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Short communication

Simultaneous speciation of selenomethionine and 2-hydroxy-4-methylselenobutanoic acid by HPLC–ICP MS in biological samples

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ABSTRACT

An analytical method was developed for the simultaneous speciation of selenomethionine (SeMet) and 2-hydroxy-4-methylselenobutanoic acid (NutraSelen®), a new SeMet precursor. The compounds could be baseline resolved by ion-pairing reversed-phase HPLC using ICP MS detection. Detection limits of 1 ng mL^{-1} (Se content) could be reached. SELM-1 reference material was used to validate the SeMet measurement. Additionally, the quantification of NutraSelen® was validated by standard addition together with checking the Se mass balance. The procedure developed was then applied to the monitoring of the conversion of NutraSelen® into SeMet by yeast.

1. Introduction

Selenium is an essential nutrient which plays a key antioxidant/catalytic role in animal and human physiology through its incorporation into enzymes involved in defence against oxidative stress, regulation of redox status and metabolic modulation of thyroid hormone activities [1–3]. Selenium supplementation is already recommended for patients requiring nutritional support and its health benefits for the general population have been recently acknowledged by the European Food Safety Authority.

At present, for historical reasons, selenium supplementation is mainly performed using a mineral form, sodium selenite, but the natural source of selenium in human and animals is selenomethionine (SeMet), its organic form. It is now well documented that SeMet is more bioavailable and less toxic than selenite [4], and it should be the favoured nutritional supplement. This orientation has recently been taken with the development and registration of various SeMet-enriched yeasts as dietary ingredients. These products offer an alternative to SeMet itself, whose access is difficult, but they remain complex and variable mixtures [5].

2-Hydroxy-4-methylselenobutanoic acid (NutraSelen®) was designed as a precursor of SeMet. It differs from SeMet by having a hydroxyl group on the alpha carbon rather than an amino group. The synthesis and spectral characterisation (NMR, MS) of this molecule were described elsewhere [6]. By analogy with its sulphur analogue, a well-known methionine precursor [7], it has been assumed that NutraSelen® could be converted into L-selenomethionine by oxidation followed by transamination. Yeasts were chosen to demonstrate this bioconversion in eukaryotic cells, and it becomes therefore of primary importance to have an analytical method for the simultaneous speciation of SeMet and NutraSelen® in those microorganisms.

Se speciation in yeast has been widely studied. Among the different methods that have been developed for selenium speciation, the coupling of HPLC with ICP MS is the most popular one in biological samples due to its low detection limits and simplicity to set up [8,9]. Numerous selenium species have been measured this way but to date and to our knowledge, no data on the speciation analysis of the new NutraSelen® molecule by this methodology are available.

The objective of this work was to improve the method used for SeMet speciation in yeast to make it usable to quantify simultaneously the new NutraSelen® molecule.

2. Materials and methods

2.1. Instrumentation

Chromatographic separations were carried out using a Model 1100 HPLC pump (Agilent, Wilmington, DE, USA) as the delivery

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system. The exit of the column was directly connected to the Meinhard nebulizer (Glass Expansion, Romainmotier, Switzerland) of the ICP MS equipped with a collision cell (Agilent 7500ce, Tokyo, Japan) by means of PEEK tubing. Injections were performed using a Rheodyne valve with a 100 μL sample loop. During extraction, samples were shaken mechanically (OLS 200, GRANT, Chelmsford, UK) and then centrifuged (Force 6, Denver Instruments, Arvada, CO, US). For the total selenium determination DigiPrep digestion system (SCP Science, Quebec, Canada) was used.

2.2. Reagents, solutions and materials

Analytical reagent grade chemicals were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Water (18 MΩ cm) obtained with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout unless specified otherwise. The chromatographic mobile phases were degassed by purging with helium. The NutraSelen® standard was obtained from Tetrahedron (Vincennes, France). A stock solution was prepared by dissolving the standard powder in water to reach a final concentration of 1 mg mL^{-1} (Se content). Working solutions were prepared daily by appropriate dilution with water. The standard powder and the stock solution were stored at 4 °C.

2.3. Samples

A strain of baker's yeast, *Saccharomyces cerevisiae*, was isolated from the Fala Backhefe commercial product. Cells were grown at 37 °C, under stirring (250 rpm), in a Yeast–Peptone–Glucose (YPG) medium containing 10 g of yeast extract, 20 g of peptone and 20 g of glucose in 1 L of water at pH 4. For selenium enrichment, a yeast culture resulting from 24 h of incubation was inoculated at 10% (v/v) into the YPG medium supplemented with 20 mg L^{-1} of NutraSelen® (expressed in selenium equivalent). Following 48 h of incubation at 37 °C under orbital stirring (250 rpm), the yeasts were harvested by centrifugation at 6500 rpm for 5 min. The yeasts obtained were washed by resuspending the pellet in physiological water. After centrifugation at 6500 rpm for 5 min, the pelleted yeasts were then lyophilized.

2.4. Analytical procedures

2.4.1. Total Se determination

50 mg of freeze-dried yeast samples (but the mass uptake could vary depending on the amount of sample available) or 500 μL of the YPG medium samples were digested with 1.5 mL of a mixture (2/1, v/v) of nitric acid (69–70%) and hydrogen peroxide (35%) in a 50 mL polypropylene tube (DigiTube, SCP Science) using the following program: up to 45 °C in 20 min, stabilization at 45 °C during 40 min, up to 85 °C in 20 min and stabilization at 85 °C during 100 min. At least 10 mL of water was added to decrease the HNO_3 concentration in the solution fed to the ICP MS but further dilution could be applied for the most concentrated samples. Total Se was measured by direct ICP MS analysis. Isotopes 76, 77 and 78 were used for quantification. The method of the standard addition was used.

2.4.2. Proteolytic extraction of yeasts

50 mg of freeze-dried yeast samples (but the mass uptake could vary depending on the amount of sample available) were incubated (16 h, 37 °C) with an aliquot of 2 mL of protease/lipase solution (20 mg of protease and 10 mg of lipase in 30 mM phosphate buffer pH 7.5). The supernatant was separated by centrifugation and the extraction was repeated twice on the residue. All the supernatants were mixed and spiked with 0.1 M DTT to prevent SeMet oxidation. If necessary, the supernatants were diluted in water before analysis. The possibility of artefactual bioconversion of NutraSelen® during the proteolytic treatment was checked by incubating NutraSelen® with the enzymatic cocktail. This control is recommended for each series of analysis.

2.4.3. Chromatographic and ICP MS conditions

An aliquot of the proteolytic extract (100 μL) was injected on a C_8 Altima column (250 mm × 4.6 mm, 5 μm) (Alltech, Deerfield, IL, US). The buffer A was 0.1% trifluoroacetic acid (TFA) in water and the buffer B 0.1% TFA in methanol. The gradient used for elution was 0–5 min (100% A isocratic), 5–20 min linear gradient up to 25% B followed by stabilization at the initial elution conditions for 5 min. The flow rate was set at 0.9 mL min^{-1}. The collision cell was pressurized with hydrogen. ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se were monitored.

3. Results and discussion

3.1. Optimization of the separation conditions of SeMet and NutraSelen®

The separation conditions of SeMet and NutraSelen® were optimized using the individual standards in aqueous solution. Preliminary experiments focused on an attempt to apply anion-exchange HPLC using ammonium citrate buffer (pH 4.8) which is routinely used for SeMet determination. However, NutraSelen® did not elute from the column with those conditions. Neither when the isocratic elution conditions were replaced by a gradient aiming the increase of the mobile phase buffer concentration.

Reversed-phase HPLC, previously used for the determination of the NutraSelen® sulphur analogue [10], was then attempted. But, because of the use of ICP MS detection instead of UV, the acetonitrile-based mobile phase was replaced by a methanol-based one. After optimization of the elution conditions, the baseline separation of the 2 species studied was achieved as shown in Fig. 1 (individual standards were injected) using a gradient of methanol in the presence of TFA as ion-pairing reactant. The recovery level of NutraSelen® from the RP column was evaluated at 97 ± 2%.

3.2. Quantitative determination of SeMet and NutraSelen®

3.2.1. Analytical figures of merit

Calibration graphs, acquired in the range of 5–100 ng mL^{-1} (Se content) for both NutraSelen® and SeMet, were linear over the whole range investigated ($R^2 > 0.995$). Reproducibility was in the range of 10%. The detection limit was calculated as three times the

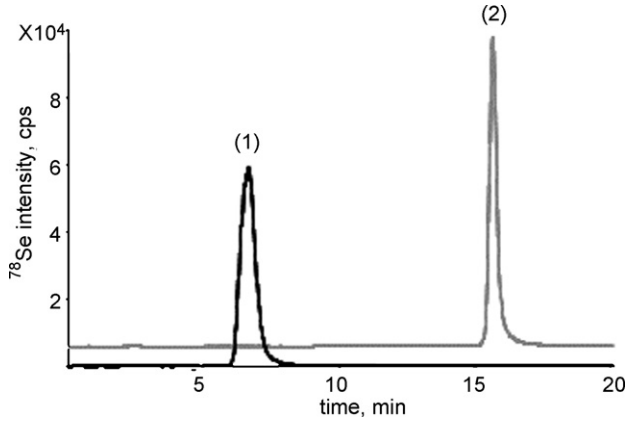

Fig. 1. RP HPLC–ICP MS chromatograms for the individual standards. Peak identification: (1) SeMet; (2) NutraSelen®. The offsets of the chromatograms were added for clarity.

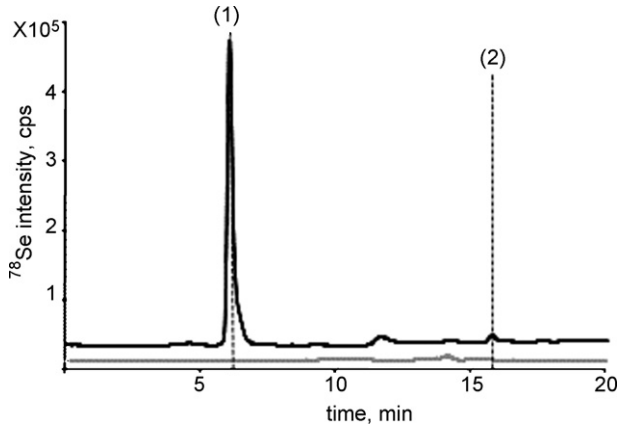

Fig. 2. Typical chromatographic patterns obtained for (—) a NutraSelen®-fed yeast proteolytic extract and (—) a corresponding control sample. Peak identification: (1) SeMet; (2) NutraSelen®. The offsets of the chromatograms were added for clarity.

standard deviation of the blank. It was evaluated at 1 ng mL^{-1} (in Se equivalent).

3.2.2. Method validation and quality control

The absence of yeast Certified Reference Material (CRM) for both SeMet and NutraSelen® content made the formal validation of the method impossible. The best option was to validate the analytical method by spiking the SELM-1 CRM (a selenized yeast certified for its total Se and SeMet content) with NutraSelen® and by checking for the recovery level. The results show (i) a good agreement between the SeMet measured, ca. 1377 ± 99 mg kg^{-1}, and the certified value (1383 ± 83 mg kg^{-1}); (ii) the stability of NutraSelen® during the analytical process. Indeed the intensity of the NutraSelen® peak is the only one that linearly increases with the amount spiked. Four series of samples from microorganisms were then analysed over a period of 5 months. The quality control was assured by the systematic analysis of the SELM-1 CRM to validate both the SeMet determination (average concentration measured of 1332 ± 50 mg kg^{-1}) and the total Se concentration that was used to check the selenium mass balance (the concentration measured of 2019 ± 54 mg kg^{-1} was in agreement with the certified value of 2059 ± 64 mg kg^{-1}).

3.3. Application to the study of NutraSelen® conversion into SeMet by yeast

Fig. 2 shows a typical chromatogram obtained from a NutraSelen®-fed yeast proteolytic extract (dark grey chromatogram). It is made of one major peak matching the retention time of the standard of SeMet that is absent from a control sample (light grey chromatogram). Some minor peaks are also detected but they do not account for more than ca. 5% of the total Se. One of them matches the retention time of NutraSelen® and accounts for ca. 1% of the total Se and probably corresponds to the residual one. The other species could not be identified on the basis of their retention time but they are unlikely to be oxides due to the presence of DTT in the extract. The sum of the individual species concentration measured by HPLC–ICP MS matched properly the total Se confirming the absence of significant matrix effect during analysis. These results demonstrate that NutraSelen® (i) is internalized in the yeast cells, (ii) is efficiently metabolized within these cells and (iii) is bioconverted into SeMet by the yeast.

4. Conclusion

The coupling of ion-pairing reversed-phase HPLC with ICP MS detection allows the quantitative measurement of SeMet and NutraSelen® down to the ng Se mL^{-1} level. Combined to a suitable extraction procedure and applied to the analysis of the intracellular content of yeasts, this selenium speciation method further allowed demonstrating that NutraSelen® is bioconverted into SeMet. This study validates the use of NutraSelen® as SeMet precursor in yeast, thereby emphasizing the potential interest of this novel selenoorganic compound in nutrition. The analytical method can be extended to other organisms to study their ability to convert NutraSelen® into SeMet. It can also be implemented on biological samples to determine their content in NutraSelen® and/or SeMet.

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References

[1] J. Köhrle, R. Gartner, Best Pract. Res. Clin. Endocrinol. 23 (2009) 815.
[2] R. Brigelius-Flohé, Biol. Chem. 387 (2006) 1329.
[3] M.A. Reeves, P.R. Hoffmann, Cell. Mol. Life Sci. 66 (2009) 2457.
[4] P.F. Suraï, Selenium in Nutrition and Health, Nottingham University Press, 2007.
[5] M.P. Rayman, Br. J. Nutr. 92 (2004) 557.
[6] Patent No. US2006105960, WO2006008190.
[7] J.J. Dibner, World's Poult. Sci. J. 59 (2003) 99.
[8] A. Polatajko, N. Jakubowski, J. Szpunar, J. Anal. Atom. Spectrom. 21 (2006) 639.
[9] C. B'Hymer, J.A. Caruso, J. Chromatogr. A 1114 (2006) 1.
[10] R.R. Ontiveros, W.D. Shermer, R.A. Berner, J. Agric. Food Chem. 35 (1987) 692.