pyridine acts as an acceptor of the pyrrole protons to accelerate the formation of [Cu(OEP)]. Without any bases, acetonitrile cannot easily accept these pyrrole protons due to its very weak basicity. A similar phenomenon was previously observed for the reaction of H₂TPP with copper(II) triflate in acetonitrile [11]. The reaction intermediate was ascribed to the so-called sitting-atop complex. Although the basicity of H₂OEP is much higher than that of H₂TPP, it is reasonably understood that the



Fig. 1. UV–Vis absorption spectra of H_2OEP and copper(II) complexes in acetonitrile. H_2OEP (A), the product after the reaction of H_2OEP with copper(II) ion (B), [Cu(OEP)] (C). Wavelengths of the absorption maximum at the Soret band are 393, 348, and 394 nm for A, B, and C, respectively.

reaction intermediate was observed for both porphyrins in acetonitrile.

The mole ratio method was employed in order to clarify the composition of the sitting-atop complex. The spectra of solutions containing a constant amount of H₂OEP and different concentrations of copper(II) ion were measured using the rapid scanning spectrophotometer. As shown in Fig. 2, the plot of the absorbance of the solution at 340 and 393 nm as a function of the ratio of the total concentrations of copper(II) ion and H₂OEP clearly demonstrates that the introduction of the first equivalent amount of copper(II) ion to the H₂OEP solution does not cause any spectral change and that the produced sitting-atop complex contains two copper(II) ions based on the inflection point at $C_{Cu}/C_{OEP} = 2$. These findings indicates the two-step reaction for the sitting-atop complex formation reaction:



Fig. 2. Dependence of the absorbance on the ratio of the total concentrations of copper(II) ion and H₂OEP for the reaction of H₂OEP and copper(II) triflate at T = 25.0 °C. Total concentration of the porphyrin is 2.94×10^{-6} M.

$$H_2OEP + Cu^{2+} \rightleftharpoons [H_2OEP \cdots Cu^{2+}]$$
(16)

$$[\mathrm{H}_{2}\mathrm{OEP}\cdots\mathrm{Cu}^{2+}] + \mathrm{Cu}^{2+} \rightleftharpoons [\mathrm{H}_{2}\mathrm{OEP}\cdots(\mathrm{Cu}^{2+})_{2}] \quad (17)$$

The mole ratio dependence of the absorbance A shown in Fig. 2 was analyzed by the least squares fitting program employing the following equation:

$$A = \frac{\varepsilon_1 + \varepsilon_2 K_1 [\text{Cu}^{2+}] + \varepsilon_3 K_1 K_2 [\text{Cu}^{2+}]^2}{1 + K_1 [\text{Cu}^{2+}] + K_1 K_2 [\text{Cu}^{2+}]^2} C_{\text{OEP}}$$
(18)

where ε_1 , ε_2 , and ε_3 represent the molar absorption coefficients of H_2OEP , $[H_2OEP \cdots Cu^{2+}]$, and $[H_2OEP\cdots(Cu^{2+})_2]$, respectively, and K_1 and K_2 are the equilibrium constants of reaction 16 and 17, respectively. The determined value of $\varepsilon_2 C_{\text{OEP}}$ is 0.071 ± 0.010 (340 nm) and 0.486 ± 0.021 (393 nm), which can be considered to be equal to that of $\varepsilon_1 C_{\text{OEP}}$ (0.074 + 0.006 at 340 nm and 0.481 + 0.006 at 393 nm)within experimental error. These findings indicate that the introduction of the first equivalent amount of the copper(II) ion to the H₂OEP solution is spectrophotometrically silent. The equilibrium constants are calculated to be $\log(K_1 \ (M^{-1})) = 9.2 \pm 0.4$ and $\log(K_2$ $(M^{-1}) = 7.8 \pm 0.4$ (T = 25.0 °C) for the copper(II) triflate system based on the assumption that ε_2 is equal to ε_1 . The solid lines in Fig. 2 are calculated curves based on the obtained equilibrium constants and explain the results very well. For copper(II) perchlorate, similar results were obtained, and the equilibrium constants were determined to be $log(K_1 (M^{-1})) = 9.4 \pm 0.3$ and $\log(K_2 (M^{-1})) = 8.2 \pm 0.3 (T = 25.0 \text{ °C})$. These findings indicate that the counter anion of copper(II) has little effect on the sitting-atop complex formation reaction of H₂OEP.

3.3. Kinetics of the sitting-atop complex formation reaction

The reaction of H_2OEP with the copper(II) ion is too fast to be followed by a stopped-flow apparatus under the pseudo-first-order conditions where the copper(II) ion exists in large excess over the porphyrin in acetonitrile. The reaction rate was, therefore, measured under second-order conditions. The reaction was spectrophotometrically followed by monitoring the absorbance change associated with the formation of the sittingatop complex under the conditions where the reaction quantitatively proceeds. Taking into account the results of the mole ratio method, several reaction mechanisms should be considered to analyze the reaction curve. The first candidate is the series of second-order reactions in which the first step (reaction 16) is the rate-determining step. In this case, the rate equation can be expressed by:

$$(b-2a)^{-1}\ln\{a(b-2x)b^{-1}(a-x)^{-1}\} = kt$$
(19)

where a and b represent the initial concentrations of H₂OEP and copper(II) ion, respectively, x is the



Fig. 3. Kinetic plots for the reaction of H₂OEP and copper(II) triflate at T = 25.0 °C. Each plot is based on Eq. (19) (A), Eq. (20) (B), and Eq. (21) (C). $C_{Cu} = 6.83 \times 10^{-6}$ M, $C_{OEP} = 2.94 \times 10^{-6}$ M.

concentration of the produced sitting-atop complex, and k is the second-order rate constant of reaction 16. The second possible mechanism is the series of second-order reactions in which the second step (reaction 17) is the rate-determining step, i.e. the mechanism including the fast pre-association equilibrium between H₂OEP and copper(II) ion. In this case the rate equation can be expressed by:

$$(b-a)^{-1} \ln\{a(b-x)b^{-1}(a-x)^{-1}\} = kt$$
(20)

where k is the second-order rate constant of reaction 17. Because the intermediate species $[H_2OEP\cdots Cu^{2+}]$ is produced immediately after mixing the solution of H_2OEP and copper(II) ion, the value of b is set at $C_{Cu}-C_{OEP}$. Another reaction mechanism is the thirdorder reaction with two reactants, i.e. $-d[H_2OEP]/dt = k[Cu^{2+}]^2[H_2OEP]$. In this case the rate equation can be expressed as:

Table 2

Second-order rate constant of the sitting-atop complex formation of H₂OEP in acetonitrile at T = 25.0 °C

Copper(II) compound	$C_{\text{OEP}} (10^{-6} \text{ M})$	$C_{\rm Cu} (10^{-6} {\rm M})$	$k (10^6 \text{ M}^{-1} \text{ s}^{-1})^{\text{a}}$
Copper(II) triflate	2.94	6.83	3.6
	2.94	8.14	3.5
	2.34	8.14	3.1
	2.94	9.54	3.5
	2.34	9.58	3.1
	2.94	10.8	3.0
	2.34	11.2	3.0
	2.39	12.9	3.0
copper(II) perchlorate	2.81	7.32	3.1
-	2.81	8.68	3.1
	2.81	10.1	3.0
	2.81	11.6	2.9

$$2(2a-b)^{-1}\{(b-2x)^{-1}-b^{-1}\} + 2(2a-b)^{-2}\ln\{a(b-2x)b^{-1}(a-x)^{-1}\} = kt$$
(21)

In Fig. 3 are shown plots of the left-hand sides of Eqs. (19), (20) and (21) as a function of time t. If the equation was correct, the plot in Fig. 3 should be a straight line. It is clear that the reaction mechanisms based on Eqs. (19) and (21) are not the case for the present reaction. On the other hand, a fairly linear plot was obtained for the reaction mechanism using Eq. (20). The second-order rate constant thus obtained under various conditions is listed in Table 2. The average value is $k = (3.2 \pm 0.3) \times 10^{-10}$ $10^{6} \text{ M}^{-1} \text{ s}^{-1} (T = 25.0 \text{ °C})$ for copper(II) triflate and $k = (3.0 \pm 0.2) \times 10^{6} \text{ M}^{-1} \text{ s}^{-1} (T = 25.0 \text{ °C}) \text{ for cop-}$ per(II) perchlorate. The activation enthalpy and activation entropy were determined from the temperature dependence of the second-order rate constant in the temperature range between 15.0 and 35.0 °C for the reaction of copper(II) triflate: $\Delta H^{\ddagger} = 58 \pm 2 \text{ kJ mol}^{-1}$, $\Delta S^{\ddagger} = 74 \pm 6$ J K⁻¹ mol⁻¹. Almost the same rate constant was obtained for both copper(II) compounds indicating that the counter anions (triflate and perchlorate ions) of the copper(II) salts do not affect the rate of the sitting-atop complex formation reaction. Since both anions have a weak coordination ability toward metal ions, they at most exist as a solvent-separated ion pair with the copper(II) ion and do not directly coordinate to the copper(II) ion even in acetonitrile. These findings are consistent with the results for the solution chemistry of the copper(II) ion in acetonitrile, where the copper(II) ion exists as a hexasolvated ion $[Cu(AN)_6]^{2+}$ [14,22].

4. Discussion

The kinetics of the metalloporphyrin formation reaction has been extensively studied for many kinds of porphyrins and metal ions in various solvents in order to clarify the reaction mechanism [23–26]. Usually the reaction of the free-base porphyrin (H₂por) with a metal ion occurs via a single step reaction to form the metalloporphyrin as expressed in Eq. (22), and two pyrrole protons in H₂por will be released after the complexation.

$$\mathbf{M}^{n+} + \mathbf{H}_2 \mathbf{por} \rightleftharpoons [\mathbf{M}(\mathbf{por})]^{(n-2)+} + 2\mathbf{H}^+$$
(22)

Metalloporphyrin formation reactions are generally interpreted to proceed via a dissociative-interchange mechanism. The metal ion and porphyrin first form an outer sphere complex and the porphyrin core deforms to present the reactive pyrrolenine nitrogen atoms having lone-paired electrons to the incoming metal ion. The metal ion then dissociates a solvent molecule to form a bond between the nitrogen atom of the porphyrin and metal ion. A sitting-atop (or activated) complex is thus formed. In the solvents with a higher Brønsted basicity, the following deprotonation should occur from the activated complex. The trend in the rate constant variation for a series of divalent metal ions including the 3d-block metal ions for the solvent exchange of DMF and for the metallation of H_2TPP and its Nmethylated derivative in DMF is very similar, although the metallation rate is slower than the solvent exchange rate by several orders of magnitude [27–29]. Therefore, the rate-determining step for the metallation of the porphyrin is thought to be the dissociation of coordinated solvent molecules from the metal ion in the outersphere association complex. The overall rate constant for the metalloporphyrin formation, $k_{\rm f}$, is given by the product of the equilibrium constant for the outer-sphere complex formation (K_{OS}) , the equilibrium constant for the porphyrin ring deformation $(K_{\rm D})$, and the rate constant for the dissociation of the coordinating solvent molecules in the inner-sphere of the metal ions (k): $k_{\rm f} =$ $K_{OS}K_{D}k$. The slow metalation rate of the porphyrin can be explained by the small value of $K_{\rm D}$.

The transient species, which includes both the incoming metal ion and two pyrrole protons, can exist as an intermediate of the complex formation reaction of the porphyrin, and this intermediate is assumed to be the sitting-atop complex first proposed by Fleischer and Wang [7]. However, in solvents such as water and DMF where the pyrrole protons of the intermediate may be easily released, the sitting-atop intermediate can hardly be observed and the metalloporphyrin is simply produced as the product during the metallation reaction. On the other hand, the existence of the sitting-atop complexes was evidenced for the protoporphyrin IX derivatives, H₂TPP, and other porphyrins including H₂OEP in the solvents such as chloroform and acetonitrile [7-11, 14, 15]. It is obvious that the low basicity of these solvents is ascribed to the stability of the sittingatop intermediate. Although the sitting-atop complex was claimed to have a composition of M^{n+} : porphyrin = 1:1 for many cases, the results of the present study clearly suggest that two copper(II) ions are included in the sitting-atop complex of H₂OEP as evidenced by the mole ratio method. The proposed structure of this sitting-atop complex is shown in Chart 1.

Concerning the stoichiometry and mechanism of the metalloporphyrin formation reaction, Hambright has proposed that two metal ions are always required for the metal ion incorporation into porphyrins, one to distort



Chart 1. Proposed structure of the sitting-atop complex.

the planar porphyrin core, and the second to incorporate from the opposite side of the porphyrin [30]. This proposal was supported by many kinetic studies. Tanaka et al. investigated the kinetics of the reaction of H₂TPP with copper(II), zinc(II), and cadmium(II) ions in DMF, and evidence for the involvement of the two metal ions in the reaction was obtained from the kinetics of the reaction [27]. The metalloporphyrin formation reaction is first-order with respect to the porphyrin, and the pseudo-first-order rate constant was obtained as:

$$k_{\text{obsd}} = (k_1 K[M^{2+}] + k_2 K[M^{2+}]^2) (1 + K[M^{2+}])^{-1}$$
(23)

On the basis of the rate law, the following reaction mechanism was proposed.

$$H_2 TPP + Cu^{2+} \stackrel{\kappa}{\rightleftharpoons} H_2 TPP \cdots Cu^{2+}]$$
(24)

$$[H_2 TPP \cdots Cu^{2+}] \xrightarrow{\kappa_1} [Cu(TPP)] + 2H^+$$
(25)

$$[H_2TPP\cdots Cu^{2+}] + Cu^{2+} \xrightarrow{\kappa_2} [Cu(TPP)] + Cu^{2+} + 2H^+$$
(26)

The k_2 path involving two metal ions is consistent with Hambright's proposal. Similar kinetic features were observed for the other metalloporphyrin formation reaction [31,32]. On the other hand, it was demonstrated that the rate of the metalloporphyrin formation reaction of the one-face-hindered fenced porphyrin with copper(II), zinc(II), and cadmium(II) ions is about the same as for the sterically unhindered porphyrin in DMF or pyridine [33], and these findings are considered to be the evidence against the Hambright mechanism. Nevertheless, the Hambright mechanism should apply to the complex formation reaction of many sterically unhindered porphyrins.

The two-step reactions of the sitting-atop complex formation of H₂OEP observed in the present study corresponded to the mechanism in which two metal ions interact with the porphyrin molecule from the opposite side of the porphyrin core as proposed by Hambright. This type of sitting-atop complex should be regarded as the intermediate of the metalloporphyrin formation reaction in the solvents such as DMF and water. What is significant is the extremely large K value for the formation of the outer sphere association complex of H₂OEP with copper(II) ion in acetonitrile. The corresponding value of the pre-association constant K was reported to be 1.6×10^4 M⁻¹ at 25.0 °C for the reaction of H₂TPP with copper(II) ion in DMF [27]. Formation of the outer sphere complex is usually postulated as the primary step for the ligand substitution reaction of the metal complexes. The formation constant of the outer sphere complex can be theoretically estimated to be 0.3 for the reaction of the noncharged ligand at the distance of closest approach of two species of 5 Å [34,35]. Although the pre-association

constant for the reaction of H_2 TPP with Cu²⁺ in DMF is much greater than the predicted value for the outersphere complex, the first step of the metalation reaction (Eq. (24)) can be ascribed to the outer sphere complex formation due to the following spectral features. Under the conditions where the outer-sphere pre-associated complex $[H_2TPP \cdots Cu^{2+}]$ is quantitatively formed, the spectral change for the metallation reaction was reported to be from the spectrum of H₂TPP to that of [Cu(TPP)], and no spectral evidence was obtained for the formation of the outer-sphere complex [27]. This fact indicates that the kinetically detected intermediate $[H_2TPP \cdot \cdot Cu^{2+}]$ has the same absorption spectrum as that of H₂TPP. A similar spectral feature was observed for the sitting-atop complex formation reaction of H₂OEP in the present study. The pre-association constant of H₂OEP with copper(II) ion in acetonitrile is much greater than that of the H₂TPP case in DMF. Taking into account the low solubility of H₂OEP in acetonitrile being on the order of 10^{-5} M, the large preassociation constant can be rationalized by the stabilization of the H₂OEP molecule by forming the outer sphere complex with copper(II) ion in the solvent.

In the present study, we determined the second-order rate constant for the reaction of H2OEP with the copper(II) ion. The rate of the sitting-atop complex formation of H₂OEP is faster than that of H₂TPP (k = $3.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) [11]. The activation enthalpy, ΔH^{\ddagger} , for the H₂OEP and H₂TPP is almost the same, i.e. 58 ± 2 kJ mol⁻¹ for the former and 56 ± 5 kJ mol⁻¹ for the latter and the activation entropy for the H₂OEP (74 \pm 6 J $K^{-1} \text{ mol}^{-1}$) is greater than that for $H_2 \overline{TPP}$ ($46 \pm 19 \text{ J}$ $K^{-1} \text{ mol}^{-1}$) [11]. The activation enthalpy for these reactions is much greater than the usual ligand substitution and solvent exchange reactions of the copper(II) ion. For example, ΔH^{\ddagger} for the reaction of the copper(II) ion with ammonia in aqueous solution is 18.8 kJ mol^{-1} [36]. The ΔH^{\ddagger} for the solvent exchange is 11.5 kJ mol⁻¹ for water [37], 24.3 kJ mol⁻¹ for DMF [38], and 17.2 kJ mol^{-1} for methanol [39,40]. The larger activation enthalpy for the reaction of the present sitting-atop complex formation can be attributed to the energetic requirement due to the distortion of the porphyrin core prior to the rate-determining step of the binding between the metal ion and porphyrin.

The difference in the reactivity of the porphyrins towards a metal ion has been often discussed in terms of the acid-base properties of the porphyrin as well as the structural properties of the molecule, especially concerning the planarity of the porphyrin core. Before discussing the relationship between the reactivity of the porphyrin with the copper(II) ion and the porphyrin basicity, we will first consider the acid-base properties of H₂OEP and some *meso*-substituted porphyrins. The pK_a value of the conjugate acid of the porphyrins was spectrophotometrically determined in acetonitrile. In

Table 1 are shown the pK_a values of the mono- and diprotonated porphyrins. It seems reasonable to assume that the higher the degree of delocalization of the charge introduced by the first proton from the center of the porphine moiety, the closer the second pK_a will be to the first. This explains why H₂OEP has the greatest $\Delta p K_a$ of the porphyrins, because the delocalizing power of the phenyl rings of TPP is greater than that of the ethyl groups of OEP. Although the phenyl groups in the free base of TPP are almost perpendicular to the porphyrin core, this does not hold for the mono- and diprotonated forms, for which the porphyrin core is also quite distorted. Such a situation can be seen in the molecular structure of the porphyrin diacid species [41-48]. The crystal structure of the porphyrin diacid species, [H₄OEP](ClO₄)₂, [H₄TPP](ClO₄)₂, [H₄TMP](ClO₄)₂, indicates that the distortion of the porphyrin core from planarity should result from the severe transannular crowding between the four (or three for the monoacid form) hydrogen atoms of the pyrrole nitrogen atoms [48]. The crystal structures of the D_{2d} -symmetry distortion of the porphyrin core depend on the nature of the peripheral substituents. The phenyl and 4-Cl-phenyl substituents can rotate to some extent under the D_{2d} symmetry distortion, so that they will be more coplanar with the porphyrin core. The mean porphyrin-phenyl group dihedral angles in $[H_4TPP](ClO_4)_2$ are $27(2)^\circ$. This enables the resonance interaction that is very efficient in charge delocalization. As a consequence, the $\Delta p K_a$ of TPP and T(4-ClP)P is far less than the OEP. TMP is in a different situation from TPP, because it has two o-methyl substituents on each aryl substituent. The eight o-methyl groups of [H₄TMP](ClO₄)₂ are positioned above and below the plane of the porphyrin ring, approximately over the meso-carbons. Such a steric hindrance significantly hinders the rotation of the aryl groups about the C_m-C_p bond. The porphyrin-aryl



Fig. 4. Relationship between the second-order rate constant k of the reaction of the sitting-atop complex formation with copper(II) triflate and the basicity of the porphyrin for H₂OEP (**A**), H₂TPP (**B**), H₂T(4-ClP)P (**C**), and H₂TMP (**D**). The p K_a value represents that of the conjugate acid of the corresponding free base porphyrin in acetonitrile. The values of the rate constants for the porphyrins other than H₂OEP are from [15].

group dihedral angles of [H₄TMP](ClO₄)₂ are less acute than those of [H₄TPP](ClO₄)₂, averaging 63(13) and $75(15)^{\circ}$ in the two forms of molecules in the crystal. Consequently, $[H_4TMP](ClO_4)_2$ show substantially smaller core distortions than $[H_4TPP](ClO_4)_2$, though saddled both are considerably more than $[H_4OEP](ClO_4)_2$. These steric effects cause a smaller charge delocalization which is given by the resonance effect than in the case of TPP. However, even without the resonance effect, the mesityl groups are more efficient in charge delocalization than the ethyl groups. As a result, the $\Delta p K_a$ of TMP is intermediate between OEP on one hand and TPP on the other hand.

The dependence of the second-order rate constant of the sitting-atop complex formation reaction on the basicity of the porphyrins is shown in Fig. 4. The rate constant decreases in the order of $H_2OEP \gg H_2TPP >$ $H_2T(4-ClP)P \gg H_2TMP$. The difference in the reactivity can be interpreted by the electronic and steric factors. It has been demonstrated how the reactivity of the porphyrins with metal ions is influenced by the porphyrin basicity. A linear relationship between the $\log k$ (k is the second-order rate constant of the metalloporphyrin formation reaction) and the pK_a of the conjugate acid of the monoprotonated porphyrin has been demonstrated, for example, for the reaction of the watersoluble porphyrins with the copper(II) and zinc(II) ions [49,50]. Also, the correlation between the porphyrin reactivity and the reduction potential of the porphyrins has been explored [26,51]. The faster metalloporphyrin formation reaction was observed for the porphyrin with a stronger basicity and a more negative reduction potential for the metal ion incorporation reaction into the water-insoluble porphyrins. The reduction potentials parallel the basicity scale of the porphyrins, and thus the porphyrin bearing the stronger basicity reacts with the zinc(II) ion faster than the less basic porphyrins [51]. As shown in Fig. 4, a similar correlation was observed in the present study except for H_2TMP , which exhibits the additional steric effect on the reaction. The interaction between the metal ion and the free base porphyrin should play an important role in the present sitting-atop complex formation reaction. The electrostatic attraction between the local negative charge on the pyrrolenine nitrogen atoms and the positive charge of the metal ion should drive the outer-sphere association between these two species. The electron density of the nitrogen atom also affects the interaction between the metal ion and the pyrrolenine nitrogen atom of the porphyrin during the rate-determining exchange of the bound solvent molecule by the incoming porphyrin ligand around the metal ion. For H₂TMP, as has already been discussed in a previous paper, the slow rate can be interpreted by the steric hindrance due to the α -methyl groups of the *meso*aryl substituents of H₂TMP [15]. The meso-aryl rings of H₂TMP become more perpendicular to the porphyrin

skeleton due to the steric interaction between the methyl groups of the aryl rings and the hydrogen atoms on the pyrrole rings. Due to this steric hindrance, the electrostatic approach between the copper(II) ion and H_2TMP is also inhibited and the more distant rupture in the bonding between the copper(II) ion and the dissociating acetonitrile molecule is also required since it is hard for the pyrrolenine nitrogen to approach closer to the copper(II) ion in the transition state. The correlation between the porphyrin reactivity and the acid-base properties of the porphyrins shown in Fig. 4 can thus be interpreted by the electronic and steric factors of the porphyrins.

5. Supplementary material

Figure showing the temperature dependence of the second-order rate constant for the sitting-atop complex formation reaction of H_2OEP with copper(II) triflate in acetonitrile (Fig. S1). The supplementary material is available from the authors on request.

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