Biosensors and Bioelectronics 85 (2016) 157–163

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An ultra-facile and label-free immunoassay strategy for detection of copper (II) utilizing chemiluminescence self-enhancement of Cu (II)-ethylenediaminetetraacetate chelate

Hui Ouyang, Qi Shu, Wenwen Wang, Zhenxing Wang, Shijia Yang, Lin Wang, Zhifeng Fu*

Key Laboratory of Luminescence and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form
27 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Copper (II)
Immunoassay
Chemiluminescence
Chelate
Ethylenediaminetetraacetate

ABSTRACT

The establishment of facile, rapid, sensitive and cost-effective protocols for the detection of heavy metals is of great significance for human health and environmental monitoring. Hereby, an ultra-facile and label-free immunoassay strategy was designed for detecting heavy metal ion by using Cu (II) as the model analyte. Cu (II) reacted previously with ethylenediaminetetraacetate (EDTA) was captured by immobilized monoclonal antibody for Cu (II)-EDTA chelate. Then Cu (II) was detected based on the self-enhancing effect of Cu (II)-EDTA chelate to luminol-H_2O_2 chemiluminescence reaction. The CL intensity is linear relative with Cu (II) concentration in a very wide range of 1.0–1000 ng/mL, with a detection limit of 0.33 ng/mL ($S/N=3$). Since the specificity of this proposed strategy relied on both the specificity of monoclonal antibody and the specificity of luminol-H_2O_2 system, it could avoid interference from most common ions. The proposed method was used successfully to detect Cu (II) in traditional Chinese medicine and environmental water samples with acceptable recovery values of 82–113%. This proof-of-principle work demonstrated the feasibility of the label-free immunoassay for heavy metal ions, and opened a new avenue for rapid screening and field assay for drug safety, environmental monitoring and clinical diagnosis.

© 2016 Elsevier B.V. All rights reserved.

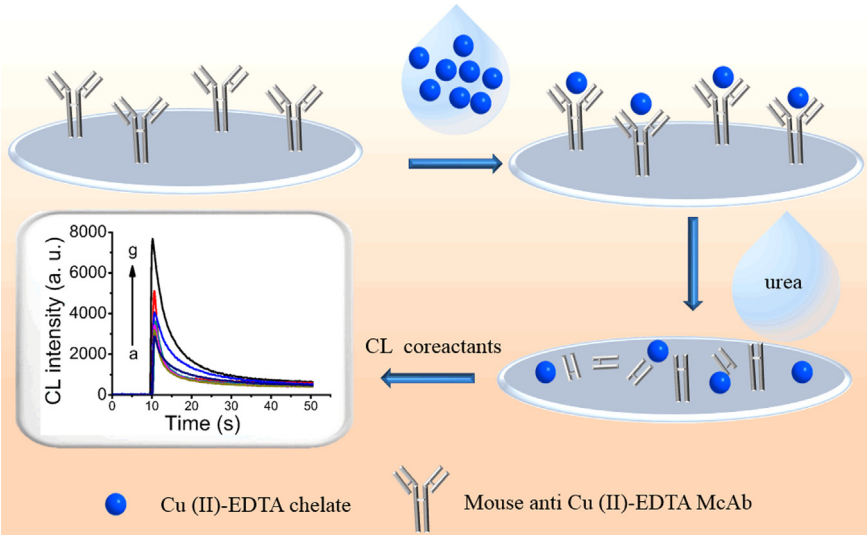
1. Introduction

Due to the massive application of heavy metals in industry and agriculture, heavy metal pollution has become an increasingly serious global environmental problem. Long-term exposure to heavy metals at even trace levels can be potentially catastrophic. For instance, some heavy metals accelerate the production of the toxic free radicals, especially reactive oxygen species, which slowly results in structure damage and function disorder of most biological molecules, including DNA and proteins (Chen and Solomon, 2004). Moreover, many seriously pathological sequences such as lung cancer, diabetes, brain dysfunction, kidney disease and infant liver damage may be related to heavy metal pollution (Barnham et al., 2004; Coen et al., 2001; Cooper et al., 2004; Yuan et al., 2014; Zietz et al., 2003). Thus, the development of sensitive, specific, rapid and low-cost assay protocols for heavy metals is of great significance in various areas such as environmental monitoring and clinical diagnosis.

Thus far, a variety of instrumental techniques have been established to detect trace amount of heavy metals, including atomic absorption spectrometry (AAS) (Anthemidis and Ioannou, 2006; Lin and Huang, 2001), inductively coupled plasma atomic emission spectrometry (Barciela et al., 2008; Liu et al., 2005), inductively coupled plasma mass spectroscopy (Otero-Romani et al., 2005; Su et al., 2014), and X-ray fluorescence spectrometry (Sitko et al., 2015). However, all these methods require sophisticated instrumentation and skilled personnel. Furthermore, they are not appropriate to be developed for field assay and rapid screening. To overcome these defects, some facile methods have also been developed for heavy metal detection. For example, a “signal off” strategy was proposed for visual detection of Cu (II) based on its blocking effect to the escape of photoelectron from quantum dots (Wang et al., 2013). Additionally, a platform was established for ultrasensitive detection of Zn (II) and Cu (II) by using a multifunctional peptide fluorescent chemosensor, in which Zn (II) and Cu (II) led to increase and decrease of fluorescence intensity, respectively (Wang et al., 2015). A fluorometric sensor was developed for the detection of Cu (II) based on the fluorescence quenching of green fluorescent protein by Cu (II) (Lei et al., 2015).

* Corresponding author.
E-mail address: fuzf@swu.edu.cn (Z. Fu).

http://dx.doi.org/10.1016/j.bios.2016.05.007
0956-5663/© 2016 Elsevier B.V. All rights reserved.



Scheme 1. Schematic illustration of the label-free CLIA protocol for Cu (II) detection.

Nevertheless, there are still some limitations in their specificity because of the interference from other heavy metal ions.

Recently, some immunoassay protocols including ELISA (Liu et al., 2013; Xi et al., 2015; Zhao et al., 2011), chemiluminescent immunoassay (CLIA) (Xu et al., 2015), microfluidic immunoassay (Date et al., 2013), fluorescent immunoassay (Sasaki et al., 2009), and resonance Rayleigh scattering immunoassay (Luo et al., 2014) have been developed for the detection of various heavy metal ions such as Cu (II), Hg (II) and Cr (III). They have shown great promise in rapid screening owing to simple manipulation, high throughput and acceptable specificity. In order to achieve ideal sensitivity, up to now, all these reported immunoassay methods for heavy metals adopted probe labelling format. Various signal probes, such as enzyme (Liu et al., 2013; Xu et al., 2015; Zhao et al., 2011), functionalized nanoparticles (Date et al., 2013; Luo et al., 2014), and fluorescent dyes (Sasaki et al., 2009) were employed to label the secondary antibodies. In all these protocols, two antibodies were required to establish the indirect competitive methods, which resulted in high assay cost. Furthermore, probe labelling process is always labor-intensive and time-consuming, and results in the loss of activity of the secondary antibody. Sometimes low labelling efficiency also led to insufficient detection sensitivity (Anderson et al., 2013).

Some metal ions (e.g., Cu (II), Pb (II), Co (II)) and their chelates (e.g., Co (II)/ethanolamine, Co (II)/ethylenediaminetetraacetate (EDTA), Cu (II)/2-[bis[2-[carboxymethyl-[2-oxo-2-(2-sulfanylethylamino)ethyl]amino]ethyl]amino]acetic acid, Cu (II)/cysteine) have been reported to catalyze strongly various chemiluminescence (CL) reaction systems such as luminol-H_2O_2 and *N*-(aminobutyl)-*N*-(ethylisoluminol)-H_2O_2 systems, to enhance dramatically signal emission (Li et al., 2014; Lin et al., 2001; Liu et al., 2014; Zhang et al., 2014). Here, Cu (II)-EDTA chelate was found to improve greatly luminol-H_2O_2 CL reaction efficiency and result in very intensive CL signal. Based on this fact, we proposed a label-free immunoassay method for ultra-facile and cost-effective detection of Cu (II) by a direct format using only one antibody. Cu (II) reacted with EDTA to form a chelate, and then was captured by an immobilized monoclonal antibody (McAb). After the antibody was denatured to release the antigen, the released chelate was quantified by luminol-H_2O_2 CL system based on a self-enhancing strategy.

2. Experimental

2.1. Reagents and instrumentations

Sulfates of Cu (II), Mg (II) and Fe (II), nitrate of Ag (I), permanganate of K (I), dichromate of K (I), persulfate of K (I), thiocyanate of NH_4 (I), iodide of K (I), bromate of Na (I), bromide of K (I), fluoride of K (I), chlorides of Na (I), K (I), NH_4 (I), Ba (II), Al (III), Cd (II), Cr (III), Fe (III), Mn (II), Ca (II), Pb (II), Zn (II) and Hg (II), EDTA, $HClO_4$, HNO_3, hexadecyl trimethyl ammonium bromide (CTAB) and urea were all obtained from Chengdu Kelong Chemical Reagent Company (China). Luminol was purchased from Sigma-Aldrich (USA). Cu (II) coupled to keyhole limpet hemocyanin (KLH) *via* 1-(4-isothiocyanobenzyl) ethylene diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (ITCE), and mouse anti Cu (II)-EDTA McAb were provided by Wuxi Determine Bio-Tech Co. Ltd. (China). Horseradish peroxidase (HRP)-labelled goat anti-mouse IgG was purchased from Boster Biotechnology Co., Ltd. (China). *Ginseng* and *Salvia Miltiorrhiza* were purchased from a local pharmacy, and the lake water was collected from Chongde Lake inside campus. Phosphate buffer saline (PBS, 0.10 M, pH 7.4) was used to dilute all immunoreagents (dilution buffer 1). A same buffer but containing 4.0 mM EDTA was used to prepare Cu (II) sample (dilution buffer 2). Carbonate buffer saline (0.05 M, pH 9.0) was adopted as the coating buffer to immobilize the antibody on microplate. The washing solution was 0.10 M PBS at pH 7.4 containing 0.05% Tween-20. The digestion solution for *Ginseng* and *Salvia Miltiorrhiza* was a mixture of concentrated $HClO_4$ and concentrated HNO_3 (v/v=1:4) All other reagents were analytical reagent grade and used without further purification.

All aqueous solutions were prepared using ultrapure water (18.2 MΩ cm) produced by an ELGA PURELAB Classic system (UK). Polystyrene high-affinity 96-well microplate was provided by Corning Incorporated (USA). AAS as a reference method for Cu (II) detection was conducted on a TAS-990 G atomic absorption spectrometer (Beijing Purkinje General Instrument Co., Ltd., China). The CL signals were collected using a MPI-A CL analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd., China) equipped with a photomultiplier operated at −800 V.

2.2. Procedure of label-free CLIA of Cu (II)

As shown in Scheme 1, a direct format was adopted in the proposed label-free CLIA of Cu (II). The microplate was coated with

Download English Version:

https://daneshyari.com/en/article/7230318

Download Persian Version:

https://daneshyari.com/article/7230318

Daneshyari.com