Talanta 138 (2015) 144–148

Contents lists available at ScienceDirect

Talanta

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

A label-free fluorescence turn-on sensor for rapid detection of cysteine

Xia Chen [a,b], Hongli Liu [a], Chen Wang [b], Hui Hu [a], Yuhui Wang [a], Xiaodong Zhou [a,*], Jiming Hu [a,*]

[a] Key laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China
[b] Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, PR China

ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form
2 February 2015
Accepted 4 February 2015
Available online 16 February 2015

Keywords:
Fluorescence
Sensor
T–Hg^{2+}–T
Cysteine

ABSTRACT

A Hg^{2+}-mediated fluorescence turn-on sensor for cysteine (Cys) detection was developed using the nucleic acid minor groove binding dye DAPI. In this work, two fully complementary DNA sequences, a T-rich single-stranded molecule (ssDNA) and an A-rich single-stranded molecule, were employed to constitute consecutive "AT/TA" base pairs, which could strongly enhance the fluorescence of DAPI. In the absence of cysteine, Hg^{2+} reacted with T-rich single-stranded DNA and "T–Hg^{2+}–T" base pairs formed, this seriously disrupted consecutive AT base pairs. As a result, the fluorescence of DAPI was not increased efficiently. However, considering that cysteine binds strongly to Hg^{2+}, the structure of the "T–Hg^{2+}–T" complexes was destroyed in the presence of cysteine, resulting in the re-formation of consecutive AT base pairs and increased DAPI fluorescence. Obviously, the amount of cysteine could be easily measured based on the enhancement of DAPI fluorescence, and it took only 20 min to complete the whole cysteine-sensing process. Therefore, a label-free fluorescent "turn-on" sensor for the rapid detection of cysteine was designed, and the detection limit of this sensor was as low as 2.4 nM, which was much lower than those of the most of the previously reported cysteine sensors.

1. Introduction

As a natural, thiol-containing amino acid and one of the most important components among the bio-relevant proteins, cysteine has attracted much attention in recent years due to its crucial role in the human body. For example, it provides the modality for the intramolecular cross-linking of proteins through disulfide bonds to support their secondary structures and functions [1]. However, a number of diseases can result from abnormalities in cysteine levels. Cysteine deficiency is known to be involved in many syndromes, such as edema, lethargy, hair depigmentation, liver damage, muscle and fat loss, slowed growth, and skin lesions [2]. Additionally, the increased cysteine levels can lead to a higher risk of cancer, Alzheimer's, and cardiovascular diseases [3,4]. Therefore, it is of considerable significance to develop rapid, sensitive, and selective cysteine sensors. A number of methods for the measurement of cysteine have been studied in recent years, including electrochemical analysis [2,5–7], chromatography [8], and colorimetric assays [9–12]. However, most of these approaches require complicated instrumentation, involve cumbersome laboratory procedures, and take a long time to complete. Further, most of these methods lack sensitivity; only micromolar concentrations of cysteine are typically detectable. As a highly sensitive, non-destructive technique, fluorescence analysis has also been applied extensively for the measurement of cysteine [13–16]. Unfortunately, fluorescence methods usually need complicated steps such as covalent labeling, modification, or immobilizations, and these steps are time-consuming and cost-intensive. Therefore, it is still meaningful to explore low-cost and time-saving methods for measuring cysteine.

It is well known that some metal ions selectively react with mismatched pyrimidine base pairs to form metal-mediated base pairs. For example, Ag^{+} was found to selectively bind cytosine and form "cytosine–Ag^{+}–cytosine" (C–Ag^{+}–C) base pairs [17]. Similarly, a strong interaction exists between Hg^{2+} and thymine bases; Hg^{2+} offsets the thymine–thymine mismatch to form "thymine–Hg^{2+}–thymine" (T–Hg^{2+}–T) base pairs [18]. Nowadays, metal-mediated base pairs has been widely applied in many researches and studies [19–22].

DAPI (4, 6-diamidino-2-phenylindol) is a nucleic acid minor groove binding dye that is known to selectively bind to consecutive AT/TA sequences in the minor groove of B-form DNA duplexes [23–27]. Upon effectively binding to AT-rich sequences of dsDNA, the fluorescence of DAPI is obviously enhanced. Moreover, the binding affinity between DAPI and dsDNA increases with the number of contiguous AT/TA base pairs [28–30]. It has also been suggested that the minor groove

* Corresponding authors. Tel.: +86 27 68752439 8701; fax: +86 27 68352136.
E-mail addresses: zhouxd@whu.edu.cn (X. Zhou), jmhu@whu.edu.cn (J. Hu).

http://dx.doi.org/10.1016/j.talanta.2015.02.012
0039-9140/

binding of DAPI requires at least four consecutive AT/TA sequences to occur. Herein, we report a new Hg^{2+}-mediated label-free sensor for fluorescence-based detection of cysteine. Hg^{2+} plays a crucial role in this technology by forming "T–Hg^{2+}–T" base pairs, and DAPI was used to give the fluorescence signal. The strong binding of the active sulfhydryl group (–SH) of cysteine with Hg^{2+} was also important in the development of this new sensor. And as a result, this "turn-on" label-free cysteine sensor was found to be cheap, highly sensitive and selective, and moreover it is quite simple and easy to use.

2. Experimental section

2.1. Materials and reagents

DAPI stock solution (5 mg mL^{-1}) was purchased from Dingguo Biotechnology Company (Wuhan, China). The two oligonucleotides (S1: 5′-AAAAAAAAAA-3′, S2: 5′-TTTTTTTTTT-3′) were purchased from Sangon Biotech Co. Ltd. (Shanghai, China). $Hg(NO_3)_2 \cdot 2H_2O$, Cysteine, and 19 other common proteinogenic amino acids (Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val) were of analytical grade and used without further purification. A 0.1 M stock solution of $Hg(NO_3)_2 \cdot 2H_2O$ was prepared in 0.5% HNO_3 solution. RU-21 was purchased from local market, and distributed by Spirit Sciences USA, Inc. The amino acids were dissolved in ultrapure water prepared with a Millipore system (18.2 MΩ). All the experiments were carried out in phosphate buffer solution (PBS buffer, 10 mM, pH 7.0).

2.2. Instrumentation

Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer with Xenon lamp excitation. The excitation wavelength was set at 360 nm and the emission wavelength was set at 460 nm.

2.3. Kinetics study of the reactions

The kinetics study was based on measurements of the reaction between Hg^{2+} and a reaction system of S1/S2/DAPI: 150 nM S1, 150 nM S2 and 1 μM DAPI fully mixed in PBS buffer (10 mM, pH 7.0) at 30 °C. 800 nM Hg^{2+} was then added to this reaction system, and S1/S2/DAPI/Hg^{2+} fluorescence spectra were recorded at different times.

Kinetics study of the reaction between cysteine and the system of S1/S2/DAPI/Hg^{2+}: 150 nM S1, 150 nM S2, 1 μM DAPI and 800 nM Hg^{2+} were fully reacted in PBS buffer at 30 °C, and then 400 nM cysteine was introduced into this system, and finally fluorescence spectra were recorded at different times.

2.4. Optimization of Hg^{2+} concentration

Different concentrations of Hg^{2+} were used to incubate with a mixture of 150 nM S1, 150 nM S2 and 1 μM DAPI under optimal experimental conditions in PBS buffer (10 mM, pH 7.0). Subsequently, fluorescence measurements were taken at room temperature.

2.5. Cysteine detection

In a typical cysteine detection process, 150 nM S1, 150 nM S2, 1 μM DAPI and 800 nM Hg^{2+} were mixed and reacted in PBS buffer (10 mM, pH 7.0) at 30 °C for 15 min. Different concentrations of cysteine were then reacted with above system under optimal experimental conditions. Finally, fluorescence spectra were acquired at room temperature.

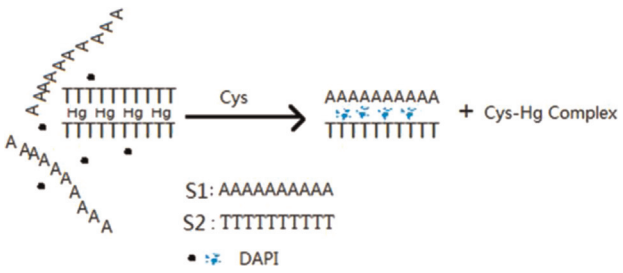
RU-21 tablets were ground and dissolved in water. The sample solutions, which, according to the label content of the tablet supplier, were equivalent to about 30 mg of cysteine were filtered and transferred to 250 mL volumetric flasks, sonicated for 15 min, and then stored for analysis.

3. Results and discussion

3.1. The strategy of this cysteine-sensing system

The principle of our work is represented schematically in Scheme 1. Two simple oligonucleotide strands (S1 and S2) were used. S1 was composed of 10 adenines (A), and S2 was composed of 10 thymine (T). S1 and S2 were able to fully match with each other and form dsDNA through AT base pairing, and the fluorescence of DAPI could be effectively enhanced by consecutive AT pairs of minor groove. When cysteine was absent from the system, the pre-existing Hg^{2+} bound to thymine of S2 and the Hg^{2+}-mediated base pairs "T–Hg^{2+}–T" were formed, so the consecutive AT sequences between S1 and S2 was seriously disrupted and the fluorescence of DAPI was effectively suppressed. However, the presence of cysteine completely changed the situation: cysteine removed Hg^{2+} far away from the structure of "T–Hg^{2+}–T" with its active sulfhydryl group, causing consecutive AT base pairing between S1 and S2 and the fluorescence of DAPI was thus successfully enhanced. As expected, the degree of fluorescence enhancement of DAPI was closely related to the amount of the cysteine present in the system, and cysteine content could be determined in this manner.

The feasibility of the above proposed label-free sensor for the fluorescence detection of cysteine was evaluated, Fig. 1 shows the pre-experimental results. It was found that free DAPI in PBS buffer showed very weak fluorescence emission intensity at 460 nm (curve 1 in Fig. 1A), and neither S1 or S2 alone would bring significant enhancement on the fluorescence of DAPI (curves 2 and 3 in Fig. 1A). However, when S1 and S2 coexisted in the same system (curve 4 in Fig. 1A), the fluorescence of DAPI was significantly enhanced owning to the continuous AT base pairs in the DNA duplexes formed by S1 and S2. According to this phenomenon, S1, S2 and DAPI were fully mixed at first to obtain strong fluorescence of DAPI (curve a, Fig. 1B). The introduction of Hg^{2+} to the system of S1/S2/DAPI weakened the fluorescence of DAPI by forming "T–Hg^{2+}–T" with S2 and disrupting the consecutive AT pairs in DNA duplexes (curve b, Fig. 1B). However, when cysteine was involved in the system of S1/S2/DAPI/Hg^{2+}, an effective fluorescence recovery of DAPI was observed (curve c, Fig. 1B), which resulted from the binding of cysteine to Hg^{2+} of the "T–Hg^{2+}–T" structure and the re-formation of consecutive AT sequences by S1 and S2. These favorable results implied that the proposed label-free fluorescent sensor based on the minor groove dye of DAPI was fully capable of sensing cysteine without any expensive reagents and instruments, or any other complicated separation steps.



Scheme 1. Schematic representation of Hg^{2+}-mediated label-free fluorescence sensor for detection of cysteine by using DAPI.