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A fluorescence turn-on detection of copper(II) based on the template-dependent click ligation of oligonucleotides

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ABSTRACT

In this work, a fluorescence turn-on method for copper(II) detection is reported. A molecular beacon (MB) was designed as a template. Cu^{2+} was reduced to Cu^{+} in the presence of a reductant (ascorbic acid). Two short single-stranded oligonucleotides one was labeled with a 5′-alkyne and the other with 3′-azide group, proceeded a template-dependent chemical ligation through the Cu(I)-catalyzed azide-alkyne cycloaddition. The newly generated click-ligated long chain oligonucleotide, which was complementary to the MB, opened the MB hairpin structure and resulted in a turn on fluorescence. The increase in fluorescence intensity is directly proportional to the amount of Cu^{2+} added to the assay solution. The present assay is quite sensitive and allows the detection of 2 nM Cu^{2+}. The described assay also exhibits high selectivity over other metal ions.

1. Introduction

Copper is one of the most abundant transition metals in the human body and is essential for human health [1]. Copper plays important biological roles as enzyme cofactors and as structural components of proteins [1–3]. The lack of copper in the human body may cause copper deficiency diseases, such as coeliac disease and anaemia [4]. However, high concentration of copper is also harmful to the human body, which may cause gastrointestinal disturbance [5], liver or kidney damage [6,7], and various neurological diseases, such as Alzheimer's disease and Parkinson's disease [8,9]. In recent years, continuous release of copper(II) into environmental water has been witnessed due to its extensive use in industry [5]. Therefore, selective and sensitive detection of copper(II) is of great importance.

Great efforts have been made for the development of techniques for selective copper(II) sensing. They include absorption spectrometry [10], atomic absorption spectrometry [11], inductively coupled plasma atomic emission spectroscopy [12], inductively coupled plasma mass spectrometry [13], and various electrochemical [14,15], colorimetric [16–19], and fluorescent methods [20–23]. Fluorescent methods have attracted more and more attentions because of their high sensitivity, simple operation, good reproducibility, and low-cost. Although a number of fluorescent sensors for copper(II) detection have been reported, simple, sensitive and selective methods without the use of harmful substances are still highly demanded.

The Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction reported by Sharpless [24] has emerged as one of the most prominent click chemistry reactions. And it has been used as a versatile, highly efficient and selective chemical tool in many fields [25,26]. It also possesses characters of high-speed and simplicity under mild conditions which earned its name "click reaction" [27]. A trace amount of Cu(I) could catalyze the Huisgen 1,3-dipolar cycloaddition reaction between an alkyne and an azide to form a 1,2,3-triazole [25], and the overall percentage of conversion is related to the amount of Cu(I) added to the reaction mixture. Based on the reduction reaction of Cu(II) to Cu(I) in the presence of a reductant, a number of methods for copper(II) sensing were proposed [28–34]. Recently, a few copper(II) sensors based on template-dependent oligonucleotide ligation were developed [35–39]. It has been shown that the CuAAC driven click reaction enables the ligation of oligonucleotides without any biological enzymes and it shows good biocompatibility [40–44]. However, these methods require the use of additional signaling unit, such as gold nanoparticles, graphene or organic dye, thus the reaction could not be monitored in a real-time fashion, and some used fluorescent turn-off sensing strategy which may increase the possibility of false positive signals [36–39].

Molecular beacon (MB) was originally designed for the sensing of oligonucleotides, nucleic acid point mutations, RNA targets in vivo, and for monitoring PCR reactions in real time [45].

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The method shows high sensitivity and selectivity. Since its first report, MB has also been used for the development of a number of novel fluorescence turn-on assays for the sensing of nucleic acid binding protein, gene expression in living cells, enzyme activity, and many aptamer targeted proteins and small biomolecules, etc [46–51].

Herein, we describe a novel fluorescence turn-on strategy for copper(II) detection based on the template-dependent click ligation (Scheme 1). In the present method, a rationally designed MB (Oligo-MB) was adopted as a template. Two short oligonucleotides (Oligo-A and Oligo-B) labeled with a 5′-alkyne and a 3′-azide groups, respectively, were used. Oligo-A and Oligo-B could hybridize to the complementary sequences of the Oligo-MB, which brought the azide and the alkyne functional groups closer to each other. In the presence of Cu^{2+} and ascorbic acid, Cu^{2+} was reduced to Cu^{+}, and the two short oligonucleotides were ligated by the formation of a triazole linkage. The Cu(I) catalyzed click reaction between the azide and the alkyne functional groups resulted in the connection of Oligo-A and Oligo-B. The newly formed long chain oligonucleotide (Oligo-A-B) opened the Oligo-MB hairpin structure and turn on fluorescence was therefore observed. The increase in intensity could be directly related to the amount of Cu(II) added to the assay solution. Our Cu^{2+} detection method is sensitive, selective, simple, and inexpensive.

2. Experimental section

2.1. Materials

The oligonucleotides (Oligo-A, Oligo-B and Oligo-MB) and L-ascorbic acid were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other chemicals were of analytical reagent grade and used without further purification. The ultra pure water used in all experiments was obtained with a Milli-Q system (Millipore, MA, USA).

2.2. Instrumentation

Fluorescence measurements were performed using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Sample solutions were excited at 480 nm and the fluorescence emission spectra were recorded with slits for excitation and emission both of 5 nm. The quantification of nucleic acids was carried out via UV–vis absorption recorded on a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA). A PB-10 pH meter (Sartorius Scientific Instrument Co., Ltd. Beijing) was used to adjust the pH value of all buffer solutions.

2.3. Assay procedures

Oligonucleotide stock solutions were prepared with Tris–HCl buffer (20 mM, pH 7.4) and stored at 4 °C. Prior to the addition of different concentrations of Cu^{2+} at room temperature, the oligonucleotides and ascorbic acid were mixed and stored at 4 °C for 10 min. The assay solutions were incubated at 25 °C for an appropriate period of time before the fluorescence measurement. The final concentrations of Oligo-A, Oligo-B and Oligo-MB were 30 nM, 30 nM and 10 nM, respectively.

2.4. Lake water sample analysis

Lake water samples were acquired from the South Lake of Changchun, Jilin province, China. The samples were first centrifuged at 12,000 rpm for 10 min and filtered through a nitrocellulose filter (0.45 μm) to get rid of the insoluble materials. 100 μL lake water sample was added into the assay solution for the quantitative analysis (final sample volume: 400 μL). Known quantities of Cu^{2+} were added to the assay solutions and their recoveries were determined. The experiments were repeated three times and the average recovery values were obtained.

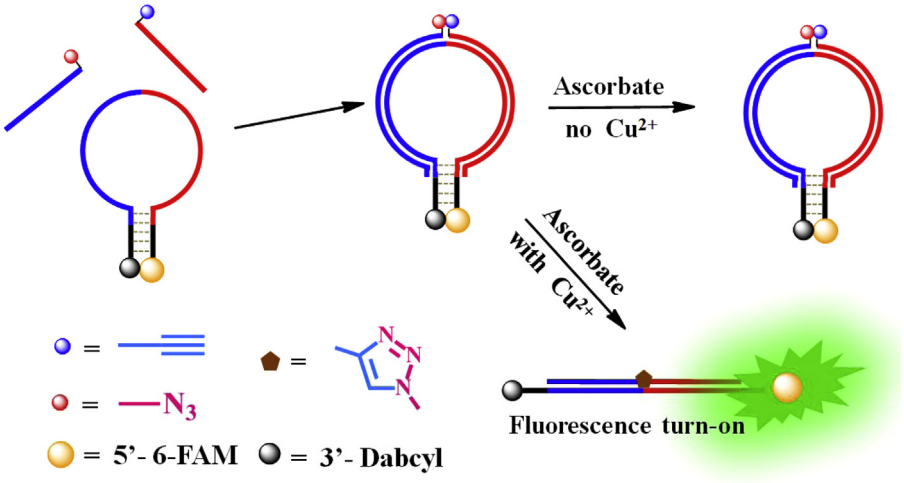
3. Results and discussion

3.1. The assay strategy

The sequences of the oligonucleotides for Cu^{2+} detection were carefully selected (Table 1). Oligo-MB with a fluorophore and a quencher at its termini was selected as a template. It was designed to be a stable hairpin structure at an ambient temperature. When Oligo-MB was in its hairpin state, the fluorophore and the quencher interacted with each other and the fluorescence of the

Table 1
The oligonucleotides employed in the current investigation.

	Oligonucleotide sequence (5′→3′)
Oligo-A	alkyne-GAA ACT GAT AG
Oligo-B	CTA AAT TCC AA-azide
Oligo-MB	FAM-TCG CTA TCA GTT TCT TGG AAT TTA GCG A-Dabcyl
Oligo-A-B	CTA AAT TCC AA-ligated-G AAA CTG ATA G



Scheme 1. Schematic representation of the copper(II) sensing strategy.