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Colorimetric sensing of malathion using palladium-gold bimetallic nanozyme

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ABSTRACT

In this work, a simple, sensitive and selective label free colorimetric assay using palladium-gold nanorod as nanozyme is reported for malathion detection. Study investigates the peroxidase potential of the nanozyme on colorimetric substrates and explores the effect of selected organophosphates on their enzyme mimetic activity. Palladium-gold nanozyme shows excellent peroxidase mimetic activity with O-phenylenediamine in the presence of hydrogen peroxide. Its Kinetic parameters Km and kcat are better than horseradish peroxidase which makes it a superior enzyme. Nanozyme is stable over a broad temperature range (4–70 °C) and shows high peroxidase activity from 2 to 6 pH. The peroxidase activity of nanozyme is selectively quenched with increasing concentration of malathion and is the principle of developed assay. Assay has a lowest detection limit of 60 ng/ml and shows no cross-reaction with other analogous organophosphates or metal salts. Validation on tap water samples spiked with different concentrations of malathion shows good recovery in the range of 80–106%. Assay also displays good intra and inter-assay precision which lie in the range of 2.7–6.1% and 3.2–5.9% respectively. This study demonstrated the catalytic potential of palladium-gold nanorods, which can be employed as nanozyme for developing highly sensitive detection methods.

1. Introduction

Recent years have witnessed a significant rise in the investigations on nanostructures acting as artificial enzymes or nanozymes owing to their advantages over natural enzymes (Wei and Wang, 2013; XiaoNa et al., 2011). A wide variety of materials including noble metals, metal-oxide, metal-organic frameworks, bimetallic nanostructures have been reported to mimic peroxidase, catalase, esterase, nuclease, superoxide dismutase and oxidase activities (Liu and Liu, 2015). Out of all, nano-gold is attracting tremendous research interest as nanozyme due to its stability, biocompatibility and easy surface modifications (Lin et al., 2014). Intrinsic peroxidase potential of naked or surface passivated gold nanoparticles has been used for detection of different analytes like antibiotics and pesticides (Salih Hizir et al., 2016; Weerathunge et al., 2014; Bai et al., 2015; Bala et al., 2016; Wang et al., 2016). Additionally, gold based hybrid nanozymes such as Vanadium-gold hybrid (Qu et al., 2014) or graphite-Au hybrid (Tao et al., 2013) have also demonstrated enhanced catalytic activity which is applied for detecting single nucleotide polymorphisms and cancer cells respectively. Recently, bimetallic nanostructures have shown to possess better catalytic properties than single metals, probably because, bimetallization combines the properties of two metals or create a new property. Pt-Au nanostructures synthesized by growing platinum nanoparticles on the surface of gold nanorods have enhanced intrinsic peroxidase activity and are used for developing a hydrogen peroxide electrochemical sensor (Feng et al., 2014), and detecting interleukin-2 (He et al., 2011) in an ELISA format. Additionally, their oxidase like activity is also employed for detecting glucose with minimized interference from ascorbic acid (Liu et al., 2012). Dispersion of Pt nanoparticles on the surface of gold nanorods obviates the problem of aggregation of nanoparticles and improves their catalytic potential. Urchin like gold core@Pt hybrids have been used as peroxidase mimetic to detect insitu amplified colorimetric detection of prostate specific antigen (Gao et al., 2015). Peroxidase activity of Au@PtCu nanostructures is also demonstrated for detection of immunoglobulins in an indirect ELISA format (Hu et al., 2014). Au@TiO_2 nanostructures have been employed for CO oxidation (Green et al., 2011). Application of peroxidase mimetic property of Pd@AuNS (core-shell spheres) also is demonstrated in a competitive inhibition based fluoroimmunoassay to detect bensulfuron-methyl herbicide using a fluorogenic substrate (Nangia et al., 2012).

Our recently reported findings have demonstrated for the first time that gold nanorods (AuNRs) itself possess peroxidase activity (Biswas et al., 2016). The highlight of the work is that this intrinsic peroxidase activity of AuNRs is selectively quenched in the presence of malathion.

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This property is employed for simple colorimetric sensing of malathion, however the limitation of the assay is its low sensitivity. Our aim to develop a sensitive and simple colorimetric assay for malathion sensing using peroxidase potential of AuNRs encouraged us to explore AuNR based bimetallic nanozymes. Very recently, Li et al. (2015), have predicted high peroxidase activity in Pd-Au nanostructures followed by Pt-Au nanostructures. Wei et al. (2015), also reported peroxidase, oxidase and catalase like activities in 2-D Pd based nanostructures. This activity is found to be structure and composition dependent. Most of these studies have used Pd with Au nanospheres or shells and hence the probability of their peroxidase potential to vary when Pd is alloyed with Au nanorods is high. Therefore, we are motivated to explore the peroxidase potential of Pd@AuNRs (palladium NPs on nanorods) on the oxidation of colorimetric substrate 3, 3′, 5, 5′tetramethylbenzidine (TMB) and o-phenylenediamine (OPD) in the presence of H_2O_2. Further, we aim to investigate whether or not the peroxidase activity of Pd@AuNR is quenched by malathion as is the case with AuNRs. With this background, the present study successfully employed Pd@AuNRs as peroxidase nanozyme and reports a simple, cheap, label-free colorimetric assay for malathion detection.

2. Materials and methods

2.1. Materials

All chemicals and reagents used in the study were of analytical grade. Gold(III) chloride hydrate ($HAuCl_4$), palladium chloride ($PdCl_2$), malathion were purchased from Sigma Aldrich, India. Cetyltrimethyl ammonium bromide (CTAB-99%), Sodium borohydride ($NaBH_4$), 3,3′,5,5′tetramethylbenzidine (TMB), and Silver nitrate (extra pure) were procured from Sisco Research Laboratory, India. Sodium bicarbonate ($NaHCO_3$), disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen phosphate (NaH_2PO_4), Hydrogen peroxide (H_2O_2-30%), Sulphuric acid (H_2SO_4-98%) all were purchased from Rankem, India. L-Ascorbic Acid (AA), was purchased from Lobachemie, India and 1, 2-phenylenediamine (OPD) was purchased from TCI, India. MilliQ water was used for preparation of all solutions.

2.2. Gold nanorod synthesis

Gold nanorods were synthesized using two step seed mediated method (Nikoobakht and El-Sayed, 2003) with modification.

2.2.1. Preparation of Seed Solution

Briefly, 2.5 ml of milliQ was added to 5 ml of 0.2 M CTAB, followed by addition of 2.5 ml of $HAuCl_4$ (1 mM) to the mildly stirred (250 rpm) solution. Finally, 600 µl of 0.01 M ice cold $NaBH_4$ solution was added, and vigorous stirring (380 rpm) was done for 2 min resulting in the formation of brownish yellow color. This seed solution was kept at room temperature and used within 2–3 h, as nucleating center in growth solution.

2.2.2. Preparation of Growth Solution

150 µl of 4 mM $AgNO_3$ was mixed with 5 ml of 0.2 M CTAB solution and the solution was gently stirred (250 rpm) for 2 min. To this solution, 5 ml $HAuCl_4$ (1 mM) and 70 µl of ascorbic acid (78.8 mM) was added with gentle stirring resulting in a change of the color of solution from yellow to colorless, this solution was stirred vigorously (380 rpm) for 2 min. After that, 12 µl of seed solution was added to the mixture. This solution was incubated overnight at room temperature, leading to the formation of purplish blue color of gold nanorods, which was centrifuged at 10,000 rpm (25 °C) and resuspended in 2 mM CTAB solution for further use.

2.3. Synthesis of Pd@ AuNR Nanostructure

Pd@AuNRs was synthesized by growing Pd nanoparticles on the surface of AuNRs (Jing and Wang, 2014) with modification. Briefly, 10 ml of the as-prepared gold nanorods (Section 2.2) were centrifuged and resuspended in 2 ml CTAB (2 mM) solution. 500 µl of resuspended AuNRs were diluted to 2 ml with milliQ followed by addition of 250 µl of H_2PdCl_4 with gentle stirring. This makes the final concentration of Pd in the reaction mixture to 0.18 mM. Finally, 500 µl of ascorbic acid (50 mM) was added to the solution and vigorously shaken (380 rpm) for 3 min. This solution was kept overnight undisturbed at room temperature followed by centrifugation at 10,000 rpm (28 °C) for 5 min. Pellet was finally resuspended in milliQ. Pd nanoparticles were also synthesized for reference purpose using this protocol except the addition of AuNRs.

2.4. Characterization of nanostructures

Physical characterization of gold nanorods and Pd@AuNRs was carried out using UV–Vis spectroscopy in the range (200–1100 nm) to determine their λmax and absorbance intensity on a CARRY60 Agilent (India) Uv–Vis spectrometer. TEM analysis was also carried out on a HITACHI (H-7500) electron microscope at 120 kV to further confirm the shape and deposition of Pd nanoparticles on the surface of nanorods by preparing the samples on a Cu grid and air drying it. Energy Dispersive Spectroscopy (EDX) analysis was done for compositional analysis on a Oxford Energy dispersive spectroscopy system. X-ray diffraction analysis was done on a Rikaku-Smart lab X-ray diffractometer at 40 kV, 30amperes at X-Ray wavelength 1.4 A° to confirm the crystal structure.

2.5. Enzyme mimetic activity of Pd@AuNRs and optimizing other reaction conditions

The enzyme mimetic peroxidase activity of Pd@AuNR was examined using OPD/H_2O_2 and TMB/H_2O_2 substrate. Checkerboard assay was carried out at different TMB and OPD/H_2O_2 concentrations. With TMB as chromogenic substrate, 100 µl of Pd@AuNR were mixed with equal volume (75 µl) of different concentrations TMB and H_2O_2. This reaction mixture was incubated in dark for 10 min followed by addition of 50 µl of 0.5 N H_2SO_4 to stop the reaction and the absorbance was recorded at 450 nm. For OPD, 100 µl of Pd@AuNR were mixed with different concentration of 150 µl OPD and 50 µl H_2O_2 in microtiter plate. After 5 min the absorbance of the oxidized product of OPD was recorded at 417 nm on a Tecan sunrise ELISA reader. MilliQ was taken as negative control. Various reaction conditions were also optimized for Pd@AuNR peroxidase assay with OPD substrate. The optimum amount of Pd@AuNRs is determined by taking various concentrations of the original stock and evaluating their peroxidase activity. Stability of the Pd@AuNRs was also investigated by adjusting their pH (2–10) and evaluating their peroxidase activity after 2 h. Temperature stability was determined by incubating an appropriate concentration of Pd@AuNRs at varied temperatures (25–70 °C) for 2 h and subsequently evaluating their peroxidase activity.

2.6. Enzyme kinetics of Pd@AuNRs

Apparent kinetic parameters of Pd@AuNR were determined by recording absorption spectra at 10 min interval. Steady-state kinetic assays were carried out at 25 °C with OPD/H_2O_2. Enzyme activity and reaction rate was calculated using absorbance at 417 nm and molar extinction coefficient of OPD product at 417 nm (Fornera and Walde, 2010). Kinetic parameters of the Pd@AuNRs were also compared with horseradish peroxidase enzyme as a positive control. The apparent kinetic parameters were determined on the basis of Michaelis Menten (Eq1) and Lineweaver-Burk plot (Eq 2):