β -Cyclodextrin as a Metal-anionic Porphyrin Complexation Accelerator in Aqueous Media

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The rate of the complexation reaction between anionic porphyrins and 11 metal ions was found to be accelerated by the presence of β -cyclodextrin (β -CD) in aqueous media at room temperature without the need for additional heating or sonication. The porphyrin complexation reaction with metal ions under aqueous conditions can be difficult due to the strong hydration energy between the metal ions and water. In this study, the specific role of β -CD as an accelerator was determined and found to enhance the typically slow reaction of the porphyrin with metal ions. A significant acceleration effect was exhibited when the model anionic porphyrin, 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-tetrasulfonic acid, and Pb(II) ions were combined in the presence of β -CD. Other than for Hg ion, the addition of β -CD decreased the metalation reaction time from 30 to 2 min. The order in the degree of acceleration was Pb >> Zn, Cd > Cu > Fe, Pd > Sn >> Ag, Co, Mn. Using Pb(II) as the model ion, it was determined that the complexation rate constant was enhanced by a factor of 2.4, while the dissociation rate constant was diminished by a factor of 135 in the presence of added β -CD relative to that in its absence. Overall, the complex was much more stable (formation equilibrium constant 324-fold greater in the β -CD medium. The formation of a ternary complex (cf. bicapped complex; (β -CD)₂-porphyrin-metal ion) was demonstrated through the use of nuclear magnetic-resonance spectroscopy and mass spectrometry. This acceleration effect is expected to be applicable systems in which porphyrin ligands are employed for determining of metal ions in chemical analysis and separation science.

Keywords β -Cyclodextrin, metal-anionic porphyrin complexation accelerator, TPPS₄

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Introduction

Porphyrin derivatives are known to serve as very sensitive photometric or fluorophotometric reagents for the determination of metal ions.¹⁻⁹ In addition, metal-porphyrin complexes have various functions that are useful in analytical chemistry, and are also of interest to many other researchers, which has led to the use of metal-porphyrin complexes in applications such as optics,¹⁰ chemical stacking,¹¹ catalysis,¹² electron transport,¹³ oxidation-reduction reactions,¹⁴ and biological mimicking substances.^{15,16} Thus, numerous reports regarding the chemical function and applications of porphyrin derivatives have been published.

Among the many useful porphyrin reagents, the anionic porphyrin 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-tetrasulfonic acid (TPPS₄) has been widely utilized and has potential applications in many fields due to its unique properties, such as a very large molar absorptivity value ($\epsilon_{413} = 4.66,^{17} 5.10,^{18} 5.30,^{19}$ or $5.24^{20} \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$). However, complexation

reactions between porphyrins, including TPPS₄, and metals often require heating,²¹ sonication,²² the addition of special additives (catalysts),²³ and/or a synthetic approaches (metal exchange reactions)²⁴ to facilitate complexation, since the rate of metalloporphyrin formation is several orders of magnitude slower than that of complexation involving many other analytical ligands. Some metal ions can be inserted into the center of the TPPS₄ porphine ring through heating, while heavy metal ions, including Cd, Pb, and Hg, are known to form complexes with TPPS₄ in a sitting-atop (SAT) structure²⁵⁻²⁹ due to their ionic radii. The sitting-atop structure of the Pb-TPPS₄ complex is particularly useful in detecting Pb ions.³⁰⁻³⁴ Although many researchers are aware of the importance of Pb analysis and its applications, the requirement of a heating step has precluded the use of this method for heat-labile samples, especially biological samples and nanomaterials.^{21,23,24,35-37}

Previously, Tabata^{23,26} reviewed five methods that can be employed to accelerate the rate of formation of metal-porphyrin complexes. These include: (1) the use of a substitution reaction for Cd(II)- or Hg(II)-porphyrins,^{20,23,38,39} (2) the use of porphyrins with substituents at the pyrrole nitrogen,^{40,41} (3) the addition of aromatic, heterocyclic bases,⁴²⁻⁴⁴ (4) the introduction of functional groups to bind metal ions in the vicinity of the

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porphyrin nucleus,⁴⁵ and (5) the use of an oxidation/reducing agent.⁴⁶⁻⁴⁸

In the present study, a new approach was developed in which β -cyclodextrin (β -CD) was employed to accelerate the complexation reaction between TPPS₄ and 11 metal ions [Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Fe(III), Mn(II), Pb(II), Pd(II), Sn(II), and Zn(II)] in aqueous media at room temperature. Spectrophotometric kinetic measurements were made to follow the complexation reaction, while the nature of the TPPS₄-Pb(II)- β -CD complex was determined using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) measurements.

Experimental

Reagents

TPPS₄, α -cyclodextrin (α -CD), β -CD, γ -cyclodextrin (γ -CD), heptakis(2,3,6-tri-*O*-methyl)- β -cyclodextrin (TMe- β -CD), and sodium tetraborate (borax) were obtained from Wako Pure Chemical Industries (Osaka, Japan). 5,10,15,20-Tetrakis(4-carboxyphenyl)-21*H*,23*H*-porphine (TCPP) [5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-tetrasulfonic acid was obtained from Tokyo Chemical Industry (Tokyo, Japan). Standard stock solutions of 12 metal ions [Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Fe(III), Hg(II), Mn(II), Pb(II), Pd(II), Sn(II), and Zn(II)] present at a concentration of 1000 ppm were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents used were analytical grade.

Measurements and experimental procedure

A JASCO V-560 spectrophotometer (JASCO Inc., Tokyo, Japan) equipped with a JASCO EHC-477 single position Peltier thermostated cuvette holder was used along with a quartz cell having an optical path length of 1 cm. A Hitachi F-7000 spectrofluorometer equipped with a thermostated cuvette holder (Hitachi High-Technology Co. Ltd., Tokyo, Japan) was employed. A JASCO SFS series rapid-scan photometer with a stopped-flow system was used. ¹H NMR spectra were acquired using a JEOL JNM-AL300 spectrometer (300 MHz). A Bruker Amazon X Ion Trap Mass Spectrometer SL was used for complex identification.

Typically, TPPS₄ (200 μ L) and disodium tetraborate (Borax) pH buffer (800 μ L, pH 10.5, 0.1 M) were mixed with β -CD in a spectrophotometer cuvette. The sample solution (500 μ L) including the metal ion was then added to the mixture in the cuvette with continuous stirring, the absorbance was immediately measured as a function of time.

Results and Discussion

Kinetic analysis of interaction of metal ions with TPPS₄

The 12 investigated metals [Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Fe(III), Hg(II), Mn(II), Pb(II), Pd(II), Sn(II), and Zn(II)] interact with TPPS₄ to form metal-TPPS₄ complexes in which the metal ion is located in the center of the porphine ring. The times required for the formation of the metal-TPPS₄ complexes in aqueous media (without any heating) in the absence and presences of added β -CD were determined. Acceleration was defined by the difference in rates between the complexation reaction in the presence of β -CD and its absence:

$$\Delta(dC/dt) = [(dC_{\text{without}}/dt)_{\text{without}} - (dC_{\text{with } \beta\text{-CD}}/dt)_{\text{with } \beta\text{-CD}}], \quad (1)$$

Fig. 1 Pb(II)-TPPS₄ complexation percent *versus* reaction time. [TPPS₄]_T, 1.7 μ M; [Pb²⁺]_T, 10 μ M; pH, 10.5; [β -CD]_T, (a) 0, (b) 10, (c) 50, and (d) 1000 μ M; temperature, 25°C; and detection wavelength λ_{max} = (a) 464 nm and (b – d) 466 nm.

where C , C_{without} , and $C_{\text{with } \beta\text{-CD}}$ are the metal-TPPS₄ complex concentrations, those in the absence of β -CD (without heat), and those in the presence of β -CD (without heat), respectively, and t represents reaction time (s).

With the exception of Hg(II), the presence of β -CD decreased the complexation reaction time for all metal ions examined. The magnitude of the acceleration effect depended upon the specific metal ion, and followed the order: Pb >> Zn, Cd > Cu > Fe, Pd > Sn >> Ag, Co, Mn. The trend in the acceleration effect roughly parallels the size (radius of the hydrated metal cation); *i.e.*, the larger is the size, the greater is the acceleration effect (In the case of Hg(II), the addition of β -CD inhibited the complexation reaction (time required increased) relative to that in its absence). The metal ion, Pb(II), was used as the model ion to more fully examine this system and to demonstrate the complexation acceleration effect of β -CD addition, as Pb ion complexation is a subject of general interest to several fields of chemistry.

The reaction of TPPS₄ and Pb(II) ions without β -CD required 30 min at room temperature. This complexation time was reduced to 2 min in the presence of β -CD, as shown in Fig. 1, with the degree of acceleration depending on the concentration of β -CD (The profile of Zn(II) with TPPS₄ is shown in Fig. S1 in Supporting Information). The greater the β -CD concentration, the faster is the metalation reaction. Consequently, β -CD was found to remarkably accelerate the formation of Pb-TPPS₄ complex. Based on these results, the pseudo-first-order reaction rates (k_0) were obtained. In addition, it was preliminarily confirmed that Pb²⁺ did not react with β -CD. To obtain the complex formation (k_+), dissociation (k_-), and equilibrium (K_m) constants, the kinetic data were treated using the following equations:

In this study, measuring the second order reaction rate with reactants (TPPS₄-(β -CD) and Pb²⁺) could be experimentally problematic, because the concentrations of two reactants must be followed simultaneously. However, the forward reaction (complexation) can be regarded as pseudo-first order, when the concentration of TPPS₄-(β -CD)₂ is much higher than that of Pb²⁺

Fig. 2 Correlation equations between the pseudo-first-order rate constant (k_0) and the concentration in (a) the absence and (b) presence of β -CD. $[\text{TPPS}_4]_T$, 1.7 to 4.2 μM ; $[\text{Pb}^{2+}]_T$, 7.5×10^{-7} to 7.5×10^{-8} M; $[\beta\text{-CD}]_T$, 1.0 mM; temperature, 25°C; detection wavelength, λ_{max} , = (a) 464 and (b) 466 nm.

and the kinetic equation is followed as below;

$$\frac{d[\text{Pb-TPPS}_4\text{-(CD)}_2]}{dt} = k_+([\text{Pb}^{2+}]_0 - [\text{Pb-TPPS}_4\text{-(CD)}_2])[\text{TPPS}_4\text{-(CD)}_2]_0 - k_-[\text{Pb-TPPS}_4\text{-(CD)}_2] \quad (3)$$

The equation was given by integration, as below;

$$\ln\left(\frac{1 - (k_+[\text{TPPS}_4]_0 + k_-)[\text{Pb-TPPS}_4\text{-(CD)}_2]}{k_+[\text{Pb}]_0}\right) = -(k_+[\text{TPPS}_4\text{-(CD)}_2]_0 + k_-)t \quad (4)$$

where $[\text{TPPS}_4\text{-(}\beta\text{-CD)}_2]_0$ and $[\text{Pb}^{2+}]_0$ were the initial concentrations of $\text{TPPS}_4\text{-(}\beta\text{-CD)}_2$ and Pb^{2+} , respectively. The $[\text{Pb-TPPS}_4\text{-(}\beta\text{-CD)}_2]$ is the concentration of $\text{Pb-TPPS}_4\text{-(}\beta\text{-CD)}_2$ on the reaction time, t . The equation can be followed by Lambert-Beer's law, as follows;

$$\ln \frac{A_{\text{eq}} - A_t}{A_{\text{eq}} - A_0} = -(k_+[\text{TPPS}_4\text{-(CD)}_2]_0 + k_-)t = -k_0t \quad (5)$$

where

$$k_0 = k_+[\text{TPPS}_4\text{-(}\beta\text{-CD)}_2]_0 + k_- \quad (6)$$

$$K_m = \frac{k_+}{k_-} \quad (7)$$

Here, A_0 , A_t and A_{eq} denote the absorbance of the reaction time at reaction times 0, t , and the equilibrium state. A pseudo-first order plot can be obtained from plotting $\ln[(A_{\text{eq}} - A_t)/(A_{\text{eq}} - A_0)]$ versus t . The observed rate constant (k_0) was provided from the linear line. In addition, the values of k_0 were obtained in various lead concentrations. Based on these results, the dependence of the observed rate constants, k_0 , upon the TPPS_4 concentrations in (a) the absence and (b) the presence of β -CD is shown in Fig. 2. The rate constants for the forward reaction (k_+) and the reverse reaction (k_-) were obtained as the value of the slope and intercept of the linear line. The individual rate constants obtained for the metal ions Pb(II) and Zn(II) are summarized in Table 1.

Table 1 Additive effect of β -CD on the complexation of Pb(II)- and Zn(II)- TPPS_4 : complex binding (k_+), dissociation (k_-), and equilibrium (K_m) constants

	Additive	$k_+/\text{M}^{-1} \text{s}^{-1}$	k_-/s^{-1}
Pb(II)	Without β -CD	9.64×10^2	2.76×10^{-2}
	With β -CD	2.27×10^3	2.03×10^{-4}
Zn(II)	Without β -CD	0.68×10^2	2.75×10^{-5}
	With β -CD	1.25×10^2	1.21×10^{-5}

Values obtained from correlation equations described in text.

The addition of β -CD enhanced the forward rate constant, k_+ , for the complexation reaction between TPPS_4 and Pb(II) by a factor of 2.4, while the reverse dissociation observed rate constant, k_- , was decreased by a factor of 135 compared to that in the absence of β -CD. Thus, the β -CD served to both suppress the dissociation reaction and to enhance the complexation reaction. These same general trends (both suppress the dissociation and enhance the complexation) held in the case for the complexation of Zn(II) with TPPS_4 . However, the magnitudes of the complexation acceleration effect and the dissociation rate diminution were much more modest (Table 1). Overall, the complexation equilibrium constant was only 1.8-fold greater due to the addition of the β -CD. In contrast, the kinetic rate constants were reported with literature values as $2.16 \times 10^2 \text{ M}^{-1} \text{s}^{-1}$ and $6.39 \times 10^{-11} \text{ M}^{-2} \text{s}^{-1}$ for k_+ and k_- in the elementary reaction of TPPS and Pb ion (in the absence of CD).⁴⁹ The equilibrium constants were also reported as being $10^{-0.43}$ and $10^{-9.97}$ for Zn and Pb, respectively.⁵⁰ In a comparison with this data, the value of k_+ is very similar; otherwise, the value of k_- is quite different. This is included not only in the elementary reaction, but also concerning other reactions; therefore, the value of k_- represents the observed reserve rate constant.

Spectroscopic behavior and impact of CD pore size

The absorption spectra of TPPS_4 and its Pb(II) complexes formed in the absence and presence of β -CD are shown in Fig. 3. The maximum absorption wavelength (λ_{max}) of TPPS_4 (free-base type) was 413 nm (Fig. 3a), which shifted to 464 nm (Fig. 3b) upon complexation with Pb(II) after 30 min. The λ_{max} of the complex was slightly shifted (to 466 nm) in the presence of β -CD (Fig. 3c) with the molar absorptivity being increased slightly compared to the Pb-TPPS_4 complex without β -CD ($\epsilon_{464} = 2.73 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$ for Pb-TPPS_4 versus $\epsilon_{466} = 3.17 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$ for $\text{Pb-TPPS}_4\text{-(}\beta\text{-CD)}_2$).

The formation of bicapped complexes between TPPS_4 and β -CD was previously reported in the literature.^{51,52} TPPS_4 contains four phenyl substituents, which are of appropriate size and shape to bind within the β -CD cavity, to form inclusion complexes. Among the possible β -CD- TPPS_4 stoichiometries, those with 2:1 CD: TPPS_4 stoichiometry in which two opposite phenyl moieties of the porphyrin ring are included into the CD cavity through its wider end have been reported to be the most stable.⁵³ The addition of other CDs with various pore sizes (*i.e.*, α -CD, γ -CD, and TMe- β -CD) did not appreciably change the complexation rates of the tested metal ions relative to that in their absence. In the absence of Pb(II), inclusion complexes between CD and TPPS_4 were observed in the spectrophotometric profiles. The values of ϵ were as follows: $\epsilon_{416} = 4.61 \times 10^5$ for $\text{TPPS}_4\text{-(TMe-}\beta\text{-CD)}_2$, $\epsilon_{413} = 4.66 \times 10^5$ for TPPS_4 , $\epsilon_{413} = 4.70 \times 10^5$ for $\text{TPPS}_4\text{-(}\alpha\text{-CD)}_2$, $\epsilon_{415} = 4.79 \times 10^5$ for $\text{TPPS}_4\text{-(}\gamma\text{-CD)}_2$, and $\epsilon_{416} = 4.85 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$ for $\text{TPPS}_4\text{-(}\beta\text{-CD)}_2$ (absorbance

Fig. 3 Absorption spectra of (a) TPPS₄, (b) Pb-TPPS₄ complex, and (c) Pb-TPPS₄ complex with β -CD. Spectrum a: [TPPS₄]_T, 1.7 μ M; pH, 10.5; and temperature, 25°C. Spectrum b: [TPPS₄]_T, 1.7 μ M; [Pb²⁺]_T, 10 μ M; pH, 10.5; temperature, 25°C; and reaction time, 30 min. Spectrum c: [TPPS₄]_T, 1.7 μ M; [Pb²⁺]_T, 10 μ M; [β -CD]_T, 10 mM; pH, 10.5; temperature, 25°C, and reaction time, 4 min. The maximum wavelengths (λ_{max}) of spectra a–c are 413, 464, and 466 nm, respectively.

spectra are shown in Fig. S2 in Supporting Information). Furthermore, fluorescence sensitization arose from the formation of TPPS₄-(CD)₂ bicapped complexes with the sensitization increasing in the following order: TPPS₄ < TPPS₄-(α -CD)₂ < TPPS₄-(γ -CD)₂ < TPPS₄-(β -CD)₂ < TPPS₄-(TMe- β -CD)₂ (emission spectra shown in Fig. S3 in Supporting Information). The TPPS₄-(TMe- β -CD)₂ complex exhibited the highest binding constant ($2.92 \times 10^{13} \text{ mol}^{-2} \text{ dm}^3$)^{54,55} among the TPPS₄-(CD)₂ complexes.^{51,52} Thus, it is considered that the use of larger CD and/or strong binding to the porphyrin rings (*e.g.*, TPPS₄-(γ -CD)₂) inhibits the insertion of metal ions into the porphyrin ring due to steric hindrance. In contrast, the cavity size of α -CD was insufficient to bind or cap TPPS₄, and thus had little impact the kinetics of metal ion binding. Thus, β -CD appears to be optimal in terms of enhancing the rate for TPPS₄-metal ion complexation reactions.

¹H NMR studies of the reaction between the TPPS₄-(β -CD)₂ complex and Pb ion

The interaction between Pb(II) ions and TPPS₄-(β -CD)₂ was investigated by ¹H NMR spectroscopy. Typically, TPPS₄ has three ¹H NMR signals corresponding to H^o and H^m (singlet at δ 8.8 and doublets at δ 8.2 and δ 7.65 ppm), as shown in Fig. S4 in Supporting Information. During the formation of TPPS₄-(β -CD)₂, the H^o doublet at δ 7.65 ppm disappeared by CD capping to TPPS₄. Under the molar ratio conditions of TPPS₄: β -CD = 1:2, while the H^m doublet remained at any β -CD concentration,^{56,57} the Hⁱ, H ^{β -py}, and H ^{β -py} singlets (in TPPS₄) appeared along with an increase of β -CD. In particular, it was found that the pyrrole of the porphyrin ring and the phenyl group of porphine were influenced by CD inclusion (capping); therefore, the pK_a values of the porphyrin ring would most likely change. In contrast, the formation of the Pb-TPPS₄ complex (in the absence of β -CD) demonstrated a lack of the three ¹H NMR signals corresponding to H^o and H^m in TPPS₄ at a molar ratio of Pb:TPPS₄ = 1:1 (Fig. S5 in Supporting Information). In addition, the singlets disappeared in the presence of excess Pb²⁺ concentration. This is considered to mean that the TPPS₄ decreases in the ionic activity with an excess concentration of Pb²⁺, and thus the

Fig. 4 Transition of ¹H NMR spectra during TPPS₄-(β -CD)₂ complexation with Pb in D₂O. [TPPS₄]_T, 1.25 mM; [β -CD]_T, 2.50 mM; borax buffer at pH 10.5, and 25°C. “i” represents the protons inside the CD cavity. Chemical shift corresponding to (A) TPPS₄ and (B) β -CD.

¹H NMR signals are influenced.

On the other hand, the interaction between β -CD and Pb(II) ion was not observed in the ¹H NMR spectra of β -CD (Fig. S6 in Supporting Information). Upon the formation of TPPS₄-(β -CD)₂, as shown in Fig. S7 in Supporting Information, the original ¹H NMR spectra of β -CD changed to the five signals in β -CD corresponding to H-1, H-3, H-6, H-5 and H-4. Based on these NMR profiles, the reaction between TPPS₄-(β -CD)₂ and Pb(II) ions was eventually tracked, as shown in Fig. 4. In the TPPS₄ region (10–7 ppm) of the ¹H NMR spectrum of TPPS₄-(β -CD)₂, a doublet (δ 8.2 ppm) for H^m in TPPS₄ remained, while the other signals disappeared with increasing Pb(II) concentration (Fig. 4(A)). Conversely, in the β -CD region (5.5–3 ppm) of TPPS₄-(β -CD)₂, one doublet for H-3 in β -CD was converted to a singlet (Fig. 4(B)). Thus, it was found that TPPS₄-(β -CD)₂ and metal ions form a bicapped ternary complex of metal-TPPS₄-(β -CD)₂. As a result of NMR tracking, obvious differences in the chemical shifts in the presence and absence of β -CD were confirmed. This is considered to involve the formation of a ternary complex (*cf.* bicapped complex; (β -CD)₂-porphyrin-metal ion). As a complementary fact, the 1:1 complexation between TPPS₄-(β -CD)₂ and Pb²⁺ was spectrophotometrically confirmed by the mole ratio method, as shown in Fig. S8, as Supporting Information. In addition, the Pb-TPPS₄-(β -CD)₂ ternary complex was supported by the mass spectrum, as shown in Fig. S9 in Supporting Information.

Impact of pH on the acceleration effect

The optimum pH for both reactions (with and without β -CD) was 10.5. However, the range of the pH in which the reaction

Fig. 5 Absorbance-pH curves in (a) the absence and (b) presence of β -CD. [TPPS₄]_T, 1.7 μ M; [Pb²⁺]_T, 10 μ M; [β -CD]_T, (a) 0 and (b) 1.0 mM; temperature, 25°C; and reaction time, 4 min.

occurred was expanded to 9.5 – 11 upon the addition of β -CD, as shown in Fig. 5. Thus, it is assumed that the pK_a values decreased slightly (position of N-H in the porphine ring), thus allowing for easier complexation of the metal ions with the porphyrin (in the presence of β -CD). Likewise, the pK_a variation, which is caused by a structural variation, was reported to slow that asymmetric porphyrins possess lower pK_a values than symmetric porphyrins.^{38,40} In addition, it was previously noted in the literature that deprotonation slightly opens (distorts) the porphyrin ring, which facilitates complexation with the incoming metal ion⁵⁸ and also that the addition of β -CD likely enhances this effect. This is supported by the results of the NMR profile corresponding to $H_{\beta-py}$ and $H^{\beta-py}$ signal transitions (in the pyrrole ring) in Fig. S4 in Supporting Information.

Utilization of another anionic porphyrin

A brief study was conducted using another anionic porphyrin, TCPP, which has four carboxyl groups. It was found that only the addition of β -CD accelerated the complexation reaction between TCPP and Pb(II) ions, as shown in Fig. 6. A similar trend with TPPS₄ was observed. The maximum absorption wavelength (λ_{max}) of TCPP was 413 nm (Fig. 6a), which shifted immediately to 416 nm (Fig. 6b) upon inclusion with β -CD. Otherwise, TCPP shifted to 464 nm (Fig. 6c) upon being complexed with Pb(II) after 30 min under 25°C. The λ_{max} of the complex was slightly shifted (to 465 nm) in the presence of β -CD (Fig. 6d).

The reaction of TCPP and Pb(II) ions in the absence of β -CD required 30 min at 25°C. This complexation time was reduced to 4 min in the presence of β -CD, as shown in the inside window of Fig. 6. Although the magnitude of the acceleration effect of TPPS₄ was slightly better than TCPP, a comparison of the acceleration effect between TCPP and TPPS₄ was not appropriate directly, due to different pK_a values of the porphyrin compound and the optimum condition of the reaction. Thus, it appears that the addition β -CD may be utilized to enhance the complexation rate between the anionic porphyrins and the metal cations, in general.

Acceleration mechanism

The results obtained under this experimental condition show that the complexation between water-miscible, anionic porphyrins, and metal ions is accelerated in the presence of β -CD, and proceeds due to the formation of a ternary complex

Fig. 6 Absorption spectra of (a) TCPP, (b) TCPP with β -CD, (c) Pb-TCPP complex, and (d) Pb-TCPP complex with β -CD. Spectrum a: [TCPP]_T, 2.0 μ M; pH, 10.5; and temperature, 25°C. Spectrum b: [TCPP]_T, 2.0 μ M; [β -CD]_T, 1 mM; pH, 10.5; temperature, 25°C; and reaction time, 4 min. Spectrum c: [TCPP]_T, 2.0 μ M; [Pb²⁺]_T, 10 μ M; pH, 10.5; temperature, 25°C; and reaction time, 30 min. Spectrum d: [TCPP]_T, 2.0 μ M; [Pb²⁺]_T, 10 μ M; [β -CD]_T, 1 mM; pH, 10.5; temperature, 25°C; and reaction time, 4 min. Inside window exhibits TCPP-Pb(II) complexation percent *versus* reaction time. [TCPP]_T, 2.0 μ M; [Pb²⁺]_T, 10 μ M; pH, 10.5; [β -CD]_T, (e) 0 and (f) 1 mM; temperature, 25°C; and detection wavelength, λ_{max} , (e) 464 nm and (f) 465 nm.

(cf. bicapped complex; (β -CD)₂-porphyrin-metal ion). This reaction proceeds according to the following sequence of reactions:

The rate constant for the binding interaction between TPPS₄ and β -CD was too rapid to be measured, because the inclusion of TPPS₄ by β -CD was faster than that of metal complexation (<1 ms) (cf. rate constants between TPPS₄ and β -CD in ethylene glycol-H₂O (3:1) was previously reported).^{56,57} The free-base TPPS₄ was immediately incorporated by β -CD in aqueous solution to form the inclusion complex, TPPS₄-(β -CD)₂.

The solubility of β -CD in water is lower than that of other CD (cf. solubility in water at 25°C: 145, 18.5, and 232 g/L for α -, β -, and γ -CD, respectively),⁵⁹ due to the relatively strong intermolecular hydrogen bonding.⁵⁹ As such, the degree of freedom of TPPS₄ in aqueous solution is reduced upon its inclusion with the CD. Furthermore, upon the addition of β -CD, the dissociation reaction of the complex was relatively limited compared to the complexation promotion (based on the results of kinetic analysis). In addition, the pK_a of TPPS₄ became lower, and the original geometric torsion⁶⁰ was slightly twisted upon CD inclusion.^{61,62} Consequently, the acceleration of metal-porphyrin complexation with CD capping was caused by several factors: (1) an improvement of the complex stability upon the formation of the ternary bicapped complex ((β -CD)₂-porphyrin-metal ion), (2) suppression of the dissociation reaction of TPPS₄-Pb by capping with β -CD, whose flexibility is limited due to low solubility in water, and (3) an improvement of reactivity between TPPS₄ and Pb(II) ion due to a distortion of the porphyrin ring upon ionization and complexation with the β -CD.

Detection ability

Under the optimized conditions ($[TPPS_4]_T = 1.7 \mu M$ and $[\beta\text{-CD}]_T = 1 \text{ mM}$; wavelength = 466 nm), quantitative linearity was obtained for the determination of Pb(II) using TPPS₄ in the presence of β -CD. Quantitative results were obtained up to $2.5 \mu M$ Pb²⁺ with a detection limit (signal-to-noise = 3) of 9.0 nM. The correlation coefficient for the calibration curve was 0.985, and the relative standard deviation contained an error of 2.0% (Pb: $1.0 \mu M$, $n = 5$). There was no difference between the detection limit for Pb(II) in either the presence or absence of the β -CD; however, the reaction time was greatly diminished, as previously noted. Thus, the same sensitivity is achieved, but in a much more rapid time frame.

Conclusions

The rate of the complexation reaction between anionic porphyrins and 11 metal ions was found to be accelerated in the presence of β -cyclodextrin (β -CD) in aqueous media at room temperature without the need for additional heating or sonication. The addition of β -CD strongly impacted on the apparent acceleration of the complexation between porphyrin and metal ions. Using Pb(II) as the model ion, the complexation rate constant was enhanced by a factor of 2.4, while the dissociation rate constant was diminished by a factor of 135 in the presence of added β -CD relative to that in its absence when the model anionic porphyrin, TPPS₄, was employed. The formation equilibrium constant of the complex was 324-fold greater in the β -CD medium. The acceleration effect was confirmed not only for TPPS but also for anionic porphyrin, TCPP. The formation of a bicapped complex was demonstrated through the use of NMR spectroscopy and MS spectrometry. This acceleration effect is expected to be applicable to systems in which porphyrin ligands are employed for the determination of metal ions in chemical analysis, separation science and sensor techniques using not only UV-Vis spectrometry, but luminescence, electrochemical measurements and chromatographic application *etc.*

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Supporting Information

Supporting Information is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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