DETERMINATION OF TRACE ELEMENTS IN SUBCELLULAR FRACTION OF HUMAN LIVER BY INAA

Zheng Jian (郑 建), Zhuang Guisun (庄圭荪) and Cheng Yuandi (成源棣)

(Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai 201800, China)

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ABSTRACT

The concentrations of 18 elements in subcellular fractions of human liver were determined by combining differential centrifugation and INAA. Samples of human liver were homogenized in a buffer. The homogenate was separated into nuclei, mitochondrial, lysosomal, microsomal and cytosol fractions by successive differential centrifugation. Biological standard reference materials were used to evaluate the accuracy of the INAA method, and the results agree with the certified values. Element levels in subcellular fractions of human liver were discussed.

Keywords: Trace elements Subcellular fractions INAA

1 INTRODUCTION

With increasing the knowledge of elemental distributions, metabolism and effects of the trace elements in the organisms, it becomes more and more apparent that there is a need to determine elemental contents in subcellular fractions of the tissues, in addition to those in the whole sample. This enables one to obtain information about specific sites of action and biological effect of trace elements and to investigate changes of elemental contents in subcellular fractions in relation to environmental, nutritional or pathological factors.

Trace element studies have been conducted for quite a long time on subcellular fractions of animal tissues, such as rat liver,[1] rat kidney,[2] rat brain,[3,4] and bovine kidney.[5] However, data on trace element levels in subcellular fraction of human tissues are little seen in the literatures.

Instrumental neutron activation analysis (INAA) is one of the most suitable techniques for determination of subcellular trace elements. Its major advantages include freedom from reagent and laboratory contamination and blank correction, multielement specificity, excellent sensitivity, precision and accuracy, and the absence of significant matrix effects. In the present study INAA was used, along with some bioanalytical techniques (particularly differential centrifugation), to determine the distributions of trace elements in subcellular fractions of normal human liver.

2 EXPERIMENTAL

2.1 *Reagents* The HEPES buffer used came from the Sigma Chemicals, and the sucrose from the Shanghai Chemical Reagent Factory. All reagents were of the highest purity available. The glassware and polyethylene vials were soaked in 4mol/l nitric acid for 48 h and rinsed many times with deionized water. Tubes for centrifugation were soaked in 1 mmol/l EDTA to remove metals. Metal contamination from all possible sources was under well control in the experiment.

2.2 *Sampling and separation of subcellular fraction* The liver sample was collected from a female of 35 y in age by the Shanghai Medical University. It was collected within 24 h after her accidental death and she had been proven to be in good health before the accident. Approximately 200 g of the liver sample were minced using a titanium knife and washed in a sucrose buffer solution at 4°C. The solution consisted of 0.25 mol/l sucrose and 10 mmol/l HEPES (pH7.4). The minced liver was homogenized in a fresh portion of the sucrose buffer at 1:4 (v/v) ratio in a blender working at low speed. The efficiency of cell rupture was checked in a microscope after staining the homogenate with a 0.1% solution of crystal violet. The homogenate was separated into different fractions by successive differential centrifugation according to procedures recommended by Sabbioni *et al.*[6] The centrifugation was done at 4°C on a Hitachi 85P-72 ultracentrifuge. Different subcellular fractions were obtained with different centrifugation speeds and durations, nuclei and nondisrupted cells at 3000 r/min (700× g) and 10 min, mitochondria at 10000 r/min (9000× g) and 10 min, lysosomal fractions at 19000 r/min (30000× g) and 25 min, and microsome and cytosol (soluble portion) fractions at 36000 r/min (100000× g) and 110 min.

2.3 *Protein assay* The protein content of different fractions were measured according to the method described by Bradford.[7]

2.4 *Standards and reference materials* Standard solutions were prepared from high purity analytical reagents. The elemental standards are prepared by placing the standard solution on an analytical grade filter paper. The standards and blank were packed into polyethylene bags for short irradiations, or wrapped in high purity aluminium foils for long irradiations. Two biological standard reference materials (SRM), Bovine liver NBS-SRM-1577a and Horse kidney IAEA H8, were used to evaluate the accuracy and precision of the analytical procedures.

2.5 *Irradiation and counting* The samples and standards were successively placed in a pneumatic transfer system and irradiated in the MNSR reactor at China Institute of Atomic Energy. The thermal neutron flux was 8×10^{11} n/(cm$^2\cdot$ s). The long irradiations were executed in a swimming pool-type reactor with a thermal neutron flux of 1×10^{13} n/(cm^{2}s). The nuclides to be studied were devided into three groups in

terms of their irradiation, decay and counting periods as shown in Table 1. The activated samples were counted using an Ortec Ge(Li) detector with 1.9 keV of resolution for 1332 keV of ^{60}Co Gamma-ray. A CANBERRA S-80 pulse-height analyzer and a PDP11/34 computer were used for data acquisition and handling. The net peak areas, statistical errors, and the elemental contents (ppm) were calculated with the SPAN code.

Table 1

The conditions of experiment for INAA

Irradiation time	Decay time	Counting time	Group of nuclides
40 s	3 s	60 s	^{77m}Se
10 min	1 min	10 min	^{80}Br, ^{49}Ca, ^{38}Cl, ^{66}Cu, ^{128}I, ^{42}K, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{37}S, ^{52}V
50 h	10-15 d	1 h	^{51}Cr, ^{59}Fe, ^{203}Hg, ^{86}Rb, ^{46}Sc, ^{124}Sb, ^{65}Zn

3 RESULTS AND DISCUSSION

The nuclear data for the elements of interest in the tissue sample are shown in Table 2. There are two significant interferences encountered in this study. One

Table 2

Nuclear data for INAA measurement of tissue samples

Elements	Product nuclides	Half-life	γ-ray energy (keV)	Elements	Product nuclides	Half-life	γ-ray energy (keV)
Br	^{80}Br	17.6 min	617	Mn	^{56}Mn	2.58 h	1811
Ca	^{49}Ca	8.72 min	3083	Na	^{24}Na	15.0 h	1369
Cl	^{38}Cl	37.2 min	1642	Rb	^{86}Rb	18.66 d	1076
Cr	^{51}Cr	27.7 d	320	S	^{37}S	5.1 min	3102
Cu	^{66}Cu	5.1 min	1039	Sb	^{124}Sb	60.9 d	602.6
Fe	^{59}Fe	44.6 d	1099	Sc	^{46}Sc	83.9 d	889.4
Hg	^{203}Hg	46.8 d	279	Se	^{77m}Se	17.4 s	162
I	^{126}I	25.0 min	442.7	V	^{52}V	3.76 min	1434
K	^{42}K	12.4 h	1525	Zn	^{65}Zn	243.8 d	1115.4
Mg	^{27}Mg	9.45 min	1014				

is the spectral interference of Se to Hg. The only useful photopeak of ^{203}Hg at 279 keV suffers interference from the 280 keV gamma-ray of ^{75}Se. Fortunately, ^{75}Se emits some other intense gamma-rays (265 and 280 keV) and the interference can be easily eliminated by subtracting the ^{75}Se contribution from the overlapping peak with the ^{75}Se γ-ray ratio that can be determined experimentally. The other interference is the interference of ^{54}Fe(n, α)^{51}Cr to ^{50}Cr(n, γ)^{51}Cr. This can be easy to resolve because the contribution of interfering reaction ^{54}Fe(n, α)^{51}Cr could be reliably determined. Cornelis *et al.*[8] have reviewed some potential interference in the NAA of biological materials.

A comparison of the results of the SRM is shown in Table 3. The data were

derived from three measurements for each SRM. The data show good agreement between our results and certified values within ±5–10%.

Table 3

Elemental content of biological standard reference materials ppm

Element	Bovine liver 1577a		Horse kidney H-8	
	This work	NBS	This work	IAEA
Br	9.18±0.36	(9)	109±5	104±11
Ca	-	120±7	890±23	924±77
Cl	2611±39	2800±100	10800±1030	11800±1800
Cu	152±3	158±7	32.2±4.5	31.3±1.8
Fe	196±2	194±20	272±12	265±15
Hg	-	0.004±0.002	0.88±0.13	0.91±0.08
K	9692±30	9960±70	11540±492	11700±749
Mg	592±26	600±15	859±19	818±75
Mn	10.9±0.1	9.9±0.8	5.64±1.52	5.73±0.28
Na	2341±51	2430±130	8827±418	9600±298
Rb	12.2±0.3	12.5±0.1	21.6±0.8	22.2±0.8
S (%)	0.77±0.02	0.78±0.01	1.004±0.021	0.9
Se	0.73±0.11	0.71±0.07	4.83±0.48	4.67±0.30
Zn	120±3	123±8	197±8	193±6

Table 4

Elemental content of homogenate ppm, dry weight

Element	Concentration	Element	Concentration	Element	Concentration
Br	12.6±0.4	Hg	0.443±0.140	Rh	13.2±0.5
Ca	<156	I	<0.938	Sb	0.011±0.001
Cl	819±26	K	3117±737	Sc(ppb)	0.832±0.175
Cr	0.306±0.040	Mg	242±51	Se	0.568±0.086
Cu	15.5±1.4	Mn	2.16±0.15	Zn	146±2
Fe	121±3	Na	72.6±4.3	V	<0.496

Table 5
Protein content of subcellular fractions

Subcellular fraction	Protein (wt %)
Nuclei	54.6±1.0
Mitochondria	67.6±3.3
Lysosomes	61.8±1.6
Microsomes	87.7±0.4
Cytosol	79.7±0.7

In order to investigate element levels in human liver prior to subcellular analysis, part of the homogenate was freeze-dried and analyzed by INAA. The results are shown in Table 4. Fifteen elements were quantitatively measured while only upper limits could be given for the other three, namely Ca, I and V. These upper limits were calculated according to the method described by Currie.[9]

The protein content of each fraction was determined with respect to a bovine-albumin standard. The results are shown in Table 5. The nuclei fraction contained the lowest amount of protein because of the presence of higher proportion

of nucleotides and cell debris in it. The microsomal fraction was the richest in protein. The protein content in mitochondria fraction is 67.6%, which is consistent with the literature values reported by Novikoff *et al.*[10]

Table 6

Elemental content of subcellular fractions of human liver by INAA ppm, dry weight

Element	Nuclei fraction	Mitochondrial fraction	Lysosomal fraction	Microsomal fraction	Cytosol fraction
Br	6.21±0.40	1.99±0.37	0.712±0.092	1.85±0.11	4.73±0.77
Ca	157±40	68.5±25.5	-	50.9±20.7	416±58
Cl	1515±68	519±9	442±25	388±31	3462±740
Cr	0.185±0.020	0.457±0.043	0.292±0.058	0.268±0.003	0.326±0.056
Cu	35.4±4.4	41.6±3.1	5.97±1.25	6.25±0.86	18.3±5.4
Fe	239±7	303±11	46.1±9.9	1042±38	61.8±8.3
Hg	0.474±0.088	0.971±0.049	0.125±0.017	0.225±0.014	2.42±0.32
I	<1.18	<1.00	<0.708	<0.741	<2.13
K	5381±331	2007±343	964±46	896±159	3180±442
Mg	233±55	464±35	162±15	74.4±31	475±34
Mn	4.58±0.13	4.67±0.35	0.773±0.193	1.45±0.20	3.02±0.46
Na	1464±174	673±109	374±15	353±9	1088±168
Rb	33.6±0.4	9.39±0.24	5.13±0.76	7.35±0.38	17.5±1.6
Sb	0.048±0.005	0.025±0.002	0.020±0.001	0.026±0.003	0.039±0.005
Sc (ppb)	-	3.14±0.74	0.81±0.15	-	-
Se	1.24±0.22	1.24±0.10	0.199±0.003	0.405±0.030	0.576±0.179
V	0.191±0.083	0.243±0.046	0.565±0.171	0.295±0.103	0.948±0.331
Zn	174±5	167±4	13.3±2.2	41.1±1.6	65.2±5.8

Concentrations of up to 18 elements in 5 fractions are shown in Table 6. The distribution of elements in subcellular fractions are similar to the data reported by Jayawickreme *et al.*[5]. However, we have found something special.

Selenium is an essential trace element necessary for human health. Normal Se concentrations in human tissues and fluids, such as liver, hair, blood and heart, are ranging from 0.05 to 0.7 ppm,[11] but so far no information on the normal Se contents in subcellular fractions of human liver is available. Our results show that the Se levels in different subcellular fractions vary between 0.24 and 1.24 ppm and it is mainly concentrated in the nuclei and mitochondria. Jayawickreme *et al.*[5] reported that Se was mostly seen in the nuclei and cytosol fractions of bovine kidney. Sabbioni and Girardi[2] found similar results in rat kidney.

Zinc is highly essential to most cells, it is required for growth, development, reproduction and other important physiological functions. Dhar *et al.*[14] found high Zn concentration in the nuclear sediments but also significant contents in the mitochondrial and microsomal fractions of human prostates. Our study shows that Zn is present in high concentration in human liver, mainly located in the nuclei and mitochondria. Furthermore, it was noted that the Se, Zn and Mn in human liver

subcellular fractions distributed in similar ways, and they showed the same variation.

Iron was detected in relatively large amounts in all subcellular fractions, in particular microsomal fraction. Similar observations in rat liver were reported by Ludany *et al.*[1], and in bovine kidney by Jayawickreme[5]. The microsomal fraction is composed mainly of broken parts of the endoplasmic reticulum. It is known that respiratory chains of the endoplasmic reticulum contain a huge amount of ferredoxins[12], this may explain the high Fe concentration in the microsomal. Higher concentrations of Ca, Cl and Hg were observed in the cytosol fraction than in the other fractions. Anghileri[13] reported fairly high Ca concentration in cytosol fraction of rat liver.

Concentration of Cr was found to vary significantly from one fraction to another with the highest percentage in the mitochondrial fraction. Copper was mostly concentrated in the nuclei and mitochondrial fractions. Saito *et al.*[3] reported that Cu was concentrated mostly in mitochondrial fraction of rat brain. Manganese was mainly concentrated in the mitochondrial and cytosol fraction. Rb was found mostly in the nuclei fraction. The concentration pattern in all subcellular fractions of human liver follows the following order:Fe > Zn > Cu. Further research will focus on making use of bioanalytical techniques and INAA to study metalloproteins and protein-bound elements in human liver.

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