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Notes

Major-to-Ultratrace Elements in Bone-Marrow Fluid as Determined by ICP-AES and ICP-MS

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The major-to-ultratrace elements in human bone-marrow fluid were determined by ICP-AES (inductively coupled plasma atomic emission spectrometry), and ICP-MS (inductively coupled plasma mass spectrometry). The bone-marrow fluid sample was centrifuged prior to acid digestion to exclude the bone piece from bone marrow, and then digested with nitric acid. As a result, 20 elements could be determined over the concentration range from 1610 μg g^{-1} for Na to 0.00043 μg g^{-1} for W. It was found that Fe, Zn and Sb were enriched by *ca*. 264-, 7- and 15-fold, respectively, in bone-marrow fluid, compared to those in human blood serum. Alkali metals (K, Rb, Cs), except for Na, were also significantly enriched in bone-marrow fluid. Furthermore, the concentrations of various elements, such as Fe, P, Al, Zn, Cu, Se, Zr, Sn, Ag and W, were significantly higher than those in open seawater.

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Introduction

In recent years, the available analytical methods for trace analysis have extensively progressed by the development of analytical atomic spectrometry, such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS).[1] As is well known, ICP-AES and ICP-MS provide excellent analytical features, such as high sensitivities for almost all elements, wide linear dynamic ranges of calibration curves and simultaneous multielement detection capabilities. These analytical features allow us to determine the elements over a wide concentration range, *i.e*., major-to-ultratrace elements, in various geochemical, biological and environmental samples.[1,2] As for biological samples, the multielement determination of major-to-ultratrace elements in whole blood, blood serum, urine, hair, and organs as well as in plants has been performed by ICP-AES and ICP-MS.[3,4]

According to the recent progress in the biomedical research on trace elements, it has been elucidated that various biological or physiological functions of organs substantially depend on the kinds and concentration levels of the elements contained in cells and organs.[5] As a consequence, various trace elements are known as bio-essential elements,[6] when they play some essential roles related to biological or physiological functions. Bone marrow is one of the essential and important organs in our human body because it produces primitive cells, such as erythrocytes (red blood cells), leukocytes (white blood cells) and small blood platelets.[6] In our literature survey, however, only a few reports about the elemental distributions in bone-marrow fluid have so far been published in terms of a limited number of elements.[7–10] Thus, in the present work, the multielement determination of the elements in bone marrow fluid was performed to elucidate the distributions of diverse elements at the major-to-ultratrace concentration levels.

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Experimental

Instruments

An ICP-AES instrument of a model Plasma AtomComp MkII (Jarrell-Ash, Franklin, MA, USA), consisting of a Pashen-Runge type of polychromator with 39 channels for simultaneous multielement detection, was used for the determination of major and minor elements. An ICP-MS instrument of Model SPQ 8000A (Seiko Instruments, Chiba), equipped with a quadrupole mass spectrometer, was used for the determination of trace and ultratrace elements. The experimental conditions for the ICP-AES and ICP-MS measurements are summarized in Table 1. The experimental conditions were chosen after optimizing the instrumental parameters as in previous studies.[11,12] The wavelengths of the emission lines and mass numbers (m/z) for the analyte elements used in the ICP-AES and ICP-MS measurements, respectively, are given in Table 2. In the ICP-MS measurement, matrix effects due to major elements in the sample were corrected by the internal-standard method,[13] where Ge, Rh, Re and Tl were used as the internal-standard elements.

Chemicals

The nitric acid used for sample digestion and preparation was of ultrapur grade and was purchased from Kanto Chemicals (Tokyo). Standard solutions for standardizing the calibration

Table 1 Instrument components and operating conditions of ICP-AES and ICP-MS instruments

ICP-AES instrument	Jarrel Ash Plasma AtomComp Mk II
Plasma conditions	
Rf frequency:	27.12 MHz
Incident Rf power:	1.0 kW
Gas conditions	
Outer gas:	Ar 20 l min^{-1}
Intermediate gas:	Ar 1.0 l min^{-1}
Carrier gas:	Ar 0.5 l min^{-1}
Sampling conditions	
Observation height:	18 mm above work coil
Sampling uptake rate:	1.2 ml min^{-1}
Polychromator	Paschen–Runge mounting
Focal length:	75 cm
Grating:	2400 grooves mm^{-1}
Entrance slit width:	25 μm
Exit slit width:	50 μm
Repetition:	3 times
Torch:	Fassel type
Spray chamber:	Single type
Nebulizer:	Cross-flow type
ICP-MS instrument	Seiko SPQ8000A
Plasma conditions	
Rf frequency:	27.12 MHz
Incident Rf power:	1.0 kW
Gas conditions	
Outer gas:	Ar 16 l min^{-1}
Intermediate gas:	Ar 1.0 l min^{-1}
Carrier gas:	Ar 0.95 l min^{-1}
Sampling conditions	
Sampling depth:	12 mm from work coil
Sample uptake rate:	0.8 ml min^{-1}
Sampling cone:	copper, 1.1 mm orifice diameter
Skimmer cone:	copper, 0.35 mm orifice diameter
Data acquisition	
Accumulation:	20 times
Dwell time:	10 ms
Repetition:	5 times
Channel width:	3 channels
Torch:	Fassel type
Spray chamber:	Scott type
Nebulizer:	Concentric type

curves were prepared by diluting the stock solutions for atomic absorption spectrometry, which were purchased from Wako Pure Chemicals (Osaka). Deionized water used throughout the present experiment was prepared by a Milli-Q water purification system (Model Milli-Q SP TOC; Nihon Millipore Kogyo, Tokyo).

Sample-preparation procedure

The bone-marrow fluid sample (*ca*. 4 g) was collected with a Teflon-coated syringe from a healthy volunteer with self-agreement at the Nagoya University Hospital. The fluid sample was first diluted with 1 ml at 0.1 M Tris–HCl (pH 7.4), and then centrifuged at 3000 rpm for 15 min prior to sample digestion to exclude bone pieces. Since bone pieces might be introduced in bone marrow fluid in the sampling process, this centrifugation was inevitable, as is discussed later. Then, the supernatant (0.3 g) after centrifugation was digested with 0.5 ml of *conc*. HNO_3 at *ca*. 100°C on a hot plate. The digested sample was once heated almost to dryness, and then dissolved with 5 ml of 0.1 M HNO_3 solution, which contained Ge, Rh, Re and Tl (10 ng g^{-1} each) added as the internal standard elements. This solution is hereafter referred to as the analysis solution. This analysis solution was subjected to the determination of major-to-ultratrace elements by ICP-AES and ICP-MS.

Results and Discussion

Determination of major-to-ultratrace elements in bone-marrow fluid

The analytical results for the bone-marrow fluid sample are summarized in Table 2, where the literature values of the elements in bone-marrow fluid obtained by NAA (neutron activation analysis)[7] and AAS (atomic absorption spectrometry)[8-10] are also shown for a comparison. It can be seen from Table 2 that 20 elements in bone-marrow fluid could be determined in the present experiment, which were in the concentration range from 1610 μg g^{-1} for Na to 0.00043 μg g^{-1} for W. In Table 2, the observed values obtained in the present experiment are expressed as the mean of 3 replicated measurements, together with their standard deviation (SD) and relative standard deviation (RSD). Since the RSDs for Al, Se, Cs and Sn were larger than 20%, their observed values are shown in paretheses in Table 2. However, the RSDs for other elements were within 10%. Especially, the RSDs for the major and trace elements including Fe, Zn and Cu were smaller than 5%. These experimental results indicate that the observed values in the present experiment were obtained with fairly good precision. The poor RSDs for Al and Sn may be caused by their hydrolysis in the analysis solution.

In Table 2, the concentrations of some elements in bone-marrow fluid obtained by NAA[7] and AAS[8-10] are also shown as literature values. Since NAA generally allows multielement analysis, the analytical values for 7 elements in bone-marrow fluid are provided in the literature for NAA.[7] It should be noted here that the concentrations of P and Ca obtained by NAA are remarkably high, compared to the analytical values obtained in the present experiment. As is well known, P and Ca are the main components of human bone. The atomic ratio of Ca/P in the literature values[7] was 1.50, which is quite similar to that (= 1.60) in human bone.[6] These results evidently indicate that the sample examined in the NAA experiment was contaminated with bone pieces consisting of bone marrow,[7] which might be introduced in the sample by crushing the wall of bone marrow in the sampling process with a syringe through the wall. As mentioned earlier, in the present experiment, the bone-marrow fluid sample was centrifuged prior to acid digestion to eliminate bone pieces. No description was given of the sampling procedures in the literature,[7] but it is quite obvious from the literature values that the centrifugation was not performed in the NAA work, which resulted in the contamination of bone pieces in the sample. On the other hand, it can be seen from Table 2 that the analytical value for Ca obtained by AAS[9] is in good agreement with the value in the present experiment.

In addition, the concentrations of Fe, Zn and Sr obtained by NAA[7] are also very high compared to the analytical values obtained in the present experiment. These results also suggest the contamination of these elements from bone pieces of the bone marrow, because these elements are usually enriched in bone.[5] It can also be seen from Table 2 that the Na concentration obtained by NAA was significantly lower than that obtained in the present experiment. This may indicate that Na was apparently diluted with large amounts of Ca and P contaminated from bone pieces.

It can also be seen from Table 2 that the limited number of elements, such as Zn and Cu, as well as alkali and alkaline earth metals in bone-marrow fluid, were determined by AAS.[8-10]

Table 2 Analytical results and literature values for bone-marrow fluid

Element[a]	Present work			Literature value			
	Wevelength[b] or *m*/*z*	Observed value[c]/ μg g^{-1}	RSD, %	NAA[d]/ μg g^{-1}	AAS[e]/ μg g^{-1}	AAS[f]/ μg g^{-1}	AAS[g]/ μg g^{-1}
Na†	589.0 nm I	1610 ±25	2	37.5 (0.0233)		2493.5	
K†	788.4 II	1130 ± 7	0.6			1173.5	
Fe†	259.9 II	314 ± 5	2	1560 (4.97)	609		
P†	213.6 I	239 ± 7	3	40500 (169)			
Ca†	317.9 II	40.5 ± 0.8	2	65200 (1610)		39.4	
Mg†	279.0 II	22.3 ± 0.6	3			61.1	
Al†	308.2 I	(6.2 ± 3.9)	**60**				
Zn†	213.8 I	4.60 ± 0.05	1	210 (45.7)	14.1		5.26
Rb	85	1.70 ± 0.01	0.7				
Cu	65	0.60 ± 0.010	2		2.4		1.94
Se	82	(0.24 ± 0.06)	**30**	0.375 (1.56)			
Ba	138	0.19 ± 0.004	3				
Sr	88	0.027 ± 0.0001	0.4	135 (5000)			
Zr	91	0.0053 ± 0.0006	10				
Cs	133	(0.0036 ± 0.0008)	**20**				
Sb	121	0.0029 ± 0.0003	10				
Sn	120	(0.0015 ± 0.0004)	**30**				
Mo	98	0.0015 ± 0.00007	5				
Ag	107	0.00066± 0.00005	8				
W	184	0.00043± 0.00004	9				

a. The elements with † were determined ICP-AES, and other elements by ICP-MS. b. I and II indicate atomic and ionic lines, respectively. c. Mean ± SD (n = 3). d. Cited from Ref. 7, analyzed by NAA (neutron activation analysis). Dry weight converted into wet weight (factor 0.75). The values in parentheses are the concentration ratios to observed value. e. Cited from Ref. 8, analyzed by AAS (atomic absorption spectrometry). f. Cited from Ref. 9, analyzed by AAS. g. Cited from Ref. 10, analyzed by AAS.

These values obtained by AAS are almost consistent with those obtained in the present experiment, although the concentrations of some of them were slightly different from each other. These results, thus, suggest that further study on the concentrations and distributions of the elements in bone-marrow fluid is required using samples from volunteers of the different ages as well as from different countries.

Comparison of elemental concentrations in bone-marrow fluid with those in blood serum and open seawater

In order to evaluate the concentration levels of the elements in bone-marrow fluid as a biological fluid, the concentrations of the elements in bone-marrow fluid obtained in the present work were compared with those in human blood serum. The results are summarized in Table 3, where the concentration ratios between bone-marrow fluid and blood serum are also shown. The concentrations of elements in blood serum are the mean values for 92 healthy volunteers obtained in a previous study.[11] As can be seen in Table 3, although the concentrations of Fe and Zn in bone-marrow fluid are significantly higher than those in blood serum, the concentration of Cu was lower in bone-marrow fluid than in blood serum. It is well known that primitive blood cells are created in bone marrow, and Fe and Zn are required in such cell development. For example, Fe is a component of hemoglobin in erythroblast produced in bone marrow, and Zn is essential, for example, as a co-factor of carbonic anhydrase in erythrocyte. Furthermore, Zn also plays essential roles in the syntheses of genes and proteins as well as for cellulation or cell division. Thus, the high concentrations of Fe and Zn in bone-marrow fluid suggest the requirement for the evolution of such potential functions mentioned above. The concentrations of alkali metals (K, Rb, Cs), except for Na, were higher in bone-marrow fluid than in blood serum. It is also known that, in animals, K is more abundant in the inner cellular fluid than in the outer cellular fluid. Since bone marrow contains primitive blood cells, it may be considered that K as well as Rb and Cs are distributed more in these primitive blood cells in the bone-marrow fluid. On the other hand, since Na is

Table 3 Comparison of the concentrations of major-to-ultratrace elements in bone-marrow fluid and human blood serum

Element	Bone-marrow fluid (C_B)/μg g^{-1}	Blood serum[a] (C_S)/μg g^{-1}	Ratio[b] C_B/C_S
Na	1610	3120	0.52
K	1130	150	7.53
Fe	314	1.19	264
P	239	119	2.01
Ca	40.5	92.5	0.44
Mg	22.3	17.5	1.27
Al	6.2		
Zn	4.60	0.65	7.04
Rb	1.70	0.17	10.0
Cu	0.60	0.75	0.80
Se	0.24	0.17	1.41
Ba	0.19		
Sr	0.027	0.030	0.90
Zr	0.0053		
Cs	0.0036	0.00061	5.90
Sb	0.0029	0.00019	15.3
Sn	0.0015		
Mo	0.0015	0.0015	1.00
Ag	0.00066	0.00018	3.67
W	0.00043		

a. Cited from Ref. 8; n = 92.
b. Concentration ratio of the element in bone-marrow fluid (C_B) to that in blood serum (C_S).

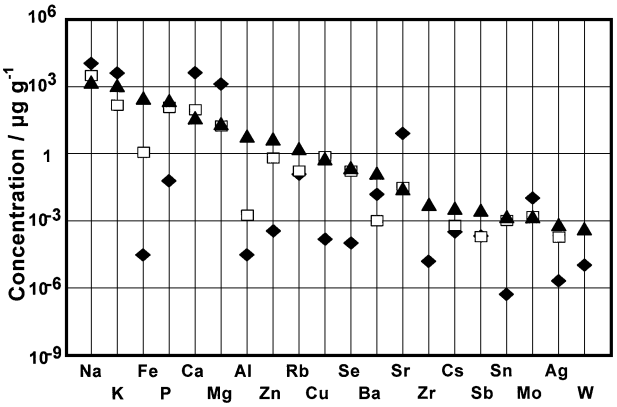

Fig. 1 Comparison of the concentrations of major-to-ultratrace elements in human bone-marrow fluid, human blood serum, and open seawater. ▲, bone-marrow fluid; □, blood serum; ◆, seawater.

generally more abundant in the outer cellular fluid of animals, it may be reasonable that the concentration of Na in bone-marrow fluid was lower than that in blood serum.

Furthermore, the concentrations of the elements in human bone-marrow fluid were compared with those in open seawater.[14] The results are summarized in Fig. 1, where the concentrations of the elements in human blood serum are also shown. Since the composition of blood serum is quite similar to that of seawater, it is often said that the sea might be the field for the origin of life on the Earth. Therefore, a comparison of the concentrations of the elements in bone-marrow fluid as well as in blood serum with those in open seawater may provide some information about the roles or functions of trace elements in biological systems. It is clearly shown in Fig. 1 that the concentrations of major elements (Na, K, Ca, Mg), Sr and Mo in seawater[14] are much higher than those in bone-marrow fluid as well as in blood serum. On the other hand, the concentrations of Fe, P, Al, Zn, Cu, Se, Zr, Sn, Ag, and W in bone-marrow fluid are higher by more than 5-times than those in seawater. These results suggest that such elements, especially bio-essential elements, such as Fe, P, Zn, Cu and Se, are accumulated in bone marrow, perhaps, to play essential roles for some biological functions in bone marrow.

Conclusion

Bone marrow is an organ that produces primitive blood cells, such as erythrocytes, leukocytes and small blood platelets. Thus, in order to know the distributions of diverse elements in bone-marrow fluid, the concentrations of major-to-ultratrace elements were determined by ICP-AES and ICP-MS, and the analytical results were compared with the concentrations of the elements in human blood serum and seawater. As a result, it was found that the concentrations of K, Fe, P, Mg, Zn, Se, Cs, Sb, and Ag in bone-marrow fluid are markedly higher than those in human blood serum as well as in seawater. In addition, the concentrations of Fe, Al, Zn, Cu, Se, Zr, Sn, Ag, and W in bone-marrow fluid were significantly higher than those in seawater. The concentration distributions of diverse elements in bone-marrow fluid obtained in the present research may provide some important or useful information to elucidate the biological or physiological functions of the elements in bone marrow as well as some blood diseases caused by bone-marrow infections.

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