Chapter 1
Metallomics: Integrated Biometal Science

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Abstract The historical aspects of metallomics, which were proposed as integrated biometal science in 2004 by the present author, are described. The significant development of analytical atomic spectrometry since the late 1960s allowed the all-element analyses of various biological samples as well as chemical speciation analysis of trace elements. Such a progress of trace metal analysis opened new era of trace metal science in various scientific fields to cooperate with omics sciences such as genomics, proteomics, and metabolomics. Under such situations in life science, it was desired to integrate biometal science as one of omics sciences for further development. So far, the international symposiums of metallomics were held five times around the world since 2007 in Nagoya, and the academic journal of metallomics has been published since 2009 from the Royal Society of Chemistry. Furthermore, essentiality and toxicity of trace metals, a simplified model of the biological system, the research fields in metallomics, and so forth are discussed in this chapter.

Keywords Metallomics • Omics science • Genomics • Proteomics • Life science • Analytical atomic spectrometry • All-Element Present Theory • Chemical speciation • Biological system • Biological essentiality

1.1 Introduction

In this paper, "Metallomics" is proposed as a new scientific field in order to integrate the research field related to biometals. Metallomics should be a scientific field in symbiosis with genomics and proteomics, because syntheses and metabolic functions of genes (DNA and RNA) and proteins cannot be performed without the aid of various metal ions and metalloenzymes. In metallomics, metalloproteins, metalloenzymes and other metal-containing biomolecules are defined as "metallomes", in a similar manner to genome in genomics and proteome in proteomics. Since identification of metallomes and the elucidation of their biological or physiological functions in the biological systems are the main research target of metallomics, chemical speciation for specific identification of bioactive metallomes is one of the most important analytical technologies to establish metallomics as

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integrated biometal science. In order to rationalize the concept of metallomics, the distributions of the elements in man, human blood serum and seawater, a challenge to all-elements analysis of one biological cell, and some other topics are introduced with emphasis on recent development of chemical speciation of trace metals in some biological samples.

This is the abstract of my paper published under the title of "Metallomics as Integrated Biometal Science" in the *Journal of Analytical Atomic Spectrometry* (*J Anal At Spectromet*) in 2004 [1]. The publication of this paper was an epoch-making event for metallomics, because metallomics was coined as a new academic field from this publication. That is, "metallomics" was proposed for the first time as the academic research field. Since then, metallomics has received great attention as the emerging new scientific field [2], and great progress has been achieved as the one of omics science [3–8]. In the present chapter, thus, the current status and perspective of metallomics will be described together with the research backgrounds before and after the proposal of metallomics.

As will be mentioned later, the advancement of analytical atomic spectrometry such as ICP-AES (inductively coupled plasma atomic emission spectrometry) and ICP-MS (inductively coupled plasma mass spectrometry) was one of the strong driving forces for me to conceive the idea for metallomics. Since the middle of the 1970s, ICP-AES and ICP-MS have been extensively developed as the highly sensitive analytical methods with excellent feasibilities of simultaneous multielement detection [9–11]. As a result, in these days, the distribution analyses of the elements in the biological fluids, cells, organs, and organisms are performed mostly by ICP-AES and ICP-MS [1, 9, 10, 12]. Furthermore, it is now possible to perform all-element analysis of almost all samples by analytical atomic spectrometry. Thus, the present author also proposed the concept of "the Extended All-Present Theory of the Elements" from the consideration on the distributions and cycles of the elements on the earth [1, 9]. In addition, chemical speciation for the identification of chemical species in the biological and environmental samples has been also developed in last two decades and now established as the important analytical technologies [1, 13–16].

It should be further noticed here that genomics [17, 18], proteomics [19], and metabolomics [20] as omics science have been significantly developed in life science and molecular cell biology for last few decades, and now these terms are popularly used, indicating the integrated biological sciences for genes, proteins, and metabolites. In fact, genes and proteins are the key materials to regulate and/or maintain the life systems of animals, plants, and microorganisms. However, the biological functions and physiology of the life system do not work only with genes and proteins, but the aid of various metals is required by the life system in nature. Thus, it was desirable to establish a new scientific field for biometals among omics science. It was "metallomics" [1]. When I conceived the idea of metallomics, I thought that it was really meaningful to integrate (1) metallomics with genomics, proteomics, and other omics sciences, (2) various independent fields in trace metal research, and (3) separated scientific communities. This was the reason why the title

of "metallomics as integrated biometal science" was employed in the original paper [1].

1.2 Progress of Analytical Atomic Spectrometry

The present author started the research carrier with analytical atomic spectrometry in 1969 [9] and reached to "metallomics" in 2004 [1]. It was a long way, but it was a great pleasure. Thus, I would like to start this chapter with description of my experience in analytical atomic spectrometry.

In the early 1960s, the instruments of flame atomic absorption spectrometry (FAAS) became commercially available as the sensitive analytical method, and it was extensively applied to trace analysis, especially to environmental analysis [9]. In those days, Japan had serious environmental problems such as Minamata disease caused by mercury and ouch-ouch disease (*itai-itai disease* in Japanese) caused by cadmium. Nowadays, it is well known that Minamata disease as the environmental issue was caused by the poisoning effect of methylmercury, which was bioaccumulated in fishes in the Minamata Bay area. Thus, the environmental criteria of total mercury and methylmercury were enacted to be "0.0005 ppm" and "not detected" by the governmental law in Japan. FAAS was also applied to the analysis of trace elements (metals and metalloids) in various biological samples such as blood, urine, and organs. Thus, FAAS was a powerful analytical tool to elucidate causative heavy metals in environmental pollution [9, 12].

After 1970, various analytical atomic spectrometric methods have been further developed for trace element analysis. Until now, the present author has had a lot of experiences in flame atomic absorption spectrometry (FAAS; 1969–1974), graphite furnace atomic absorption spectrometry (GFAAS;1974–1975), atomic fluorescence spectrometry (AFS; 1975–1977), inductively coupled plasma atomic emission spectrometry (ICP-AES; 1977–2000), and inductively coupled plasma mass spectrometry (ICP-MS; 1990–2010) [1, 3, 9]. During these four decades, the analytical detection limits of those methods have been extensively improved from the ppb (10^{-9} g/mL) level down to the ppt (10^{-12} g/mL) or ppq (10^{-15} g/mL) level. Furthermore, it should be emphasized that ICP-AES and ICP-MS allow simultaneous multielement analysis with wide dynamic ranges of the working calibration curves, while AAS was mostly used for single-element analysis. The instrumental specifications and analytical feasibilities of those methods can be understood from the literatures [9–11]. The analytical characteristics of ICP-AES and ICP-MS will be briefly reviewed in the following paragraphs.

In ICP-AES and ICP-MS, the sample solution is introduced into the high-temperature argon plasma through a nebulizer. The photo of an argon inductively coupled plasma (argon ICP) is shown in Fig. 1.1. The characteristics of the ICP are the formation of the *so-called* doughnut structure, in which the temperature in the central channel of the plasma is lower than that of the surroundings. This doughnut structure is a very important property of the inductively coupled argon plasma for

Fig. 1.1 A photo of an inductively coupled argon plasma used for excitation and ionization sources in ICP-AES and ICP-MS, respectively

the efficient sample introduction into the plasma by pneumatic nebulization. In the case of ICP-AEC, the emission intensities of atomic or ionic lines are observed as the emission signals. Usually, the ionic emission lines provide the better sensitivities than the atomic lines, because most metallic elements are significantly ionized (more than 90%) and efficiently excited in the argon plasma at 6000–8000 K. However, since efficient excitation of ions often causes analytical errors due to serious spectral interferences because of the emission line overlapping [10], the spectral interference correction must be properly performed in the ICP-AES measurements.

In ICP-MS, on the other hand, the ions produced in the argon plasma are let into a mass spectrometer through the sampling cone and the skimmer cone and detected by a secondary electron multiplier [9, 11]. Two types of mass spectrometers such as a low-resolution quadrupole mass spectrometer and a high-resolution (HR) double-focusing mass spectrometer are available as the ICP-MS instruments. The former is called Q-ICP-MS and the latter is HR-ICP-MS. As is well known, many elements are composed of several stable isotopes, and the information for intrinsic isotope ratios corresponding to natural abundances of atoms can be obtained in ICP-MS, since ICP-MS can detect the mass of each isotope (m/z). These isotope ratio measurements, thus, are helpful for the identification of the elements [11]. In addition, isotope dilution mass spectrometry (IDMS) can be performed in ICP-MS by adding some enriched isotopes [16, 21, 22], since IDMS provides most precise and reliable analytical data in quantification of trace metals.

The detection limits obtained by commercially available ICP-AES and ICP-MS instruments are summarized in Fig. 1.2 [23], which were obtained by Q-ICP-MS and HR-ICP-MS. In the figure, the elements are plotted at the positions

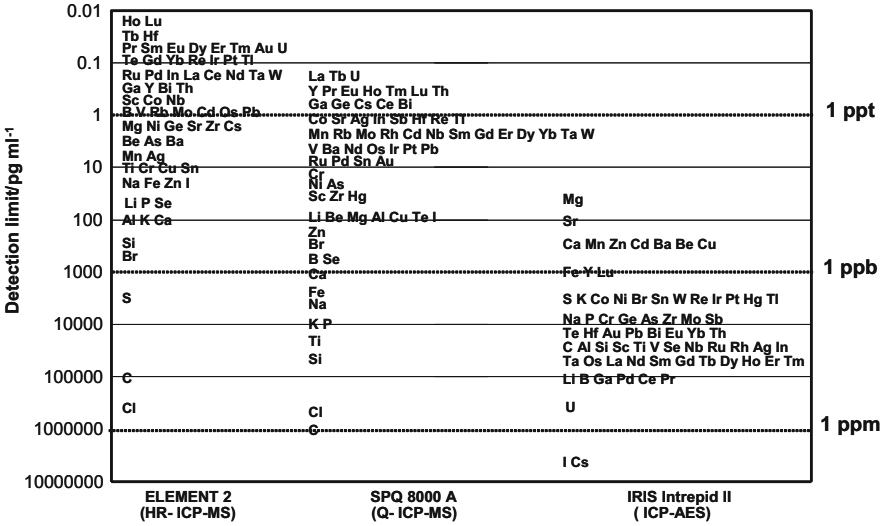

Fig. 1.2 Comparison of the detection limits obtained by HR-ICP-MS, Q-ICP-MS, and ICP-AES [23]. HR-ICP-MS: high-resolution ICP-MS with double-focusing mass spectrometer from Thermo Fisher Scientific Co., Q-ICP-MS (SPQ 8000A): ICP-MS with quadrupole mass spectrometer from Seiko Instruments Co., ICP-AES: ICP-AES with a CID (Charge Injection Device) multielement detector from Thermo Fisher Scientific Co.

corresponding to the detection limits on the vertical axis (concentration unit). It can be seen from Fig. 1.2 that the detection limits at the ppb level are generally obtained by ICP-AES, while the detection limits obtained by ICP-MS are much more improved down to the ppt (pg/mL) or sub-ppt level. Then many elements can be directly detected at the sub-ppt level by HR-ICP-MS.

It is also noted here that ICP-AES and ICP-MS provide the wide linear dynamic ranges of working calibration curves (four to six orders of magnitude), which provide excellent analytical feasibilities for the multielement detection of the elements in the concentration range from ppb up to 1000 ppm in ICP-AES and that from sub-ppt up to 1 ppm in ICP-MS [9]. Thus, if both ICP-AES and ICP-MS instruments are available, analyte elements (metallic and metalloid elements including S, P, Cl, Br, I) in the concentration range from sub-ppt up to 1000 ppm, i.e., over 10^{10} orders of magnitude, can be simultaneously determined for any biological samples. These characteristics of plasma spectrometry make it possible to perform all-element analysis of biological samples as well as geochemical and environmental samples [1, 9], as will be described in the next section.

1.3 All-Element Present Theory

In 1936, I. Noddack [24] published the article entitled "Concerning the ubiquitous nature of the chemical elements" (its original title in Germany was "Über die Allgegenwart der chemischen Elemente") in *Angewandte Chemie* and suggested that all elements in the periodic table might be present in all rock and mineral samples on the earth. Kuroda supported the Noddack's idea (hypothesis) of ubiquitous presence of all elements in geological samples in his book *The Origin of the Chemical Elements and the Oklo Phenomenon*, published in 1982, and he named the Noddack's concept "the All-Present Theory of the Elements" [25].

In the 1930s, the number of elements measurable in rock and minerals was quite limited because the analytical methods available in those days, such as flame emission spectrometry, arc/spark emission spectrometry, electrochemical methods, and so forth, were not sensitive enough to detect the low-abundant elements. Thus, many scientists were thinking that the elements whose concentrations were unknown were not contained in the samples of interest. On the contrary, Noddack believed that even the elements whose analytical values had been unknown might be contained in any geological samples, and she insisted that the existence of all elements in all geochemical samples would be elucidated, when the sensitive analytical techniques for trace elements would be developed in the future.

These days, according to great progress in analytical methodology, as described before, almost all elements are able to be determined or detected not only in geochemical samples but also in biological and environmental samples [9]. Since 1990, thus, the Haraguchi's research group in Nagoya University challenged all-element analyses of the representative samples collected from the atmosphere, lithosphere, hydrosphere, biosphere, and urbanosphere (urban area), for example, airborne particulate matter, rocks, seawater and lake water, biological samples from humans and plants, and bottom/fly ashes from the waste incinerators [1, 9, 12]. Based on such experimental results, Haraguchi proposed a new concept of "the Extended All-Present Theory of the Elements" [9], as follows:

> The elements contained in rocks and minerals are dissolved into water because of the weathering processes on the earth, and plants growing in soil absorb the elements in water for nutrition, and then animals ingest plants for food, and humans drink water and ingest plants and animals as food because humans are at the top of food chain. Consequently, it is obvious to consider that plants and animals, even their organs and blood, contain all elements through the elemental cycles on the earth, in another words, the All-Present Theory of the Element stands up all right not only for rocks and minerals, but also for all biological systems on the earth. Then, the Extended All Present Theory of the Elements is more suitable term to express the situations.

This concept indicates that all elements in the periodic table are ubiquitously contained not only in geochemical samples but also in all biological systems, such as humans, plants, and microorganisms. Humans are of course the object of the all-present theory. Thus, the All-Present Theory of the Elements proposed by Noddack should be extended to the new concept with the wide viewpoint, and then the Extended All-Present Theory of the Elements was newly proposed. Since

the newly proposed term is a little long, a slightly simple term of "**the All-Element Present Theory**" is recommended here for the use as the academic term.

Haraguchi is further thinking that the final goal of the All-Element Present Theory is to prove the existence of all elements in single biological cell. This concept "the existence of all elements in single biological cell" may be referred to as "cell microcosm" [1]. If the cell microcosm is scientifically elucidated for the living biological cells, such knowledge of the elemental distributions in the biological cells may provide important information for the study on life science as well as global cycles of the elements in the whole universe including the earth. From the viewpoint of the All-Element Present Theory, the elemental distributions in humans, human blood serum, cherry blossom, and salmon egg cell will be discussed in the following description.

(a) **The elements contained in humans and their components/functions**

In analytical chemistry, the elements in the concentration ranges of 100–1%, 1–0.01%, 0.01–0.0001%, and below 0.0001% are defined as major, minor, trace, and ultratrace elements, respectively. Since 0.0001% is equal to 1 ppm (part per million = μg/g), trace elements are the elements in the concentration range of 1–100 ppm. In the case of metals, they are called "trace metals." It is noted here that, in medical and biological sciences, ultratrace elements are usually considered as the same as trace elements, and thus, all the elements whose concentrations are less than 100 ppm are generally called trace elements hereafter. As will be seen in Table 1.1, the amounts of Fe and Al are about 6 g and 60 mg per 70 kg (the body weight), which correspond to 85.7 μg/g and 0.857 μg/g in the concentration, respectively. According to the definition above, both of Fe and Al are conveniently trace elements, although, exactly to say, Al is ultratrace element.

In Table 1.1, the average amounts of major, minor, trace, and ultratrace elements contained in a human body (70 kg weight) are summarized together with their components or biological functions [1, 26]. As for essential elements, major, minor, and trace (ultratrace) elements established for humans and experimental mammals are marked with *, **, and ***, respectively, in Table 1.1. It is seen from Table 1.1 that a variety of elements in the wide concentration range are contained in our human body and also that many of metallic elements play essential roles as metalloproteins and/or metalloenzymes in the blood and organs of the biological systems (*see* the discussion in "1.8.2 Essentiality and toxicity of the elements"). For example, the antioxidation functions of Se (glutathione peroxidase) and Fe (catalase) are important to protect the biological systems from oxidation caused by peroxides. Superoxide dismutase, which usually contains Mn, Fe, Cu, and/or Zn, acts as an antioxidant against superoxide. In addition, it is known that Na and K act as a Na-K pump across the cell to perform material transportation through cell membranes. Sodium and K are extracellular and intracellular electrolytes, respectively, which means that the Na concentration is higher outside the cell than inside, while the K concentration is higher inside the cell than outside. Such concentration differences of Na and K produce the concentration gradients across the cell membranes, which result in producing osmotic pressure to provide the field for

Table 1.1 The elements contained in humans and their components/functions[a]

	Element[b]	Amounts, (70 kg weight)	Components/functions in body
Major elements	O*	45.5 kg	Water and organics
	C*	12.6	Organics
	H*	7.0	Water and organics
	N*	2.1	Amino acids, proteins, nucleic acids
	Ca*	1.05	Bones, cell membrane, blood
	P*	0.70 (98.5 %)[c]	Bones, nucleic acids, ATP
Minor elements	S*	175 g	Amino acids, Vitamin B_1, heparin
	K*	140	Inner-cell electrolyte, blood
	Na*	105	Outer-cell electrolyte, blood
	Cl*	105	Main anions inside and outside cells
	Mg*	105 (99.4%)[c]	Co-factors of enzymes, chlorophylls
Trace elements	Fe**	6	Transportation and storage of oxygen and iron, catalase
	F***	3	Concomitants of bone and teeth
	Si***	2	*Unknown*
	Zn**	2	Co-factors of enzymes (DNA & RNA polymerases), cell division
	Sr***	320 mg	*Unknown*
	Rb	320	*Unknown*
	Pb***	120	Metabolism of iron, hematopoiesis
	Mn**	100	Co-factors (superoxide dismutase, pyruvate kinase)
	Cu**	80	Redox reactions, electron transfer, oxygen addition
Ultratrace elements	Al	60	Alzheimer disease
	Cd	50	Ouch-ouch disease
	Sn***	20	Redox reactions
	Ba	17	*Unknown*
	Hg	13	Minamata disease (methylmercury)
	Se**	12	Antioxidation (glutathione peroxidase), mercury detoxication
	I**	11	Activation of thyroid gland
	Mo**	10	Co-factors of enzymes (xythanthin oxydase, nitrate reductase),
	Ni***	10	Co-factor of enzyme (urease), stabilization of RNAs
	Cr**	2	Glucose tolerance factor, metabolisms of fats and proteins
	As***	2	Metabolic activation of zinc
	Co**	1.5	Hematopoiesis (vitamin B12)

(continued)

Table 1.1 (continued)

	Element[b]	Amounts, (70 kg weight)	Components/functions in body
	V***	1.5	Metabolism of cholesterols, inhibitors of Na^+- and K^+-ATPase

[a]Cited from Ref. [26] with some revision
[b]*Essential major and minor elements, ** Essential trace elements in humans, *** Essential trace elements in experimental mammals
[c]Cumulative amounts

active or passive transport of various biomaterials across the cell membrane. Although the functions of metallic elements are not explained anymore here, there is no doubt that most of biological systems cannot maintain their activities without the aid of a variety of metallic elements [1, 26, 27]. Thus, metallomics should be systematically established as biometal science in order to appreciate the biological systems from the viewpoint of the biological functions for syntheses and metabolisms.

(b) **The concentrations of the elements in human blood serum**

According to the All-Element Present Theory, human blood serum supposed to contain all elements in the periodic table, too. The concentrations of major-to-ultratrace elements in human blood serum so far reported are summarized in Table 1.2 [1]. It is seen from Table 1.2 that 53 kinds of elements in the concentration range of 12 orders of magnitude are contained in blood serum, where essential elements for humans and animals are indicated in bold letters. In human blood serum, Na, K, Mg, and Ca are contained as major elements. These concentration characteristics of major elements in human blood serum are similar to those in seawater, as will be seen later in (d) of this section. Thus, it is often said that such a similarity of the concentration levels of major elements in blood serum and seawater might be one of evidences for the origin of life in the sea [28].

Here, the concentration levels of essential trace elements in blood serum are compared with those in seawater. It is remarkable that the concentrations of essential trace elements such as Fe, Cu, Zn, Se, Mn, and Co are much higher in blood serum than those in seawater, while the concentrations of I, Mo, and Cr are significantly lower in blood serum than those in seawater. These facts indicate that Fe, Cu, Zn, Se, Mn, and Co are enriched in blood serum by bioaccumulation, because these elements exist as a variety of metalloproteins to express the specific biological roles required by the life systems. Blood serum is also taking important roles to transport such elements to the cells and organs for nutrition and metabolism by circulating our whole body. It is also mentioned here that trace elements such as Pb, Sn, Hg, Cd, and Ag, which are considered to be rather toxic or hazardous elements, are contained at the higher concentration in blood serum rather than in seawater, while these elements does not cause any toxicity at the concentration level in humans and other animals.

Table 1.2 The concentrations of major-to-ultratrace elements in human blood serum[a]

Element[b]	Blood serum concentration (ng/g)	Element[b]	Blood serum concentration (ng/g)
Cl	3,200,000	W	0.34
Na	3,130,000	U	0.31
K	151,000	**Ni**	0.23
P	119,000	*Ce*	0.21
Ca	93,100	Ag	0.20
Mg	17,500	Cd	0.15
Br	4440	**Co**	0.11
Fe	1200	Be	0.09
S	1110	**Cr**	0.069
Cu	750	*La*	0.063
Zn	651	*Nd*	0.034
Rb	170	**V**	0.031
Se	160	*Yb*	0.013
Si	140	*Pr*	0.011
Sr	33	*Dy*	0.0096
Sb	2.3	*Er*	0.0095
B	2.1	*Gd*	0.0072
Al	1.8	**I**	0.007
Pb	1.2	*Sm*	0.0058
Mo	0.95	*Ho*	0.0026
Cs	0.95	*Lu*	0.0025
Y	0.73	*Tm*	0.0017
Mn	0.57	Sc	0.0017
Hg	0.55	Pt	0.0014
Sn	0.51	*Tb*	0.0013
Th	0.50	*Eu*	0.00082
Ba	0.48	Au	0.00003
As	0.45		

[a]Cited from Table 1.4 in Ref. [1], and the references are there
[b]The essential elements for humans and animals are indicated in bold letters (*See* Table 1.1), and rare earth elements are indicated in italic letters

In 2000, Inagaki and Haraguchi reported the existence of all rare earth elements in blood serum [29]. As is seen Table 1.2, the concentrations of rare earth elements are extremely low at the ppt or sub-ppt level, which are almost at the same concentration level as those in seawater. The biological roles or functions of rare earth elements in the life system have not been known so far.

(c) **Multielement analyses of cherry blossom petals and leaves**

The elemental distributions in cherry samples are described as the next topics. It is well known that the cherry blossoms are the symbolic flower of Japan. Usually, the cherry blossoms start flowering late March or early April. Exactly to say, the cherry flowers are cherry blossom petals, which are kept shortly on the tree only for

Table 1.3 Multielement determination of major-to-ultratrace elements in cherry samples by ICP-AES and ICP-MS[a] [30]

	Concentration (μg/g)			Concentration (μg/g)	
Element[b]	Petals[c] (4/9)	Leaves[c] (8/11)	Element[b]	Petals[c] (4/9)	Leaves[c] (8/11)
K	19,200	19,800	Pr	0.27	0.23
Ca	2530	11,500	Zr	0.26	0.80
P	2520	1870	Sm	0.17	0.11
Mg	1170	2370	Gd	0.15	0.093
Al	179	37	Sn	0.12	0.032
Fe	115	58.9	Cs	0.11	0.032
Na	69.8	13.5	**Co**	0.102	0.044
Mn	76.7	49.4	Dy	0.093	0.048
B	31.4	35.9	Cd	0.046	0.012
Rb	30.2	29.2	W	0.034	0.011
Zn	22.7	15.2	Eu	0.033	0.024
Cu	7.01	7.01	Th	0.030	0.008
Ba	3.50	25.6	Er	0.027	0.020
Ce	1.85	1.81	Yb	0.019	0.011
Ni	1.60	0.86	Tb	0.019	0.010
La	1.46	1.54	Tl	0.013	0.015
Sr	1.30	18.6	Ho	0.011	0.009
Nd	1.02	0.78	U	0.0073	0.0025
Pb	0.97	0.89	Tm	0.0034	0.0023
Y	0.70	0.34	Lu	0.0028	0.0017
Mo	0.47	0.54			

[a]The elements in bold letters are the essential elements for plants
[b]The samples were digested by using HNO_3 and HF
[c]The sampling dates; petals on April 9, 1996, and leaves on August 11, 1996

about 2 weeks. Soon after the petals fall down, the cherry leaves come out and grow until the end of August. The analytical data for petals collected on April 11 and leaves collected on August 9 are summarized in Table 1.3 [30]. In the present experiment, petals and leaves were collected and analyzed every week from the beginning of April to the end of August in 1996. These samples were analyzed by ICP-AES and ICP-MS after the acid digestion. In addition, the chlorophyll contents in leaves were analyzed every 2 weeks, although the data are not shown. In the year 1996, the cherry trees were almost in full bloom around April 11, while the chlorophyll contents in leaves were almost at the maximum around August 9, indicating that photosynthesis in leaves was most active around the middle of August.

The analytical data were obtained for 41 elements, as is seen in Table 1.3, where the essential elements for plants are indicated in bold letters. The number of the elements measured was limited to about 40, because the quadrupole-type ICP-MS instrument which was in low resolution and less sensitive was only available in this experiment. It is noticeable that the elemental concentrations in short-lived petals were almost the same as those in leaves collected about 4 months later. However, if

the data are examined carefully, we can find some characteristic behaviors of the elements by comparing the data between petals and leaves. It is just pointed out here that the concentrations of Mg and Ca are remarkably higher in leaves rather than in petals. In the case of Mg, Mg increased along with the increase of chlorophyll contents in leaves because Mg was a component of chlorophyll. It was also observed that the Mg concentration in leave decreased after the middle of August. This phenomenon may be interpreted by the fact that Mg moved from leaves to other parts of the tree, maybe to trunks and roots for preservation. Calcium also increased in leaves through the experimental period, but its concentration did not decrease even after the middle of August. These results suggest that Ca taken into leaves was accumulated as non-movable components therein, maybe because of some interactions with cell proteins in leaves. As for other elements, their concentrations were almost the same levels in petals and leaves, although some essential elements such as P, Fe, Na, Mn, Zn, and Ni provided the higher concentrations in petals than in leaves.

(d) **A challenge to all elements analysis of salmon egg cells**

All-element analysis of salmon egg cells was also challenged in order to examine the concept of cell microcosm, i.e., all-element presence in single biological cell. The analytical results are summarized in Table 1.4 [1, 31], together with the concentrations of the elements in seawater. In salmon egg analysis, three or five salmon eggs were digested with nitric acid by using a microwave digestion method. In the present experiment, rare gas elements and natural/artificial radioactive elements were not measured because the specific gas sampling systems as well as the skillful gas treatment techniques were necessary in rare gas analysis and the specially designed experimental facilities for protection from harmful radioactivity were required for treatment of radioactive elements. As a result, 78 elements in the periodic table were the target of all-element analysis in the standard laboratory.

The analytical results for 66 elements among 78 are shown in Table 1.4, where the metallic and metalloid elements were measured by ICP-AES and ICP-MS and nonmetallic elements such as H, C, N, O, and Cl were determined by the conventional elemental analysis method, while other seven elements (Li, Zr, Nb, Hf, Ir, Bi) were just detected because of their low abundances in salmon egg cell. Fluorine, Rh, Te, Ta, and Re in salmon egg cells could not be determined or detected at this experiment, maybe because of their extremely low concentrations. However, these elements except for F were detected by HR-ICP-MS in the recent experiment, although the results have not been reported yet.

Many interesting facts can be found from the results in Table 1.4, but here it is just pointed out that Fe, Zn, Cu, Co, Mn, Se, and P provided the bioaccumulation factors larger than 10,000 [31], which indicated the large enrichment of these elements in salmon egg cell from seawater. In the next section, chemical speciation of the elements in salmon egg cell will be described in relation with the data shown in Table 1.4.

Table 1.4 Concentrations of the elements in salmon egg cells and seawater [31]

Element[a]	Salmon egg cell concentration (ng/g)	Seawater concentration (ng/ml)
O	600,000,000	–
C	250,000,000	–
H	100,000,000	–
N	42,000,000	–
P[†]	3,580,000	62
S	2,990,000	898,000
Cl	2,800,000	19,350,000
K[†]	1,860,000	399,000
Ca[†]	432,000	412,000
Na[†]	247,000	10,780,000
Mg[†]	222,000	1,280,000
Br	20,000	67,000
Zn[†]	13,600	0.35
Fe[†]	10,700	0.03
Cu[†]	8900	0.15
I	3700	58
Sr[†]	3600	7800
Se	1970	0.16
Ti	1420	0.0065
Rb	523	120
Mn[†]	512	0.02
As	192	1.2
Ba	47.3	15
Ni	26.5	0.48
V	18.8	2.0
Co	12.5	0.0012
Hg	12.0	0.00014
Ag	10.7	0.002
Pd	8.0	0.00006
B	7.8	4500
In	7.0	0.0001
Al	6.44	0.31
Mo	6.43	10
Ge	5.83	0.005
Cs	5.58	0.31
Cd	1.1	0.07
Sn	0.96	0.0005
Be	0.80	0.00021
Cr	0.73	0.21
U	0.66	3.2
Pb	0.48	0.0027
Ru	0.19	0.000005

(continued)

Table 1.4 (continued)

Element[a]	Salmon egg cell concentration (ng/g)	Seawater concentration (ng/ml)
Sb	0.12	0.2
Y	0.11	0.017
La	0.075	0.0056
Nd	0.074	0.0033
Au	0.054	0.0066
Tl	0.050	0.013
W	0.033	0.01
Gd	0.016	0.0009
Ce	0.014	0.0007
Pr	0.010	0.0007
Sm	0.010	0.00057
Th	0.009	0.00002
Dy	0.0074	0.0011
Sc	0.0059	0.0007
Pt	0.0043	0.0002
Er	0.0031	0.0012
Yb	0.0027	0.0012
Ga	0.0023	0.0012
Tb	0.0021	0.00017
Os	0.0019	0.000002
Ho	0.0017	0.00036
Eu	0.0011	0.00017
Lu	0.00078	0.00023
Tm	0.00037	0.0002

[a]The elements with † were determined by ICP-AES, and other elements were determined by ICP-MS. In addition, H, C, N, O and Cl were determine by the conventional elemental analysis method

1.4 Chemical Speciation of Trace Metals in Biological Samples

As described earlier, the analytical sensitivities for metallic elements (including metalloids) were substantially improved down to the ppt or sub-ppt level according to the progress of ICP-MS. However, the direct information for the elements obtained by ICP-MS is only their total concentration data, since the molecular information is lost because chemical species are decomposed in the high-temperature argon plasma at around 7000–8000 K. In biological and environmental analysis, however, the information for chemical or molecular species is generally more important than that of total concentrations of the elements, because the impacts and toxicities to the biological systems are dependent on their chemical forms. For example, inorganic arsenic (arsenite and arsenate) are toxic and/or carcinogenic, while methylated arsenicals are nontoxic, as is well known [32]. In

the case of chromium, Cr(III) is bio-essential, but Cr(VI) is toxic because of its strong oxidation ability. In addition, the causal substance of Minamata disease was methylmercury, not inorganic mercury. Thus, the identification and quantification of chemical species including oxidation states of the elements are really required to elucidate their biological/physiological functions and activities to the biological systems. The analytical technology for identification and quantification of chemical species is defined as "chemical speciation" or "speciation" in analytical chemistry [13–16].

Trace metals (mostly ionic forms) are usually bound to large molecular proteins to form metalloproteins in biological cells and fluids, but their concentrations in biological samples are generally very low. Then, the highly sensitive and element-selective analytical methods are required for the detection of trace chemical species like metalloproteins. In addition, the chemical compositions of biological samples are very complicated. In another word, trace metallic ions exist in very complex matrices controlled by the complicated chemical equilibria [33], so that the element-selective and highly sensitive detection methods of trace metals are necessary in speciation analysis. At present, in order to fulfill such requirements for chemical speciation analysis, a hyphenated system combined with efficient species separation and highly sensitive element detection (e.g.,HPLC/ICP-MS) is most suitable for speciation analysis. Various types of chromatographic methods are used for efficient separation of chemical species, and ICP-MS is used for the highly sensitive and element-selective detection of metallic and metalloid elements.

In recent years, various combined methods for chemical speciation have been developed and extensively applied to the elucidation of the chemical forms even for biometals. In Fig. 1.3, the present state-of-the-art hyphenated methods for chemical speciation are summarized [34], and there are many excellent review articles concerning with chemical speciation so far published [13–16, 32]. It can be seen in Fig. 1.3 that the analytical systems for chemical speciation generally consist of three main parts such as separation shown in the left-hand side, detection in the right-hand side, and identification of chemical species in the center. Nowadays, a variety of separation methods can be selected, depending on the kinds of chemical species from small molecules to large molecules (proteins and other biopolymers) as well as their chemical properties. As the detection method, ICP-MS is the most frequently used, where a quadrupole-type mass spectrometer (Q-MS), time-of-flight mass spectrometer (TOF-MS), and sector field or high-resolution mass spectrometer (SF-MS or HR-MS) are employed for the detection of analyte elements.

As mentioned before, ICP-MS can provide the element information, but it cannot provide the molecular information. Thus, in chemical speciation using the hyphenated systems, chemical species or molecules are generally identified by referring to the retention times for standard (known) compounds observed in the chromatograms. MALDI-MS (matrix-assisted laser desorption/ionization MS) and ESI-MS (electrospray ionization mass spectrometry) are often used for direct identification of chemical or molecular species, but they are not so sensitive enough to provide the information for trace metals in various biomolecules.

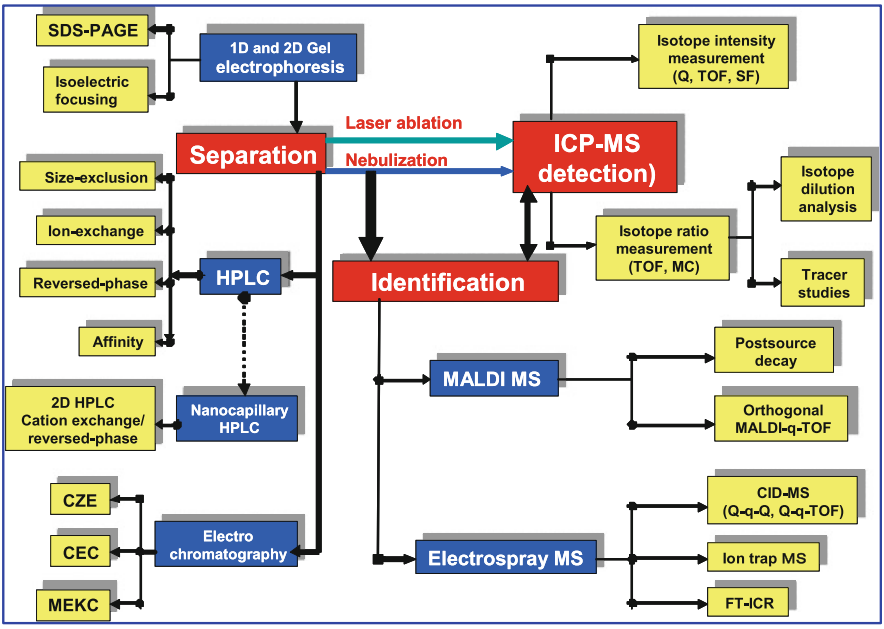

Fig. 1.3 Various hyphenated methods for chemical speciation analysis, cited from Ref. [34] with some revision

As an example, a schematic diagram of SEC (size exclusion chromatography)/ ICP-MS system for chemical speciation is shown in Fig. 1.4, where a UV absorption detector is equipped for monitoring protein elution. Such a SEC/ICP-MS system is conveniently used for the identification and molecular weight calibration of metalloproteins.

(a) **Chromatogram of standard proteins**

In Fig. 1.5, a chromatogram of standard proteins obtained by using the SEC column with the UV absorption detection (measured in absorbance) is shown together with the retention times of proteins. The colored zone in Fig. 1.5 indicates the permeation range of the SEC column, and the numbers (1–7) in the chromatogram correspond to proteins examined (proteins and their molecular weights are indicated in the figure caption). In the SEC chromatogram, of course, the larger molecules are eluted earlier than the smaller molecules because of the size effects of the SEC column.

(b) **Chemical speciation of arsenicals in salmon egg cell**

The HPLC chromatograms for arsenicals (arsenic species) in salmon egg cell cytoplasm and membrane are shown in Fig. 1.6a and b, respectively [32], where arsenic was detected at m/z 75 by ICP-MS. Cell cytoplasm (intracellular fluid) and cell membrane were separately collected from whole egg cells by using a Teflon

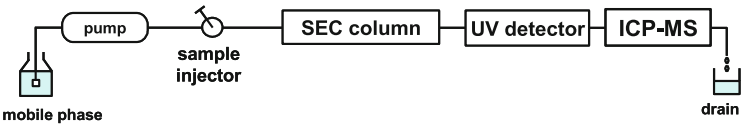

Fig. 1.4 A schematic diagram of SEC/ICP-MS with a UV absorption detector for chemical speciation of metalloproteins

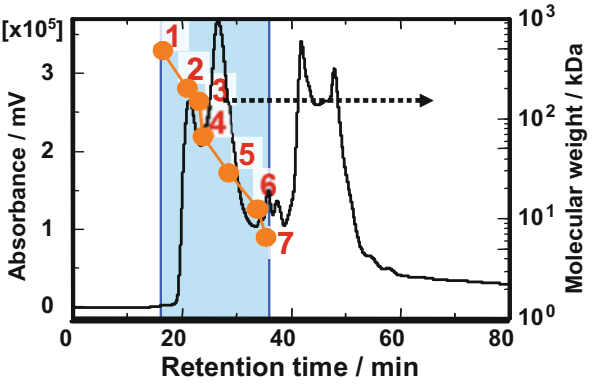

Fig. 1.5 Chromatogram of standard proteins obtained by a hyphenated system of SEC/ICP-MS. Stationary phase: SuperoseTM 12 10/300 GL (molecular permeation range 1–300 kDa); mobile phase, 0.1 M Tris-HCl (pH 8.0); flow rate of mobile phase, 0.5 mL/min; sample injection volume, 100 μL; wavelength of UV absorption detection, 254 nm; standard proteins, (1) ferritin (440 kDa), (2) β-amylase (200 kDa), (3) alcohol dehydrogenase (150 kDa), (4) albumin (66 kDa), (5) carbonic anhydrase (29 kDa), (6) cytochrome c (12 kDa), (7) aprotinin (7 kDa)

tweezers and a Teflon needle. Arsenic species were extracted from cell cytoplasm and cell membrane as follows. Approximately 1 g of cell cytoplasm or cell membrane sample was taken in a centrifugation tube, and 10 mL of 50% methanol was added. The sample was sonicated for 30 min and then centrifuged at 4000 rpm for 5 min. The supernatants as the analysis sample were collected two times, put together in the rotary evaporator, and evaporated almost to dryness at 40°C. The residue was dissolved in 1 mL of HPLC mobile phase solution and then filtered with a membrane filter (pore size; 0.45 μm). The dissolved solution was subjected to the HPLC/ICP-MS analysis. In the case of cell membrane analysis, before the extraction procedure mentioned above, 0.5 mL of TMAH (tetramethylammonium hydroxide) was added as an alkali reagent into the sample to dissolve arsenic species from lipids or proteins in cell membrane.

It is seen in Fig. 1.6a that a large peak of AB (arsenobetaine) and three small peaks of iAs^V (arsenate), DMA (dimethylarsinic acid), and TMAO (trimethylarsine oxide) were observed for cell cytoplasm. The concentrations of these AB, iAs^V, DMA, and TMAO were estimated to be 17.6, 1.7, 0.4, and 3.0 ng/g as As, respectively, on the wet-weight basis. On the other hand, as is seen in Fig. 1.6b, iAs^V, MMA (monomethylarsonic acid), DMA, and AB were found in cell membrane,

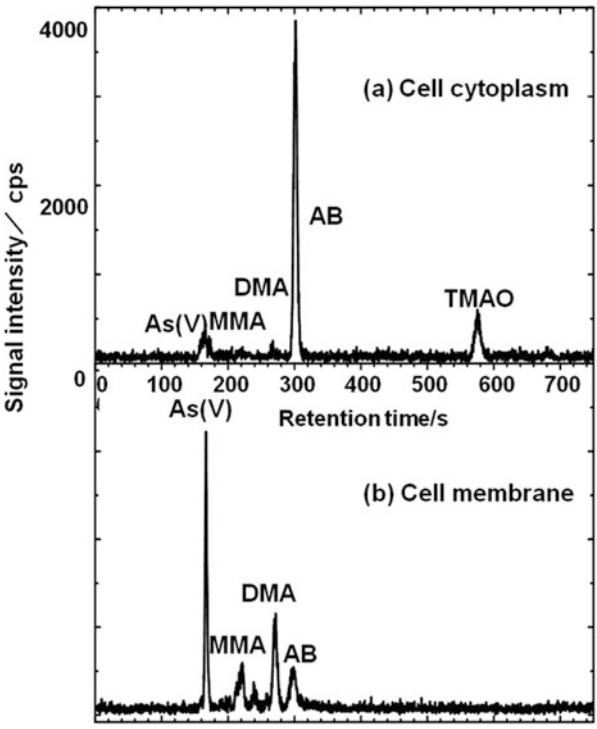

Fig. 1.6 HPLC chromatogram for arsenic species in (**a**) cell cytoplasm and (**b**) cell membrane of salmon egg detected by ICP-MS at m/z 75 [32]. Column: CAPCELL PAK C18 (ODS column); mobile phase, 4 mM malonic acid + 4 mM TMAH + 10 mM 1-butane sulfonic acid sodium salt + 0.05% methanol (pH 3.0); flow rate, 0.75 ml/min; sample injection volume, 10 μL

whose concentrations were 15.4, 8.1, 12.3, and 8.4 ng/g as As, respectively. As a result, the total concentrations of arsenic species in the extracts from cell cytoplasm and cell membrane were 22.7 ng/g and 44.2 ng/g, respectively, which corresponded to ca. 12% and 35% of the total amounts of arsenic in cell cytoplasm and cell membrane, respectively. These results suggest that the rest of arsenic species may exists as large molecular arsenic species such as protein- or sugar-binding arsenic in both cytoplasm and membrane, because only small molecular arsenic species could be identified by HPLC/ICP-MS using the ODS column in ion-pair mode. Inorganic arsenic (iAs^V) was the main arsenic species in cell membrane, while AB (arsenobetaine) was the main species in cell cytoplasm. These facts suggested that methylation of inorganic arsenic to AB occurred on passing through the cell membrane or after penetrating into cell cytoplasm.

(c) **Fractionation analysis of the elements in salmon egg cell cytoplasm**

As one of the examples for chemical speciation, the fractionation analysis of the elements in salmon egg cell cytoplasm was carried out by HPLC/ICP-MS, where the CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; zwitterionic surfactant)-coated ODS (octadecyl silica) column (4.6 mm i.d. x 250 mm long) was used in order to examine the protein bindings of the elements [35]. The CHAPS-coated ODS column was prepared by the dynamic coating

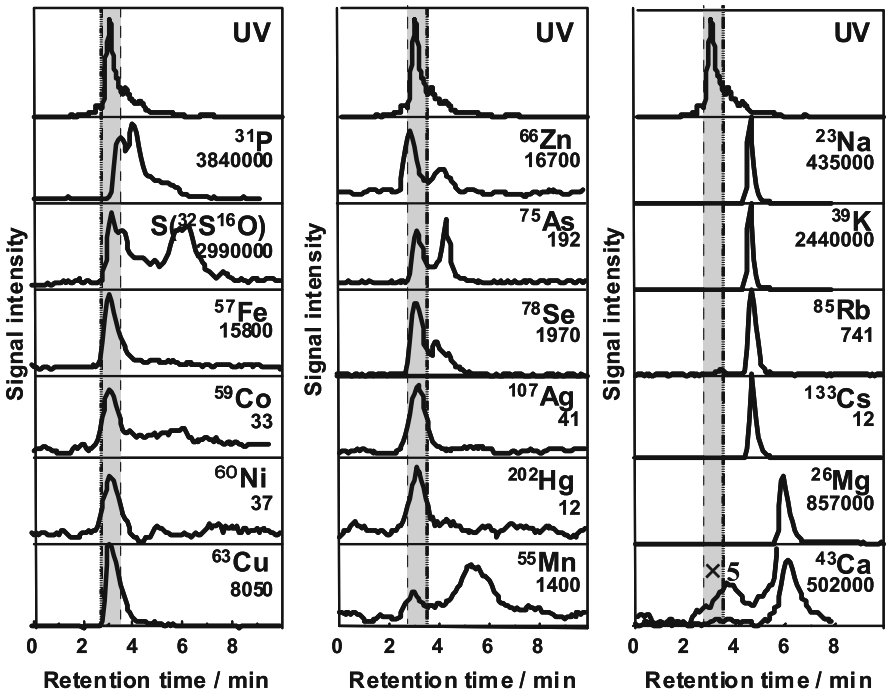

Fig. 1.7 The element-selective chromatograms for salmon egg cytoplasm obtained by HPLC/ICP-MS. [31]. Sample: salmon egg cell cytoplasm diluted five-fold with 0.1 M Tris buffer (pH 7.4); column, CHAPS-coated ODS column; mobile phase, 0.1 M Tris buffer solution (pH 7.4); ICP-MS detection, m/z shown in the figure; UV absorption detection, 254 nm. The numbers below the elements indicate the concentrations (ng/mL) of the elements in salmon egg cell. The retention time range between 3.0 and 3.5 min (*gray* zone) corresponds to the protein elution zone

method, where 20 mM CHAPS aqueous solution was passed through the ODS column at 0.7 ml min^{-1} for 2 h, as described in the previous papers [35, 36]. After coating, the column was rinsed with pure water for 1 h. Since the CHAPS-coated ODS column has unique characteristics for simultaneous separation of ions/small molecules and large molecules (e.g., proteins) [36, 37], the useful information about the elements binding or nonbinding with proteins is easily obtained.

The chromatograms for the elements in salmon egg cell cytoplasm obtained by HPLC/ICP-MS using the CHAPS-coated ODS column are shown in Fig. 1.7 [31]. In Fig. 1.7, the chromatograms with the element-selective detection are illustrated together with those with the UV absorption detection at 254 nm, where the concentrations of the elements determined by ICP-MS are also indicated in each chromatogram. The retention range of 3.0–3.5 min shown as the gray zones in Fig. 1.7, which is almost consistent with the elution zones for proteins detected by UV absorption shown in the upper parts of Fig. 1.7, indicates the elution zone for large molecules with molecular weight (MW) larger than *ca.* 10,000 Da. On the

other hand, the retention range after 3.5 min indicates the elution zone for small ions and molecules [37]. However, the information about the molecular weights cannot be estimated from the chromatograms obtained by the CHAPS-coated ODS column, since the CHAPS-coated ODS column has no size exclusion property.

It is seen in Fig. 1.7 that heavy metals such as Fe, Co, Ni, Cu, Ag, and Hg, which are essential or toxic elements, provided single broad peaks within the protein elution zone. These results indicate that these heavy metals mostly exist as protein-binding species. On the other hand, Zn and Mn provided two main peaks within and after the protein elution zone, which suggests that Zn and Mn exist partly as small molecules/ions, maybe free ions or amino acid complexes, in addition to protein-binding species. Furthermore, alkali and alkaline earth elements, such as Na, K, Rb, Cs, and Mg, provided single peaks in the small molecules/ions zone in Fig. 1.7. These experimental results suggest that Na, K, Rb, Cs, and Mg in salmon egg cell exist as the ionic forms. It is interesting that Ca provided small peaks in the large molecular zone (*see* expanded *chromatogram*), which indicated that small amount of Ca was binding with proteins. As for arsenic as well as selenium, two separated peaks in the small and large molecular ranges were observed in a similar manner to the cases of Zn and Mn. These results suggest that arsenic and selenium in salmon egg cell cytoplasm exist not only as small molecules (e.g., arsenate and selenate) but also as protein-binding molecules. Thus, the results in Fig. 1.7 suggest existence of protein-binding molecules of arsenic in cell cytoplasm. In recent years, arsenic-binding proteins have been reported with great attention from the viewpoint of toxicity and drug design [38, 39]. In the case of selenium, glutathione peroxidase, antioxidant, is known as a large molecule (tetramer; MW *ca*. 84 kDa) in animals.

1.5 Human Genome Project and the Rapid Rise of Omics Science

The term of "genomics" emerged as the scientific term in 1988 [40], and in genomics the entirety of genes was defined as "genome" [41]. As is well known, the Human Genome Project had been conducted under the international cooperative research since 1991 in order to determine the sequence of human genome [17, 18]. In 2003, it was declared that the human genome was completely determined.

In the late 1990s, it was considered in the science community that in the early 2000s, the human genome sequence analysis would be completed, and then the post-genome project would be the next big international program, in which protein identification should be performed together with elucidations of their structures and functions [42]. Such a research field was called as "proteomics" in a similar manner to genomics, and the term of "proteomics" appeared as the scientific term in the literature for the first time in 1997 [19]. The entirety of proteins is defined as "proteome," in a similar manner to genome in genomics.

Then, in 2002, "metabolomics" emerged for the first time as the scientific term in the literatures [20]. Metabolomics is defined as the total science of metabolism in biological cell, tissue, organ, or organism (whole body). In metabolomics, the term of "metabolome" is used to express the entirety of all metabolites in analogy to genome in genomics [43]. In the metabolomics study, the LC-MS (liquid chromatography-mass spectrometry) system is extensively used for the profiling analysis of metabolites.

The emergence of genomics, proteomics, and metabolomics in biological science has strongly stimulated to create the new research trends in each field with cross correlation, and they are now called totally as "omics science." The progress of omics science since the late 1980s also gave great impact to the scientific fields other than omics science, of course, including trace metal chemistry, and at last in 2002, the term of "metallomics" as a new scientific field came to my mind, as will be described in the next section.

1.6 Proposal of Metallomics as Integrated Biometal Science—Historical Aspects

The abstract of my paper "Metallomics as Integrated Biometal Science" [1] is shown on the top page of this chapter, because it was the beginning of "metallomics" as the emerging scientific field of biometal science. As is described there, in these last few decades, various scientific fields such as analytical atomic spectrometry, omics science, as well as cell biology have progressed rapidly, and they had given great impacts to promotion of the study on metals in biology. Thinking about such advances of biological science, the idea of "metallomics" came into my mind suddenly one day. It was just like an inspiration, suggesting that metallomics should be a new scientific field among omics science. In such a time, it was very fortunate that good chances to introduce my idea were given to me in the following domestic seminar and international symposiums in 2002. As a result, the year of 2002 was the milestone for the proposal of "metallomics."

The first chance was the Tokushima Seminar on Chemical Engineering, held in Tokushima, Japan, in June 2002. The title of my invited lecture in the seminar was "A Challenge to Pico-World Science and Metallomics: A New Frontier of Trace Element Chemistry." This was the first time to introduce the idea of "metallomics" as the new scientific field, although the lecture was given in Japanese. The abstract of the lecture in the Tokushima Seminar was as follows [44]:

> In recent years, the analytical detection sensitivities have been increasingly improved to pico (10^{-12}) gram in the absolute amount or sub-ppt (10^{-12} g/ml) level in the concentration, according to the development of ICP-MS. As a result, now we have a good chance to challenge the research on the pico-technology or pico-science, which may be called "Pico-World Science".
>
> Such a progress of analytical atomic spectrometry will lead to another interesting and important research on bio-trace elements in the biological systems including our "human

> beings", because all-elements might be contained in all the biological systems. This concept is referred to as "Extended All Present Theory of the Elements". Furthermore, various trace elements play important roles in the biological systems, as metalloproteins and/or metalloenzymes. Then, now is the good time to challenge to trace element biochemistry to open our new scientific world "metallomics."

As is seen from the above sentences, my first idea of metallomics was on the basis upon the progress of analytical atomic spectrometry and the concept of the Extended All-Present Theory of the Elements, about which some explanation will be given later. However, my idea was just an inspiration at that time.

The International Symposium on Bio-Trace Elements 2002 (BITREL 2002) was the second chance for proposal of metallomics. This symposium was held as the Joint Symposium of RIKEN (Institute of Physical and Chemical Research) and YIES (Yamanashi Institute of Environmental Sciences) from October 28 to November 2, and it was co-organized by Shuichi Enomoto in RIKEN (he is now a professor in Okayama University) and Yoshiyuki Seko in YIES. The present author delivered the invited lecture with the title of "Trace Element Speciation for Metallomics," and metallomics was formally proposed as a new scientific term on this occasion. The abstract of the invited lecture is shown below, which was cited from the Proceedings of BITREL 2002 [45].

> In this paper, "metallomics" is newly proposed as a new scientific field in order to integrate the research fields related to bio-trace metals. Metallomics might be the scientific field of post-genomics and post-proteomics, where metal-containing compounds are defined as metallomes, in a similar manner to genome in genomics and proteome in proteomics. Since the elucidation of the biological or physiological functions of metal-containing species in the biological systems is the main research target of metallomics, elemental speciation is important as one of analytical technologies to promote metallomics.

As is seen in the abstract above, the importance of speciation analysis (species analysis) concerning with biotrace metals in the biological samples as well as in the biological systems was emphasized in the lecture, because most of biotrace metals (actually exist as the ionic forms in the biological systems) are contained in metalloproteins and/or metalloenzymes, and in particular biotrace metals play essential roles as the active centers of metalloenzymes, expressing the biological and physiological functions. On the other hand, it is also known in environmental pollution that some of metals and metalloids are seriously toxic or hazardous to humans and living organisms. Thus, it should be understood that "trace element chemistry" concerns with double natures of the elements; one is essential and the other is toxic.

In BITREL 2002, the distinguished scientists working in the field of biometal science were invited from various countries; they were Ryszard Lobinski (Warsaw University of Technology, Warsaw, Poland; now CNRS, Pau, France), Zhifang Chai (Institute of High Energy Physics, Chinese Academy of Science, Beijing, China), Wolfgang Maret (Harvard Medical School, USA; now Imperial College, London, UK), Joanna Szpunar (Group of Bioinorganic Analytical Chemistry, CNRS, Pau, France), Bibudhendra Sarkar (University Toronto, Ontario, Canada), David Brown (University of Bath, UK), and so forth. In the symposium, some of the

participants proposed the term of "metalloproteomics," while I proposed "metallomics." As a result, hot discussion was made about the nomenclature for biometal science.

On the occasion of BITREL 2002, Lobinski asked me to submit a paper about metallomics to *Journal of Analytical Atomic Spectrometry* (J Anal At Spectrom), published from the Royal Society of Chemistry, UK, because the journal was planning to publish the special issue on "Metals in Biology," [46] and he was one of the editorial board members of the special issue. In 2004, then, my paper entitled "Metallomics as Integrated Biometal Science" was published in J Anal At Spectrom [1], which was the summary of the lecture presented in BITREL 2002. The abstract of the paper in J Anal At Spectrom [1] was already introduced on the first page of this chapter, because it provided important suggestion for future direction of biometal science.

Another surprising thing happened in 2009. It was the publication of the journal of *Metallomics* from the Royal Society of Chemistry (frankly, I did not know the plan for publication!). In the first issue of the journal, J. Caruso (chair of the Editorial Board, Cincinnati University) and N. O'Conor (editor of *Metallomics*, RSC) announced their welcome and acknowledge message [2] as follows (*see* also 1.8.1):

> We would like to acknowledge the work of those whose vision has led to the establishment of this field, including Bob Willams (R. J. P. Williams, Coord. Chem. Rev., 2001, 216–217; 583–595), Hiroki Haraguchi (H. Haraguchi, J. Anal. At. Spectrom., 2004, 19, 5–14) and Joanna Szpunar (J. Szpunar, Anal. Bioanal. Chem., 2004, 378, 55–56), and to all those who continue to contribute to the emergence of metallomics, without whom we would not be launching this exciting new journal.

Williams published his paper entitled "Chemical selection of elements by cells" in Coord Chem Rev in 2001 [33]. This paper was the summary of his lecture in the 34th International Conference on Coordination Chemistry (ICCC34) held in the University of Edinburgh, Edinburgh, Scotland, July 09–14, 2000. The abstract of his paper is cited here [33]:

> The selection of the chemical elements by a particular cell from the environment involves a series of steps, the complexity of which depends upon the organism. There are usually two membranes to be crossed (bacteria) but there may be as many as ten (higher animals which distribute elements from intake fluid, through cells of organs to circulating fluids through a further set of cells to fixed locations in particular parts of space). The individual steps can be thermodynamically controlled or kinetically managed. In the second case energy can be used. The elements may remain in relatively fast exchange in their final condition or in non-exchanging chemical combination. The variety of paths which individual elements follow in any organism adds to the specific character of the organism. Clearly the paths have evolved to create an element distribution which we shall call the **metallome**, to parallel the nomenclature of protein distribution, the proteome.

As is seen in the above abstract, William used the term "metallome" for the first time in 2000, but he never mentioned "metallomics" in his papers.

Szpunar published her paper entitled "Metallomics: a new frontier in analytical chemistry" in Anal Bioanal Chem in 2004 [47]. The following sentences are part of her paper [47]:

> Recently, Haraguchi and Matsuura suggested the term"metallomics" to denote metal-assisted function biochemistry and postulated it to be considered at the same level of scientific significance as genomics or proteomics [45]. The metallomic information will comprise the identities of the individual metal species (qualitative metallomics) and their concentrations (quantitative metallomics). As such, metallomics can be considered as a subset (referring to cellular biochemistry) of speciation analysis understood as the identification and/or quantification of elemental species [13]. Species of interest for metallomics will include complexes of trace elements and their compounds (e.g. metalprobes) with endogenous or bio-induced biomolecules such as organic acids, proteins, sugars or DNA fragments. [15]

Szpunar was a participant in the International Symposium of Bio-Trace Elements (BITREL 2002) held in 2002. Then she knew my proposal of metallomics in the symposium as well as the publication of the symposium proceedings before publication of her paper, and so she cited our paper in the proceedings [38]. It is appreciated from these facts that Szpunar just followed my proposal, and she was not a proposer of metallomics. Even so, I would like to say that she recognized the importance of "metallomics" earlier than anyone, because she had been so enthusiastic to develop the chemical speciation methods for the biological systems [14, 15].

1.7 International Symposium on Metallomics

After publication of my paper in 2004, "metallomics" received great attention as the newly emerging scientific field [3, 5–7]. Such situations were the same in Japan, and then in 2004, the present author got the Grant-in-Aid for Specially Promoted Research (2004–2007; 3-year term) with the project title of "Creation of Metallomics as the New Scientific Field," which was supported by the Ministry of Education, Science, Culture, and Sports of Japan. In the proposal of the grant, I promised the organization of the International Symposium of Metallomics, when the project would be terminated. Thus, 3 years later in 2007 (November 28–December 1), I organized as the chairman the International Symposium on Metallomics 2007 (ISM 2007), which was held in Nagoya as the first international symposium on metallomics. Professors Kazuo Suzuki, Hiromu Sakurai, and Naoki Furuta helped me as vice-chairmen of the symposium. In this symposium, about 350 scientists participated and more than 250 lectures and posters were presented [48]. The front cover of the program book for ISM 2007 is shown in Fig. 1.8.

The International Advisory Board members of ISM 2007 are listed in Table 1.5, and they concluded that ISM 2007 was the successful meeting and "metallomics" should be promoted as a newly emerging scientific field thereafter. Furthermore, it was also agreed in the board meeting that the symposium would be held regularly in

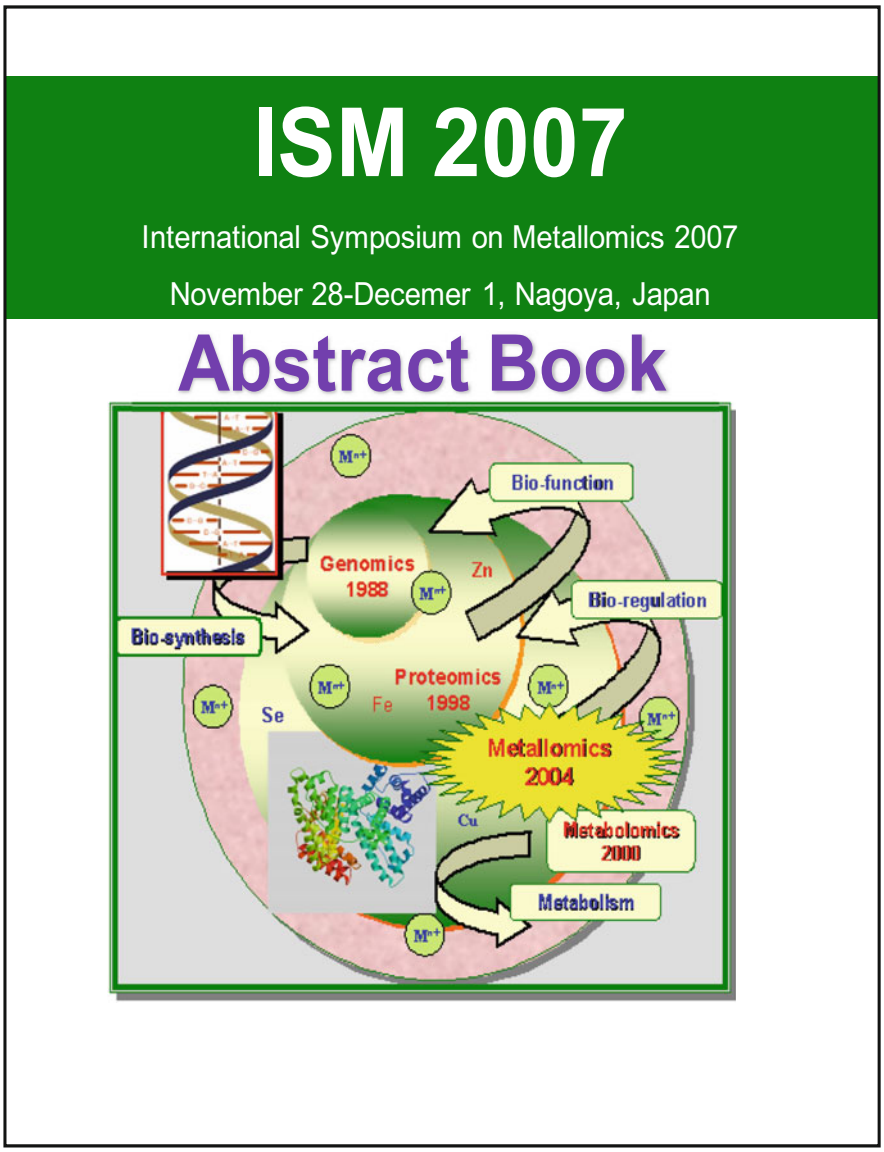

Fig. 1.8 The front cover of the Abstract Book for ISM 2007

every 2 years in the world. In addition, the Proceedings of ISM 2007 was published as the special issue of *Pure and Applied Chemistry* from the International Union of Pure and Applied Chemistry (IUPAC) in 2008 [49].

So far, the ISM has been held five times, that is, at Nagoya, Japan, in 2007; at Cincinnati, USA, in 2009; at Münster, Germany, in 2011; at Oviedo, Spain, in 2011;

Table 1.5 International advisory board members in ISM 2007

Dr. Becker, J. Sabine: Research Center Juelich, Germany
Prof. Caruso, Joseph A: University of Cincinnati, USA
Prof. Chai, Zhifang: Institute of High Energy, Physics, China
Prof. Francescon, Kevin: Karl-Franzens University Graz, Austria
Prof. Hieftje, Gary M: Indiana University, USA
Prof. Qiuquan Wang: Xiamen University, China
Dr. Jakubowski, Norbert: ISAS – Institute for Analytical Sciences, Germany
Prof. Kim, Hasuck: Seoul National University, Korea
Dr. Koppenaal, David W: Pacific Northwest National Laboratory, USA
Prof. Lobinski, Ryszard: CNRS, France
Prof. Lucchini, Roberto: University of Brescia, Italy
Prof. Maret, Wolfgang: The University of Texas Medical Branch, USA
Dr. McArdle, Harry J.: Rowett Research Institute, UK
Prof. McLeod, Cameron W: University of Sheffield, UK
Prof. Natile, Giovanni: Università degli Studi di Bari, Italy
Prof. Prange, Andreas: GKSS Research Centre, Germany
Prof. Sanz-Medel, Alfredo: University of Oviedo, Spain
Prof. Sarkar, Bibudhendra: The Hospital for Sick Children, Canada
Prof. Sun, Hongzhe: The University of Hong Kong, Hong Kong
Prof. Tanner, Scott D:. University of Toronto, Canada
Prof. Haraguchi Hiroki: Nagoya University, Japan---Chairman
Prof. Furuta Naoki: Chuo University, Japan---Vice-chairmen
Prof. Sakurai Hiromu: Kyoto Pharmaceutical University, Japan---Vice-chairmen
Prof. Suzuki Kazuo T.: Chiba University, Japan---Vice-chairmen

Table 1.6 The years, conference place and organizers of the International Symposium on Metallomics

Year	Places	Organizers
2007	Nagoya, Japan	H. Haraguchi
2009	Cincinnati. USA	J. Caruso & G. Hiefje
2011	Munster, Germany	U. Karst & M. Sperling
2013	Oviedo, Spain	A. Sanz-Medel
2015	Beijing, China	Z. Chai & Z. Xinrong
2017	Wien, Austria (scheduled)	G. Köllensperger

and at Beijing, China, 2015. These symposiums are listed in Table 1.6 together with the years and places (It was sad that Prof. Caruso, who organized the second symposium and the first Editorial Board Chair of *Metallomics* journal, passed away in November, 2015.). The proceedings of these symposiums were published as the special issues in the journal *Metallomics* [50–52] from the Royal Society of Chemistry. According to these symposiums, metallomics has been receiving more attention as the emerging scientific field, and in recent years, it has been taken up as the hot topics in other scientific fields, not only in the journals but also in the conferences.

It is additionally mentioned here that in Japan the Metallomics Research Forum has been held every even-numbered year since 2008 as the domestic meetings, and some selected papers presented in the Metallomics Research Forum were published as the themed issues in *Metallomics* [53, 54]. Shuichi Enomoto (RIKEN, Tokyo), Hiroyuki Yasui (Kyoto Pharmaceutical Univ., Kyoto), Yasumitsu Ogura (Showa Pharmaceutical Univ., Kanagawa), and Masahiro Kawahara (Musashino Univ., Tokyo) organized the forums in 2008, 2010, 2012, and 2014, respectively.

1.8 Progress of Metallomics Research

1.8.1 The Scientific Journal of Metallomics

It was a wonderful news and our pleasure that the scientific journal of *Metallomics-Integrated Bimetal Science* (in this chapter it is just called *Metallomics*) was launched in January 2009, from RSC. In the beginning part of the first issue of *Metallomics*, Caruso (the chair of Editorial Board) and O'Connor (editor of RSC) gave the welcome message for celebrating the publication of the new journal as follows [2]:

> Welcome to the first issue of Metallomics: Integrated Biometal Science. The study of metals in biological systems is an increasingly important area of research. Metallomics is a newly emerging scientific field that is receiving great attention as a new frontier in the study of trace elements in the life sciences. It is a global discipline encompassing many areas including biology, chemistry, geology, medicine, physics, and pharmacy.
>
> As this field brings together researchers from such diverse areas, we anticipate that Metallomics will help to bridge the gap between researchers from different backgrounds so that ideas can be shared and the field progresses to the benefit of all. Our journal will serve as a focus for the community of metallomics researchers to come together and gain new perspectives and insights. It is our aim to reflect the interests of the emerging community and to support you as your community grows.

Until 2015, seven volumes (Vol.1–Vol.7) of the journal *Metallomics* have been distributed, in which more than 1000 articles and review papers have been published. It should be highly evaluated that the publication of *Metallomics* has accelerated the progress of the metallomics research as a multidisciplinary academic science. Recently the Editorial Board of *Metallomics* announced the guideline for the publication requirement of the research papers required [8].

1.8.2 Essentiality and Toxicity of the Elements

In trace element chemistry, the terms of essential elements and toxic (or hazardous) elements are often used, when the biological influences/functions of the elements are explained. In such cases, the biological influences of the elements on the

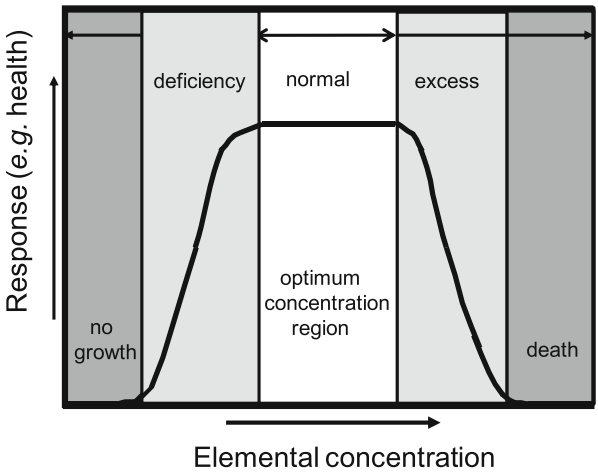

Fig. 1.9 Response curve of the biological system to the dose of the elements as the nutrients

biological systems (humans, animals, plants, and microorganisms) are generally examined as the response curve of the biological systems to the dose of the elements (generally contained in nutrients, foods, chemicals, and so forth). As an example, the dose-response curve is shown in Fig. 1.9, where the concentration of the element as a nutrient is taken on the horizontal axis and the biological response (e.g., health condition) is taken on the vertical axis. In the figure, the health condition is taken as the indicator of the response to the dose of the elements. As the biological response, body height or weight, cell growth, cell proliferation, medical indexes, and so forth are also taken into account for the response curve, where the upper position of the vertical axis in the curve indicates the better healthy condition (more normal).

It is well known that most of biological systems show the response curve in a trapezoid-shaped curve, as shown in Fig. 1.9. In such a biological response curve in the trapezoid shape, there are three regions such as deficiency region, normal region (or optimum concentration region), and excessive region, when the amount of element administered is increased from the low to higher concentration (from the left to the right). In the normal region, our health is physiologically or functionally maintained to be normal without any disease or dysfunctions. When the nutrient concentration becomes lower than the lower limit of the normal region, some specific symptom or disease is caused, depending on the kind of the element, for example, iron deficiency causes anemia. In the extreme case, no growth of the living systems is observed; a microorganism such as *Escherichia coli* (*E. coli*) does not grow completely, when the zinc concentration is below 1 ppb in the culture medium. On other hand, various symptoms are observed in the excessive region, where an element is administered more than the upper limit of the normal region. Especially, heavy metals such as Hg, Cd, Pb, and Sn cause serious damages or dysfunctions in the relatively low concentration, and in the extreme cases, such

symptoms become fatal. Thus, such heavy metals are classified to toxic or hazardous metals. Most of environmental issues due to metals are caused as serious adverse effects by such toxic heavy metals. It should be pointed out here that any elements (and their compounds) cause some toxic effects on the biological systems if the living organisms are exposed to the excessive conditions by overdose of the elements, as in the right-hand side of Fig. 1.9. If an element causes some adverse effect or dysfunction by the smaller amount of dose, it is said that the toxicity of that element is greater.

According to definition, an element which is indispensable in our life system is called "essential element." In general, if the element satisfies with one of the following two cases, that element is classified to "essential element." The first case is that some abnormal diseases or physiological dysfunctions are observed because of the deficiency of a specific element, and the life system is recovered to normal condition by the dietary supplementation of that specific element. The second case is that a specific element is contained in biomaterials (mostly metalloproteins and/or metalloenzymes) with an important biological function for our life system, for example, Fe in hemoglobin, Zn in carboxypeptidase, and Se in glutathione peroxidase.

In Table 1.1, the essential elements contained in humans are indicated by the marks *, **, and ***. It can be seen from Table 1.1 that 11 elements such as H, C, N, O, Na, Mg, P, S, Cl, K, and Ca are essential major and minor elements. The cumulative amount of these 11 elements is 99.4% of the total, and they are mostly used to construct the whole-body structures of the living or biological systems. The cumulative amount of the rest of the elements is only 0.4% of the total, and all of them belong to trace elements, because their concentrations are below 100 ppm. Among those trace elements, eight elements (Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, I) are essential for humans, and seven elements (F, Si, V, Ni, As, Sr, Sn, Pb) are essential for experimental mammals [26]. It is noted here that B is essential for plants (*see* Table 1.3), but not for animals.

In recent years, some people say that the trace elements such as Li, Rb, Cs, Ba, Al, Ge, Br, Sb, W, Ag, Au, Cd, and Hg might be essential for humans and animals, in which the toxic or hazardous elements such as Cd and Hg are included. As expected from the All-Element Present Theory described in Sect. 1.3, almost all elements can be determined or detected in the biological samples (*see* Table 1.3). These facts suggest that any elements are ubiquitously distributed in the biological systems including humans, so that some scientists are now thinking that even the hazardous or toxic elements might be necessary for maintenance of our life systems at the concentration level as low as the background concentration in nature. This is the reason why it is considered that other elements except for the well-established essential elements are possibly essential to the biological systems. However, since biological essentiality of such elements has not well elucidated so far, further extensive research on their essentiality as biological trace elements as well as their physiological functions should be carefully carried out in future research.

1.8.3 A Simplified Model of the Biological System

The biological cells containing various cell organs (organelles) are composed of numerous internal structures, so that the cell structures in the biological system (either prokaryotes or eukaryotes) are of complex assemblies, but well organized. At present, such cell structures composed of various organelles have been revealed by the advanced analytical technologies, especially electron microscopy. Taking into account of such cell structure, a simplified model of biological system is schematically illustrated in order to gain an insight into the scientific aspects of "metallomics" in Fig. 1.10 [1, 7], where the dotted line (inside) and continuous line (outside) indicate a biological cell unit and an organ/whole body, respectively, and biological fluids (e.g., blood) circulate between the cell membrane and organ. Some biological substances and their functions in the biological system are also indicated in Fig. 1.10.

On the left-hand side of the simplified model in Fig. 1.10, the scientific terms such as genomics, proteomics, glycomics, and metabolomics are shown along with metallomics to indicate their research areas in the biological system. Such a simplified model is helpful to understand the relationship of metallomics with genomics, proteomics, metabolomics, and glycomics. As is well known, genomics deals with the genetic information of DNAs and RNAs encoded as the sequences of nucleobases. The entirety of DNAs and RNAs called "genome" contains the information for synthesis of proteins and also for regulation of protein structures/

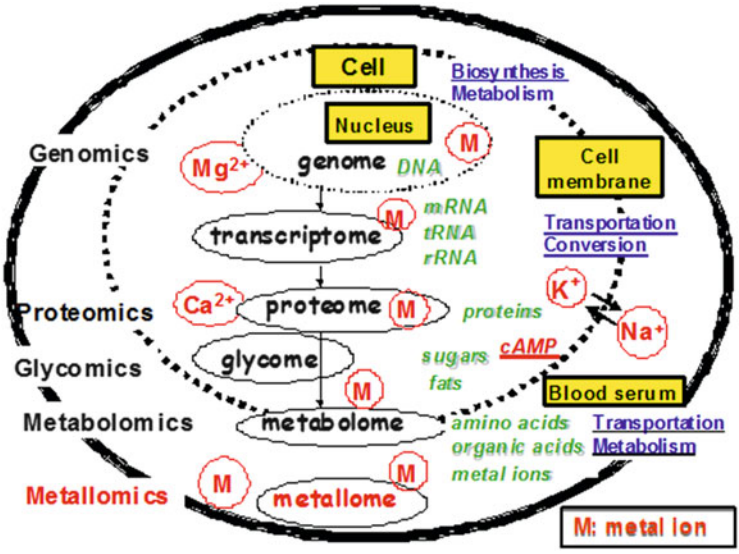

Fig. 1.10 Simplified model of biological system, showing the relationship of *omics* sciences [1, 4]. The *outer line* and the *inner dotted line* indicate organ (or whole body) and biological cell, respectively

functions. A large number of proteins are distributed inside and outside the cell as well as in the membranes, and they work as enzymes for synthesis and metabolism of various biological substances inside the cell. It is well known, for example, that DNAs and RNAs are synthesized by DNA polymerase and RNA polymerase, both of which are zinc enzymes. Since various proteins play essential roles to regulate and maintain the life system through the biosyntheses and metabolisms, "proteomics" as protein science has been receiving great attention as post-genome science linked with genomics.

In addition, many biological substances as well as metal ions are transported inside and outside the cell through the membrane. In general, since material conversion is actively occurring inside the cells and also often in the cell membranes, the scientific field for material conversion and transportation/exclusion processes are now called "metabolomics." Biological substances such as amino acids, organic acids, and other organic substances produced by metabolism are defined "metabolome" in a similar manner to genome in genomics [55].

As is seen from Fig. 1.10, metal ions are ubiquitously distributed inside and outside the cell to assist the physiological functions of genome, transcriptome, proteome, glycome, and metabolome, maybe, due to strong interactions and/or weak interactions. Since the limited space is available for the chapter, the detailed discussion about the roles of biometals is impossible, and then it is recommended to refer to the literatures [1, 26, 27, 56] in order to understand the functions of biometals in the biological systems.

1.8.4 Scientific Fields in Trace Metal Science

Biosciences concerned with metallic elements have been studied independently in many scientific fields such as physics, chemistry, biology, medicine, pharmacy, agriculture, environmental science, and other applied scientific fields. Such situations of trace metal studies are illustrated in Fig. 1.11, where the various scientific fields are shown in three groups: (i) basic science such as physics, chemistry, biology, and so on; (ii) applied science such as toxicology, food science, nutritional science, public hygiene, and others; and (iii) future science consisting of health science and environmental/green science. Since health science and environmental/green science are very important fields for sustainability of humans and nature, they are shown apart from basic and applied sciences in Fig. 1.11. It is seen in Fig. 1.11 that metallomics is located at the center as the integrated biometal science and linked with all of basic and applied sciences. Then, this figure suggests that metallomics should be appreciated as multidisciplinary science in wide diversity.

As already mentioned, all scientific fields shown in Fig. 1.11 have complementary relationship with each other. Therefore, it is desirable that biometal science, that is, metallomics, will be promoted with the cooperative work of the metal-related scientific fields in the diverse communities. This is the reason why the present author proposed "metallomics as integrated biometal science." The most

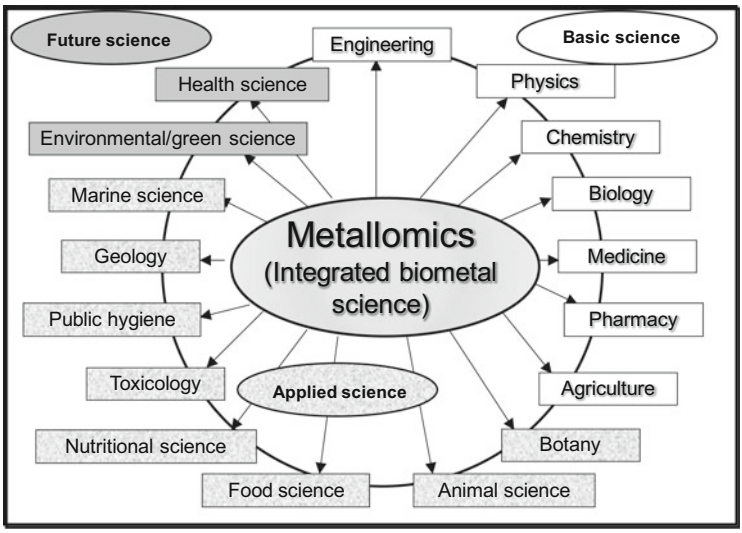

Fig. 1.11 Various scientific fields as studied in relation with metallomics

important research target in metallomics is the elucidation of the physiological roles and functions of biomolecules binding with metal ions for regulation of the life systems, so that metallomics may be considered, in another words, as "metal-assisted function bioscience".

Short comments about the difference between metallomics and metalloproteomics are added here briefly. In the literatures, the term of metalloproteomics is seen quite often. In fact, most of transition metal ions bind with proteins to form metalloproteins, which express the important biological or physiological functions in biological synthesis and metabolism. However, there are many other metal-binding compounds except for metalloproteins, for example, vitamin B12(Co) and chlorophylls(Mg), which also work as important bioactive substances. In addition, metal ions such as alkali and alkaline earth metal ions also play many important roles to regulate the physiological conditions in the biological systems. Thus, it is preferable that metals (metallic ions) not only in metalloproteins but also in nonprotein biomolecules should be included as metallome in metallomics. In that sense, metallomics, rather than metalloproteomics, may be more proper term as the academic terminology to represent biometal science.

1.8.5 Research Subjects in Metallomics

As an example, the research subjects to be performed in metallomics study are listed in Table 1.7 [1], which was summarized with taking into consideration the biological roles of biometals in life science. It can be seen from Table 1.7 that a

Table 1.7 Research subjects in metallomics [1]

(1)	Distributions of the elements in the biological fluids, cell, organs *etc*
(2)	Chemical speciation of the elements in the biological samples
(3)	Structural analysis of metallome (metal-binding molecules)
(4)	Elucidation of reaction mechanisms of metallome using model metal complexes (bioinorganic chemistry)
(5)	Identification of metalloproteins and metalloenzymes
(6)	Metabolisms of biological molecules and metals (metabolome and metabolites)
(7)	Medical diagnosis of health and disease related to trace metals on the multielement basis
(8)	Design of inorganic drugs for chemotherapy
(9)	Chemical evolution of the living systems and organisms on the earth
(10)	Other metal-assisted function biosciences in medicine, environmental science, food science, agriculture, toxicology, biogeochemistry *etc*

variety of research subjects related to biometals are involved in metallomics from the fundamental to the applied fields. Among the subjects listed in Table 1.7, Subject 1 "the distributions of the elements in the biological fluids, cell, organs, etc." and Subject 2 "chemical speciation of metals and metalloids (metallome) in the biological samples," about which the detailed description was given in the earlier part of this chapter, are important to obtain the fundamental information about biological species in the biological systems. It is really desirable that some regularity (general principle) of the distributions of metals (and metalloids) in the biological systems, in analogy to DNA sequences in genes and amino acid sequences in proteins, are found from the analytical data for further progress of metallomics. Of course, it is a difficult task, but such data may be helpful in the study especially on chemical evolution of the living system not only on the earth but also on other planets.

Subjects 3–5 in Table 1.7 have been extensively performed in the field of biological inorganic chemistry, and many interesting articles can be found in the *Journal of Biological Inorganic Chemistry (JBIC)* published from Springer. As for Subject 6, great progress has been achieved by applying the analytical technology for the profiling analysis of metabolites (metabolome) such as GC-MS and LC-MC to analysis of urine and blood serum. The results are also used for medical diagnosis of health and disease in Project 7.

Subject 8, i.e., the design of inorganic drugs for chemotherapy, is one of the most active research areas in metallomics. In order to understand such situations, the top 10 ranked articles (citation ranking) are listed in Table 1.8, which were selected from *Metallomics* by the literature survey using the keyword "metallomics" in the Web of Science (Thomson Reuters). In the citation ranking, the articles are arranged in the order of the times of citation referred to the Web of Science. It can be seen from the citation ranking that the researches related with drug design such as anticancer drugs, antitumor compounds, therapeutic agents, and biomedicine are actively performed in metallomics. That means the development of metal-containing drug design and chemotherapy is the most interested research area in

Table 1.8 Top-10 citation ranking of the articles published in *Metallomics*

The titles of articles[a]	Citation[b]	References
Recent developments in ruthenium anticancer drugs	263	[57]
Inhibition of transcription by platinum antitumor compounds	187	[58]
Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium	132	[59]
Cytosolic zinc buffering and muffling: Their role in intracellular zinc homeostasis	129	[60]
Gold compounds as therapeutic agents for human diseases	123	[61]
Advances in metal-carbene complexes as potent anticancer agents	104	[62]
Role of metal dyshomeostasis in Alzheimer's disease	93	[63]
Interactions of Zn(II) and Cu(II) ions with Alzheimer's amyloid-beta peptide. Metal ion binding, contribution to fibrillization and toxicity	87	[64]
Trace metal imaging with high spatial resolution: Applications in biomedicine	76	[65]
Molecular and genetic features of zinc transporters in physiology and pathogenesis	68	[66]

[a]Total of publication 1020 on the date of February 29, 2016
[b]Times of citation were referred to the Web of Science of Thomson Reuters

metallomics now. Of course, other metal-assisted function biosciences in Subject 10 should be carried out in cooperation with Subjects 1–9.

1.9 Summary

The historical aspects of metallomics before and after the proposal in 2004 were described in order to understand how metallomics came into the academic research field and how it is going on and where it will go. Since metallomics is the interdisciplinary academic research field, it should be promoted as the integrated research field for biometal science, in cooperation with other omics sciences such as genomics, proteomics, and metabolomics. In genomics, all DNA sequences in a variety of biological species have been surveyed, and in proteomics, all amino acid sequence analysis of proteins in the biological systems has been promoted to understand their biological roles and functions in our life system. In that sense, it is really hoped that in metallomics the existence of all elements in single biological cell is proved from the viewpoint of the All-Element Present Theory to further understand our life systems.

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