Biosensors and Bioelectronics 53 (2014) 71–75

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A highly selective sensor of cysteine with tunable sensitivity and detection window based on dual-emission Ag nanoclusters

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ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form
12 September 2013
Accepted 17 September 2013
Available online 25 September 2013

Keywords:
Fluorescence sensor
Dual-emission
Ag nanoclusters
Cysteine

ABSTRACT

An effective dual-emission fluorescent Ag nanoclusters (NCs)-based probe have been constructed for rapid and selective detection of cysteine (Cys) with tunable sensitivity. Electrostatically induced reversible phase transfer method is employed to synthesize Ag nanoclusters with tunable emission intensity at 430 nm and 630 nm by controlling molar ratio between Ag and glutathione. The fluorescence of the Ag nanoclusters could be selectively quenched in the presence of Cys with a detection limit as low as 10 nM. Good linear correlations are obtained over the concentration range from 0.5 to 55 μM (quenched emission at 630 nm), 55 to 120 μM and 120 to 220 μM (enhanced emission at 555 nm) and 120 to 200 μM (quenched emission at 430 nm), respectively. The long-wavelength emission of the Ag nanoclusters can avoid the interference of the autofluorescence of the biosystems, which facilitated their applications in monitoring Cys in urine.

1. Introduction

Biological thiols are essential for maintaining the appropriate redox status of cells, because the alternations in the level of cellular thiols have been linked to a number of diseases, such as leukocyte loss, psoriasis, liver damage, cancer, and AIDS (Wood et al., 2003; Schulz et al., 2000; Townsend et al., 2003; Herzenberg et al., 1997). Cysteine (Cys), a sulfur containing amino acid, facilitates crosslinking of bio-macromolecules through the formation of disulfide bonds in a biological system, which is important in both the structure and function of secondary order proteins (Voet and Voet, 1995). In light of the important roles that Cys plays in a variety of fundamental physiological processes in organisms, it is of considerable significance to develop highly sensitive and selective probes for Cys.

Thus far, various efficient and reproducible methods, such as the colorimetric method (J.S. Lee et al., 2008; K.S. Lee et al., 2008; Rusin et al., 2004), fluorescence spectrophotometry (Zhang et al., 2007; Wang et al., 2005; Yuan et al., 2013; Cao et al., 2013; Tang et al., 2013), electrochemistry (Zen et al., 2001; Shahrokhian, 2001; Tseng et al., 2006; Zhao et al., 2003; Hignett et al., 2001), and high performance liquid chromatography (Ozyurek et al., 2012; Xiao et al., 2013) have been developed for the detection of Cys. Among these detection techniques, the fluorescence method is very important due to its distinct advantages of high sensitivity, specificity, and ease of operation (Zhang et al., 2007; Wang et al., 2005; Yuan et al., 2013; Cao et al., 2013; Tang et al., 2013). Therefore, increasing attentions have recently been focused on the design of fluorescent probes for Cys, including those based on organic fluorophores (Tanaka et al., 2004; Lin et al., 2008; J.S. Lee et al., 2008; K.S. Lee et al., 2008; Shibata et al., 2008; Yang et al., 2011), complex (Chen et al., 2007; Huang et al., 2013), and quantum dots (Wang et al., 2011; Negi and Chanu, 2008; Zhang et al., 2009). Although these probes open up new avenues in the development of high-performance sensors for the detection of Cys, it is still a great need for the development of successful nanoprobes for the determination of Cys with not only high sensitivity and selectivity but also low cost in operation.

Inorganic nanoclusters (NCs), especially those made of gold and silver, are a new class of nanomaterials consisting of only tens of atoms. They provide a bridge between the larger metal nanoparticles and the molecular scale characteristics. Compared with common fluorophores such as organic dyes and semiconductor quantum dots (QDs), where practical applications can be limited by relatively poor photostability (for organic fluorophores) or toxicity concerns (e.g., QDs), fluorescent metal NCs are promising

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http://dx.doi.org/10.1016/j.bios.2013.09.036

alternatives for the design of novel fluorescence probes for cysteine (Yuan et al., 2013; Liu et al., 2013; Han and Wang, 2011; Cui et al., 2012; Shang and Dong, 2009).

Recently, Xie's group reports a simple and versatile electrostatically induced phase transfer method for the synthesis of thiol-protected metal NCs (Yuan et al., 2011; Yuan et al., 2012). Here, we employ and modify this method to synthesize Ag nanoclusters with tunable dual-emission intensity at 430 nm and 630 nm by controlling molar ratio between Ag and glutathione (GSH). The tunable dual-emission is ascribed to the tunable particle size and ligand loading amount of GSH-Ag NCs. The dual-emission Ag nanoclusters sensor is applied for rapid and ultrasensitive detection of Cys in aqueous solution and urine. Our method showed better linear ranges or detection limits than the other method. This new method allows detection of Cys with tunable sensitivity and detection window and offers high selectivity for Cys over other amino acids.

2. Materials and methods

2.1. Materials

All chemicals are of at least analytical grade. Silver nitrate ($AgNO_3$), decanoic acid (DA) and tetramethylammonium hydroxide pentahydrate (TMAH) are purchased from Aladdin (Shanghai, China). Methanol (CH_3OH), ethanol (C_2H_5OH), sodium hydroxide (NaOH) and chloroform are purchased from Bodi Chemical Co., LTD (Tianjin, China). L-glutathione reduced (GSH) and toluene are purchased from Biosharp (Japan) and Tianli Chemical Co., LTD (Tianjin, China), respectively. Sodium borohydride ($NaBH_4$) and cetyltrimethyl ammonium bromide (CTAB), L-cysteine and other amino acids are purchased from Sinopharm Chemical Reagent Co., LTD (Shanghai, China). All solutions are freshly prepared before use.

2.2. Synthesis of the Ag NCs in toluene

Aqueous solutions with different molar ratios of Ag (20 mM) to GSH are prepared with ultrapure water. An aqueous solution of $NaBH_4$ (112 mM) is freshly prepared by dissolving 43 mg of $NaBH_4$ in 8 mL of ultrapure water, followed by the addition of 2 mL of 1 M NaOH solution. The addition of a controlled quantity of NaOH to the $NaBH_4$ solution is used to improve the stability of the borohydride ions against hydrolysis. In a typical synthesis of GSH-Ag NCs in aqueous solution, GSH solution (200 μL, 50 mM), $AgNO_3$ solution (125 μL, 20 mM) and $NaBH_4$ solution (50 μL, 112 mM) are added sequentially to water (4.85 mL) under vigorous stirring. The GSH-Ag NCs in aqueous solution (5 mL) are collected after 5 min, followed by the addition of 5 mL of CTAB in ethanol (100 mM). The mixture is stirred for 1 min. Hydrophobic CTAB-protected GSH-Ag NCs are formed. Toluene (5 mL) is then added and stirred continuously for one more minute. The CTAB-protected GSH-Ag NCs are completely transferred to toluene within 5 min. The CTAB-protected GSH-Ag NCs in toluene are incubated at room temperature for 5 h. The aged toluene solution could then produce both strong blue and red emission.

2.3. Phase transfer the Ag NCs from toluene to aqueous solution

Hydrophobic tetramethylammonium decanoate (TMAD) is used to transfer the Ag NCs back to the aqueous phase. Stock methanolic TMAD solution is prepared by dissolving DA (1.7 g) and TMAH (1.8 g) in 100 mL of methanol. In a typical phase transfer process, the Ag NCs in toluene (5 mL) were collected, followed by the sequential addition of chloroform (5 mL), water (5 mL), and TMAD (5 mL). The mixture is stirred for 1 min. The Ag NCs are then transferred back to the aqueous phase. The Ag NCs are stored in the fridge (4 °C) without inert gas protection.

2.4. Sensitivity of the GSH-AgNCs for Cys detection

Cys aqueous solution with different concentrations are freshly prepared before use. Solutions of GSH-AgNCs and Cys with different concentrations are mixed and equilibrated for 2 min before the spectral measurements. The preliminary investigation shows that the interaction could reach equilibrium within 2 min, and further incubation would result in negligible spectral changes.

2.5. Selectivity and interference measurements for Cys

To investigate the selectivity of Ag NCs to cysteine over other α-amino acids, the following amino acids are used: L-Alanine (Ala), L-arginine (Arg), L-asparagine monohydrate (Asn), L-aspartic acid (Asp), L-glutamic acid (Glu), L-glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr), L-valine (Val). A 2 mM stock solution of amino acids is prepared. Then the amino acid solution with appropriate volume is mixed with GSH-AgNCs for the spectral measurements.

2.6. Instruments

The morphologies of the prepared samples are examined using JEM-100SX electron microscope (Nicolet, Japan). Fourier Transform Infra-Red (FTIR) spectra are taken with a spectrum one FTIR spectrophotometer (Perkin-Elmer, America) at room temperature. The UV–vis absorption measurements are performed on Lambda 35 UV analyzer (Perkin-Elmer, America). The fluorescence intensity is recorded on a LS55 fluorescence spectrometer (Perkin-Elmer, America).

3. Results and discussion

3.1. Synthesis and charaterization of dual-emission GSH-Ag NCs

GSH-Ag NCs are synthesized according to reports from Xie's group with slight modification (Yuan et al., 2011, 2012). To have a full understanding of the influence of molar ratio between the Ag and GSH on the synthesis, different ratios of Ag to GSH at 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:8 and 1:10 are applied to synthesize GSH-Ag NCs, respectively. Fig. 1a displays the fluorescence spectra of GSH-Ag NCs in the toluene synthesized with different molar ratio between the Ag and GSH. The fluorescence intensity at 430 nm increases as the molar ratio varied from 1:1 to 1:10, while the fluorescence intensity at 660 nm increases as the molar ratio varied from 1:1 to 1:2, then levels off from 1:2 to 1:10. However, when the ratios of Ag to GSH at 1:1 and 1:2, the fluorescence intensity at 430 nm is very weak. Therefore, the ratio of Ag to GSH at 1:4 is chosen for synthesizing GSH-Ag NCs to get the strong fluorescence emission both at 430 nm and 660 nm. Fig. 1b displays the fluorescence spectra of the returned GSH-Ag NCs in the aqueous phase synthesized with the different ratio of Ag to GSH. The variation tendency of the two emission peaks at 430 nm and 660 nm are in consistent with that of the GSH-Ag NCs in the toluene.

The etching of GSH-Ag NCs in toluene is much slower than in the aqueous phase. The influence of incubation time in toluene on the fluorescence intensity of GSH-Ag NCs is also investigated in Fig. S1. The fluorescence intensity of GSH-Ag NCs at 430 nm (λ_{ex}=350 nm) gradually increases with incubation time from 1 h to 10 h, while the fluorescence intensity of GSH-Ag NCs at 660 nm