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Ultrasensitive and non-labeling fluorescence assay for biothiols using enhanced silver nanoclusters

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ABSTRACT

A label-free, ultra-sensitive and turn-off fluorescence method for detecting cysteine (Cys) has been developed using enhanced DNA-templated silver nanoclusters (DNA-AgNCs) as the fluorescence probe. The method is based on the specific interaction between Cys and DNA-AgNCs *via* robust Ag-S bonds and the fluorescence quenching ability of Cys to DNA-AgNCs. Using this method for Cys assay, it was found that the change of fluorescent intensity has a good linear relationship with Cys concentration in the range from 0.1 nM to 100 nM ($R^2 = 0.991$). The detection limit of Cys was 0.05 nM. Furthermore, the method was successfully used for the detection of Cys in human serums, the result of which was confirmed using clinical colorimetric assay. In summary, our study shows that this simple, sensitive and rapid detection method can be hopefully used for theranostics.

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1. Introduction

As a biological thiol, Cys plays a critical role for its participation in the process of reversible redox reaction, detoxification and metabolism [1]. Many evidences have indicated that the imbalance of the cellular biothiols often resulted in a variety of disease [2]. For example, a lack of Cys can cause retarded growth in children, leukocyte loss, liver damage, hematopoiesis decrease, skin lesions and weakness, whereas excess Cys leads to neurotoxicity [3]. Thus, developing sensitive and specific methods for Cys assay in the human plasma or fluids is in high demand especially for the early diagnosis of a variety of diseases. Until now, a variety of developed methods including chromatography [4,5], capillary electrophoresis [6] and mass spectroscopy [7], have made great contribution for biothiols assay. However, these approaches involve cumbersome laboratory procedures, require expensive and sophisticated instrument in different degree. Furthermore, the relatively low sensitivity of some methods limited their practical application.

Recently, new methods for biomolecules assay, which were based on nanomaterials, have attracted great attention due to their significant advantages. Among of them, DNA strand has become an extremely favorable tool in nanotechnology and material science owing to its remarkable molecular recognition properties and flexible structure [8]. For example, a large amount of phosphate groups, amino groups and heterocyclic nitrogen atoms in DNA molecules offered multiple binding sites for several metal ions to form metallic cluster following the contour of the DNA template [9]. Moreover, the optical and physical properties of these metal clusters can be conveniently tuned by the change of base sequence, temperature and pH [10]. Recently, Yeh et al. synthesized a Ag cluster, the fluorescence intensity of which was enhanced only when placed in proximity to G-rich seqeunces [11]. Compared with the semiconductor quantum dots or dye molecules, this kind of nanomaterial showed low toxicity, ultrasmall size, prominent photostability, good biocompatibility and water solubility. Until now, silver nanoclusters have been widely used in many fields such as biosensing [12–14], molecule image[15–19] and anti-bacteria[20], multiplexed genes analysis [21]; DNA-targeted anticancer drugs *in vitro* [22] and latent fingerprint visualization [23]. However, these reported Ag clusters often exposed their weak anti-inference ability and low sensitivity when they were used for complicated biosample assay due to the weak fluorescence signal. In order to overcome these drawbacks and further widen application of Ag clusters, we synthesized a new Ag clusters nanomaterial with significantly improved fluorescence signal using G-richsequences and further developed a turn-off and label-free approach for biothiols detection.

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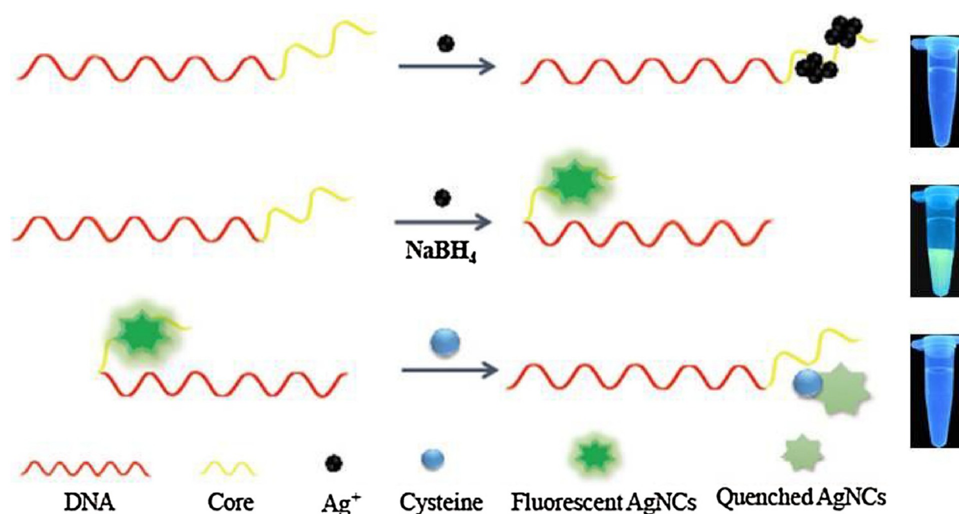

Scheme 1. Schematic illustration of the fluorescence assay for Cys by using DNA-AgNCs.

2. Experimental section

2.1. Materials and regents

The oligonucleotides sequences involved were listed in the Table S1 and all oligonucleotides were synthesized and purified by HPLC (Takara Biotechnology Inc., Dalian). Sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), dibasic sodium phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4), L-Cys and other amino acids were purchased from Sinopharm Chemical Reagent Co., Ltd. All reagents were of analytical reagent grade without further purification. All solutions were prepared using distilled water and stored at 4 °C before use.

2.2. Apparatus

Fluorescence measurements were carried out on the FL-2500 Fluorescence Spectrophotometer (Japan). CD spectra were determined using a MOS-500 Circular Dichroism Spectrometer (France). AFM images were carried out on the bioscope system Atomic Force Microscope (America). UV–vis absorption spectra were recorded by using UV-1800 Ultraviolet spectrophotometer (Japan). Fluorescence life assays were measured on FLS920 single-photon counting (TSCPC) spectrofluorometer (United Kingdom).

2.3. Preparation of fluorescence silver nanoclusters

The ultrasmall DNA-AgNCs were prepared according to the literature reported method [24] with minor modification. The ssDNA-templated silver deposition was synthesized by reduction of $AgNO_3$ with $NaBH_4$ in the presence of DNA. Briefly, DNA was mixed with $AgNO_3$ solution and vortexed for 30 s, then, incubated for 20 min in the dark. Finally, according to the molar ratio Ag:DNA:$NaBH_4$ of 6:1:6, $NaBH_4$ was added to the mixture to reduce Ag ions. The final mixture was kept in the dark for 12 h before use.

2.4. Optimization of temperature and pH

10 μL of 5 μM DNA-AgNCs was added into 89 μL phosphate buffer saline (PBS, pH6.6). The mixture solution was equilibrated for 0.5 h under different temperature or pH. Then, 1 μL of 10 μM Cys was added into the above solutions and incubated 0.5 h under different temperature or pH. Fluorescence intensities of all samples at 535 nm were recorded with the excitation wavelength of 450 nm.

2.5. Detection of Cys

Cys samples with different concentrations were freshly prepared before use. After mixing the 10 μL of 5 μM DNA-AgNCs with 89 μL PBS (pH6.6), the mixture was equilibrated for 0.5 h. Then, different concentrations of Cys was added and incubated for 0.5 h. Fluorescence intensities of all samples at 535 nm were recorded with the excitation wavelength of 450 nm. In the following experiment, other amino acids including alanine (Ala), Cys, leucine (Leu), lysine (Lys), methionine (Met), proline (Pro), serine (Ser), tyrosine (Tyr) and valine (Val) were used to investigate the selectivity of DNA-AgNCs for Cys assay. Meanwhile, the stability of DNA-AgNCs was investigated by monitoring its fluorescence change in 7 days.

2.6. Analysis of human serum samples

Human serum samples were obtained from the third Xiangya Hospital of Central South University. The disulfide bonds of samples were reduced according to the literature [1]. 40 μL of hydrochloric acid (HCl, 0.2 M) and 20 μL of triphenylphosphine (PPh_3) (400 mM in H_2O- acetonitrile;methyl cyanide (CH_3CN) 20:80 v/v and 2 M HCl) were added to 500 μL of plasma and incubated for 15 min at 25 °C to hydrolyze the disulfide bonds. Then, the same volume (500 μL) of CH_3CN was mixed with hydrolyzed plasma to precipitate plasma proteins [25,26] followed by centrifugation at 3000 g for 20 min. The supernatant containing the reduced biothiols was used for further analysis. In the recovery study experiment, Cys solutions with known concentration were added to the samples and the total biothiol concentration was determined combing with the constructed standard curve. Before measurement, the plasma samples were appropriately diluted with PB buffer so as to keep consistence with the dynamic range of our method.

3. Results and discussion

3.1. Strategy for Cys detection

The working principle for Cys assay is represented in the Scheme 1. The detection system consists of DNA- templated AgNCs and detection target of Cys. The synthesis of AgNCs was achieved using a single-stranded DNA containing core sequences as the tem-

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