

Chemical Speciation of Trace Metals in Seawater: a Review

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The recent development of the chemical speciation of trace metals in seawater is described. The speciation studies reveal that metal ion complexation is one of the most important processes in seawater; especially, most bioactive trace metals, such as Fe(III) and Cu, exist as complexes with ligands in dissolved organic matter. The organic ligands in seawater are characterized with metal ions selected by the HSAB concept. A strong organic ligand, which originates from marine microorganisms, is classified as a hard base including carboxylates. The free organic ligand concentrations in seawater are buffered by complexation with excess amounts of Ca and Mg in seawater. The chemical equilibrium model suggested that the concentrations of bioactive free metal ions are at an optimal level to activities of marine microorganisms. For chemical speciation, it is important to have a better understanding of the ecological roles of trace metals in seawater.

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1 Introduction

The chemical speciation of elements in aquatic environments is one of the most important topics in the fields of analytical chemistry, geochemistry, toxicology, and environmental chemistry.¹ Chemical speciation is essential for discussing the chemical reactivity of trace constituents in the environment, such as biological availability and toxicity, and the geochemical behavior of chemical species. The term “chemical speciation” has been defined in IUPAC Recommendations 2000.² In this recommendation, the term “chemical species” is utilized as a specific form of an element: isotopic composition, electronic or oxidation state, and complex or molecular structure. “Speciation analysis” is an analytical process for identifying and/or measuring the quantities of one or more individual chemical species in a sample, and “speciation of an element” implies to know the distribution of an element amongst defined chemical species in a system. The term “fractionation”, which is distinguished from speciation, is a process involving the separation of an analyte or a group of analytes from a certain sample according to physical (*e.g.*, size, solubility) or chemical (*e.g.*, binding, reactivity) properties. In this review, our attention is focused on the speciation analysis of trace metals in seawater, and appropriate topics of fractionation are also included.

Many analytical techniques have been developed for the chemical speciation of elements in environmental samples: typically, sequential leaching methods,³⁻⁷ hyphenated techniques,⁸⁻¹² such as GC-ICP-MS (analysis of organometallic

elements), X-ray spectroscopic techniques and others.¹³⁻¹⁵ These methods provide significant information on the chemical forms of elements in the environment. However, they are not sufficient to discuss the ecological roles of chemical species in seawater, because natural systems are generally very complicated and the concentrations of target species are extremely low.¹⁶

Seawater is a chemically complicated system because many kinds of inorganic and organic chemical species are dissolved in solution of high salt concentrations. In the 1970s and 1980s, the concentration levels of trace metals in seawater were established as a result of the development of analytical techniques.¹⁶⁻²² The concentration of bioactive trace metals, such as Cu, Zn, Fe, is on the order of nM or less in the open ocean. On the other hand, most of the dissolved organic matter in seawater, which is 40–60 μM in the oceanic surface waters, has not yet been identified.²³

The chemical speciation of trace inorganic and organic constituents in oceanic water samples has been studied for more than four decades.²⁴⁻²⁶ Until the 1970s, the subject of study had been confined to the interaction of metal ions with inorganic ligands, such as halogens, carbonate and hydroxide. However, these studies on inorganic speciation are evidently insufficient to understand the ecological roles of trace metals in the marine environment. The purpose on chemical speciation is to clarify the chemical forms of trace metals in seawater and their controlling processes from both chemical and geochemical aspects. It is also important to elucidate the chemical form of trace metals related to their acquisition by phytoplankton in seawater.

There has been interest in the effect of trace metals on the growth of phytoplankton in natural water. A typical metal is copper, which is a bioactive element as a component of respiratory proteins and oxidases.^{27,28} However, higher Cu²⁺ concentrations have caused reduction of the cell-division rates of phytoplankton.^{27,29,30} In the 1970s, laboratory studies^{31,32} revealed that Cu toxicity is related to the free metal ion concentration; chelation by chelators reduces the Cu toxicity to phytoplankton growth. The Cu toxicity depends on the species of marine microorganisms; cyanobacteria are one of the most sensitive species to Cu toxicity in the laboratory.^{28,30} Another representative trace metal in the marine environment is Fe, which is one of the most important bioactive elements for phytoplankton growth.³³ In contrast to Cu, Fe deficiency occurs in the wide-area of the open ocean.³⁴⁻³⁶ Especially, Fe is a limiting factor in the primary production of phytoplankton in high-nutrient low chlorophyll (HNLC) regions. These findings suggest that trace metals play an essential role in the marine ecosystem.

In this paper, the recent development of chemical speciation of trace metals, *i.e.*, Cu, Zn, Fe and others, in seawater is summarized. Sound knowledge on metal complexation in solution is essential for understanding trace metal species in seawater and the chemical properties of the relevant ligands.

2 Organic Complexes of Trace Metals

Strong metal complexes with organic substances had been recognized in seawater before 1980.³⁷⁻⁴¹ The development of bioinorganic chemistry has revealed the presence of strong organic metal complexes in biological systems;^{27,28} some metals (Fe, Cu, Zn, *etc.*) occupying the reactive center in enzymes, and highly stable complex, such as Fe(III) complexes of siderophores,⁴² were also discovered.

The most important parameter to characterize metal complexation with organic ligands is the stability constant, which is thermodynamically unique for a complex between a metal ion and an organic ligand.⁴³ The stability constant is determined by the chemical properties of both organic ligands and metal ions. If we can determine a rigorous stability constant of the complex of an organic ligand with a metal ion, we can evaluate the chemical properties (a kind of donor atoms, number of donor atoms and others) of the organic ligand and the relevant metal complex by a comparison with the values determined for metal complexes with well-known ligands. The concentrations of the organic ligands in seawater can be determined as well as the conditional stability constants of natural metal complexes. The concentration level of the organic ligand in seawater is another significant parameter to know sources of the organic ligands and their ecological roles.

Since 1980, the complexing characteristics of seawater have been widely studied for several transition metals (*e.g.* Cu, Zn) using two distinct methods, *i.e.* metal titration and ligand titration; studies are mostly focused on copper because of its strong complexation property and usefulness as an analytical tool. For metal titration, MnO₂ adsorption,^{44,45} electrochemical,⁴⁶⁻⁵⁵ chromatographic,^{56,57} bioassay,⁵⁸ solvent extraction,^{59,60} and chemiluminescence techniques⁶¹ have been used to measure the concentrations of free metal ions in solution. However, recent studies have focused on the electrochemical technique with ligand competition.⁶²⁻⁶⁶ On the other hand, ligand titration, namely competitive reactions between natural and artificial organic ligands, has hardly been developed until initial work with EDTA (ethylenediaminetetra-

acetic acid) carried out in 1982.⁶⁷

Recent experimental studies on copper speciation in seawater have revealed more than 99% of copper to be present as organic complexes, of which the conditional stability constants are in the range between 10⁹ and 10¹⁵ M⁻¹. In these studies, the conditional stability constant, K_{ML}^c , is defined as

$$K_{ML}^c = [ML][M^{n+}]^{-1}[L']^{-1}, \quad (1)$$

where [ML], [Mⁿ⁺] and [L'] denote the metal complex, free metal ion and total ligand concentrations, respectively. The ligand in seawater is present as complexes with other metal ions in the presence of excess amounts of the other metal ions (*e.g.*, Ca²⁺, Mg²⁺ and others). Therefore, the total ligand concentration can be expressed as the product of the free ligand concentration and the side-reaction coefficient of the ligand. The side-reaction coefficient of the ligand in natural waters, $\alpha_{L(H,N)}$, is given as

$$\alpha_{L(H,N)} = 1 + \sum \beta_{HL}[H^+]^i + \sum K_{NL}[N^{n+}], \quad (2)$$

where β_{HL} and K_{NL} show the protonation constants of the ligand and the stability constant of complexes with other metal ions (Nⁿ⁺), respectively. It must be noted that seawater is regarded as a ligand buffer⁶⁸ of L in large excess concentrations of Ca and Mg. In this case, the conditional stability constants of a trace metal complex in seawater may be significantly lower than that in fresh water.

Hirose⁶⁹ has attempted to classify strong organic ligands in seawater by means of the conditional stability constants of metal complexes and the actual ligand concentration. According to the conditional stability constants of organic Cu(II) complexes, organic ligands in seawater are conveniently divided into three classes: the strongest, L₁, ($\log K_{CuL}^c > 13$);^{53,58,61,67,70-72} the strong, L₂, ($\log K_{CuL}^c \sim 12$);^{58,59,62,67} and the weak ligand, L₃, ($\log K_{CuL}^c < 10$).^{44,46,53} It must be noted that we have found no sample having all of these kinds of organic ligands. As a general trend, the concentrations of the organic ligands complexed with Cu(II) are in the following order: L₁ < L₂ < L₃. Because the total concentration of Cu(II) dissolved in seawater is usually less than that of organic ligands, L₂, L₁, or L₁ + L₂, it is evident that most of the organic ligand, L₃, is not bounded with Cu(II) under the conditions of seawater; in other words, most of the Cu(II) is complexed with the organic ligands, L₁ and/or L₂. This situation is consistent with the fact that the organic ligand, L₃, cannot be detected by the ligand titration method.⁶⁷

The chemical speciation of Zn(II) in seawater has been studied by several workers,⁷³⁻⁷⁷ although inorganic speciation of Zn(II) in seawater has been carried out from the initial stage.^{25,26,78,79} The conditional stability constants of organic Zn(II) complexes are divided into three classes, as for Cu(II) complexes. The logarithmic conditional stability constant of Zn(II) with the L₁-type, L₂-type and L₃-type ligands is around 11,^{67,75,76} 9,^{67,73,77} and 7.5,⁷⁴ respectively. The conditional stability constants of organic Zn(II) complexes are systematically lower than those of Cu(II). This tendency is reasonable according to Irving-Williams series for transition metals,⁴³ although there is no discussion about whether the organic ligands associated with Zn(II) are the same as those of Cu(II) or not.

In the 1990s, chemical speciation of iron in seawater has been extensively studied because iron is a biologically essential trace metal related to phytoplankton growth in oceanic water.³⁴⁻³⁶ Hudson *et al.*⁸⁰ suggest that organic complexation of Fe(III) is

possible in seawater from the results of complexation between Fe and the microbial siderophore and the synthetic chelator in synthetic and coastal seawater. Hudson and Morel⁸¹ have studied Fe transport *via* marine phytoplankton cell surface, in which a half-saturation constant for the Fe cell surface complex of phytoplankton was measured kinetically. The conditional stability constant of the Fe³⁺ cell surface complex was evaluated to be 20.2 (= $\log K_{\text{FeL}}$).⁸¹ Studies on the chemical speciation of Fe(III)^{64,65,82,83} revealed that the conditional stability constant of the Fe(III) complexes dissolved in seawater is very high ($20.5 < \log K_{\text{FeL}} < 22.5$). It should be noted that the concentration of the strong organic ligand determined for Fe complexation^{64,65,82,83} was on the order of nM, which is the same order of magnitude as that detected for Cu and Zn.^{53,58,61,67,70-72,75,76} This finding strongly supports that the stronger organic ligand, which is not specific to Fe(III), such as siderophores, exists at the order of nM in seawater.

Thorium in seawater has been extensively studied in the field of chemical oceanography, in which a major topic has been removal processes of thorium isotopes by sinking particles in the ocean interior.⁸⁴⁻⁸⁶ A typical feature for Th in seawater is that the concentration level (0.02 – 2 pM) of ²³²Th⁸⁷⁻⁹² is like that of the supersaturation of an inorganic salt (solubility of thorianite, about 0.01 pM).⁹³ In order to understand the mechanism of scavenging and dissolution phenomena of Th in the ocean, it is also significant to see the chemical form of both particulate and dissolved Th in seawater. A sequential leaching study⁶ suggests that Th in oceanic particulate matter is present as organic complexes. Dissolved Th is also considered to be complexes with humic substances.^{94,95} The speciation of thorium in oceanic particulate matter⁹⁶ has revealed that Th in oceanic particulate matter exists as a complex with the L₁-type organic ligand, of which the conditional stability constant was estimated to be $10^{23.8} \text{ M}^{-1}$. Natural organic metal binding sites in dissolved organic matter and particulate matter are of a kind of polyaminopolycarboxylate ligands containing many numbers of carboxyl groups, such as DTPA (diethylenetrinitriolpentaacetic acid) and EDTA. Santschi *et al.*⁹⁷ have suggested that carboxyl groups in colloidal organic matter in seawater are responsible for the adsorption of thorium.

The most important question is whether the strongest L₁-type ligand reacting with Cu is chemically the same ligand complexing with other metals, such as Fe(III) and Zn. Linear free energy relationship (LFER) techniques for metal complexation have been developed for the determination of missing values of the metal complex formation constant in natural water.^{98,99} The LFER techniques can be used to characterize the chemical properties of unknown ligands by comparisons with the stability constants of metal complexes having known synthetic ligands. Figure 1 shows the log-log relationship between the stability constants of metal complexes with DTPA and the L₁-type ligand. The fairly linear relationship with a slope of unity indicates that the L₁-type ligand reacting with Cu, Zn, Fe, Th and others in seawater is chemically similar to DTPA.

We now summarize speciation studies of trace metals in seawater. In order to characterize the chemical species of trace metals in seawater, methodologies involving both metal-exchange and ligand-exchange reactions have been applied. It must be noted that these methodologies implicitly contain the assumption of reversibility to reactions of natural organic ligands with metal ions. As a result, most bioactive trace metals in seawater exist as complexes with natural organic ligands. Therefore, the existence of strong organic ligands is common in marine environments.

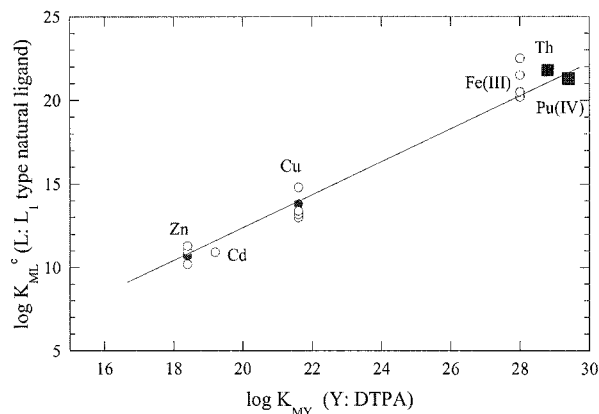


Fig. 1 Log-log plots of the conditional stability constant of metal complexes with the L₁-type ligand to the stability constants of metal complex with DTPA. The slope is unity. Dissolved complexes (closed circle, Ref. 67; open circle, Refs. 53, 58, 61, 64, 65, 75, 76, 82, 83, 146), particulate complexes (closed square, Refs. 96, 147).

3 Chemical Properties of Organic Ligands in Seawater

Metal ions are useful to characterize organic ligands dissolved in seawater. Copper is a candidate due to its strong complexation ability. The study of Cu-complexing capacity of dissolved organic matter in natural water has been developed since about 1970.^{100,101} Measurements of the metal complexing capacity of natural water have been widely adopted as a methodology for chemical speciation. The complexing capacity of a sample, defined as moles of added metal (usually Cu²⁺), which are complexed per liter of sample, is a measure of the metal-buffering capacity, and is of fundamental importance for a quantitative assessment of the fate of polluting metals in natural water.¹⁰⁰ The complexing capacity is also a measure of the abundance of the organic ligand in natural water. However, Cu is insufficient to specify organic ligands because Cu²⁺, belonging to boundary metal ions in Pearson's classification,¹⁰² forms complexes with ligands having oxygen, nitrogen and sulfur as a donor atom. In order to characterize organic ligands in natural water, the use of typical hard or soft metals is effective. Recently, organic speciation studies based on this concept have been carried out.

Thorium is a typical hard acid, which reacts with hard bases having oxygen as a donor atom.¹⁰² According to thermodynamic considerations,⁹³ the affinity of Th(IV) to organic ligands in acidic solutions is larger compared to other metals because the effect of side-reactions of hydrolysis for Th is larger than that for the other metal ions, such as Cu(II), Fe(III) and others. In the respect, the reactivity of organic ligands for metal ions is largely suppressed by the protonation on basic sites of organic ligands. These findings suggest that an experiment under acidic conditions is an effective approach for studying the chemical interactions of Th with natural organic ligands. Acidic experimental conditions yield some merits; the system can be simplified to a singular reaction between Th and organic ligands without other complicated and complexing side-reactions. The effects of side reactions due to complexation with endogenously occurring metals in natural organic matter as well as contaminated metals are eliminated during the experimental processes.

Taking into account the merit of using thorium isotopes for

organic characterization, Hirose and Tanoue¹⁰³ characterized organic ligands in oceanic particulate matter, and introduced a new parameter for estimating the complexation abilities of particulate matter, namely, the Th-complexing capacity of particulate matter. An adsorption experiment to particulate matter in a 0.1 M solution of HCl revealed that thorium reacts with a strong organic ligand in particulate matter, and forms a 1:1 complex with the ligand in particulate matter under the experimental conditions. The Th-complexing capacity (ThCC) is defined as the total concentration of a strong organic ligand in particulate matter reacting with Th in an acidic medium. Since there is only a single reaction process for the Th adsorption onto particulate matter in 0.1 M HCl solution, the Th complexing capacity of particulate matter is expressed as

$$\text{ThCC} = [\text{ThL}_p] \{1 + (K_{\text{ThL}}[\text{Th}^{4+}])^{-1}\}, \quad (3)$$

where K_{ThL} shows the conditional stability constant of an organic Th complex in particulate matter, and $[\text{ThL}_p]$ and $[\text{Th}^{4+}]$ denote the concentrations of the adsorbed Th and the free Th ion, respectively. The conditional stability constant of the organic Th complex in particulate matter was evaluated to be $10^{6.6} \text{ M}^{-1}$ in the 0.1 M HCl solution. It is noted that the ThCC, the concentration of the strong organic ligand, is a powerful tool as a new quantitative indicator to characterize organic matter in seawater.

Metal exchange reactions are useful tools to understand the chemical properties of the strong organic ligand identified by Th. Hirose and Tanoue¹⁰³ examined the metal-exchange reactions between typical hard acids, *i.e.*, Th and Fe(III). The metal-exchange equilibrium constant between Fe(III) and Th for the binding site in particulate matter can be directly compared with the corresponding constant for the natural organic ligand in seawater, because the side-reaction coefficients of proton and other metals for the organic ligand are not different for Th and Fe(III), and side-reaction coefficients of hydrolysis for Th and Fe(III) are negligible in 0.1 M HCl solution. Furthermore, a theoretical consideration¹⁰⁴ suggests that the conditional stability constant of the metal complex dissolved in seawater should be equal to the corresponding constant for the binding site in particulate matter, provided that the ligand in dissolved organic matter is chemically the same as that in particulate matter. Hirose and Tanoue¹⁰³ experimentally obtained the logarithmic value of -1.7 for the metal exchange equilibrium constant of Fe(III) with Th. The speciation study of Th provides estimates of the conditional stability constants ($10^{21.8} \text{ M}^{-1}$: defined by the Eq. (1)) of the Th complex in oceanic particulate matter.⁹⁶ The conditional stability constant of the Fe(III) complex with the ligand in particulate matter is estimated to be $10^{20.1} \text{ M}^{-1}$ from the metal-exchange equilibrium constants of Fe(III) with Th, which is in good agreement with that determined for dissolved Fe complex in seawater ($20.5 < \log K_{\text{FeL}} < 22.5$).^{64,65,82,83} On the other hand, the logarithmic metal exchange equilibrium constants of Fe(III) with Th for the L_1 -type and L_2 -type were calculated to be -1.0 and 1.9 from the estimated conditional stability constants of Th and Fe(III) complexes in seawater, respectively.^{69,103} Taking into account of some uncertainties due to the difference of the experimental conditions and in the model, the chemical properties of the binding site in particulate matter identified by Th are more similar to that for the L_1 -type organic ligand in seawater. This type of organic ligand corresponds to the strongest one for the complexation of Cu and Zn, which were determined by using ligand competitive metal titration techniques.^{53,58,61,67,70-72,75,76}

Soft acids, such as Ag(I), Hg(II), are also useful tools to

characterize organic ligands in natural water. Most soft metal ions, such as Hg(II) and CH_3Hg^+ , have high biological toxicity. The complexation of natural ligands with soft donors is important to mitigate any biological toxicity of soft metal ions. Scott Smith *et al.*¹⁰⁵ determined strong ligands in sewage effluents-impacted water and terrestrial water by competitive ligand titration with Ag. Silver ion can be used as a probe to determine the ligand concentrations and formation constants of Ag complexes. There are two advantages in using Ag(I) as a probe metal: one is that Ag has only one oxidation state under both mild oxidizing and reducing conditions; the other is that Ag reacts preferentially with S(-II). As a result, it was suggested that metal complexes with ligands containing reduced sulfur exists in natural oxic aquatic environments. In the marine environment, Lagrela and van den Berg¹⁰⁶ indicated that Cu complexes with thiol compounds exist in estuarine water using the cathodic stripping voltammetry (CSV) technique and the determination of the free thiol concentration.

Acid base characters, *i.e.*, the number of acidic and basic sites and protonation constants, are important chemical properties of ligands. The complexation reactions with metal ions under different pH conditions provide information on the acid-base properties of ligands. The thorium adsorption ability onto the oceanic particulate matter has been examined under strongly acidic conditions.¹⁰³ The results reveal that the Th adsorption capacity of a particulate is present even in a 6.9 M HCl solution, although the fraction of Th adsorbed to particulate matter decreased when the acidity of the solution increased. This finding suggests that the binding site in particulate matter shows the property of a strong acid, whose protonation constant is estimated to be $10^{-1.1} \text{ M}^{-1}$. The presence of a strong acid site in the ligand is a characteristic of aminopolycarboxylic acids, such as EDTA and DTPA.

A comparison of the conditional stability constants of the metal complex under different pH conditions provides information about the acid-base properties of the ligand. Hirose⁹⁶ determined the conditional stability constants of the Th complex with the ligand in particulate matter in seawater (pH = 8.2) and in a 0.1 M HCl solution. The difference between two conditional stability constants is due to side-reactions involving protonation on the ligand in particulate matter because the ligand possesses several weak acid residues. The conditional stability constant of the Th complex is given as follows using the side-reaction coefficients of the organic ligand:

$$K_{\text{ThL,SW}} = K_{\text{ThL}} \alpha_{\text{L(H,SW)}} \quad \text{and} \quad K_{\text{ThL,pH=1}} = K_{\text{ThL}} \alpha_{\text{L(H,pH=1)}}, \quad (4)$$

where K_{ThL} is the stability constant of the Th complex with the ligand. The terms $\alpha_{\text{L(H,SW)}}$ and $\alpha_{\text{L(H,pH=1)}}$ are the side-reaction coefficients of protonation to the ligand in seawater and pH = 1 solution, respectively. Hirose⁹⁶ calculated the ratio of the value of $K_{\text{ThL,pH=1}}$ to $K_{\text{ThL,SW}}$ for the L_1 -type ligand. In order to compare the acid-base property of a natural ligand with that of known chelators (aminopolycarboxylic acids), the ratios ($= K_{\text{ThL,SW}}/K_{\text{ThL,pH=1}} = \alpha_{\text{L(H,SW)}/\alpha_{\text{L(H,pH=1)}}$) were also calculated from the protonation constants of known chelators.¹⁰⁷ The result is the following order: L-alanine (8.5) < citric acid (9.3) < NTA (9.6): nitrilotriacetic acid < EDTA (15.1) < CyDTA (15.9): *trans*-1,2-cyclohexylenedinitrilotetra-acetic acid < The natural ligand (17.3) < DTPA (19.8) < TTHA (25.5): triethylenetetranitriolhexa-acetic acid, where the value in parenthesis is the logarithmic ratio ($= \log(K_{\text{ThL,SW}}/K_{\text{ThL,pH=1}})$). Taking into account the LFER between the L_1 -type ligand and DTPA,⁶⁹ and the presence of a strong acid site¹⁰³ in addition to this finding, it may be likely that the L_1 -type ligand is a typical chelator with five acid sites.

Table 1 Conditional stability constants of the Th complex with a strong ligand in marine organisms under the conditions of 0.1 M HCl solution and the concentrations of the strong organic ligand in marine organisms

Organisms	Concentration in organisms (gC/g d.w.)	log K_{ThL} (pH = 1)	Concentration of the ligand (nmol/mg d.w.)	Ratio of ligand to OC (mmol/mol C)
Bacteria				
<i>Shewanella algae</i> ^a	0.148	6.63	51.6	4.2
<i>Alteromonas tetraodonis</i> ^b	0.285	6.88	60.5	2.5
<i>Alteromonas macleodii</i> ^b	0.302	6.81	58.4	2.3
<i>Vibrio alginolyticus</i> ^b	0.215	7.07	56.0	3.1
<i>Flavobacterium marinotypicum</i> ^b	0.273	6.88	96.6	4.3
Zooplankton (copepods) ^a				
91-3A NXX13	0.381	6.74	10.5	0.34
91-3A NGG54	0.409	6.68	7.8	0.23
Phytoplankton (diatom) ^a				
93-4A NXX13	0.292	6.59	8.1	0.33
Particulate matter ^c				
		6.6		Surface 0.9 – 2.4 ^d
				Deep 0.2 – 0.6 ^d
Dissolved organic matter				
		6.7		~0.04 ^e

a. Ref. 133. b. Ref. 134. c. Ref. 103. d. Ref. 148. e. Ref. 135.

4 Ligands in Microorganisms

Bacteria are the most abundant microorganisms on the earth. Such a microorganism has potential ability to change chemical environments: it adsorbs metal ions, transforms their chemical forms by redox reactions and biomineralization, and transports chemical substances.^{108,109} Both living and non-living microorganisms, including microalgae and bacteria, have strong affinity to trace metals.^{110,111} Sakaguchi *et al.*¹¹² have found that some microalgae, such as *Chlorella regularis*, can accumulate large amounts of uranium from fresh water. The adsorption reaction of uranium onto microorganisms is reversible with a time scale of several days. The results suggest that metal ions are associated with the binding site in microorganisms by complexation. Speciation studies on microorganisms¹¹³⁻¹¹⁸ have indicated that microorganisms, especially bacteria, possess a ligand, like carboxylates, reacting with metal ions, such as Th⁴⁺, UO₂²⁺, Fe³⁺, Am³⁺ and other hard acids.¹⁰² Furthermore, it is revealed that bacteria can adsorb cadmium by complexation.¹¹⁹ Beveridge¹²⁰ has shown that the metal binding site in bacteria exists on the cell surface, *i.e.*, cell wall, by using transmission-electron microscope techniques.¹²¹

The source of the strong organic ligands in the ocean is believed to be marine organisms, such as phytoplankton and bacteria. Three production pathways have been proposed for organic ligands produced by marine organisms. The first type is intracellular chelators, such as phytochelatin, induced by exposure to toxic metals.¹²²⁻¹²⁵ The second is the synthesis and release of extracellular chelators, such as siderophores, to enhance metal assimilation.¹²⁶⁻¹²⁸ The marine cyanobacterium *Synechococcus* sp. produces an extracellular chelator with binding characteristics comparable to the strong ligands in seawater (log $K_{CuL} \approx 13$).^{129,130} Finally, adsorption on surface complexation sites can also facilitate metal-ion uptake. In relation to cell-surface chelators, Anderson and Morel¹³¹ have suggested that iron uptake is regulated by Fe complexation at the cell membrane-bound site (phytotransferrin), which is in *pseudo-equilibrium* with free iron ion in the bulk solution. Hudson and Morel⁸¹ demonstrated that cell surface-bound Fe is directly transported into the cell. This result implies that for certain marine phytoplankton Fe transport occurs from specific,

surface-associated sites. Metal uptake experiments¹³² also suggest that phytoplankton, diatoms and dinoflagellate have a metal-reactive cell surface. One might, therefore, hypothesize the presence of cell surface chelators, which play a significant role for the uptake of trace metals. These chelators are evolutionary products of marine organisms adapting to an environment with extremely low levels of bio-available elements, such as the open ocean.

The interaction between marine microorganisms and thorium was studied experimentally in an acidic medium.^{133,134} This speciation study demonstrated that heterotrophic bacteria, as well as phytoplankton and zooplankton,¹³³ possess specific chelators, which may be present in the cell membrane. These chelators form a 1:1 complex with Th in 0.1 M HCl solution. The conditional stability constants of the organic Th complexes in marine bacteria range from 10^{6.63} to 10^{7.07} M⁻¹ in a 0.1 M HCl medium, which are the same order of magnitude as that of dissolved organic matter,¹³⁵ particulate matter and phytoplankton (Table 1). The levels of the conditional stability constants are for the strongest ligands, classified as the L₁-type ligands¹³⁶ or the DTPA type ligand,⁶⁹ which chemically correspond to hard bases^{43,102} showing high affinity to hard acids, such as Th⁴⁺, UO₂²⁺, Fe³⁺, Am³⁺ and others. Therefore, the strong ligands found in marine organisms, particulate matter and dissolved organic matter are candidates for chelators related to iron uptake of marine organisms. It is also suggested that heterotrophic bacteria concentrates the chelator more than one order of magnitude greater than phytoplankton and zooplankton, as the used concentration unit of ligand per biomass carbon. The conditional stability constants and the ligand concentration per organic carbon for particulate matter and marine organisms^{103,134} may suggest a marine microbial source for the L₁ (DTPA) type ligand associated with particulate and dissolved organic matter in seawater.

Recent knowledge on the interaction of microorganisms with metal ions suggests that microorganisms, which possess metal binding sites on the cell surface, are universally present in the earth environment. It is likely that the ligand on the cell surface of marine microorganisms is a possible candidate for the source of the ligands in oceanic dissolved organic matter and particulate matter.

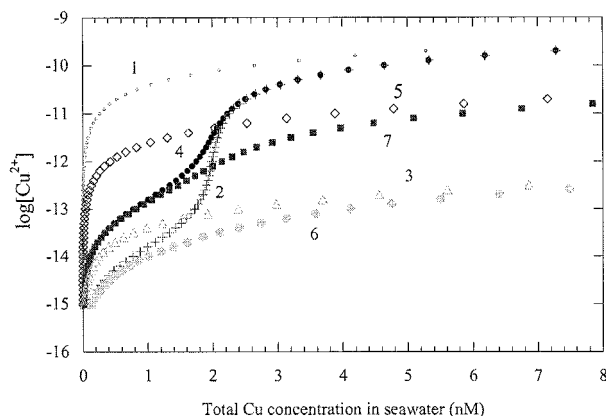


Fig. 2 Relationship between the total Cu and the free Cu^{2+} concentrations in the presence of Ca and Mg. 1, Inorganic Cu species exist in solution without a ligand and Ca and Mg. 2, Cu complex with the L_1 -type ligand (2 nM) exists without Ca and Mg. 3, Cu complex with the L_2 -type ligand (40 nM) exists without Ca and Mg. 4, Cu complex with the L_1 -type ligand exists with Ca and Mg. 5, Cu complex with the L_2 -type ligand exists with Ca and Mg. 6, Cu complexes with two dissolved organic ligands exist in solution without Ca and Mg. 7, Cu complexes with two dissolved organic ligands exist in solution with Ca and Mg.

5 Chemical Model of Trace Metals

One of the purposes for the chemical speciation of trace metals is application to chemical modeling of the ocean. Many kinds of models for ocean systems have been proposed to have a better understanding of ocean systems and to predict the future of the oceanic environments; it includes physical models *i.e.*, general ocean circulation model,¹³⁷ biogeochemical models^{138,139} and ecological models.¹⁴⁰ There are a few oceanic models including chemical reactions, especially, the complexation of bioactive trace metal ions with ligands in dissolved organic and particulate matter. Recently, a chemical model including metal complexation has been proposed.¹⁴¹

Chemical speciation studies^{58,67,99,136} have revealed that two types of dissolved organic ligands are related to the complexation of trace metals, such as Cu and Zn, in seawater. However, there is little information on the ecological and biogeochemical roles of each ligand, except for the metal buffering capacity.¹⁴² In order to elucidate the ecological roles of dissolved organic ligands in the marine environment, therefore, a chemical model, including the relationship between the total Cu and free Cu^{2+} concentrations in the presence of each ligand, is effective.

According to our present knowledge of dissolved organic ligands,⁶⁹ the concentrations in seawater are 1–3 nM for L_1 and 20–60 nM for L_2 . The free Cu^{2+} concentration in seawater reflects the reactivity of Cu, and is directly related to the ecological role to marine microorganisms.^{31,32} The free Cu^{2+} concentration in seawater was calculated as a function of the total Cu concentration based on the chemical equilibrium model,¹⁴¹ in which the effects of Ca and Mg were taken into account. The result is shown in Fig. 2. For the presence of only a stronger organic ligand (L_1), the free Cu^{2+} concentration showed a sigmoid curve in the range of natural occurring Cu concentrations in seawater; the free Cu^{2+} concentration was maintained at a lower level (less than 1 pM) when the total Cu concentration was less than that of the stronger organic ligand,

whereas the free Cu^{2+} concentration steeply increased at a Cu toxic level (more than 10 pM)^{30,143,144} when the total Cu concentration exceeded that of the stronger organic ligand. On the other hand, for the weaker organic ligand (L_2), the free Cu^{2+} concentration showed a different behavior, in which the free Cu^{2+} concentration was maintained at lower level (less than 10 pM) within the natural occurring concentration range of the total Cu in seawater. The increase in the ligand concentration shifts the free Cu^{2+} concentration to lower levels. These findings suggest that a weaker ligand plays a more significant role than a stronger ligand to reduce Cu toxicity to marine microorganisms under higher Cu conditions. This result is consistent with the finding that a weaker ligand can dominate Cu speciation in coastal water with higher Cu concentrations.¹⁴⁵

There has been little attention on the effects of alkali earth metals for reacting with the ligands dissolved in seawater. Calcium and magnesium, which are major components in seawater, can be complexed with the organic ligands in seawater; especially, most of the L_2 -type ligand is associated with Ca and Mg.⁶⁹ A chemical model¹⁴⁰ revealed that the reactivity of the organic ligands reacting with trace metals is mitigated by complexation with Ca and Mg (Fig. 2). In other words, the concentrations of the ligands in seawater are buffered by excess amounts of Ca and Mg (ligand buffer): the free (no metal-bound) ligand concentration is regulated at a low level when the total ligand concentration is changed in solution.⁶⁸

Although Fe is an essential element for marine organisms, it is well known that Fe deficiency occurs in a marine environment, especially in HNLC regions.^{34–36} The causes of Fe deficiency in the marine environment have been considered to be low Fe fluxes *via* atmosphere and active Fe export from the euphotic layer to the ocean interior. Hirose¹⁴¹ suggested the hypothesis that the Fe limitation in the marine ecosystem occurs due to chemical constraints, such as a possibility that marine organisms cannot possess sufficient Fe by competitive reactions between Fe and Cu to the cell surface ligand, which may be related to Cu toxicity. The chemical model suggests that competitive reactions between Fe and Cu to the dissolved organic ligand reduce the level of the organic Fe concentration in seawater because solubility of Fe hydroxide controls the free Fe(III) concentration at an extremely low level in seawater. This may lead to a decrease of the reactive Fe concentration in the marine environment, provided that reactive Fe consists of organic Fe complexes and reactive inorganic species, such as free Fe(III) ion.

6 Conclusion

The current understanding of the chemical speciation of trace metals in seawater is as follows.

1. Methodologies for the strong organic ligands, based on mass balance techniques, have revealed that reactions between natural strong organic ligands and metal ions, which occurs in the ocean, are reversible; ligand-exchange reactions as well as metal-exchange reactions are powerful tools to characterize the chemical forms of trace metals in seawater.

2. Major chemical species of bioactive trace metals, such as Cu and Fe, are complexes with organic ligands dissolved in seawater.

3. The strong organic ligands dissolved in seawater can be classified into three groups by using the conditional stability constant of metals complexes and the concentration of the corresponding organic ligand. The LFER relationships are useful to characterize the strong organic ligands. Two types of

the non-specific strong organic ligands coexist in seawater: one is the L₁-type, whose concentration is relatively low; the other is the L₂-type, which is a major ligand.

4. Metals become a good tool to characterize the chemical properties of natural organic ligands by taking into account the HSAB concept. Strong organic ligands are commonly found in marine environments. Especially, the L₁-type ligand, which exists in both particulate matter and dissolved organic matter, originates from marine microorganisms. The natural strong organic ligands in the ocean have several functional groups, including carboxylates.

5. Chemical models including organic complexation are essential to have a better understanding of the biogeochemical and ecological roles of trace metals in marine environments.

A purpose of chemical speciation is to elucidate the chemical forms of species in seawater. The most important issue of chemical speciation of trace metals in seawater is to construct chemical models, which was initially conducted by Sillén, who successfully specified chemical species. Knowledge of the conditional stability constants and the ligand concentrations relevant to chemical species is required for a chemical model, in which the free metal ion concentrations can be calculated as the most significant parameter. The concentrations of free trace metal ions in seawater provide information on better understanding of roles of trace metals in the field of toxicology, geochemistry and environmental sciences.

Chemical speciation studies have revealed that metal complexation plays significant roles regarding the biogeochemical behaviors of trace metals in marine environments. The remaining problem on concerning studies of the strong organic ligands related to metal complexation in seawater is to isolate and chemically identify the strong DTPA and EDTA-type organic ligands. Another important aspect is a kinetic approach for the speciation of trace metals in seawater, in which it has been pointed out that knowledge of dynamic speciation is necessary to understand the nonequilibrium properties, such as transport processes.²

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