Theoretical Approaches to Determination of Copper Complexation of Strong Ligands in Natural Waters

II. Kinetics of competitive reactions for copper complexation

by

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Abstract

The kinetics of competitive effects of side reactions for the complexation reaction between copper and the strong organic ligand isolated from seawater is examined with regard to other metals that were possibly present in the equilibrium systems and also a chelating agent, EDTA (ethylenediaminetetraacetic acid) that was added exogenously in the procedure developed by Midorikawa and Tanoue (1994). The kinetic problems in the ligand-exchange reaction of the strong ligand with EDTA is negligible because of quantitative removal of the alkaline earth metal ions in the pretreatment of seawater samples. It is found that the assumption for the concentration of copper-EDTA complex in the sample, introduced by Midorikawa and Tanoue (1994) in their equilibrium model, may lead to a conservative estimate of the conditional stability constant of the strong ligand, taking into account the kinetic reactivity of the strong ligand for copper complexation.

When trace metals other than copper are present, the model calculation demonstrates that these metal-competition effects involving the side reactions of EDTA finally lead to an overestimation of the conditional stability constant for the strong ligand. The magnitude of the overestimation is as small as the errors in its estimation based on the uncertainties of measurements. Possible metal competitions for the strong ligand may result in the conservative estimate of the total concentration of the strong ligand and would have no serious influence on the estimation of its conditional stability constant.

1. Introduction

Copper organic complexation in natural waters has been studied extensively due to the great interest in its biological and geochemical influences. In recent years, it has become apparent that complexation reaction with natural organic ligands dominates copper speciation in natural waters. There are only a few studies in which the influence of metals other than copper on copper complexation of natural ligands has been investigated thermodynamically in terms of the possible competition for the same binding sites between these

metals. The results found in the relevant literature are at times controversial. The possibilities of such competitive reactions for aquatic humic substances were pointed out by Stumm and Morgan (1970) and Reuter and Perdue (1977). Significant competitive effects or humate-binding have been observed among Cu, Pb, Cd, Zn and Ca (Guy and Chakrabarti 1976), between Cu and Ca (Truitt and Weber 1981) and between Cu and Cd (Fish, 1984). By contrast, it has been reported that the competitive behavior of calcium in the copper titration of humic substances in fresh waters is negligible (Buffle et al., 1980) and only slight

(Sunda and Hanson, 1979; Cabaniss and Shuman, 1988). Cabaniss and Shuman (1988) examined copper binding abilities of humic substances from fresh waters and suggested that the strong ligands for copper are more Cu-specific than the weaker ligands in the presence of magnesium.

With regard to strong ligands in seawater, their specific preferences for copper have been suggested from the results based on equilibrium studies which showed that no effects were found by the addition of other metals during the course of the copper titration (Sunda and Hanson, 1987; Coale and Bruland, 1988; Apte et al., 1990). Besides copper, the occurrence of strong ligands with strong specificity has been reported for trace metals, such as zinc (Bruland, 1989), lead (Capodaglio et al., 1990) and cadmium (Bruland, 1992). If we try to characterize natural ligands from the knowledge based on coordination chemistry of synthetic chelating reagents, such strong specificity is difficult to understand, because chelating agents generally make interactions with most transition metals.

It has been indicated that the strong ligand in seawater may predominantly exist as a calcium complex from the thermodynamic calculations of ligand speciation on synthetic chelating reagents used as a model ligand for the strong ligand (*e.g.*, Motekaitis and Martell, 1987; Hirose, 1993). Hirose (1993) has noted that the addition of a small amount of other metals would not significantly affect the results of the metal-titration method because the strong ligand would be under "the condition of ligand buffered" by calcium in equilibrium systems of seawater. Thus, he has concluded that no influence of added metals in such experiments would not provide a convincing evidence for the strong specificity of the ligand for each metal.

Another possible reason for the controversial results in the literature is insufficient knowledge about the kinetics of copper complexation of natural ligands and of the competitive reactions of other metals for their complexation with copper. Kramer (1986) has reported that ligand concentrations (C_L) after

equilibration for more than 12h were 19–63% higher than that for less than 20min. In his experiments, no large differences in the conditional stability constants (K'_{CuL}) were observed between the two values in the ranges of $10^{7.8}$ – $10^{8.6}$. Coale and Bruland (1988) have reported that no significant differences in ligand concentrations or conditional stability constants were observed when copper additions to three sets of aliquots of the same sample were made and the samples were allowed to equilibrate for 5.5 min, 24h, or 96h, with regard to two types of the ligands with K'_{CuL} of 10^{13} and 10^{10} .

Hering and Morel (1988a, b and 1989) have reported, based on their kinetic studies, that the rate of complexation of copper by nitrilotriacetic acid (NTA: $K'_{CuL}=10^{8.5}$ in seawater) is only slightly affected by high alkaline-earth concentrations, whereas the copper complexation reaction of a more strong ligand, ethylenediaminetetraacetic acid (EDTA: $K'_{CuL}=10^{10.4}$ in seawater), is kinetically hindered greatly at the concentrations of calcium in seawater and/or in the presence of significant levels of other ambient metals that can interact at the same ligand sites as copper. They have indicated that, on addition of copper ions to equilibrium systems in seawater, strong ligands can paradoxically be slower to react than weaker ligands and the rate of metal-exchange reaction between added copper and the other metal that has complexed with the strong ligand is very slow: *e.g.*, for EDTA as a model ligand, its half-life is ~ 8 days for the Ca complex and ~ 10 years for the Cd complex. Thus, they have suggested that strong ligands in seawater may be underestimated or some ligands may be undetectable not only by metal-into-ligand titrations but also by measurements involving addition of ligands, as artifacts due to slow kinetics of complexation reactions under the conditions of seawater.

On the basis of the results by Hering and Morel (1988 and 1989), the paradoxical results between Kramer (1986) and Coale and Bruland (1988) could be interpreted as follows. In the study by Coale and Bruland (1988), they could not detect the occurrence of the metal-exchange reaction between added copper and

calcium complex because the dissociation of Ca complex of the strong ligand is very slow and added copper was at a low concentration; consequently, Ca complex of the strong ligand was substantially inert for copper addition. The fraction of strong ligands complexed with calcium has not been determined by their measurements and their values for the ligand concentration may be underestimated. On the other hand, Kramer (1986) could observe relatively rapid metal-exchange reactions of Ca complexes with relatively low K'_{CaL} and high concentrations by a relatively high level of added copper.

It is of great interest to quantify the extent to which the ligand has higher affinity for copper than other metals, and to elucidate the mechanism of reaction and the nature of the ligand that may be responsible for its specificity. Judging from the scanty information as mentioned above, however, it is not necessary to propose such strong specificity of the strong ligand for copper to explain the observation concerning its complexation equilibria with other metals, but it is possible to think that the reported specificity may be attributable to kinetic effects on the metal-exchange reaction. Therefore, insufficient consideration of the kinetic effects would lead to an incorrect interpretation of the experimental results. In the present paper, with respect to the strong ligand, the kinetic effects of possible competitive reactions for its copper complexation are discussed on the estimation of its complexing ability.

2. Background and data

The theory of kinetics of complexation reaction has been extensively reviewed and empirical reaction mechanisms and rate constants obtained in laboratory studies have been compiled by Margerum et al. (1978). On the subject of EDTA used in the present study, for example, it is shown that the complexation reaction between Cu(II) and free (or protonated) EDTA (Y), namely,

can attain equilibrium very rapidly in a variety of synthetic media, such as $NaClO_4$ and KNO_3 . In addition, both the replacement of one metal in a complex by another metal, that is, the metal-exchange reaction, such as the following one:

and the replacement of one ligand in a complex by another ligand, that is, the ligand-exchange reaction, such as the following one:

are relatively well characterized on their reaction rates and pathways. However, little laboratory model based on the kinetic study in simpler systems has been extended to application to actual natural samples of enormous complexity (e.g., Buffle, 1988; Langford and Gutzman, 1992). We have extremely insufficient information on the kinetics, actual reactivities, side reactions and associated species, with reference to complexation reaction of natural organic ligands (Li) in seawater, represented by the following equilibrium equation:

Midorikawa et al. (1990) developed the procedure that involves the removal of both inorganic salts and metals bound to natural ligands, in order to minimize the influence of simultaneous side reactions for competition with coexisting metal ions and contamination by metals. The procedural sequence is schematically shown in Figure 1. The seawater sample was desalted by dialysis and subsequent electrodialysis after concentration by lyophilization. Metals bound to natural ligands isolated from seawater was removed through the ligand-exchange reaction in the presence of a large excess of EDTA (demetalization process). In both processes, a dialysis membrane with a 1000 molecular weight cut-off was used. When EDTA (Y) is added to the concentrated

Fig. 1 Schematic representation of the procedure.

sample at the demetalization step, the following competitive reaction takes place in addition to the reaction described by the equilibrium equation (4):

The addition of a large excess of EDTA shifts this equilibrium (5) to the right. Thus, metal ions are removed from the natural ligand (L_i) by the ligand-exchange reaction and are transferred to EDTA. After equilibration of the reactions, the metal-EDTA complex (MY) and the excess EDTA (Y) are separated from the solution in the dialysis bag (referred to as the bag, hereafter) by dialysis and subsequent electrodialysis (Fig. 1).

During the course of the demetalization procedure, the treatment of samples was always performed in the identical cell in parallel with exactly the same treatment of the control, and corrections were made for every measurement using the control data to compensate for the effect of possible adsorption of copper ions onto the dialysis membrane and variations in the conditions of electrodialysis. A control bag contains a solution of Cu(II) and EDTA without a sample of seawater. The changes in the concentrations of copper and EDTA in the bag during the demetalization process were examined.

On the basis of the experimental results obtained by using this method under the simpler conditions (at an ionic strength lower than $10^{-5}M$, pH5.71, and a temperature of $4^\circ C$) defined by the process of electrodialysis, the occurrence of a low level of a strong ligand (designated L_N) was detected and its conditional stability constant was estimated (Midorikawa and Tanoue, 1994). For the ligand L_N , the following equilibrium equation is written as an analogue of the ligand-exchange reaction (equation 5), with $i = N$ and $M = Cu$,

Therefore

$$K_N = \frac{K'_{CuY}}{K'_{CuL_N}} = \frac{[L_N'] [CuY]}{[CuL_N] [Y']} \quad (7)$$

For K'_{CuL_N} , equation (7) can be rewritten as follows:

$$K'_{CuL_N} = \frac{K'_{CuY} \cdot [CuL_N] [Y']}{[CuY] [L_N']} \quad (8)$$

In equation (8), the concentrations of the copper-EDTA complex $[CuY]$ and of free EDTA $[Y']$ are obtained from the control experiment (Midorikawa and Tanoue, 1994). The concentration of the CuL_N complex $[CuL_N]$ in the sample solution is calculated as the difference

in C_{Cu} between the control and sample bags after demetalization, assuming that the value of $[CuY]$ in the sample bag is the same as that in the control bag. The conditional stability constant of copper-EDTA, K'_{CuY} , is taken from the literature (Turner and Whitfield, 1987) and corrected for the conditions of the present experiment ($\log K'_{CuY} = 13.78$). Only the concentration of free L_N $[L_N']$ in equation (8) could not be measured experimentally. The upper limit of $[L_N']$ was estimated based on the sensitivity limit of detection technique in the separate experiment of copper titration for the identical sample of seawater under the conditions at an ionic strength of 0.7M, pH8.15 and 25°C. Thus, the lower limit of K'_{CuL_N} was estimated.

The ligand L_N is stronger than two ligands (designated L_1 and L_2 ; Midorikawa et al., 1990) that were determined by copper titration. Copper speciation in the sample bag after demetalization (Fig. 1) indicated that the concentrations of inorganic complexes and natural organic complexes with L_1 and L_2 were negligible as compared with the level of complexes of L_N and EDTA. In part I of this series (Midorikawa and Tanoue, 1993a), the thermodynamic aspect of complexation behavior of the strong ligand (L_N) was examined, which indicated that the ligand L_N was maintained quantitatively and reproducibly in the form of the complex with copper during demetalization. It was ascertained that such a copper complex is remarkably stable. By contrast, the ligands L_1 and L_2 have relatively low affinities for copper and can be demetalized quantitatively by our procedure (Midorikawa and Tanoue, 1994). The potentialities of applying this approach to determine complexing abilities of organic ligands in natural waters from a variety of environments will be discussed in part III of this series (Midorikawa and Tanoue, 1993b). This paper describes the kinetic effects on both the procedure employed by Midorikawa and Tanoue (1994) and equilibrium speciation of copper by their model. In addition, we analyze competitive reactions for copper complexation of the strong ligand, especially, with other transition metals from the

viewpoints of both thermodynamics and kinetics. In the present paper, the kinetic influence of competitive reactions on the estimation of complexing ability of the strong ligand is discussed on the models by Midorikawa and Tanoue (1993a, 1994).

Data sets used in the present study are based on those published in four papers (Midorikawa et al., 1990, 1992; Midorikawa and Tanoue, 1993a, 1994). These data are given in detail in part I of this series (Midorikawa and Tanoue, 1993a). Symbols used in the present study and their definitions are also listed in part I of this series (Midorikawa and Tanoue, 1993a). Copper represents Cu(II) unless otherwise noted.

3. Results and discussion

3. 1. Kinetics of demetalization

The complexation reaction (equation 1) between Cu(II) and free EDTA is very rapid (Margerum et al., 1978). However, the kinetics of the ligand-exchange reaction (equation 5) in the present procedure for demetalization could be an important problem in the estimation of complexing abilities of natural ligands and in the interpretation of the results. Hering and Morel (1989) reported that the rate of the complexation of a strong ligand with an added metal is very slow in seawater during metal titrations. In our procedure, almost all inorganic salts are separated from the sample solution by the dialysis and subsequent electrodialysis (Midorikawa et al., 1990). At the equilibration of the ligand-exchange reaction in the demetalization process, EDTA added in excess is predominantly in the form of $Hedta^{3-}$ in the solution at pH8. After the decrease in $[Y']$ as a result of the electrodialysis, any EDTA not bound to copper is in the form of H_2edta^{2-} and $Hedta^{3-}$ in the solution at pH5.71. Consequently, EDTA not bound to copper remains in the protonated form through the entire demetalization process; complexes of alkaline earth metals and EDTA, such as CaY , are at low levels. The concentration of calcium in the solution of the seawater sample fell to below $10^{-5}M$ before

demetalization and calcium was not detected in the solution after demetalization (Midorikawa et al., 1990). This result indicates that the complete ligand-exchange reaction occurs and natural ligands are free from calcium after demetalization. This observation is consistent with the general kinetic property of synthetic chelating reagents that the rate of the metal-exchange reaction (equation 2) of one metal complex with another metal added in excess is slow, whereas the rate of the ligand-exchange reaction (equation 3) of a metal complex with a free or protonated ligand (chelating agent) is rapid in the case of labile metals (Margerum et al., 1978). It is unlikely that we need to concern ourselves with kinetic problems in the ligand-exchange reaction with EDTA in the demetalization procedure.

3. 2. Effects of the kinetic reactivity of the ligand L_N on estimations of $[CuY]$ and K'_{CuL_N}

For evaluation of K'_{CuL_N} , the value of $[CuY]$ in the sample solution inside the bag was postulated *a priori* to be the same as that obtained from the control experiments, on the assumption of a rapid response of all copper species (Midorikawa and Tanoue, 1994). Since $[Cu']$, $[CuL_1]$ and $[CuL_2]$ in the sample bag were negligible as compared with $[CuY]$, the effects of both inorganic ligands and organic ligands except the ligand L_N on the change in $[CuY]$ can be ignored. The principal species responsible for the equilibrium with the ligand L_N in the sample solution can be considered to be the four species shown in equation (6). From equation (7), the copper complexation of the ligand L_N has the following relation with that of EDTA:

$$\begin{aligned} \frac{[CuL_N]}{[L_N']} &= K'_{CuL_N} \cdot \frac{[CuY]}{K'_{CuY} \cdot [Y']} \\ &= K'_{CuL_N} \cdot [Cu] \end{aligned} \quad (9)$$

The value of $[CuL_N]$ was determined as the concentration of excess copper remained in the sample over the control after demetalization (Midorikawa and Tanoue, 1994). The changes in the conditions of electro dialysis are partly

responsible for those in the values not only of $[CuY]$ and $[Y']$, but also of $[CuL_N]$ and $[L_N']$ in the bag after electro dialysis. Thus, the effects of the distribution of the L_N species on the change in $[CuY]$ (the behavior of CuY) are closely associated with both the process of electro dialysis and the kinetic reactivity of the ligand L_N .

Let us now consider the effects of conditions of electro dialysis on $[CuL_N]$ by reference to the results of the control experiments based on equation (9), assuming a rapid response of each species of the ligand L_N . These effects are determined by the values of $[CuY]$ and $[Y']$ in the sample bag after electro dialysis as follows. In the equilibrium system in the sample bag, copper is under "the condition of metal buffered" predominantly by the high level of EDTA and the concentration of free metal is controlled by free EDTA; the level of free $Cu(II)$ is suppressed to be very low, depending upon the concentration of free EDTA. Equation (9) implies that the distribution of the two forms of the ligand L_N is dependent on $[Cu]$, which is controlled by EDTA. The $[Y']$ and $[CuY]$ decrease with increasing duration of dialysis at the demetalization step (Midorikawa and Tanoue, 1994), keeping the equilibrium in equation (1), because EDTA is quantitatively in the form of free Y' and the components in equation (1) can attain equilibrium rapidly as mentioned above. The rate of removal of Y' from inside the bag to the outer medium through the dialysis membrane is higher than that of CuY because $[Y']$ is overwhelmingly higher than $[CuY]$. In addition, the removal rate of Y' through the dialysis membrane is dependent on $[Y']$ itself. Thus, copper speciation in the bag is predominantly controlled by $[Y']$. In the sample bag, therefore, the equilibrium involving the ligand L_N is similarly controlled by EDTA; not only copper speciation but also ligand speciation of the ligand L_N in the sample bag is defined by that of EDTA. Consequently, the dialysis rates of Y' and CuY are the same in the solutions of both the control and the sample.

If we take into account the possible kinetic reactivity of the ligand L_N , the assumption for

[CuY] in the sample may lead to a conservative estimate of the conditional stability constant of the ligand L_N for the following reasons. The estimations of [CuY] and K'_{CuL_N} depend on the rate of the response of the equilibrium reaction (4) for CuL_N to the increase in [Cu] that is due to the decrease in [Y'] in the sample solution, in the following three ways. First, when its response is very rapid, the equilibrium in equation (4) for CuL_N can be kept together with that in equation (1). The [CuL_N] at equilibrium can be measured. The concentrations of all copper species including CuY and CuL_N are controlled by [Y'], which is the highest among the concentrations of the components permeable to the dialysis membrane. The [CuY] is kept the same as that in the control bag. In this case, K'_{CuL_N} can be estimated correctly. Second, when the rate of the response of reaction (4) is comparable with the rate of decrease in [Y'], the equilibrium in equation (4) for CuL_N shifts to the right and the equilibrium in equation (1) shifts to the left. These shifts are associated with the formation of additional CuL_N and a decrease in the level of CuY as compared with the control. Consequently, [CuY] in the sample solution is lower than that in the control solution. Because reaction (4) does not attain equilibrium completely, the value of [CuL_N] measured is conservative. Therefore, the use of both the observed data of [CuL_N] and the control value of [CuY] in equation (8) for K'_{CuL_N} leads to an underestimation of K'_{CuL_N} . Third, when the response of reaction (4) in terms of CuL_N is slow compared with the rate of removal of Y' by dialysis, [CuY] remains the same as that in the control bag and the equilibrium in equation (6) apparently does not shift. This absence of a shift in the equilibrium leads to a measurement of [CuL_N] that is lower than that after equilibration and also leads to an underestimation of K'_{CuL_N} . In either case, the evaluation of K'_{CuL_N} is relatively conservative.

If Y' and/or CuY interacted specifically with natural ligands in the sample, the interpretation of the results of experiments would be more complicated, although such types of interaction have been suggested in the case of the humic acid from soil (Guy and Chakrabarti,

1976). However, even if they occurred, such interactions would not give rise to a serious error in our calculation as follows. First, it is unlikely that the ternary bridged complex (*i.e.*, mixed ligand complex, Y-Cu-L) forms in the low levels of EDTA (Y) in the latter half of electrodialysis. In this case, if such a complex occurs even in a moment, all the coordination sites of copper would promptly be occupied by either of the two ligands that is profitable to complex in the aspects of its concentration, configuration and complexing ability, by "wrapping" it around copper in accordance with the characteristic mechanism of multidentate ligands. Second, if EDTA could be adsorbed on an organic ligand (or dissolved organic matter) by the electrostatic interaction (as Y-L), protonated species of Y^{4-} and HY^{3-} would be more susceptible to adsorption than CuY^{2-} , depending upon the magnitude of the electric charge. Such effects would give rise to a greater concentration of EDTA (especially, HY^{3-} at pH5.71) in the sample, compared with that in the control. When the K'_{CuY} for this type of EDTA is maintained on a high level, its occurrence will lead to the underestimation of [Y'] in the sample bag, and consequently, to a conservative value of K'_{CuL_N} in equation (8) in the model. Even if it occurs, our estimation again stands on the safe side with regard to such effects.

3. 3. Influence of other metals on estimations of [L_N'] and K'_{CuL_N}

(1) *The model by Midorikawa and Tanoue (1994)*

When trace metals other than copper are present, they may possibly interact with EDTA and the ligand L_N , which are not bound to copper in the equilibrium reaction in equation (6). In the original model by Midorikawa and Tanoue (1993a, 1994), such interactions were not parameterized in the equilibrium systems. In this section, the model by Midorikawa and Tanoue (1994) is extended to the equilibrium study in more complex systems and reassessed in more detail with regard to the competitive reactions with other metals, including their

kinetic effects on the estimation of $[L_N']$ and K'_{CuL_N} .

Trace metals compete with copper for the complexation reaction with EDTA. Such reactions reduce the levels of protonated forms of EDTA and give rise to an overestimation of K'_{CuL_N} when it is calculated from equation (8). In addition to the protonation reaction involving EDTA in the model, such competitive reactions can also be taken into account as side reactions involving free EDTA (Y) in the equilibrium (6). With regard to the effects of these competitive reactions, only their thermodynamic aspects need to be examined because the complexation reactions between EDTA and trace metals other than copper are rapid (Margerum et al., 1978). These side reactions were evaluated to be insignificant from the thermodynamic calculation using the results of the measurements of other metals (Appendix I). Therefore, these metal-competition effects involving the side reactions of EDTA would similarly have no serious influence on the kinetics during the demetalization.

Another potential problem arises if there is significant competition between copper and other metals in complexing with the ligand L_N . In the model by Midorikawa and Tanoue (1994), only the concentration of the ligand L_N not bound to copper, $[L_N']$, in equation (8) was not measurable experimentally for the estimation of K'_{CuL_N} under the conditions of electro dialysis (pH5.71). The range of $[L_N']$ was assumed to be lower than the sensitivity limit, $C_{Cu}^{min'}$ of the Cu-ISE measurement under the conditions of titration (pH8.15) because no ligand of the L_N type was detected by the copper-titration method (Midorikawa et al., 1990). The effects of metal competition for the ligand L_N on the estimation of K'_{CuL_N} need to be considered only when the sum of concentrations of the competing metals is not significantly lower than $C_{Cu}^{min'}$. If their complexes with L_N are stable and their concentrations are significant, any ligand L_N not bound to copper will be distributed via the formation of complexes with other metals. In such cases, $[L_N']$ may include the concentration of the ligand L_N complexed with metals other than copper as well as

those of free L_N and protonated forms of L_N (Appendix II). The value of K'_{CuL_N} based on $[L_N']$ that includes the concentration of the ligand L_N derived from such metal complexes is dependent on not only pH but also the free ion concentrations of such coexisting metals. Our purpose in the present study is to estimate the conditional stability constant K'_{CuL_N} (*i.e.*, $K'_{CuL_N(H)}$) described in Appendix II) that is controlled by the protonation reactions but is independent of the existence of other metals. Since it is impossible to determine the value of $[L_N']$ accurately, it should be examined how the upper limit of $[L_N']$ estimated from the results of the copper-titration method (Midorikawa and Tanoue, 1994) is related to the composite concentration of free and protonated forms of L_N .

In the presence of other metals, we estimated such metal-competition effects for two extreme cases of the kinetics of the metal-exchange reactions during the copper titration. In the first, we assumed that other metals complexed with the ligand L_N are kinetically susceptible to the displacement by the exogenously added copper during the copper titration. In this case, because all the ligand L_N can be determined by the copper-titration method, the measured value of $[L_N']$ includes the concentration of all the ligand L_N complexed with the competing metals as well as those of free and protonated forms of L_N . In estimating the conditional value for K'_{CuL_N} on the protonation alone, therefore, the use of this value of $[L_N']$ leads to an underestimation (Appendix II). Its magnitude corresponds to the value of the total concentration of L_N complexes with the competing metals other than copper. In other words, the value of $[L_N']$, which includes the concentrations of free and protonated forms of the ligand L_N alone, is lower than $C_{Cu}^{min'}$ taken as its upper limit. Consequently, these metal-competition effects result in the underestimation of K'_{CuL_N} by the value corresponding to side reactions of the ligand L_N accompanied by the formation of other metal- L_N complexes. The quantification of the magnitude of underestimation of K'_{CuL_N} has not been accomplished. It is required for the corrections not only to determine chemical speciation of

other metals but also to estimate pH-dependence of $K'_{M_jL_N}$ for all complexes (M_jL_N) with other metals (and to distinguish the ligand of L_N type for each metal).

In the second case, we assumed that other metals complexed with the ligand L_N are not displaced at all by the exogenously added copper during the copper titration. In this case, the ligand L_N in the forms of complexes with other metals cannot be determined but the free and protonated forms of L_N can be determined by the copper-titration method. Therefore, the value of the sensitivity limit $C_{Cu}^{min'}$ corresponds to the upper limit for the composite concentration of free and protonated forms of L_N , irrespective of the presence of the ligand L_N complexed with other metals. This value of $C_{Cu}^{min'}$ was able to be calculated from the value estimated under the conditions of titration, namely, at pH8.15 (Midorikawa and Tanoue, 1994). The complexation equilibrium with regard to the ligand L_N in the bag after demetalization was, however, examined under the conditions of electrodialysis (namely, at pH5.71) used in the present study. The total concentration of free and protonated forms of L_N at pH5.71 is higher than that at pH8.15 because the protonation involving the ligand L_N would be enhanced with decreasing pH in such a range of pH, in accordance with the general properties of organic ligands (Midorikawa and Tanoue, 1993 b). Therefore, we should reconcile the value of $[L_N']$ estimated from $C_{Cu}^{min'}$ to that under the conditions of electrodialysis. The thermodynamic consideration (Appendix III) suggested that the increment of the total concentration of free and protonated forms of L_N derived from the difference in pH would be insignificant. The upper limit for the total concentration of free and protonated forms of L_N at pH 5.71 is estimated to be not appreciably higher than the value deduced from the sensitivity limit $C_{Cu}^{min'}$ in the copper-titration experiment. It is concluded that metal-competition reactions have no serious influence on the estimation of the lower limit for K'_{CuL_N} . Thus, the net effect of these competitions is considered to be insignificant, regardless of the concentrations of other metals.

With regard to most of the heavy metals, their kinetics of the metal-exchange reactions during the copper titration would correspond to the second case. Because the kinetic reactivities of the sluggish metal complexes, such as those of Cr(III) and Fe(III), are intrinsically so low (Margerum et al., 1978), metal-exchange reactions between such metals and the exogenously added copper could not occur at all. The ligand L_N complexed with the fast-reacting metals (*i.e.*, with high water-exchange rate constants), such as Pb(II) and Cd(II), also could hardly be titrated by copper because of the slow metal-exchange reaction (Hering and Morel, 1989). In conclusion, it is considered that these metal-competition effects would not have any serious influence on the estimation of K'_{CuL_N} at pH5.71 in any case. Such competitive effects of other metals lead to the underestimation of the total concentration of L_N (C_{L_N}). We do not know whether or not the ligand " L_N " for each metal is the same as that for copper, although consideration on the coordination chemistry of the synthetic chelating reagents suggests that perhaps by analogy the ligand L_N for copper can interact with Zn(II), Cd(II), Pb(II), Co(II) and Mn(II) similarly. The interactions of the L_N -type of ligand with a variety of metals need to be investigated further.

(2) *The model by Midorikawa and Tanoue (1993a)*

In the model presented in part I of this series (Midorikawa and Tanoue, 1993a), the behavior of the strong ligand in the sample solution was investigated by means of thermodynamic calculations based on the experimental results of copper speciation after demetalization at pH5.71 and copper titration at pH8.15. A more realistic value for the free ligand concentration of the strong ligand, $[L_N']$, which was not measurable experimentally among species in equation (8), under the conditions at pH5.71 was tentatively estimated from the difference between the value for C_{L_N} estimated at pH8.15 and that for $[CuL_N]$ measured at pH5.71. With respect to the value for $[CuL_N]$, the occurrence of other metals and the

effect of pH have no influence on its value since $[\text{CuL}_N]$ was calculated only from the results of copper determination: C_{Cu} in the sample and $[\text{CuY}]$ in the control at pH5.71. Although the value of C_{L_N} is independent of the change in pH, the competitive reactions for L_N with other metals need to be considered on the similar effects of the kinetics of their metal-exchange reactions during copper titration as discussed above. In this section, the model by Midorikawa and Tanoue (1993a) is reassessed in detail on the effects of other metals and associated effects of pH on the evaluation of K'_{CuL_N} .

When other metals complexed with the ligand L_N are completely displaced by the exogenously added copper, the observed value for C_{L_N} corresponds to the actual total concentration of L_N present in the sample. In this case, however, $[\text{L}_N']$ obtained as the difference between C_{L_N} and $[\text{CuL}_N]$ includes the concentrations of the free and protonated forms of L_N , and L_N in the forms of complexes with other metals at pH5.71. When the significant levels of other metals are present, this overestimation of $[\text{L}_N']$ may lead to the underestimation of K'_{CuL_N} by the value corresponding to the total concentration of L_N complexes with other metals (displaceable by copper) at pH5.71.

When other metals complexed with the ligand L_N are not displaced at all by the exogenously added copper, the observed C_{L_N} is an underestimate. The value calculated for $[\text{L}_N']$ corresponds to the total concentration of the free and protonated forms of L_N at pH8.15, which is lower than that at pH5.71. Although this effect with decreasing in pH may lead to the overestimation of K'_{CuL_N} , its magnitude is concluded to be insignificant for the same reason that was discussed above on the model by Midorikawa and Tanoue (1994).

The effects of other metals on EDTA species ($[\text{CuY}]$ and $[\text{Y}']$) are the same as those in the model by Midorikawa and Tanoue (1994) mentioned above. Consequently, it was found that, in the model presented in part I of this series (Midorikawa and Tanoue, 1993a), the metal competitions with other metals have the same influence on the estimation of K'_{CuL_N} as in the model by Midorikawa and Tanoue

(1994). It is considered that such effects would not lead to serious errors to the final estimation of K'_{CuL_N} , although the magnitude of the errors would become relatively large as compared with that in the model by Midorikawa and Tanoue (1994) because the estimated values of $[\text{L}_N']$ were at a very low level in the model by Midorikawa and Tanoue (1993a).

4. Conclusions

With regard to both the procedure of demetalization employed by Midorikawa and Tanoue (1994) and equilibrium speciation of copper by their model, their kinetic aspects were examined. It is unlikely that the slow coordination reactions reported by Hering and Morel (1989) exert their influence on the ligand-exchange reaction between the strong ligand and EDTA in the demetalization process. The competitive reactions for copper complexation of the strong ligand with both EDTA and other metals seem to have no serious influence on the estimation of the lower limit of the conditional stability constant of the strong ligand, although their effects were not quantitatively estimated because of the paucity of information on the kinetics of complexation reactions concerning natural ligands in seawater.

It was confirmed that most of the assumptions introduced by Midorikawa and Tanoue (1994) in their equilibrium model may lead to a conservative estimate of the conditional stability constant of the strong ligand. With regard to the tentative calculation of more realistic values of the conditional stability constant of the strong ligand by Midorikawa and Tanoue (1993a), it was concluded that such competitive reactions would similarly have no serious effect on its estimation. The quantification of more realistic complexing ability of the strong ligand requires the more detailed studies on both thermodynamics and kinetics of its complexation and its chemical characterization.

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銅に対して強い錯形成能を有する天然水中の有機配位子の定量法に関する理論的解析

II. 銅と天然有機配位子との錯形成に対する競合反応の速度論的評価

緑川 貴・田上英一郎

銅に対して強い錯化能力を有する天然有機配位子の錯形成と競合する反応の速度論的效果を評価する。本研究において海水から濃縮・脱塩した有機配位子の銅との錯形成に関する副反応としては、海水由来の銅以外の微量金属との錯形成反応及び試料操作 (Midorikawa and Tanoue, 1994) の際添加されたキレート試薬エチレンジアミン四酢酸 (EDTA) との置換反応について検討する必要がある。海水試料の前処理においてアルカリ土類金属は定量的に除去されているので、強い有機配位子とEDTAとの配位子置換反応に関する速度論的な問題はない。強い有機配位子の銅との錯形成反応を速度論的に考察すると、Midorikawa and Tanoue (1994) の平衡モデルにおける試料溶液中の銅EDTA錯体濃度に関する仮定は、強い有機配位子と銅との錯体の条件安定度定数の評価に対して内輪の見積もりを与えることが分かった。また、銅以外の微量金属が共存する場合、銅とEDTAとの錯形成反応に対するこれらの金属の競合効果が、測定精度と同程度の過大評価をもたらすことを、モデル解析により見積もることができた。強い有機配位子自身への他金属の効果は、その配位子濃度の見積もりに対しては過小評価を与え、また条件安定度定数の見積もりに対しては大きな影響は与えないことを評価することができた。

Appendix I: Equilibria between EDTA and other metals

Competitive reactions between EDTA and other metals can be taken into account as side reactions involving free EDTA (Y) in the equilibrium (6), in addition to the protonation reaction involving EDTA in the above model. The concentration of EDTA not bound to copper, $[Y']$, is obtained by combining the corresponding equilibrium constants with the mass-balance equation for EDTA:

$$[Y'] = [Y] + \sum_p [H_p Y] + \sum_j [M_j Y] \quad (M_j \neq \text{Cu})$$

$$= [Y](1 + K_1[H] + K_1 K_2 [H]^2 + \dots + \sum_j K_{M_j Y} [M_j])$$

$$= [Y] \cdot \alpha_Y \quad (10)$$

$$\alpha_Y = \alpha_{Y(H)} + \sum_j K_{M_j Y} [M_j] \quad (11)$$

where $H_p Y$ and $M_j Y$ are the protonated and metal-complexed species of EDTA other than copper-EDTA complex, respectively; K_1 and K_2 are the stepwise protonation constants for EDTA; $K_{M_j Y}$ is the stability constant for the complexation of the j th type of metal ion, M_j , with EDTA; α_Y is the overall side-reaction coefficient (Ringbom and Still, 1972) of EDTA; and $\alpha_{Y(H)}$ is the side-reaction coefficient for the protonation of EDTA.

The preliminary ICP-AES measurement of levels of metals was performed using a control solution, which included EDTA, without the addition of any metal ions other than copper initially. Although it is difficult to estimate the control values for most of the metals from small differences in metal concentrations

Table 1 Parameters for EDTA speciation and estimated concentrations of EDTA complexes in the control bag at an ionic strength of zero, pH5.71, and a temperature of 4°C.

Mj	$\alpha_{M_j}^a$	$\log K_{M_j Y}^b$	C_{M_j} (nM)	Estimated ^c [MjY] (nM)
Cu	1.0012	20.74	5.3	5.3
Zn	1.0001	18.30	8.1	8.1
Cd	1.00001	18.45	1.4	1.4
Pb	1.0023	20.20	3.9	3.9
Co	1.00002	18.08	1.9	1.9
Ni	1.00002	20.53	4.3	4.3
Mn	1.000002	15.65	1.6	1.5
Ag	1.0000001	7.32	2.0	2×10^{-7}
ΣM_j ($M_j \neq \text{Cu}$)			23.2	21.1

^a Inorganic side-reaction coefficient, α_{M_j} , at pH5.71 was calculated by using the literature values of Turner and Whitfield (1987) for copper and those of Turner et al. (1981) for other metals; the thermodynamic stability constants of hydroxides and carbonate for each metal at infinite dilution and at 25°C were used after correction for a temperature of 4°C, using the respective values of the enthalpy, ΔH , estimated by the method of Byrne et al. (1988).

^b The thermodynamic stability constant of EDTA complex, $K_{M_j Y}$ at infinite dilution and at 4°C was calculated as follows: the constant for copper, K_{CuY} , was taken from the literature (the value at infinite dilution and at 25°C; Turner and Whitfield, 1987) and was corrected for a temperature of 4°C, using the value of the enthalpy, ΔH , given by Martell and Smith (1974); for other metals, the conditional constants at an ionic strength of 0.1 M and at 25°C by Martell and Smith (1974) were corrected for ionic media (Na or K; Turner and Whitfield, 1987) and for temperature in a similar manner to that in the case of copper.

^c This result yields $[Y'] = 55.7$ nM, $[Y]$. $\alpha_{Y(H)} = 34.6$ nM and $\alpha_Y = 10^{7.17}$.

between the control and the blank for the digestion with acids, the following metals were detected in the control solution at significant levels: Zn, Cd, Pb, Co, Ni, Mn and Ag (Table 1). The concentration of each metal-EDTA complex was estimated by a combination of the experimental results from measurements of total concentrations (C_{M_i}) of metals and the calculations using the literature values of the stability constants of their EDTA complexes and inorganic species after corrections for the present conditions at an ionic strength lower than $10^{-5}M$, pH 5.71 and a temperature of $4^\circ C$ (Table 1, footnote a, b). It was found that Zn, Cd, Pb, Co and Ni were completely complexed with EDTA, Mn was partially dissociated from such a complex and Ag was completely dissociated from such a complex in the control solution. These results of EDTA speciation for the control solution may be applicable to the sample containing additional other metals originated from seawater without any correction because $[CuY]$ detected in the control after demetalization was not dependent on the initial C_{Cu} (Midorikawa and Tanoue, 1994). The composite concentration of metals complexed with EDTA, apart from copper, in the control solution after demetalization was calculated to be $21nM$. This value yields an overall side-reaction coefficient, α_Y , of $10^{7.17}$, which is not very different from the side-reaction coefficient for the protonation reaction alone, $\alpha_{Y(H)}$, of $10^{6.96}$ used for the correction of K'_{CuY} in equation (8) in the estimation of K'_{CuL_N} by Midorikawa and Tanoue (1994). These metal-competition effects finally lead to an overestimation of $\log K'_{CuL_N}$ by the difference between $\alpha_{Y(H)}$ and α_Y , namely, 0.21 (the logarithmic value). The effect of competition by trace metals for EDTA on the estimation of K'_{CuL_N} was found to be comparable in magnitude to the errors in the estimation of K'_{CuL_N} (Midorikawa and Tanoue, 1994). This relatively small effect is due to the high value of $\alpha_{Y(H)}$ ($10^{6.96}$) because the concentration of free EDTA, $[Y]$, is predominantly controlled and maintained at a low level by the protonation reaction for Y, that is, EDTA is under "the condition of ligand buffered" by protonation at the relatively low pH (5.71)

used in the present study.

Appendix II: *Equilibria between the ligand L_N and other metals in the case of rapid metal competition*

When there is significant competition between copper and other metals in complexing with the ligand L_N , such metal-competition effects can also be considered as side reactions of the ligand L_N in the equilibrium (6) for copper as well as the protonation of L_N . The concentration of the ligand L_N not bound to copper, $[L_N']$, is given, in analogy with equation (10) in the case of EDTA, as follows:

$$\begin{aligned} [L_N'] &= [L_N] + \sum_q [H_q L_N] + \sum_j [M_j L_N] \quad (M_j \neq Cu) \\ &= [L_N] \cdot \alpha_{L_N} \end{aligned} \quad (12)$$

where $H_q L_N$ and $M_j L_N$ are the protonated and metal-complexed species of L_N other than CuL_N complex, respectively. The overall side-reaction coefficient of L_N , α_{L_N} , is represented by using the side-reaction coefficients of L_N for the protonation, $\alpha_{L_N(H)}$, and for the competition with other metals, $\alpha_{L_N(M)} = \sum_j K_{M_j L_N} [M_j]$, as follows:

$$\alpha_{L_N} = \alpha_{L_N(H)} + \alpha_{L_N(M)} \quad (13)$$

The conditional stability constant for the copper complex of the ligand L_N , K'_{CuL_N} , is defined as follows:

$$\begin{aligned} K'_{CuL_N} &= \frac{[CuL_N]}{[Cu][L_N']} \\ &= \frac{[CuL_N]}{[Cu][L_N] \cdot \alpha_{L_N}} \end{aligned} \quad (14)$$

In the case of rapid metal-exchange reactions during the copper titration, the measured value of $[L_N']$ includes the concentration of all the ligand L_N complexed with the competing metals as well as those of free and protonated forms of L_N . From equation (13), therefore,

$$K'_{CuL_N} = \frac{[CuL_N]}{[Cu][L_N] (\alpha_{L_N(H)} + \alpha_{L_N(M)})} \quad (15)$$

The conditional stability constant $K'_{CuL_N(H)}$ that is controlled by the protonation reactions but is independent of the existence of other metals, which is our purpose in the present study, is written as follows:

$$K'_{\text{CuL}_N(\text{H})} = \frac{[\text{CuL}_N]}{[\text{Cu}][\text{L}_N] \cdot \alpha_{\text{L}_N(\text{H})}} \quad (16)$$

In estimating the conditional value for $K'_{\text{CuL}_N(\text{H})}$ on the protonation alone, the use of the value of $[\text{L}_N']$, which includes the metal-competition effects, obtained from copper titration leads to an underestimation. A comparison between equations (15) and (16) indicates that the magnitude of the underestimation corresponds to the effect of the side-reaction coefficient for the competition with other metals than copper, $\alpha_{\text{L}_N(\text{M})}$.

Appendix III: Equilibria between the ligand L_N and other metals in the case of slow metal competition

When other metals complexed with the ligand L_N are not displaced at all by the exogenously added copper during the copper titration, only the free and protonated forms of L_N can be determined by the copper-titration method, irrespective of the presence of the ligand L_N complexed with other metals. In this case, in estimating the total concentration of free and protonated forms of L_N in equilibrium at pH5.71 under the conditions of electro-dialysis, based on the results obtained from copper titration at pH8.15, the increase in the concentration with decreasing pH needs to be considered as well as the metal-competition effects. In order to estimate the increment of this concentration resulting from the difference in pH, we must determine the extent to which other metals are complexed with the ligand L_N at both pH's as well as the dependence of K'_{CuL_N} on pH. Lack of knowledge on the acid-base properties of the ligand L_N and the extent of organic complexation of these metals allows us only to speculate on it. However, the following analysis based on the side-reaction coefficient of the ligand L_N at each pH may provide useful information on such effects.

The distribution of L_N species between protonated forms and other metal complexes is defined by the magnitude of $\alpha_{\text{L}_N(\text{M})}$ and $\alpha_{\text{L}_N(\text{H})}$ at each pH, as represented in equations (12) and (13). The metal-competition effects are exceedingly significant in the case where

$$\alpha_{\text{L}_N(\text{H})} \text{ at pH8.15} \ll \alpha_{\text{L}_N(\text{M})} \ll \alpha_{\text{L}_N(\text{H})} \text{ at pH5.71} \quad (17)$$

In other cases, such effects have little influence on the estimation of K'_{CuL_N} because the changes in the total concentration of other metal complexes with L_N accompanied with alternations in pH are very small. In the case shown in equation (17), the difference in the value of α_{L_N} between the two pH's approximately corresponds to that between $\alpha_{\text{L}_N(\text{M})}$ at pH8.15 and $\alpha_{\text{L}_N(\text{H})}$ at pH5.71; the ligand L_N not bound to copper is predominantly in the forms of complexes with other metals at pH8.15 and in the protonated form at pH5.71. In this case, therefore, the estimation of K'_{CuL_N} at pH5.71 based on the low value of $[\text{L}_N']$ at pH8.15 may lead to overestimation. However, the above conditions require a great difference in the value of $\alpha_{\text{L}_N(\text{H})}$ between the two pH's, as well as the occurrence of high levels of free metal ions, which can complex with L_N , at pH5.71. It is unlikely that the ligand L_N is of this type because the difference is only 2.97 (as a logarithmic value) even for EDTA. Indirect evidence was also found from experimental results. When the sample solution demetalized at pH5.71 was titrated by copper at pH 8.15, additional formation of the CuL_N complex did not occur to any significant extent during the titration (Midorikawa and Tanoue, 1993a). This result indicates that the increment of the total concentration of free and protonated forms of L_N derived from the dissociation of the CuL_N complex with decreasing pH (*i.e.*, from 8.15 to 5.71) is not significant. With regard to other metals, these complexation reactions are similarly controlled by the side reaction for protonation of the ligand L_N , whose effect is identical to that in the case of copper [*i.e.*, the common effect of $\alpha_{\text{L}_N(\text{H})}$ in equation (13)]. Thus, it is anticipated that the degree of dissociation of these complexes with decreasing pH would also be insignificant.

The ligand L_N is not in forms of any unstable complexes with other metals because there exist significant amounts of free EDTA in the demetalized solution at pH5.71. It is impossible that high levels of heavy metals are in the form

of free metal ion in the presence of excess EDTA (*i.e.*, under "the condition of metal buffered" analogously to that in the case of copper mentioned in section 3. 2). Even if the total concentration of the ligand L_N complexed with these metals was high, $[M_j]$ at pH5.71 is suppressed to be very low as well as copper. The composite concentration of other metal- L_N complexes formed at pH8.15 corresponding to their respective low values of $[M_j]$ at pH 5.71 would be low; as the reverse process, the

contribution of dissociation of such complexes with decreasing pH to the total concentration of free and protonated forms of L_N would also be small. These effects result from the removal of metals and metal-buffering capacity by EDTA in the demetalization process. Although the values of side-reaction coefficients of L_N have not been known, it is concluded that the ligand L_N would not be in the case shown in equation (17).