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Metallomics study on all-elements analysis of salmon egg cells and fractionation analysis of metals in cell cytoplasm*

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Abstract: Salmon egg cells were the subject of investigation for all-elements analysis in a single biological cell, where the elements in egg cells were simultaneously determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted acid digestion. As a result, 74 elements among 78 stable isotope elements were determined or detected, although H, C, N, and O were determined separately by conventional CHN elemental analysis. In addition, the survey of protein-binding metallic elements as well as metalloid elements in salmon egg cell cytoplasm was performed by the fractionation analysis with a hyphenated system of high-performance liquid chromatography (HPLC)/ICP-MS using a 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS)-coated octadecylsilyl silica (ODS) column and a size exclusion chromatography (SEC) column.

Keywords: metallomics; salmon egg cells; all-elements analysis; cell microcosm; fractionation analysis.

INTRODUCTION

In the last few decades, the analytical sensitivities of various metallic elements have been increasingly improved down to ppt or sub-ppt levels, in response to improving detection limits due to the development of inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) [1–3]. In addition, these methods have analytical capability, such as wide dynamic ranges in the working calibration curve and the capability for simultaneous multi-element detection. Such progress in analytical methodology has allowed us to determine almost all elements in biological, geochemical, and environmental samples [1,4]. As a consequence, the concept of "extended all present theory of the elements" was proposed [1], which postulates that all elements in the periodic table may be contained in all materials or substances (rocks, minerals, sediments, soils, sea and fresh waters, etc.) including biological systems, such as animals, plants, and microorganism, on the earth. Of course, human beings are also the subject of the extended all present theory of the elements.

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Furthermore, it is considered that the final target of the research on the extended all present theory of the elements is to elucidate the presence of all-elements in a single biological cell, which was denoted by a new and challenging concept as "cell microcosm" [4].

The bio-trace metals in the biological systems have been receiving great attention because of their essential roles in biological or physiological functions [5,6]. Accordingly, it is necessary that such biological functions of metals, including metalloid and nonmetallic elements, be comprehensively elucidated to understand the biological systems and to establish bio-metal science as a part of life science. The foregoing background prompted the present author to propose "metallomics" as an integrated biometal science in 2004 [4,7]. Since the proposal, metallomics has received great and growing attention as an emerging scientific field [8–14].

The following research subjects were recommended [4] as the main topics in metallomics: (1) distributions of the elements in the biological fluids, cell, organs, etc.; (2) chemical speciation of the elements in the biological samples and systems; (3) structural analysis of metallomes (metal-binding molecules); (4) elucidation of reaction mechanisms of metallomes using model compounds (bioinorganic chemistry); (5) identification of unknown metalloproteins and metalloenzymes; (6) metabolisms of biological molecules and metals (metabolomes, metabolites); (7) medical diagnosis of health and disease-related trace metals on a 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 sciences in medicine, environmental science, food science, agriculture, toxicology, biogeochemistry, etc. Among the subjects proposed [4], the distributions and chemical species (metallome) of metals and metalloids in biological systems are the basic information for metallomics research, because such information would constitute fundamental codes of metals (and metalloids) encoded in biological systems, in analogy with base sequences in genes and amino acid sequences in proteins. As has been reviewed by many workers [1,4,8,9,15–17], the distribution and speciation analyses of the elements in the biological fluids, cells, organs, and organisms are generally performed by highly sensitive analytical methods such as ICP-AES and ICP-MS. In such cases, all-elements analysis of a single biological cell is still a challenging subject in metallomics research [4], together with identification of metalloproteins and metalloenzymes to elucidate biological and metabolic functions of metals in biology. As a contribution to the challenge, this paper describes all-elements analysis of salmon egg cells (*ikura* in Japanese) and fractionation analysis of the protein-binding elements in salmon egg cell cytoplasm.

EXPERIMENTAL

Sample preparation

Salmon egg cells were decomposed with conc. HNO_3 by the microwave-assisted acid digestion method; firstly, conc. HNO_3 (5 mL) was added to a vessel containing 3 egg cells, and the sample was kept overnight. Then, further HNO_3 (5 mL) and H_2O_2 (5 mL) were added, and the vessel was heated in a microwave oven for almost 1 h. After cooling to room temperature, the decomposed sample was allowed to evaporate almost to dryness on a hot plate at ~220 °C. The residue was then dissolved in 1 M HNO_3 to prepare the analysis solution, which was subjected to the determination of major and minor elements by ICP-AES and of trace and ultratrace elements by ICP-MS. Nonmetallic elements, such as H, C, N, and O, were determined separately by the conventional CHN elemental analyzer.

In fractionation analysis of the elements in cell cytoplasm, egg cell cytoplasm was extracted by squeezing egg cells with Teflon needle and Teflon tweezers. Cytoplasm was then diluted 5- or 10-fold with 0.1 M Tris buffer solution (pH 7.4), and subjected to fractionation analysis by high-performance liquid chromatography (HPLC)/ICP-MS using a 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS)-coated octadecyl silica (ODS) column or a size exclusion column (SEC).

Instrumentation

An ICP-AES instrument of model Plasma AtomComp Mk II (Jarrell Ash, Franklin, MA, USA) was used under the usual operating conditions [18] to determine the major and minor elements, as summarized in Table 2. Two types of ICP-MS instruments were used for trace and ultratrace elements in the present experiment; one (ICP-Q-MS) was of model Agilent HP4500 (Agilent Technologies, Tokyo, Japan) with a quadrupole mass spectrometer and the other (HR-ICP-MS) was of model Finnigan Element 2 (Thermo Fisher Scientific) with a double-focusing mass spectrometer. The operating conditions for the Agilent HP 4500 instrument were the same as usual [18], whereas those for the Element 2 HR-ICP-MS instrument are summarized in Table 1.

Table 1 Operating conditions of HR-ICP-MS (Finnigan element 2).

Plasma conditions:	
Incident power	1.0 kW
Coolant gas flow rate	Ar 16.0 L/min
Auxiliary gas flow rate	Ar 0.8 L/min
Carrier gas flow rate	Ar 0.9 L/min
Nebulizer: concentric-type	
Sample uptake rate	0.2 mL/min
Data acquisition:	
Mass window	150 %
Integration window	80 %
Sampling points per peak	15 points
Integration times (runs × passes)	7 × 5 times

Fractionation analysis by HPLC/ICP-MS

The HPLC system consisted of a pump (model LC-10Ai; Shimadzu, Kyoto, Japan), a sample injector (model 7725; Rheodyne, Cotani, CA, USA) with a 20 μL loop, an ODS column (L-column, 4.6 mm i.d. × 250 mm long; Chemicals Evaluation and Research Institute, Tokyo, Japan) and a UV absorption detector (model UV 970; Jasco, Tokyo, Japan), where the ICP-MS was also used as the element-selective detector. The CHAPS-coated ODS column was prepared by the dynamic coating method, as described in previous papers [4,19–21], and has unique characteristics for simultaneous separation of large and small ions/molecules, as mentioned later. The size exclusion chromatography (SEC) column used for protein separation was of Superose 12HR 10/300 GL (Amersham Bioscience, Piscataway, NJ, USA) with a molecular permeation range of 1–300 kDa, whose calibration for molecular weights (MWs) was performed using standard proteins, as usual [22,23].

RESULTS AND DISCUSSION

Multielement determination of major-to-ultratrace elements in salmon egg cells

The concentrations of the elements in salmon egg cells were determined by ICP-AES and ICP-MS after the microwave-assisted acid digestion, as described in the experimental section. The experimental results are summarized in Table 2, together with the data for sea water and human blood serum for comparison. In Table 2, the elements with † were determined by ICP-AES and other elements were done by ICP-MS in the case of salmon egg cells. The concentrations of the elements in salmon egg cells are also presented in a periodic table display (Fig. 1) to facilitate and contextualize the range of elements determined or detected. In Fig. 1, the elements in gray boxes of the periodic table indicate natural or artifi-

cial radioactive elements and rare gas elements, which are difficult to treat in the conventional standard laboratory. Therefore, such radioactive and rare gas elements were not determined in the present experiment. Thus, the number of the stable isotope elements (including U and Th) for quantification analysis was 78. It means that these 78 elements are the target elements for all-elements analysis in the present experiment. As is seen in Fig. 1, 74 of this total of 78 elements have been determined or detected in salmon egg cells.

Table 2 Concentrations of the elements in salmon egg cells, seawater, and human blood serum.

Element	Salmon egg cell Concentration, ng/g	Seawater [4] Concentration, ng/ml	Blood serum [4] Concentration, ng/ml
P†	3 580 000	62	119 000
S†	2 990 000	898 000	1110
Cl	2 800 000	19 350 000	3 200 000
K†	1 860 000	399 000	151 000
Ca†	432 000	412 000	93 100
Na†	247 000	10 780 000	3 130 000
Mg†	222 000	1 280 000	17 500
Br	20 000	67 000	4440
Zn†	13 600	0.35	651
Fe†	10 700	0.03	1200
Cu†	8900	0.15	750
I	3700	58	0.007
Sr†	3600	7800	33
Se	1970	0.16	160
Ti	1420	0.0065	–
Rb	523	120	170
Mn†	512	0.02	0.57
As	192	1.2	0.45
Ba	47.3	15	0.48
Ni	26.5	0.48	0.23
Si	25.1	2800	140
V	18.8	2.0	0.031
Co	12.5	0.0012	0.11
Hg	12.0	0.000 14	0.55
Ag	10.7	0.002	0.2
Pd	8.0	0.000 06	–
B	7.8	4500	2.1
In	7.0	0.0001	–
Al	6.44	0.03	1.8
Mo	6.43	10	0.95
Ge	5.83	0.005	–
Cs	5.58	0.31	0.95
Cd	1.1	0.07	0.15
Sn	0.96	0.0005	0.51
Be	0.80	0.000 21	0.09
Cr	0.73	0.21	0.069
U	0.66	3.2	0.31
Pb	0.48	0.0027	1.2
Ru	0.19	0.000 005	–
Sb	0.12	0.2	2.3

(*continues on next page*)

Table 2 (*Continued*).

Element	Salmon egg cell Concentration, ng/g	Seawater [4] Concentration, ng/ml	Blood serum [4] Concentration, ng/ml
Y	0.11	0.017	0.73
La	0.075	0.0056	0.063
Nd	0.074	0.0033	0.034
Au	0.054	0.0066	0.000 03
Tl	0.050	0.0130	–
W	0.033	0.01	0.34
Gd	0.016	0.0009	0.0072
Ce	0.014	0.0007	0.21
Pr	0.010	0.0007	0.0111
Sm	0.010	0.000 57	0.0058
Th	0.0090	0.000 02	0.50
Dy	0.0074	0.0011	0.0096
Sc	0.0059	0.0007	0.0017
Pt	0.0043	0.0002	0.0014
Er	0.0031	0.0012	0.0095
Yb	0.0027	0.0012	0.013
Ga	0.0023	0.0012	–
Tb	0.0021	0.000 17	0.0013
Os	0.0019	0.000 002	–
Ho	0.0017	0.000 36	0.0026
Eu	0.0011	0.000 17	0.000 82
Lu	0.000 78	0.000 23	0.0025
Tm	0.000 37	0.0002	0.0017

†Determined by ICP-AES.

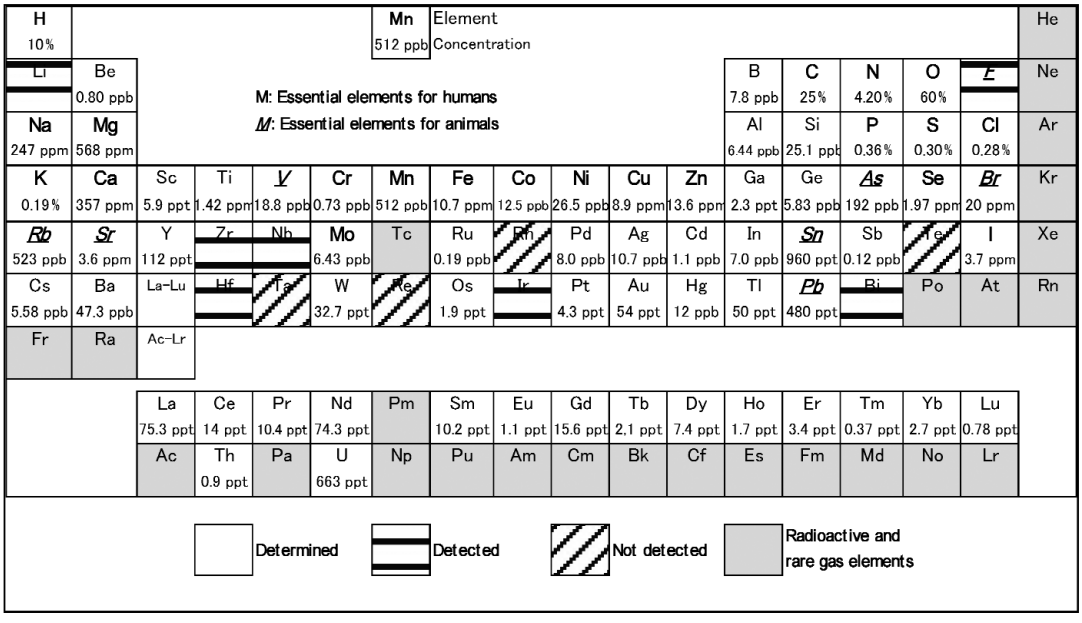

Fig. 1 The elements and their concentrations in salmon egg cells, determined or detected by ICP-AES and ICP-MS. The concentrations of H, C, N, O were determined by the conventional elemental analyzer.

In a previous paper [4], the quantitative data for 39 elements were reported, which were determined by ICP-AES and ICP-Q-MS. In the present paper, as is seen in Table 2, the quantitative data for 63 elements are shown in the concentration range from 0.359 % for P to 0.000 37 ng/g for Tm. These new data for 24 elements were obtained by using HR-ICP-MS, which generally provided detection limits improved by 2–3 orders of magnitude in comparison with ICP-Q-MS. In addition, the peak signals of 7 elements (Li, F, Zr, Nb, Hf, Ir, and Bi) could be detected on the mass spectra of HR-ICP-MS, but the rest of 4 elements (Rh, Te, Ta, and Re) could not be detected even by HR-ICP-MS. These results indicate that the concentrations of the former 7 elements in salmon egg cells are close to the detection limits obtained by HR-ICP-MS, while those of the latter 4 elements are below the detection limits.

Except for typical nonmetallic elements (H, C, N, O), nonmetallic elements of P (0.36 %) and S (0.30 %) were significantly abundant in salmon egg cells, and also the concentrations of alkali elements (K and Na), alkaline earth elements (Ca and Mg) and halide elements (Cl and Br) were significantly higher. The concentrations of these abundant elements were almost similar to those in sea water, as is seen from Table 2, and may be present as simple ionic forms or composite electrolyte solutions in cell cytoplasm, as will be described later. It is noted here that the concentrations of the biologically essential trace elements, such as Zn, Fe, Cu, and Se, were higher than 1 ppm. It is well known that these trace elements play important roles in biological or physiological functions as metalloproteins and metalloenzymes, for example, such as electron transfer and anti-oxidants (catalase, superoxidodismutase, and glutathione peroxidase) as well as DNA polymerase and RNA polymerase [4,5], which are essential to regulate living cells and cell growth.

Among the elements in Table 2, the concentrations of As (193 ppb) and Hg (12 ppb), which are usually considered as hazardous or toxic elements, were significantly high in salmon egg cells. However, as was reported previously, most As in salmon egg cells exists as arsenobetain, organoarsenic species [24], and ca. 4 ppb and ca. 8 ppb of Hg exist as cysteine- and protein-binding species, respectively [21]. The existence of organoarsenic species, amino acid, and protein complexes suggests that salmon egg cells have access to detoxification mechanisms for these toxic elements.

Bioaccumulation of the elements in salmon egg cells

In general, marine organisms, such as sea animals, fish, and shellfish accumulate trace elements present in seawater. Such accumulation of trace elements is generally estimated as the bioaccumulation factor (BAF), which is usually used as an index of pollution by hazardous metals and chemicals in sea animals and fish in environmental science. However, essential trace elements are also accumulated in sea animals and fish to sustain their lives by regulating various biofunctions. Then, the BAFs of the elements in salmon egg cells were estimated as the ratios between the concentrations of the elements in salmon egg cells and seawater, using eq. 1.

$$\mathrm{BAF} = (M_{\text{salmon egg cell}})/(M_{\text{seawater}}) \quad (1)$$

where $M_{\text{salmon egg cell}}$ and M_{seawater} are the concentrations of the elements in salmon egg cells and seawater, respectively, whose data are summarized in Table 2. The estimated BAFs are shown in Fig. 2. The BAF of Fe was ca. 500 000, which was the largest among those of all the elements examined. Furthermore, bioaccumulation factors of Cu, Zn, Co, Mn, P, and Se were substantially larger than 10 000. As is known, these are all bioessential elements, which have important roles in the biological systems [4,5]. Since salmon egg cells grow salmon after fertilization and incubation for ontogeny, it is suggested that the elements with the large accumulation factors play essential roles in the ontogeny process.

In addition, the accumulation factor of Hg was also larger than 10 000. Ag, Sn, Au, and Ge provided accumulation factors larger than 1000, although the biological functions of these elements are unknown at present. It is also seen in Fig. 2 that the BAFs of rare earth elements are in the range of 10–100, which shows that rare earth elements are slightly enriched in salmon egg cells.

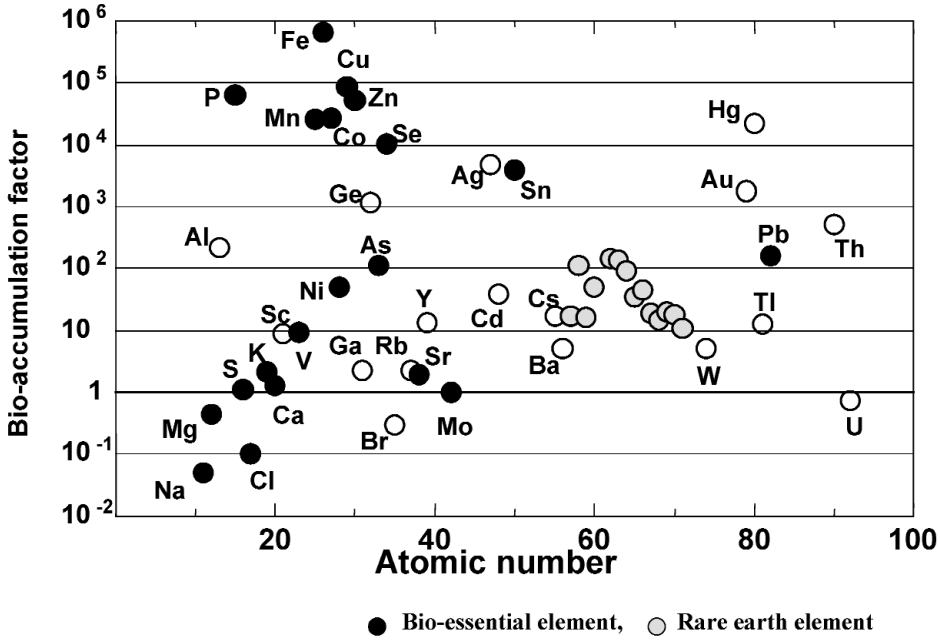

Fig. 2 BAFs of the elements in salmon egg cells. The BAF is defined as the ratio of the concentration of the elements in salmon egg cells to those in sea water.

On the other hand, the concentrations of major elements in salmon eggs, such as Na, K, Ma, Ca, Cl, and S, were almost the same levels as those in sea water, which results in BAFs close to or less than 1.

Fractionation analysis of protein-binding elements in salmon egg cell cytoplasm by surfactant-mediated HPLC/ICP-MS

Next, fractionation analysis of the elements in salmon egg cell cytoplasm was examined by HPLC/ICP-MS in order to elucidate the elements binding with proteins. Two separation columns were employed for molecular separation; one was a CHAPS (zwitterionic surfactant)-coated ODS column and the other was an SEC column. As described in previous papers [19–22], the CHAPS-coated ODS column has unique characteristics for simultaneous separation of ions/small molecules and large molecules (e.g., proteins), which provides useful information for metals binding or not binding with proteins.

The chromatograms obtained by using the CHAPS-coated ODS column with the detections of UV-absorption and ICP-MS are shown in Fig. 3. In Fig. 3, the chromatograms with UV-absorption detection at 254 nm are shown in the upper columns of each row, together with the element-selective chromatograms, where isotopes detected and elemental concentrations determined are also indicated in each chromatogram. It should be noted here that the retention range of 3.0–3.5 min shown as the gray zones in Fig. 3 indicates the elution zone of large molecules with MWs larger than ~10 000 Da, while the retention range after 3.5 min indicates the elution zone of small ions and molecules. However, the MWs of the eluted molecules cannot be estimated from the chromatograms obtained by the CHAPS-coated ODS column. In Table 3, the percentages of the protein-binding elements in cell cytoplasm are summarized for some elements, which were estimated from the chromatograms shown in Fig. 3.

Table 3 Percentages of protein-bindings of metals and metalloids in salmon egg cell cytoplasm.

Element	Protein-bindings (%)
P	20
S	22
Se	58
As	52
Mg	0
Ca	2.2
Sr	0.4
Ba	13
Mn	54
Fe	79
Co	78
Ni	78
Cu	80
Zn	66
Ag	86
Hg	65
Mo	0
W	0
I	5.3

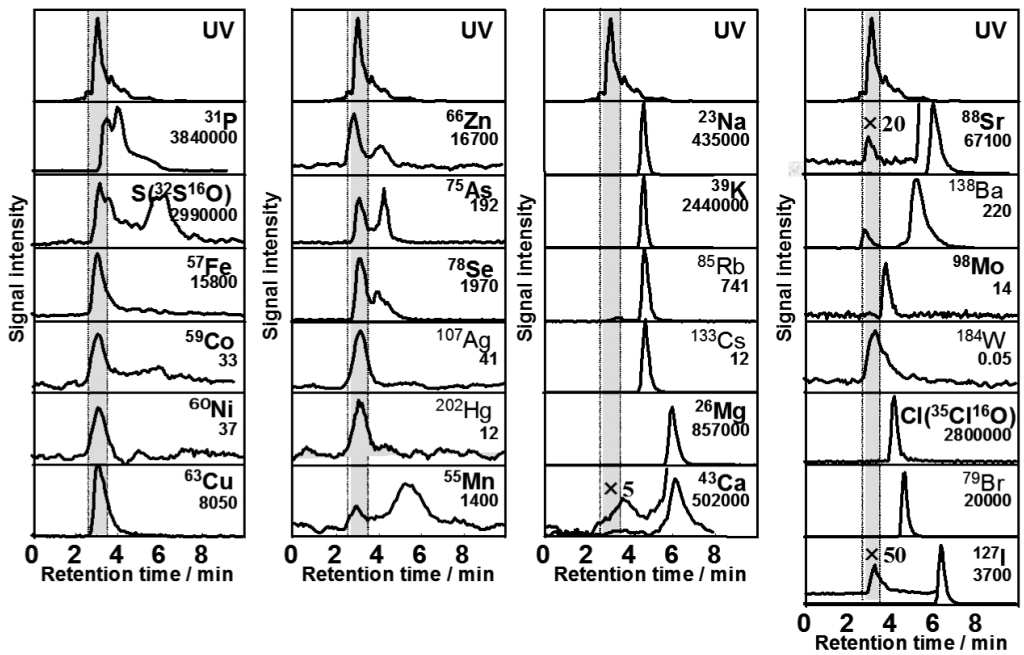

Fig. 3 Chromatograms for salmon egg cell cytoplasm diluted 5-fold with 0.1 M Tris buffer (pH 7.4), which were obtained by HPLC using a CHAPS-coated ODS column. Mobile phase: 0.1 M Tris buffer solution (pH 7.4), detection: UV absorption at 254 nm and ICP-Q-MS at *m*/*z* shown in the figure. The numbers under the elements indicate the concentrations of the elements in salmon egg cells. The retention time range between 3.0–3.5 min (gray zone) corresponds to the protein fraction.

It is seen from the chromatograms in Fig. 3 that a small part of P peak overlaps with the protein peak, while the large part of P are eluted in the small ion/molecule range. The largest peak of P near 4 min corresponds to phosphate ion (PO_4^{3-}) [7]. In the case of S, two large peaks are mainly observed in the S-selective chromatogram in Fig. 3; the first peak mostly overlaps with the protein peaks, which indicates that S-containing amino acids, such as cysteine and methionine, are incorporated in proteins. The second large peak near 6 min is attributed to sulfate ion, SO_4^{2-} [7].

Figure 3 shows that heavy metals such as Fe, Co, Ni, Cu, Au, and Hg mostly exist as protein-binding complexes, while Zn and Mn exist partly as small molecules, maybe free ions or amino acid complexes. Mo was eluted clearly after the protein-elution zone, which indicates that Mo mostly exists as small molecules, possibly molybdate ions, in cytoplasm [23]. On the other hand, the peak of W overlaps with those of proteins. However, W in cytoplasm was tungstate ion, WO_4^{3-}, not protein-binding W, which was found by the spiked experiment in the present experiment. As for metalloid elements, As and Se provided two main peaks corresponding to the protein-binding and small molecules. These results suggest that As and Se are partly incorporated with proteins in cell cytoplasm. In the case of As, it was reported previously [24] that small molecules in cell cytoplasm and cell membrane were arsenobetain and arsenate ion, respectively.

As is seen in Fig. 3, Na, K, Rb, Cs, Mg, Cl, and Br provided single peaks after the protein-elution zone. These experimental results indicate that these elements exist solely as simple ions in egg cell cytoplasm. On the contrary, Ca, Sr, Ba, and I provided small peaks overlapped with the protein peaks, although their main peaks appeared as simple ions after the protein-elution zone. These results suggest that Ca, Sr, Ba, and I are partly bound with proteins in cell cytoplasm.

Metal-profiling chromatograms for salmon egg cell cytoplasm obtained by SEC/ICP-MS

The CHAPS-coated ODS column provides the information just about large molecules or small ion/molecules for various elements, and such information is useful for the preliminary survey of the existing states of the elements, as described above. In general, separation of proteins is performed by using the SEC column. Therefore, in order to ensure detection of protein-binding metals and metalloids, the SEC chromatograms were measured with UV-absorption and ICP-MS detectors. Such chromatograms are shown in Fig. 4, as the metal-profiling chromatograms with element-selective detection. The permeation range of the SEC column used was 1–300 kDa, which corresponded to the elution range of 16–38 min in the chromatograms in Fig. 4, indicated as the gray zone.

The chromatograms detected by UV absorption are shown at the top of each column in Fig. 4, where several peaks of proteins are observed, although they are not well separated. It is seen in Fig. 4 that P, S, and Se provided several peaks in the protein-elution range, which are seemingly consistent with those in the UV-detected chromatograms. These results suggest that P exists as phosphorylated proteins, and S and Se are incorporated in proteins as S-containing amino acids (cysteine and methionine for S and selenocysteine and selenomethionine for Se). It is noted here that Ca and Mn, which have highly ionic properties, showed the several peaks consistent with those of proteins, although their peak intensities were different from each other. As mentioned in the previous section, a part of Ca (2.2 %) is bound to protein. The experimental results in Fig. 4, therefore, support the existence of protein-binding Ca. Although not experimentally verified, it is speculated that bindings of Ca with proteins may be through ion-pair formation between Ca^{2+} and the terminal carboxylic groups of proteins. The case of Mn seems similar to that of Ca, but of course not all of Mn is not so.

Heavy metals such as Fe, Cu, Zn, Co, and Ni provided rather sharp peaks with several small peaks in the SEC chromatograms in Fig. 4. These results may suggest that these sharp peaks are caused by some rigid binding metal complexes as metalloproteins and metalloenzymes. The enzymatic activity of catalase was experimentally proved for the Fe protein fraction in Fig. 4. Alkaline phosphatase (Zn protein) was found in the Zn protein fraction [25]. However, it is noted here that hydrophobic and hydro-

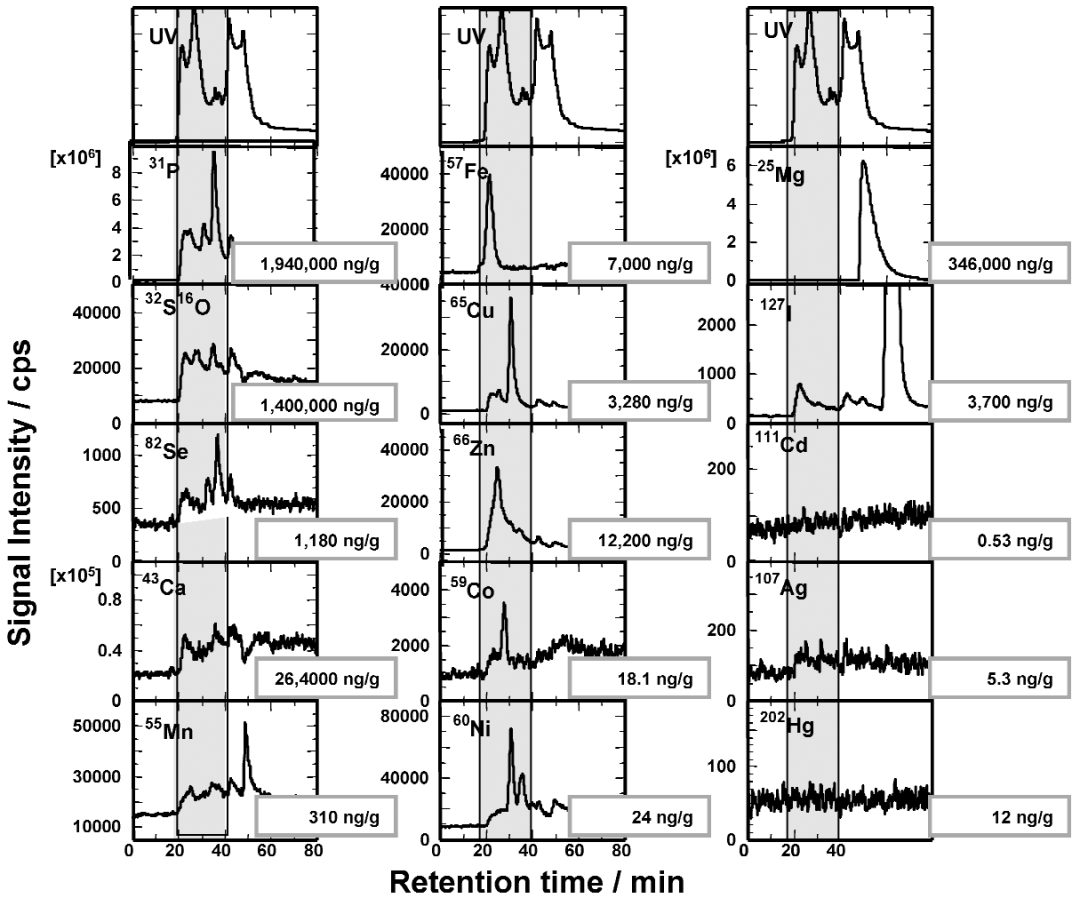

Fig. 4 Metal-profiling chromatograms for salmon egg cell cytoplasm obtained by SEC/UV/ICP-Q-MS. The concentrations of the elements in cell cytoplasm are shown in each element column. The retention time range between 16–38 min (gray zone) corresponds to the permeation range (1–300 kDa) of the SEC column used. Mobile phase: 0.1 M Tris buffer solution (pH 7.4), detection: UV absorption at 254 nm and ICP-Q-MS at *m*/*z* shown in the figure.

philic alkaline phosphatase exist in the Zn protein fraction and that hydrophobic alkaline phosphatase is much more abundant (ca. 80 %) than hydrophilic alkaline phosphatase (~20 %) [23]. It was difficult to identify proteins in cell cytoplasm by matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOFMS) and electrospray ionization (ESI)-MS due to the existence of hydrophobic proteins.

It is seen in Fig. 4 that Mg displays no characteristics of protein-bindings, while 5.3 % of I is bound to protein. Since no clear peaks of Cd, Ag, and Hg were observed in the chromatograms obtained by ICP-Q-MS, the chromatograms were observed separately with the sensitive detection by HR-ICP-MS, which are shown in Fig. 5. Clear peaks for Ag and Hg were observed in Fig. 5, although a broad signal was observed for Cd in the protein-elution range. As reported previously, 8 and 4 ppb of Hg (total 12 ppb) were found in salmon egg cell cytoplasm as protein- and cysteine-binding complexes, respectively [21]. In addition, protein-binding Hg provided mainly three peaks in SEC chromatogram, and their peak positions were detected at the same peak positions as S and Se [21].

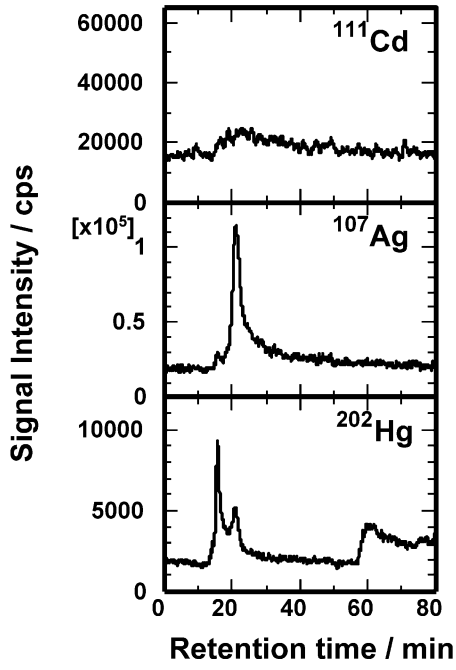

Fig. 5 Metal-profiling chromatograms for Cd, Ag, and Hg in salmon egg cytoplasm, obtained by SEC/HR-ICP-MS. The separation conditions were the same as in Fig. 4.

The SEC chromatogram for salmon egg cell cytoplasm with ^{78}Se ICP-MS detection is shown in Fig. 6. In this case, only a hydrophilic fraction of cytoplasm was injected into the SEC column, because hydrophobic proteins deteriorated the resolution of SEC chromatogram. The hydrophilic fraction could be prepared by diluting cytoplasm 10-fold with pure water, where hydrophobic proteins were precipitated by pure water dilution. As is seen in Fig. 6, five peaks were observed in the ^{78}Se-selective SEC chromatogram. According to the spiked experiment, they were assigned to (1) unknown protein I (MW ~200 kDa), (2) glutathione peroxidase (MW ~87 kDa), (3) unknown protein II (MW ~50 kDa), (4) selenite, (5) selenocysteine. Furthermore, the SEC chromatogram for commercially available glutathione peroxidase with the UV absorption-, ^{78}Se-, and ^{202}Hg-detections are shown in Fig. 7. It should be noted here that glutathione peroxidase provided one broad peak with some small peaks in the UV absorption-detected chromatogram, but three peaks corresponding to tetramer, dimer, and monomer were clearly separated in the ^{78}Se-detected chromatogram. The existence of four units of commercially available glutathione peroxidase was examined by MALDI-TOF-MS, but only three of them were detected in the Se-detected SEC chromatogram shown in Fig. 7. It is further noticed here that Hg signals were detected at the peak positions of three glutathione peroxidase units. These facts suggest that Hg is binding with glutathione peroxidase, which results in detoxification effect of glutathione peroxidase for Hg in salmon egg cell cytoplasm.

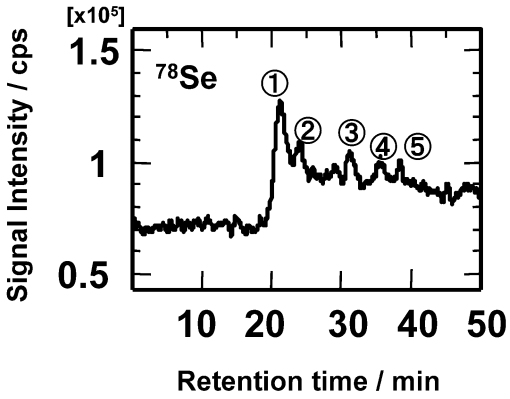

Fig. 6 SEC chromatogram for the hydrophilic fraction of salmon egg cell cytoplasm with the detection by ^{78}Se HR-ICP-MS. The separation conditions were the same as in Fig. 4. (1) unknown protein I (MW 200 kDa); (2) glutathione peroxidase (87 kDa); (3) unknown protein II (MW 50 kDa); (4) selenite ion; (5) selenocysteine.

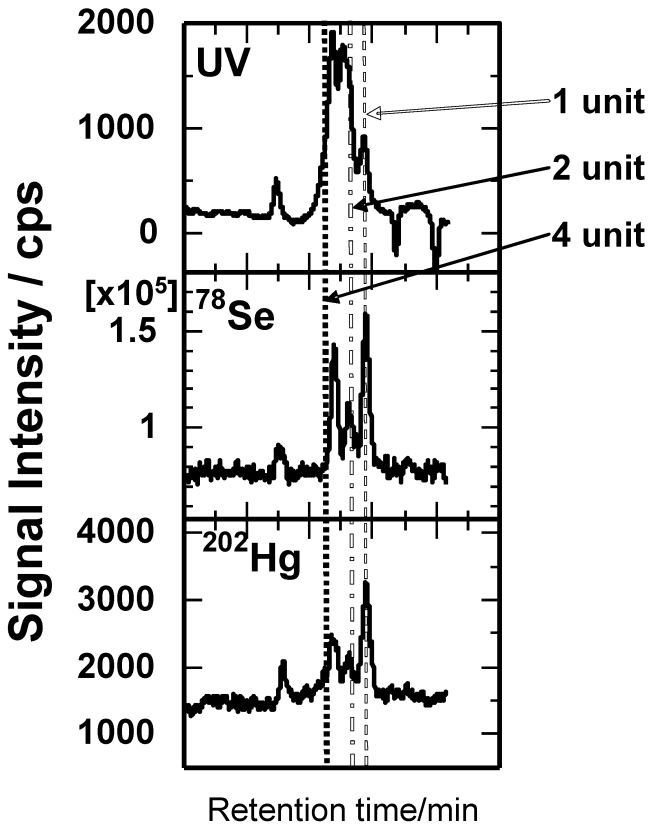

Fig. 7 SEC chromatograms for commercially available glutathione peroxidase, obtained with detection by UV absorption at 254 nm, and ^{78}Se- and ^{202}Hg-HR-ICP-MS. The separation conditions were the same as in Fig. 4. In the figure, 1, 2, and 4 units indicate monomer, dimer, and tetramer of glutathione peroxidase.

CONCLUSION

All-elements analysis of salmon egg cells and fractionation analysis of the elements in egg cell cytoplasm were performed in relation to metallomics research. Almost all elements (74 elements among 78 stable isotope elements) were determined or detected in salmon egg cells. This result strongly supports the concept of "cell microcosm", which indicates the existence of all elements in a single biological cell. Fractionation analysis of salmon egg cell cytoplasm by the CHAPS-coated ODS column/ICP-MS hyphenated system provided information about the existing states of the elements for protein-binding or not. Metal-profiling chromatograms obtained with the SEC column with element-selective detections are also useful to elucidate protein-binding metals on the molecular basis, such as metalloproteins and/or metalloenzymes. It is highly desirable to promote metallomics research on human tissue/organ cells as well as plant cells, in a similar experimental procedure to those described here, in order to establish the concept of all present theory of the elements in a single biological cell, i.e., "cell microcosm", which may constitute a useful data library for life sciences in the future.

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