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A redox-mediated chromogenic reaction and application in immunoassay☆

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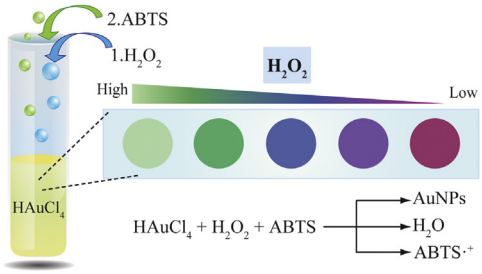
HIGHLIGHTS

- A novel kind of chromogenic reaction was demonstrated which could be mediated by redox substance.
- The mechanism based on the reaction between HAuCl4 and ABTS.
- It has been successfully adapted to the sensitive detection of both catalase and disease biomarkers

GRAPHICAL ABSTRACT



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ABSTRACT

A novel redox-mediated chromogenic reaction was demonstrated based on the reaction between $HAuCl_4$ and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), which generate various color responses from red to green in the resulting solutions. Various redox substance could be used to mediate the reaction and trigger a distinct color response. We established a sensitive hydrogen peroxide colorimetric sensor based on the redox-mediated chromogenic reaction and depicted the application both in detection of enzyme and in an immunoassay. Combining the traditional chromogenic reagent with gold nanoparticles, our assay has the advantage in short response time (within three minutes), high sensitivity (10^{-12} g mL^{-1} for HBsAg) and stability.

1. Introductions

Colorimetric assay has been widely applied to detect metal ions [1,2], and [3], anions [4], biomolecules [5], disease biomarkers [6–9], and [10], etc. due to its simple and convenient readout by the naked eye. Compared with other detection methods, no advanced instruments or skilled operators are needed in colorimetric detection, making it an attractive and affordable detection method around the world [11]. To achieve this sensitive detection by colorimetric assay, the selection of the chromogenic reagent or reaction as well as the effective transformation of the detection events into color change, is of vital importance [12].

In recent years, besides traditional chromogenic reagents such

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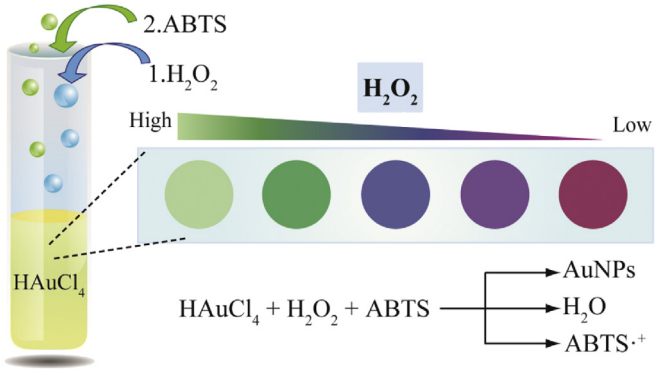
as TMB (3,3′,5,5′-tetramethylbenzidine) [13,14], and [15] and ABTS, gold nanoparticles have been rapidly developed as a new member of chromogenic reagents, providing a more sensitive method for colorimetric detection [16] and [17]. The gold nanoparticle-based colorimetric assays are attributed to the aggregated process [18–20], and [21], where a red-to-blue color variation is performed through the transformation of detection target-induced molecular events, thus could be distinguished by the naked eye. Improper external factors would cause the undesirable aggregation of gold nanoparticles which would resulted in unreliable results.

Herein, we reported a redox-mediated chromogenic reaction based on the mixture of $HAuCl_4$ and ABTS, which could generate gold nanoparticles and $ABTS^{\cdot+}$, thus inducing various color responses from red to green in the resulting solutions. Though the reaction between $HAuCl_4$ and ABTS has been reported [22], the mediation of this reaction by redox substance and the applications in enzyme or biomarkers have not been reported yet. It is noteworthy that the resulting color was dependent on the dominance of generated gold nanoparticles or $ABTS^{\cdot+}$. Combining the traditional chromogenic reagent with gold nanoparticles, our assay has the advantage in short response time (within three minutes), sensitivity and stability. To the best of our knowledge, this is the first example in which two different chromogenic reagents are used together to realize a series of color responses. Moreover, this kind of redox-mediated chromogenic reaction could be tuned via the addition of other reagents, for instance, hydrogen peroxide or NADH (nicotinamide adenine dinucleotide), making it a potential to broaden applications in many fields (Scheme 1). For example, the addition of hydrogen peroxide at high concentrations could lead to a green-colored solution whereas the low concentrations of hydrogen peroxide could result in a red-colored solution. Once the detection events induce the decrease of hydrogen peroxide, the resulting color could thus turn green to red, therefore establishing a new colorimetric method.

2. Experimental

2.1. Reagents

All reagents were used as received, without further purification. Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, >99.0%), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt (ABTS, 98%), hydrogen peroxide (H_2O_2, 30%), sodium phosphate monobasic (NaH_2PO_4, 99%), and sodium phosphate dibasic (Na_2HPO_4, 99%) were obtained from Aladdin. β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH, ≥97%), peroxidase from horseradish (HRP), catalase (CAT), albumin from bovine serum (BSA) were purchased from Sigma-Aldrich. Antigen (Hepatitis B surface antigen, HBsAg) and antibodies (goat IgG and mouse IgM) used here were obtained from Shanghai Yemin Biotech Inc. [SM(PEG)$_{24}$] (24-unit ethylene glycol functionalized with succinimidyl and maleimido ends) and desalting columns were obtained from Thermo Scientific. Streptavidin and biotinylated secondary antibody (anti-mouse IgG(H + L)) were obtained from Jackson Immuno Research Laboratories, Inc. Label free second antibody (goat anti-mouse IgG(H + L)) was purchased from Abcam. Polystyrene plate was obtained from Corning Incorporated. Ultrapure water system (Millipore) was used to produce ultrapure water for the preparing of solutions.



Scheme 1. Schematic and equation of redox-mediated chromogenic reaction tuned by H_2O_2. Different concentrations of H_2O_2 were firstly added to avoid finish of the reaction between $HAuCl_4$ and ABTS.

2.2. Novel chromogenic reaction of $HAuCl_4$ and ABTS

1% (wt) $HAuCl_4 \cdot 3H_2O$ was prepared by adding 1 g $HAuCl_4 \cdot 3H_2O$ to 100 mL H_2O. The prepared $HAuCl_4 \cdot 3H_2O$ was added (100 μL) to the wells at different concentrations of 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.002%, 0.001%. After the addition of 100 mL H_2O, we finally added 5 μL of ABTS (4 mM). Photographs and UV–Vis spectra were illustrated the resulting color variation.

2.3. H_2O_2-mediated chromogenic reaction

0.1% (wt) $HAuCl_4 \cdot 3H_2O$ was prepared and added to the wells. Hydrogen peroxide was diluted at different concentrations of 0.5 mM, 0.4 mM, 0.3 mM, 0.2 mM, 0.1 mM, 0.09 mM, 0.08 mM, 0.07 mM, 0.06 mM, 0.05 mM, 0.04 mM, 0.03 mM, 0.02 mM, 0.01 mM, 0.001 mM, 0.2 mM and added to the wells. Finally, 5 μL of 4 mM ABTS was added. The last well was set as a control without adding ABTS. Photographs and UV–Vis spectra were illustrated the resulting color variation.

2.4. Catalase detection with H_2O_2-mediated chromogenic reaction

Different concentrations of catalase (5 μL) was added to 0.2 mM hydrogen peroxide (100 μL, 10 mM PBS without sodium chloride), thus making the final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 0 g mL^{-1}, 37 °C, 30 min. Then, 0.1% $HAuCl_4$ (100 μL) and 4 mM ABTS (5 μL) was added to the wells. Photographs and UV–Vis spectra were illustrated the resulting color variation.

2.5. Conjugation of streptavidin to catalase

First, 1 mL streptavidin (1 mg mL^{-1}, dissolved in PBS) was activated by 4 μL [SM(PEG)$_{24}$] (250 mM in DMSO) for 30 min at room temperature. Then desalting column was used to removed the excess linker ([SM(PEG)$_{24}$]). 5 mg catalase was added to the functionalized streptavidin and the mixed solution was kept at 4 °C for 10 h. The streptavidin-catalase conjugate was thus obtained and stored at 4 °C before use. In the conjugation, the concentration of catalase was higher than streptavidin and the concentration of streptavidin-catalase could be approximately regarded the same as streptavidin (2.0×10^{-5} M). The excess catalase could be completely removed in ELISA detection through steps of washing.

2.6. Verification of streptavidin-catalase conjugate

Biotinylated and label-free anti-mouse IgG(H + L) (this kind of secondary antibody has specificity to the whole primary antibody) were added in polystyrene plate and incubated for 1 h at room temperature. The plate was washed three times with PBS and then 1 mg mL^{-1} BSA was fully added into each well for blocking. After steps of washing the streptavidin-catalase conjugate was added at