Analysis of selenoproteins in rat serum by Triple Quadrupole ICP-MS

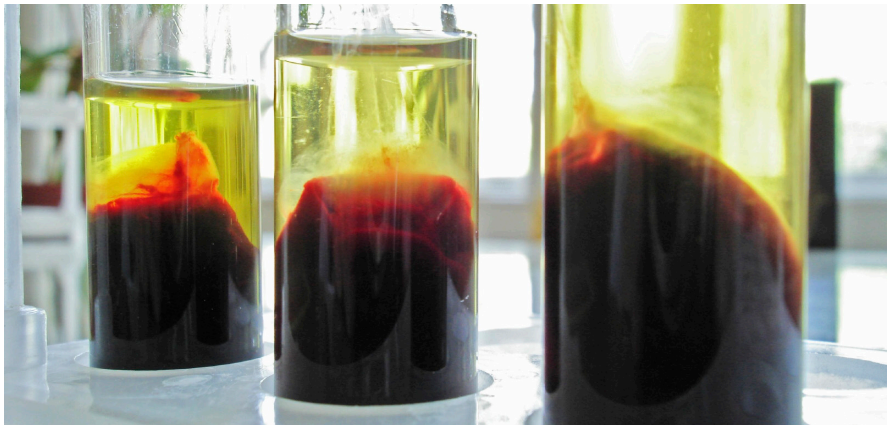
Application note

Proteomics/Metallomics

Authors

Yasumi Anan
Yoshiko Hatakeyama
Maki Tokumoto
Yasumitsu Ogra

Laboratory of Chemical Toxicology and Environmental Health,
Showa Pharmaceutical University,
Machida, Tokyo 194-8543, Japan.



Introduction

The importance of selenium (Se) as an essential micronutrient in animals is illustrated by the fact that it is present in several of the key proteins found in plasma. Two selenoproteins which contain Se as selenocysteine (SeCys) in their primary structures, extracellular glutathione peroxidase (eGPx, GPx-3) and selenoprotein P (Sel P), have been detected in animal plasma.[1-3] Other Se-containing proteins which have Se incorporated into their peptide sequence as selenomethionine (SeMet), are also detected because animals are unable to discriminate SeMet from methionine (Met).[4] The most abundant Se-containing protein in human plasma is albumin.[5] However, some studies have indicated that no or little Se-containing albumin is detected in the blood plasma of experimental animals compared to human plasma.[6-8] This can be explained by the fact that humans ingest Se mainly as SeMet, whereas the major Se species in the feeds given to experimental animals is inorganic Se, such as selenite and selenate.

Verified for Agilent
8900 ICP-QQQ

Agilent Technologies

HPLC-ICP-MS is often used for the determination of Se species because it provides high sensitivity and selectivity.[9] Various studies report the separation of selenoproteins/Se-containing proteins in plasma/serum of human and experimental animals using HPLC-ICP-MS.[4, 10, 11] Our research group has detected two major selenoproteins in rat serum with good separation on a multi-mode size-exclusion HPLC column.[12]

While ICP-MS is often used to determine Se, the three most abundant Se isotopes, ^{80}Se (49.6%), ^{78}Se (23.8%), and ^{76}Se (9.36%), suffer from interference by several polyatomic ions originating from the Ar plasma, namely, $^{40}Ar^{40}Ar^+$, $^{40}Ar^{38}Ar^+$, and $^{38}Ar^{38}Ar^+$, respectively. In addition, ^{77}Se is also subject to interference by $^{40}Ar^{37}Cl^+$ when chloride is present in the sample matrix, as is the case with biological samples. Thus, the less abundant ^{82}Se isotope (8.73%) was frequently used for Se detection by ICP-MS. To overcome the polyatomic ion overlaps, an ICP-MS equipped with a collision/reaction cell (CRC) can be used. Several CRC methods have been used for the detection of Se.[13] For instance, hydrogen (H_2), helium (He), and methane (CH_4) can be used individually or in combination as the collision/reaction gas.[14-16] The use of H_2 cell gas gives a dramatic reduction in the intensity of the Ar-based polyatomic ions that overlap the main Se isotopes, yielding single ng/L detection limits for Se. However, H_2 reaction mode can lead to a new interference problem in the detection of Se in extracellular fluid, such as plasma/serum and urine. Because extracellular fluid contains substantial amounts of bromine (Br), newly generated polyatomic interferences may be formed from the combination of Br and the H_2 reaction gas. These new polyatomic ions $^{79}Br^1H^+$ and $^{81}Br^1H^+$, adversely affect the detection of ^{80}Se and ^{82}Se, respectively. This causes a significant problem in tracer studies using enriched Se isotopes, or isotope dilution (ID) studies for the accurate quantification of Se containing compounds such as Se peptides and Se proteins. A previous study demonstrated the use of D_2 to avoid the BrH interferences on ^{80}Se and ^{82}Se using ICP-MS[17]. However, a triple quadrupole mass spectrometer (ICP-QQQ) has been introduced, which offers some advantages over the single quadrupole instruments (ICP-QMS)[18]. Namely, ICP-QQQ can operate in mass shift mode using oxygen to shift the analyte ions for detection to M+16 amu e.g. $^{78}Se^+$ is measured as $^{78}Se^{16}O^+$ at 94 amu; $^{80}Se^+$ is measured at 96 amu; and $^{82}Se^+$ is measured at 98 amu. Consequently ICP-QQQ is expected to give more precise detection for Se than ICP-QMS. The aim of this study is to evaluate the performance of ICP-QQQ for the speciation of Se in rat serum.

Experimental

Reagents

Standard Se solution (1000 µg/mL) was purchased from Kanto Chemicals (Tokyo, Japan) and diluted with 0.1 M nitric acid prior to use. Tris(hydroxymethyl) aminomethane (TRIZMA base and TRIZMA HCl) were purchased from Sigma (St. Louis, MO, USA). Deuterium gas (>99.6 atom %) was purchased from Showa Denko (Tokyo).

Animal experiments

All animal experiments were carried out according to the "Principles of Laboratory Animal Care" (NIH version, revised 1996) and were approved by the Animal Investigation Committee, Showa Pharmaceutical University, Japan. Specific pathogen free (SPF) male Wistar rats (5 weeks of age; Sankyo Labo Service Corporation, Inc., Tokyo, Japan) were purchased. The animals were housed in a humidity-controlled room maintained at 22–25 °C with a 12 h light–dark cycle and fed a commercial diet and tap water ad libitum. After a one-week acclimatization period, blood was collected under light ether anesthesia and clotted blood was centrifuged at 1600 x g for 10 min to separate the serum. Serum samples were preserved at -30 °C prior to use.

HPLC-ICP-MS and HPLC-ICP-QQQ analyses

An Agilent 7500ce ICP-MS equipped with an Octopole Reaction System (ORS) and an Agilent 8800 Triple Quadrupole ICP-MS were used. The operating conditions are summarized in Table 1.

Table 1. Operating conditions for ICP-QMS and ICP-QQQ for the speciation of Se

	Agilent 7500ce ICP-QMS	Agilent 8800 ICP-QQQ
Plasma setting		
RF power (W)	1450	1550
Nebulizer type	Babington	MicroMist
Nebulizer gas flow (L/min)	1.15	0.90
Make-up gas flow (L/min)	0.11	0.25
Plasma gas flow (L/min)	15.0	14.0
Reaction/Collision cell		
D_2 gas flow (mL/min)	3.0	-
O_2 gas flow (mL/min)	-	0.3
Data acquisition		
m/z monitored	76 to 84	
		94 shifted from 78
		96 shifted from 80
		98 shifted from 82

The HPLC system consisted of an on-line degasser, an HPLC pump (PU713; GL Science Co., Ltd., Tokyo, Japan), a Rheodyne six-port injector with a 200 µL sample loop, and a column. A multi-mode gel filtration column, Shodex Asahipak GS-520HQ (7.5 i.d. x 300 mm, with a guard column, 7.5 i.d. x 75 mm, Showa Denko, Tokyo, Japan), was used. The column was injected with a 200 µL aliquot of serum sample and then eluted with 50 mmol/L Tris-HCl, pH 7.4, at a flow rate of 0.6 mL/min. The eluate was introduced directly into the nebulizer of the ICP-QMS or ICP-QQQ, and Se signals were monitored at *m/z* 78, 80, and 82 using D_2 reaction mode in ICP-QMS, or as SeO^+ at *m/z* 94, 96, and 98 using O_2 mass shift mode in ICP-QQQ.

Results and Discussion

Two well-separated Se peaks were detected at retention times of 11.7 and 14.3 min (Figure 1). The former and latter peaks were assignable to eGPx and Sel P, respectively, per our previous literature.[19] It was reported that albumin was eluted at the retention time of 15.0-16.0 min on this column.[12] However, we did not detect a Se peak at a retention time of 15.0-16.0 min, suggesting that SeMet was not incorporated into albumin in place of Met.

The most problematic interferences affecting Se detection in serum by ICP-MS are argon-based polyatomic ions from the ICP plasma gas e.g., $^{38}Ar^{38}Ar^+$, $^{40}Ar^{37}Cl^+$, $^{38}Ar^{40}Ar^+$, and $^{40}Ar^{40}Ar^+$.

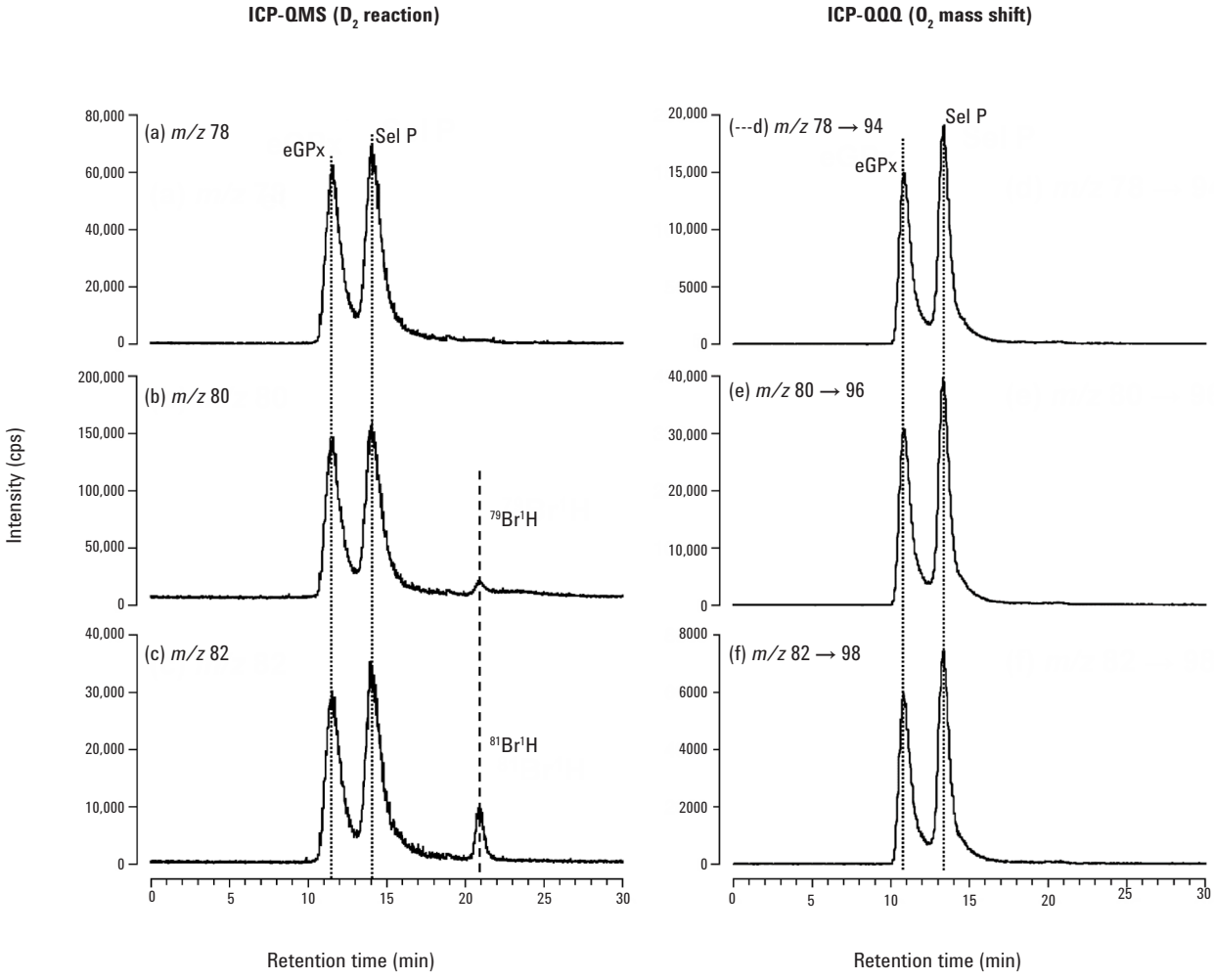


Figure 1. Elution profiles of Se in rat serum. A 200-µL aliquot of a rat serum sample was injected into a GS-520HQ column and the eluate was monitored by ICP-MS (a-c) or ICP-QQQ (d-f) at *m/z* 78 (a), 80 (b), 82 (c), 94 (d), 96 (e), and 98 (f).

Thus, ^{82}Se is the least affected isotope in the detection by ICP-MS without a CRC (Figure 2a). H_2 is frequently used as the reaction gas for Se detection. However, when Se in extracellular fluid, such as serum, is analyzed, interferences originating from Br are formed (Figure 2b). Indeed, the quantification and speciation of Se in urine and serum samples using multiple isotopically enriched tracers is not possible under H_2 reaction mode due to severe interference from BrH. An alternative technique to avoid the interference is to use D_2 in place of H_2[17]. When D_2 is used as the reaction gas, the Br interference is reduced. However, the commercially available D_2 gas that we used contained substantial amounts of H_2 (<0.5%); hence, the interference could not be excluded completely though the interference originating from BrH did not restrict Se speciation of serum. As shown in Figure 1b and 1c, the peak corresponding to BrH is well separated from the peaks of the two selenoproteins. There was a problem caused by D_2 based interference (by SeD) as shown in Figure 2c, and this interference was unavoidable. Indeed, the signal at *m/z* 80 overlapped with the signal for $^{78}SeD^+$, and the signal at *m/z* 82 was more severely affected because ^{80}Se has a larger abundance than ^{78}Se (Figure 3a).

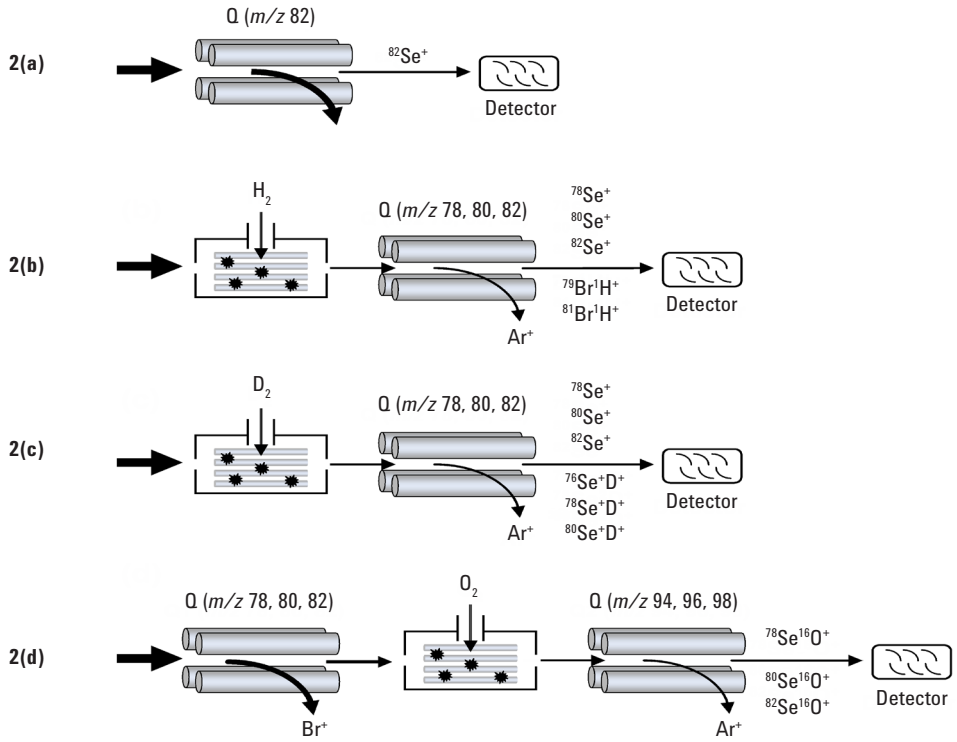


Figure 2. Schematic diagram of the detection of Se in extracellular fluid by ICP-MS under normal (a), H_2 (b), and D_2 (c) cell gas modes, and ICP-QQQ using O_2 mass shift mode (d).

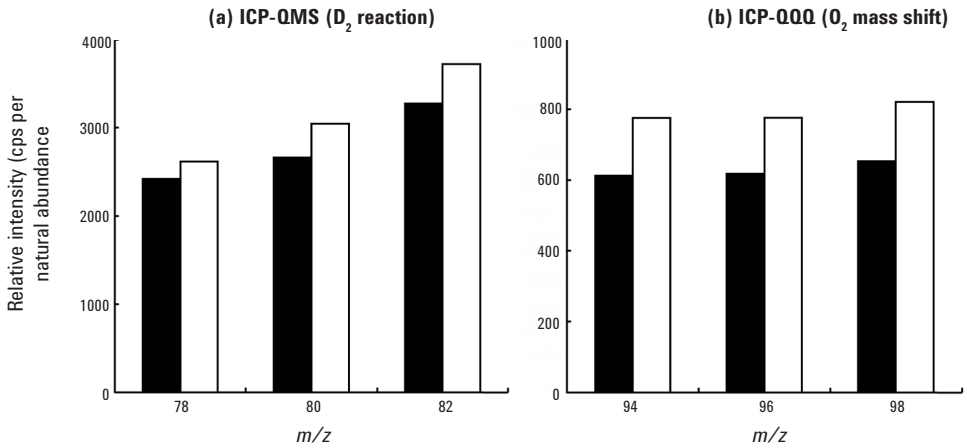


Figure 3. Effect of O_2 mass shift mode by ICP-QQQ on the speciation of Se. Relative intensity was defined by counts per second (cps) of the peak heights of GPx (closed columns) and Sel P (open columns) divided by the isotope ratio of each Se isotope.

ICP-QQQ using O_2 mass shift mode has been used successfully for the low level detection of sulfur and phosphorus[19]. In this study, we used the ICP-QQQ in O_2 mass shift mode for Se speciation (Figure 2d). The first quadrupole (Q1) was set to allow ions only at *m/z* 78, 80, and 82 to pass to the CRC thereby eliminating Br at *m/z* 81 and 83. No interference originating from BrH was detected in the elution profiles obtained by ICP-QQQ under O_2 mass shift mode (Figures 1d-1f). In addition, the relative peak heights of the two serum selenoproteins were more consistent when measured using the ICP-QQQ method than by ICP-QMS (Figure 3b). Consequently, ICP-QQQ is a more powerful tool than ICP-QMS for the speciation of Se in biological samples.

Conclusions

Two major selenoproteins, eGPx and Sel P, in rat serum were well separated on an HPLC column. ICP-QQQ was a more accurate detector for the speciation of serum selenoproteins than ICP-QMS because it was completely free of interference originating from the plasma source Ar and the matrix elements.

Acknowledgements

We thank Mr. Naoki Sugiyama (Agilent Technologies International Japan, Ltd.) for facilitating the use of the ICP-QQQ. This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (Nos. 23390032 and 24659022 to Y. O., and 25870739 to Y.A.), and the financial support from Takeda Science Foundation, Japan.

References

1. M. Borglund, A. Akesson, and B. Akesson, *Scand J Clin Lab Invest*, 1988, 48, 27.
2. B. Akesson, and B. Martensson, *J Inorg Biochem*, 1988, 33, 257.
3. M. Persson-Moschos, W. Huang, T. S. Srikumar, B. Akesson, and S. Lindeberg, *Analyst*, 1995, 120, 833.
4. P. Jitaru, H. Goenaga-Infante, S. Vaslin-Reimann, and P. Fisicaro, *Anal Chim Acta*, 2010, 657, 100.
5. L. A. Daniels, *Biol Trace Elem Res*, 1996, 54, 185.
6. P. Òscar, and R. Łobiński, *Talanta*, 2007, 71, 1813.
7. Y. Kobayashi, Y. Ogra, and K. T. Suzuki, *J. Chromatogr. B Biomed. Sci. Appl.*, 2001, 760, 73.
8. H. Koyama, Y. Kasanuma, C. Y. Kim, A. Ejima, C. Watanabe, H. Nakatsuka, and H. Satoh, Tohoku *J Exp Med*, 1996, 178, 17.
9. Y. Ogra, and Y. Anan, *J. Anal. At. Spectrom.*, 2009, 24, 1477.
10. G. A. Jacobson, A. M. Featherstone, A. T. Townsend, R. Lord, and G. M. Peterson, *Biol Trace Elem Res*, 2005, 107, 213.
11. K. T. Suzuki, M. Itoh, and M. Ohmichi, *J Chromatogr B Biomed Appl*, 1995, 666, 13.
12. Y. Tsuji, T. Mikami, Y. Anan, and Y. Ogra, *Metallomics*, 2010, 2, 412.
13. J. J. Sloth, and E. H. Larsen, *J. Anal. At. Spectrom.*, 2000, 15, 669.
14. J. J. Sloth, E. H. Larsen, S. H. Bügel, and S. Moesgaard, *J. Anal. At. Spectrom.*, 2003, 18, 317.
15. J. Darrouzès, M. Bueno, G. Lespès, and M. Portin-Gautier, *J. Anal. At. Spectrom.*, 2005, 20, 88.
16. W. Guo, S. Hu, Y. Wang, L. Zhang, Z. Hu, and J. Zhang, *Microchem. J.*, 2013, 108, 106.
17. Y. Ogra, K. Ishiwata, and K. T. Suzuki, *Anal. Chim. Acta*, 2005, 554, 123.
18. S. Diez Fernandez, N. Sugishama, J. Ruiz Encinar, and A. Sanz-Medel, *Anal Chem*, 2012, 84, 5851.
19. Y. Anan, Y. Hatakeyama, M. Tokumoto, and Y. Ogra, Anal. Sci., 2013, 29, 787.

Verified for Agilent
8900 ICP-QQQ

Results presented in this document were obtained using the 8800 instrument, but performance is also verified for the 8900 ICP-QQQ

www.agilent.com

Published March 7, 2017
Publication number: 5991-2750EN

Agilent Technologies