Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 193 (2018) 349–356

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Highly selective apo-arginase based method for sensitive enzymatic assay of manganese (II) and cobalt (II) ions

Nataliya Stasyuk [a], Galina Gayda [a], Andriy Zakalskiy [a], Oksana Zakalska [a], Abdelhamid Errachid [b], Mykhailo Gonchar [a,*]

[a] Department of Analytical Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Str. 14/16, Lviv 79005, Ukraine
[b] Université de Lyon, Analytical Sciences Institute, UMR CNRS 5180, CNRS, Université de Lyon 1, ENS Lyon, 5 rue de la Doua, F-69100 Villeurbanne Cedex, France

ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form 27 November 2017
Accepted 5 December 2017
Available online 06 December 2017

Keywords:
Recombinant arginase I
Apoenzyme
Holoenzyme
Enzymatic analysis
Manganese (II) and cobalt (II) ions
L-arginine

ABSTRACT

A novel enzymatic method of manganese (II) and cobalt (II) ions assay, based on using apo-enzyme of Mn^{2+}-dependent recombinant arginase I (arginase) and 2,3-butanedione monoxime (DMO) as a chemical reagent is proposed. The principle of the method is the evaluation of the activity of L-arginine-hydrolyzing of arginase holoenzyme after the specific binding of Mn^{2+} or Co^{2+} with apo-arginase. Urea, which is the product of enzymatic hydrolysis of L-arginine (Arg), reacts with DMO and the resulted compound is detected by both fluorometry and visual spectrophotometry. Thus, the content of metal ions in the tested samples can be determined by measuring the level of urea generated after enzymatic hydrolysis of Arg by reconstructed arginase holoenzyme in the presence of tested metal ions. The linearity range of the fluorometric apo-arginase-DMO method in the case of Mn^{2+} assay is from 4 pM to 1.10 nM with a limit of detection of 1 pM Mn^{2+}, whereas the linearity range of the present method in the case of Co^{2+} assay is from 8 pM to 45 nM with a limit of detection of 2.5 pM Co^{2+}. The proposed method being highly sensitive, selective, valid and low-cost, may be useful to monitor Mn^{2+} and Co^{2+} content in clinical laboratories, food industry and environmental control service.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

The importance of monitoring ions of heavy and transitional metals spans all areas of science and technology. A system that can provide selective detection of these ions in aqueous solutions will find numerous applications in such fields as environmental monitoring, biomedical research, clinical chemistry and pharmacology. Ions of heavy and transitional metals in trace quantities are essential mineral components for the human body and all other living organisms, but they exhibit the high level of toxicity at higher concentrations [1–2].

Manganese (Mn) and cobalt (Co) are considered as the most abundant trace elements in the biosphere, being widely distributed in soil, sediment, water and live organisms [3–6]. In human, chronic manganese excess affects the central nervous system, with the symptoms resembling those of Parkinson's disease and autism [7–11]. The mean total content of Mn in human adult is about 15 mg (typically seen in nucleic acids), and the requirement of in this element is about 2–5 mg/day [6]. Mn acts as an activator of enzymes and as a component of metalloenzymes, taking part in oxidative phosphorylation, fatty acids and cholesterol metabolism, mucopolysaccharide metabolism, and urea cycle [6].

Co is an essential element for life in minute amounts [5]. The human adult contains about 1.1 mg Co with the daily requirement of 0.1 μg/day. Co is a component of vitamin B_{12}. Co induces erythropoietin and blocks iodine uptake by the thyroid, controls the transfer of enzymes like homocysteine methyltransferase in methionine metabolism [6]. Contact dermatitis, cardiomyopathy, vision or hearing impairment, hypothyroidism and polycythemia, have been attributed to chronic of cobalt ions due to dietary supplements, occupation and medical devices (Co-containing implants). The knowledge of steady state Co concentrations in tissues in combination with the data on its adverse health effects in humans should help in the characterization of potential hazards associated with increased blood Co concentrations due to exposure to dietary supplements or cobalt chromium (Co-Cr) containing implants [12].

Thus, the possibility to monitor Mn^{2+} and Co^{2+} ions can represent a helpful tool for clinical diagnostics, food industry and environmental control service. The analysis of the localization and distribution of essential and beneficial metals (including Mn and Co) in biological tissues and liquids (e.g. blood and urine) is a challenging task for medicine and other life sciences. Blood concentrations as well as urinary excretion rates of metallic ions are reliable biomarkers for systemic exposure to these compounds [1,7,13].

Many instrument-based techniques have been applied for quantitative analysis of metal ions. Analytical methods such as high performance liquid chromatography (HPLC), gas chromatography, atomic absorption

* Corresponding author.
E-mail address: gonchar@cellbiol.lviv.ua (M. Gonchar).

https://doi.org/10.1016/j.saa.2017.12.031
1386-1425/© 2017 Elsevier B.V. All rights reserved.

and emission spectroscopy, mass spectrometry (MS) are currently and widely used [14–15]. The classical approaches of spectrophotometry and fluorometry, especially in combination with micro- and nanotechnologies [4,16–19] are very popular too.

Over the past years, the development and application of different MS imaging techniques for metals' analysis, including laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), has been rapidly growing in the life sciences in order to investigate the uptake and the transport of both essential and toxic metals in cells and tissues [20]. Although the high-tech analytical techniques for detection of heavy and transition metals are highly sensitive and accurate, they are, however, time-consuming, expensive, require special skills and show limited capability for real-time measurements in biological samples.

As an attractive choice to the mentioned above methods, the electrochemical analytical approaches are also extensively used for metal ions assay. Highly specific, sensitive, simple, fast and cheap biosensors based on electrochemical transducers (amperometric and conductometric electrodes, potentiometric pH-sensitive field effect transistors) and different biorecognition molecules as sensitive elements were developed for inhibitory analysis of heavy metals ions [21–22]. Different microbial cells [23–24], enzymes [22,25–27], DNA and antibodies [28–33], as well as biomimics (molecularly imprinted polymers) [22,34] were used as bioelements. These analytical devices are promising tools for needs of health care, environmental control, biotechnology, agriculture and food industries. Improvement of their analytical characteristics (e.g. sensitivity, selectivity, stability etc.) may be achieved by using negatively or positively charged additional semi-permeable membranes, micro- and nanomaterials of different origin, genetically modified enzymes [22].

Being rather simple, inexpensive, and portable, bio- and chemosensors are promising for real-time measuring for online and continuous analysis of natural samples for detection of metallic ions. The number of chemosensors, namely, based on platinum nanoparticles and 3,3,5,5-tetramethylbenzidine as chromogene with fiber optic detection [35] as well as based on gold clusters [36] and fluorescence graphene-based quantum dots [37–39] were proposed in recent years.

Thus, a majority of known chemical and physico-chemical methods of metallic ions assay has a number of disadvantages, such as a low sensitivity and selectivity, high costs and complexity of the equipment. Therefore, the development of simple cost-effective sensitive methods of quantitative analysis of metallic ions, including Mn^{2+} and Co^{2+}, is an important task of analytical chemistry. Thus, metal-ion-dependent enzymes [22,25–27] and catalytic nucleic acids – DNA-zymes or RNA-zymes [28–33,40] present promising tools for elaboration of such methods.

The use of functional nucleic acids as bioelements for the lab-on-a-chip (LOC) biosensors for detection of heavy metal ions have made a great step forward in recent years. A number of metal-ion-dependent DNAzymes and metal-ion-binding DNA structures have been obtained through combinatorial selection and rational design [33]. These molecules have been used as bioselective elements of sensors with fluorescent, colorimetric, electrochemical, and surface Raman detection of correspondent metal ions. For selective sensing metal ions in complex biological samples and live cells, a facile and stable biosensor based on non-biological enantiomer (L-DNAzyme) was proposed by Kue et al. [32]. With its highly sensitivity (with a detection limit down to 11 ppt) and selectivity (up to millions-fold) toward specific metal ions, these sensors have been applied for on-site and real-time environmental monitoring, point-of-care medical diagnostics and for in situ cellular imaging [41].

According to the degree of automation and system integration, modern analytical devices may be classified as: microfluidic LOC system, microchip, lateral flow dipstick, personal glucose meter, disc-based analytical platform and microfluidic paper-based (MFPB) sensors [42–44]. MFPB sensors are inexpensive, simple, low-cost, portable, easy to use and usually naked-eye quantitative methods, so these analytical devices are promising for use in developing countries and for field measurements.

Analysis of literature data suggests that the further development of novel highly selective and sensitive methods for clinical diagnostics and industry, including enzymatic ones, is necessary. Recently we have demonstrated the possibility to use apoenzyme of Mn^{2+}-dependent recombinant human liver arginase I as a Mn^{2+}-sensitive bioelement in biosensor analysis [45]. The bi-enzyme amperometric biosensor based on arginase, isolated from the recombinant yeast cells, and commercial urease revealed a high sensitivity to Mn^{2+}-ions (9200 ± 20 A M^{-1} m^{-2}) and a low detection limit (0.15 μM).

Arginase I (EC 3.5.3.1; L-arginine amidinohydrolase) is an Mn^{2+} containing enzyme of the urea cycle. It catalyses the final cytosolic reaction of urea formation in the mammalian liver — the conversion of arginine to ornithine and urea. Arginase I (further – arginase) has recently been considered as a prospective pharmaceutical in enzymotherapy for some kinds of auxotrophic cancers for L-arginine (further - Arg) as well as an analytical instrument for assay of the own substrate — Arg [46].

In this paper, we describe the development of a novel enzymochemical method for differential Mn^{2+} and Co^{2+} ions determination and its application on the real samples of wastewaters. The proposed method, based on apoenzyme of recombinant arginase, revealed a high sensitivity and selectivity to Mn^{2+} and Co^{2+} ions. The binding of Mn^{2+} or Co^{2+} with apoenzyme of arginase, at experimentally estimated optimal pH values, induces holoenzyme reconstruction, followed by recovering arginase activity and generation of urea from Arg in arginase-catalysed reaction at different pH-values: 9,1 and 7,1 for Mn^{2+} and Co^{2+}, respectively. Analytical signal is the result of the formation of a stable product between urea and 2,3-butanedione monoxime (DMO) in an acidic medium [47]. The resulted compound can be spectrophotometrically and fluorometrically monitored. The advantages of the proposed method are the simplicity and the fastness (if compared to HPLC and other instrumental methods) of analytic procedure. Being highly sensitive, selective, valid and cost-effective, the proposed analytical method will be promising for Mn^{2+} and Co^{2+} ions assay in different fields of science and technology, including environmental chemistry, plant and animal biochemistry, nutrition, and medicine.

2. Experimental Section

2.1. Reagents and Materials

2,3-butanedione monoxime (DMO), disodium salt of ethylenediamine tetraacetic acid (EDTA), Ni-NTA Superflow beads and sulfuric acid (95–98%) were purchased from Sigma-Aldrich. L-arginine (Arg) and inorganic salts were obtained from Merck (Darmstadt, Germany).

Chemical reagents were prepared using Millipore milli-Q water pretreated with sorbent Chelex 100 (Bio-Rad, Hercules, CA, USA) to remove traces of transient metal ions.

2.2. Apparatus

The fluorescence of the end product of analytical reactions was measured using a Tecan Infinite M-200 spectrofluorometer (Thermo Scientific, United States) at an excitation wavelength of 360 nm and an emission wavelength of 515 nm. Optical density was measured at 350 nm using a Shimadzu UV1650 PC spectrophotometer (Japan).

2.3. Arginase Isolation

Recombinant human arginase I (further – arginase) has been expressed in *Saccharomyces cerevisiae* yeast cells under the control of the inducible *CUP1* promoter. Isolation and purification of $(His)_6$-tagged arginase from cell-free extract of cultivated cells using affinity

Download English Version:

https://daneshyari.com/en/article/7669990

Download Persian Version:

https://daneshyari.com/article/7669990

Daneshyari.com