Importance of the surface chemistry of nanoparticles on peroxidase-like activity

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1. Introduction

In terms of peroxidase activity, natural enzymes are outstanding catalysts which could drive prompt and specific oxidation of the substrates. The most intensively used peroxidase is horseradish peroxidase (HRP) which is extracted from the root of horseradish and could amplify the weak signal via cascade reactions. The distinct stereo conformation imparts catalytic activity, but also some drawbacks to HRP [1]. Its activity is restricted to quite specified conditions; maintenance of the activity is challenging, especially during transfer and storage phases that include unexpected exposure to mechanical or thermal stress. To circumvent these drawbacks, a considerable amount of research has turned to exploration of artificial enzymes which may have higher resistance to environmental alterations on aspects such as temperature, pH, and ionic strength.

Artificial enzymes are designed to mimic natural ones in many aspects; for instance on specific affinity towards substrates, facilitation of electron transfer from donor to recipient, and stabilization of intermediates [2]. Recent decades have witnessed rapid catalytic nanomaterial advancements, creating a rather dynamic research field called “nanozymes” [3]. In contrast to natural enzymes, nanozymes are easily produced in the laboratory or on an industrial scale, insensitive to external environment, tunable in catalytic activity with activators or inhibitors, and reusable, thereby exhibiting great promise in biosensing and biomedicine [4].

Gold nanoparticles have received enormous attention, benefitting from the intrinsic advantages in chemical inertness, biocompatibility, and peroxidase activity. This unique peroxidase-like catalytic activity is one of the brightest accomplishments in biotechnology, environmental chemistry, and medicine. However, it still faces several obstacles to extensive application, such as moderate catalytic activity and substrate specificity [5]. In order to

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Abstract

We report the studies on origin of peroxidase-like activity for gold nanoparticles, as well as the impact from morphology and surface charge of nanoparticles. For this purpose, we have synthesized hollow gold nanospheres (HAuNS) and gold nanorods (AuNR) with different morphology and surface chemistry to investigate their influence on the catalytic activity. We found that citrate-capped HAuNS show catalyzing efficiency in oxidation reaction of 3,3′,5,5′-tetramethylbenzidine (TMB) by hydrogen peroxide (H2O2) and it is superior to that of cetyltrimethylammonium bromide (CTAB)-capped AuNR. The kinetics of catalytic activities from HAuNS and AuNR were respectively studied under varied temperatures. The results indicated that surface chemistry rather than morphology of nanoparticles plays an important role in the catalytic reaction of substrate. Furthermore, influencing factors such as pH, amount of nanoparticle and H2O2 concentration were also investigated on HAuNS-catalyzed system. The great impact of nanoparticle surface properties on catalytic reactions makes a paradigm in constructing nanozymes as peroxidase mimic for sensing application.

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improve the accessibility of substrates and the consequent catalytic efficiency, many researchers are seeking to diversify the surface of gold nanoparticles. Some groups have combined gold nanoparticles with other functional nanomaterials such as graphene oxide [6], single-walled carbon nanotubes [7], and mesoporous silica [8] to gain strengthened activity compared to that of unsupported gold nanoparticles. Nevertheless, there is a lack of information regarding the relation between gold nanoparticle morphology and their capacity to act as a peroxidase enzyme.

We synthesized hollow gold nanospheres (HAuNS) and gold nanorods (AuNR), and studied their peroxidase-like activity in catalytic reaction of 3,3′,5,5′-tetramethylbenzidine (TMB). The kinetics of HAuNS and AuNR peroxidase activity under environmental changes with altered pH and temperature were investigated, and the influence of nanoparticle and hydrogen peroxide (H₂O₂) concentrations were also studied. Our study shows that the catalytic activity of nanoparticles is strongly dependent on their surface chemistry, rather than on morphology, which originates from the chemical affinity to the substrate. We believe that this work would enrich our understanding of gold nanoparticles as a stable and effective nanomaterial-based peroxidase mimetic in catalytic activities and be potentially useful in bioassays and biodiagnostics.

2. Materials and methods

2.1. Synthesis of gold nanostructure

The HAuNS were synthesized by sacrificial galvanic replacement of cobalt nanoparticles in the presence of chloroauric acid as reported by Schwartzberg et al. [9] with modification. Briefly, under nitrogen atmosphere 150 mL of deoxygenated water was placed into a three-neck flask followed by addition of 0.1 M solution of sodium citrate (450 μL) and 1 M sodium borohydride solution (500 μL). Under rapid magnetic stirring, 0.4 M cobalt chloride solution (100 μL) was added. Solution color changed from pale pink to gray, indicating the reduction of Co²⁺ ions into cobalt nanoparticles. After ensuring the complete hydrolysis of sodium borohydride, 0.1 M solution of chloroauric acid (250 μL, 50 μL/addition) was added. After the reaction was completed, the nitrogen flow was replaced by air flow to oxidize any remaining cobalt metal. The AuNRs were prepared by a non-seeded method. Briefly, a mixture of 1 mM chloroauric acid and 0.1 M cetyltrimethylammonium bromide (CTAB) was prepared and followed by addition of 0.2 mM AgNO₃, 1 M ascorbic acid (2.4 mL) and 0.01 M NaBH₄ (1.2 mL). The obtained suspension of nanostructures were treated with centrifugation and washing against deionized water.

2.2. Characterization

The morphology of nanoparticles was characterized by transmission electron microscopy (TEM; JEOL JEM-2100 F, Tokyo, Japan). All peroxidase reactions were carried out under standard reaction conditions and absorbance spectra were monitored in time scan mode at 652 nm using a SpectraMax M5 plate reader spectrometer.

3. Results and discussion

3.1. Synthesis and characterization of nanoparticles as peroxidase mimic

The HAuNS were synthesized using Co nanoparticles as a template. The Co²⁺ ions have a lower oxidation state than Au³⁺ ions, which facilitates the reduction of Au³⁺ ions via a galvanic reaction forming the inner shell. And the outer shell of HAuNS was reduced by the citrate. The TEM images in Fig. 1a show the hollow spherical
of HAuNS with a mean diameter of 16.7 ± 2.6 nm and a shell thickness of approximately 3–5 nm. It is also noticeable that there are deformed shells, which can be explained by Au3+ ion fast diffusion in the solution; the shells were therefore not uniformly grown. Fig. 1b shows the morphology of AuNR synthesized via a non-seeded method using a CTAB micelle template. The AuNRs have lengths in range of 20–40 nm and aspect ratio (length/width) of 3–5. The insets of high resolution TEM images exhibit high crystallinity for both gold nanoparticles.

3.2. Peroxidase-like catalytic activity of gold nanoparticles in H2O2-mediated oxidation of a substrate

To investigate the peroxidase-like behavior of synthesized HAuNS and AuNR, TMB was chosen as a peroxidase substrate in the presence of H2O2 for examining the catalytic oxidation by gold nanoparticles. The typical blue color reaction with a maximum absorbance at 655 nm originates from the oxidation of TMB. To avoid the influence of chemical residues (e.g., positive effect of HAuCl4 directly oxidizing TMB, negative effect of excess citrate ions diminishing the peroxidase activity of gold nanoparticles), it is important that the synthesis protocol should include several washing cycles to exclude the remaining chemicals in the suspension. Fig. 2 shows time dependent (kinetics) absorbance evolution at 652 nm of the TMB oxidation system in the presence of 10 μg of HAuNS (Fig. 2a) and AuNR (Fig. 2b) at different temperatures (24 °C, 30 °C, and 40 °C). Fig. 2a shows absorbance at 652 nm increasing dramatically in the initial stage and reaching maximal absorbance at 540 s (24 °C), 580 s (30 °C), and 900 s (40 °C) respectively for HAuNS catalyzed reaction. A higher catalytic activity was observed at higher temperatures. The absorbance at three different temperatures decreases and gradually stabilizes to a similar value. The catalytic kinetics for AuNRs, however, exhibit a different pattern (Fig. 2b), where the absorbance at three different temperatures shows no increments, just slowly increases to a small extent during the whole experiment period. There is no apparent temperature-dependent effect. The results indicate that HAuNS have higher efficiency than AuNRs regarding both the rate and extent of TMB oxidation catalysis. These can be related to the charge characteristic of peroxide substrate, TMB, which has two amino groups and bears a strong affinity to anionic citrate-capped gold nanoparticles compared with cationic CTAB-capped AuNR. Similar variation of peroxidase-like activity due to surface modification and superficial atoms of gold nanoparticles was reported previously [10].

3.3. Investigation of the impact of environmental factors on catalytic activity of HAuNS

Furthermore, the effects of environmental alteration on the catalytic activity of HAuNS were examined at different pH, particle concentrations, and H2O2 concentrations (Fig. 3). We have recorded the absorbance of the oxidation reaction during 2400 s at pH 3.54, 4.6, 5.46, and 6.4, respectively. The results show that under pH 3.54, absorbance increases only slightly over the period of 2400 s. When pH was increased to 4.6, a dramatic increase in absorbance was observed, reaching maximum at 1000 s before gradually dropping. At pH 5.6, the absorbance quickly increased and reached maximum at 150 s followed by a fast drop until reaching a static state. The absorbance at pH 6.4 exhibited a different pattern, where the absorbance started to drop to a steady state shortly after the zero time point (Fig. 3a). There is a clear trend in the range of pH 5.46 to pH 6.4 that the catalytic efficiency by HAuNS on TMB oxidation reaction will increase and then decrease following the increment increases of pH, leaving a narrow range of pH for optimization of the TMB oxidation reaction. For the system using different amount of HAuNS (5 μg, 10 μg, 20 μg, and 40 μg), we found that the absorbance increased along the reaction time and higher amount of HAuNS induced faster increase in absorbance (Fig. 3b). The increase of concentration of H2O2 (from 100 mM to 200 mM, 385 mM, and 500 mM) also induced increment of absorbance (Fig. 3c).

Citrate-capped HAuNS have been shown to have intrinsic peroxidase-like activity, which exhibits significantly higher catalytic activity toward TMB compared with cationic CTAB-capped AuNR. This different activity indicates that surface charge characteristics of nanoparticles, rather than morphology, play an important role in the catalytic reaction of specific substrate. Kinetic analysis on the reaction catalyzed by HAuNS shows that there is a...
strong dependence of catalysis on pH, particle concentration and H₂O₂ concentration. Under different pH, the catalytic efficiency can be enhanced only in a narrow pH range. For the system with increased amount of particle concentration or H₂O₂ concentration, the catalytic efficiency by HAuNS also increased. The present study will facilitate the investigation and control of the peroxidase-like activity of nanoparticles for biotechnology and medical applications.

Disclosure

All authors declare no financial competing interests.

Conflict of interest

The authors declare no conflict of interest.

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