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An indicator-displacement assay based on the Murexide-Hg^{2+} system for fluorescence turn-on detection of biothiols in biological fluids

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ABSTRACT

In this paper, we present a novel, rapid and sensitive sensor for biothiols including detection of glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) based on an indicator displacement assay (IDA) for turn on the fluorescence of Murexide-Hg^{2+} system. In this IDA approach, a commercially available dye, murexide (Mu), using as the indicator and selective detection of biothiols was achieved based on the competition between the indicator and biothiols for the binding with Hg^{2+}. The fluorescence of Mu could be quenched in the presence of Hg^{2+} due to the coordination between the indicator and Hg^{2+}. Subsequently, the fluorescence of the Mu-Hg^{2+} system was recovered gradually with the addition of biothiols such as GSH, Cys or Hcy due to their stronger affinity with Hg^{2+}. A good relationship was obtained from 0.1–40 μM for GSH, from 0.5–30 μM for Cys and from 0.5–50 μM for Hcy, respectively. This method has been successfully applied to the trace detection of GSH, Cys and Hcy in human urine and serum samples with satisfactory results. Remarkably, the sensing process is able to be observed by the naked eye under UV irradiation which provides an instrument-free platform for point-of-care monitoring.

1. Introduction

Biothiols, such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) play important roles in biological systems [1–4]. For instance, the abnormal levels of Cys and Hcy are linked to a number of health disorders including renal failure, liver damage, developmental retardation, Alzheimer's disease, *etc.* [5–7]. GSH, the most predominant non-protein thiol within the human cellular system, performs vital biological functions that are involved in combating oxidative stress and maintaining redox homeostasis [8,9]. The level of thiol-containing amino acids and peptides in serum is also linked to AIDS [10,11]. Thus, the rapid, sensitive and selective detection of biothiols in biological matrix is of considerable importance and significant interest [4,12].

To date, numerous conventional analytical techniques for the detection of biothiols have been developed for the determination of biothiols, including high performance liquid chromatography, capillary electrophoresis, electrochemical, colorimetric and fluorimetric analysis [13–16]. However, most of the methods currently used in research settings are not optimal for frequent diagnostic applications. These methods usually suffer from more or less inherent drawbacks such as requirement of complicated and expensive instrumentations, involvement of cumbersome laboratory procedures. Fluorimetric sensors are especially promising due to their intrinsically high sensitivity, simplify and ease of operation [17–25]. Most of the fluorimetric sensors for biothiols have been constructed by exploiting transition metal-affinity of the thiol group or the high nucleophilic reactivity which involve specific reactions between probes and thiols [22–25]. These strategies, however, require complicated and time-consuming for synthesis of fluorescent materials or expensive organic fluorophores. Furthermore, the techniques developed based on the fluorescence quenching responsive could cause undesired false positive signals due to the presence of external quenchers or other environmental factors.

As a consequence of these deficiencies, a great incentive skill exists for the development of new, turn-on fluorescence procedures for biothiols detection in a convenient and cost effective manner [26–30]. The indicator displacement assay (IDA) has attracted great attention for the advantages as follow: (1) requires no tedious synthesis to incorporate the chromophore; (2) is easily adapted to different receptors and analytes for rapid analysis; (3) works well in both organic and aqueous media [31]. The IDA has been successfully used in a simple process for detection of diverse analytes such anions, saccharides, amino acids and other functional group molecules [31–35]. In an IDA, an indicator is first allowed to bind reversibly to a receptor, and a competitive analyte is then intro-

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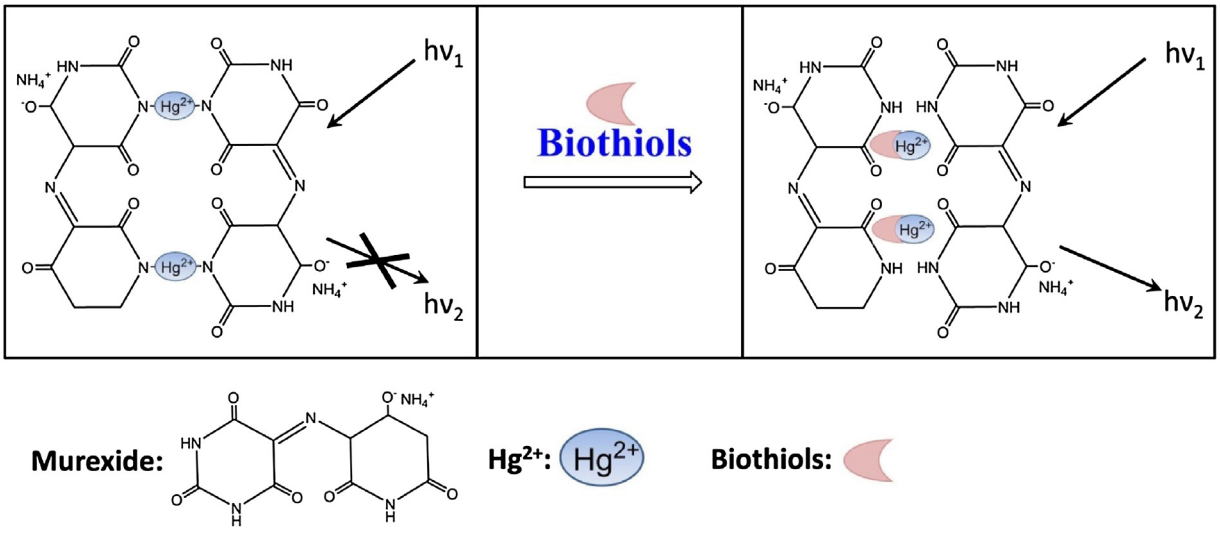

Scheme 1. Schematic illustration of the IDA based on the Mu-Hg^{2+} system for biothiols detection.

duced into the system to displace the indicator from the host with an accompanying colorimetric or fluorescence signal change. The high affinity of Hg^{2+} to biothiols has long been recognized and well explored in the construction of sensing system of thiol-containing peptides and thiol-tagged proteins in modern biotechnology and nanotechnology [29,36].

Inspired by the developments mentioned above, we reported a simple and efficient IDA-based fluorescence turn-on sensor for the detection of biothiols in biological fluids. Important biothiols of GSH, Cys and Hcy were employed as targets. In this IDA approach, a commercially available dye, murexide (Mu) was used as the indicator and the selective detection of biothiols was achieved based on the competition between the indicator and biothiols for binding with Hg^{2+}. The design of the IDA-based fluorescence turn-on sensor, optimization of main factors, evaluation of the performance, and application to analysis of human urine and serum samples were studied in detail.

2. Experimental

2.1. Materials and instrumentation

Murexide (Mu) was purchased from Beijing Chemical Reagent Co. (Beijing, China). All natural amino acids and reduced glutathione (GSH) were purchased from Aladdin Ltd. (Shanghai, China). Mercury(II) acetate (97%) was purchased from Sinoreagent Co., Ltd. (Beijing, China). Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Beijing Chemical Factory (Beijing, China). Human total thiols ELISA Kit and Human cysteine ELISA Kit were purchased from Yuduo Biotechnology Co., Ltd. (Shanghai, China). Phosphate buffer solutions (PBS) of various pH were prepared with different ratios of Na_2HPO_4, NaH_2PO_4, and ultrapurewater. Ultrapure water (18.2 MΩ cm^{-1}) was obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO, USA). The murexide solution was freshly prepared just before experiments. All chemicals were at least of analytical grade and used without further purification.

UV–vis absorption spectra were recorded using a TU-1901 UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China). Fluorescence emission spectra were measured on Hitachi F-4500 spectrophotometer equipped with 1 cm quartz cell (Hitachi, Japan). The pH values were measured with an Oakton pH510 benchtop meter (Thermo Fisher, USA).

2.2. Fluorescence measurements

The fluorescence detection was performed at room temperature in PBS buffer (pH 6.0). In a typical test, to study the quenching effect of Hg^{2+} on the fluorescence intensity of Mu, 1 mL of 0.2 M PBS buffer solution (pH 6.0), 250 μL of 1 mM Mu solution and different amounts of Hg^{2+} standard solution were sequentially added to a 10-mL calibrated test tube. The mixture was diluted to volume with ultrapure water. The solution was mixed thoroughly, and left for 5 min to measure the fluorescence spectra.

Then we investigated the effect of GSH, Cys or Hcy on the fluorescence recovery of the Mu-Hg^{2+} system. Herein, 1 mL of 0.2 M PBS buffer solution (pH 6.0), 250 μL of 1 mM Mu solution and 400 μL of Hg^{2+} standard solution were sequentially added to a 10-mL calibrated test tube. The mixture was diluted to about three-quarters of the total volume of the test tube with ultrapure water, and left for 5 min. Different amounts of analytes were added to the above mixture solution, and the mixture was further dilute to volume with ultrapure water. The resulting solution was shaking thoroughly and set for a certain time before recording the spectra.

All the fluorescence detections were under the same condition: the slit widths of the excitation and emission were both 5 nm. The fluorescence intensity at the maximum emission peak with the excitation wavelength of 335 nm was used for quantitative analysis.

2.3. Samples

The human urine samples employed in this study were provided by the members in our group and the human serum samples were collected from healthy adult volunteers from local hospital. For determination of total biothiols, the disulfide bonds were reduced in order to release free thiols by addition of triphenylphosphine (PPh3) [20]. The collected urine or serum samples were spiked with different concentrations of Cys separately to prepare the spiked samples. The following procedure was carried out: the human urine samples were firstly treated with excess PPh3 and then centrifuged at 8000 rpm for 15 min. The supernatant liquid was used for further analysis. 500 μL of serum sample was first reduced by adding 40 μL of 0.2 M HCl and 20 μL of 0.4 M PPh3 (in water-acetonitrile 20:80 v/v) [18]. After votexing for 10 min, the hydrolyzed serum