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Microchemical Journal 85 (2007) 329 – 340
MICROCHEMICAL JOURNAL
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Towards the characterization of metal binding proteins in metal enriched yeast

Tamer Shoeib [a,b,*], Zoltán Mester [a]

[a] Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R9
[b] The British University in Egypt, El Sherouk City, Postal No.11837, P.O. Box 43, Egypt

Received 20 April 2006; received in revised form 1 August 2006; accepted 1 August 2006
Available online 25 September 2006

Abstract

This paper presents size exclusion chromatography data with on-line coupling to UV and inductively coupled plasma mass spectrometry (ICP-MS) of water soluble metal-binding compounds present in zinc, copper, chromium and iodine enriched yeast nutritional supplements. Molecular weight estimates of the extracted metal-containing compounds are given and are shown to vary substantially from 1.2 kDa to larger than 668 kDa. Seven proteins suspected of containing chromium were identified from one of the chromium-containing fractions. Four of these identified proteins are known to form complexes with other metal ions. The metal chromatographic profiles of zinc, copper and chromium-enriched yeasts were compared to their respective native metal profiles in non-enriched yeast samples. The chromium profiles are shown to be markedly different while those of zinc and copper are qualitatively similar. Only iodide ions or weakly bound, non-aromatic, low molecular weight (~1.2 kDa) iodine species were observed in the iodine-enriched yeast samples.
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Keywords: ICP-MS; ESI-MS; Yeast; Metal; Size Exclusion

1. Introduction

The use of nutritional supplements is becoming increasingly common and is growing at a rapid pace. Nutritional supplements adhere to far fewer and significantly less stringent regulations than prescription drugs. Despite this, mineral supplements, in particular, are often recommended to ensure the sufficiency of endogenous and essential trace elements that may be missing or deficient in the modern diet. These trace elements, usually metals and metalloid species, are naturally found in living organisms as a consequence of the biochemical evolution and bio-induction of metal-binding ligands. Imbalances in the concentrations of these metals often represent significant health risks [1].

Typically two forms of mineral supplements are commercially available; inorganic metal salts and metal species that are bound to organic substrates. The latter form has the metal attached to a molecule such as a vitamin (e.g., niacin in polynicotinate versions) or an amino acid derivative (e.g., picolinic acid, a derivative of tryptophan in picolinate versions). This attachment is often claimed to make the metal more efficiently absorbed by the body [2]. It is also suggested that organically bound metals have greater biological activity [2] and are generally less toxic at higher concentrations compared to their respective inorganic salts [3]. Due to its ability to incorporate metals within its cells; yeast biomass is increasingly being used as a delivery vehicle in over-the-counter metal supplements. The bioavailability, desired activity and toxicity of such supplements are functions of the metal concentration, its oxidation state and the chemical forms of its complexes [4,5]. Metal enrichment in yeast is obtained by the inclusion of an inorganic salt of the desired metal in the cultivation medium followed by a heat treatment process to stop yeast growth and inhibit enzymatic activity. The metal is incorporated into the yeast in a variety of ways, including the production of metal-binding proteins (including metallothioneins) [6–8], mineralization [9–11] and sequestration to vacuoles [12–14].

While the vast majority of studies in the area of elemental speciation in nutritional supplements have been devoted to selenium, recently fractionation of soluble species of P, Mn, Fe,

* Corresponding author.
E-mail addresses: tamer.shoeib@nrc.ca, tshoeib@bue.edu.eg (T. Shoeib).

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doi:10.1016/j.microc.2006.08.001

Co, Ni, Cu, Zn, Se, and Mo in pea and lentil seeds with size exclusion chromatography followed by inductively coupled plasma mass spectrometry detection were reported [15]. Here we present the results of some initial and exploratory experiments on commercial yeast supplements enriched with chromium, zinc, copper and iodine.

2. Experimental

2.1. Reagents

A NANOpure mixedbed ion-exchange system (Barnstead Thermolyne, Dubuque, IA) was used to obtain deionized water at a resistivity of 18.2 MΩ cm^{-1}. All analytical grade reagents were used as purchased without any further purification. Sodium dodecyl sulfate (SDS) was purchased from Fluka (Oakville, ON, Canada). Tris(hydrosymethyl)amino-methane (Tris–HCl), ammonium bicarbonate, acetonitrile, formic acid, proteomics grade trypsin (T6567) and rabbit liver metallothionine (MT) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Homogenized yeast powders were provided by Lallemand-Institut Rosell (Montreal, QC, Canada).

2.2. Protein extraction procedure

Samples of ca. 0.5 g of enriched yeast were used in each extraction. After the addition of 10 ml of extraction solvent, the samples were placed in a sonicating bath (Model 3510, Branson, Danbury, CT, USA) for 30 min then centrifuged (Thermo IEC, USA) at 7200 rpm for 60 min. The solid residue was collected for further analysis and to 5 ml of the resulting supernatant solution an equal volume of cold (4 °C) acetonitrile was added to induce the precipitation of water soluble proteins. Samples were then re-centrifuged at 4800 rpm for 10 minutes. The final precipitate was then re-dissolved in 1 ml of water for analysis.

2.3. Microwave digestion for total chromium determination

The solid residues obtained after the two stages of centrifugation and the remaining supernatant solution from the extraction procedure described previously were quantitatively transferred into individual pre-cleaned Teflon digestion vessels. Five milliliter of high purity nitric acid and 0.2 ml of H_2O_2 were then added to each vessel. The vessels were then sealed and heated in a CEM MDS-2100 microwave oven according to the conditions shown in Scheme 1.

After cooling, the caps were removed and rinsed while keeping the rinse solution in the vessels. The contents of the vessels were then evaporated on a hot plate to a volume of about 0.5 ml and subsequently quantitatively made up to 10 ml in water. The samples were finally serial diluted by 100-fold for analysis by ICP-MS.

2.4. Size-exclusion chromatography ICP-MS

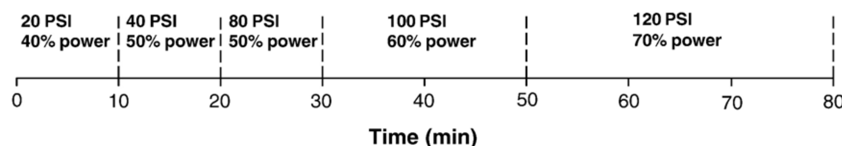
Initial separations were accomplished by an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA, USA) employing a Superdex G200 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) size exclusion column. Elemental detection was achieved using an ELAN 6000 (SCIEX, ON, Canada) ICP-MS equipped with a Ryton spray chamber and cross-flow nebulizer. The ICP-MS parameters were optimized daily but typical operating conditions were 0.7 L min^{-1} for nebulizer gas flow, 1100 W for ICP RF power and 10 V for the lens voltage. The injection size was 20 μL and the elution was accomplished using 30 mM of Tris–HCl adjusted to a pH of 7.5 as mobile phase at a flow rate of 0.6 ml min^{-1}. The effluent was directed into a UV detector set at 254 nm coupled on-line to the ICP-MS for elemental detection. Fractions from several runs were collected based on the retention times of the metal-containing species and added together to be concentrated through freeze-drying for further analysis.

2.5. In-solution enzymatic hydrolysis

The freeze-dried metal-containing protein fractions collected from size exclusion chromatography were re-dissolved in 100 μL of 100 mM ammonium bicarbonate. A sample of 20 μg of proteomics grade trypsin was dissolved in 20 μL of 1 mM HCl. The re-dissolved metal-containing protein fractions were then added to the 20 μL trypsin solution and were incubated at 37 °C for 24 h.

2.6. Reverse phase chromatography ES-MS/MS

Injections of 20 μL were made on a 5 μm, 150 × 2.0 mm, Luna C18 column (Phenomenex, Canada). The mobile phases A (95:4.9:0.1) H_2O/ACN/formic acid and B (4.9:95:0.1) H_2O/ACN/formic acid were used in a gradient from 0% to 60% B over 70 min at a constant flow rate of 0.15 ml min^{-1}. Identification and detection was accomplished using a TSQ Quantum triple quadrupole and a DecaXP ion trap mass spectrometers (ThermoFinnigan, San Jose, CA) both employing electrospray ionization. In the case of the triple quadrupole instrument, about ten scans were averaged at unit resolution (FWHM = 0.7) to produce each mass spectrum. Precursor ion spectra were taken using the following optimized conditions: electrospray voltage at 4000 V, collision energy at 10 V, collision cell pressure at 0.5 mTorr of argon. The ion trap generated data dependent MS/MS spectra were collected at the following conditions: isolation width set at 2 amu, activation Q



Scheme 1. Experimental conditions of the CEM MDS-2100 microwave oven.

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