An approach to the identification of selenium species in yeast extracts using pneumatically-assisted electrospray tandem mass spectrometry

Corinne Casiot, Veronique Vacchina, Hubert Chassaigne, Joanna Szpunar, Martine Potin-Gautier and Ryszard Łobiński

CNRS EP132, Université de Pau et des Pays de l'Adour, Hélioparc, 64053 Pau-Pyrénées, France

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An approach to the identification of unknown signals in selenium speciation analysis of yeast by reversed-phase chromatography with ICP-MS detection is described. The analytical strategy was based on: (*i*), heart-cutting of a Se-containing fraction in the reversed-phase chromatographic eluate followed by its lyophilization; (*ii*), pneumatically-assisted electrospray (ESI) MS and ESI tandem MS of the lyophilizate; and (*iii*) confirmation of the fragmentation pattern obtained using the sulfur analogue of the seleno compound that was expected to have been identified. The approach developed allowed the identification of Se–adenosyl*homo*cysteine as the major selenium species in an extract of a selenized yeast sample.

Introduction

There has been much interest recently in the speciation analysis of selenium in materials of plant origin, and, in particular, in selenized yeast-derived food supplements.[1–6] The majority of these studies were based on HPLC of water or methanol–water sample extracts with on-line element specific detection of selenium by ICP-MS. Various separation mechanisms, including ion-exchange (anion and cation) and anion-pairing reversed-phase (for an exhaustive list of references see refs. 4 and 7), have been optimized to separate four commercially available (Se^{IV}, Se^{VI}, selenomethionine, selenocystine) selenium species.

The HPLC-ICP-MS coupling brought into evidence the presence of a considerable number (7–20) of selenocompounds in yeast extracts.[3–6] No information, however, could be obtained regarding the identity of the majority of the species detected. The lack of convenient methods of structure determination and confirmation remains a major barrier to the mechanistic understanding of Se functions, toxicity and bioavailability.[8] Access to structural information for the identification of known, unknown or unexpected compounds is a great challenge to biochemical speciation analysis, especially because the improving sensitivity of ICP-MS instruments will inevitably increase the number of selenium species detected.

The few attempts at the identification of selenocompounds in yeast were based on the synthesis of the anticipated target compound and the expectation that the retention time of a synthetic standard will match that of a compound in the yeast extract chromatogram.[3,4,8] The mixed success of such an approach was due to the fact that the analyst needed to predict which compound he had detected, which was not always obvious. Also, even if the retention time of a synthetic standard matches by chance that of a signal from the sample, a doubt remains about the chromatographic purity of the signal. Further, the co-extracted ion-pairing or complexing agents present in the sample may change the retention time of the analyte species, but this phenomenon can be controlled by spiking the standard on a sample matrix.

An alternative is the use not of an element-specific but of a species (moiety)-specific detection technique such as, for example, mass spectrometry. Because of the poor volatility of selenocompounds in yeast extracts, electrospray MS seems to be the most attractive choice. The technique offers the possibility of the determination of the molecular mass and the isotopic composition; the fragmentation of the molecular peak by collision induced dissociation (tandem MS mode) brings additional structural information.

The shortcomings of ESI-MS include the relatively (in comparison with ICP-MS) poor detection limits and, especially, the dramatic loss of sensitivity in the presence of matrix components, such as, for example, inorganic salts. Probably for this reason no application of ESI-MS has, to our knowledge, been successful for signal identification in the speciation analysis of selenium from real samples despite the well demonstrated potential of this technique for commercial selenoaminoacids preparations[9] and for synthetic preparations of the precursors of alkylselenides.[8] The combined use of HPLC-ICP-MS and ESI-MS was proposed for speciation of arsenic.[10]

The purpose of this paper is to demonstrate that the sensitivity of ESI-MS can be sufficient for the confirmation of peak identity in chromatograms of yeast extracts provided that this technique is employed following a purification/preconcentration procedure. Further, we propose ESI tandem MS for the identification of an unknown selenium species and postulate the confirmation of the fragmentation pattern with the readily available sulfur analogues of selenocompounds.

Experimental

Instrumentation

Electrospray MS experiments were performed using a PE-SCIEX API 300 Ion-spray triple-quadrupole mass spectrometer (Thornhill, ON, Canada). The HPLC-ICP-MS chromatogram was acquired using a 9012 Varian HPLC pump (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to the Model HP4500 (Yokogawa Analytical Systems Inc., Tokyo, Japan) *via* a Scott double-pass spray chamber fitted with a Babington-type nebulizer. Injections were done using a Model 7725 injection valve (Rheodyne, CA, USA) with a 100 μl injection loop. Separation was done using a 4.6 × 150 mm × 5 μm Inertsil ODS-2 (Interchim, Montluçon, France) column.

Standards and samples

DL-Selenomethionine and *S*-adenosyl*homo*cysteine were purchased from Sigma (St. Quentin Fallavier, France) and were

used without further purification. Stock solutions 1 mg ml^{-1} in deionized water (Millipore, Bedford, MA, USA 18 MΩ) were stored in the dark at 4 °C. A sample of industrially produced selenium enriched yeast was used. *Saccharomyces cerevisiae* was grown in the presence of sodium selenite, out of which it naturally synthesizes organic selenocompounds. It was then pasteurized and dried.

Procedure

A sample of 0.2 g of dry yeast was leached at 85–90 °C with 5 ml of water for about 1 h and centrifuged at 4000 rpm. The extract was filtered through a 0.45 μm filter and freeze-dried. The lyophilizate was dissolved in a minimum amount of water; an aliquot of 100 μl of this solution was chromatographed on a reverse-phase column using the conditions specified in Table 1. The fraction with retention times between 10 and 20 min was collected and lyophilized. The residue was dissolved in 100 μl of a water–methanol mixture (70 : 30, v/v) containing 0.6% of 0.1 M hydrochloric acid. The mixture was analysed by ESI-MS and ESI-MS-MS using the conditions summarized in Table 1.

The optimization of the ESI-MS conditions was carried out using a 10 μg ml^{-1} solution of selenomethionine in a water–methanol mixture (70 : 30, v/v) containing 0.6% 0.1 M hydrochloric acid.

Results and discussion

The application of ESI-MS to the identification of selenocompounds detected in yeast extracts by ICP-MS suffers from two major shortcomings. The first is the discrepancy between the detection limits of ESI-MS (10–100 ng ml^{-1}) and those of ICP-MS (0.1 ng ml^{-1}). The second is the vulnerability of ESI-MS (even in the pneumatically assisted mode) to the presence of salts that suppress the ionization of the analyte compounds and reduce the signal intensity. Because of these two reasons no success could be achieved with the identification of selenocompounds by running HPLC-ESI-MS in parallel in the same conditions as HPLC-ICP-MS.

It was attempted to overcome the above shortcomings by the preconcentration of a reversed-phase chromatographic fraction containing the selenocompound(s) to be identified. In order not to preconcentrate the salts from the chromatographic buffer, separations with salt-free mobile phases were optimized. The best results were obtained with dilute (0.01 M) acetic acid. This was preferred to the trifluoroacetic acid (TFA) used earlier for HPLC-ICP-MS studies[6] in view of the ESI-MS measurements (TFA is known to suppress the signal).

A HPLC-ICP-MS chromatogram obtained for an extract of selenized yeast is shown in Fig. 1 (*cf.* our previous study[6]). It shows a number of small signals followed by a huge peak. The retention time of the major peak does not correspond to any of the commercially available Se-standards (selenite, selenate, selenomethionine, selenocystine, selenoethionine). The presence of such a pattern in the HPLC-ICP-MS of selenized yeast supplement extracts was also reported by Zheng *et al.* without giving any clue to the identity of the compound detected.[5]

Electrospray mass spectrometry

Fig. 2 shows an electrospray mass spectrum of the heart-cut fraction shown in Fig. 1. The fraction was freeze-dried and the residue was dissolved in 100 μl of 30% methanol containing 0.6% of 0.1 M HCl. This medium was found in preliminary experiments to provide the maximum signal for ESI-MS of selenoaminoacid standards. The mass spectrum shows an ion-cluster centred at *m/z* 433 which exhibits a characteristic natural abundance Se isotope pattern. It corresponds to the molecular ion cluster of a protonated ^{80}Se-compound with a molecular mass of 432.0. No other cluster showing a similar pattern can be

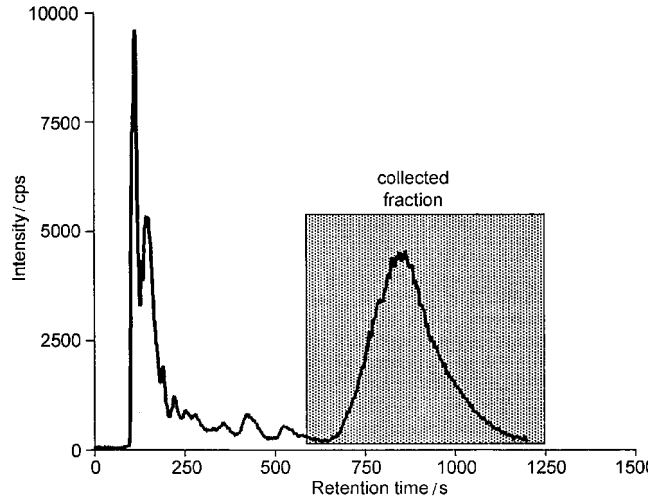

Fig. 1 A reversed-phase HPLC-ICP-MS chromatogram of an extract of selenized-yeast prepared as described in the Procedure. The heart-cut fraction is shadowed.

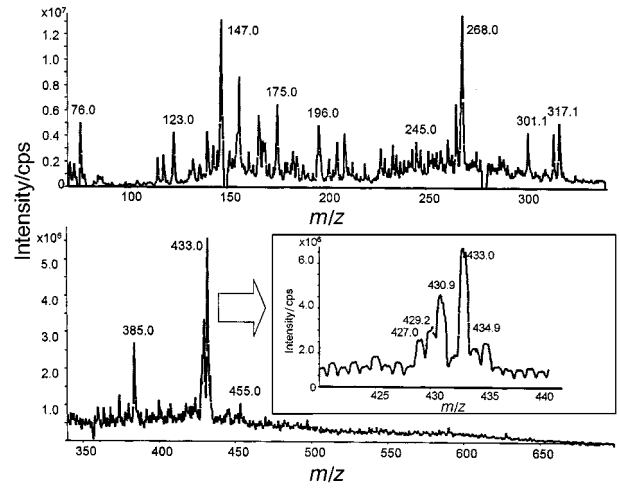

Fig. 2 An electrospray MS spectrum of the shaded fraction in Fig. 1 after preconcentration. The part of the spectrum containing the Se-characteristic isotope pattern is expanded in the inset.

Table 1 Experimental conditions

HPLC conditions	
Column	4.6 × 150 mm × 5 μm Inertsil ODS-2
Mobile phase	0.1% aq. acetic acid
Flow rate	1 ml min^{-1}
ICP MS conditions	
Forward power	1350 W
Nebulizer gas flow rate	1.05 l min^{-1}
Isotope monitored	^{82}Se
Dwell time	100 ms
ESI MS conditions	
Orifice	20 V
Ionspray voltage	4100 V
Scan range	70–700 u within 5 s
Dwell time	10 ms
Step size	0.5 u
MS CID MS conditions	
Parent ion	431, 433
Collision energy	20 eV
Product ion scan range	70–450 within 7 s
Dwell time	10 ms
Step size	0.5 u
Multiplier voltage	2400 V

seen, which confirms the observation from reversed-phase HPLC that the isolated fraction contains only one selenium species. The molecular mass may be an indication of the species identity. A deeper insight into this identity can be obtained by fragmenting the molecular peak by a collision induced dissociation (CID) process. It should be noted that the isolated fraction contains a number of other organic compounds of which the origin remains unknown.

Electrospray tandem MS

Fig. 3a and b shows CID mass spectra of ions with *m/z* 433 and 431 u, corresponding to the two most abundant selenium isotopes ^{80}Se and ^{78}Se, respectively. The comparison of these spectra allows the differentiation between fragments that contain selenium (the *m/z* signals in both spectra appear at a difference of 2 u) and fragments that do not contain selenium (the *m/z* signals in both spectra have the same value). In this way two ^{80}Se-containing fragments with masses of 182.0 and 298.0 u can be identified in addition to the molecular ion (433.2 u) which obviously contains selenium. The fragments that do not contain selenium can be seen as peaks at 136.0 u and a peak at 250.0 u. This fragmentation allows one to put forward a hypothesis on the identity of the peak in the HPLC-ICP-MS chromatogram (Fig. 1). The fragmentation pattern shown in Fig. 4 corresponds with a high probability to Se–adenosyl*homo*selenocysteine. This compound has, to our knowledge, only once been reported in the literature in the context of the transmethylation and polyamine synthesis pathway in the metabolism of selenomethionine in mammalians.[11]

Confirmation of the fragmentation pattern by ESI-MS-MS of the sulfur-analogue of Se–adenosyl*homo*selenocysteine

The ultimate confirmation of the identity of the seleno-compound can be obtained by comparing its CID fragmentation pattern with that of its sulfur analogue. This seems to be a fairly universal approach since standards of many sulfur-compounds having selenium analogues are commercially available. The CID mass spectrum of the molecular peak of ^{32}S-adenosyl-*homo*cysteine (*m/z* 385.0) shows intense signals at *m/z* 136.0 and *m/z* 250.0 corresponding to the Se-free fragments in the spectrum in Fig. 3b. A peak at *m/z* 134.0 corresponding to the selenium fragment at *m/z* 182.0 can also be identified. Note that the mass difference between ^{80}Se and ^{32}S is 48 u.

Conclusions

Pneumatically-assisted electrospray tandem MS is an attractive instrumental technique for peak identification in HPLC-ICP-MS chromatograms of selenium species in biological materials. It may offer the long-sought-for key to many problems related

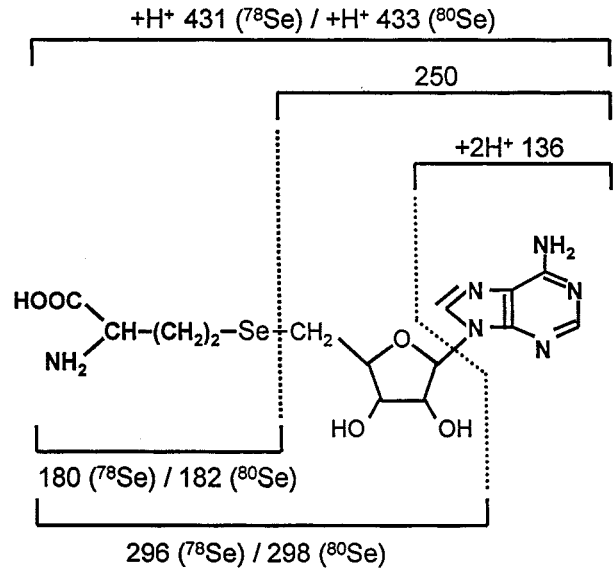

Fig. 4 Fragmentation pattern of the identified compound: Se-adenosyl-*homo*cysteine.

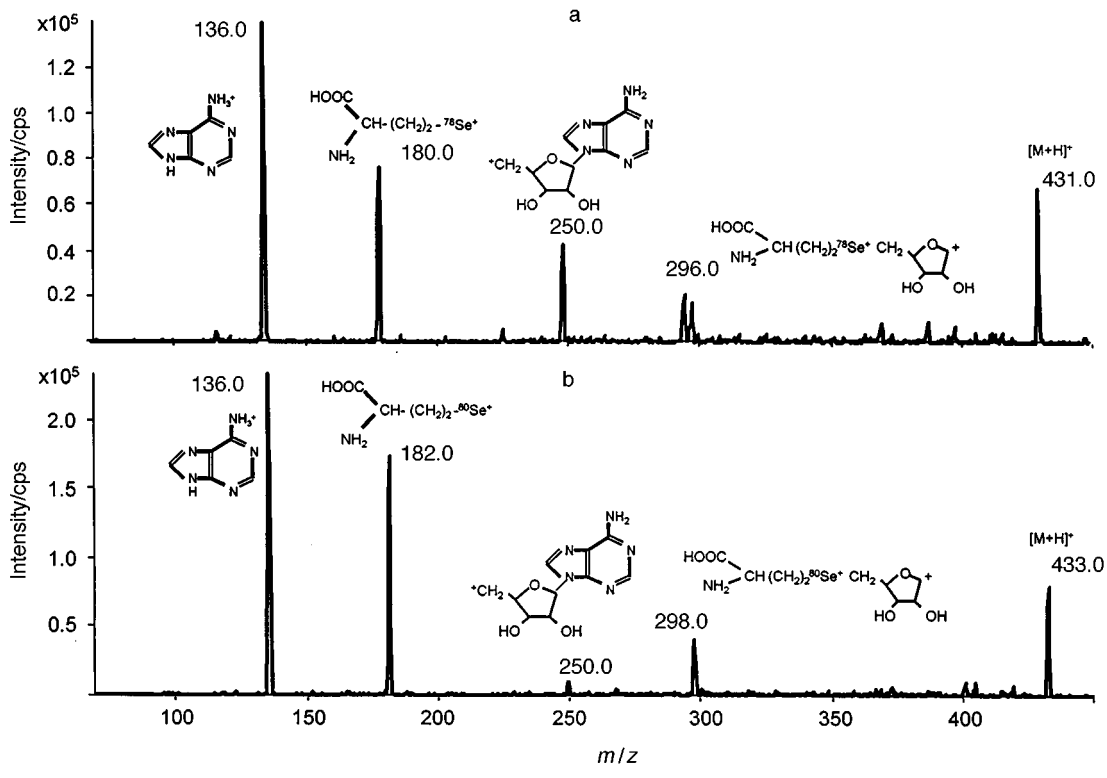

Fig. 3 Collision induced dissociation spectra (product ion scan) of the Se-containing molecular ions. (a) 431 u (^{78}Se); (b) 433 u (^{80}Se).

to studies of the metabolism of this element that are now hampered by the virtual impossibility of identification and/or structure confirmation of the increasing number of selenocompounds detected in HPLC-ICP-MS studies. Electrospray MS detection limits of 10–100 ng ml^{-1} may appear rather high but a volume of 5 μl of an analyzed solution is fully sufficient to complete a structural analysis. The latter is facilitated by the ease of identifying peaks of selenocompounds in an ESI-MS spectrum owing to its characteristic isotopic pattern. The expansion of this technique for speciation studies will depend on the progress in purification and preconcentration of selenocompounds, and in handling microvolumes of solutions to be analysed.

References

1 G. Kölbl, K. Kalcher, K. J. Irgolic and R. J. Magee, *Appl. Organomet. Chem.*, 1992, **7**, 443.
2 N. Gilon, A. Astruc, M. Astruc and M. Potin-Gautier, *Appl. Organomet. Chem.*, 1995, **9**, 623.
3 S. M. Bird, P. C. Uden, J. F. Tyson, E. Block and E. Denoyer, *J. Anal. At. Spectrom.*, 1997, **12**, 785.
4 S. M. Bird, H.-h. Ge, P. C. Uden, J. F. Tyson, E. Block and E. Denoyer, *J. Chromatogr.*, 1997, **789**, 349.
5 J. Zheng, W. Gössler and W. Kosmus, *Trace Elem. Electrolytes*, 1998, **15**, 70.
6 C. Casiot, J. Szpunar, R. Łobiński and M. Potin-Gautier, *J. Anal. At. Spectrom.*, submitted for publication.
7 W. Gössler, C. Kühnelt, C. Schlagenhaufen, K. Kalcher, M. Abegaz and K. Irgolic, *J. Chromatogr.*, 1997, **789**, 233
8 T. W.-M. Fan, A. N. Lane, D. Martens and R. M. Higashi, *Analyst*, 1998, **123**, 875.
9 H. M. Crews, P. A. Clarke, D. J. Lewis, L. M. Owen, P. R. Strutt and A. Izquierdo, *J. Anal. At. Spectrom.*, 1996, **11**, 1177.
10 E. Larsen, *Spectrochim. Acta, Part B*, 1998, **53**, 253.
11 E. O. Kajander, R. L. Pajula, R. J. Harvima and T. O. Eloranta, *Anal. Biochem.*, 1989, **179**, 396.

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