Analytica Chimica Acta xxx (2017) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Simultaneous fluorescent detection of multiple metal ions based on the DNAzymes and graphene oxide

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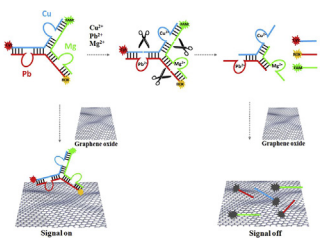
HIGHLIGHTS

- A novel fluorescent detection strategy for simultaneous detection of Cu^{2+}, Pb^{2+} and Mg^{2+} was presented.
- This strategy was successfully used for simultaneous detection of Cu^{2+}, Mg^{2+} and Pb^{2+} in human serum.
- It had potential application in the detection and imaging of multiple metal ions in living cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 May 2017
Received in revised form
30 June 2017
Accepted 11 July 2017
Available online xxx

Keywords:
DNAzyme
Graphene oxide
Metal ions
Fluorescent

ABSTRACT

A novel fluorescent detection strategy for simultaneous detection of Cu^{2+}, Pb^{2+} and Mg^{2+} based on DNAzyme branched junction structure with three kinds of DNAzymes and graphene oxide (GO) was presented. Three fluorophores labeled DNA sequences consisted with enzyme-strand (E-DNA) and substrate strand (S-DNA) were annealed to form DNAzyme branched junction structure. In the presence of target metal ion, the DNAzyme was activated to cleave the fluorophore labeled S-DNA. The S-DNA fragments were released and adsorbed onto GO surface to quench the fluorescent signal. The detection limit was calculated to be 1 nM for Cu^{2+}, 200 nM for Mg^{2+}, and 0.3 nM for Pb^{2+}, respectively. This strategy was successfully used for simultaneous detection of Cu^{2+}, Mg^{2+} and Pb^{2+} in human serum. Moreover, it had potential application for simultaneous detection of multiple metal ions in environmental and biological samples.

1. Introduction

Metal ions play an important role in many biological processes and environmental systems [1]. Metal ions including lead (Pb^{2+}), magnesium (Mg^{2+}) and copper (Cu^{2+}) are essential for human health. Normally the concentrations and distributions of such metals are tightly controlled. It has been proven that metal ion

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http://dx.doi.org/10.1016/j.aca.2017.07.015
0003-2670/

imbalances can lead to a plethora of disease states, and many diseases are related to the abnormal accumulation of metal ions in cell [2]. For example, lead is the most prevalent heavy metal contaminant. Even at low concentration, lead can cause severe damage to the brain, central nervous system and kidneys in adults or children [3,4]. Mg^{2+} ion is an essential enzyme activator for neuromuscular excitability and cell permeability. In addition, it is also important in cellular proliferation and apoptosis processes [5]. Cu^{2+} is a critical micro-nutrient element in cellular homeostasis, gene transcription and neural signal transmission [6–8]. Moreover, the cooperative and synergistic effects of metal ions in cell level are critical for the regular biological processes and functions [9]. Some papers indicated that various diseases are related to the imbalance of metal ions at the same time such as cancers, genetic disorders and metabolic diseases [10,11]. Consequently, development of strategy for the simultaneous detection of these metal ions to reduce the detection time and cost is highly desirable and has significantly impact on chemical, environmental and biological area.

DNAzymes are catalytic DNA sequences which have been isolated by SELEX (systematric evolution of ligands by exponential enrichment) procedure [12,13]. In the presence of specific metal ion, it can coordinate with enzyme strand (E-DNA), catalyzing the cleavage of substrate strand (S-DNA) at ribo-adenine (rA) site, which is 10^6 fold more liable to cleavage than deoxyribonucleotides [14,15]. DNAzymes have attracted significant attention due to their excellent programmability, stability, and activity [16,17]. DNAzyme can easily be modified and labeled with very low immunogenicity. Moreover, DNAzymes own high affinity and specificity toward metal ions [18]. These unique characters make DNAzyme as a promising platform for metal ions detection in therapeutic and diagnostic applications. So far, DNAzymes have conjugated with various signaling transduction strategies for various metal ions detection including Zn^{2+}, Pb^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, and UO_2^{2+} by using colorimetric, fluorescent, electrochemical and Raman methods [6,19–26]. Recently, there are some reports about simultaneous detection of two kinds of metal ions based on DNAzyme strategies. Li group immobilized two kinds of DNAzyme on gold nanoparticles for simultaneous imaging of Zn^{2+} and Cu^{2+} in living cell [6]. Bi group designed a colorimetric logic gates by using Mg^{2+} and Pb^{2+} DNAzymes [27]. Xiang group reported an approach for multiplexed sensing of UO_2^{2+} and Pb^{2+} based on dual-color encoded DNAzyme nanostructures [28]. Although great improvements have been made for multiple metal ions detection, it is still significantly challengeable and meaningful for developing a strategy for multiple metal ions detection.

Some paper have proven the systemic effects of metal ions such as copper, lead and magnesium in serum were related with cancers [29]. These metal ions also have significant functions and roles in cause, progression, treatment and diagnosis of some grave diseases and disorders. Here in, we designed a DNAzyme branched junctions structure with three kinds of DNAzymes for simultaneous fluorescent detection of Cu^{2+}, Pb^{2+} and Mg^{2+}. Each DNAzyme consisted with both E-DNA and S-DNA and labeled with different fluorophore, respectively. The complementary relationships between three DNA sequences were specified, making them to hybridize with each other to form designed DNAzyme branched junction structure. The formed branched junction mainly consisted with double stand DNA (dsDNA) was stiffer and had week binding force with graphene oxide (GO). In the presence of target metal ions, the fluorophore labeled S-DNA of target's DNAzyme was cleaved at rA site. The S-DNA fragments were released and adsorbed onto GO surface via $\pi-\pi$ stacking to quench the fluorescent signal [30–32]. This proposed strategy provides a sensitive approach for simultaneous detection of three kinds of metal ions in homogeneous solution. Importantly, this DNAzyme-GO system has good water-solubility, superior fluorescence quenching ability and biocompatibility. These advantages make it as a promising platform for simultaneous detection of multiple metal ions in environmental and biological samples.

2. Experimental

2.1. Materials and chemicals

DNA sequences were purchased from Sangon Biotech Company, Ltd (Shanghai, China). Table S1 was the list of fluorophore labeled DNA sequences. Tirs-HCl (pH 8.0) was obtained from Beijing Leagene Biotech. Co, Ltd. (Beijing, China). All other chemicals were of analytical grade and were used without further purification. All solutions were prepared with ultrapure water with resistivity of 18.2 MΩ cm.

2.2. Apparatus

Fluorescence measurements were carried out on a LS55 fluorometer (PerkineElmer, USA). The fluorescence emission spectra of FAM were excited at 492 nm and scanned from 505 nm to 600 nm with a step of 1 nm. The fluorescence emission spectra of Cy5 were excited at 640 nm and scanned from 650 nm to 750 nm with a step of 1 nm. The fluorescence emission spectra of ROX were excited at 580 nm and scanned from 590 nm to 700 nm with a step of 1 nm.

SPA300-HV atomic force microscopy (AFM) (Seiko, Japan) was used to characterize the morphology of the prepared GO nanosheets by the tapping mode. The sample was prepared through depositing a droplet of GO dispersion (10 μL, 10 μg/mL) on a freshly cleaved mica surface and then dried at room temperature.

2.3. Synthesis and characterization of GO

GO was synthesized with a modified Hummer's method. Typically, 2 g graphite power was added and stirred with concentrated H_2SO_4 for 2 h. Then, 10 g $KMnO_4$ was gradually added under the temperature of 20 °C. The mixture solution was then transferred into 35 °C water bath and stirred for 4 h vigorously. Then 600 mL of deionized water was added to dilute the solution. After that, 20 mL of 30% H_2O_2 was added drop by drop. Finally, the solution was washed with 0.1 M HCl and deionized water in turn. Graphene oxide was sonicated for exfoliation at least 1 h. The resulted product was centrifuged at 5000 rpm for 10 min, and then took off the upper supernatant for further experiments.

2.4. Fluorescence measurement

DNA sequences were heated to 95 °C for 5 min and then allowed to cool down to room temperature for at least 2 h to form designed branched junctions structure before use. In a typical metal ions detection assay, metal ions were mixed with 100 nM formed DNAzyme branched junctions in 10 mM Tris-HCl (pH 8.0) with 50 mM NaCl, then GO was added into the above-mentioned mixture. The fluorescence signal of the mixed solution was detected after 25 min incubation.

3. Results and discussion

3.1. Multiple fluorescent detection principle of Cu^{2+}, Pb^{2+} and Mg^{2+}

The principle of simultaneous fluorescent detection of three kinds of metal ions based on DNAzyme branched junctions was shown in Scheme 1. All DNA sequences were designed with E-DNA part and S-DNA part of different DNAzymes. Three different kinds

Please cite this article in press as: W. Yun, et al., Simultaneous fluorescent detection of multiple metal ions based on the DNAzymes and graphene oxide, Analytica Chimica Acta (2017), http://dx.doi.org/10.1016/j.aca.2017.07.015