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Colorimetric detection of glutathione in cells based on peroxidase-like activity of gold nanoclusters: A promising powerful tool for identifying cancer cells

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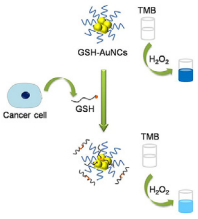
HIGHLIGHTS

- A novel and facile colorimetric assay of cellular glutathione (GSH) level was developed based on the inhibition effect of GSH on the peroxidase-like activity of GSH stabilized gold nanoclusters.
- This assay can be potentially used to identify normal and cancer cells by accurately evaluating cellular GSH level.
- This assay bears some advantages in terms of ease operation, time saving, low cost, and good durability.

GRAPHICAL ABSTRACT



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ABSTRACT

Glutathione (GSH), the most abundant biothiol in cells, not only plays a pivotal role in protective and detoxifying functions of the cell, but also serves as a very important mediator in many cellular functions. Especially, the difference of GSH level between cancer cells and normal cells is regarded as one of most important physiological parameters for cancer diagnosis. It is thereby extremely necessary to develop a simple, sensitive, and reliable analytical method for detection of GSH in cells. On the basis of the inhibition effect of GSH on the peroxidase-like activity of GSH stabilized gold nanoclusters, here a novel and facile strategy for colorimetric detection of cellular GSH level was well established. In this sensing system, GSH can effectively inhibit the oxidation of peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) to produce a blue colored product. Under the optimized conditions, the absorbance at 652 nm against GSH concentration shows a linear relationship within a range from 2 to 25 μM with detection limit of 420 nM. This excellent property allows our approach to be used to accurately evaluate the cellular GSH levels, and it is revealed that the overall GSH level in cancer cells was much higher than that in normal cells. The presented assay will enable a powerful tool for identifying cancer cells in a simple manner for biomedical diagnosis associated with GSH.

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1. Introduction

Low molecular weight biothiols such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), play crucial roles in

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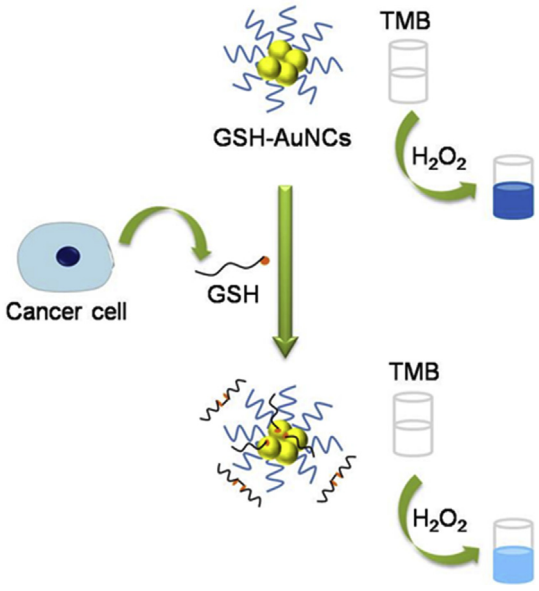
maintaining the appropriate redox status in cellular systems through scavenging reactive oxygen species and against many toxins, mutagens, and drugs [1,2]. Glutathione (GSH) is the most abundant biothiol in cells, which consists of a tripeptide of glutamic acid, cysteine, and glycine [3]. GSH not only plays a pivotal role in protective and detoxifying functions of the cell, but also serves as a very important mediator in many cellular functions [4]. In particular, as one of the most essential endogenous antioxidants, its abnormal level is closely associated with diseases including aging and cancers [5]. As is reported that GSH concentration in cancer cells is much higher than that in normal cells [6–10], it may offer an opportunity to assess whether it is a cancer cell or a normal cell, which holds great promise for cancer diagnosis at an early stage.

A simple, efficient, and reliable method to evaluate cellular GSH levels is thereby of significant importance for cancer diagnosis and other biomedical applications. To date, a variety of analytical methods including high-performance liquid chromatography [11,12], spectrophotometry [13,14], electrochemistry [15–17], and fluorescent approaches [18–20], have been established for the determination of GSH. However, these presented assays have their limitations, such as complex and expensive instruments, time-consuming laboratory operations, and/or complicated synthetic procedures, which are disadvantageous for the development of a practical method. Some commercial GSH kits based on enzyme-mediated cascade reactions have also been developed [13]. Though widely employed in practice, enzyme inactivity still remains the issue to be mainly focused on. Thus, it is still necessary to develop a feasible approach that enables cellular GSH evaluation in a straightforward way.

As a significant colorimetric tool, artificial enzyme mimetics is a current research interest for the detection of targets [21–24]. Natural enzymes have the susceptibility to environmental conditions and high costs in preparation and purification, which largely limits their applications. Therefore, tremendous efforts have been made to develop inorganic nanomaterials as enzyme mimetics due to their advantages over conventional natural enzymes including low cost, high stability, and ease of preparation [25,26]. Noble metal nanoclusters (NCs) comprising several to tens of atoms have gathered enormous attention in recent years. Because of their distinct electronic structures and properties such as ultrasmall size, large stokes shift, and good biocompatibility and photostability, the NCs show great potential as the candidate in many applications [27,28]. While most of analytical applications of the NCs focus on their fluorescence properties, little attention has been paid to their catalytic properties [21,29]. Therefore, it is very important to explore new sensing platforms based on the catalytic activity of the NCs.

In this paper, we report a novel and simple strategy for colorimetric detection of cellular GSH level based on peroxidase-like activity of GSH stabilized gold NCs (GSH-AuNCs) (Scheme 1). The AuNCs can catalyze the oxidation of the peroxidase substrate 3,3′,5,5'-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a blue-colored reaction, however, GSH can inhibit such reaction very efficiently, resulting in a blue color fading. On the basis of this, the proposed method enabled us to develop a facile and fast way for colorimetric detection of GSH with the naked eye. Very importantly, we successfully applied it to evaluate the cellular GSH levels, which revealed that cellular GSH level in cancer cells was higher than that in normal cells. This approach may pave a new route to identifying cancer cells in a simple fashion for related biomedical diagnosis.



Scheme 1. Schematic diagram of colorimetric detection of GSH in cancer cells based on the inhibition effect of GSH on the peroxidase-like activity of GSH-AuNCs.

2. Experimental section

2.1. Chemicals and reagents

Chemicals were of analytical grade and used without further purification. Hydrogen tetrachloroaurate hydrate ($HAuCl_4 4H_2O$), 3,3′,5,5'-tetramethylbenzidine (TMB), 30% H_2O_2, and glutathione (GSH) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Amino acids including L-Phenylalanine (Phe), L-Alanine (Ala), L-Methionine (Met), L-Glycine (Gly), L-Glutamic (Glu), L-Glutamine (Gln), L-Lysine (Lys), L-Arginine (Arg), L-Tyrosine (Tyr), L-Proline (Pro), L-Tryptophane (Try), L-Serine (Ser), L-Threonine (Thr), L-Asparagine (Asp), L-Valine (Val), L-Isoleucine (Ile), L-Histidine (His), L-Cysteine (Cys) and L-Homocysteine (Hcy) were all bought from Shanghai Jingchun Technology Co. Ltd. (Shanghai, China). NaAc, $ZnCl_2$, and glacial acetic acid were obtained from Shanghai Qingxi Technology Co. Ltd. (Shanghai, China). The commercial GSH assay kit was purchased from Shanghai Beyotime Biotechnology Co. Ltd. (Shanghai, China). The pH value of the solution was adjusted by mixing different volume ratios of NaAc and HAc. All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm^{-1}).

2.2. Apparatus and measurements

UV–vis absorption spectra were conducted at room temperature on a UV-2550 spectrophotometer (Shimadzu, Japan). Absorbance of the oxidized TMB (oxTMB) at 652 nm was monitored for quantitative analysis.

2.3. Synthesis of GSH-AuNCs

GSH-AuNCs were prepared according to a literature procedures [30]. Briefly, the freshly prepared aqueous solution of $HAuCl_4$ (4 mM, 10 mL) was added into a 6 mM (10 mL) GSH aqueous solution, and the mixture was under gentle stirring at 90 °C for 6.50 h. Yellow-colored and orange-emitting Au clusters were obtained. The resulting AuNCs solutions were purified by the acetonitrile with a centrifugation at 1200 rpm for 10 min, and the remaining

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