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Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging

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ABSTRACT

Gold nanoparticles have received a great deal of interest due to their unique optical and catalytic properties and biomedical applications. Developing applications as well as assessing associated risks requires an understanding of the interactions between Au nanoparticles (NPs) and biologically active substances. In this paper, electron spin resonance spectroscopy (ESR) was used to investigate the catalytic activity of Au NPs in biologically relevant reactions. We report here that Au NPs can catalyze the rapid decomposition of hydrogen peroxide. Decomposition of hydrogen peroxide is accompanied by the formation of hydroxyl radicals at lower pH and oxygen at higher pH. In addition, we found that, mimicking SOD, Au NPs efficiently catalyze the decomposition of superoxide. These results demonstrate that Au NPs can act as SOD and catalase mimetics. Since reactive oxygen species are biologically relevant products being continuously generated in cells, these results obtained under conditions resembling different biological microenvironments may provide insights for evaluating risks associated with Au NPs.
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1. Introduction

During the rapid developments in nanoscience and nanotechnology, intense interest in gold nanostructures has emerged due to their unique optical, electronic and catalytic properties [1]. Bulk gold has been long recognized as an inert material, yet Au particles at the nano scale have been demonstrated to act as catalysts for a number of chemical reactions under a range of experimental conditions. For example, gold clusters can catalyze the oxidation of CO at or even below room temperature with extraordinary efficiency [2]. In addition, Au NPs have been studied intensively as promising materials for medical applications due to their superior optical and photothermal conversion properties, easy synthesis and surface modifications with multifunction [3,4]. Many efforts have been made to extend the use of Au NPs with different shapes (sphere, rod and cage-like) in bioimaging [5], biosensors [6], photothermal therapy and drug delivery [7,8].

Recently, a number of nanomaterials have been recognized as enzyme mimetics. Metals [9,10], metal oxides or sulfides [11–14], and carbon based nanostructures [15,16] have been found to exhibit intrinsic oxidase- and peroxidase-like activities observed in Fenton-like reactions and decomposition of hydrogen peroxide, respectively. Owing to their enzyme-like activity, nanoparticles of noble metals have been used to fabricate biosensors for detection of hydrogen peroxide, glucose, enzyme inhibitors, and antigen/antibody [9–16]. For example, Au NPs with different coatings and BSA-stabilized Au clusters exhibited peroxidase-like activity along with good biocompatibility, which may make gold nanoparticles promising candidates for use in biosensors [17–19].

These widespread applications of Au NPs in medical and consumer products inevitably result in increased human exposure. Therefore, it is essential to have adequate information about the safety of these materials. Studies have found that Au NPs larger than 10 nm are inert and biologically safe. For example, Au NPs have been reported to be biocompatible and have not elicited cytotoxicity or immunogenic effects after being taken up by cells [20,21]. However, Au NPs have been shown to induce DNA damage and in turn inhibit cell proliferation in human embryonic lung fibroblasts [22].

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Investigators also observed that Au NPs with negatively charged coatings and smaller size were more toxic than Au NPs with positively charged coatings and larger size [23,24]. Others have demonstrated that shape appears to play a key role in mediating the cellular response to Au NPs. Au nanospheres (coated with mercaptopropane sulfonate) were found to be nontoxic to HaCaT cells even at a dose of 100 μg/mL. In contrast, Au nanorods (polyethylene glycol-coated), significantly decreased the viability of HaCaT cells at 25 μg/mL and caused a significant production of reactive oxygen species (ROS) and up-regulation of several genes involved in cellular stress and toxicity [25]. These studies suggest that the biological effect of Au NPs not only depends on their particle size and shape, but also the surface modification and concentrations. In addition, the reported enzyme-like activities of Au NPs may affect their biocompatibility. A thorough and systematic assessment of the biological effects of Au NPs is needed, especially for Au NPs larger than 10 nm. To understand the overall biological activities of nanoparticles, it is critical to examine the multiple interactions between Au NPs and biological systems. Reactive oxygen species (ROS), including hydroxyl radicals, superoxide, hydrogen peroxide and singlet oxygen, have been recognized as prevailing contributors in oxidative stress and toxicity induced by nanoparticles [26]. Although Au NPs have been reported to induce ROS, it remains unclear which ROS are produced. ESR is the most reliable and direct non-destructive method for identification and quantification of short-lived free radicals [27,28]. We have employed ESR to investigate the generation of reactive species facilitated by Au NPs in a biologically relevant model system [29–31].

In biological systems, ROS such as H_2O_2, hydroxyl radicals, and superoxide are continuously generated and their levels must be controlled to maintain redox homeostasis in cells and tissues. Overproduction of these ROS can cause oxidative stress and in turn major adverse effects. Among these ROS, hydroxyl radicals are considered as the most reactive species. In addition, oxygen is an essential component in living organisms and subtle changes in oxygen availability can have a profound impact on physiological and homeostatic processes [32]. In the current study, we have examined the interactions between well characterized commercial gold particles (10–100 nm) and hydrogen peroxide in both acidic and alkaline conditions. This study provides a model system for understanding the effects of Au NPs under biologically relevant conditions. Also, ESR spin trap and spin label techniques have been employed to determine generation of hydroxyl radical and oxygen, respectively.

2. Materials and methods

2.1. Materials

Au nanoparticles (10, 20, 30, 40, 50, 60, 75, 100 nm) with a spherical morphology and coated with polyvinylpyrrolidone (PVP) or tannic acid were purchased from nanoComposix, Inc. (San Diego, CA) and were used as received. Au nanorods (Au NRs) with aspect ratio of ~4.0 (longitudinal surface plasmon resonance peak around 800 nm) coated with polystyrenesulfonate (PSS) or poly(diallyldimethylammoniumchloride) (PDDAC) were prepared according to reported methods [33]. Spin traps 5, 5-dimethyl N-oxide pyrroline (DMPO) and 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO) were purchased from Radical Vision (Marseille, France) and Bioanalytical Labs (Sarasota, FL), respectively. Spin label 3-carbamoyl-2,5-dihydro-2,2,5,5-tetramethyl-1H-pyrrol-1-yloxyl (CTPO), spin trap α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4 - POBN), 30% H_2O_2, dimethyl sulfoxide (DMSO), ethanol, diethylene triamine pentaacetic acid (DTPA), xanthine, xanthine oxidase, and standard buffer solutions were all purchased from Sigma–Aldrich (St. Louis, MO). Each buffer stock solution (pH 1.2 HCl-KCl, pH 3.6 HAc-NaAc, pH 4.6 HAc-NaAc, pH 5.5 HAc-NaAc, pH 6.5 HAc-NaAc, pH 7.4 PBS, pH 9.5 borax and pH 11.0 borax) was 0.1 M. Before use, each buffer was treated with Chelex® 100 molecular biology grade resin from Bio-Rad Laboratories (Hercules, CA) to remove trace metal ions. Milli-Q water (18 MΩ cm) was used for preparation of all solutions. Artificial perspiration containing lactic acid, uric acid, urea, minerals, and amino acids was obtained from Pickering Laboratories (Mountain View, CA).

2.2. Characterization

Transmission electron microscopy (TEM) images were captured on a JEM 2100 FEG (JEOL) transmission electron microscope at an accelerating voltage of 200 kV located at the NanoCenter, University of Maryland, College Park, MD. To observe the morphological evolution of the nanoparticles after reaction, 0.2 mg/mL Au NPs, 10 mM buffer solutions and 5 mM H_2O_2 were mixed in 0.5 mL H_2O for 30 min and twice centrifuged (12,000 rpm, 5 min). After the supernatants were decanted, 20 μL water was added to redisperse the precipitates. The samples for TEM analysis were prepared by adding drops of the redispersed colloidal solutions onto standard holey carbon-coated copper grids, which were then air dried at room temperature.

UV–Vis absorption spectra were obtained using a Varian Cary 300 spectrophotometer. The UV–Vis spectra of Au NPs in the presence of H_2O_2 at different pHs were obtained as follows: 5 mM H_2O_2 (freshly prepared) was mixed with 10 mM different pH buffers and made up with H_2O to 1.0 ml H_2O. Then, 0.1 mg/mL 10 nm PVP coated Au NPs were added into the above mixture. To monitor the evolution of the reaction, absorption spectra were collected at 1 min intervals using the scanning kinetics mode of the spectrophotometer.

2.3. Electron spin resonance

All ESR measurements were carried out using a Bruker EMX ESR spectrometer (Billerica, MA) at ambient temperature. Fifty microliter aliquots of control or sample solutions were put in glass capillary tubes with internal diameters of 1 mm and sealed. The capillary tubes were inserted into the ESR cavity, and the spectra were recorded at selected times. Other settings were as follows: 1 G field modulation, 100 G scan range, and 20 mW microwave power for detection of spin adducts using spin traps and 0.04 G field modulation, 5 G scan range, and 1 mW microwave power for ESR oximetry using the spin label CTPO.

The spin traps DMPO, BMPO and POBN were employed to verify the formation of hydroxyl radicals (•OH) during the degradation of H_2O_2 in presence of Au NPs under various conditions. The amount of hydroxyl radicals was quantitatively estimated by the ESR signal intensity of the hydroxyl radical spin adduct (DMPO/•OH) using the peak-to-peak height of the second line of the ESR spectrum. The hydrogen peroxide solution was mixed with DMPO in buffers of different pHs and the reaction was initiated by addition of Au NPs. ESR spin label oximetry is a quantitative approach to measure oxygen content using the water soluble spin label CTPO whose ESR spectrum has a super hyperfine structure. Since the change of super hyperfine structure is sensitive to the oxygen molecule, the O_2 concentration can be sensitively monitored. The detailed method for calculation of the O_2 concentration has been described in a previous review [34]. The sample mixture, containing CTPO, H_2O_2, buffer with or without Au NPs, was deoxygenated by aerating with N_2. The final concentration of each component is described in each figure caption.

To verify the ability of Au NPs to scavenge superoxide anions, xanthine and xanthine oxidase (XOD) were mixed together in a PBS buffer (pH 7.4) to generate superoxide and BMPO was used to trap superoxide in the form of spin adduct BMPO/•OOH. The control sample contained 25 mM BMPO, 0.05 mM DTPA, 1 mM xanthine and 0.2 U/mL XOD in 10 mM pH 7.4 PBS, to which SOD or Au NPs was additionally introduced to scavenge radicals. The reaction was started by adding XOD. ESR spectra were recorded at selected time intervals.

3. Results and discussion

3.1. Au NPs interaction with hydrogen peroxide: generation of hydroxyl radicals

In most available reports, the production of ROS induced by Au NPs has been demonstrated by indirect spectroscopic methods [35–37]. Definitive identification of short-lived ROS, such as hydroxyl radical and superoxide, with these methods is difficult since these reactive species can be quickly scavenged by endogenous reductants or scavengers. However, these ROS readily react with diamagnetic nitrone spin traps, forming a stable free radical (spin adduct) that can be identified from the magnetic parameters of the ESR spectrum. Therefore, herein we used this ESR technique to identify the ROS generated in the presence of Au NPs. To confirm the generation of hydroxyl radicals induced by Au NPs, we chose three spin traps that are often used to capture hydroxyl radicals, POBN, BMPO and DMPO. Fig. 1 shows the ESR spectra obtained for solutions at pH 1.2 containing different spin traps in the absence and presence of Au NPs. In samples containing H_2O_2 and a spin trap (Fig. 1A), characteristic ESR spectra of spin adducts attributable to hydroxyl radical were weak. In contrast, strong ESR spectra of the hydroxyl radical spin adduct appeared upon the addition of