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Regular Article

Gold core/ceria shell-based redox active nanozyme mimicking the biological multienzyme complex phenomenon

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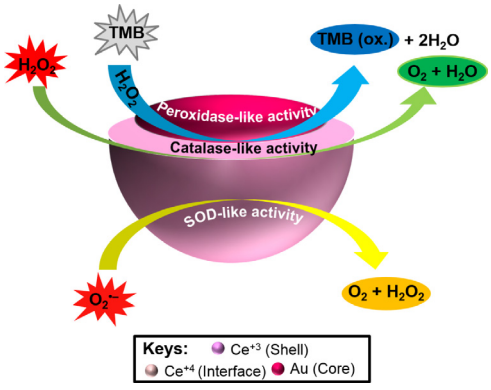
GRAPHICAL ABSTRACT



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ABSTRACT

Catalytically active individual gold (Au) and cerium oxide (CeO_2) nanoparticles (NPs) are well known to exhibit specific enzyme-like activities, such as natural catalase, oxidase, superoxide dismutase, and peroxidase enzymes. These activities have been maneuvered to design several biological applications such as immunoassays, glucose detection, radiation and free radical protection and tissue engineering. In biological systems, multienzyme complexes are involved in catalyzing important reactions of essential metabolic processes such as respiration, biomolecule synthesis, and photosynthesis. It is well known that metabolic processes linked with multienzyme complexes offer several advantages over reactions catalyzed by individual enzymes. A functional nanozyme depicting multienzyme like properties has eluded the researchers in the nanoscience community for the past few decades. In the current report, we have designed a functional multienzyme in the form of Gold (core)-CeO_2 (shell) nanoparticles (Au/CeO_2 CSNPs) exhibiting excellent peroxidase, catalase, and superoxide dismutase enzyme-like activities that are controlled simply by tuning the pH. The reaction kinetic parameters reveal that the peroxidase-like activity of this core-shell nanozyme is comparable to natural horseradish peroxidase (HRP) enzyme. Unlike peroxidase-like activity exhibited by other nanomaterials, Au/CeO_2 CSNPs showed a decrease in hydroxyl radical formation, suggesting that the biocatalytic reactions are performed by efficient electron transfers. A significant enzyme-like activity of this core-shell nanoparticle was conserved at extreme pH (2–11) and temperatures (up to 90 °C), clearly suggesting the superiority over natural enzymes. Further,

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the utility of peroxidase-like activity of this core-shell nanoparticles was extended for the detection of glucose, which showed a linear range of detection between (100 μM to 1 mM). It is hypothesized that the proximity of the redox potentials of Au^{+}/Au and Ce (III)/Ce (IV) may result in a redox couple promoting the multienzyme activity of core-shell nanoparticles. Au/CeO_2 CSNPs may open new directions for development of single platform sensors in multiple biosensing applications.

1. Introduction

Nanomaterials exhibiting biological enzyme-like activity (Nanozymes) are inevitably employed in multiple biomedical applications such as biosensing, immunoassays, organic pollutant degradation, magnetic separation and protection of cells from reactive oxygen species (ROS) [1–6]. The superior enzyme-like response and affluent synthesis, storage and overall low cost of production have attracted enormous excitement of the researchers worldwide. Significant efforts have been made towards developing efficient nanozymes by controlling the particle size, shape, composition and redox states of materials [7,8]. Recently, gold nanoparticles (AuNPs) were reported to exhibit peroxidase enzyme-like properties, which have been used to design and develop several applications in bionics, biosensing, and biomedicines [9,10]. The biological peroxidase-like activity of AuNPs is considered due to their unique physicochemical and optoelectronic properties. It is suggested that peroxidase-like activity of AuNPs also depends on the speedy degradation of hydrogen peroxide used in the reaction. However, the AuNPs-based peroxidase mimetic system suffers from many shortcomings such as lower substrate affinity and specificity, compared with the natural biological enzyme, probably due to inorganic nature of the Au-based catalyst. This issue leads to the lower catalytic performance of AuNPs-based peroxidase nanozymes, which further limits their biomedical applicability. Several efforts made to address this concern have met with limited success. Therefore, additional efforts are imperative to improve the catalytic efficiency of AuNPs as peroxidase nanozyme.

CeO_2 nanoparticles are attractive for several applications ranging from wafer polishing to biomedicines [11,12]. Among its biomedical uses, antioxidant therapy has been tested in several diseases involving ROS such as autoimmune diseases that involve oxidative stress and multiple sclerosis [13]. CeO_2 NPs exhibit redox state-dependent enzyme-like activities wherein "Ce" (III) and "Ce" (IV) oxidation states show biological superoxide dismutase (SOD)-like and catalase-like activities, respectively [14,15]. Self and co-workers first observed this activity and reported that analogous to biological SOD enzyme, CeO_2 NPs catalyzes the disproportionation of superoxide radicals into hydrogen peroxide and molecular oxygen [16,17].

In biological systems, multienzyme complexes are involved in catalyzing important reactions of essential metabolic processes such as respiration, biomolecule synthesis, and photosynthesis. It has been clearly observed that metabolic processes linked with multienzyme complexes offer several advantages over reactions catalyzed by individual enzymes [18,19]. These natural complexes are generally assembled into sophisticated three-dimensional arrangements with well-controlled geometries that lead to a sequential catalytic conversion of respective substrates into products [20,21]. Although the individual enzyme reactions are well orchestrated, the lack of spatial proximity synergy may diminish the cascade activities. These issues may decrease the overall efficiency of substrate catalysis as well as the end and intermediate products. Within this context, an inorganic nanozyme exhibiting multienzyme-like activities is expected to be a better alternative for controlling the metabolic processes. Hydrogen peroxide and superoxide radicals are two important ROS controlling the redox nature of cell cytoplasm. Therefore, in the context of enzyme-like activities of AuNPs and CeO_2 NPs, it is expected that intimate integration of AuNPs and CeO_2 NPs in form of core-shell would perform their respective nanozyme activities efficiently. Various core-shell or nanocomposites of varying materials have been developed showing biological peroxidase and oxidase-like activities. Both of these activities produce hydroxyl radicals, needed to oxidize the substrate at similar pH (4.0). Synthesizing an artificial structure that mimics the complexity and function of a natural multienzyme system are considered as a significant challenge. In this context, Lin et al. [22] have developed a self-activated, enzyme-mimetic catalytic cascade consisting of mesoporous silica-encapsulated gold nanoparticles (EMSN-AuNPs) as both glucose oxidase and peroxidase mimetic enzymes. Although authors were able to demonstrate the oxidase-peroxidase coupled enzyme system, the developed method is complicated with limited sensitivity. Zheng et al. [23] have reported the self-assembled three-dimensional graphene-magnetic palladium nanohybrids exhibiting intrinsic peroxidase-like and oxidase-like activity. Chen et al. have demonstrated the peroxidase and catalase-like activities in iron oxide nanoparticles [24]. It was revealed that the peroxidase and catalase-like activity was pH-dependent and exposure to human glioma cells NPs showed a concentration-dependent cytotoxicity by enhancing H_2O_2-induced cell damage. Additionally, the catalase-like activity in NPs was activated at neutral pH and degraded the excess of H_2O_2 leading to significantly reduced toxicity to cells. Both peroxidase (Eqs. (1a) and (1b)) and catalase (Eq. (2)) mimetic activities use hydrogen peroxide as a substrate and either break it down to benign water and oxygen molecules (in catalase activity) or generate a cascade of hydroxyl and perhydroxyl radicals (in peroxidase activity) at acidic pH values.

$$\text{AuNPs} + H_2O_2 \rightarrow \cdot OH + \cdot OH \quad (1a)$$

$$\text{TMB(red.)} + \cdot OH \rightarrow \text{TMB(ox.)} + H_2O \quad (1b)$$

$$2Ce^{4+} + H_2O_2 + 2OH^{-} \rightarrow 2Ce^{3+} + 2H_2O + O_2 \quad (2)$$

The subtle changes in pH of the medium can control the redox potential of participating nanomaterials that can engage in single electron oxidation or reduction of the hydrogen peroxide. We have now demonstrated a third enzymatic activity from a single nanoparticle system to reduce the superoxide radicals (Eq. (3)) to less harmful peroxide ions.

$$O_2^{\cdot -} + Ce^{3+} + 2H^{+} \rightarrow H_2O_2 + Ce^{4+} \quad (3)$$

Inorganic materials showing a combination of peroxidase, catalase, and SOD enzyme-like properties, as those demonstrated in the current study have not been reported so far. Additionally, this study could be helpful in providing a better understanding of the electron transfer mechanism of inorganic nanomaterials mimicking multienzymes-like properties and their potential applications in the biomedical fields.

Apart from multi-enzyme activity, such hybrid nanomaterials could also be used for the sensitive detection of multiple analytes simultaneously [25,26]. Au and carbon nanotubes based hybrid