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Colorimetric detection of urea, urease, and urease inhibitor based on the peroxidase-like activity of gold nanoparticles

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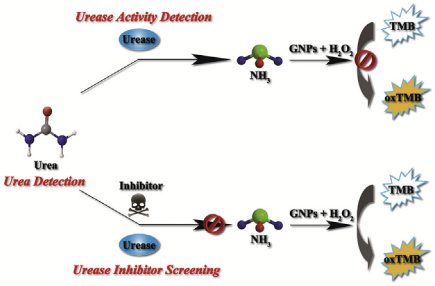
HIGHLIGHTS

- GNPs-catalyzed TMB-H_2O_2 reporting system is used as an ultrasensitive pH indicator.
- A_{450} exhibits a linear fashion over the pH range of 6.40–6.60.
- A platform is established for the detection of urea, urease, and urease inhibitor.

GRAPHICAL ABSTRACT



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ABSTRACT

Herein, we reported for the first time that gold nanoparticles-catalyzed 3,3′,5,5′-tetramethylbenzidine-H_2O_2 system can serve as an ultrasensitive colorimetric pH indicator. Gold nanoparticles acted as a catalyst and imitated the function of horseradish peroxidase. The absorbance at 450 nm of the yellow-color product in the catalytic reaction exhibited a linear fashion over the pH range of 6.40–6.60. On the basis of this property, we constructed a novel sensing platform for the determination of urea, urease, and urease inhibitor. The limit of detection for urea and urease was 5 μM and 1.8 U/L, respectively. The half-maximal inhibition value IC_{50} of acetohydroxamic acid was found to be 0.05 mM. Urea in human urine and urease in soil were detected with satisfied results.

1. Introduction

Urea and urease play a significant role in the development of chemistry and biochemistry. Urea is widely distributed in living species and it is the main nitrogen component of urine and the end-product of protein metabolism, so urea determination are very important in food industry, environmental monitoring and clinical

Abbreviations: GNPs, gold nanoparticles; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine; AHA, acetohydroxamic acid; PB, phosphate buffer.

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chemistry [1,2]. Urease (urea amidohydrolase, E.C.3.5.1.5) can specifically hydrolyze urea to ammonia, a basic molecule, leading to the rise of medium pH. Urease is the molecule distinct in the development of heme protein chemistry and enzymology. Medically, bacterial ureases are most often the mode of pathogenesis for some clinical conditions. In agriculture, high urease activity generally causes serious environmental and economical problems. Urease inhibitors have been studied extensively because of their potential uses such as therapy against bacterial urease, protecting soil from pH elevation and loss of nitrogen, and understanding of enzyme kinetics [3]. Although many analytical approaches have been utilized to the detection of urea, urease, and urease inhibitor, the design of reliable methods with sensitivity, simplicity, and low-cost is still appealing.

Recently, Fe_3O_4 nanoparticles have been found to possess intrinsic enzyme mimetic activity similar to that of naturally occurring horseradish peroxidase (HRP), which can catalyze the oxidation of peroxidase substrate by hydrogen peroxide to form colored products [4]. Since then, nanoscaled peroxidase mimetics such as cupric oxide nanoparticles [5], silver nanoparticles [6], platinum nanoparticles [7], carbon nanotube [8], V_2O_5 nanowires [9], grapheme oxide [10], and WS_2 nanosheets [11] have been reported. These nanomaterials-based peroxidase mimetics (peroxidase nanomimetics) exhibit enormous advantages over HRP in terms of low cost, good stability, design flexibility, simple preparation, easy modification and long-term storage [12]. However, their analytical applications are now still very limited, generally for the detection of H_2O_2 and the relative analytes. Over the past few years, much effort has been dedicated to broadening their use. To this end, many studies have suggested that target-induced shielding against or target-stimulated enzyme mimetic activity of peroxidase nanomimetics is an effective way, and based on this effect, quite a few approaches have been successfully developed for determination of DNA [13], nuclease [14], kanamycin [15], dopamine [16], pesticide [17], Hg^{2+} [18,19], Pb^{2+} [20], and S^{2-} [21]. Besides, target-induced aggregation or anti-aggregation of nanomaterials has also been demonstrated to be a good alternative for extending their potential applications [8,22,23]. Until now, searching new outlets for the use of peroxidase nanomimetics is still of great importance and a challenging topic for researchers.

3,3′,5,5′-tetramethylbenzidine (TMB) is a popular chromogenic substrate of peroxidase and usually employed to evaluate the peroxidase-like activity of nanomaterials. Similar to HRP, the catalytic activity of peroxidase nanomimetics is closely related to the environmental pH value. Typically, the catalytic activity is higher in acidic condition than that in neutral or basic condition, resulting from the acid-promoted colorimetric reaction of TMB [24]. Such feature makes nanomaterials-mediated TMB chromogenic reaction as a promising tool for pH sensing. More recently, gold nanoparticles (GNPs) were discovered for their ability to oxidize the peroxidase substrate TMB in the presence of H_2O_2 to generate colored product [25]. Our previous study revealed that the superficial gold atom plays a dominant role in the observed peroxidase-like activity, confirming the activity is indeed contributed by GNPs [26]. In this study, GNPs-catalyzed TMB-H_2O_2 reporting system was utilized as an ultrasensitive colorimetric pH indicator. The absorbance at 450 nm (A_{450}) of the yellow-color product in the catalytic reaction exhibits a linear fashion over the pH range of 6.40–6.60. This new-constructed colorimetric pH sensor is ultrasensitive ($\Delta pH \leq 0.2$ between ON/OFF states) and more importantly, the pH-responsive range is within the range of physiological dimension, showing great potential application in biosensing for urea, urease, urease inhibitor, glucose (by following gluconic acid produced by the glucose oxidase cascade) and so on.

2. Materials and methods

2.1. Materials

All chemicals and solvents were of analytical grade and commercially available. TMB, $HAuCl_4 \cdot 4H_2O$, urea, and acetohydroxamic acid (AHA) were obtained from Aladdin Reagent Company (Shanghai, China). Urease (34.31 U/mg) was purchased from Sigma–Aldrich (Shanghai, China). Trisodium citrate dihydrate, H_2O_2 (30%, wt.), and H_2SO_4 were brought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). TMB was dissolved in dimethyl sulfoxide solution (3%, v/v). To avoid the possible interference from metal ions, deionized water was used throughout experiments.

2.2. Apparatus

UV–visible absorption spectra were measured on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). A 1 mL-capacity cuvette of 1 cm path length was used to measure absorbance and deionized water was employed as the reference solution in the

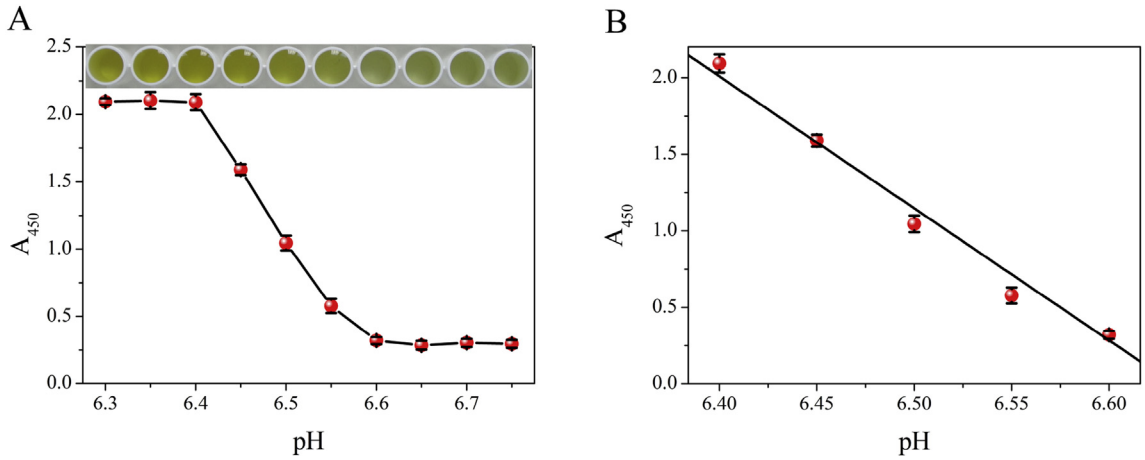

Fig. 1. (A) The plot of A_{450} versus pH value. Inset: visual observation of the corresponding color changes in white microplate. (B) Linear relationship between A_{450} and pH value. Error bars represent standard deviations of three repeated experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)