

Distribution of Selenium in Human Plasma Detected by High Performance Liquid Chromatography-Plasma Ion Source Mass Spectrometry

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NAKATSUKA, H. and SATOH, H. *Distribution of Selenium in Human Plasma
Detected by High Performance Liquid Chromatography-Plasma Ion Source Mass
Spectrometry.* Tohoku J. Exp. Med., 1996, 178 (1), 17-25 — The distribution of
selenium in human plasma has been investigated by high performance liquid
chromatography (HPLC) connected directly to inductively coupled plasma mass
spectrometry (ICP-MS). Human plasma was loaded on to a size exclusion column
and eluted with 0.01 M sodium phosphate buffer (pH 7.0) at a flow rate of 0.6 ml/
min. Four peaks of selenium were detected in the chromatogram. The first
selenium peak was obtained in the void volume. The retention time of the third
peak was in accord with that of bovine serum albumin as a standard. The forth
peak was thought to be a ghost. The method was applied to identify the chemical
form of selenium in blood plasma immediately after intestinal absorption. The
chromatographic pattern of selenium in postprandial human plasma was compared
with that in fasting plasma. The first and third peaks in the postprandial plasma
sample were slightly higher than those in the fasting plasma sample. This finding
suggests that absorbed selenium is associated with the high molecular weight
fraction and mercaptalbumin in blood plasma. ——— high-performance liquid
chromatography; inductively coupled plasma mass spectrometry; selenium;
human plasma

Selenium is an essential trace element, first recognized in 1957 (Schwarz and
Foltz 1957). Its clinical significance and selenium-related diseases have been
identified (Lockitch 1989). It has recently been proposed that selenium acts to
prevent malignant transformations of cells (Schrauzer 1992). In several

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TABLE 1. *Selenoproteins and selenium binding molecules in blood plasma*

1. Selenoproteins
Selenoprotein-P
Glutathione peroxidase
2. Proteins contain as selenomethionine
Albumin
3. Others
Lipoproteins
Peptides
Heavy metals

epidemiological studies (Willett et al. 1983; Salonen et al. 1984, 1985; Menkes et al. 1986; Kok et al. 1987; Nomura et al. 1987; Schober et al. 1987; Virtamo et al. 1987; Coates et al. 1988; Burney et al. 1989; Helzlsouer et al. 1989; Knekt et al. 1990) selenium levels in serum were determined to examine their relationship to the subsequent development of cancer. However, the results obtained were inconsistent.

These studies appear to ignore what is known about the nature of tissue selenium (Burk 1989). Selenium in plasma can be divided into three groups, two of which are selenium-containing proteins (Table 1). The first group of selenium-containing proteins is referred to as selenoproteins, which contain selenium as selenocysteine, where the selenocysteine is encoded by a UGA in mRNA (Stadtman 1991). This type of selenium is thought to account for the biologically active forms of the element. The other group of proteins contains selenium as selenomethionine (Burk and Hill 1993), which is incorporated into proteins in place of methionine and has no selenium-related function. The third group of selenium in plasma is thought to bind to heavy metals, peptides, or lipoproteins (Burk and Hill 1993).

It is necessary to determine which types of selenium in plasma are related to the prevention or development of the disease. A fast and meaningful technique for the analysis of selenium distribution in plasma is required in the field of epidemiology. In the present study, serum ingredients were separated with size-exclusion chromatography and the selenium contents were directly monitored by inductively coupled plasma mass spectrometry (Koyama et al. 1995a). The method was then applied to identify the chemical form of selenium in blood plasma immediately after intestinal absorption (Koyama et al. 1995b).

EXPERIMENTAL

Instrumentation

The schematic diagram of the instruments is shown in Fig. 1. A high-performance liquid chromatography (HPLC) system (Integral 4000; Perkin-

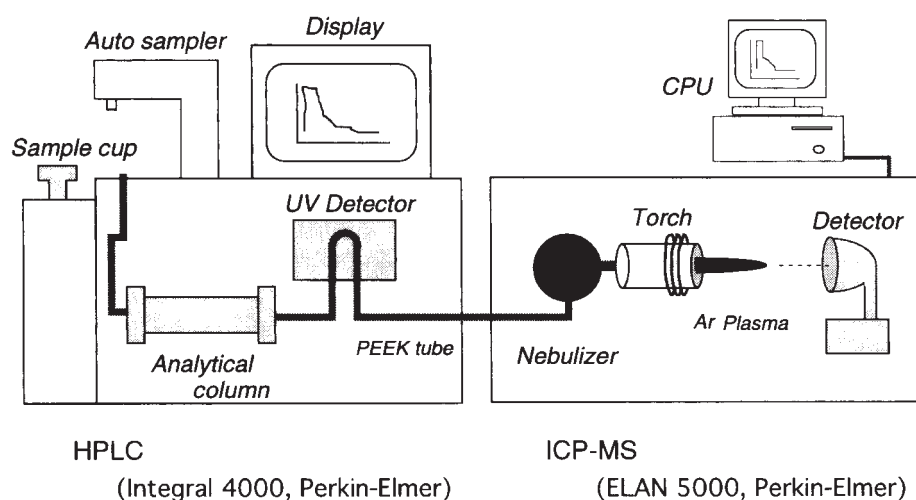


Fig. 1. Schematic diagram of the HPLC-ICP-MS interface.

Elmer Japan, Yokohama) equipped with a pre-packed analytical column (TSKgel3000sw; Tosoh, Tokyo) for size-exclusion chromatography or a multi-mode column (Asahipak GS520HQ; Showa Denko, Tokyo) was used. Sodium phosphate buffer was used at a flow rate of 0.6 or 1.0 ml/min. A built-in UV monitor was used at a wavelength of 280 nm.

ICP-MS (Elan 5000; Perkin-Elmer Japan) was connected directly to the HPLC system. The eluted solution from the outlet of the UV monitor was connected with PEEK tubing (Waters Japan Ltd., Tokyo) to the inlet of the ICP-MS nebulizer. The diameter of the tubing was 0.127 mm and the length was 1.4 m (the minimum necessary), resulting in a tubing dead volume 0.018 ml. The time delay from the UV monitor to the ICP-MS was estimated to be 1.06 sec. The graphics mode of the ICP-MS was used for time-resolved element monitoring. Operating conditions for the HPLC and ICP-MS instruments and detailed information on the mode of operation are shown in Table 2.

TABLE 2. *Operating conditions for HPLC and ICP-MS*

Eluent	0.01 M sodium phosphate buffer pH = 7.0, degassed and filtrated (See text)
Flow rate	0.6 ml/min
Sample	Human serum 30 μ l
Connection to ICP-MS	PEEK tube (0.127 mm ID)
R.F. Power	1300 KW
Nebulizer	Cross-flow type
Gas Flow	
Nebulizer	1.1 liter/min
Auxiliary	0.8 liter/min
Plasma	13.0 liter/min

TABLE 3. *Recipe of the experimental diet and its selenium content.*

	Food No.*	Weight (g)	Se conc. ($\mu\text{g}/100\text{ g}$)	Se cont. (μg)
Spaghetti (dry)	1 34a	100.0	21	21
Egg, Chicken	10 5a	66.7	57	38
Bacon	9 85a	28.9	0	0
Cream	11 7a	22.2	2	0.4
Cheese (Parmesan)	11 22 h	3.9	31	1.2
Garlic	12 95	1.1	0	0
Oil (vegetable)	5 1	0.2	0	0
Pepper (black)	17 18a	0.06	11	0.007
Wine (white)	16 3a	16.7	0.05	0.008
			Calculated	60.7 μg
			Measured	77.6 μg

*Resources Council, Science and Technology Agency, Japan (1982)

Reagents and materials

Analytical reagent grade water ($>18\text{ M}\Omega/\text{cm}$) purified with a Milli-Q system (Milli-Q Labo; Nihon Millipore Kogyo, Yonezawa) was used throughout. Analytical grades of sodium dihydrogenphosphate and disodium monohydrogenphosphate were obtained from Wako Pure Chemicals (Osaka). The sodium phosphate solutions (0.01 M each) were mixed to form an elution buffer with a pH of 7.0. The buffer was degassed by the bubbling of helium gas for 10 min before use. The elution buffer were prefiltered with an HV or Durapore HV13 filter (both $0.45\text{ }\mu\text{m}$, Nihon Millipore Kogyo) before being used in the analyses.

Postprandial blood plasma

Eight healthy adults participated to the dietary study to identify the chemical form of selenium in blood plasma immediately after intestinal absorption. After 12 hr fasting, the participants took a formula diet. The recipe of the diet is shown in Table 3. The calculated selenium content of the diet using the trace element table (Suzuki and Tanushi 1993) was $60.7\text{ }\mu\text{g}$. The selenium content of the diet measured by the Watkinson method was $77.6\text{ }\mu\text{g}$. Blood was obtained before and 30 min after the beginning of the meal by venipuncture using a Venoject VP-H (Terumo, Tokyo) and centrifuged at 3,000 rpm for 10 min.

RESULTS

The results of the separation of serum selenium by size-exclusion HPLC using a TSKgel3000sw column followed by ICP-MS are presented in Fig. 2. Four selenium peaks were detected. The chromatograms of copper and zinc are also

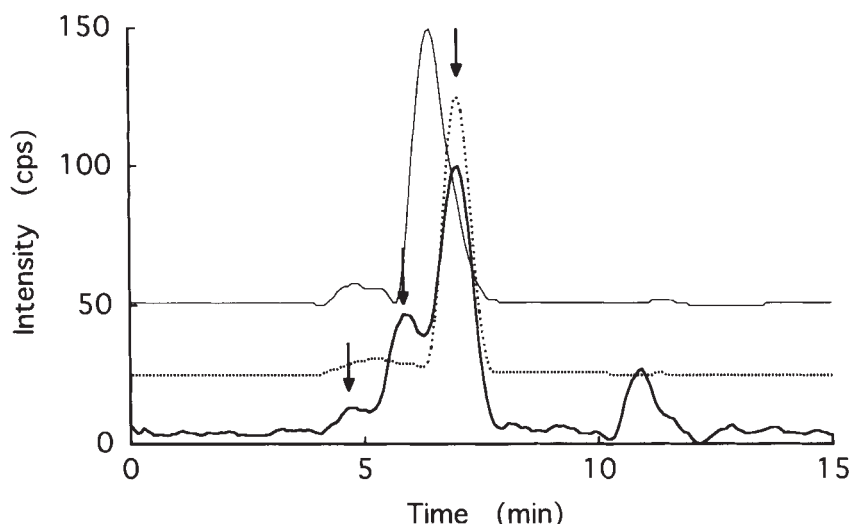


Fig. 2. Size-exclusion chromatograms of copper (—), zinc (···) and selenium (—) in human serum.

shown in the same figure. Approximately 90% of plasma copper is contained in ceruloplasmin (molecular weight 132,000) and more than 60% of plasma zinc binds to albumin (molecular weight 69,000). The peaks of these elements can be used as molecular-size markers. The third serum selenium peak is in accord with the zinc peak in retention time. It appears that the third peak is due to selenium contained in albumin. The fourth selenium peak is thought to be a ghost due to interference from molecular species, because chromatographic patterns of the fourth peak monitored with mass numbers of 77 and 82 are inconsistent.

The eluate from the column was divided into 30-sec fractions and the activity of glutathione peroxidase in each fraction was measured. Fig. 3 shows that the peak of glutathione peroxidase activity appears at a point midway between the

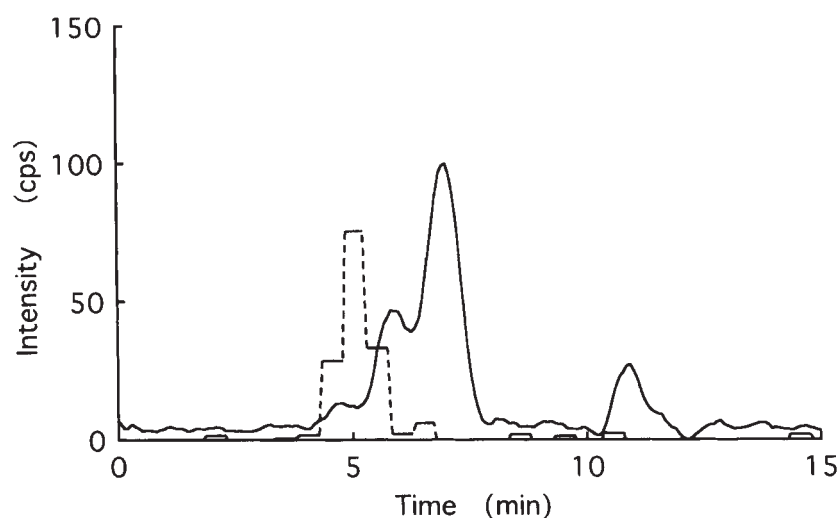


Fig. 3. Size-exclusion chromatograms of serum selenium (—) and glutathione peroxidase (---) activity.

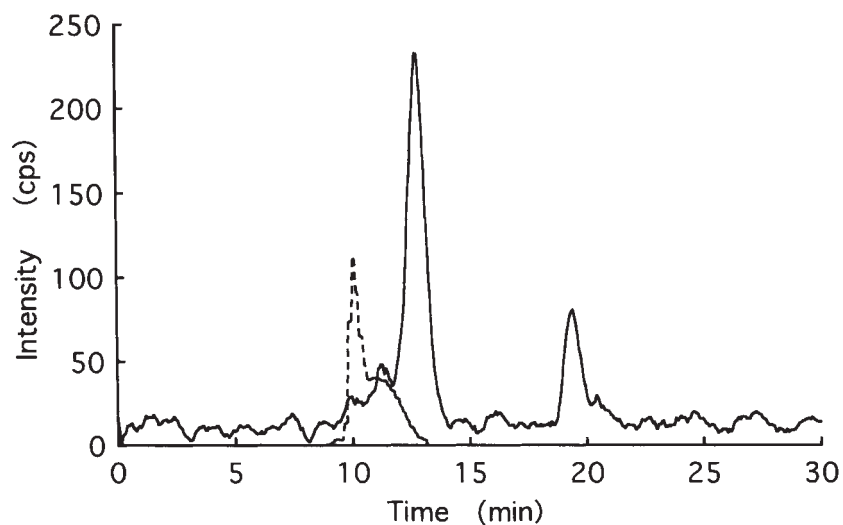


Fig. 4. Size-exclusion chromatograms of serum selenium (—) and cholesterol (---) concentrations.

first and second peaks of serum selenium. Thus, neither the first nor second selenium peak of the chromatogram seems to represent selenium contained in glutathione peroxidase.

Cholesterol contents in the fractions collected with the same procedure described above were also measured and are presented in Fig. 4. The cholesterol peak had the same retention time as the first selenium peak and has a shoulder. The cholesterol peak represents large lipoproteins, such as very low density lipoproteins (VLDL) and low density lipoproteins (LDL) and the shoulder is likely to be a cholesterol contained in high density lipoprotein (HDL). The first peak of selenium is thought to be selenium associating with the high molecular

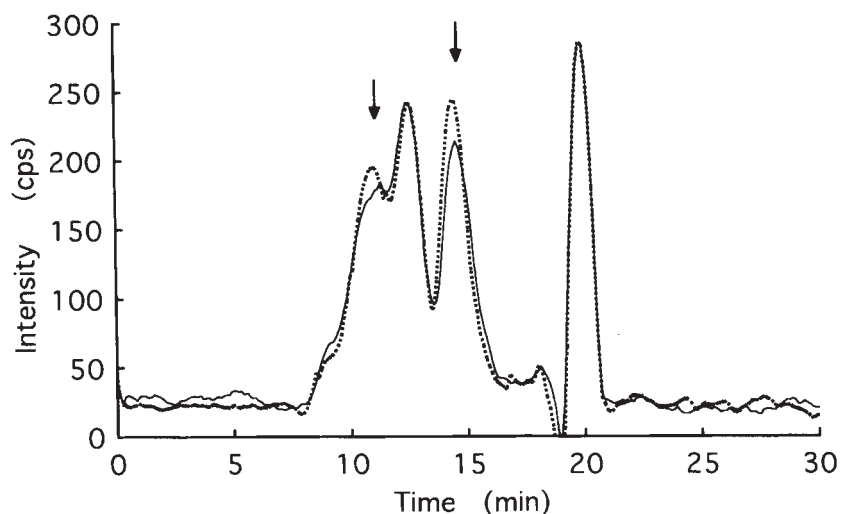


Fig. 5. Chromatograms of fasting (—) and postprandial (---) plasma selenium. The arrows indicate the first and third peaks of selenium which were increased in postprandial plasma.

weight fraction in blood plasma, which includes large lipoproteins.

The chromatogram of fasting and postprandial plasma selenium obtained with the HPLC ICP-MS method is shown in Fig. 5. The column used in this study was a multi-mode column (Asahipak GS520HQ) which is basically a size-exclusion column and is excellent for the separation of serum albumin. Both fasting and postprandial plasma selenium were divided into four peaks. The fourth is thought to be a ghost peak. The first and third peaks of postprandial plasma were slightly higher than those of fasting plasma. This finding suggests that absorbed selenium associates with the high molecular weight fraction and mercaptalbumin in blood plasma.

DISCUSSION

Several techniques have been used to separate the components of serum selenium. Deagen et al. (1993) were partly successful with affinity column chromatography. They showed that serum selenium was distributed between selenoprotein-P, glutathione peroxidase (GSH-Px), and albumin. However, the technique is time consuming.

The coupling of HPLC with an element detector was first done with flame atomic absorption spectrometry and then with ICP-atomic emission spectrometry (Shibata et al. 1992). However, selenium was not measured by these methods because they were not sufficiently sensitive to detect selenium. The detection limit of the HPLC ICP-MS system used in the present study was 50 pg of selenium (50 μ l of a 1.0 ppb solution) estimated from 3- σ of background fluctuations.

The detection of serum selenium distribution using size-exclusion HPLC ICP-MS has two major advantages. The first is that ICP-MS can be used as a multi-element detector. Thus, copper, zinc and iron can be used as molecular-size markers. The other advantage is that size-exclusion HPLC can avoid the influence of serum minerals, which disturb the detection of selenium by ICP-MS. Serum minerals are retained in the HPLC column and elute far behind the last selenium peak.

The results of the present study show that serum selenium can be divided into three fractions. The first selenium peak is thought to be selenium associating with the high molecular weight fraction in blood plasma, which includes large lipoproteins. The second selenium peak has not been identified. This peak was not in accord with glutathione peroxidase activity. The third peak seems to be selenium contained in albumin, because the retention time of the third peak is in accordance with that of the serum albumin standard. This method was applied to identify the chemical form of selenium in blood plasma immediately after intestinal absorption. The first and third peaks of postprandial plasma were slightly higher than those of fasting plasma. This finding suggests that absorbed selenium associates with the high molecular weight fraction and mercaptalbumin in blood plasma.

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References

- 1) Burk, R.F. (1989) Selenium and myocardial infarction. *JAMA*, **262**, 775.
- 2) Burk, R.F. & Hill, K.E. (1993) Regulation of selenoproteins. *Ann. Rev. Nutr.*, **13**, 65-81.
- 3) Burney, P.G.J., Comstock, G.W. & Morris, J.S. (1989) Serologic precursors of cancer: Serum micronutrients and the subsequent risk of pancreatic cancer. *Am. J. Clin. Nutr.*, **49**, 895-900.
- 4) Coates, R.J., Weiss, N.S., Daling, J.R., Morris, J.S. & Labbe, R.F. (1988) Serum levels of selenium and retinol and the subsequent risk of cancer. *Am. J. Epidemiol.*, **128**, 515-523.
- 5) Deagen, J.T., Bultler, J.A., Zachara, B.A. & Whanger, P.D. (1993) Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma. *Anal. Biochem.*, **208**, 176-181.
- 6) Helzlsouer, K.J., Comstock, G.W. & Morris, J.S. (1989) Selenium, lycopene, α -tocopherol, β -carotene, retinol, and subsequent bladder cancer. *Cancer Res.*, **49**, 6144-6148.
- 7) Knekt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R.-K., Hakama, M., Hakulinen, T., Peto, R. & Teppo, L. (1990) Serum selenium and subsequent risk of cancer among Finnish men and women. *J. Natl. Cancer Inst.*, **82**, 864-868.
- 8) Kok, F.J., Bruijn, A.M. de, Hofman, A., Vermeeren, R. & Valkenburg, H.A. (1987) Is serum selenium a risk factor for cancer in men only? *Am. J. Epidemiol.*, **125**, 12-16.
- 9) Koyama, H., Watanabe, C., Kasanuma, Y., Ejima, A., Kim, C.-Y., Nakatsuka, H. & Satoh, H. (1995a) Identification of increased fractions of selenium in postprandial blood plasma by HPLC-ICP-MS. *Biomed. Res. Trace Elements*, **6**, 145-146. (in Japanese with English abstract)
- 10) Koyama, H., Watanabe, C., Kasanuma, Y., Ejima, A., Kim, C.-Y. & Satoh, H. (1995b) Serum selenium fractionation analysis by HPLC-ICP-MS. *Jpn. J. Hyg.*, **50**, 154. (in Japanese)
- 11) Lockitch, G. (1989) Selenium: Clinical significance and analytical concepts. *Cri. Rev. Clin. Lab. Sci.*, **27**, 483-541.
- 12) Menkes, M.S., Comstock, G.W., Vuilleumier, J.P., Helsing, K.J., Rider, A.A. & Brookmeyer, R. (1986) Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. *N. Engl. J. Med.*, **315**, 1250-1254.
- 13) Nomura, A., Heilbrun, L.K., Morris, J.S. & Stemmermann, G.N. (1987) Serum selenium and the risk of cancer, by specific sites: Case-control analysis of prospective data. *J. Natl. Cancer Inst.*, **79**, 103-108.
- 14) Resources Council, Science and Technology Agency, Japan (1982) Standard Tables of Food Composition in Japan, 4th ed.
- 15) Salonen, J.T., Alfthan, G., Huttunen, J.K. & Puska, P. (1984) Association between serum selenium and the risk of cancer. *Am. J. Epidemiol.*, **120**, 342-349.
- 16) Salonen, J.T., Salonen, R., Lappetelainen, R., Maenpaa, P.H., Alfthan, G. & Puska, P.

- (1985) Risk of cancer in relation to serum concentrations of selenium and vitamin A and E: Matched case-control analysis of prospective data. *Br. Med. J.*, **290**, 417-420.
- 17) Schober, S.E., Comstock, G.W., Helsing, K.J., Salkeld, R.M., Morris, J.S., Rider, A.A. & Brookmeyer, R. (1987) Serologic precursors of cancer. I. Prediagnostic serum nutrients and colon cancer risk. *Am. J. Epidemiol.*, **126**, 1033-1041.
- 18) Schrauzer, G.N. (1992) Selenium: Mechanistic aspects of anticarcinogenic action. *Biol. Trace Elem. Res.*, **33**, 51-62.
- 19) Schwarz, K. & Foltz, C.M. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Sci.*, **79**, 3292-3293.
- 20) Shibata, Y., Morita, M. & Fuwa, K. (1992) Selenium and arsenic in biology. *Adv. Biophys.*, **28**, 31-80.
- 21) Stadtman, T.C. (1991) Biosynthesis and function of selenocysteine-containing enzymes. *J. Biol. Chem.*, **266**, 16257-16260.
- 22) Suzuki, Y. & Tanushi, S. (1993) *Table of Trace Element Contents in Japanese Foodstuffs*. Dai-ich Shuppan, Tokyo. (in Japanese)
- 23) Virtamo, J., Valkeila, E., Alfthan, G., Punsar, S., Huttunen, J.K. & Karvonen, M.J. (1987) Serum selenium and risk of cancer. *Cancer*, **60**, 145-148.
- 24) Willett, W.C., Morris, J.S., Pressel, S., Taylor, J.O., Polk, B.F., Stampeer, M.J., Rosner, B., Scheider, K. & Hames, C.G. (1983) Prediagnostic serum selenium and risk of cancer. *Lancet*, **2** (8342), 130-134.
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