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Accelerating peroxidase-like activity of gold nanozymes using purine derivatives and its application for monitoring of occult blood in urine

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ABSTRACT

Gold nanoparticles (AuNPs) is an important kind of nanozymes and a variety of its artificial enzymatic activities have been reported, such as oxidase, peroxidase, and superoxide dismutase. The DNA with poly purine-modified AuNPs shows an enhancement peroxidase activity compared with poly pyrimidine-modified AuNPs. In this work, purine derivatives are modified on the surface of AuNPs. The peroxidase-like activity of AuNPs is dependent on the chemical structure of the molecules capped on the surface. We find that ferric ions can accelerate the mimic enzymatic ability of 2, 6-diaminopurine (DAP) modified AuNPs. The enhanced catalytic activity comes from the synergistic reaction of AuNPs and ferric ions. The cooperative system can be applied to measure hemoglobin and red blood cells in urine with better sensitivity. Generally, there is no or few red blood cells in human urine. The presence of blood in the urine is closely related with serious diseases, for example chronic nephrotic syndrome and urinary system tumors. Compared the commercial urine test paper, the method based on DAP-AuNPs has a good sensitivity and wider quantitative range and will be helpful to do urine test at home.

1. Introduction

Researchers explore artificial enzymes as stable and low-cost alternatives to natural enzymes in a wide range of appliations [1–6]. A variety of materials including cyclodextrins [7], metal complexes [8], porphyrins [9], polymers [10], dendrimers [11] and biomolecules [12] have been extensively explored to mimic the structures and functions of enzymes. With the development of nanotechnology, the nanomaterial-based artificial enzymes (nanozymes) have already found wide applications in numerous fields, including chemical analysis, catalysis, biosensing, pollutant removal, cancer diagnostics and therapy [1,5,13–17]. Among these nanozymes, gold nanoparticles (AuNPs) is an important kind of nanozymes and a variety of its artificial enzymatic activities have been reported, such as oxidase, peroxidase, and superoxide dismutase [18]. Particularly, its peroxidase-like activity has attracted a significant attention for biochemical studies [1,19,20].

AuNPs show many applications in different fields, for example chemical sensors, immunoassays, diagnosis and industrial catalysts due to its good physical and optical properties [1,21–24]. The surface chemistry of AuNPs is related with its application. Generally, Gold has strong interaction with sulfur, nitrogen and oxygen. So the surface of AuNPs is likely to absorb various components to reduce surface energy, and at the same time these adsorbed species can be easily displaced [25]. AuNPs can be modified with various compounds, especially the ones with S, N and O, like organic molecules, DNA, aptamer, polymers, and dendrimers [23,26–29]. Since the citrate-capped AuNPs are reported to have peroxidase activity, DNA, proteins, and amino acids capped AuNPs are discussed to show different enzymatic activities [25,26,31]. Peroxidase-like ability of AuNPs has attracted a significant attention for biochemical studies. Recently, some literatures are focus on the discussion of the surface chemistry of AuNPs, and figure out how the surficial ligands influent the native peroxidase-like activity. Among these researches, DNA capped AuNPs are studies carefully, because oligonucleotides are ordinary biopolymers with multi-function due to their targeting, biocompatibility, structure-switching and the strong interaction on the interface [25,26,30–32]. While, the effect of DNA for the enzymatic activity of AuNPs is not unique. Some studies have demonstrated that DNA inhibits the enzymatic activity due to the physical hindrance or electrostatic repulsion [33–35]. On the contrary, DNA at the nanointerface enhance the enzymatic activity of the nanozymes are also reported [26,36,37]. Some data have identified that adenine has the stronger adsorption of the surface of metal (gold, silver and copper),

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and show more stable bonding compared with the pyrimidine derivatives [30,38,39]. The DNA with poly purine-modified AuNPs shows an enhancement peroxidase activity compared with poly pyrimidine-modified AuNPs [26]. The lengths of DNA shows no obvious effect on the enzyme activity of AuNPs. On the basis of the fore mention research, we think that purine structure will be helpful to enhance the enzyme activity of AuNPs. Here, we apply five purine derivatives to modify AuNPs on the surface, and value the peroxidase activity of these nanozymes. Among them, 2, 6-diaminopurine (DAP) modified AuNPs display the best enzymatic activity. 2, 6-Diaminopurine have two amine groups outside of the purine ring, and can stable AuNPs more well. Moreover, we find ferric ions can accelerate the peroxidase-like activity of DAP-capped AuNPs dramatically. This exploration triggers a quick new method to monitor red blood cells (RBC) in urine. The presence of blood in the urine is closely related with traumatic damage to the kidneys or the genitourinary organs. So the monitoring of occult blood in urine is important. Our system shows a better sensitivity and wider quantitative range of RBC compared with the commercial test paper, and will be helpful for diagnosis and monitor.

2. Experiment section

2.1. Reagents

H_2O_2 (30%), 2,6-diaminopurine (DAP), 6-mercaptopurine (MP), adenine (AD), purine (PU), 6-hydrazine (HY) bovine serum albumin (BSA), globulin, glucose, creatinine, glycine, histidine, lysine, serine, taurine, urea and uric acid were purchased from Beijing Kehuajingwei Scientific (Beijing, China) Co. Ltd. $NaBH_4$, Tween 80, $HAuCl_4$, dialysis bag, $NaCH_2COOH$ (NaAc), CH_3COOH (HAc) and $FeCl_2$ were purchased from Beijing InnoChem Science & Technology (Beijing, China) Co. Ltd. Hemoglobin, 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) and 3,3′,5,5′-Tetramethyl -benzidine (TMB) were purchased from Sigma-Aldrich. Artificial urine is purchased from Dongguan Kehong Chemical Reagent Co. Ltd. All other chemicals are analytical reagent grade and used without further purification. Deionized water is used throughout the experiment.

2.2. Apparatus and characterization

A U-3900/3900H spectrophotometer was used to implement kinetic experiments and scan spectrum. A Tecnal $G^2$20 S-TWIN transmission electron microscope (TEM) was used for measuring the morphologies. Zeta potential measurements of AuNPs were performed with a Malvern Nano-ZS apparatus. X-ray Photoelectron Spectroscopy (XPS) patterns were obtained by ESCALAB 250 X-ray photoelectron spectrometer. ICAP 6000 SERIES spectrometer was used for inductively coupled plasma (ICP) test. Fourier transform infrared spectrometer (FTIR) spectra was characterized by Nicolet 6700. DTG-60A was used for thermogravimetric analysis (TGA). JES-FA200 ESR Spectrometer was used for analyzing hydroxyl radicals.

2.3. Preparation of 2, 6-diaminopurine modified AuNPs

2, 6-Diaminopurine modified AuNPs were prepared using a liquid-phase synthesis method. Briefly, 2,6-diaminopurine (22.5 mg, 150 μM) was dissolved in deionized water (50 mL) in an ice bath, followed by the addition of Tween 80 (200 μL), $HAuCl_4$ (155 μL, 15 μM) and $NaBH_4$ (0.5 mL, 2.5 mg) and the solution color turned to brown. The mixture was stirred rapidly for 45 min. The gold nanoparticles were then dialysized for 24 h and renewal deionized water every 8 h. The synthesis steps of other AuNPs were similar as DAP-AuNPs.

2.4. Peroxidase-like activity of purine derivatives capped AuNPs

AuNPs with different ligands were added into solution contained with 2 mM H_2O_2, 1 mM TMB in HAc-NaAc buffer at pH 3.0 and then incubated at 40 °C for 30 min. The solution was mixed up to make it uniform and used for recording UV–vis spectra.

2.5. Optimization of pH, temperature and concentration of H_2O_2 in solution

For selecting the optimal pH in solution, 50 mM HAc-NaAc buffers with different pH were added into solution contained with 0.8 μM DAP-AuNPs, 2 mM H_2O_2, 1 mM TMB and then incubated at 40 °C for 30 min. For selecting the optimal temperature, the sample solutions were kept in different temperature bathes for 30 min contained with 0.8 μM DAP-AuNPs, 2 mM H_2O_2, 1 mM TMB in HAc-NaAc buffer at pH 3.0 and the UV–vis spectra were recorded. For selecting the optimal concentration of H_2O_2, different concentrations of H_2O_2 were added into solution contained with 0.8 μM DAP-AuNPs, 1 mM TMB in pH 3.0 HAc-NaAc buffer at 40 °C for 30 min, then the UV–vis spectra of the samples were recorded.

2.6. Detection of Fe^{2+} by DAP-AuNPs

Different concentrations of Fe^{2+} range from 0 μM to 0.1 μM were added into solution contained with 0.8 μM DAP-AuNPs, 2 mM H_2O_2, 1 mM TMB in HAc-NaAc buffer at pH 3.0 incubated for 30 min and the UV–vis spectra were recorded.

Various cations (Na^+, Mg^{2+}, Al^{3+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Hg^{2+}, Ag^+, Fe^{2+}, Fe^{3+}) were added into the solution contained with 0.8 μM DAP-AuNPs, 2 mM H_2O_2, 1 mM TMB in HAc-NaAc buffer at pH 3.0, and incubated for 30 min and the UV–vis spectra were recorded. The concentration of Fe^{2+} is 0.4 μM and the concentration of other cations are 10 μM.

2.7. Detection of $\cdot OH$ produced by Fe^{2+} and DAP-AuNPs

The samples (10 nM Fe^{2+}, 0.8 μM DAP-AuNPs, DAP-AuNPs & Fe^{2+}) were added into the mixture of HAc-NaAc buffer (pH 3.0), H_2O_2 (2 mM) and DMPO (5,5-Dimethyl-1-pyrroline N-oxide, 50 mM), then incubated at 40 °C for 30 min for electron spin resonance (ESR) analysis.

2.8. Detection of HB by DAP-AuNPs

Different concentrations of HB range from 0 μM to 0.225 μM were added into solution contained with 0.8 μM DAP-AuNPs, 2 mM H_2O_2, 1 mM TMB in HAc-NaAc buffer at pH 3.0 incubated for 30 min and the UV–vis spectra were recorded.

2.9. Detection of red blood cells (RBC) by DAP-AuNPs

Blood with certain amount of RBC was diluted to different concentrations using HAc-NaAc buffer and incubated at 40 °C for 30 min. Then, the RBC were added into the mixture of 0.8 μM L^{-1} DAP-AuNPs, 2 mM H_2O_2 and 1 mM TMB in HAc-NaAc buffer at pH 3.0, then incubated for 30 min and the UV–vis spectra were recorded.

The original blood sample was diluted for 1000 folds using saline and then counted by hemacytometer counting method. Both sides of the hemacytometer (with cover slip in place) were filled with the diluted sample, and the hemacytometer was left undisturbed for approximately 3–5 min to allow the red blood cells to settle to a uniform focal plane. Then red blood cells were counted with a microscope at 200–400 magnification.

Various components (H_2O, Cl^-, F^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, Na^+, Ca^{2+}, NH_3, urea, uric acid, bovine serum albumin, globulin, glucose, creatinine, glycine, histidine, lysine, serine, taurine, hemoglobin) were added into DAP-AuNPs (0.8 μM), and the stability of DAP-AuNPs was recorded for 24 h. H_2O_2 (2 mM), TMB (1 mM), HAc-NaAc buffer (pH 3.0) were then added into the solutions, incubated for 30 min and recorded by UV–vis spectra. (The concentration of HB was 0.15 μM; the

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