A Turn-On Fluorescent Sensor for Glutathione Based on Bovine Serum Albumin-Stabilized Gold Nanoclusters

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Received 30 September 2018; Accepted 4 November 2018; Published 2 December 2018

Academic Editor: Neil D. Danielson

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A fluorescence sensor for the detection of glutathione based on the fluorescence recovering of the bovine serum albumin-stabilized gold nanoclusters is reported. This study indicates that glutathione restores the copper-ion-quenched fluorescence by coordinating the bound copper ion in the bovine serum albumin molecule used for stabilizing the gold nanocluster through its sulfydryl. Under the experimental conditions, the fluorescence response showed a linear relationship with the concentration of glutathione over the range from 10⁻⁶ M to 400 µM. The fluorescence sensor successfully detected glutathione in commercial drug products.

1. Introduction

Development of glutathione (GSH) assay methods has received attention due to its diverse functions in organisms and extensive market prospects. GSH, an important non-enzymatic antioxidant, is found in almost all cell types. GSH protects cells from damage of reactive oxygen species such as hydroxyl radical, hydrogen peroxide, and lipid peroxides, directly by eliminating free radicals, and indirectly by serving as a cofactor for glutathione peroxidase [1, 2]. GSH also participates in other physiological processes such as control of cell proliferation and nucleotide metabolism [3, 4]. Based on its essential role in the health of organisms, GSH is used in clinic to treat kinds of diseases such as liver disease and uremia and reduce the side effects correlated with chemoradiotherapy.

Many analytical methods, such as high performance liquid chromatography, capillary electrophoresis, fluorophotometry, and electrochemistry, have been developed for detection of GSH [5–8]. Among these methods, fluorophotometry has advantages over the other techniques at sensitivity, simplicity, and costs. In recent years, fluorescent probes for detection of GSH have been designed and investigated for overcoming the disadvantages of traditional fluorometric assays [9–12]. Although these fluorescent probes successfully detected GSH from various samples, including aqueous solutions, human serum, bovine serum album (BSA), and liposome, they suffered from complicated and tedious synthesis procedures.

Bovine serum albumin-protected fluorescent gold nanoclusters (AuNCs-BSA) reported by Xie et al. have given rise to research interest in sensing applications owing to the advantages of facile preparation, high fluorescence quantum yield (~6%), favorable photostability, and good biocompatibility [13]. Xie’s research group developed a simple label-free method for the selective and sensitive detection of Hg²⁺ based on fluorescence quenching of AuNCs-BSA triggered by Hg²⁺-Au⁺ interactions [14]. Liu et al. reported a AuNCs-BSA-based fluorescent sensor for the recognition and determination of cyanide in aqueous solution, which was based on the fluorescence quenching of AuNCs-BSA induced by the Elnser reaction between cyanide and gold atoms of AuNCs-BSA [15]. Durgads et al. demonstrated the AuNCs-BSA can be used as a selective fluorescence “turn-off” sensor for Cu²⁺ in live cells based on fluorescence quenching of AuNCs-BSA resulting from intersystem crossing of the excited electron from the gold cluster stimulated by the bound Cu²⁺ in the BSA molecule [16]. Their paper also showed that the
copper-ion-quenched emission was reversible with a copper chelator glycine.

A previous study demonstrated that the fluorescence of GSH-capped gold nanoparticles was quenched by Cu²⁺ due to the complexation between Cu²⁺ and GSH [17]. Thus, we assumed that GSH might be able to retrieve the copper-ion-quenched fluorescence of AuNCs-BSA by coordinating Cu²⁺. GSH was found to be much more effective than glycine on restoring the fluorescence quenched by copper ions in our study. Thus, we have developed a fluorescence “turn-on” sensor for GSH based on the AuNCs-BSA-Cu system.

2. Materials and Methods

2.1. Reagents. Glutathione (98%), HEPES (99%), and amino acids (≥98%) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). HAuCl₄ (AR), bovine serum albumin (BR), NaOH (AR), and metal nitrates (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Reduced glutathione for injection and reduced glutathione tablets were from YaoPharma Co., Ltd. (Chongqing, China). Ultrapure water with 18.2 MΩ-cm resistivity was used for preparing the solutions.

2.2. Preparation of AuNCs-BSA. The AuNCs-BSA was synthesized based on a modified Xie method [13]. In brief, HAuCl₄ solution (25 mL, 10 mM) was mixed with BSA solution (25 mL, 30 mg·mL⁻¹) under vigorous stirring at 37°C. Two minutes later, NaOH solution (3 mL, 1 M) was introduced, and the mixture was incubated at 37°C for 12 h.

2.3. Detection of GSH. For fluorescent detection of GSH, varying volumes of 10 mM GSH solutions were mixed with the AuNCs-BSA solution containing Cu²⁺ which was prepared by adding 30 μL 10 mM Cu²⁺ solution to 250 μL AuNCs-BSA solution, and the mixtures were diluted to 5 mL with HEPES buffer (pH=7.2). Fluorescence emission spectra of the as-prepared solutions were measured under 480 nm excitation.

2.4. Sample Preparation. A bottle of reduced glutathione powder for injection was dissolved and diluted to 100 mL with ultrapure water. After four reduced glutathione tablets were ground, the powder was dissolved in ultrapure water and filtered. The filtrate was finally diluted to 100 mL with ultrapure water.

3. Results and Discussion

3.1. Mechanism for Fluorescence Recovering of AuNCs-BSA. The deep brown solution of AuNCs-BSA emits a red fluorescence under 480 nm excitation. The fluorescent emission peak at 648 nm was found to disappear upon addition of 300 μM Cu²⁺. It was further observed that the AuNCs-BSA solution containing Cu²⁺ emitted strong fluorescence again after treatment with of 1.6 mM GSH (Figure 1).

3.2. Optimization of Conditions for GSH Sensing. Concentration-dependent effects of AuNCs-BSA and Cu²⁺ on the detection of GSH were investigated. High concentrations of Cu²⁺ were required for high fluorescence quenching efficiency at high concentrations of AuNCs-BSA, which means low detection sensitivity for GSH. On the other hand, too low a concentration of Cu²⁺ would increase background
fluorescence and narrow the allowing quantitative range of GSH due to low fluorescence quenching ability. In a solution with a total volume of 5 mL, 250 μL AuNCs-BSA and 60 μM Cu²⁺ were finally selected for GSH sensing.

The acid effect on the sensing system was studied over a pH range from 6 to 11. When the pH value increased in the tested range, diminutive change in the fluorescence intensity of AuNCs-BSA was observed, whereas the fluorescence intensity of the AuNCs-BSA in the presence of Cu²⁺ increased, indicating the fluorescence quenching efficiency of Cu²⁺ decreased with increasing of the pH value. It was also observed that the fluorescence recovering efficiency of GSH changed with the pH value. The fluorescence quenching and recovering efficiency are represented with $F_0/F_1$ and $F_2/F_1$ respectively, where $F_0$ and $F_1$ correspond to the fluorescence intensity of the AuNCs-BSA in the absence and presence of Cu²⁺, respectively. $F_2$ represents the fluorescence intensity of the AuNCs-BSA in the presence of Cu²⁺ and GSH. As shown in Figure 2, the fluorescence recovering efficiency of GSH is stabilized and maximized at physiological pH. The HEPES buffer solution was finally employed to adjust the pH of solutions used in the measurement to 7.2.

Time-dependent fluorescence signals of the sensing system were observed. The change in fluorescence properties of AuNCs-BSA in the absence and presence of Cu²⁺ was not obvious within 30 minutes. However, the fluorescence intensity of the AuNCs-BSA in the presence of Cu²⁺ and GSH slowly decreased with time, and thus the fluorescence recovering efficiency decreased with time (Figure 3). Therefore, the fluorescence of the sensing system should be measured immediately upon adding GSH to the solution of AuNCs-BSA in the presence of Cu²⁺.

### 3.3. Selectivity and Sensitivity for GSH Sensing

Although the presence of Pb²⁺, Co²⁺, or Ni²⁺ with the same concentration of Cu²⁺ (60 μM) showed a quenching effect on the fluorescence of the AuNCs-BSA, their quenching efficiencies were much lower than that of Cu²⁺ (Figure 4). The degree of interference of other metal ions, including K⁺, Ca²⁺, Mg²⁺, Zn²⁺, Cd²⁺, Mn²⁺, and Fe³⁺, for the detection of GSH was further investigated. On the basis of a relative error range from -5% to 5% in detecting 50 μM GSH, the tolerance concentrations were as follows: 1 mM for K⁺, Ca²⁺, Mg²⁺, 500 μM for Zn²⁺, Mn²⁺, Cd²⁺, and 100 μM for Fe³⁺. Some amino acids were also used to evaluate the selectivity of the sensing system. As shown in Figure 5, only cysteine could result in significant fluorescence recovery of the AuNCs-BSA, whereas no obvious changes in the quenched fluorescence were observed in the presence of other amino acids such as glycine, lysine, proline, glutamic acid, tryptophan, and phenylalanine at the same concentration of GSH (50 μM).

Under the optimum detection conditions, the relationship between the fluorescence recovering efficiency ($F_2/F_1$) and the concentration of GSH over the range from 10 μM to 400 μM could be expressed by a linear equation ($R^2 = 0.996$).
4. Conclusions

We found that GSH restored effectively the copper-quenched fluorescence from the AuNCs-BSA and therefore develop a new fluorescence "turn-on" sensor for GSH detection. The sensor shows advantages such as fast and sensitive response to GSH, simplicity in preparation and usage, and environmental friendliness. The recovery and precision obtained from commercial GSH drug products indicate the potential application of the GSH sensor.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was supported by Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules, Hubei University (no. 2015-KL-009).

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