Biosensors and Bioelectronics 39 (2013) 268–273

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Label-free G-quadruplex-specific fluorescent probe for sensitive detection of copper(II) ion

Libing Zhang [a,b], Jinbo Zhu [a,b], Jun Ai [a,b], Zhixue Zhou [a,b], Xiaofang Jia [a,b], Erkang Wang [a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, PR China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 12 April 2012
Received in revised form
25 July 2012
Accepted 27 July 2012
Available online 5 August 2012

Keywords:
Label-free
G-quadruplex
PPIX
Fluorescent probe
Sensitive

ABSTRACT

An effective G-quadruplex-based probe has been constructed for rapid and sensitive detection of Cu^{2+}. In this probe, an anionic porphyrin, protoporphyrin IX (PPIX) served as a reference signal, which binds to G-quadruplex specifically and the fluorescence intensity increases sharply. While, in the presence of Cu^{2+}, the G-quadruplex can catalyze the related Cu^{2+} insertion into the protoporphyrin, the fluorescent intensity is decreased. The fluorescence of the response ligand could be selectively quenched in the presence of Cu^{2+} and not interfered by other metal ions. The probe provided an effective platform for reliable detection of Cu^{2+} with a detection limit as low as 3.0 nM, the high sensitivity was attributed to the strong metalation of PPIX with Cu^{2+} catalyzed by G-quadruplex (PS5.M). Linear correlations were obtained over the logarithm of copper ion concentration in the range from 8×10^{-9} M to 2×10^{-6} M (R=0.998). The G-quadruplex-based probe also could be used to detect Cu^{2+} in real water samples. Additionally, these striking properties endow the G-quadruplex-ligand with a great promise for analytical applications.

1. Introduction

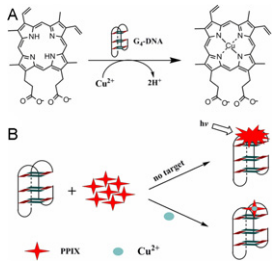
The determination of metal ions in biological systems and environment is of tremendous interest due to their quite beneficial or hazardous effects on the ecosystem and the human health (Xiang et al., 2009). Copper is one of the heavy metals, on the one side, which is essential for all plants and animals as it plays an important role in various physiologic processes (Mayr et al., 2002). As a transition element, it is essential to human health for its key role in cooperating with certain proteins to produce numerous enzymes critical for life. But on the other side, Cu^{2+} ions are toxic when its level exceeds cellular needs. Without regulation and intervention, copper ions would lead to the disturbance of the cellular homeostasis, which will cause serious neurodegenerative diseases, such as Menkes disease, Wilson disease, and Alzheimer's disease (Kim et al., 2008). Copper contamination and its potential toxic effects on human beings are challenging problems throughout the world due to the widespread use of Cu^{2+} in agriculture and industry. Thus far, several efficient and reproducible methods have been reported to determine trace amounts of Cu^{2+} ions, such as atomic absorption spectroscopy (Chan and Huang, 2000; Lin and Huang, 2001; Pourreza and Hoveizavi, 2005), inductively coupled plasma mass spectrometry (ICP-MS) (Becker et al., 2007; Wu and Boyle, 1997) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Liu et al., 2005; Otero-Romani et al., 2005). However, these methods are usually complicated, time-consuming and costly. It is, therefore, of considerable significance to develop highly sensitive and selective probes for Cu^{2+} determination.

G-quadruplexes result from the association of four oligonucleotides strands held together by the hydrophobic stacking of large, planar, hydrogen-bonded DNA base quartets coordinated to a monovalent cation (Bourdoncle et al., 2006). Although the basic features of G-quartets have been known for more than 40 years (Gellert et al., 1962), it is only in the past decade that the interest in these peculiar structures has increased, due to growing evidences for the implication of G-quadruplex structures in key biological processes (Duquette et al., 2004; Paeschke et al., 2005; Rangan et al., 2001). G-quadruplex applied in a biosensor can function as a versatile signaling component in combination with other small molecules, for example, catalytic DNAzyme and fluorescent reagent (Li et al., 2010a, 2009; Xiao et al., 2004; Zhang et al., 2011; Zhu et al., 2011). Compared with the utility of G-quadruplex as DNAzyme, fluorescence enhancement based on G-quadruplex has its own unique characteristics, such as higher stability and reproducibility.

Protoporphyrin IX (PPIX) as a photodynamic agent in cancer therapy (Bedwell et al., 1992; Grant et al., 1993; Malik and Lugaci, 1987) has been widely investigated over the past half century, with the ability to interact with many proteins (Brancaleon and

* Corresponding author. Tel.: +86 431 85262003; fax: +86 431 85689711.
E-mail address: ekwang@ciac.jl.cn (E. Wang).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.07.058



Scheme 1. (A) Protoporphyrin metalation catalyzed by a G-quadruplex DNA. (B) Schematic representation of label-free G-quadruplex-specific fluorescent probe for the detection of Cu^{2+}.

Moseley, 2002; Guo et al., 2007; Tipping et al., 1978; Treffry and Ainswort, 1974). In addition, some literatures about the metalation of PPIX have been reported. Human ferrochelatase is a membrane-associated mitochondrial protein which catalyzes the insertion of Fe(II) into protoporphyrin IX, the final step in the biosynthesis of heme (Yoon and Cowan, 2004). Sen group has reported that the G-quadruplex (PS5.M) catalyzed the related Cu(II) insertion into protoporphyrin IX resulting in the metalation of PPIX (Geyer and Sen, 2000; Li and Sen, 1997). Scheme 1A shows the process of protoporphyrin metalation catalyzed by a G-quadruplex DNA. Generally, PPIX is subjected to aggregation into micelles with low fluorescence in aqueous solution (Brown et al., 1976; Melo and Reisaeter, 1986), while its fluorescence intensity is enhanced via binding to G-quadruplex (Li et al., 2010b).

Here, we present a simple, rapid and label-free assay for highly sensitive and selective detection of Cu^{2+} using G-quadruplex (PS5.M) and PPIX. This new strategy is based on fluorescence quenching of G-quadruplex binding ligand to detect cupric ion. The fluorescence intensity of PPIX is low in aqueous solution, unless it comes into a hydrophobic environment, such as binding to the G-quadruplex that enhances its fluorescence intensity dramatically. While, in the presence of Cu^{2+}, the G-quadruplex can catalyze the related Cu^{2+} insertion into the protoporphyrin. Although the G-quadruplex still can bind to PPIX-Cu complex, the fluorescent intensity is decreased. The whole detection strategy is clearly illustrated in Scheme 1B. In the absence of the Cu^{2+}, PPIX serves as a fluorescent probe binds to G-quadruplex and the fluorescence intensity of this probe increases sharply. In the presence of Cu^{2+}, this probe shows very weak fluorescence. This signaling mechanism makes it possible to detect Cu^{2+} by fluorescence spectroscopy.

2. Experimental details

2.1. Materials

The PS5.M (5′-GTG GGT CAT TGT GGG TGG GTG TGG-3′) was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The stock solution of oligonucleotide was prepared in 40 mM HEPES buffer and accurately quantified using UV–Vis absorption spectroscopy with the following extinction coefficients ($\varepsilon_{260\ nm}$, $M^{-1}\,cm^{-1}$) for each nucleotide: A=15,400, G=11,500, C=7400, T=8700. PPIX was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals were of analytical reagent grade and were used as received without further purification. Solutions were prepared with deionized water processed with a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA). 40 mM HEPES buffer (100 mM NaCl, 25 mM KCl, pH 7.4) was used throughout the experiments.

2.2. Fluorescence spectroscopic analysis

The fluorescence emission spectra of G-quadruplex–PPIX complexes (1 μM) in the HEPES buffer were collected from 550 to 750 nm by using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France). The excitation wavelength was set at 410 nm. The time-resolved fluorescence was measured by using FLSP920 Combined Steady State and Lifetime Spectrometer (Edinburgh, England).

2.3. UV–Visible spectroscopic analysis

The binding interaction between G-quadruplex and PPIX in the HEPES buffer was investigated by UV–Vis absorption spectroscopy, which is reflected by the hyperchromicity of the Soret band of PPIX. The absorption spectra were collected with a Cary 500 scan UV–Vis–NIR spectrophotometer (Varian) in the wavelength range from 300 to 500 nm.

2.4. Circular dichroism analysis

A JASCO J-820 spectropolarimeter (Tokyo, Japan) was utilized to collect the circular dichroism (CD) spectra in the Tris–HCl buffer. The optical chamber (1 mm path length, 0.3 mL volume) was deoxygenated with dry purified nitrogen (99.99%) before use and kept the nitrogen atmosphere during experiments. The background of the buffer solution was subtracted from the CD data automatically.

2.5. Binding assays

Fluorescence titration measurement was performed by keeping the concentration of PPIX at 1.0 μM while varying the DNA PS5.M concentrations in HEPES buffer. According to the literature (Kong et al., 2009; Wang et al., 1997), the association constant K_α and binding stoichiometry n are obtained by the following equation:

$$[DNA]_T = \frac{F-F_0}{F_\infty-F_0}\times\frac{[PPIX]_T}{n}+\frac{(F-F_0)(F_\infty-F_0)^{n-1}}{nK_\alpha(F_\infty-F)^n[PPIX]_T^{n-1}} \quad (1)$$

where $[PPIX]_T$ and $[DNA]_T$ are the initial concentrations of the PPIX and PS5.M, respectively; F_0, F_∞, and F are the fluorescence intensities (at 630 nm) for analyzing uncomplexed PPIX (in the absence of DNA), fully bound PPIX (in the presence of extremely excess DNA), and PPIX bound partially by DNA (in the presence of appropriate concentrations of DNA), respectively.

2.6. Performance of Cu^{2+} detection

The stock PS5.M solution was heated at 88 °C for 10 min and gradually cooled to room temperature. Then, freshly prepared PPIX solution and copper cation were added into the DNA solution. The mixture was kept at room temperature in the dark for 1 h to achieve binding, and then the fluorescence intensity was measured. The concentrations of PS5.M and PPIX in the working solution are both 1.0 μM.

2.7. Detection of Cu^{2+} in real samples

The lake water sample was obtained from South Lake (Changchun, China) and used by filtration. The sewage was obtained from the plant for wastewater treatment and industrial wastewater was obtained from China FAW (First Automobile Works) Group Corporation (Changchun, China), in which a simple treatment was performed to remove large solids and main organic pollutants. We filtered twice