Biological Selenium Species and Selenium Speciation in Biological Samples

Katarzyna Bierla, Joanna Szpunar, Ryszard Lobinski

Chapter 35

Biological selenium species and selenium speciation in biological samples

Katarzyna Bierla, Joanna Szpunar and Ryszard Lobinski

CNRS-UPPA, UMR 5254, Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE-IPREM), Hélioparc, 2, Avenue Pr. Angot, 64053 Pau, France

e-mail: ryszard.lobinski@univ-pau.fr

Keywords: Electrospray MS Orbitrap; Food supplements; Foodstuffs; HPLC- ICP MS; Human health; Selenium speciation and Selenoproteins

Abstract

The chapter summarizes the state of the art of the analytical methodology for the speciation of selenium (Se) in biological samples relevant to human health (body fluids, cell cultures, tissues, food supplements). Selenoproteins (with genetically encoded selenocysteine (Sec), Se-containing proteins (with Met substituted by selenomethionine, SeMet) and Se-containing metabolites are discussed. Whereas gel electrophoresis followed by radiography of ^{75}Se is the benchmark for selenoproteins and Se-containing protein detection, the recent advances in laser ablation-ICP MS allow the scanning of the gels for stable Se isotopes, increasing the number of biological systems to be investigated and enhancing the depth of the studies. The democratization of proteomics approaches opens the way to the high throughput identification of selenoproteins, although several bottlenecks, such as loss of Sec during sample preparation, identification on the basis of the part of the proteins without Se, and insufficient sensitivity, are still demanding considerable improvements of analytical methodology. In the field of metabolomics, the combined use of multidimensional HPLC with the combined Se-specific ICP MS detection and

Orbitrap MS^n detection, seems to be an ultimate tool for the comprehensive quantitative and qualitative Se speciation analysis.

35.1 Introduction: Interest in Selenium Speciation Analysis in Biology

The knowledge of the speciation: the identity and the concentration of the exact chemical form(s) in which selenium (Se) is present in a sample is fundamental to describe its molecular mechanisms of the biological activity of this element and to delineate the specific metabolic pathways in cells and tissues. The processes concern medical, physiological and nutritional research: the role of Se in health and disease, the metabolism and activity of Se-containing active therapeutic molecules and nanoparticles, the biotechnology of Se-enriched food and feed supplements [1-3], and Se bioavailability and metabolism in food [1,2].

35.2 Selenium Species of Interest

There are numerous foods and food supplements. The most important Se species of interest are described below.

35.2.1 Selenoamino acids: Selenomethionine (SeMet) and Selenocysteine (Sec)

The SeMet concentration is an important parameter to evaluate the degree of conversion of inorganic Se into organic Se. It is produced by the replacement of sulfur with Se in the biosynthesis of Met. The SeMet concentration is used for the characterization of the efficiency of the Se-rich yeast biotechnology and of that of the conversion of soil inorganic Se by plants. SeMet represents >60 % in yeast food supplements [4] and ca. 80 % of the total Se in staple food [5,6]. SeMet is the primary form of Se in storage proteins produced during the production of Se-rich food of animal origin (meat, milk, eggs, etc.). In humans, the concentration of SeMet in plasma (and the SeMet/Sec ratio) increases with an increase of total Se [7].

Sec, referred to as the 21st proteinogenic amino acid [8], concentration is a measure of selenoprotein content, as demonstrated in the microanalysis of gel bands and spots [9,10]. Note that Sec can also be introduced into proteins in a similar way as SeMet, i.e., by a simple substitution of sulfur by Se, without a gene coding for a selenoprotein biosynthesis pathway being present. Substantial concentrations of Sec can be found in some yeasts [9], plants [5,6] and bacteria [11]. Therefore a caution should be made when the speciation between SeMet and Sec is used as a measure of the "true" intracellular selenoprotein content.

35.2.2 Selenoproteins and Se-Containing Proteins

Selenoproteins (proteins containing genetically inserted Sec) are believed to be of paramount importance in health and disease [12] and to be responsible for the beneficial effects of Se [13,14]. The most important ones are known for their catalytic activity, e.g., glutathione peroxidase (GPX) and thioredoxin reductase (TXNRD), or are used by mammals as storage protein, e.g., selenoprotein P (SEPP1). The maximum GPX3 activity in plasma was used as the basis for the current Recommended Dietary Allowance (RDA) of 55 μg Se/day of Se [15]. Fundamental work by Gladyshev's group [16] allowed the prediction of selenoproteomes in a number of mammals, including man, and in other organisms. Studies using both cell culture and animal models have shown that expression of selenoproteins is differentially regulated by Se availability. Whereas the expression of a subclass of selenoproteins called stress-related selenoproteins, e.g., GPX1, MSRB1, SEPW, and SELH, is significantly decreased under Se-deficient conditions the expression of the other subgroup, housekeeping selenoproteins, e.g., TXNRD1 and TXNRD2, is less regulated by dietary Se [17]; thus, demonstrating the dependence of stress-related selenoprotein expression on dietary Se.

In contrast to true genetically encoded selenoproteins, the mechanism of incorporation of Se into Se-containing proteins is chemically mediated and consists in the replacement of sulfur in Met and/or in Cys by Se. Examples of such proteins include, selenoalbumin in mammals [18], and the storage proteins, glutenins and gliadins, in plants, [5].

35.2.3 Selenometabolites

This generic category of molecules includes low molecular weight (<1000 Da) Se compounds (Fig. 35.1). The largest characterized pool of metabolites is known to accompany the production of SeMet in yeast and can be divided in several sub-categories including selenols ($R_1 – CH_2 – Se-H$), diselenides and Se-sulfides, di- and tripeptides containing SeMet and Sec, selenoethers (R_1-CH_2-Se-CH_2-R_2) and selenoxides (R_1-CH_2-Se(O)-CH_2-R_2) [4]. The beneficial health effects of a number of natural (grown on seleniferous soils) or cultivated (Se-rich garlic) Se-rich plants are assigned to a number of non-proteinaceous low molecular weight selenium species [19]. Selenometabolites (e.g., selenoneine) account for a minor fraction of Se in blood and serum [20,21]. Low molecular weight species including tetramethylselenonium (TMSe) and selenosugars (Se-methylseleno-N-acetyl-galactosamine, Se-methylseleno-N-acetyl-glucosamine and Se-methylseleno-galactosamine) can be found in urine which is a major excretory route for Se [22,23].

35.2.4 Nanoparticles and Selenodrugs

An exhaustive list of Se compounds studied for their cytotoxic effects is given in a recent review [3]. Se-containing nanoparticles have recently attracted attention as potential cancer therapeutic payloads, due to their biological activity and low toxicity [3]. The metabolic pathways between different Se compounds differ significantly and can produce various Se metabolites [3].

35.3 Speciation of Selenoaminoacids: Determination of SeMet and Sec

35.3.1 SeMet

Chemical protocols, typically used for amino acid determination often fail as the conditions are too harsh and do not always preserve the integrity of SeMet leading to biased results. The most popular have become protocols based on the enzymatic digestion including multiple incubation of a sample with different types of proteases [4]. The procedures do preserve the integrity of SeMet but may not be sufficiently aggressive to release all the SeMet present. SeMet is subsequently determined by HPLC-ICP-MS or, after derivatization, by GC-MS. A standard reference yeast material with a certified concentration of SeMet is available (SELM-1, NRCC, Canada), but caution is necessary to extrapolate the validity of a method developed for SELM-1 to other biological materials, including other types of yeast. The mass balance (the sum of the concentrations of the species in comparison with the total Se content) of all the Se forms present is the *sine qua non* condition for the method validation.

35.3.2 Sec

Accurate determination of Sec is still subject to controversy because of its by far lower stability resulting in its oxidative conversion to dehydroalanine or reactivity with derivatizing reagents. Derivatization is, however, necessary in order to stabilize Sec. It should be carried out on the purified protein fraction, otherwise a number of artefacts resulting from side-reactions with selenometabolites interfere with the analysis. The simultaneous derivatization of SeMet may occur leading to the splitting of its content between the native and the derivatized form; and degradation can be minimized by optimizing the procedure for a given matrix and in a given analyte concentration range. The risk of the potential loss of Sec, especially at the low and

sub-ppm levels (e.g., in meat, milk, eggs) has to be controlled by the Se mass balance [24-28]. On the basis of the identification of 10 proteins richest in Se, it was demonstrated that Se was incorporated by the *Lactobacillus reuteri* strain exclusively as Sec; its exact location within the primary sequences was determined [11].

35.4 Speciation of Protein-Bound Se and Selenoproteomics

The three types of analytical approaches to identifying Se-containing proteins include the targeted analysis of known selenoproteins, Se-filtered exploratory analysis of selenoproteins, and generic (e.g, SWATH) proteomics.

35.4.1 Targeted Analysis of Known Selenoproteins

The classical approach for analysis of known proteins is Western blot analysis, which, although sensitive does not guarantee the presence of Se in the detected species. For example, two potential isoforms of SEPP1 have been identified by Western blot analyses (51 kDa and 61 kDa); the biological relevance of the smaller isoform has been called into question by several studies confirming the presence of at least one Sec in SEPP1 structure [29-31] and thus suggesting that the shorter 51 kDa one might be an artifact of protease activity during the purification process. This ambiguity demonstrates limitations of Western blot analyses providing no information regarding the Se content of the potential isoforms [32]. Also, the feasibility of the analysis for a particular selenoprotein depends on the availability of an antibody.

Indicative levels of the most abundant selenoproteins (GPX, SEPP1) and selenoalbumin) in a commercially available human serum (BCR-637 CRM) with a certified level of total Se were calculated using the results obtained by 13 different analytical methods on the basis of (affinity) high-performance liquid chromatography coupled to ICP-MS [33]. The methodology was further developed to allow the

simultaneous speciation of GPX, SEPP, selenoalbumin and selenometabolites (eluting as a single peak) [21].

35.4.2 Se-targeting Proteomic Techniques

35.4.2.1 Bottom-Up Approach

A preliminary comprehensive screen for the presence of Se-containing proteins can best be carried out by gel electrophoresis (GE), either one (isoelectric focusing, IEF, or SDS PAGE) or two dimensional (2D). Classical procedures, especially for model studies, used radioactive ^{75}Se-labeling [34,35]. They can be replaced by the Se-specific ICP MS detection [36] via a coupling with laser ablation, thus avoiding problems with handling of radioactive isotopes. Quantitative analysis is possible upon calibration of the in-gel detection by commercial or in-house prepared protein standards [37,38]. The use of ICP MS has also the advantage of the isotopic specificity allowing the use of stable Se isotopes for tracer studies and isotope dilution quantification.

As LA-ICP MS is destructive and the ablated parts of the gel cannot be further analyzed, the proteomics analysis should be carried out using spots in a gel prepared in parallel or for a non-ablated part of bands in 1D gels [5,6,11,39]. The workflow is shown in Fig. 35.2.

Canonical HPLC – MS/MS proteomics protocols can be used for the protein identification [40], but it should be noted that they may easily miss Sec because of H_2Se-elimination leading to dehydroalanine, especially for proteins with a terminal Sec. Also, the protein identification is often based on the part of the protein sequence which does not contain Se; hence, the interest in the parallel ICP MS and ESI MS detection (Fig. 35.2).

35.4.2.2 Shotgun Approach

The parallel HPLC-ICP MS and HPLC ESI MS can be applied to a tryptic digest of the whole proteome which has the advantage of combining the elemental and molecular information on Se-containing peptides [6,41]. Because of the complexity of the samples, initial fractionation of the samples based on their solubility [6] or molecular weight [41] was used for real samples.

35.4.2.3 Top-Down Approaches and Structural Studies

The state-of-the-art protocols still do not address some challenges such as the identification of truncated isoforms, post-transitional modifications, possible formation of dimers [42], and the formation of bonds with selenite. The elucidation of reaction mechanisms [42] or structural studies of isoforms of SEPP1, the only human selenoprotein containing multiple Sec residues [32] remains a challenge.

35.4.3 High Throughput Non-targeting Approaches

A future trend in analytical proteomics is non-focused data acquisition of the whole proteomes after their digestion with trypsin and identification of all the produced peptides. The data obtained in this way can be reanalyzed later for the presence of useful information. Se-containing species that were found included, e.g., (i) TXNRD1 and SEP15 identified among 450 proteins in bovine colostrum after high-speed centrifugation and 2D-HPLC fractionation [43], and (ii) SEPP1 among 311 proteins identified in the study of biomarker candidates of alcohol abuse [44]. Because of the instability of Sec in the protocols not optimized for this purpose and the consequent lack of the link between Se and the protein other than prediction, no difference between Sec, Cys, and serine proteins in the active site (crucial from the point of view of insight into the role of Se) could be detected. Mutant enzymes with Cys instead of Sec had reduced hydroperoxidase activity, thus Se is required for the catalytic

activities of TXNRD explaining the essential role of this trace element for the cell growth. [45].

High throughput approaches, when optimized, should allow estimating Met/SeMet and Cys/Sec amounts and thus the identification of the privileged or random replacement of sulfur.

35.5 Speciation Analysis of Selenometabolites

The information on Se speciation is essential in animal and plant physiology [19]; it can also be useful for the control of the technological enrichment processes and the determination of the origin of the commercial products [46]. Special interest concerns studies on Se detoxification and/or extraction. Moreover, in food science, low molecular weight of a species is considered as favorable for its bioaccessibility.

A critical step in the analysis for selenometabolites is the sample preparation procedure. For solid samples, it is expected that extraction with water removes the metabolites, but dedicated extractions with less polar solvents may be essential to recover more hydrophobic species. Generally, immediate analysis to avoid possible changes in speciation due to oxidation is recommended. Risk of volatilization is significant for some samples, in particular for garlic and allium plants [47]. Deproteinization and desalting are necessary for body fluids.

The principle of a typical analytical approach consists of the parallel elemental and molecular MS detection in HPLC allowing one to take advantage of both techniques: the quantitative character and matrix-independent response of ICP MS and the structural information provided by ESI MS^n (Fig. 35.3). The optimization of the HPLC separation in the context of subsequent MS detection is important for assuring the compatibility of eluent with the requirements of both detectors (note that plasma and ESI ionization conditions are changing during the gradient elution). The

identification by molecular mass spectrometry is based on the accurate mass and the characteristic isotopic pattern (*cf* inset in Fig. 35.3). The high accuracy and precision together with the multistage fragmentation capability of state-of-the-art molecular spectrometers are necessary for de novo species identification [48]. In contrast to selenoproteomics studies, where predictions can be made on the basis of the genome, structures of selenometabolites are difficult to predict and their full elucidation is a complex task often beyond the capacity of mass spectrometry.

The major compounds found in the plant samples enriched with Se(IV) were MeSec, SeMet and γ-Glu-MeSec; accumulation of these organic forms, particularly MeSeCys and γ-Glu-MeSec, may also be increased by genetic modification [49]. Selenohomolanthionine was identified in the water extract of pungent Japanese radish [50]. The comprehensive list of selenometabolites identified in Se-enriched yeast includes more than 60 compounds [39,51].

An assessment study of the analytical methods used for identification of urine Se metabolites revealed assignment of incorrect structures to several compounds; especially, the long-held view that trimethylselenonium ion is a major human urinary metabolite was judged unjustified [23]; the major forms being selenosugars [22], e.g., Se-methyl-N-acetylselenohexosamine The Se speciation in blood serum is dominated by selenoproteomics; the selenometabolite fraction (as a whole) was quantified [21] in a global approach focused on selenoproteins. However, the low molecular weight species detected together as a single chromatographic peak may have resulted from, e.g., degradation of selenoproteins. The key metabolite seems to be selenoneine [52,53] (Fig. 35.1A) and its methylated derivative [20] which were identified in tuna [52] and human blood [20,53].

35.6 Solid-State Speciation of Se (X-Ray Absorption Spectrometry, XAS)

Although the coupled techniques offer excellent sensitivity and the ability to identify unequivocally individual compounds of trace and ultratrace levels, they require extensive sample preparation of biological tissues. The digestion and acidification of samples can alter the speciation of Se, particularly with regard to its oxidation state. Note that, except for body or plant fluids, a significant fraction of the Se often remains insoluble. It can be accessed by soft protocols that degrade the original species in a controlled way in order to dissolve it, while preserving to a certain level speciation information (e.g., digestion of selenoproteins to Sec or SeMet). However, there remain a number of questions that can only be answered by the direct (solid-state) techniques, such as X-ray absorption spectrometry (XAS), including Extended X-Ray Absorption Fine Structure, (EXAFS) and X-ray Absorption Near Edge Structure (XANES).

X-ray absorption spectrometry also offers high spatial resolution that can be used to acquire information on the distribution of Se within organs which is lost during the homogenization of the samples [54]. In comparison with hyphenated techniques, XAS lacks sensitivity at low concentrations and is unable to detect trace components. Also, it is limited to the determination of the coordination and oxidation environment of Se, rather than giving the exact identity of the Se compounds. The speciation of Se in Se-enriched yeast was investigated using both XAS and HPLC-ICP-MS; the results were in agreement, although the restricted model compound library and absence of an EXAFS spectrum limited the information that could otherwise be derived from XAS [55]. XANES was applied to the study of Se speciation in tissues from rainbow trout exposed to SeMet [56]. SeMet- and MeSec-treated cancer cells showed XAS spectra distinct from selenite-treated cells [57]. Although sample preparation is minimal compared to the preparation required for HPLC-ICP-MS, it may still interfere with

the sample by selectively mobilizing, redistributing or washing out sample components [57].

35.7 Conclusions

Considerable progress has been made during the last decade in the analytical methodology allowing qualitative and quantitative Se speciation in biological samples. Valid quantitative methods for the determination of SeMet and Sec exist in a variety of samples. The coupling of HPLC with the parallel ICP MS and electrospray MS^n detection is a generic method allowing the global speciation of Se on the metabolome levels.

The use of canonical high throughput protocols for proteomics such as shotgun or data independent analysis protocols gives the illusion of producing meaningful data for selenoproteins as well. However, the prerequisite of the validity of the selenoproteomics method is the detection of the selenoproteins via the Se–containing peptide(s) and a rigorous control of the Se mass balance during the sample preparation aiming at the preservation of the Sec-containing entity. Solid-state speciation techniques, such as XAS, offer the spatial resolution at the micrometer level, but their applicability is limited to fairly high Se concentrations and simple systems such as Se(IV), Se(VI), SeMet, and Sec.

References

1. M Bodnar et al 2016 *Critical Reviews in Food Science and Nutrition* 56:36
2. MP Rayman et al 2008 *British Journal of Nutrition* 100:238
3. AP Fernandes, V Gandin 2015 *Biochimica et Biophysica Acta* 1850:1642
4. K Bierla et al 2012 *TrAC - Trends in Analytical Chemistry* 41:122
5. J Bianga et al 2013 *Analytical Chemistry* 85:2037
6. P Cheajesadagul et al 2014 *Metallomics* 6:646

7. MJ Christensen 2014 *Cancer Prev Res (Phila)*, 7:781

8. A Böck et al 1991 *Molecular Microbiology* 5:515

9. D Behne et al 1998 *Analyst* 123:871

10. D Behne et al 1994 *Biological Trace Element Research* 43:287

11. E Galano et al 2013 *Molecular and Cellular Proteomics* 12:2196

12. MP Rayman *The Lancet* 379:1256

13. SJ Fairweather-Tait et al 2011 *Antioxidants and Redox Signaling* 14:1337

14. PR Hoffmann, MJ Berry 2008 *Molecular Nutrition and Food Research* 52:1273

15. Io Medicine. 2000 "Selenium. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids," (Washington, D.C.)

16. GV Kryukov et al 2003 *Science* 300:1439

17. VM Labunskyy et al 2014 *Physiological Reviews* 94:739

18. P Jitaru et al 2010 *Analytica Chimica Acta* 657:100

19. Y Ogra, Y Anan 2009 *Journal of Analytical Atomic Spectrometry* 24:1477

20. M Klein et al 2011 *Metallomics* 3:513

21. MA García-Sevillano et al 2014 *Analytical and Bioanalytical Chemistry* 406:2719

22. Y Ogra et al 2002 *Journal of Chromatography B* 767:301

23. KA Francesconi, F Pannier 2004 *Clinical Chemistry*, 50:2240

24. K Bierla et al 2008 *Analytical and Bioanalytical Chemistry* 390:1789

25. K Bierla et al 2008 *Analytica Chimica Acta* 624:195

26. E Lipiec et al 2010 *Analytical and Bioanalytical Chemistry* 397:731

27. DT Juniper et al 2008 in *Trace Elements in Animal Production Systems,* p 304

28. DT Juniper et al 2009 *Livestock Science* 122:63

29. RF Burk, KE Hill 2009 *Biochimica et Biophysica Acta - General Subjects* 1790:1441

30. S Himeno et al 1996 *Journal of Biological Chemistry* 271:15769

31. S Ma et al 2002 *Journal of Biological Chemistry* 277:12749

32. G Ballihaut et al 2012 *Metallomics* 4:533

33. P Jitaru et al 2010 *Accreditation and Quality Assurance* 15:343

34. SV Novoselov et al 2002 *EMBO Journal* 21:3681

35. S-J Han et al 2014 *PLoS ONE* 9:e95518

36. J Bianga et al 2012 *Journal of Analytical Atomic Spectrometry* 27:25

37. G Ballihaut et al 2007 *TrAC - Trends in Analytical Chemistry* 26:183

38. G Ballihaut et al 2005 *Journal of Analytical Atomic Spectrometry* 20:493

39. K Bierla et al 2013 *Journal of Proteomics* 87:26

40. Y Zhang et al 2013 *Chemical Reviews* 113:2343

41. M Dernovics et al 2007 *Journal of Analytical Atomic Spectrometry* 22:41

42. J Liu et al 2014 *FEBS Letters* 588:3311

43. A Nissen et al 2012 *PROTEOMICS* 12:2866

44. X Lai et al 2009 *ELECTROPHORESIS* 30:2207

45. LW Zhong, A Holmgren 2000 *Journal of Biological Chemistry* 275:18121

46. S Gil Casal et al 2010 *Metallomics* 2:535

47. S Mounicou et al 2009 *Talanta* 77:1877

48. M Dernovics, R Lobinski 2008 *Analytical Chemistry* 80:3975

49. K Pyrzynska 2009 *Food Chemistry* 114:1183

50. Y Ogra et al 2007 *Journal of Analytical Atomic Spectrometry* 22: 1390

51. C Arnaudguilhem et al 2012 *Analytica Chimica Acta* 757:26

52. M Yamashita et al 2013 *Biological Trace Element Research* 156:36

53. Y Yamashita, M Yamashita 2010 *Journal of Biological Chemistry* 285:18134

54. IJ Pickering et al 2000 *Proc. Natl. Acad. Sci. USA* 97:10717

55. L Yu-Feng et al 2010 *Journal of Analytical Atomic Spectrometry* 25:426

56. S Misra et al 2012 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 155:560

57. CM Weekley et al 2013 *Nutrients* 5:1734

58. J Bianga et al 2014 *Journal of Proteomics* 108:316

Figure legends

Fig. 35.1. Summary of the most popular selenometabolites found in (a) body fluids (b) plants and yeast. Compound structures and names are shown in the figure.

Fig. 35.2. Analytical workflow for bottom up selenoproteomics using (a,b) 1D IF protein separation and LA-ICP MS detection of Se-containing bands followed by (c) in-gel tryptic digestion and HPLC separation of tryptic peptides by HPLC with parallel (d) ICP and (e) ESI MS detection; the example shown is the identification of GPx with the following peptides: multi-ion XIC chromatogram for all the ions corresponding to the target protein (1 - DYTQM(OX)NELQR, 2 – NEEILNSLK, 3 – NDVAWNFEK, 4 – LITWSPVCR, 5- FLVGPDGVPLR, 6 – GLVVLGFPCNQ FGHQENAK, 7 – VLLIENVASLUGTTVR, 8 – YVRPGGGFEPNFMLFEK) [58]

Fig. 35.3. Analytical workflow for selenometabolomics. The cation-exchange HPLC chromatograms shown in the insets were obtained for ammonium acetate extracts of maize (the species eluted: methylseleno-Se-pentose-hexose, deamino Sec-Se-hexose, SeMet).

Fig. 35.4. Library of Se K-edge X-ray absorption spectra of model Se compounds used in the linear combination fitting of experimental spectra. XANES spectra of cancer cells treated with 100 µM SeMet (blue), 100 µM MeSec (red) or 5 µM selenite (orange) are also shown for comparison [57].

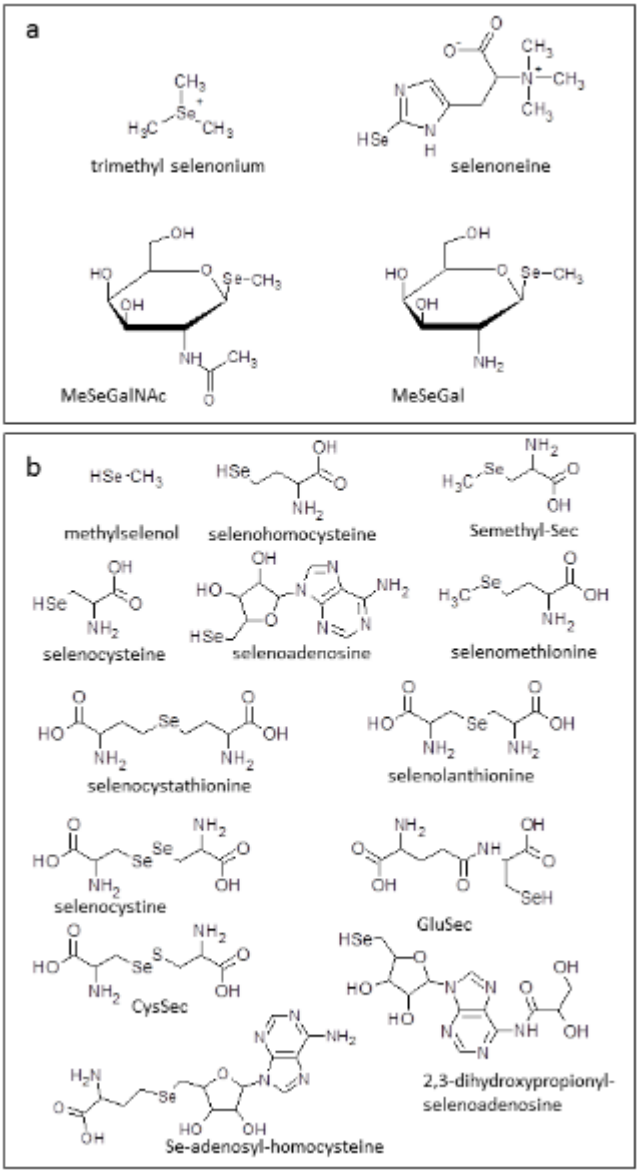
a
trimethyl selenonium
selenoneine
MeSeGalNAc
MeSeGal
b
methylselenol
selenohomocysteine
Semethyl-Sec
selenocysteine
selenoadenosine
selenomethionine
selenocystathionine
selenolanthionine
selenocystine
GluSec
CysSec
2,3-dihydroxypropionyl-
selenoadenosine
Se-adenosyl-homocysteine

Fig. 35.1

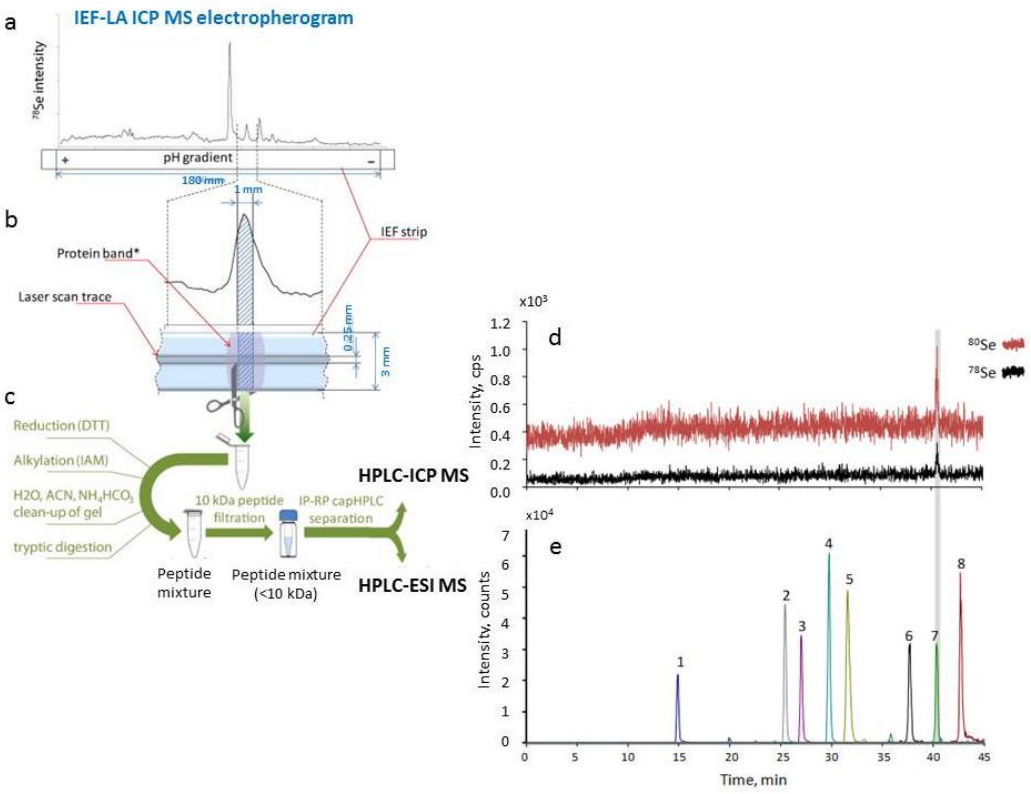
a
IEF-LA ICP MS electropherogram
78Se intensity
pH gradient
+
-
180 mm
1 mm
b
IEF strip
Protein band*
Laser scan trace
0.25 mm
3 mm
c
Reduction (DTT)
Alkylation (IAM)
H2O, ACN, NH4HCO3
clean-up of gel
tryptic digestion
10 kDa peptide filtration
IP-RP capHPLC separation
HPLC-ICP MS
HPLC-ESI MS
Peptide mixture
Peptide mixture (<10 kDa)
d
x10³
Intensity, cps
80Se
78Se
e
x10⁴
Intensity, counts
Time, min

Fig. 35.2

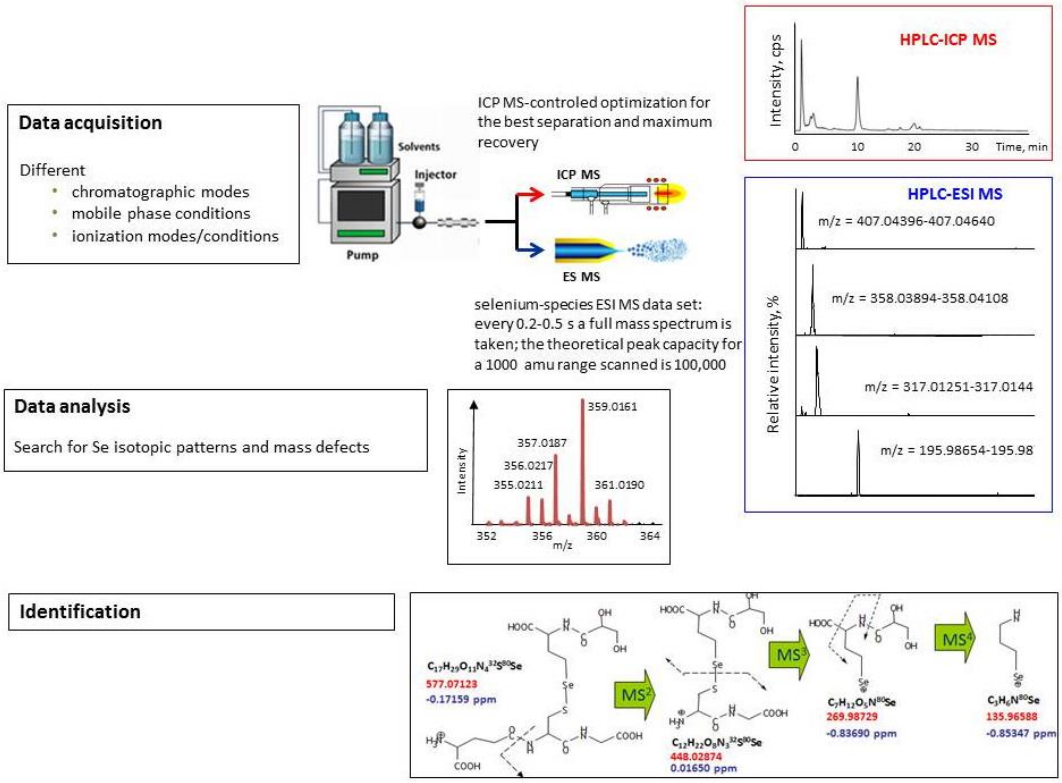
Data acquisition
Different
chromatographic modes
mobile phase conditions
ionization modes/conditions
Solvents
Injector
Pump
ICP MS-controled optimization for the best separation and maximum recovery
ICP MS
ES MS
HPLC-ICP MS
Intensity, cps
0
10
20
30
Time, min
HPLC-ESI MS
m/z = 407.04396-407.04640
m/z = 358.03894-358.04108
m/z = 317.01251-317.0144
m/z = 195.98654-195.98
Relative intensity, %
selenium-species ESI MS data set: every 0.2-0.5 s a full mass spectrum is taken; the theoretical peak capacity for a 1000 amu range scanned is 100,000
Data analysis
Search for Se isotopic patterns and mass defects
359.0161
357.0187
356.0217
355.0211
361.0190
Intensity
352
356
360
364
m/z
Identification
MS2
MS3
MS4
577.07123
-0.17159 ppm
448.02874
0.01650 ppm
269.98729
-0.83690 ppm
135.96588
-0.85347 ppm

Fig. 35.3

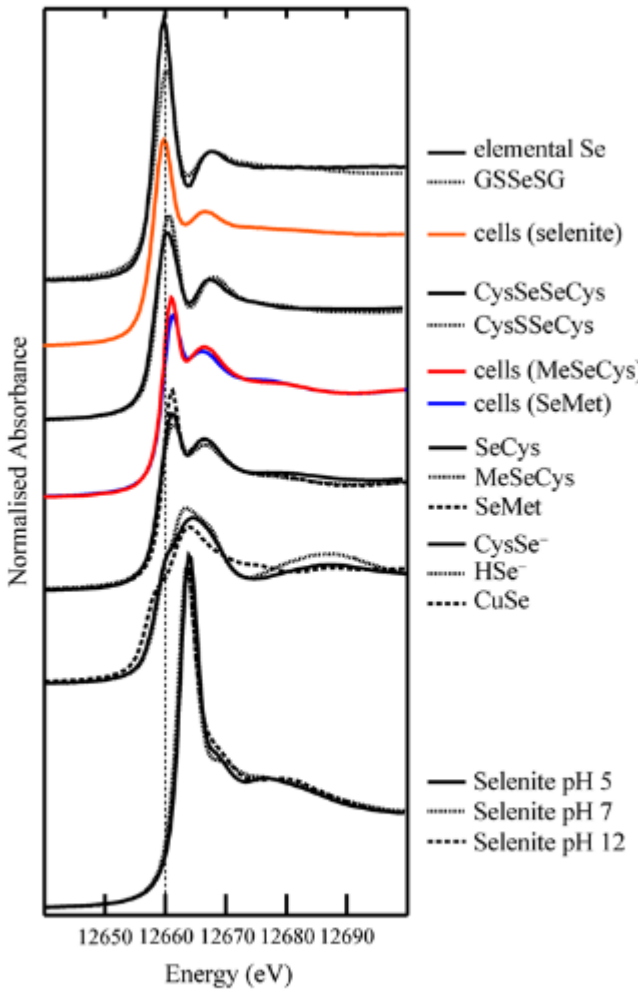
Normalised Absorbance
12650 12660 12670 12680 12690
Energy (eV)
elemental Se
GSSeSG
cells (selenite)
CysSeSeCys
CysSSeCys
cells (MeSeCys)
cells (SeMet)
SeCys
MeSeCys
SeMet
CysSe⁻
HSe⁻
CuSe
Selenite pH 5
Selenite pH 7
Selenite pH 12

Fig. 35.4