Bull. Chem. Soc. Jpn., **73**, 121—127 (2000)

Chemical Stability of Large Organic Molecule–Metal Complexes Dissolved in Natural Water as Studied by Size Exclusion Chromatography/Inductively Coupled Plasma Mass Spectrometry

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(Received August 25, 1999)

The chemical stability of large organic molecule–metal complexes (LOMMCs) dissolved in pond water were investigated by a hyphenated system of size exclusion chromatography/UV absorption detection/inductively coupled plasma mass spectrometry (SEC/UV/ICP-MS) after ultrafiltration using a filter with a molecular permeation limit larger than 10000 Da. LOMMCs dissolved in the preconcentrated pond water sample were observed at the retention times corresponding to molecular weights larger than 300000 Da (Peak I) and that of 10000—50000 Da (Peak II) in the chromatograms measured by UV absorption and ICP-MS. When the pH of the sample solution was varied from 7.0 to 0.5 by adding the HNO_3 solution, the peak intensities of both Peak I and Peak II in these chromatograms decreased with the decrease in pH of the sample solution. These results indicate that LOMMCs were decomposed by addition of HNO_3. Peak I was rapidly decomposed below pH 4, while Peak II was gradually decomposed over the pH range from 7.0 to 0.5. The decomposition curve of Peak I was similar to the solubility characteristics of hydroxides of Fe and Al. On the other hand, that of Peak II suggested the dissociations of metal complexes (Cu and Zn) with some biogenic organic molecules. It was concluded from these results that some colloidal particles of hydroxides of Fe and Al may be core materials of LOMMCs for Peak I, while some metal complexes with biogenic protein-like compounds may be LOMMCs for Peak II. The possible models for LOMMCs in pond water were proposed as "a string-ball model", which might interpret as the removal and preservation mechanisms for trace elements in natural water.

In water quality analysis, the total concentrations of toxic or hazardous elements are usually determined on the elemental basis. However, it has been pointed out that the total concentrations of trace elements are not enough even for environmental risk assessment because the biological functions or toxicities are different, depending on the chemical forms of the elements.[1,2] Therefore, speciation analyses of the elements even at the trace or ultratrace concentration level are required in various scientific fields. Recent development of highly-sensitive analytical techniques allows one to explore the analytical methodologies for speciation of trace and ultratrace elements in natural water.[1,2]

Nowadays, there are many approaches for speciation analysis of trace metals in natural water.[3] Most of these speciation analyses have been focused on the identification and determination of stable and specific chemical species of organometallic compounds such as As, Se, Sn, and Hg.[4] On the contrary, the present authors have been engaged in speciation analyses of trace elements in natural water with particular interest in their interactions with large organic molecules.[5—7] As a result, the existence of large organic molecule–metal complexes (LOMMCs) for various kinds of trace metals has been elucidated in lake and pond water.[1,8]

A combined system of size exclusion chromatography (SEC) with a UV absorption detector and inductively coupled plasma mass spectrometry (ICP-MS), which is hereafter referred to as a SEC/UV/ICP-MS system, is often used for speciation of metal-binding proteins in blood and other biological samples.[9,10] However, such a combined system can not be directly applied to speciation analysis of LOMMCs in natural water[11] because of the extremely low concentrations of trace elements in LOMMCs. Thus, the present authors have employed an ultrafiltration technique to preconcentrate large organic molecules with molecular weight larger than 10000 Da before the measurements of LOMMCs by the SEC/UV/ICP-MS system.[5,6] As a result, it has been found that a large number of trace metals (30—40 elements) are dissolved as the forms of LOMMCs in pond and lake water. In addition, some studies on the identification of large organic molecules in natural water have also been performed by using NMR[12] and GC-MS,[13] but their chemical forms and characteristics have not been well elucidated yet.

In the present paper, the chemical stability of LOMMCs dissolved in natural water was investigated by the SEC/UV/ICP-MS system, with adding the acid solution into the ultrafiltration-preconcentrated pond water. Two types of LOMMCs in natural water showed the different chemical behaviors in the acidic media.

Experimental

Instrumentation. A SEC/UV/ICP-MS system composed of a size exclusion chromatograph (SEC), a UV absorption detector and an ICP-MS instrument was used for molecular separation and detection of LOMMCs in pond water. The SEC system was consisted of a high performance liquid chromatograph (LC-9A, Shimadzu, Kyoto) and a size exclusion column (Superose 12HR, Pharmacia, Uppsala, Sweden). The molecular weight permeation range of the SEC column used was 1000—300000 Da. The UV absorption detector (model 870-UV, JASCO, Tokyo) was used for the detection of organic molecules separated by the SEC column. The ICP-MS instrument of Model SPQ 8000A (Seiko Instruments, Chiba) was set after the UV detector for the element-selective detection of metallic elements in the SEC chromatograms. The operating conditions of the SEC/UV/ICP-MS system are summarized in Table 1; they are almost similar to those described in the previous paper.[6]

An ultrafiltration system of a tangential flow type (Pellicon cassette system, Millipore, Bedford, MA, USA) was used for preconcentration of large organic molecules in pond water. The ultrafilters with the permeation limit of 10000 Da in molecular weight were purchased from Nihon Millipore Kogyo Co. (Tokyo).

Chemicals. The 0.1 M (M = mol dm^{-3}) tris-HNO_3 buffer solution (pH 7.0) used as the mobile phase was prepared from tris(hydroxymethyl)aminomethane (Tris; Katayama Kagaku Co., Osaka) and HNO_3 (electronics grade; Kanto Kagaku Co., Tokyo). The nitric acid solutions (0.1 and 1 M) were used for pH adjustment of all the sample solutions. Pure water used throughout the present experiment was prepared by a Milli-Q de-ionization system (Millipore, Bedford, MA, USA).

Sample Collection and Pretreatment. In the present experiment, the pond water samples were collected from Kagami-ga-ike pond located in the campus of Nagoya University. They were filtered with a glass filter (pore size 0.6 μm) and then with a membrane filter (pore size 0.45 μm) immediately after sampling. The filtered sample solutions were concentrated to 20- or 150-fold in volume by ultrafiltration to preconcentrate large organic molecules. The 150-fold ultrafiltration-preconcentrated sample solution was subjected to speciation analysis of LOMMCs by the SEC/UV/ICP-MS system. In order to investigate the chemical stability of LOMMCs in natural water, the 20-fold ultrafiltration-preconcentrated sample solutions were analysed by the SEC/UV/ICP-MS system after their pH adjustment in the range from 7.0 to 0.5.

Results and Discussion

As mentioned earlier, the pond water samples were filtered with a membrane filter, and then subjected to preconcentration by ultrafiltration. Thus, it should be noted that large organic molecule-metal complexes as the dissolved species in pond water were principally examined in the following experiment. The concentrations of trace metals in the original pond water sample after filtration by the membrane filter were also determined by ICP-AES and ICP-MS in a similar manner to that of the previous paper,[6] and they are summarized in Table 2.

Speciation of Large Organic Molecule–Metal Complexes Dissolved in Pond Water. The SEC chromatograms for ultrafiltration-preconcentrated pond water are shown in Fig. 1; they were measured first by UV absorption for organic molecules and then by ICP-MS for the elements. In the UV absorption-detected chromatogram shown in the upper part of Fig. 1, two peaks are mainly observed at the retention times of about 800 s (Peak I) and 1500—1800 s (Peak II), which correspond to molecular weight larger than 300000 Da and that of 10000—50000 Da, respectively. These results indicate that at least two different kinds of LOMMCs exist in pond water, as described previously.[6]

The element-selective chromatograms for Mn, Fe, Cu, Zn, and Pb measured by ICP-MS are also shown in Fig. 1, as some typical examples. Similar chromatograms were obtained for 26 other elements in the present experiment, too. As is seen in Fig. 1, the elements except for Mn provided one or two peaks at the peak positions corresponding to the retention times of Peak I and Peak II observed in the UV absorption-detected chromatogram, although their peak intensities were significantly different from each other. Manganese gave another peak at the retention time near 1300 s in addition to that at the Peak I position. It is noted here that Fe provided

Table 1. Instrumental Components and Experimental Conditions

HPLC:	
Column	Superose-12 (Pharmacia Biotech)
Mobile phase	50 mM Tris-HNO_3 (pH 7.3)
Flow rate	0.5 ml min^{-1}
Sample injection volume	200 μL
UV absorbance	254 nm
ICP-MS:	
Instrument	SPQ-8000A (Seiko Instruments Inc.)
RF power	1.3 kW
Carrier gas flow rate	Ar 1.0 L min^{-1}
Auxiliary gas flow rate	Ar 1.0 L min^{-1}
Coolant gas flow rate	Ar 16 L min^{-1}
Nebulizer	Concentric type
Sampling depth	10 mm above load coil
Data accquisition (multielement mode by peak hopping) in chromatogram measurement	
Measurement positions	15 m/z per 1 measurement
Dwell time	100 ms at each m/z
Data plot	1 point per 5 s measurements
Measurement time	2700 s (540 points)

Table 2. Concentration of Trace Elements Dissolved in Kagami-ga-ike Pond Determined by ICP-MS and ICP-AES with Chelating Resin Preconcentration

Element	Concentration / μg L^{-1}
Al	25
Mn	69
Fe	193
Cu	4.6
Zn	15.3
Y	63
Mo	1.6
Cd	0.036
La	35
Ce	48
Pr	6.4
Nd	28
Sm	6.8
Eu	5.5
Gd	7.2
Tb	1.4
Dy	6.9
Ho	12
Er	4.3
Tm	0.74
Yb	3.5
Lu	0.79
Pb	3.0
U	14.0

only one peak at Peak I, while Cu, Zn, and Pb provided two peaks at both Peak I and Peak II. The peak intensities of Peak II for Cu and Zn were significantly higher than those of Peak I, while the peak intensity of Peak I for Pb was larger than that of Peak II.

From the chromatographic patterns as shown in Fig. 1, the elements observed as LOMMCs in the pond water samples were categorized into the following 3 groups.

(i) The elements observed only at Peak I position:

Al, Fe, P, Sr, and Ba

(ii) The elements observed at both Peak I and Peak II positions, but preferentially at Peak I position:

Pb, Ti, Mn, Y, Cd, W, and rare earth elements (REEs)

(iii) The elements observed at both Peak I and Peak II positions, but preferentially at Peak II position:

Cu, Zn, Co, Ni, Mo, and I

In addition, it was found that Si and Ca were also categorized into Group (i) from the results analysed by the SEC/UV/ICP-AES (inductively coupled plasma atomic emission spectrometry) system.[5]

The elements categorized into Group (iii), except for Mo and I, belong to the first transition elements, and they were preferentially observed at the Peak II position in the chromatograms for the ultrafiltration-preconcentrated pond water. Molybdenum and I are known to dissolve mainly as the oxo-anion forms such as MoO_4^{2-} and IO_3^- in natural water,[14] but some parts of these elements were found to bind with organic molecules in pond water.

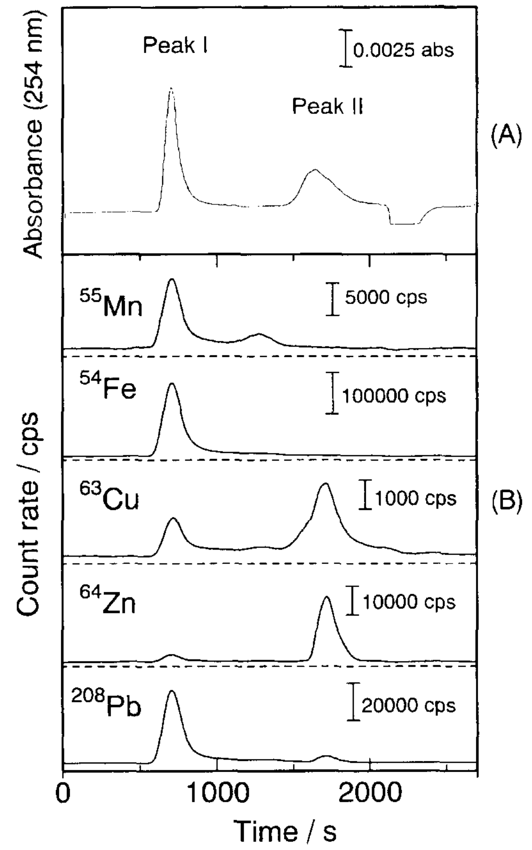

Fig. 1. Size exclusion chromatograms for ultrafiltration-preconcentrated pond water. (A) UV absorption detection, (B) ICP-MS detection. The preconcentration factor was 150-fold, and the molecular permeation limit of the ultrafiltration filter was 10000 Da.

In summary, it can be stated that various kinds of metallic elements dissolved in pond water are binding with large organic molecules, which provide Peak I and Peak II in the UV absorption-detected chromatogram. These results indicate that metallic elements may exist in the forms of LOMMCs in pond water, and that there may be at least two types of LOMMCs. One of them includes the elements preferentially observed at the Peak I position, and the other does the elements preferentially observed at the Peak II positions. Thus, the chemical stability of such LOMMCs is further examined in the following sections to suggest their structures in the dissolved states.

Chemical Stability of Large Organic Molecule–Metal Complexes. In order to investigate the chemical stability of LOMMCs in pond water, the behaviors of their chromatographic peak intensities at the different pH were examined by the SEC/UV/ICP-MS system. The pHs of the ultrafiltration-preconcentrated pond water sample solutions were changed from 7.0 to 0.5 by adding 0.1 M HNO_3 solution, and the UV-absorption and element-selective chromatograms for each sample solution were measured in a similar manner to that of the previous experiment. The results are shown in Fig. 2. It is clearly seen in Fig. 2a that the peak intensities of both Peak I and Peak II in the UV absorption-detected chromatogram became gradually smaller at the lower pH of the sample solu-

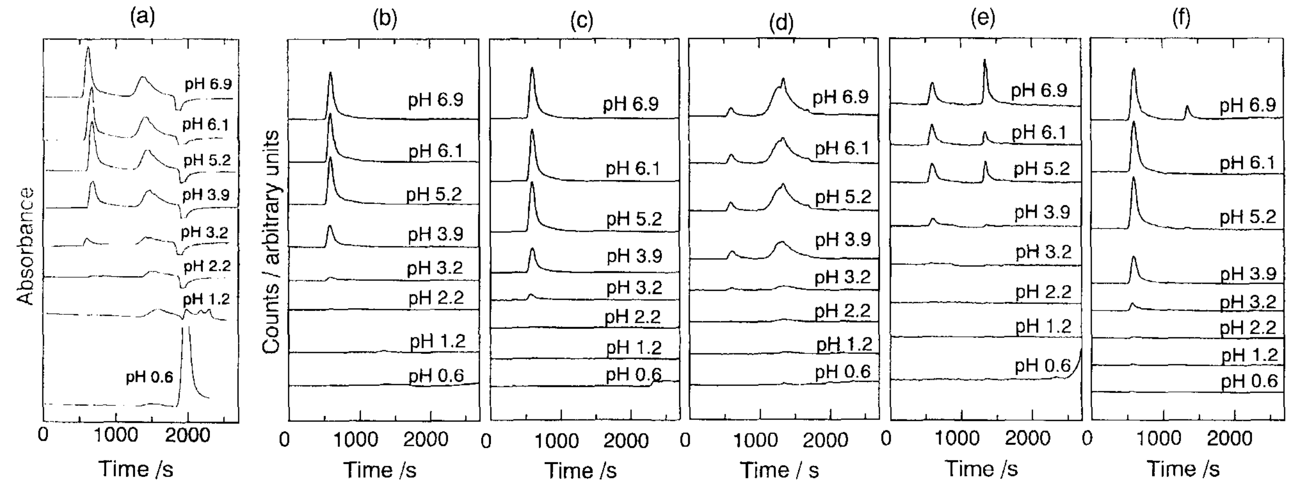

Fig. 2. Changes of SEC chromatograms for ultrafiltration-preconcentrated pond water at various pH measured by the UV absorption (a) and ICP-MS (b—f). (a) UV absorption detection, (b) Al, (c) Fe, (d) Cu, (e) Zn, (f) Pb. The samples were the same as Fig. 1.

tion, although their retention times were unchanged through the pH examined. It should be noted here that Peak I disappeared near pH 2.2, more quickly than Peak II. A large peak at pH 0.6, which newly appeared after the permeation limit (ca. 2000 s) of the present SEC column, is ascribed to HNO_3 added to the sample solution. However, no other peak appeared at the retention time before 2000 s in the UV absorption-detected chromatogram, when the pH of the sample solution was lowered from neutral to strong acidity.

The element-selective chromatograms for Al, Fe, Cu, Zn, and Pb measured by ICP-MS are shown in Figs. 2b, 2c, 2d, 2e, and 2f, respectively. It is seen in the figures that the peak intensities of each element gradually decreased with the decrease in the pH of the sample solutions, similar to the decrease of the peak intensities in the UV absorption-detected chromatograms. These results suggest that LOMMCs were dissociated into metal ions and small organic molecules, when the solution was acidified.

The chemical stability of LOMMCs was further investigated quantitatively by measuring the relative peak area intensities in the chromatograms shown in Fig. 2. The relative peak area intensity was defined as the ratio of the peak area observed at each pH to that of the original concentrated solution (pH 7.0). The variations of the relative peak area intensities with the pH change in the UV absorption-detected chromatograms are illustrated in Fig. 3. As is seen in Fig. 3, the relative peak area intensity of Peak I, which corresponded to molecular weight larger than 300000 Da, was almost constant in the pH range from 7.0 to 4.0, but it rapidly decreased in the pH range from 4.0 to 2.0, and no peak was observed below pH 2.0. On the contrary, the relative peak area intensity of Peak II, which corresponds to a molecular weight of 10000—50000 Da, gradually decreased to zero in the pH range from 7.0 to 0.5.

The difference of the chemical stability between two large organic molecules suggests that they are in quite different chemical forms. The gradual decrease of the peak intensity of Peak II may be ascribed to the dissociation of organic molecule–metal complexes. On the other hand, the rapid change of the peak intensity of Peak I in the pH range from 4.0 to 2.0 suggests that hydroxide compounds of Al and Fe, which may be the core materials of LOMMCs corresponding to Peak I, were decomposed, as will be described later.

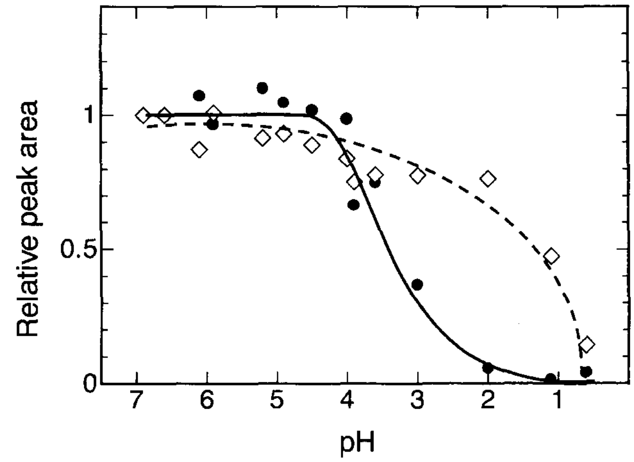

Fig. 3. Changes of the relative peak area intensities of LOMMCs in ultrafiltration-preconcentrated pond water obtained by UV absorption-detection with the addition of HNO_3.
● Peak I, ◇ Peak II.
The preconcentrated factor was 20-fold.

Decomposition Curves of Large Organic Molecule–Metal Complexes with Addition of HNO_3. The chemical stability of LOMMCs was further examined by measuring the variations of the peak intensities in the element-selective chromatograms for metallic elements with the pH change. The changes of the relative peak area intensities of Peak I for Fe and Al, which were calculated in a similar manner to those in the UV absorption-detected chromatograms, are shown in Fig. 4, together with the change of the relative peak area intensities of Peak I observed in the UV absorption-detected chromatogram. The relative peak area intensities of Peak I for these elements decreased with the decrease in pH, as did those in the UV absorption-detected chromatogram.

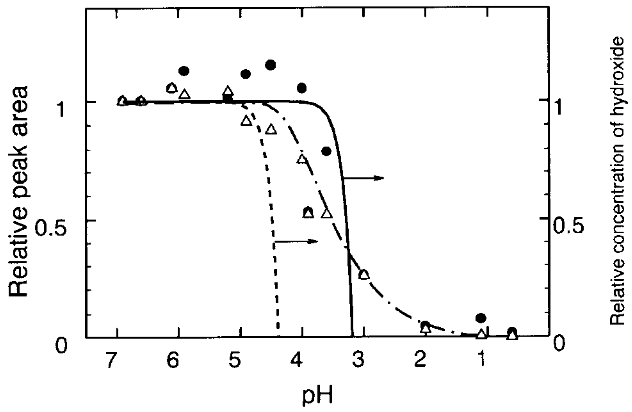

Fig. 4. Changes of the relative peak area intensities of Fe and Al in LOMMCs for Peak I in the element-selective chromatograms with the addition of HNO_3, and the solubility curves of $Fe(OH)_3$ and $Al(OH)_3$.

—: the relative concentration of $Fe(OH)_3$ calculated from solubility constant of $Fe(OH)_3$. ($K_{so} = 10^{-37}$ M^2, total concentration of Fe = 2.7×10^{-5} M).

- - -: the relative concentration of $Al(OH)_3$ calculated from solubility constant of $Al(OH)_3$. ($K_{so} = 10^{-33.5}$ M^2, total concentration of Al = 2.2×10^{-5} M)

–·–: change of the relative peak area intensity of Peak I observed by UV absorption shown in Fig. 3.

● Fe, △ Al.

The samples were the same as Fig. 3.

These results suggest that LOMMCs in pond water were decomposed with addition of HNO_3, and then metal ions in LOMMCs were released as free metal ions.

Figure 4 also shows that the peak area intensities of Fe and Al rapidly decrease in the pH range of 5—3, although the decomposition curves of these elements are slightly different from each other. The relative peak area intensity of Peak I for Al started to decrease near pH 5, and then that of Fe decreased near pH 4. In addition, these elements could not be observed below pH 2 in the element-selective chromatograms measured by ICP-MS, where the detection limits obtained by the present SEC/UV/ICP-MS system were 10 $\mu g\,dm^{-3}$ for Fe and 27 $\mu g\,dm^{-3}$ for Al.

As reported previously,[6] Fe and Al are observed only at the Peak I position, and thus they are seemingly the major metal elements in LOMMCs for Peak I. Iron and Al seem to form not only metal–organic complexes, but also colloidal hydroxides or oxides in the neutral and weakly-acidic pH region. Thus, the solubility curves of $Fe(OH)_3$ and $Al(OH)_3$ in the concentrated pond water were calculated from the total concentrations and the solubility constants of $Fe(OH)_3$ and $Al(OH)_3$, where the solubility constants of $K_{so} = 10^{-37}$ M^2 for $Fe(OH)_3$ and $K_{so} = 10^{-33.5}$ M^2 for $Al(OH)_3$ were used for calculation.[15] The results are shown in Fig. 4 as the relative concentrations of hydroxides of Fe and Al drawn by the solid and broken lines, respectively. The relative concentration of hydroxide of Fe or Al was defined as the ratio of the hydroxide concentration at each pH to that of the original concentrated pond water at pH 7. In addition, the total concentrations of Fe and Al in the original concentrated pond water were 2.7×10^{-5} M for Fe and 2.2×10^{-5} M for Al, respectively. As is seen in Fig. 4, the relative hydroxide concentrations of Fe and Al were almost constant in the pH ranges of 7.0—5.0 and 7.0—3.7, respectively, but they started to decrease significantly from pH 5.0 and 3.7, respectively, and they became close to zero below pH 4.3 and 3.2, respectively. These facts indicate that $Fe(OH)_3$ and $Al(OH)_3$ start to be dissolved near pH 5.0 and 3.7 and almost completely dissolved below pH 4 and 3, respectively.

It is noted here that the solubility characteristics of $Fe(OH)_3$ and $Al(OH)_3$ derived from their solubility constants explain well the initial changes of the relative peak area intensities of Peak I for Fe and Al near pH 4 and 5, respectively, although they were not in agreement with the present experimental results in lower pH ranges. It is also seen in Fig. 4 that the change of the relative peak area intensities of Al is quite similar to that of Peak I observed by the UV absorption detection through the pH region examined. The change of Fe is also similar to that of Peak I observed by the UV absorption detection below pH 3. These results strongly support the conclusion that the main constituents of LOMMCs for Peak I are some colloidal particles composed of aluminum and iron hydroxides. In addition, the changes of the relative peak area intensities of Peak I for Cu, Zn, and Pb showed the similar behaviors to those for Fe and Al, respectively. In pond water, some biogenic organic molecules originating from microorganisms and plants are certainly dissolved. These biogenic organic molecules may be adsorbed physically and chemically on the surfaces of colloidal particles of hydroxides to increase the particle sizes of such colloidal materials in natural water.[16,17] In the present experiment, as is seen in the UV absorption-detected chromatograms shown in Fig. 2a, no UV absorption was observed for such biogenic organic molecules after decomposition at the lower pH. These results suggest that biogenic organic molecules, which adsorb on the particles, may be non-absorbing compounds such as hydrocarbons and lipids consisting of cell membranes. Therefore, the apparent peak detected as Peak I by UV absorption may be caused by the light scattering of colloidal particles.

The changes of the relative peak area intensities of Peak II for Cu and Zn are shown in Fig. 5, together with that of Peak II observed by UV absorption. The relative peak area intensity of Peak II for Cu showed quite similar change to that observed in the UV absorption-detected chromatogram. It is also seen in Fig. 5 that the relative peak area intensity of Cu started to decrease at ca. pH 5, while that of Zn decreased near pH 6. These differences between Cu and Zn may be attributed to their different complexing abilities with large organic molecules in pond water. The results in Fig. 5 indicate that Zn is easily dissociated from large organic molecules in LOMMCs for Peak II. Therefore, the curves for the changes of the relative peak area intensities of Peak II for Cu and Zn may correspond to the dissociation curves for Cu and Zn complexing with large organic molecules. According to the Irving–Williams series[18] concerning the complexing abilities of the first transition metals, the complexing abilities of Cu with organic ligands are generally stronger than those of Zn.

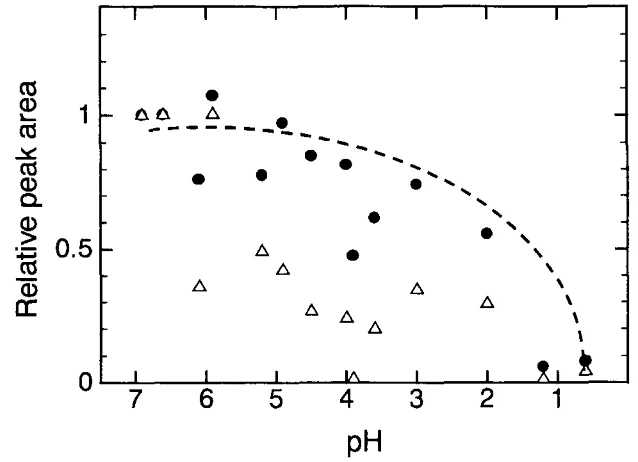

Fig. 5. Changes of the relative peak area intensities of Cu and Zn in LOMMCs for Peak II in the element-selective chromatograms with the addition of HNO_3.
● Cu, △ Zn.
- - -: change of the relative peak area intensity of Peak II observed by UV absorption shown in Fig. 3.

The trends in the dissociation curves of Peak II for Cu and Zn are consistent with those in the Williams–Irving series. Then, it is concluded that the main chemical forms of Cu and Zn for Peak II may be certain kinds of metal complexes with large organic molecules dissolved in pond water. It was also suggested by other researchers[19] that organic ligands in seawater have the primary amine groups as copper-binding sites. Therefore, it may be reasonable that some metalloprotein-like compounds originating from aquatic organisms exist in pond water as the constituents for Peak II.

As mentioned in the previous section, the elements observed preferentially at the Peak II position were Cu, Zn, Co, Ni, Mo, and I, all of which are now known as essential trace elements for animals and plants.[1] It is interesting to point out that such biologically-essential trace elements are preserved as LOMMCs in natural water, perhaps for maintenance of life in the aquatic environment. In fact, further research should be performed to elucidate the roles of biologically-essential trace elements for biological activities in natural water.

Conclusion

The existences of large organic molecule–metal complexes (LOMMCs) dissolved in pond water were elucidated by speciation analysis with the SEC/UV/ICP-MS system. Almost all the elements provided one or two peaks corresponding to Peak I and/or Peak II observed in the UV absorption-detected chromatogram. The experimental results for the chemical stability of LOMMCs examined here indicate that some colloidal particles of hydroxides of Fe and Al may be core materials of LOMMCs for Peak I, while some metal complexes with biogenic protein-like compounds may be LOMMCs for Peak II. The possible models for LOMMCs dissolved in pond water are schematically shown in Fig. 6. The colloidal LOMMCs for Peak I may grow in size as the particulates by adsorbing some organic compounds and trace elements as well as by aggregating other colloidal particles. Thus, the present authors here propose the model for Fig. 6A as a *string-ball model.* Aggregations of colloidal particles may result in sedimentation onto the bottom in pond or lake, and consequently trace elements and organic compounds contained in the particulates may be removed from the aquatic system. On the other hand, LOMMCs for Peak II could be dissolved stably in natural water, so that trace elements and biogenic organic compounds contained in such complexes are likely to be preserved in water for rather a long time. These two mechanisms, i.e. removal and preservation, may be in an equilibrium, which determines the concentration levels of trace elements in natural water.

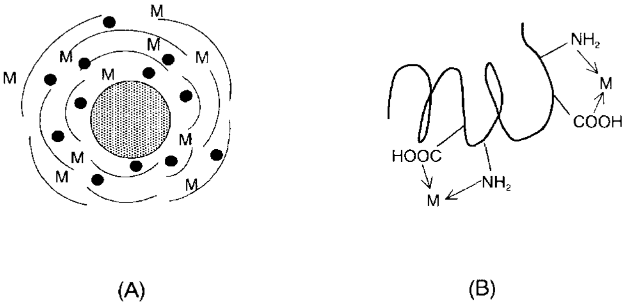

Fig. 6. Proposed models for large organic molecule-metal complexes (LOMMCs) dissolved in natural water.
(A) LOMMCs for Peak I (MW $>$ 300000 Da)
(B) LOMMCs for Peak II (MW 50000—10000 Da)
Model (A): The hatched large circle indicates core materials of colloidal particulates composed of hydroxides of Fe and Al. In addition, the black small circles show small colloidal particles of Fe and Al, and the curved lines are large organic molecules adsorbed on the core materials and/or organic substances. M is trace metal ion adsorbed on colloidal particulates of hydroxides or complexed with organic molecules on colloidal particulates.
Model (B): The long curved line indicates biogenic protein-like compounds, which contain the amino and carboxyl groups. M is trace metal ion, which forms the complexes with organic molecules.

The present research was supported by the Grant-in-Aid for Scientific Research No. 10680499 from the Ministry of Education, Science and Culture, by the Grant-in-Aid for the Future Research Problem from the Japan Society for Promotion of Science, and by the Showa Shell Oil Foundation Aid for Environment Problem (1998).

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