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ATP-enhanced peroxidase-like activity of gold nanoparticles

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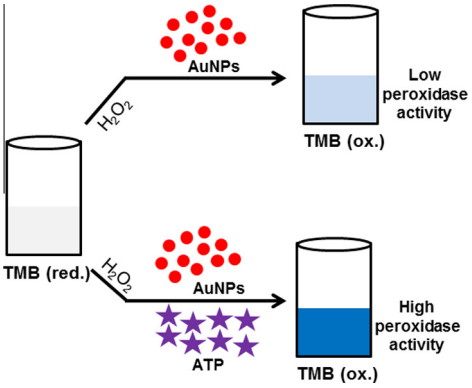
GRAPHICAL ABSTRACT



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ABSTRACT

Gold nanoparticles (AuNPs) are known to possess intrinsic biological peroxidase-like activity that has applications in development of numerous biosensors. The reactivity of the Au atoms at the surface of AuNPs is critical to the performance of such biosensors, yet little is known about the effect of biomolecules and ions on the peroxidase-like activity. In this work, the effect of ATP and other biologically relevant molecules and ions over peroxidase-like activity of AuNPs are described. Contrary to the expectation that nanoparticles exposed to biomolecules may lose the catalytic property, ATP and ADP addition enhanced the peroxidase-like activity of AuNPs. The catalytic activity was unaltered by the addition of free phosphate, sulphate and carbonate anions however, addition of ascorbic acid to the reaction mixture diminished the intrinsic peroxidase-like activity of AuNPs, even in the presence of ATP and ADP. In contrast to AuNPs, ATP did not synergize and improve the peroxidase activity of the natural peroxidase enzyme, horseradish peroxidase.

1. Introduction

The catalytic activity of natural enzymes is sensitive to environmental changes that often lead to their denaturation and digestion. The high cost involved in synthesis and purification of these enzymes and strict working conditions have created an opportunity for the use of nanomaterials as biological enzyme mimics [1,2]. Due to the high surface energy and large surface-to-volume ratios, nanomaterials offer better catalytic efficiencies than natural enzymes [3]. In contrast to natural enzymes, nanomaterial based enzyme mimics exhibit higher stability in harsh reaction conditions, have lower cost of production, demonstrate higher flexibility

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in composition, shape and size control, shelf-life and can be designed for tunable catalytic efficiency [4]. Several reports on using nanomaterials as biological enzyme mimics exist in literature for example, nanoparticles of gold [5–8], cerium oxide [9–13], iron-oxide [14,15], Co_3O_4 [16], CuO [17] and V_2O_5 nanowires [18–20] and carbon based nanomaterials [21–24], bi-metallic nanohybrids such as Au–Pd [25,26], Au–carbon [27] and graphene hybrids [28] etc are the most studied nanomaterial-based models that demonstrate peroxidase-like catalytic activity. Despite these advantages the nanomaterial based peroxidase mimics suffer from several shortcomings such as the lower substrate affinity and specificity, low stability in biologically relevant buffers and low catalytic activity as compared to that of natural biological enzymes. The above issues can affect the catalytic performance of enzyme mimicking nanomaterials, limit their usefulness in real systems and hinder further development and application of these enzyme biomimetics. Limited efforts have been explored to improve the peroxidase mimicking activity. In one such example the use of ionic liquids improved the thermal stability of product, however, completely inhibited the catalytic activity of nanomaterial based artificial enzyme due to its high viscosity and ionic strength [29]. Therefore, it is imperative to find new alternatives that are convenient and effective to improve the peroxidase-like activity exhibited by inorganic materials.

AuNPs has been explored as peroxidase mimic in recent years due to their unique physical, chemical and electronic properties [30–33]. Colloidal particles are known to be stabilized by electrostatic repulsion and van der Waals attraction forces operating between the charged particles controlled by ionic strength of suspension [34,35]. The disruption of these forces by addition of ions can cause aggregation of the suspended particles, leading to the alteration of peroxidase-like activity of nanoparticles. Additionally, due to the high surface to the volume ratio, nanomaterials tend to strongly adsorb selective molecules from immediate environment that can hinder access to the surface of nanomaterials for other molecules/ions. Such preferential interaction can either inhibit or promote the peroxidase-like activity of nanomaterials. Recently, Wang et al. [36] have reported that unmodified gold nanoparticles exhibit better peroxidase activity than corresponding amino modified or citrate stabilized gold nanoparticles. They considered the surface gold atoms to be the main factor which significantly affected the catalytic activity towards peroxidase substrate. Lien et al. [37] showed that in presence of metal ion such as Ag^+, Bi^{3+} and Hg^{2+} ions, the peroxidase-like activity of AuNPs was improved substantially. It was suggested that the main reason was the deposition of these ions over AuNPs surface, which lead to the enhanced peroxidase-like activity through competitive and synergistic interactions between ions and AuNPs. Further cellular behaviours and functions could also be controlled by signalling events produced by inorganic materials such as ATP. Recently, this strategy has been used to show that ATP and intracellular calcium signalling activities were observed during the astrocytes attachment and spreading on micro-patterned surfaces [38].

Therefore, exposure of AuNPs to the biological system may allow them to interact with biomolecules and thus influence their catalytic activity. To elucidate the effect of biologically relevant molecules and ions on the peroxidase-like activity, citrate coated AuNPs were exposed to ATP, ADP, carbonate, sulphate and free phosphate anions and the resultant peroxidase-like activity has been investigated in this work. Compared to ADP, free phosphate, sulphate and carbonate anions, incorporation of ATP resulted in significant enhancement in peroxidase-like activity of AuNPs. Furthermore, some other factors such as size dependent peroxidase-like activity of AuNPs and the origin of enhancement by ATP are also addressed.

2. Materials and methods

2.1. Materials

3,3′,5,5′-Tetramethylbenzidine (TMB) was obtained from Acros Organics (Geel, Belgium). 30% w/v hydrogen peroxide (H_2O_2) and chloroauric acid ($HAuCl_4{\cdot}3H_2O$) were purchased from SD fine chemicals (Mumbai, India). Adenosine triphosphate disodium salt (ATP), adenosine diphosphate disodium salt (ADP), di- sodium hydrogen phosphate, sodium carbonate (anhydrous), magnesium sulphate heptahydrate, L-ascorbic acid, Tri-sodium citrate dihydrate and citric acid monohydrate were obtained from Hi-Media (Mumbai, India). The chemicals were used as received unless otherwise reported.

2.2. Characterization of AuNPs

Absorbance spectra of AuNPs was acquired using a UV–visible spectrophotometer (Biotek, Synergy HT spectrophotometer) at room temperature in a quartz cuvette of 1 cm path length. High resolution transmission electron microscope (HR-TEM) images were obtained using JEOL 2010-F TEM on a Cu-coated TEM grid. Zeta potential and hydrodynamic diameters of AuNPs were carried out using dynamic light scattering measurement from Zeta sizer nano (Malvern instruments) using a laser with wavelength of 633 nm.

2.3. Methods: synthesis of AuNPs

15 nm AuNPs: AuNPs were prepared according to the method reported by Turkevich et al. [39]. In brief, 150 μL of $HAuCl_4$ solution was added in 14.85 mL Milli-Q water and was heated until it started boiling. 1.8 mL of 38.8 mM trisodium citrate was added drop wise, and solution was boiled for 5 min with continuous stirring until solution turns red. *30 nm AuNPs:* AuNPs were prepared as described previously, 1.0 mL of 38.8 mM trisodium citrate was added drop wise and solution was boiled for 5 min with continuous stirring until solution turns dark-red. *50 nm AuNPs:* AuNPs were prepared as described previously, 700 μL of 19.4 mM trisodium citrate was added drop wise and solution was boiled for 5 min with continuous stirring until solution turns dark purple. *70 nm AuNPs:* AuNPs were prepared as described previously, 450 μL of 19.4 mM trisodium citrate was added drop wise and solution was boiled for 5 min with continuous stirring until solution turns pink purple. The synthesized AuNPs were stable for at least 3 months at room temperature.

2.4. Preparation of buffer

Citric acid and trisodium citrate solution was prepared in 100 mL of Milli-Q water. From this stock citric acid and trisodium citrate was added in 59:41 ratio to obtain the citrate buffer solution of 0.1 M concentration. The buffer was further diluted to get the concentration of 0.01 M which was used in the peroxidase-like activity determination.

2.5. Peroxidase activity

Oxidation of TMB was used to measure the peroxidase like activity of AuNPs in presence of H_2O_2. In a typical reaction, TMB (1 mM) accompanied by AuNPs (35 μg/mL) was mixed. H_2O_2 (2 M) was added in the end to start the catalytic reaction. The total reaction volume of 500 μL was maintained and the resultant increase in absorbance was monitored at 652 nm. All reactions