ARTICLE

JAAS
www.rsc.org/jaas

A systematic approach to selenium speciation in selenized yeast

Aleksandra Połatajko, Magdalena Śliwka-Kaszyńska,† Mihaly Dernovics,‡ Rafał Ruzik,§ Jorge Ruiz Encinar and Joanna Szpunar*

Equipe de Chimie Analytique Bio-inorganique, CNRS UMR 5034, Hélioparc 2, av. Pr. Angot, F-64053 Pau, France

Received 25th July 2003, Accepted 3rd September 2003
First published as an Advance Article on the web 30th September 2003

A systematic approach to the characterization of selenized yeast supplements in terms of the speciation of selenium was developed. The optimized fractionation procedures included the sequential leaching of water soluble, cell-wall bound, and membrane-protein selenium followed by a further fractionation of each extract by high-resolution size-exclusion chromatography. The stability of fractions collected as chromatographic peaks was investigated in the presence of a proteolytic enzyme (pronase XIV) and trypsin in order to discriminate between selenium-containing peptides and other selenocompounds. Reversed-phase HPLC of tryptic digests of size-exclusion chromatographic fractions allowed the identification of selenopeptides by MALDI and electrospray MS. The complexity of the speciation of the water-soluble selenium in yeast was confirmed. Surprisingly, selenomethionine in the water insoluble fraction was found to be bound physically to cell wall constituents rather than being incorporated chemically into the protein structure, in contrast to former studies.

Introduction

Selenized yeast has probably been the most widely investigated natural product containing selenium.[1] The interest in these studies was triggered off by the study of Clark *et al.*,[2] who indicated a possible role of selenized yeast (one of the most economic sources of organic forms of Se) in cancer prevention.[3] During the growth of *Saccharomyces cerevisiae* yeast, selenite, which is a potentially toxic and poorly bioavailable species, is converted to safer and highly bioactive species with improved nutritional properties.[4] The knowledge of the identity of these biosynthesized compounds is of paramount importance for the understanding of the mechanisms of selenium incorporation, assuring the batch-to-batch reproducibility of the produced selenized yeast, prevention of fraud and, especially, for the understanding of the beneficial and anticancer activity of selenized yeast.

The literature on the selenium speciation in selenized yeast is fairly abundant and has been reviewed recently.[1,5] The analytical methods used have been based on the coupling of chromatography,[6–10] capillary electrophoresis,[11] and 2-D gel electrophoresis (for Se-containing proteins),[12,13] with selenium-specific detection, usually by inductively coupled plasma mass spectrometry (ICP-MS). Electrospray MS, either as an HPLC detector,[14–16] or in infusion mode using triple quadrupole[7,10] and quadrupole-TOF tandem systems,[6] have been used for the identification of selenocompounds detected with mixed success.

Despite a large number of works, the information available on the identity of the molecules incorporating or binding selenium in selenized yeast is still scarce. Solutions to a number of analytical problems are required. Indeed, most of the to-date characterization studies have focused on the water extract that accounts only for 10–15% of the total selenium in yeast.[6,7,17–19,23] The results were presented in the form of chromatograms or electropherograms with very limited information regarding the peak purity or species identity, especially concerning polypeptide compounds. The water-insoluble selenium fraction has usually been determined as selenomethionine based on proteolytic (pronase–lipase) digestion procedures.[14,16,20–22] It was assumed that this selenomethionine was bound to proteins; no ultimate proof for this, however, has ever been provided. Multidimensional chromatographic approaches were recommended to prove the chromatographic purity of a selenium peak and to isolate a selenocompound prior to electrospray MS/MS.[18] The sample preparation procedure by multidimensional chromatography often includes freeze drying and redissolution of the selenium containing fractions.[23] The stability of the different compounds is unsure and some losses of selenium from selenoproteins during purification were reported.[24]

Solubilization of selenocompounds without destroying their original identity (or with their controlled degradation) is the prerequisite for their further characterization by chromatography combined with MS detectors. In our former work a step of pectinolytic digestion with a mixture of cellulases and hemicellulases was introduced to recover selectively selenium bound to cell walls. However, the chromatographic resolution was far from optimal and the quality of chromatograms did not allow a detailed characterization of the fractions obtained.[25]

In this paper the above procedure was revisited with high-resolution size-exclusion HPLC followed by a second chromatographic dimension in the form of reversed-phase HPLC. It was preceded, if possible, by controlled degradation of the isolated fractions with trypsin which allowed the preservation of most of the structural information. A deeper insight into the identity of selenocompounds in yeast was possible by verification of the stability of selenium-containing fractions isolated in the presence of different proteolytic enzymes, followed by mass spectrometry of the reaction products.

Experimental

Apparatus

An ICP-MS (Elan 6000, PE-SCIEX, ON, Canada) fitted with a cross-flow nebulizer and double-pass Scott spray chamber was used for the analysis of fractions from size-exclusion chromatography and for on-line SEC-ICP-MS measurements. For on-line RP HPLC-ICP-MS analysis the interface consisted of a

† On leave from: Department of Chemistry, Technical University of Gdansk, Gdansk, Poland.
‡ On leave from: Department of Applied Chemistry, Szent István University, Budapest, Hungary.
§ On leave from: Department of Analytical Chemistry, Warsaw University of Technology, Warsaw, Poland.

DOI: 10.1039/b308756p

