Biosensors and Bioelectronics 45 (2013) 65–69

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

A label-free method for detecting biological thiols based on blocking of Hg^{2+}-quenching of fluorescent gold nanoclusters

Ki Soo Park, Moon Il Kim, Min-Ah Woo, Hyun Gyu Park*

Department of Chemical and Biomolecular Engineering (BK21 Program), KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

ARTICLE INFO

Article history:
Received 27 November 2012
Received in revised form
11 January 2013
Accepted 11 January 2013
Available online 4 February 2013

Keywords:
Biological thiols
Fluorescent biosensors
Fluorescence quenching
Gold nanoclusters
Metallophilic Hg^{2+}–Au^{+} interactions

ABSTRACT

A novel, label-free, fluorescent, turn-on sensor for biological thiol detection that uses highly fluorescent gold nanoclusters (AuNCs), prepared by a bovine serum albumin (BSA)-templated green synthetic route, has been developed. The assay relies on blocking Hg^{2+}-induced quenching of the fluorescence of AuNCs, caused by metallophilic Hg^{2+}–Au^{+} interactions, through selective coordination of biological thiols with Hg^{2+} ions. Biological thiols entrap added Hg^{2+} ions via a robust Hg–S interaction. This phenomenon prevents Hg^{2+}-induced quenching and results in fluorescence from AuNCs. By employing this turn-on sensor, biological thiols, such as cysteine (Cys), glutathione (GSH) and homocysteine (Hcy), are successfully detected at concentrations as low as 8.3 nM for Cys, 9.4 nM for GSH, and 14.9 nM for Hcy. The diagnostic capability and potential in practical applications of this method have been demonstrated by detecting biological thiols in human blood serum.

1. Introduction

In recent years, the detection of biologically relevant thiols (biothiols), such as cysteine (Cys), glutathione (GSH), and homocysteine (Hcy), has attracted significant interest because of the important roles these substances play in biological systems (Han and Wang, 2011; Han et al., 2009; Hu et al., 2011; Huang et al., 2011; Lee et al., 2008; Lin et al., 2011; Pu et al., 2010; Ros-Lis et al., 2004; Shang and Dong, 2009; Shao et al., 2006; Sreejith et al., 2008). Cys is a critical amino acid that governs three-dimensional structures of proteins through the formation of disulfide bonds, and it serves as an antidote for the negative effects of alcohol and a rate-limiting precursor in the biosynthesis of GSH and taurine (Kleinman and Richie, 2000). GSH, the most abundant intracellular nonprotein thiol, participates in a number of cellular processes that include maintaining the reducing environment within cells, neutralizing free radicals and peroxides, and regulating the nitric oxide cycle (Ha et al., 1999; Scholz et al., 1989). Hcy is a critical regulatory intermediate in the methionine pathway that produces methionine via methylation or creates Cys through an alternative trans-sulfuration pathway (Kleinman and Richie, 2000). Altered levels of these three important thiols have been implicated in a number of pathological conditions, including Alzheimer's and Parkinson's diseases for Cys (Heafield et al., 1990), diabetes and HIV disease for GSH (Samiec et al., 1998; Staal et al., 1992), and Alzheimer's and cardiovascular diseases for Hcy (Woo et al., 2011). Therefore, measuring the levels of biothiols is very important for early stage monitoring of the corresponding health problems.

A number of methods to detect biothiols have been described, including those that rely on capillary electrophoresis (Chen et al., 2004), high-performance liquid chromatography (Cao et al., 2004), and mass spectrometry (Burford et al., 2003). However, all of these procedures have limitations that include the need for sophisticated instrumentation and technical expertise, as well as long analysis times. With the aim of developing alternative approaches that overcome these limitations, growing interest has focused on fluorescence-based strategies, owing to their intrinsically high sensitivity, simplicity, and ease of operation. Efforts with this focus have led to the development of a number of new fluorescence-based methods to detect biothiols. For instance, Xu and Hepel (2011) recently described a molecular beacon-based fluorescence strategy for the detection of GSH and Cys. The method is based on competitive binding of Hg^{2+} ions to biothiols and a thymine–thymine (T–T) mismatched base pair, strategically installed in the stem part of a molecular beacon probe containing a fluorophore and quencher at both ends. When present in a sample, biothiols interact with Hg^{2+} ions leading to recovery of the fluorescence signal of the molecular beacon probe. Although this technique has good sensitivity and selectivity for biothiols, it requires expensive chemical modification of the molecular beacon probe to introduce the fluorophore and quencher and heating at 52 °C for 15 min to maximize detection. Recently, a single-walled carbon nanotube (SWNT) based fluorescent biosensor for

* Corresponding author. Tel.: +82 42 350 3932; fax: +82 42 350 3910.
E-mail address: hgpark@kaist.ac.kr (H.G. Park).

0956-5663/$ - see front matter Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.01.047

Cys detection was devised. This method takes advantage of the specific interaction of Cys with Ag^+ ions, complexed with a cytosine–cytosine (C–C) mismatched base pair (Zhao et al., 2010). Unfortunately, this method requires the preparation of fluorophore-labeled DNA and because it is turn-off fluorescence responsive the presence of external quenchers or other environmental factors could cause undesired false positive signals (Han and Wang, 2011; Shang and Dong, 2009).

Sensing systems that have turn-on fluorescence responses in the presence of target biothiols are highly attractive. Designs have been described for a number of methods of this type that rely on biothiol-induced displacement of fluorescent materials, such as conjugated polymers (Shang et al., 2007), PEGylated pyrene (Xu et al., 2010), Nile red (Chen and Chang, 2004), near-infrared fluorescent FR 730 (Shang et al., 2009), and fluorescein isothiocyanate (Hu et al., 2011). These strategies, however, require complicated and time-consuming procedures for synthesis of fluorescent materials or expensive organic fluorophores. Furthermore, the techniques developed thus far involve pre-incubation steps for absorption of fluorescent substances on the quenching materials as well as separation steps to remove unbound fluorescent agents.

As a consequence of these deficiencies, a great incentive still exists for the development of new, turn-on fluorescence procedures for assaying biothiols that operate in a convenient and cost-effective manner. In an investigation aimed at this goal, we have designed a new turn-on fluorescence sensor method that can be employed for the sensitive and selective detection of biothiols. The assay system utilizes highly fluorescent, bovine serum albumin (BSA)-stabilized gold nanoclusters (AuNCs), prepared by using a simple, eco-friendly green synthetic route (Xie et al., 2009). Fluorescence from the AuNCs is quenched by Hg^{2+} ions via high-affinity metallophilic Hg^{2+}–Au^+ interactions (Xie et al., 2010). Importantly, this quenching effect is reversed by selective coordination of Hg^{2+} ions to biothiols. The attributes of the fluorescent AuNCs used in this study include their nontoxicity, ultrafine size and ease of synthesis compared to the preparation of semiconductor quantum dots (QDs) (Kawasaki et al., 2011; Xie et al., 2009). Finally, the system developed in this effort represents one of only a few that utilizes AuNCs as fluorescence-based biosensing platforms (Hu et al., 2010; Li et al., 2011; Liu et al., 2010; Wen et al., 2011; Xie et al., 2010).

2. Materials and methods

2.1. Materials

Cysteine (Cys), glutathione (GSH), glutathione disulfide (GSSG), homocysteine (Hcy), $Hg(NO_3)_2$, 3-(N-morpholino)propanesulfonic acid (MOPS), N-ethyl-maleimide (NEM), triphenylphosphine (PPh3), human serum (H4522), and other 19 amino acids used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification (Park et al., 2012a, 2012b). Aqueous solutions were prepared using ultrapure DNase/RNase-free distilled water purchased from Invitrogen (Park et al., 2010, 2011, 2012a, 2012b).

2.2. Synthesis of BSA-stabilized gold nanoclusters

BSA-stabilized AuNCs were prepared using the previously reported procedure (Xie et al., 2009). Briefly, 5 mL of an aqueous $HAuCl_4$ solution (10 mM, 37 °C) was added to 5 mL of the BSA solution (50 mg/mL, 37 °C) under magnetic stirring. Then, 0.5 mL of a 1 M NaOH solution was added and the resulting mixture was incubated under N_2 purging while stirring at 37 °C for 12 h. The concentration of the as-prepared AuNCs solution is denoted as "1 ×".

2.3. Biological thiol detection procedure

A sample solution containing the target biothiol is added to the 0.00025 × as-prepared AuNCs in a MOPS buffer (0.01 M, pH 7), and the resulting solution is incubated at room temperature for 3 min. To this solution is added 400 nM of Hg^{2+} ions and the resulting solution is incubated at room temperature for 3 min.

2.4. Preparation of human serum samples

A human serum sample (500 μL) was first reduced by adding 40 μL of 0.2 M HCl and 20 μL of 0.4 M PPh3 (in water–acetonitrile 20:80v/v and 2 M HCl). After incubating for 20 min under vigorous stirring, the hydrolyzed plasma was mixed with 500 μL of

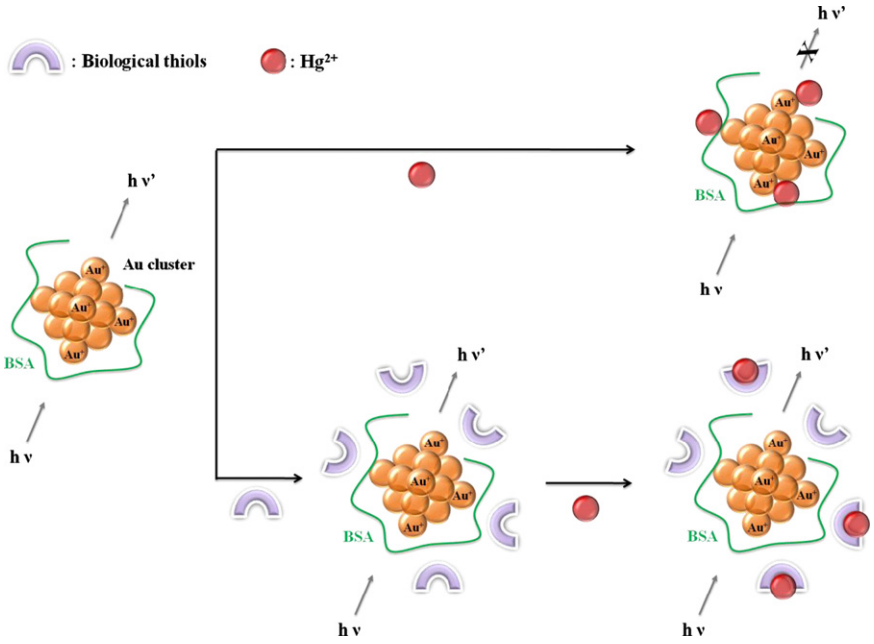

Fig. 1. Schematic illustration of the biological thiol detection system based on the prevention against Hg^{2+}-induced quenching of fluorescent gold nanoclusters (AuNCs).