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Dissociation Kinetics and Complexation Equilibrium Studies of a Zn^{II} Complex with 1,5-Bis(2-hydroxy-5-sulfophenyl)-3-cyanoformazan during Capillary Electrophoretic Separation Process

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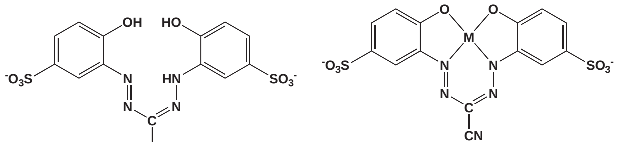
The dissociation kinetics and the complexation equilibrium of a Zn^{II} complex with 1,5-bis(2-hydroxy-5-sulfophenyl)-3-cyanoformazan (hscf: H_5L) during capillary electrophoretic (CE) separation process were studied. The solvolytic dissociation reaction rate constants of the Zn^{II}–hscf complex ($[ZnL]^{3-}$) (k_d) in the pH range of 6.4 to 8.0 were determined with a CE-based dissociation kinetic analysis method. Small k_d values on the order of $10^{-5}\,s^{-1}$ showed the remarkable kinetic inertness of $[ZnL]^{3-}$, which was detectable in the CE system. The stability constant of $[ZnL]^{3-}$ (K_{ZnL}) at 293 K and $I = 0.10$ was estimated to be $[ZnL^{3-}]/\{[Zn^{2+}][L^{5-}]\} = 10^{24.2\pm0.3}$ through the spectrophotometric titration. The thermodynamic stability of $[ZnL]^{3-}$ in the pre-capillary complexation stage was thus demonstrated. In contrast, it was shown that the CE separation environment is thermodynamically unsuitable for the metal complexes based on the conditional stability constant of $[ZnL]^{3-}$, which was determined under CE separation conditions, and the concentration of free HSCF in the complex band in the capillary. Thus, the "stability" of metal complex in the CE system strongly depends on kinetic inertness rather than thermodynamic stability, though their thermodynamic stability is necessary for the pre-capillary derivatization step.

Today, capillary electrophoresis (CE) is an universally accepted technique for the separation and determination of metal ions.[1,2] The pre-capillary chelation CE system employing an electrophoretic buffer solution without chelating reagent, known as kinetically differentiation mode (KD-) CE, has about two attractive features: (1) kinetically controlled selective detection of metal chelates, and (2) extremely high sensitivity with minimized background fluctuations due to base-line resolution with an excess.[3,4] Therefore, specific detection of a few metal ions at femto mol level have been achieved without any concentration technique.[5–8]

In KD-CE separation process, because of the absence of the free chelating reagent in the electrophoretic buffer solution, metal complexes, which migrate in their isolated bands along a capillary from those of free ligand and metal ions, are exposed to an overwhelming force causing dissociation upon CE resolution.[9,10] Therefore, only "stable" metal complexes are detectable. Furthermore, the stability of the metal complexes in a pre-capillary chelation CE system is important because it affects the sensitivity or resolution of the system.[11] However, there has been, to the best of our knowledge, no work that clarifies details concerning the stability of metal complexes during the CE separation process. In regards to the discussion on the stability of metal complexes, kinetic stability (lability or inertness) is sometimes confused with thermodynamic stability. Additionally, not all metal complexes with a large equilibrium constant are kinetically inert.[12] In this work, we attempted to study the dissociation kinetics and the complexation equilibrium of a metal complex migrating in an electrophoretic buffer solution without a complexation reagent in order to identify the origin of the "stability" in the CE separation process.

The coordination environment provided by 3-cyanoformazan analogues, which behave as a nearly planar tetradentate ligand, have been thought to be usable in KD-CE system, because of a strong "chelate-effect."[13,14] In this report, 1,5-bis(2-hydroxy-5-sulfophenyl)-3-cyanoformazan (hscf: H_5L) (Chart 1) was examined as a pre-capillary chelation reagent for KD-CE simultaneous separation and determination of metal ions. Surprisingly, although Zn^{II} ion has been generally thought to form labile complexes, the Zn^{II} complex with hscf ($[ZnL]^{3-}$) has been detected. The dissociation kinetics and complexation equilibrium of $[ZnL]^{3-}$ were, therefore, studied to clarify whether the "stability" of $[ZnL]^{3-}$ during CE separation process is kinetic or thermodynamic. The dissociation kinetics and the complexation equilibrium parameters of $[ZnL]^{3-}$ were obtained by the CE-based dissociation kinetic



H_3hscf

tetradentate metal complex with hscf; $[ML]^{3-}$
(M denotes divalent metal ion)

Chart 1.

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analysis method[9,10] and the analysis of the spectrophotometric titration data, respectively. The key factor for determining the detection selectivity for the metal complexes in KD-CE is also discussed. Furthermore, from this work, we could acquire important knowledge for designing new derivatization reagents for KD-CE system for other "labile" metal ions.

Experimental

Apparatus. A DKK-TOA (Tokyo, Japan) HM-30V pH meter was used. Electrophoresis were performed on a Photal (Hirakata, Japan) CAPI-3200Q capillary electrophoresis system with constant voltage operation mode at 20 kV, and using a GL Science (Tokyo, Japan) fused silica capillary (51 cm × 50 µm id., length to detector 39 cm). Absorption spectra and absorbance were recorded on a Shimadzu (Kyoto, Japan) Model UV-1600PC spectrophotometer.

Reagent and Solutions. Metal ion standard solutions (each 0.01 M) were prepared by dissolving the chlorides or the nitrates in ca. 0.01 M hydrochloric acid solution. A solution of 0.01 M Zn^{II} was prepared by dissolving metal Zn in 0.01 M hydrochloric acid solution. A solution of V^{V} (0.01 M) was prepared by dissolving ammonium metavanadate in 0.01 M nitric acid solution. Accurate concentrations of the metal ion standard solutions were checked via edta titrations.[15] The pre-capillary derivatization reagent hscf was prepared the same as described[13] and purified by repeated precipitation from ethanol. A solution of hscf was prepared by dissolving hscf into doubly distilled water. The concentration of hscf solution was 0.01 M. A solution of 0.1 M edta was prepared by dissolving a commercially available edta (disodium salt, Dojindo, Kumamoto, Japan) in doubly distilled water.

Procedures. Procedure for KD-CE Separation of the Metal hscf Complexes: To an acidic metal ion solution containing Al^{III}, Co^{II}, Cu^{II}, Ga^{III}, Mn^{II}, Ni^{II}, V^{V}, and Zn^{II} ions, a hscf solution and 3-morpholinopropanesulfonic acid (MOPS)/NaOH pH buffer solution (pH 7.0) were added. The mixture was heated at 60 °C for 10 min, and the volume of the solution was adjusted to the specified volume after cooling. Before every run, the capillary were conditioned with 0.1 M NaOH, followed by doubly distilled water and electrophoretic buffer. The electrophoretic buffer solutions containing 0.01 M phosphate/borate buffer (pH 8.8) was used. Sample injection was performed hydrostatically by elevating the sample at 25 mm for 60 s (5 nL) at anodic side of the capillary. Direct photometric detection at 600 nm was used.

Procedure for CE-Based Dissociation Kinetic Analysis of the Zn^{II}–hscf Complex: A sample solution containing Zn^{II} ion (6.30×10^{-5} M), HSCF (6×10^{-4} M), phosphate buffer (5 mM, pH 6.9), and bromphenol blue (BPB) (4×10^{-5} M) as internal standard for peak height standardization was used. Electrophoretic buffer solutions containing 5 mM phosphate (pH 6.4–8.0) were used. The capillary was conditioned before each run as described above. Several CE experiments with a variety of migration times while controlling the applied voltage from 5 to 20 kV were performed. Then, the migration times and the peak height of $[ZnL]^{3-}$ and BPB for each of electropherograms were recorded. The dissociation degree-time profile of $[ZnL]^{3-}$ was obtained by plotting the normalized peak height signals of $[ZnL]^{3-}$ with that of BPB against migration time. The solvolytic dissociation rate constant of $[ZnL]^{3-}$ (k_d) was obtained by fitting the plot with the equation obeying a first-order rate raw.[9,10] k_d's at various pH (6.4 to 8) were obtained.

Procedure for Equilibrium Study of the Zn^{II}–hscf Complex: The complexation equilibrium was examined spectrophotometrically under the conditions of $I = 0.10$ and 293 K. The absorption spectra were recorded for an aqueous solution containing 0.1 M of HCl, 2.0×10^{-5} M of Zn^{II} ion, and 2.0×10^{-5} M of HSCF at appropriate pH intervals, which was adjusted by adding KOH solution.

Results and Discussion

KD-CE Separation of Metal hscf Complexes. Figure 1 shows the electropherograms from the KD-CE separation of a sample solution containing eight different metal ions [Al^{III}, Co^{II}, Cu^{II}, Ga^{III}, Mn^{II}, Ni^{II}, V^{V}, and Zn^{II}] using four kinds of electrophoretic buffer solutions with different pH values. The pH values were varied in the range of 5.1 to 9.4, in which all of the metal ions tested formed 1:1 complexes with hscf in the pre-capillary derivatization step. However, as shown in Figs. 1b and 1c, only complexes of Al^{III}, Cu^{II}, Ga^{III}, Mn^{II}, V^{V}, and Zn^{II} were detected at pH 7.2 and 8.8. In addition, Mn^{II} and Zn^{II} complexes were not detected at pH 9.4 and 5.1, respectively (Figs. 1a and 1d). Co^{II} and Ni^{II} complexes were not detected at any pH. The reason why these two complexes were not detected is not clear. However, these complexes might not be stable, though both Co^{II} and Ni^{II} ion form the complexes in pre-capillary chelation stage; stabilization by chelation may not occur, because hscf might function as a tridentate ligand to these metal ions. In addition, hscf with a O, O, N, N donor set is classified as kind of a "hard" ligand,[16] which may have contribute above detection selectivity. As seen in Fig. 1c, 0.01 M phosphate/borate buffer (pH 8.8) was the most suitable for the simultaneous determination of 6 metal ions, i.e., Al^{III}, Cu^{II}, Ga^{III}, Mn^{II}, V^{V}, and Zn^{II} since well shaped and well resolved peaks are acquired. In other word, it should be possible to use hscf as a pre-capillary chelating reagent for the KD-CE determination system for these metal ions. The detection limits, defined as 3 times of the standard deviation of base-line noise were 5.58×10^{-7} M for Cu^{II} and 2.30×10^{-6} M for Zn^{II}, or on an absolute amount basis, 2.79 femto mol and 11.5 femto mol in a 5 nL sample, respectively.

In KD-CE, because of employing the electrophoretic buffer solution without a derivatization reagent, the metal complexes during the CE separation process would be exposed to a non-equilibrium environment, in which there are no free ligands and metal ions in the vicinity of the metal complexes. Therefore, kinetically unstable, i.e., labile, complexes will decompose in the capillary before arriving at the detector and thus will not be detectable. It is noteworthy that a Zn^{II} complex was observed in the electropherograms because Zn^{II} ion is thought to form kinetically labile complexes in general. This unusual kinetic stability $[ZnL]^{3-}$ during the CE separation process is exemplified in the discussion below. The importance of the kinetic inertness as the attributes of the detectable species in the KD-CE system is discussed later.

Determination of the Solvolytic Dissociation Rate Constant of Zn^{II}–hscf with CE-Based Dissociation Kinetic Analysis Method. To estimate the kinetic inertness of $[ZnL]^{3-}$ during the CE separation process, its k_d values in the pH range from 6.4 to 8.0 were determined with the CE-based dissociation kinetic analysis method.[9,10] Figure 2 shows

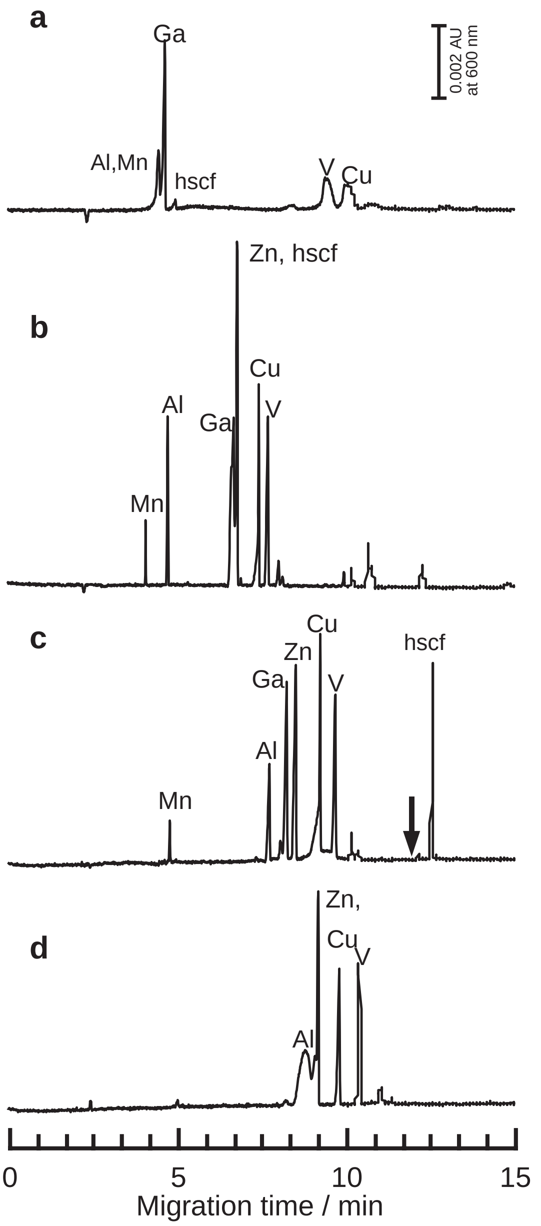

Fig. 1. Typical electropherograms of hscf chelates. Sample: [M] $= 3 \times 10^{-5}$ M (M = Al^{III}, Co^{II}, Cu^{II}, Ga^{III}, Mn^{II}, Ni^{II}, V^{V}, and Zn^{II}), [hscf] $= 1 \times 10^{-3}$ M, [MOPS] = 0.05 M (pH 7.0). Electrophoretic buffer solutions; a: 0.01 M acetate buffer (pH 5.1); b: 0.01 M Na_2HPO_4 (pH 7.2); c: 0.01 M phosphate/borate buffer (pH 8.8); d: 0.01 M borate buffer (pH 9.4). The allow in electropherogram c shows the position being apart at ca. 6σ from the HSCF peak. Where, σ is the standard deviation of hscf peak measured in min.

the typical electropherograms of [ZnL]$^{3-}$ at pH 6.45 at different applied voltages. Leading in the peak for [ZnL]$^{3-}$ in both electropherograms showed [ZnL]$^{3-}$ partially dissociated in the CE separation process. The peak height of [ZnL]$^{3-}$ was smaller than that of hscf in the electropherogram of Fig. 2b, while it is larger than that of hscf in Fig. 2a. In other words, the concentration of [ZnL]$^{3-}$ decreased with an increase in the migration time.

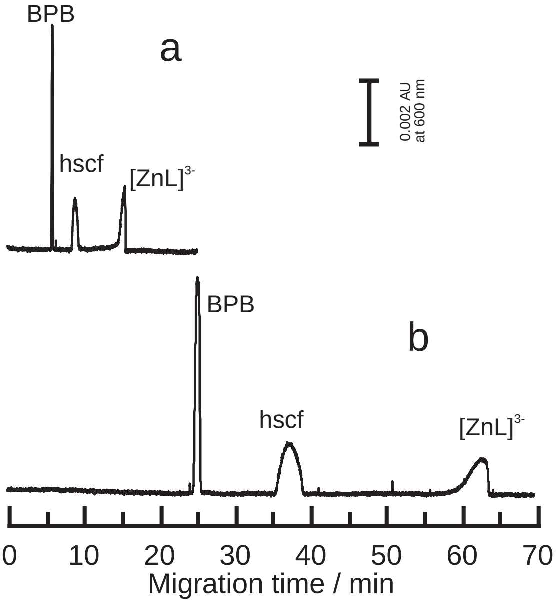

Fig. 2. Typical electropherograms of the Zn^{II}–hscf complex with varying the applying voltage. Applying voltage; a: 20 kV; b; 5 kV. Sample: [Zn^{II}] $= 6.30 \times 10^{-5}$ M, [hscf] $= 6 \times 10^{-4}$ M, [BPB] $= 4 \times 10^{-5}$ M, [Na_2HPO_4] = 5 mM. Electrophoretic buffer solution: [Na_2HPO_4] = 5 mM (pH 6.4).

The solvolytic dissociation reaction of ML during CE separation follows the first-order kinetics and the reaction given as follows:

$$[\mathrm{ML}] \rightarrow \mathrm{M} + \mathrm{L}, \tag{1}$$

where solvent molecules are omitted, and [ML], M, and L denote metal complex, metal ion, and free ligand, respectively. The basic idea of CE-based dissociation kinetic analysis method is to measure the residue of the metal complex with increasing migration time (t_m) in order to obtain a first-order decay curve for the dissociation. For the kinetic analysis of [ZnL]$^{3-}$, the peak height signals were employed because the peak area data can give unreliable results due to asymmetric peak profiles arising from the dissociation reaction. To estimate the dissociation degree of metal complexes, the decrease in the peak height absorbance of metal complex as a function of reaction time and its first-order decay profile were obtained from several CE experiments with different migration times. The dissociation degree–time profile for the complex can be quantitatively described using internal standards, BPB, for the peak signal standardization. When the ratio of the initial concentrations (subscript 0) of [ZnL]$^{3-}$ and BPB is taken as A, we obtain,

$$[\mathrm{ZnL}^{3-}]_0 = A[\mathrm{BPB}]_0. \tag{2}$$

Also, assuming that [BPB] does not decompose during electrophoresis,

$$[\mathrm{BPB}]_t = [\mathrm{BPB}]_0, \tag{3}$$

therefore,

$$[\mathrm{ZnL}^{3-}]_0 = A[\mathrm{BPB}]_t. \tag{4}$$

The rate law is given by,

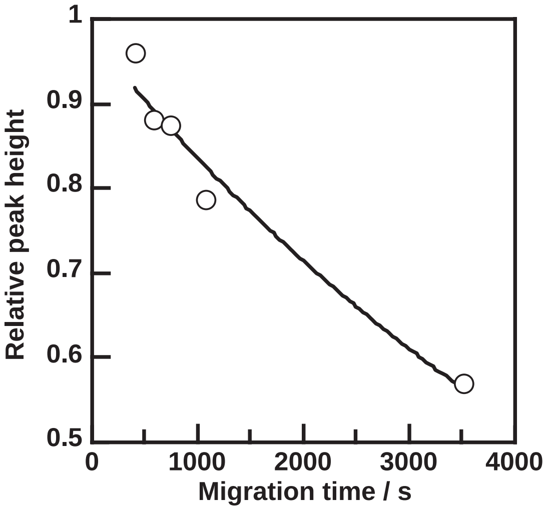

Fig. 3. Typical reaction profile of the Zn^{II}–hscf complex obtained by capillary electrophoretic kinetic analysis method at pH 6.4.

$$\frac{d[ZnL^{3-}]}{dt} = k_d[ZnL^{3-}]. \tag{5}$$

Integrating Eq. 3 from $t = 0$ to t_m yields Eqs. 4 and 5.

$$\ln([ZnL^{3-}]/[ZnL^{3-}]^0) = -k_d t_m, \tag{6}$$

$$[ZnL^{3-}]/[ZnL^{3-}]^0 = \exp(-k_d t_m). \tag{7}$$

Thus, k_d was obtained by analyzing the dissociation degree-time profile of $[ZnL]^{3-}$ as a plot of the normalized peak height signals of $[ZnL]^{3-}$ with that of BPB against the migration time, as shown in Fig. 3.[9,10] A simple first-order decay profile for $[ZnL]^{3-}$ was observed, and plots fitted well to Eq. 5. The values of k_d obtained in the pH range of 6.4 to 8.0 are shown in Fig. 4. We could not obtained the value of k_d above pH 8.0, because the dissociation reaction of $[ZnL]^{3-}$ was too slow to determine the k_d by the CE-based approach. As shown in Fig. 4, as the proton concentration increased (the value of pH decreases), the value of k_d increased. This showed that the dissociation reaction of $[ZnL]^{3-}$ is accelerated with an increase in the proton concentration of the electrophoretic buffer solution. It is thus suggested that $[ZnL]^{3-}$ is more kinetically inert under the optimal CE conditions (pH 8.8), as shown in Fig. 1c, than in the pH range of 6.4 to 8.0. Small k_d values on the order of $10^{-5}\,s^{-1}$ obtained showed that $[ZnL]^{3-}$ is kinetically innert. This remarkable kinetic "stability" of Zn^{II}–hscf complex originates from the relatively rigid coordination environment created by hscf.[13,14]

Using the k_d value at pH 8.0 ($3.2 \times 10^{-5}\,s^{-1}$), the half-life period of $[ZnL]^{3-}$ was estimated to be about 6 h. Furthermore, less than 7% of $[ZnL]^{3-}$ dissociated during KD-CE separation period, if the migration time of the complex was 10–30 min. This means that $[ZnL]^{3-}$ is kinetically inert enough to be detected in the KD-CE system; the dissociation reaction of $[ZnL]^{3-}$ is slow enough for $[ZnL]^{3-}$ to be detected before it dissociates completely in the capillary. In addition, the key factor for the detection selectivity in KD-CE is kinetic inertness.

Estimation of the Thermodynamic Stability of Zn^{II}–hscf Complex during KD-CE Separation Processes. In the above discussion, it was shown that kinetic inertness is a key factor for detection in the KD-CE system. To verify that the thermodynamic stability is also important, the thermodynamic stability of $[ZnL]^{3-}$ during KD-CE separation processes was estimated. At first, the complexation equilibrium of $[ZnL]^{3-}$ was examined by using the spectrophotometric titration. The pH-absorbance curve for $[ZnL]^{3-}$ is shown in Fig. 5. The reaction scheme for the formation of a four-coordinated 1:1 complex is presumed to follow:

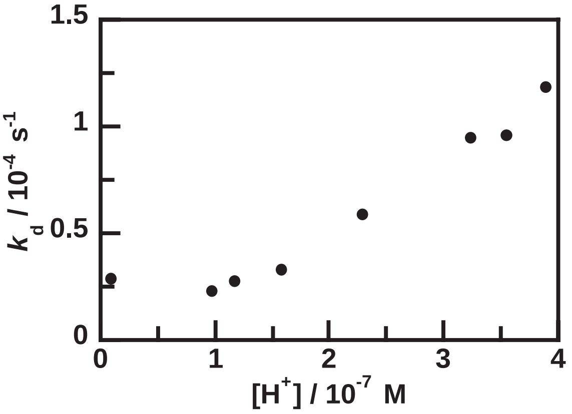

Fig. 4. Effect of $[H^+]$ on k_d obtained by the CE-based kinetic analysis method.

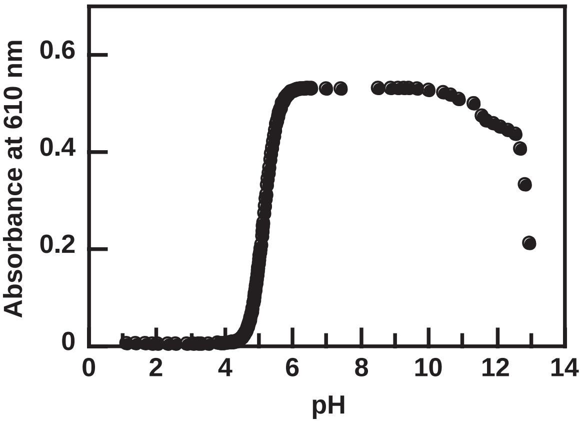

Fig. 5. pH vs absorbance curve of the Zn^{II}–hscf complex obtained by spectrophotometric titration. $[Zn] = [hscf] = 2 \times 10^{-5}$ M, $I = 0.1$.

$$Zn^{2+} + H_3L^{2-} \overset{K}{\rightleftarrows} ZnL^{3-} + 3H^+, \tag{8}$$

where K is defined as

$$K = \frac{[ZnL^{3-}][H^+]^3}{[Zn^{2+}][H_3L^{2-}]}. \tag{9}$$

The logarithm of both sides of Eq. 9 is taken and arranged, it becomes the following:

$$\log\frac{[ZnL^{3-}]}{[Zn^{2+}][H_3L^{2-}]} = 3pH + \log K. \tag{10}$$

K was determined to be -10.1 as $\log K$ by analyzing the increase in absorbance in the pH range form 4 to 6 in Fig. 5 with Eq. 10.[12] And, the formation constant of $[ZnL]^{3-}$ (K_{ZnL}) defined as

$$\mathrm{Zn}^{2+} + \mathrm{L}^{5-} \overset{K_{\mathrm{ZnL}}}{\rightleftarrows} \mathrm{ZnL}^{3-}, \tag{11}$$

$$K_{\mathrm{ZnL}} = \frac{[\mathrm{ZnL}^{3-}]}{[\mathrm{Zn}^{2+}][\mathrm{L}^{5-}]}, \tag{12}$$

was calculated as $\log K_{\mathrm{ZnL}}$ to be 24.2 ± 0.3, using the above $\log K$ value and the reported values of $\mathrm{p}K_{\mathrm{a1}} = 7.3$, $\mathrm{p}K_{\mathrm{a2}} = 12.3$, and $\mathrm{p}K_{\mathrm{a3}} = 14.6$.[13] This is much greater than those for other Zn$^{\mathrm{II}}$ complexes with complexan compounds, such as edta and other polyaminocarboxylates of related structures, that have a tendency to form 1:1 complexes with Zn$^{\mathrm{II}}$ ion.[17] The remarkable thermodynamic stability of $[\mathrm{ZnL}]^{3-}$ in pre-capillary complexation stage appears to originate from the stabilization due to chelation.

An attempt to estimate the thermodynamic stability of $[\mathrm{ZnL}]^{3-}$ during KD-CE separation processes was performed by calculating the concentration profile of free hscf in the capillary unedr optimal CE conditions (pH 8.8). In the case of the Zn$^{\mathrm{II}}$–hscf complex, its conditional stability constant (K_{ZnL}') can be defined as follows:

$$K_{\mathrm{ZnL}}' = \frac{[\mathrm{ZnL}^{3-}]}{[\mathrm{Zn}'][\mathrm{L}']}, \tag{13}$$

where $[\mathrm{Zn}']$ and $[\mathrm{L}']$ are the total concentration of Zn$^{\mathrm{II}}$ ions in the side reactions and hscf in all of its forms, respectively. The side reaction coefficients of Zn$^{\mathrm{II}}$ ion, and hscf, α_{Zn}, and α_{L}, are defined as follows:

$$\alpha_{\mathrm{Zn}} = \frac{[\mathrm{Zn}']}{[\mathrm{Zn}^{2+}]}, \tag{14}$$

$$\alpha_{\mathrm{L}} = \frac{[\mathrm{L}']}{[\mathrm{L}^{3-}]}. \tag{15}$$

Therefore, K_{ZnL}' can be rewritten as

$$K_{\mathrm{ZnL}}' = \frac{K_{\mathrm{ZnL}}}{\alpha_{\mathrm{Zn}}\alpha_{\mathrm{L}}}. \tag{16}$$

As the side reactions of Zn$^{\mathrm{II}}$ ion, complexation reactions with hydroxide ions should be considered. $[\mathrm{Zn}']$ is given as

$$\begin{aligned}[\mathrm{Zn}'] = {} & [\mathrm{Zn}^{2+}] + [\mathrm{Zn(OH)}^{+}] + [\mathrm{Zn(OH)}_2] \\ & + [\mathrm{Zn(OH)}_3{}^{-}] + [\mathrm{Zn(OH)}_4{}^{2-}]. \end{aligned} \tag{17}$$

α_{Zn} is thus defined by using $[\mathrm{Zn}']$ as follows:

$$\begin{aligned}\alpha_{\mathrm{Zn}} = {} & 1 + \beta_1[\mathrm{OH}^-] + \beta_2[\mathrm{OH}^-]^2 \\ & + \beta_3[\mathrm{OH}^-]^3 + \beta_4[\mathrm{OH}^-]^4, \end{aligned} \tag{18}$$

where β_n are the stability constants for the complexation reactions of Zn$^{\mathrm{II}}$ ions with hydroxide ions, and given as follows:

$$\beta_n = \frac{[\mathrm{Zn(OH)}_n{}^{2-n}]}{[\mathrm{Zn}^{2+}][\mathrm{OH}^-]^n} \quad (n = 1,\ 2,\ 3,\ \text{and}\ 4). \tag{19}$$

In the same way, as the side reactions of hscf, protonation reactions should be considered. $[\mathrm{Zn}']$ is given as

$$[\mathrm{L}'] = [\mathrm{H_3L}^{2-}] + [\mathrm{H_2L}^{3-}] + [\mathrm{HL}^{4-}] + [\mathrm{L}^{5-}]. \tag{20}$$

Therefore, α_{L} is given as follows:

$$\alpha_{\mathrm{L}} = 1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{a3}}} + \frac{[\mathrm{H}^+]^2}{K_{\mathrm{a2}}K_{\mathrm{a3}}} + \frac{[\mathrm{H}^+]^3}{K_{\mathrm{a1}}K_{\mathrm{a2}}K_{\mathrm{a3}}}. \tag{21}$$

α_{Zn} and α_{L} at pH 8.8 were calculated to be $10^{9.33}$ and $10^{0.25}$, respectively.[18,19] Thus, K_{ZnL}' at pH 8.8 under the CE separation conditions were calculated to be $10^{14.6}$. In the complexation reaction of Zn$^{\mathrm{II}}$–hscf system, if the equilibrium lies in the direction of the complexation, $[\mathrm{ZnL}]^{3-}$ exists in a large excess ($>10^3$) compared to free Zn$^{\mathrm{II}}$ ion. According to above calculated K_{ZnL}' value, $[\mathrm{L}']$ should be $10^{-11.6}$ M or more at pH 8.8 to be consistent. Then, as a condition for the thermodynamic stability of the Zn$^{\mathrm{II}}$–hscf complex in KD-CE separation process, it is necessary that $10^{-11.6}$ M or more of the free ligand should be present in its isolated band in a capillary. On the other hand, according to the Gaussian peak profile approximation,[20] the concentration of free ligand ($C_{\Delta z}$) at Δz from its peak can be calculated by the following equation:

$$C_{\Delta z} = C_{\mathrm{peak}} \exp\left[\frac{-(\Delta z)^2}{2\sigma^2}\right], \tag{22}$$

where C_{peak} and σ are the reagent concentration at its peak and the standard deviation of the peak measured in min, respectively. If the concentration of the free hscf in its isolated band is assumed to be ca. 1×10^{-3} M, the distance from the hscf peak to the point where free ligand concentration should be equal to $10^{-11.6}$ M is calculated to be 6.3σ by using Eq. 22. However, as shown in Fig. 1c, $[\mathrm{ZnL}]^{3-}$ and hscf are much more than 6.3σ apart from each other. From a thermodynamic point-of-view, $[\mathrm{ZnL}]^{3-}$ is inevitably decomposing due to low concentration of ligand in its vicinity, althougt it is thermodynamically stable in pre-capillary chelation step. It is clear that the KD-CE separation process environment is not the thermodynamically unsuitable for $[\mathrm{ZnL}]^{3-}$. It is also suggested that $[\mathrm{ZnL}]^{3-}$ is not detectable in KD-CE from above equilibrium estimation. This contradicts the fact that $[\mathrm{ZnL}]^{3-}$ is kinetically inert enough to be detectable in KD-CE system, as shown in the preceding section. Thus, the "stability" of metal complexes in the CE system strongly depends on kinetic inertness rather than thermodynamic stability, though it is necessary in the pre-capillary derivatization step.

Conclusion

The dissociation kinetic and complexation equilibrium studies for $[\mathrm{ZnL}]^{3-}$ shed light on the chemical basis to elicit the detection selectivity in KD-CE system. From the estimation of k_{d}, $[\mathrm{ZnL}]^{3-}$ was obviously inert enough to be detectable in the KD-CE system. The concentration profile of the free ligand in the capillary showed that the KD-CE separation process environment is the thermodynamically unsuitable for the metal complexes. It was concluded that the detection selectivity for metal complexes in the KD-CE system depends on the kinetic inertness of the metal complexes rather than their thermodynamic stability. However, thermodynamic stability is important in pre-capillary derivatization stage. In addition, the kinetic inertness of $[\mathrm{ZnL}]^{3-}$ can be used for designing of kinetic stable complexes. Interestingly, $[\mathrm{ZnL}]^{3-}$ was quite kinetically stable judging from its k_{d} value, although Zn$^{\mathrm{II}}$ ion has been reported to have a tendency to form labile complexes, because it has a large rate constant for water exchange on aqua metal ions, similar to predictions from the Eigen mechanism,[21,22] in which the reactivity solely depends on the property of metal ions.[9] This suggests the character of ligand is a primary factor to determine the kinetic stability of its metal complexes and that one can construct other kinetically inert metal complexes by designing their suitable ligand systems. Furthermore, the remarkable kinetic "stability" of $[ZnL]^{3-}$ appeared to originate from the relatively rigid coordination environment made by hscf. Therefore, KD-CE determination system for "soft" metal ions, such as Cd^{II} and Hg^{II} ions, which are much more "labile" than Zn^{II} ion, using other formazan derivatives is now under investigation.

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Supporting Information

Absorption spectra of hscf and its metal complexes. This material is available free of charge on the Web at: http://www.csj.jp/journals/bcsj/.

References

1 E. F. Hilder, M. Miroslav, D. P. Bogan, P. R. Haddad, *Anal. Commun.* **1997**, *34*, 63.

2 R. Timerbaev, *J. Chromatogr., A* **1997**, *792*, 495.

3 N. Iki, *Bunseki Kagaku* **2002**, *51*, 495.

4 H. Hoshino, Y. Saitoh, K. Nakano, T. Takahashi, T. Yotsuyanagi, *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1279.

5 N. Iki, H. Hoshino, T. Yotsuyanagi, *Chem. Lett.* **1993**, 701.

6 N. Iki, H. Hoshino, T. Yotsuyanagi, *J. Chromatogr., A* **1993**, *652*, 539.

7 T. Saitoh, H. Hoshino, T. Yotsuyanagi, *Anal. Sci.* **1991**, *7*, 495.

8 S. Saito, H. Hoshino, *Anal. Bioanal. Chem.* **2004**, *378*, 1644.

9 N. Iki, H. Hoshino, T. Yotsuyanagi, *Anal. Chem.* **2000**, *72*, 4812.

10 T. Takahashi, K. Ohtsuka, N. Iki, H. Hoshino, *Analyst* **2005**, *130*, 1337.

11 M. Macka, P. Nesterenko, P. Anderson, P. R. Haddad, *J. Chromatogr., A* **1998**, *803*, 279.

12 K. Ueno, *Chelate Chemistry, An Introduction*, Nankodo, Tokyo, **1969**.

13 B. W. Budesinsky, J. Svec, *Inorg. Chem.* **1971**, *10*, 313.

14 H. Hoshino, K. Nakano, T. Yotsuyanagi, *J. Chromatogr.* **1990**, *515*, 603.

15 K. Ueno, *Chelate Chemistry*, Nankodo, Tokyo, **1989**.

16 S. F. A. Kettle, *Physical Inorganic Chemistry: A Coordination Chemistry Approach*, Oxford University Press, New York, **1998**, Chap. 5.

17 K. L. Cheng, K. Ueno, T. Imamura, *CRC Handbook of Organic Analytical Reagents*, CRC Press, Boca Rotan, FL, **1982**.

18 α_{ZnL} and α_{L} was calculated using the stability constants of the complex of Zn^{II} ion with hydroxide ions[18] and the reported pK_a values,[13] respectively.

19 *Critical Stability Constants*, ed. by E. Martell, R. M. Smith, Plenum Press, New York, **1974**, Vol. 5, 1st Supplement.

20 J. N. Done, J. H. Knox, J. Loheac, *Application of High-Speed Liquid Chromatography*, John Willy & Sons, London, **1974**, Chap. 3.

21 M. Eigen, R. G. Wilkins, *Adv. Chem. Ser.* **1965**, *49*, 55.

22 M. Eigen, *Pure Appl. Chem.* **1963**, *6*, 97.