Biosensors and Bioelectronics 50 (2013) 447–452

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Determination of tetracycline in milk by using nucleotide/lanthanide coordination polymer-based ternary complex

Hongliang Tan*, Chanjiao Ma, Yonghai Song, Fugang Xu, Shouhui Chen, Li Wang*

Key Laboratory of Functional Small Organic Molecule, Ministry of Education, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang, 330022, PR China

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form
5 July 2013
Accepted 9 July 2013
Available online 17 July 2013

Keywords:
Coordination polymer
Europium ion
Ternary complex
Fluorescence
Tetracycline
Milk

ABSTRACT

The meta-organic coordination polymers have been emerged as fascinating nanomaterials because of their tunable nature. In this work, we employed lanthanide coordination polymer self-assembled from adenosine monophosphate (AMP) and europium ion (Eu^{3+}) as receptor reagent and citrate (Cit) as ancillary ligand to construct a fluorescent sensor for the detection of tetracycline (Tc) in milk. The co-coordination of Cit and Tc with Eu^{3+} on the surface of the coordination polymer AMP/Eu leads to the formation of ternary complex which emitted strong fluorescence due to the removal of coordinated water molecules and an intramolecular energy transfer from Tc to Eu^{3+}. The fluorescent intensity of Eu^{3+} displayed a good linear response to Tc concentrations in the range of 0.1–20 μM with a detection limit of 60 nM. This method was successfully applied to determine the levels of Tc in milk, which is the first application of coordination polymer as a fluorescent sensor in real sample. Compared with other Eu^{3+}-based fluorescent methods for Tc detection, the presented method allows simple, direct analysis of Tc without requiring special reaction media or complicated prepreparation processes. This straightforward strategy could be extended to the preparation of other lanthanide coordination polymer-based fluorescent probes for applications in biosensing, imaging, drug delivery, and so on.

1. Introduction

Tetracycline (Tc) is a class of broad spectrum antibiotics, which possesses a wide range of antimicrobial activity against Gram-positive and Gram-negative microorganisms. Due to its broad spectrum activity, good oral absorption, and cost-effectiveness, Tc has been widely used in animal husbandry for the treatment of bacterial infections and also used as a feed additive to promote the growth of livestock (Schnappinger and Hillen, 1996). An estimated 5000 metric tons of Tc are consumed per year in the globe (Behal and Hunter, 1995). However, such extensive applications might result in Tc residues remaining in food products, such as milk, meat and honey, which could promote the development and distribution of bacterial resistance to antibiotics (Zhang et al., 2006). The Tc residues could also provoke allergic reactions in some hypersensitive individuals (Traviesa-Alvarez et al., 2007). Therefore, it is an important issue for examining of trace Tc residues in food products before use.

In recent years, various analytical methods have been developed for the detection of Tc, such as microbiological inhibition test (Kurittu et al., 2000), immunoassays (Aga et al., 2003; Jeon and Rhee Paeng, 2008), high performance liquid chromatography (HPLC) (Ng and Linder, 2003), capillary electrophoresis (CE) (Kowalski, 2008), and chemiluminescence (Townshend et al., 2005). These methods are sensitive and highly specific, but certain drawbacks still exist. For example, the HPLC and CE methods are time-consuming and require expensive and sophisticated instruments because they are often performed in conjunction with separating techniques. The specificity of immunoassays may be limited by potential cross-reactivity of substances with similar chemical structures, leading to false-positive test results (Aga et al., 2005). Moreover, the preparation processes of monoclonal antibody are rather complicated and difficult and non-specific conjugation of antibody with enzymatic labels often results in a low productivity of enzyme-linked antibody. Fluorescent measurement of Tc would be a more desirable method due to its easy preparation of sample and high sensitivity, especially europium ions (Eu^{3+})-based fluorescent detection (Georges and Ghazarian, 1993; Ibañez, 2008; Tikhomirova et al., 2008; Wenzel et al., 1988). Tc can coordinate with Eu^{3+} and transfer its excitation energy to Eu^{3+} to sensitize the Eu^{3+} emission in a process known as the antenna effect. The long lifetime up to millisecond of EuTc complex allows the detection of Tc via the time-delay mode, which can eliminate efficiently the interferences from background and scattering fluorescence. However, most of the Eu^{3+}-based fluorescent methods for Tc detection have been limited to molecular lanthanide compounds.

* Corresponding authors. Tel./fax: +86 791 88120861.
E-mail addresses: hltan@jxnu.edu.cn (H. Tan), lwanggroup@yahoo.com.cn (L. Wang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.07.011

Recently, lanthanide-containing metal-organic coordination polymers, which combine the characteristics of organic and inorganic components at the nanoscale, are emerging as a hot topic owing to their tunable nature (Carne et al., 2011). Compared with molecular lanthanide compounds, such polymers can offer the advantages of superior mechanical property, better processability, and thermal stability (Qiao et al., 2011). Up to now, these coordination polymers have already shown promise in a number of applications, including gas storage and separation (Li et al., 2009; Suh et al., 2012), heterogeneous catalysis (Cunha-Silva et al., 2009; Park et al., 2006), biomedical imaging (Della Rocca and Lin, 2010; Liu et al., 2011), and drug delivery (Imaz et al., 2010; Rieter et al., 2008). Nevertheless, most studies of lanthanide coordination polymers focus on their exceptional porosity and luminescent properties, less work concerns with using lanthanide coordination polymers as fluorescent probes to detect ions or molecules, especially in aqueous solution (Tan and Chen, 2011; Tan et al., 2012a; Yang et al., 2012).

In the previous work, we reported that the fluorescence of coordination polymer (AMP/Eu) built from adenosine monophosphate (AMP) and Eu^{3+} could be switched on by the encapsulation of Tc (Tan et al., 2012b). The AMP/Eu encapsulated Tc in aqueous solution displayed a very strong fluorescence due to its hydrophobic interior environment. While, the fluorescence of AMP/Eu in a solution containing the same amount of Tc was very weak due to the quench effect caused by vibrational modes of the coordinated water molecules. Although the coordination polymers based on antenna ligands possess great potential in the applications of time-resolved fluorometric assays, the time-consuming preparation processes make them unsuitable for rapid and direct analysis of analytes. It is known that significantly greater emission intensities of lanthanide complexes can be obtained upon displacement of the coordinated water molecules by suitable chelate agents, which results in the formation of ternary complex (Fu and Turro, 1999). This principle has been probed extensively for potential applications of molecular lanthanide compounds as optical sensors, especially the time-resolved fluorometric assays based on EuTc complex (Lin et al., 2004, 2006; Wolfbeis et al., 2002). However, reports about lanthanide coordination polymer-based ternary complex as fluorescent probes for bioanalysis are relatively rare.

Inspired by the fact that displacement of water molecules by ancillary ligands leads to the formation of fluorescent ternary complex, we here attempt to design a fluorescent probe for the detection of Tc by employing lanthanide coordination polymer AMP/Eu as receptor reagent and citrate (Cit) as ancillary ligand. As shown in Scheme 1, AMP/Eu itself exhibited no fluorescence because the triplet level of AMP is below that of the resonance level $^5D_0$ of Eu^{3+}, and energy transfer from the excited AMP to the emissive $^5D_0$ state of Eu^{3+} cannot occur (Tan et al., 2012b). Cit is a tridentate ligand, which can coordinate to the Eu^{3+} through the oxygen atoms of the carboxy and hydroxy groups, resulting in the displacement of water molecules that occupy the eight to nine coordination sites of the Eu^{3+} (Lin et al., 2004). Due to large coordination numbers and high coordination flexibility of Eu^{3+}, the coordination of Tc with Eu^{3+} on the surface of coordination polymer AMP/Eu leads to the formation of AMP/Eu-Cit-Tc ternary complex. Therefore, the ternary complex AMP/Eu-Cit-Tc is expected to emit strong fluorescence due to an intramolecular energy transfer from Tc to Eu^{3+} and removal of the coordinated water molecules from the coordination sphere of Eu^{3+}.

2. Materials and methods

2.1. Chemicals and solutions

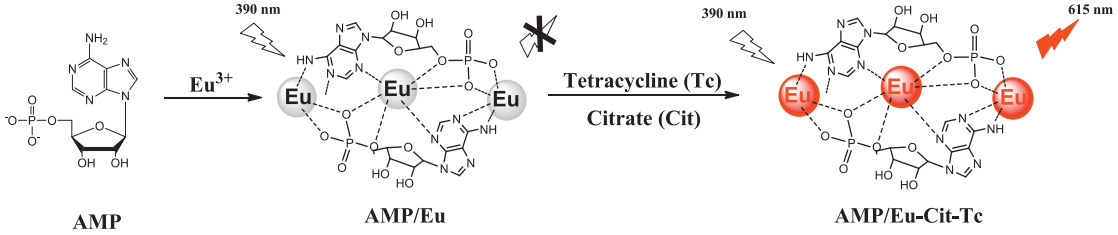
Europium nitrate (99.99%) was purchased from Rewin Rare Earth Metal Materials Co., Ltd (Baotou, China); Adenosine-5′-monophosphate disodium (AMP), 4-morpholine propanesulfonic acid (MOPS) and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China); Tetracycline (Tc) was obtained from Aladdin (Shanghai, China). Amino acids (Ala, Arg, Asp, Glu, His, Lys, and Cys), reduced glutathione (GSH), ascorbic acid, and glucose were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HEPES buffer (100 mM, pH 7.5) was prepared by dissolving HEPES in ultrapure water; 10 M NaOH was used to adjust pH to 7.5. To prepare a 10 mM MOPS buffer, 0.23 g of MOPS was dissolved in 100 mL of ultrapure water; concentrated HCl was used to adjust pH to 6.9. The pH value was calibrated with a pH meter (Sartorius). Ultrapure water (18 MΩ cm) was used for the preparation of all aqueous solutions. Unless otherwise stated, all chemicals are of analytical reagent grade and used without further purification.

2.2. Instruments and determinations

The morphology of coordination polymer was examined by transmission electron microscopy (TEM, JEM-2100, Japan). Fluorescence spectra and emission intensity were recorded on an LS 55 luminescence spectrometer (PerkinElmer, UK), with a xenon lamp as excitation source. The detection solution was placed in a quartz micro cuvette with 100 μL capacity. The light path of the quartz cuvette is 2 mm. For the emission spectra of AMP/Eu-Cit-Tc, excitation wavelength was set at 390 nm; a delay time of 0.05 ms and a gate time of 2 ms were used. Excitation spectra were recorded by observing the emission intensity of Eu^{3+} at 615 nm. UV–visible absorption spectra were recorded with a UV-3150 spectrophotometer (Shimadzu, Japan). For the measurement of emission lifetime of AMP/Eu-Cit-Tc ternary complex, the fluorescent intensities at 615 nm were recorded under different delay times and fitted with an exponential function. All the experiments were performed at room temperature. All error bars represent standard deviations from three repeated experiments.

2.3. Preparation of AMP/Eu coordination polymer

According to the previous method (Nishiyabu et al., 2009), AMP/Eu coordination polymer was prepared by self-assembling of AMP and Eu^{3+} in HEPES buffer (100 mM, pH 7.4). Briefly, l mL of Eu $(NO_3)_3$ aqueous solutions (10 mM) was added to 1 mL of AMP disodium salt solution (10 mM) dissolved in HEPES buffer under



Scheme 1. The detection of Tc based on the formation of AMP/Eu-Cit-Tc ternary complex.