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Assessment of Behavior of Rice Root Peroxidase in the Presence of Silver Nanoparticles

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Abstract

Background: Silver Nanoparticles (AgNPs) can change proteins function and structure. The increased production and high surface reactivity of silver nanoparticles, has interested researchers to study the interactions of these particles with biomolecules.
Objectives: The present study aimed to show the effects of AgNPs on rice plant root peroxidase enzyme and the interaction quality between silver nanoparticles and the enzyme.
Materials and Methods: Extracted peroxidase enzyme of rice plant root was treated by AgNPs at concentrations of 0, 20, 40, 80, 100mg/L for 2, 7 and 24 hours. The experiment was done with 15 treatments for measuring the peroxidase enzyme activity using the spectrophotometry method at a wavelength of 470.
Results: Low concentrations of AgNPs and short incubation times can have the maximum positive impact on the peroxidase activity, and in the present study the highest activity was seen at a concentration of 40 mg/L and two hours of incubation time.
Conclusions: This study suggests that changes of enzyme activity can occur as a result of the effect of silver nanoparticles on enzyme conformation, increase of reactive environment pH, and amount of substrate and enzyme stability.

Keywords: Silver Nanoparticles, Corona, Protein Conformation, Enzyme Assays, Guaiacol Peroxidase

1. Background

Many improvements have occurred regarding nanoparticles synthesis with exact sizes and specific characteristics (1). The most essential parts of nanotechnology are comprehensive understanding of nanoparticles interaction with proteins, and response of biological systems for analysis of nano medicine and nano biotechnology experimental results (2). One of the most important nanoparticles in industry is silver nanoparticles with production rate of 500 tons per year (3). The interactions of these particles with biomolecules and controlling these interactions especially with proteins, has become one of the main issues of research in this field, and the use of silver nanoparticles has been the subject of attention by different industries especially medicine, agriculture, food and production of disinfectants (4-7). The general reaction of physiological environments against the entrance of foreign substances into cells is by adjoining of biomolecules like proteins to these particles that create coronas (8). Forming a corona is a competitive process, and these entities start to grow in biological solutions quickly and upon the clash of nanoparticles with proteins and under influence of hydrodynamic, electrodynamics and electrostatic forces (9, 10). The created corona quickly becomes complete in a way that at first the proteins with high concentration absorb quickly to these particles, yet during the time lapse their positions are substituted with those proteins, which have the highest tendency (1, 11). These particles can have deep impacts on the proteins at conformation and performance levels, protein tertiary structure (by decoration of a helixes and beta sheets), compact construction of the central hydrophobic amino acids, a sharp reduction in entropy and electrostatic repulsion are controlled; the reaction of the protein with the charged levels and interaction with foreign forces can make some changes in the three dimensional structure of the protein (12, 13). The created changes in the three dimensional structure of the protein can be considered as the main reason of nanoparticles entrance in enzymes and their function in the increase of activity and stability of the enzyme. Most studies have been done on proteins with due attention to the protein sensitivity to the surroundings environment (14, 15). Song et al. reported that the activity of peroxidase enzyme, adsorption to graphite, single-layer carbon nanotubes and fullerenes were increased in terms of denaturation. Other studies reported that the six-fold activity increased the absorption of myoglobin on SBA-15 (2, 16). The proteins stabilization on the suitable supporters can also increase

the proteins activity and stability; the thermal stability of the glucose oxidase enzyme absorption to the nano-silica is much more than the free enzyme (14); however, reversed results may be observed. Peroxidase, as a general enzyme among fungi, plants and vertebrates has many functions in different areas and it is used in the biology and industry areas as a hydrogen peroxide indicator (13), and in Enzyme-Linked Immmunosorbent Assay (ELISA) systems, for polymerization and deposition of liquid phenol (15).

2. Objectives

The aim of this research was to study the interaction of silver nanoparticles with the peroxidase enzyme regarding enzyme activity and the state of nanoparticles interaction with the protein.

3. Materials and Methods

The AgNPs suspension: The silver nanoparticles were created by the chemical reduction method of silver nitrate aqueous solution by sodium citrate, in the presence of 0.1% polyvinyl alcohol (PVA) stabilizer under stirring at a speed of 1000 rpm and 97 ± 2°C the optical properties of AgNPs were evaluated using a 2501PC Shimadzu Co UV-Vis spectrophotometer. Size of the prepared AgNPs was studied using a Nanophox DLS equipped with 632.8 nm HeNe-laser from Sympatec Co. (Clausthal-Zellerfeld, Germany). For the X-ray powder diffraction study, the powder microcrystalline sample was loaded to an aluminum sample holder that was rotated during data collection to improve particle statistics and to minimize preferred orientation effects. Diffraction data were collected at a range of 1 - 80, (2 [U+019F]), on an STOE STADI P diffractometer equipped with a scintillation detector, secondary monochromator and Cu Ka1 radiation ($\lambda = 1.5406$ A°) (17).

Plant samples: the rice seeds (Orzya sativa, cv. IR651) were obtained from the international rice research institute (IRRI, Iran) and seeds were settled on Yoshida broth by a screen grid and in a growth chamber with distinct temperature (27°C at days and 25°C at nights) and 16 hours photoperiod and relative humidity. Then, after proper growth of the plant, the samples were taken and the roots were kept at the temperature of minus 70°C.

Enzyme extraction: in order to extract the peroxidase enzyme, the plant root was crushed in 0.1 M Tris-HCl buffer pH 8 and Polyvinylpyrrolidone (PVP) at 4°C. The crushed tissue was centrifuged at 20000 g at 4°C during 20 minutes, and the obtained supernatant was used as the enzyme source (18).

The peroxidase enzyme activity in the presence of the silver nanoparticles was measured by the spectrophotometry method (18). The enzyme activity was measured by 15 treatments for measuring the guaiacol peroxidase enzyme activity with the spectrophotometry method and by measuring the absorption at a wavelength 470 nm. For each treatment, the reaction was started by adding 1.5 μL of treated enzyme source with AgNPs at concentrations of 0, 20, 40, 80 and 100 mg/L and durations of 2, 7 and 24 hours to 800 μL of reactive buffer containing 5 mM guaiacol and 5 mM hydrogen peroxide in 0.2 M phosphate buffer and pH 5.8. The absorption of tetraguaiacol, as the product of the reaction, was measured at 470 nm for calculating the peroxidase enzyme activity. The activity in each treatment was reported as a percentage of maximum activity. The silver nanoparticles stability is the main determining factor of their influence, and controlling the accumulation and deposition of nanoparticles in the aquatic environment plays a basic role in this process. In order to control these events, the buffer used for this study had a simple combination and its components showed less interaction with the silver nanoparticles. Besides, at the beginning, the nanoparticles were diluted in double distilled water and all of the experimental stages were done at 4°C and on a shaker. According to Dynamic Light Scattering (DLS) reports, the nanoparticles did not show notable changes in size (12, 19).

4. Results

In order to investigate the effects of nanoparticles on enzyme, in this study the silver nanoparticles' impact on guaiacol peroxidase enzyme was studied. In the present study, peroxidase activity was examined in the presence of five different concentrations of AgNPs (0, 20, 40, 80 and 100 mg/L) and three different incubation times (2, 7 and 24 hours). The results showed that AgNPs are able to increase the peroxidase enzyme activity (Figure 1). The peroxidase activity responses to different concentrations and incubation times with AgNPs was determined by considering the fact that there were clear differences between the obtained results of the two AgNPs concentrations at different durations of time. Low concentrations (< 100 mg/L) and short incubation time (two hours) had the maximum positive effect on this enzyme activity. The most activity was seen at the concentration of 40 mg/L and the incubation time of two hours. The peroxidase enzyme showed activity reduction when incubation time was increased at the mentioned concentration. The 20 mg/L concentration significantly increased peroxidase activity. The increase of incubation time did not have a negative impact on the activity level; the low interaction level of nanoparticles with the enzyme and lack of intense impact on the enzyme structure

at this concentration can be one of the reasons for the lack of activity reduction with the increase of treatment time. The highest activity reduction was reported at the concentration of 100 mg/L silver nanoparticles and two hours treatment time; at the primary hours of treatment, the enzyme activity is intensely under the influence of nanoparticles yet the enzyme structure is partly restored by increasing the incubation time in a way that most activity is seen at the concentration of 100 mg/L after 24 hours of incubation.

The physical, chemical and biological characteristics of silver nanoparticle were characterized. Particles smaller than 20 nm exhibited absorption only below 430 nm, as can be seen from the UV spectra in Figure 2A ; colloidal AgNPs had the maximum absorbance (lmax) at 426 nm. DLS analysis Figure 2B of synthesized AgNPs, demonstrates that their size, volume mean diameter (VMD), and surface mean diameter (SMD) were 18.34 nm (X99), 4.10 nm and 2.26 nm, respectively. Based on the TEM images (Figure 2C), spherical AgNPs with relatively uniform in size distribution (Figure 2) (17).

5. Discussion

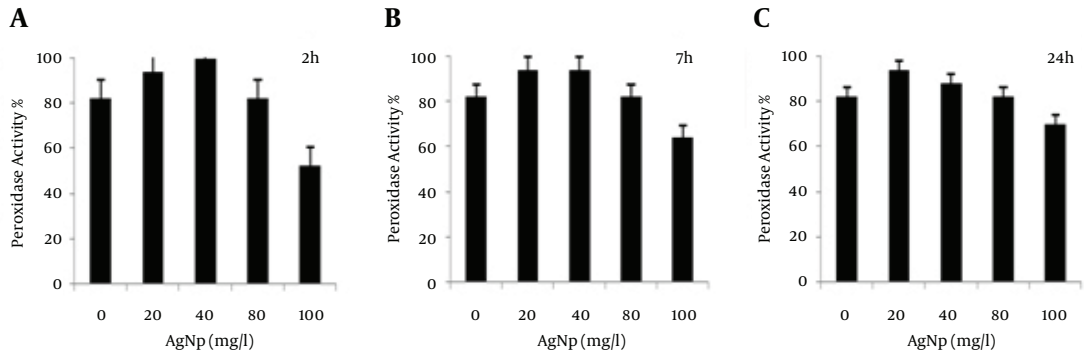
Peroxide production, change in environmental pH reaction and increase of substrate: oxidation of AgNPs to the silver ion is not accomplished with direct reduction of oxygen to water, yet it is an oxidation-reduction (redox) reaction that occurs along with peroxide production as an intermediate. The hydrogen peroxide is the easiest peroxide that is produced during the release of silver ions. In solutions containing silver ions as a control, no peroxide was produced that suggest the fact that its production depends on the release of silver ions from nanoparticles. This reaction is shown in the Figure 3 (4-6, 15, 16).

The first reaction shows the silver nanoparticles interaction path with water molecules; this reaction is spontaneous and with released energy of -91.3 kJ/mol and at 298°K (3, 20). In this reaction, in addition to the peroxide production that is enumerated as the raw material of the reaction at the second reaction, the path can cause reactive environment pH increase by hydrogen consumption. The proteins absorption has a high dependence on the reaction's environmental pH, in a way that at intense acidic and basic environments, the proteins tendency lowers towards nanoparticles and these acidity changes could have deep impacts on the peroxidase enzyme construction (12, 19, 20).

In order to separate the peroxide that is produced in reaction 1, and assessment of its impact on the products produced in reaction 2, an experiment was conducted in which the buffer had no hydrogen peroxide; this method was then applied on each of the 15 treatments with the obtained results presented in Figure 4. As observed from Figure 4A - C the produced hydrogen peroxide was not high enough for significant effects on the amount of produced tetraguaiacol, and there were no regular changes in the diagrams. The shown changes in Figure 2 can be attributed to peroxidase activity changes.

Restoration of enzyme activity by increasing the incubation time of each treatment could suggest that the peroxidase enzyme upon clashing with the silver nanoparticles catches remarkable conformational changes for forming nanoparticle-protein complexes. The peroxidase enzyme has a reflexive conformation status on the nanoparticles and shows a fairly wide activity range; the highest activity level was with the 40 mg/L treatment of the silver nanoparticles and incubation time of two hours and the lowest activity level occurred with 100 mg/L treatment of silver nanoparticles and incubation time of two hours. The lack of noticeable peroxide production in the reaction environment by AgNPs could indicate that the enzyme activity increase was due to changes in enzyme structure. However, the medium components such as PVA, citrate, and tris-HCl can influence the chemical species of AgNPs, thus indirectly affect enzyme activity. High reactivity of AgNPs can have the most effect on oxidized guaiacol production.

Figure 1. The Peroxidase Enzyme Activity With Different Treatments of Silver Nanoparticles



A, The highest activity was measured with two hours of incubation time and 40 mg/L concentration of silver nanoparticles while the lowest one was with two hours of incubation and 100 mg/L concentration; B, there was no significant difference in comparison with two hours of treatment; C, significant restoration of lost enzyme activity was observed after 24 hours of incubation time and 100 mg/L silver nanoparticles concentration.

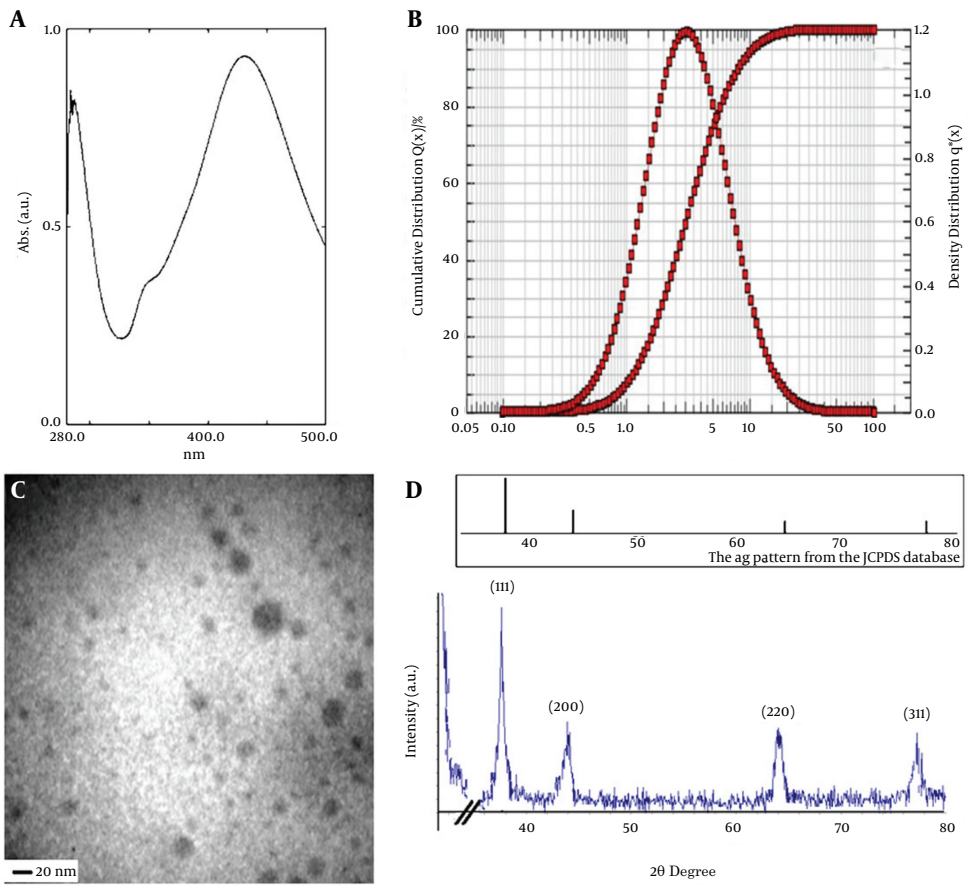


Figure 2. A, UV absorbance spectra in the range of 280-500 nm; B, dynamic light scattering and size distribution at 632.8 nm; C, TEM image and (d-bottom) X-ray powder diffraction patterns of aqueous colloidal AgNP; the Ag pattern from the JCPDS database is also shown for comparison (d-top).

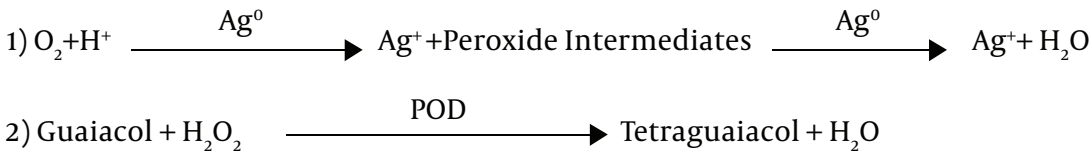
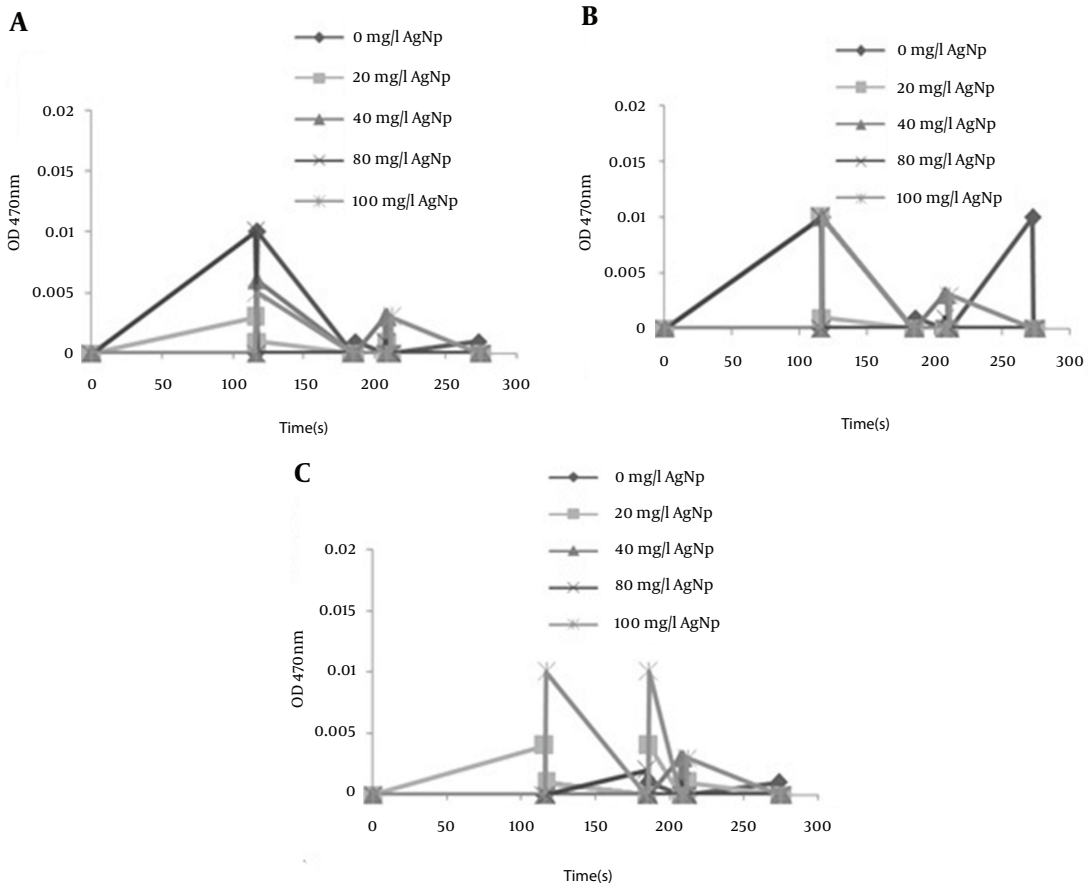


Figure 3. Chemical Reaction

Figure 4. The Peroxidase Enzyme Activity in the Absence of Peroxide in the Reactive Buffer



A, The lack of significant oxidized guaiacol production at the all concentrations of AgNPs (0, 20, 40, 80 and 100 mg per liter) and incubation time of two hours; B, no significant oxidized guaiacol production at all concentrations of AgNPs (0, 20, 40, 80 and 100 mg per liter) and incubation time of sever hours; C, no significant oxidized guaiacol production at all concentrations of AgNPs (0, 20, 40, 80 and 100 mg per liter) and incubation time of 24 hours.

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