Peroxidase-like activity of apoferritin paired gold clusters for glucose detection

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ABSTRACT

The discovery and application of noble metal nanoclusters have received considerable attention. In this paper, we reported that apoferritin paired gold clusters (Au–Ft) could efficiently catalyze oxidation of 3.3′,5.5′-tetramethylbenzidine (TMB) by H_2O_2 to produce a blue color reaction. Compared with natural enzyme, Au–Ft exhibited higher activity near acidic pH and could be used over a wide range of temperatures. Apoferritin nanocage enhanced the reaction activity of substrate TMB by H_2O_2. The reaction catalyzed by Au–Ft was found to follow a typical Michaelis–Menten kinetics. The kinetic parameters exhibited a lower K_m value (0.097 mM) and a higher K_{cat} value ($5.8 \times 10^4\,s^{-1}$) for TMB than that of horse radish peroxidase (HRP). Base on these findings, Au–Ft, acting as a peroxidase mimetic, performed enzymatic spectrophotometric analysis of glucose. This system exhibited acceptable reproducibility and high selectivity in biosening, suggesting that it could have promising applications in the future.

1. Introduction

In recent years, enzyme-based biosensing has attracted considerable attention due to its low cost, simplicity, and practicality (Li et al., 2013). It arises from the unique catalytic properties of enzymes with highly efficient and selective catalysis under mild conditions (Mireia et al., 2004; Wolfenden and Snider, 2001). Therefore, there has for thousands of years existed a great interest in utilizing enzymes in food processing, agriculture, the chemical industry and medicine. Peroxidases, especially horse radish peroxidase (HRP), can activate hydrogen peroxide to perform a variety of chemical reactions such as oxidation, which has been extensively investigated and applied in many different areas (Veitch, 2004). However, the most practical applications of natural enzymes are limited by the preparation techniques, reaction conditions and storage requirements (Breslow, 1995; Shoji and Freund, 2001). To minimize these limitations, researchers have devoted great efforts to construct highly stable and low-cost alternatives to enzymes (Kotov, 2010; Murakami et al., 1996). Recently, advances in nanotechnology and progress in designing functional nanomaterials, provide exciting new opportunities for catalysis. The concept of "nanozyme" was initially proposed by Scrimin, Pasquato, and co-workers to represent their thiol monolayer protected gold clusters with ribonuclease-like activity (Manea et al., 2004). Some nanozymes possess intrinsic peroxidase-like activity, such as ceria nanoparticles (NPs) (Jiao et al., 2012), carbon NPs (Dong et al., 2012; Li et al., 2013), Pt NPs (Fan et al., 2011; Gao et al., 2013), and Fe_3O_4 magnetic NPs (Dong et al., 2012; Gao et al., 2007; Wu et al., 2011). Gold NPs have also been considered as one of the most important nanozymes because of their unique properties, such as high chemical stability, feasible surface modifications, and excellent biocompatibility (Burda et al., 2005). In addition, they possess intrinsic peroxidase-like activity and provide a new route for colorimetric detection of H_2O_2, Hg^{2+}, xanthine, etc. (Jv et al., 2010; Wang et al., 2011; Long et al., 2011).

Glucose, as a source of energy for the living cells and metabolic intermediate, is very important for the public health. Maintaining blood glucose levels between 3.0 mM and 8.0 mM (Xu et al., 2007) is critical as diabetes mellitus may result in heart disease, kidney failure, blindness, etc. (Si et al., 2011; Lu et al., 2011). This necessitates the development of a fast, reliable, sensitive, and selective method for glucose determination. Many approaches including surface plasmon resonance (Al-Ogaidi et al., 2014), fluorescence (Deng et al., 2014), electrochemiluminescence (Xiao et al., 2014), and colorimetry (Tabrizi and Varkani, 2014) have been performed for glucose detection. These assays can be based on

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enzymatic activity of new nanomaterials or nanostructures within the sensor. Nanozyme is becoming a new tool for glucose detection due to its low cost, simplicity, and practicality. Moreover, the catalytic ability of nanozyme can be tuned by many important factors (Daniel and Astruc, 2004; Wang et al., 2012). According to previous studies, gold nanozymes can be divided into two major categories based on the structural features of nano-gold. One category utilizes monolayer protected gold particles as catalysts and biomimetic catalytic activities, which derive from the functional groups of the modification shell (Bonomi et al., 2008; Kisailus et al., 2005). Another category involves small and stable gold NPs with various surface states and the intrinsic activities, which are attributable to the nanomaterial core (Luo et al., 2010). For example, the charge properties of the coating, which affect the interaction between nanoparticles and substrates, plays an important role in their activity (Jv et al., 2010). Additionally, ultrasmall metal clusters exhibit superior catalytic activity due to their high surface energy that makes surface atoms fairly active (Biswas et al., 2012; Wang et al., 2014).

Apoferritin, is a 24-subunit spherical protein complex (450 kDa) with a nanoscale hollow interior and good biocompatiblity (Arosio et al., 2009). It is a physiological protein that impacts iron metabolism and oxidative stress regulation, and also shows unexpected enzymatic activities (Friedman et al., 2011). Recently, apoferritin has been used as a size-constrained reaction vessel to direct nanostructure synthesis. We have already successfully synthesized apoferritin paired gold clusters (Au–Ft) according to the "points of control" synthesis strategy (Sun et al., 2011). Within the apoferritin nanocage, the ferroxidase center is composed of six amino acid residues: histidine (His), aspartic acid (Asp), glutamine (Gln), and three glutamic acids (Glu). Gold clusters can bind strongly with the His residues at ferroxidase center of H-ferritin (Ueno et al., 2009). This can produce an enzyme active center that simulates natural enzyme micro-environment, thereby facilitating substrate molecular binding ability and stabilizing the enzyme–substrate complex. Therefore, this model could be a great mimicry system of enzymes. Protein–inorganic nanozymes designed by utilizing nature's strategy could insure biocompatibility and enable controllable catalysis. This gold nanozyme with paired ultrasmall size could be a novel platform for peroxidase-like activity.

In this work, we discovered that apoferritin paired gold clusters (Au–Ft) can act as enzymatic mimics possessing intrinsic peroxidase-like activity. The clusters can catalyze the reaction of peroxidase substrate 3.3.5.5-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a color reaction. Compared to larger colloidal gold nanoparticles, it was found that Au–Ft exhibit highest catalytic activity. Moreover, kinetic studies demonstrated that the catalytic reaction followed a ping-pong mechanism and Au–Ft had even higher catalytic activity to TMB than natural HRP. Combining the catalytic reaction by glucose oxidase (GO_x) and Au–Ft, this novel mimicry enzyme was used successfully to detect glucose detection (as shown in Fig. 1). This method exhibits a relatively high selective response to glucose detection and could also have potential applications in the fields of environmental chemistry and biomedicine.

2. Materials and methods

2.1. Reagents and materials

Horseradish peroxidase (HRP) and glucose oxidase were purchased from Roche. 3.3′,5.5′-tetramethylbenzidine dihydrochloridewere (TMB) was obtained from Boston Biomedical Inc. (USA). The BCA protein assay kit, horse spleen apoFt, and $HAuCl_4$ were
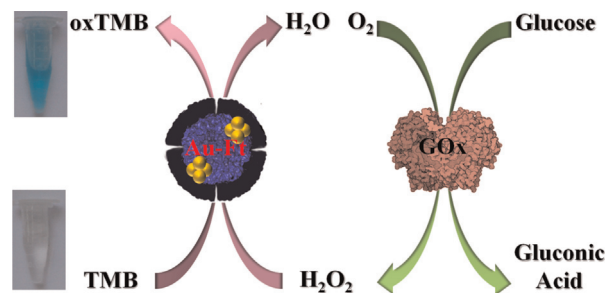

Fig. 1. Schematic illustration of colorimetric determination of glucose using glucose oxidase (GO_x) and Au–Ft catalyzed reactions.

purchased from Sigma-Aldrich. All other chemicals, such as H_2O_2 solution (30 wt% aqueous), NaOH, HOAc, NaOAc, and HCl were obtained from Beijing Chemicals Reagent Company (Beijing, China). All reagents were of analytical grade and used without further purification. All solutions were prepared with ultrapure water purified by a Millipore water purification system (≥ 18.2 MΩ, Milli-Q, Millipore).

2.2. Synthesis and characterization of apoferritin paired gold clusters

The preparation of Au–Ft was carried out in an aqueous solution as previously described (Sun et al., 2011). 300 µL of 5 mM $HAuCl_4$ was added to 300 µL of horse spleen apoFt (49 mg/mL). To avoid apoFt subunit dissociation under acidic conditions, $HAuCl_4$ was first adjusted to pH 7 before mixing with apoFt. After the mixture was mixed for 2 min, 20 µL of 1 M NaOH was added to the solution, followed by incubation for 12 h at 37 °C. The reaction solution was ultrafiltered with a centrifugal filter device (Amicon Ultra-15; 30,000 molecular weight cut off) and washed three times with 3 mL of ultrapure water. The concentration of Au–Ft was measured by the bicinchoninic acid method (BCA). An aliquot of 4 µL of Au–Ft was applied to a glow-discharged grid coated with a layer of amorphous carbon film, and excess fluid was gently blotted off with filter paper. The HRTEM images were recorded on an FEI Tecnai F20 U-TWIN electron microscope. TEM analysis was performed on a JEOL JEM-1200EX model transmission electron microscope. For negative staining, a dispersion of Au–Ft clusters was dropped onto a carbon-coated copper grid, dried inair at room temperature, and stained with 2% uranyl acetate.

2.3. Mimetic peroxidase activity assays

HRP-like activity was examined using TMB as a chromogenic substrate. Experiments were carried out using 0.58 µg Au–Ft or 0.50 ng HRP in a reaction volume of 1 mL, in 0.2 M HOAc–NaOAc buffer with 300 mM TMB. The concentration was 300 mM for Au–Ft and 4 mM for HRP. The pH was 4.0 for both Au–Ft clusters and HRP. Before the reaction, the mixture was incubated at 37 °C for HRP and 45 °C for Au–Ft clusters. After 3 min, the color formation was monitored at 652 nm using Shimadzu UV-2550 spectrophotometer after adding H_2O_2 to the reaction. To compare the influence of the reaction buffer pH on the relative activity of Au–Ft and HRP, 0.2 M NaOAc buffer solutions from pH 2.0 to 12.0 were investigated at 45 °C and 37 °C, respectively. To examine the influence of incubation temperature on the relative activity of Au–Ft and HRP, catalytic reactions incubated in water baths from 4 to 90 °C were investigated at pH 4.0.

2.4. Reaction mechanism and kinetic analysis

The reaction kinetics for the catalytic oxidation of TMB was carried out by recording the absorption spectra at 652 nm with a