Microchim Acta 155, 373–377 (2006)
DOI 10.1007/s00604-006-0520-2

Microchimica Acta
Printed in Austria

Original Paper

Selenomethionine Extraction from Selenized Yeast: an LC-MS Study of the Acid Hydrolysis of a Synthetic Selenopeptide

Shona McSheehy, Lu Yang, and **Zoltán Mester***

Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario K1A 0R9, Canada

Received October 27, 2005; accepted January 9, 2006; published online May 11, 2006

Abstract. A synthetically prepared seleno-peptide (AHPDVLTV**X**LQMLDDGR) was used as a model system for the acid hydrolysis of selenized yeast proteins. The seleno-peptide is a tryptic peptide of a heat shock protein 104 from *Saccharomyces cerevisiae*, was subjected to acid hydrolysis using methanesulfonic acid over a time period of 8 hours. Aliquots of the solution were sub-sampled at predetermined time intervals and the peptide fragments characterized by reversed phase LC MS^n. Similarly, the appearance of amino acid residues in the solution was monitored. It was found that after about 8 hours the synthetic peptide completely hydrolyzed. The use of a selenopeptide as a model for hydrolysis of selenized yeast hydrolysis was validated by comparing the decomposition time profile of the synthetic peptide with that of a selenized yeast sample. The rate of hydrolysis was identical in both systems, suggesting that the employed acid hydrolysis yields to the complete decomposition of the Se containing proteins in yeast and consequently to the liberation of selenomethionine.

Key words: Selenomethionine; yeast; speciation.

In recent years selenium supplementation has increased dramatically as a result of the numerous health claims including protection of cells against the effects of free radicals, the normal functioning of the immuno system and thyroid gland as well as protection against various forms of cancers. However the necessity of Se supplementation is still active area of research. Yeast is a popular supplement medium wherein selenomethionine (SeMet) is usually the dominant Se species, it possessing higher bioavailability and lower toxicity than inorganic selenium. Significant efforts have been made in the development of analytical methods for the speciation of Se in yeast in recent years.

The incorporation and biotransformation of inorganic selenium into yeast growing on enriched media is well known. Yeast synthesizes selenomethionine (SeMet) as a major compound *via* an intrusion or mimicking of the sulfur metabolic pathway. It is generally accepted that the SeMet is then non-specifically incorporated into the proteome due to the inability of the initiator codon of methionine (Met) to distinguish the difference between the two amino acids [1]. However, SeMet incorporation has only recently been established by the identification of SeMet-containing proteins from yeast with mass spectrometry [2, 3]. There is still no conclusive evidence that the incorporation route is global in the proteome.

In-house efforts to determine the level of incorporation of SeMet in the proteome have led to studies involving the hydrolysis of yeast protein to its

* Author for correspondence. E-mail: zoltan.mester@nrc.ca

constituent amino acids [4–6]. A hydrolysis procedure developed by Wrobel et al. [7] was determined to be the most efficient and cost effective. The amino acids were quantified by species-specific isotope dilution with GC MS [4, 5] or LC MS [8] to establish a ratio between the Met and SeMet level of incorporation. Thus far, our results indicate that for every 2 Met residues, at least one SeMet residue is incorporated into the studied yeast proteome.

This study investigates the hydrolysis procedure by using a synthetically prepared seleno peptide as a model compound. The peptide was selected because it is a tryptic peptide of a known protein and it contains two Met residues. One of the Met residues was replaced with SeMet. The peptide corresponds to residues 691–707 of the heat shock protein 104 (HSP104). Degradation of the seleno-peptide and formation of smaller peptides and amino acids was monitored over time using LC MS and GC MS.

Experimental

Instrumentation

A Thermo Finnigan LCQ deca Ion Trap instrument (Thornhill, ON, Canada) was used for LC ESI MS analysis of peptides. A Thermo Finnigan TSQ quantum AM triple quadrupole mass spectrometer (San Jose, CA, USA) was used for reverse phase LC ESI MS analysis of amino acids. ESI MS conditions (e.g., capillary voltage, lens voltage, multipole offset and entrance voltage) were optimized for the synthetic peptide (Ion Trap) or SeMet (Triple Quad) using standard tuning procedures. Reversed phase separations were undertaken using a Prevail C18 ($150 \times 2.1\,mm \times 5\,\mu m$) column (Alltech, Deerfield, IL). Chromatographic eluent was provided with a Hewlett-Packard HP 1100 pump with autosampler.

A Hewlett Packard HP 6890 GC (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) fitted with a DB-5MS column (Iso-Mass Scientific Inc., Calgary Alberta, Canada) was used for the separation of the Met and SeMet in yeast extract. Detection was achieved with an HP model 5973 mass selective detector (MS) using single ion monitoring.

Reagents and Solutions

Analytical reagent grade chemicals were used throughout. Water was purified to 18.2 MΩ-cm resistivity using a NANOpure mixed bed ion exchange system fed with reverse osmosis domestic feed water (Barnstead/Thermolyne, Dubuque, Iowa, USA). Methanesulfonic acid (98% purity) was purchased from Fluka (Oakville, ON, Canada). OmniSolv® acetonitrile and formic acid (98% purity) were purchased from EM Science (Gibbstown, NJ, USA). Eluents A and B for reversed phase chromatography were prepared by adding the appropriate amount of formic acid to 1 L water and 1 L acetonitrile, respectively. The gradient elution programs employed for reversed phase chromatography are outlined in Table 1.

Table 1. Amino acids and their one and three letter codes, molecular weight and their weight difference observed with a loss of water

Amino acid	1 letter code	3 letter code	MW	MW residue (MW-H_2O)
Alanine	A	Ala	89.09	71.07
Arginine	R	Arg	174.20	156.18
Asparagine	N	Asn	132.12	114.10
Aspartic acid	D	Asp	133.10	115.08
Cysteine	C	Cys	121.16	103.14
Glutamic acid	E	Glu	147.13	129.11
Glutamine	Q	Gln	146.15	128.13
Glycine	G	Gly	75.07	57.05
Histidine	H	His	155.16	137.14
Isoleucine	I	Ile	131.18	113.16
Leucine	L	Leu	131.18	113.16
Lysine	K	Lys	146.19	128.17
Methionine	M	Met	149.21	131.19
Phenylalanine	F	Phe	165.19	147.17
Proline	P	Pro	115.13	97.11
Serine	S	Ser	105.09	87.07
Threonine	T	Thr	119.12	101.10
Tryptophan	W	Trp	204.23	186.21
Tyrosine	Y	Tyr	181.19	163.17
Valine	V	Val	117.15	99.13
Selenomethionine*	X	SeM	196.11	178.09

* The code for SeMet have been chosen by the authors.

Standard stock solutions ($1000\,\mu g\,mL^{-1}$) of natural abundance L-methionine and seleno-DL-methionine (Sigma Aldrich, Oakville, ON, Canada) were prepared by dissolving the target compound in water. A standard solution ($3\,mg\,mL^{-1}$) of the costume synthetised Se-peptide (AnaSpec, San Jose, CA) used in this study (AHPDVLTV**X**LQMLDDGR) was prepared by dissolving the target compound in water. (Table 1 summarizes the one and three letter codes for canonical amino acids and for selenomethinone) Working solutions were prepared on the day of analysis by appropriate dilution of the stock solutions with water. The stock solutions were kept at 4 °C in the dark.

Acid Hydrolysis

The extraction procedure employed for the yeast was based on a method developed by Wrobel et al. [7]. A 20 mg subsample of the seleno-peptide was weighed into a clean, dry conical flask. The appropriate volumes of water and methanesulfonic acid were added to yield a concentration of 4 M methanesulfonic acid (12 mL). The sample was subjected to reflux on a hot plate for 8 h with glass beads acting as anti-bumping granules. At regular intervals, 50 µL aliquot of the solution was sampled and immediately refrigerated to prevent further hydrolysis of the peptide.

Results

In earlier work, we reported on a methanesulfonic acid based hydrolysis of selenized yeast proteins [5, 6] used to liberate SeMet which had been incorporated into the yeast proteome in the place of methionine [3]. The typical hydrolysis time was varied between 8 and

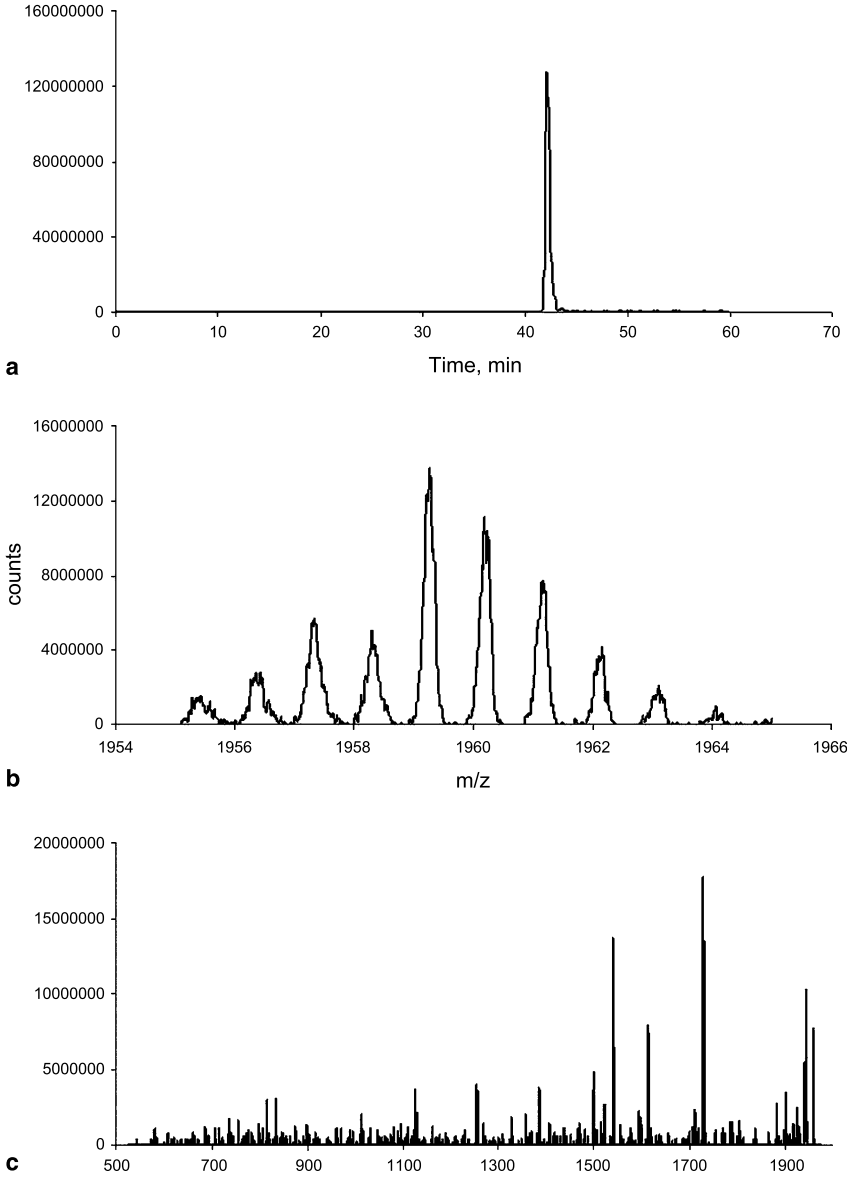

Fig. 1. LC MS chromatogram (a), high resolution mass spectrum (b) and MS/MS spectrum (c) of the selenium containing synthetic peptide AHPDVLTV**X**LQMLDDGR

16 h. Earlier studies confirmed that the isotopically labeled SeMet is neither decomposed nor absorbed into the matrix during this process [5]. However, there are no simple analytical tools available to assess the efficiency of the acid hydrolysis itself. In order to gain insight into the hydrolysis process and to confirm that the chosen 8–16 h hydrolysis time leads to the complete liberation of Met/SeMet from the yeast proteome, a SeMet containing artificial peptide was subjected to acid hydrolysis and the resulting peptide fragments and ultimately free amino acids were monitored as a function of time.

Figure 1 shows the LC ESI MS chromatogram and mass spectra of the synthetic peptide. The use of the ion trap mass analyzer precludes detection of any fragments having m/z less than $1/3$ of the molecular ion. The sequence of the peptide was confirmed by both de-novo sequencing and data base search/matching. Once the nature of the peptide was confirmed it was submitted to acid hydrolysis. Table 2 summarizes the peptides detected. A total of 16 peptide and 4 free amino acids were monitored in order to confirm the full hydrolysis of the peptide. As an example, Fig. 2 shows the chromatogram, high resolution MS spectra of the molecular ion and the high resolution MS spectra of the AHPDVLTVXL peptide fragment resulting from the cleaving of seven C terminal amino acid residues of the original peptide.

Table 2. Peptide fragments detected and monitored throughout the hydrolysis protocol

	A	H	P	D	V	L	T	V	X	L	Q	M	L	D	D	G	R
AHPDVLTVXL QMLDD																	
AHPDVLTVXL QMLD																	
AHPDVLTVXLQ																	
AHPDVLTVXL																	
AHPDVLTVX																	
VLTVXLQ																	
AHPDVL																	
TVXLQ																	
HPDVL																	
AHPDV																	
TVXL																	
PDVL																	
XLQ																	
VXL																	
TVX																	

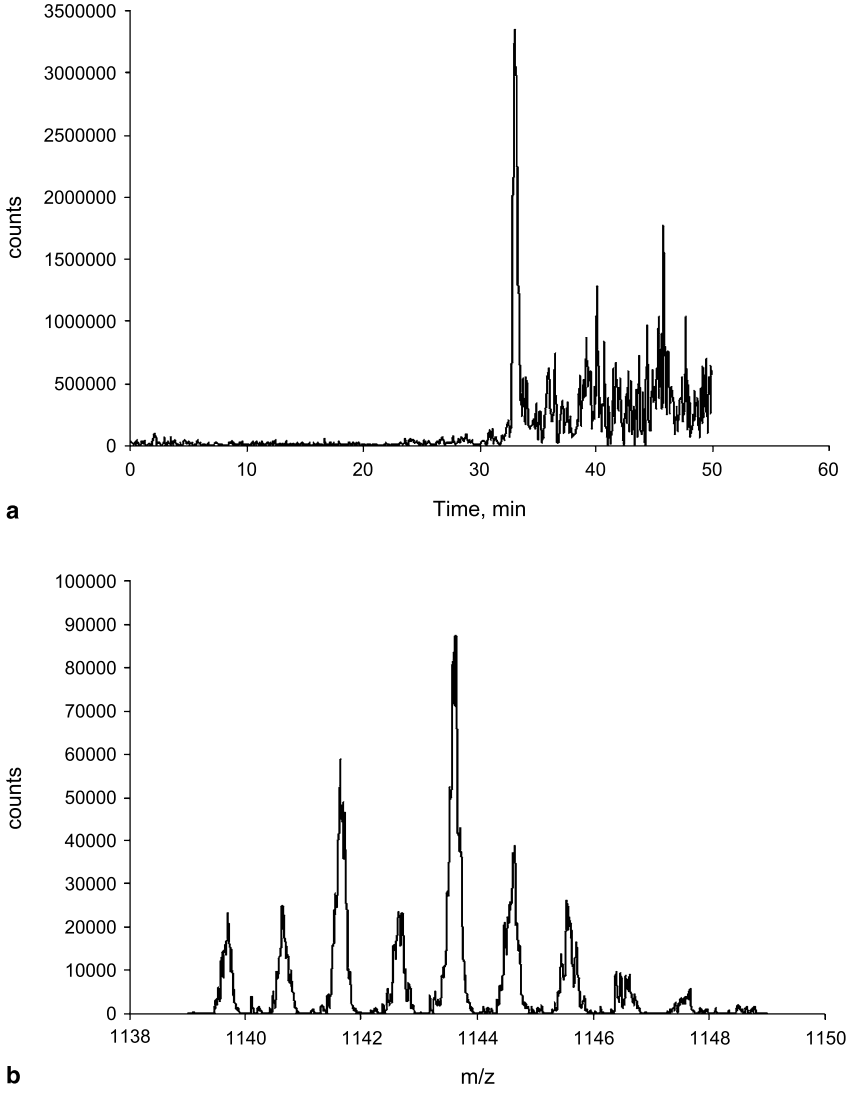

Fig. 2. LC MS chromatogram (a), high resolution mass spectrum (b) of AHPDVLTVXL which is a N-terminal fragment of the original peptide

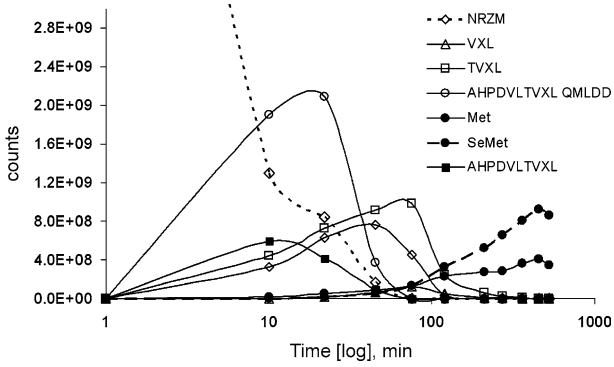

Fig. 3. Peptide and amino acid concentration profiles as a function of hydrolysis time. (NRZM corresponds to the intact peptide)

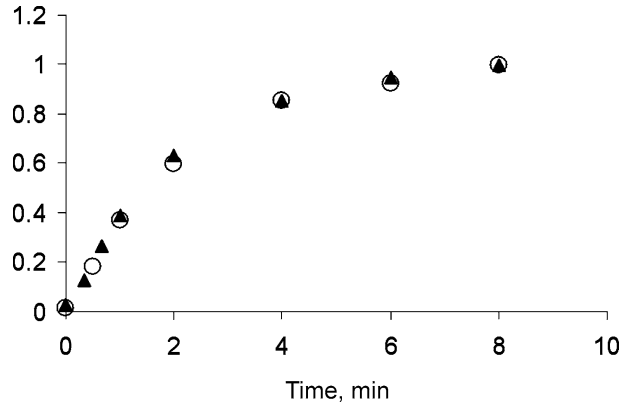

Fig. 4. Evolution of SeMet as a function of hydrolysis time. *SeY* SeMet from yeast, *SeSt* SeMet from the synthetic peptide. ▲ SeSt; ○ SeY

Figure 3 shows the ESI MS response from select peptide fragments and amino acids as a function of time. As expected, the larger peptide fragments are produced early in the hydrolysis process and they gradually give way to smaller fragments and, finally free amino acids. After 8 hours, the majority of the products (by area count) is the free amino acids, proving that the hydrolysis process is complete. It is also interesting to note that at the beginning of the hydrolysis process the peptides are almost exclusively N terminal peptides.

The liberation of Met and SeMet from the synthetic peptide was also followed by amino acid analysis based on their determination by GC MS following derivatization with methylchloroformate [5]. The results of these experiments are summarized in Fig. 4. It is clear that the digestion reaction is complete in about 8 h. The decomposition products of the selenized yeast sample were also monitored over time and it is clear from Fig. 4 that the rates of decomposition are identical, within the limits of measurement uncertainty, for both the synthetic peptide and the selenized yeast. This is quite remarkable, considering the differences in complexity and size between the intact yeast proteins and a short synthetic peptide. The identical rate of decomposition may offer the possibility of employing short SeMet containing peptides as calibration standards for the determination of SeMet in various samples. This could potentially compensate for any incomplete hydrolysis of proteins, which is probably the single largest source of error in the determination of SeMet [6].

The use of synthetic selenium containing peptides may also be extremely useful for the evaluation of the various enzymatic digestion methods currently in popular use.

Conclusions

These observations suggest that the methanesulfonic acid based hydrolysis leads to virtually complete liberation of the studied amino acid residues. This fact, coupled with our earlier observations that free SeMet spiked to the sample is stable to acid hydrolysis, leads to the conclusion that this acid hydrolysis protocol is highly efficient and does not destroy the liberated SeMet or the SeMet spike present in the sample.

It is clear that SeMet containing synthetic peptides can also be used as calibration standards for standard additions experiments or, when stable isotope labeled SeMet peptides are available for isotope dilution measurements.

References

[1] Hatfield D L (2001) Selenium: its molecular biology and role in human health. Kluwer Academic Publishers
[2] Encinar J R, Ouerdane L, Buchmann W, Tortajada J, Lobinski R, Szpunar J (2003) Anal Chem 75: 3765
[3] McSheehy S, Kelly J, Tessier L, Mester Z (2005) Analyst 130: 35
[4] Yang L, Sturgeon R E, Wolf W R, Goldschmidt R J, Mester Z (2004) J Anal At Spectrom 19: 1448
[5] Yang L, Mester Z, Sturgeon R E (2004) Anal Chem 76: 5149
[6] Yang L, Sturgeon R E, McSheehy S, Mester Z (2004) J Chromatogr A 1055: 177
[7] Wrobel K, Kannamkumarath S S, Caruso J A (2003) Anal Bioanal Chem 375: 133
[8] McSheehy S, Yang L, Sturgeon R, Mester Z (2005) Anal Chem 77: 344