Application of a new capillary HPLC-ICP-MS interface to the identification of selenium-containing proteins in selenized yeast

Application note

Food supplements

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Introduction

Diet is the major source of selenium (Se) to man, and intake of this essential element depends on the amount of food consumed and the Se concentration in food products. Since the levels of Se in agricultural products are low in many countries, Se deficiency is a significant problem. A number of supplementation strategies have been developed to increase human Se intake; Se-enriched yeast is one of the most popular sources of Se used for human and animal supplementation.

The estimation of Se adequacy requires information not only on the total Se, but also on the presence of Se species. However, most of the research carried out so far has focused on water-soluble yeast fraction containing only ca. 12 to 20% of the total Se. The insoluble Se fraction

of yeast remains largely unexplored; the very few attempts to characterize this major fraction included the work of Chassaigne and Chéry [1] who presented Se-specific laser ablation (LA-) ICP-MS mapping of yeast proteins and of Tastet et al [2] using a laboratory-made nanoHPLC-ICP-MS interface for the Se-selective detection of selenium peptides. In routine approaches, the total selenomethionine determination is used as a means of yeast quality assessment in terms of Se-containing protein content.

This work proposes an ICP-MS-assisted proteomic approach to the identification of Se-containing proteins present in selenized yeast. The insoluble Se-containing proteins were separated by 2D gel electrophoresis. The Se-containing protein spots, identified by LA-ICP-MS, were excised and digested with trypsin; the resulting peptides were analyzed by capillary HPLC-ICP-MS and then characterized by electrospray ionization (ESI-) MS/MS leading to the identification of glyceraldehyde-3-phosphate dehydrogenase-3, the major Se-containing protein in selenized yeast. The use of capillary chromatography was essential due to the low amount of the tryptic digestate available.

Experimental

Sample

A commercial selenized yeast sample with a total Se content of 2.3 mg/g was studied.

Sample preparation

Standard proteomics procedures described in detail elsewhere [3] were used for protein extraction, their 2D gel electrophoretic separation and tryptic digestion of the spots of interest.

HPLC-ICP-MS and ESI-MS/MS analysis

An Agilent 1100 LC fitted with a capillary pump (part number G1376A) and manual valve (100 nL loop) was used. An aliquot of 8 µL of the tryptic digest was loaded onto an Agilent Zorbax 300 SB-C18 35 x 0.5 mm 5 µm peptide cartridge using isocratic flow of 20 µL/min of 2% acetonitrile (ACN) - 0.1% formic acid (FA). The sample was washed with the mobile phase for 2 min, and then backflashed onto an Agilent Zorbax 300SB-C18 150 x 0.3 mm x 3.5 µm separation column. Peptides were separated using a gradient elution of A: water 0.1% FA; B: ACN 0.1% FA with a flow of 4 µL/min. The elution program is given in Table 1.

Table 1. HPLC elution program

Step	Eluant (%B)	Time (min)
1	2	0–2
2	2–5	2–5
3	5–25	5–35
4	25–40	35–40
5	40–97	40–45
6	40–97	45–50
7	97–2	50–55

The outlet of the separating column was connected to the ICP-MS or ESI-MS/MS instrument. ICP-MS operating conditions are given in Table 2.

Table 2. Agilent 7700x ICP-MS operating conditions

Parameter	Value
Spectrometer	
Nebulizer/spray chamber	Capillary LC Interface Kit (part number G3680A)
Torch ID	1 mm
Cones	Platinum
Plasma	
RF power	1560 W
Sampling depth	7.5 mm
Carrier gas flow rate	780 mL/min
Optional gas (O_2) flow rate	39 mL/min
Lens	
Extract 1	3.2 V
Extract 2	–200 V
Cell	
Octopole bias	–100 V
He flow	10 mL/min
Kinetic energy discrimination	7 V

Plasma conditions and detection parameters were optimized with 20 ppb solution of Y, Li, Tl, Ce in 2% of nitric acid. High energy He collision cell mode was used to exclude polyatomic interferences that can occur for isotopes of Se. Signals of ^{77}Se, ^{78}Se, ^{80}Se were acquired with the dwell time of 250 ms each.

The ESI LTQ Orbitrap Velos mass spectrometer was operated in positive ionization mode; the heater and capillary temperatures were 50 and 280 °C, respectively. The measurements were carried out in the 300–1100 *m/z* mass range with CID activation in the MS SIM mode (normalized collision energy of 15%); the product ions were generated in the HCD cell at a collision energy setting of 55%.

Results and discussion

The gel showing the separation of proteins is displayed in Figure 1. The spot chosen to demonstrate the feasibility of the proposed ICP-MS-assisted proteomic approach is marked with a circle. According to the LA-ICP-MS measurement, this protein spot contained the highest Se level among all the spots present in the gel.

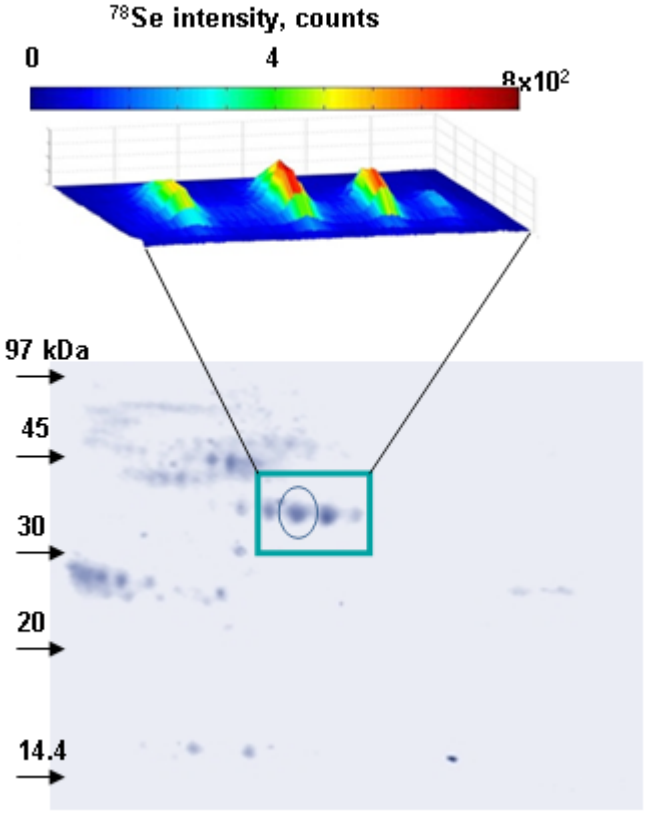

Figure 1. 2D Gel electrophoretic separation of selenized yeast proteins (LA-ICP-MS intensity image shown above the gel)

The chromatograms of the tryptic digest of the protein spot of interest are shown in Figure 2. The capillary HPLC-ICP-MS allowed detection of six peaks (Figure 2a), five of them could be identified by ESI Orbitrap MS/MS. The ICP-MS detection was essential in identifying retention times of selenopeptides, which facilitated the search for the (minor) Se species in the mass spectra as indicated in Figure 2d. The limit of detection (LOD) was calculated as a 3x the standard deviation of 20 points of the base line. This value was compared with the signal of 1 ppm SeMet. The LOD obtained for Se isotope 80 (for 30% ACN, 0.1% FA) was 0.2 pg. A list of amino acid abbreviations is given in Table 3.

The sequences of identified selenopeptides are given in Table 4. A perfect matching of retention times of signals obtained with ICP-MS detection and in a selected ion mode (Figure 2c) of ESI Orbitrap MS at *m/z* values corresponding to the identified selenopeptides was demonstrated. The data obtained allowed the identification of selenized glyceraldehyde-3-phosphate dehydrogenase-3 (Table 5). The incorporation of Se by the sulfur-selenium substitution pathway in the protein chain was demonstrated, both in methionine (M) and cysteine (C) residues, the latter for the first time in a yeast sample.

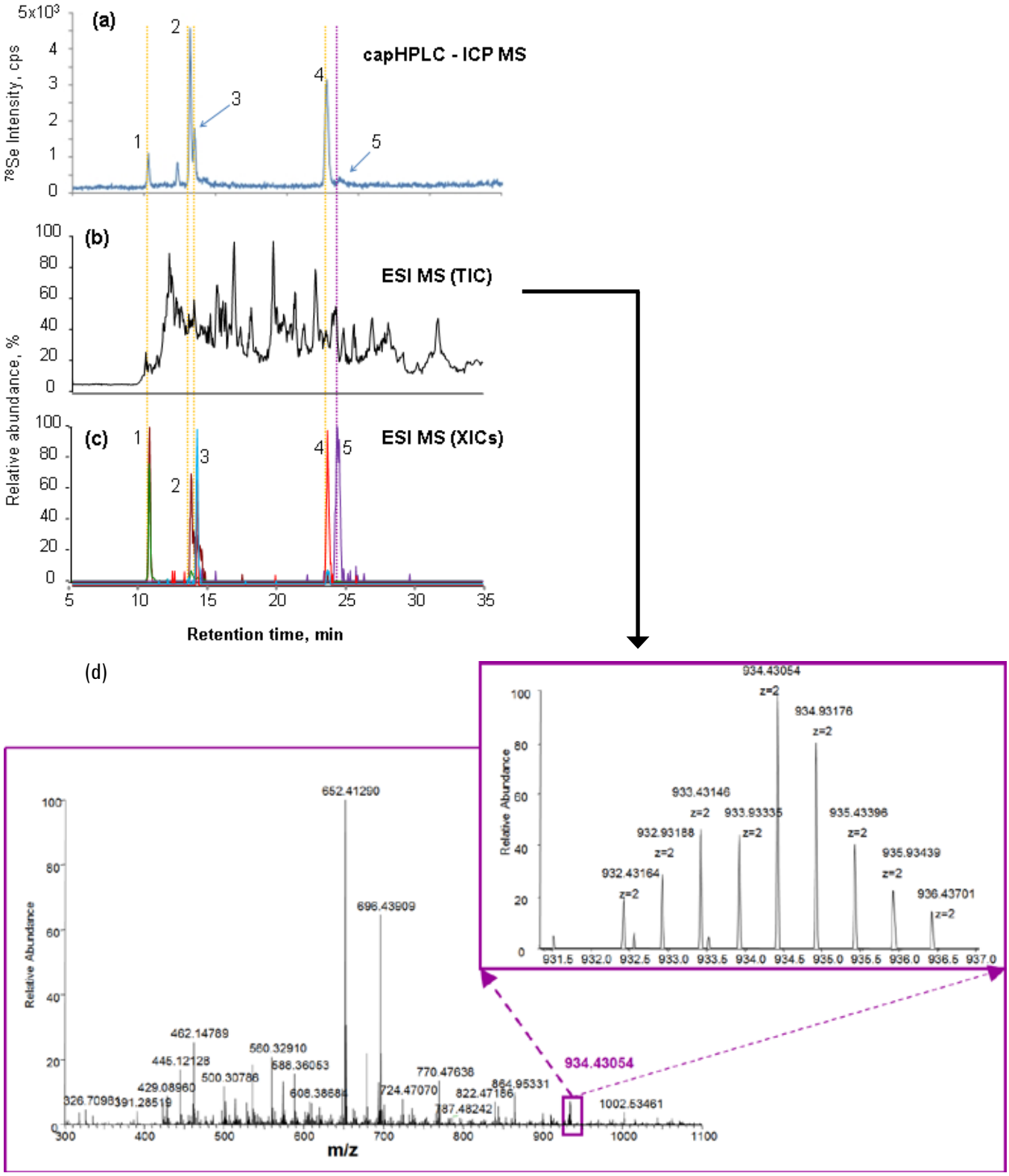

Figure 2. Capillary HPLC of selenium-containing peptides of tryptic digestate of the protein spot marked in Figure 1. a) ICP-MS detection. b) ESI Orbitrap MS detection (TIC). c) ESI Orbitrap MS detection (selected ion chromatograms). d) ESI mass spectra of (minor) Se species. Peak identities are given in Table 4.

Table 3. Amino acid abbreviations

Name	Abbreviation
Alanine	A
Arginine	R
Asparagine	N
Aspartic acid	D
Cysteine	C
Glutamic acid	E
Glutamine	Q
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

Table 4. Sequences of selenium-containing peptides identified in tryptic digestate of the protein spot marked in Figure 1

Peak	Sequence	Theoretical mass	Experimental mass	Δ mass
1	LV**SeM(ox)**R	582.2523	582.2513	-0.00104
2	LV**SeM**R	566.2573	566.2564	-0.00094
3	LTG**SeM(ox)**AFR	430.1793	430.1837	0.004395
4	LTG**SeM**AFR	422.1822	422.1859	0.00369
5	IVSNASCTTN**SeC**LAPLAK or IVSNAS**SeC**TTNCLAPLAK	934.4278	934.4279	0.00013

Table 5. Sequence of glyceraldehyde-3-phosphate dehydrogenase-3, a selenium-containing protein identified in the protein spot marked in Figure 1. Peptides marked in bold correspond to selenopeptides detected in the chromatogram (Figure 2) and listed in Table 4.

	Peptides
1	MVRVAINGFG RIGR**LVMR**IA LSRPNVEVVA LNDPFITNDY AAYMFKYDST
51	HGRYAGEVSH DDKHIIVDGK KIATYQERDP ANLPWGSSNV DIAIDSTGVF
101	KELDTAQKHI DAGAKKVVIT APSSTAPMFV MGVNEEKYTS DLK**IVSNASC**
151	**TTNCLAPLAK** VINDAFGIEE GLMTTVHSLT ATQKTVDGPS HKDWRGGRTA
201	SGNIIPSSTG AAKAVGKVLP ELQGK**LTGMA FR**VPTVDVSV VDLTVKLNKE
251	TTYDEIKKVV KAAAEGKLKG VLGYTEDAVV SSDFLGDSHS SIFDASAGIQ
301	LSPKFVKLVS WYDNEYGYST RVVDLVEHVA KA

Conclusions

An effective ICP-MS-assisted proteomic method has been developed for the identification of Se-containing proteins present in the insoluble Se fraction of a commercial selenized yeast sample. The Se-containing proteins were separated using 2 dimensional gel electrophoresis and digested using typsin. The digests were identified, analyzed and characterized using a combination of LA-ICP-MS, capillary HPLC-ICP-MS and ESI MS/MS. The use of capillary chromatography was essential due to the low amount of the tryptic digestate available. The method was used to identify glyceraldehyde-3-phosphate dehydrogenase-3, the major Se-containing protein in selenized yeast. The incorporation of Se by the sulfur-selenium substitution pathway in the protein chain was demonstrated, both in methionine (M) and, for the first time in a yeast sample, in cysteine (C) residues.

References

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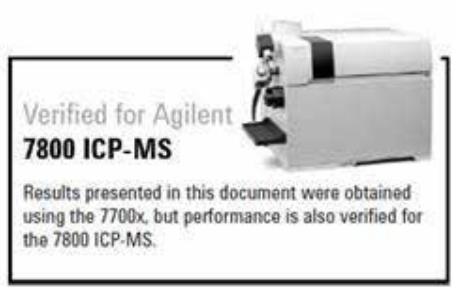

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