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Visual detection of melamine based on the peroxidase-like activity enhancement of bare gold nanoparticles

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ABSTRACT

In this study, a facile method to sensitively detect melamine and highly improve the peroxidase-like activity of bare gold nanoparticles (Au NPs) at the same time is proposed for the first time. It is interesting to find that the addition of melamine could improve the peroxidase-like activity of Au NPs. By coupling with 3,3′,5,5′-tetramethylbenzidine (TMB)–H_2O_2 chormogenic reaction, a novel method for colorimetic detection of melamine is developed. The detection limit of this method is as low as 0.2 nM with the help of UV–vis spectroscopy and 0.5 μM by naked-eye observation, both which are far below the US food and Drug Administration estimated melamine safety limit of 20 μM. In addition, the present method is successfully applied for the detection of melamine in raw milk and milk powder. More importantly, the proposed method could also improve the peroxidase-like activity of Au NPs, which may not only provide a new approach to develop effective nanomaterials-based mimetic enzyme, but also irradiative to develop new applications for Au NPs in varieties of cost-effective and simple sensors in medicine, biotechnology and environmental chemistry.

1. Introduction

Recently, tremendous efforts have been made to develop nanoscaled peroxidase mimics since the first Fe_3O_4 magnetic nanoparticles-based peroxidase was reported (Gao et al., 2007). A variety of nanomaterials, such as Au NPs (Jv et al., 2010; Wang et al., 2012), Pt nanoparticles (Gao et al., 2013), Ag nanoparticles (Jiang et al., 2012), BSA-stabilized Au clusters (Wang et al., 2011), carbon-based nanomaterials (Shi et al., 2011; Tian et al., 2013; Zheng et al., 2013), Co_3O_4 nanoparticles (Mu et al., 2012), $CoFe_2O_4$ nanoparticles (Fan and Huang, 2012; Hao et al., 2013), V_2O_5 nanowires (André et al., 2011), CuO nanoparticles (Chen et al., 2012a), etc., have been also demonstrated to possess intrinsic peroxidase-like activity. These peroxidase-like nanomaterials have shown the obvious advantages of simple preparation, good stability and low cost compared to natural enzyme (Wei and Wang, 2013). However, to one's disappointment, some of these peroxidase mimics have low catalytic activity (Long et al., 2011). Thus, it is highly necessary to improve the peroxidase-like activity of these nanomaterials.

Melamine, with a chemical formula of $C_3H_6N_4$, has been extensively used in the production of melamine formaldehyde resins for manufacturing plastics, coatings, adhesives, laminates and commercial filters (Cao et al., 2010). In the past few years, melamine was illegally added into human and animal food to increase apparent protein content due to its high nitrogen level (66% by mass). Although melamine has slight acute toxicity, the combination of melamine with its hydrolysate, cyanuric acid, results in insoluble crystals in the renal tubules, which may cause the renal failure and even death (Vasimalai and John, 2013; Zhu et al., 2009). Therefore, there is an urgent need to develop a simple, reliable, and low-cost method for the analysis of trace melamine in real samples with high sensitivity and selectivity.

Nowadays, a number of detection techniques have been utilized for the detection of melamine, such as mass spectrometry (MS) (Yang et al., 2009), gas chromatography/mass spectrometry (GC–MS) (Xu et al., 2009), liquid chromatography/mass spectrometry (LC–MS) (Filigenzi et al., 2008), capillary electrophoresis (Chang et al., 2010), surface-enhanced Raman scattering spectrometry (SERS) (Sun et al., 2013; Xu et al., 2011), matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) (Tang

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et al., 2009) and enzyme-linked immunosorbent assay (ELISA) (Liu et al., 2010). Though these techniques have high sensitivity, they suffer from the disadvantages of high costs, time-consuming sample pretreatment and sophisticated instruments. Consequently, there is a great need to develop sensitive, simple and low-cost methods for the detection of melamine.

Herein, we propose a new method to sensitively detect melamine and significantly improve the peroxidase-like activity of Au NPs at the same time. The Au NPs can conjugate with melamine to form Au NPs–melamine aggregates which could selectively improve the ability of bare Au NPs to catalyze 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 and lead to the significant enhancement of the absorbance. Therefore, melamine concentration-dependent enhancement of Au NPs catalytic activity provides a method for the semi-quantitative detection of melamine via visual observation and quantitative detection of melamine via absorbance enhancement. The proposed method shows high selectivity and sensitivity for the detection of melamine. Moreover, the proposed method could also improve the peroxidase-like activity of Au NPs, which is not only helpful to develop effective nanomaterials-based mimetic enzyme, but also irradiative to develop new applications for Au NPs in varieties of cost-effective and simple sensors in medicine, biotechnology and environmental chemistry.

2. Experimental

2.1. Chemicals and materials

$HAuCl_4 \cdot 4H_2O$ was obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). TMB and acetic acid (HAc) were purchased from Aladdin Reagent Company (Shanghai, China). Thymine was supplied by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Melamine, benzoquinone and $NaBH_4$ were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Vitamin C, glycine, L-cystenine and L-lysine were purchased from Sigma-Aldrich. Glucose, lactose, $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, $Zn(NO_3)_2 \cdot 6H_2O$, H_2O_2, *t*-butyl alcohol and sodium acetate (NaAc) were obtained from Beijing Chemical Reagent Company (Beijing, China). All of these reagents were analytical grade and used as received. Ultrapure water produced by a Milli-Q system was used throughout this work.

2.2. Synthesis of bare Au NPs

All glassware used in the following procedure was soaked in newly prepared aqua regia for 12 h and rinsed thoroughly with water prior to use. The bare Au NPs were prepared according to a reported method with a minor modification (Patil et al., 1999). Firstly, 250 μL of 48.6 mM $HAuCl_4$ solutions was diluted using 39.75 mL of water, and then 1 mL of 1% $NaBH_4$ was injected within 5 min under vigorous stirring. Finally, the mixed solution was stirred in the dark for 1 h.

2.3. Detection of melamine

A stock solution of melamine (10 mM) was prepared in water and various concentrations of melamine were obtained by serial dilution of the stock solution. For the detection of melamine, 160 μL 2.1×10^{-8} M of Au NPs was firstly pipette into a 1.5 mL vial, and then 10 μL of melamine solutions with different concentrations, 330 μL of 0.05 M NaAc buffer solutions (pH 4.5), 300 μL of 8.3 mM TMB and 200 μL of 1.4 M H_2O_2 were added sequentially. After that, the mixture was mixed thoroughly and transferred for UV–vis scanning after incubating for 10 min at 25 °C.

To investigate the effect of melamine on the peroxidase-like activity of Au NPs, 800 μL of 2.1×10^{-8} M Au NPs was firstly pipette into a 1.5 mL vial, and then 50 μL of 0.05 mM melamine was added. The mixture was centrifugated at different speeds for 10 min. Then, 170 μL of the supernatant, 330 μL of 0.05 M NaAc buffer solutions (pH 4.5), 300 μL of 8.3 mM TMB and 200 μL of 1.4 M H_2O_2 were added sequentially into a 1.5 mL vial. After that, the mixture was mixed thoroughly and transferred for UV–vis scanning after incubating for 10 min at 25 °C.

2.4. Treatment of raw milk and milk powder

Milk samples were prepared following a previous method with a minor modification (Li et al., 2013). Briefly, 5.0 mg of milk powder or 5.0 mL of raw milk was placed in a 7 mL centrifuge tube, and 1.5 mL of 2 M trichloroacetic acid was introduced. After ultrasonication for 10 min, the mixture was centrifuged at 10,000 rpm for 10 min. The supernatants were adjusted to pH 7.0 with NaOH, filtered with 0.22 μm membrane and diluted 25-fold before use.

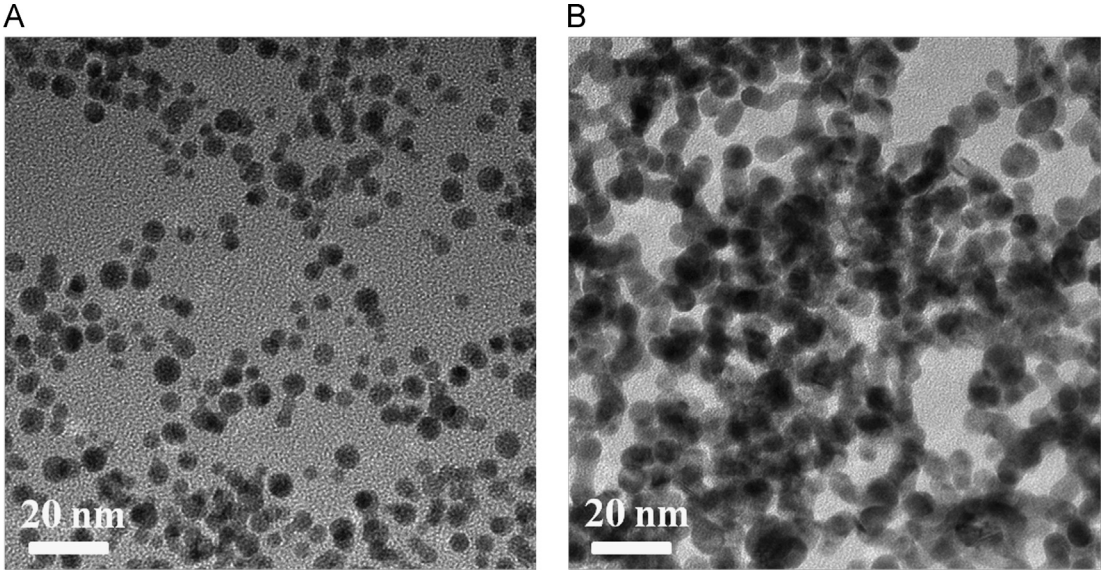

Fig. 1. (A) TEM images of the Au NPs (A) and Au NPs in the presence of 6 μM melamine (B).