Anticancer and Antioxidant Properties of Ag NPs Coated with BSA NPs

M. Azizi¹, H. Ghouarchian¹, F. Yazdian², F. Dashtestani¹

¹- Institute of Biochemistry and Biophysics (IBB), University of Tehran, Iran
²- Faculty of New Science and Technology, University of Tehran, Iran

Abstract

There has been considerable interest in developing albumin nanoparticles as drug delivery devices. Albumin is an important endogenous antioxidant due to its potential of acting as reactive oxygen species scavenger. On the other hand, toxicity of silver nanoparticles had been demonstrated on cancer cell lines. In the present study, Ag NPs coated with BSA NPs were synthesized by silver nanoparticles which were coated with bovine serum albumin (BSA) via desolvation technique. The Ag NPs coated with BSA NPs formation was confirmed by UV-Vis spectroscopy and Dynamic Light Scattering (DLS). Human breast cancer cells (MCF7 cells) were then cultured in the presence of the nanoparticles to evaluate the cytotoxicity of Ag NPs coated with BSA NPs by the MTT colorimetric technique. The antioxidant activities of Ag NPs coated with BSA NPs were evaluated in terms of their inhibition of autoxidation rate of pyrogallol as superoxide. The effect of Ag NPs coated with BSA NPs on MCF7 exhibit a dose-dependent toxicity for the cell tested and the viability of MCF-7 decreased to 50% (LD50) at the concentration of 5 μg/mL. The IC50 value of antioxidant activities of Ag NPs coated with BSA NPs were 8 μg/mL which demonstrated that Ag NPs coated with BSA NPs were good superoxide scavengers. In conclusion, our data show that Ag NPs coated with BSA NPs had antioxidant and anticancer activities in MCF-7 cells.

Keywords: Silver Nanoparticles, bovine serum Albumin, Breast Cancer cells, MCF-7

1. Introduction

Antioxidants are chemical compounds which contain monohydroxy/polyhydroxy phenol; their primary objective is to decrease the lipid peroxidation [1]. The free radicals can also be generated in biological systems in the form of reactive oxygen species (ROS), such as superoxide anion radicals (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH⁻), and the singlet oxygen (1O₂). The mentioned reactive ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA. Antioxidants have low activation energy to donate hydrogen atom and hence, cannot initiate the second free radicals. Albeit normal cells possess antioxidant defense systems

* Corresponding author: yazdian@ut.ac.ir
against ROS, the continuous accumulation of damage to the cells induces diseases such as cancer and aging. The continuous exposure to antioxidant also plays a preventive role against such diseases by removing the ROS in biological systems [2]. The free radical electrons are stable and thus, slow down the oxidation. The non-enzymatic antioxidants are actually the scavengers of ROS and reactive nitrogen species (RNS); these involve glutathione, vitamin E and C (inhibits oxidation of membrane lipid), uric acid is the scavenger of peroxynitrite in plasma, human serum albumin (HSA), bilirubin, N-Acetyl cysteine (NAC), melatonin which directly reacts with ROS and forms disulfides [1]. Serum albumin, a dominant component of the total blood protein, plays an important role in maintaining osmotic pressure and blood pH, transport of endogenous and exogenous molecules [3]. Albumin’s high stability, solubility, long circulatory half-life, and binding sites for a large number of drugs have attracted considerable attention of the formulation scientists. Bovine serum albumin (BSA), which has more than 70% similarity to the human serum albumin (HSA), is stable, biocompatible and readily available at a much lower cost than HAS [4,5]. Moreover, BSA is more susceptible to heat driven by aggregation than HAS [6]. A variety of protocols have been reported in literature for the preparation of albumin nanoparticles for encapsulating a wide range of drugs [7]. Recently, disulfide bond reducing methods using various chemical cross linkers have been investigated for the encapsulation of hydrophobic drugs [8-10].

Anticancer agents, on the other hand, are mainly related to their curative role in a damaged system. Under normal conditions, the cells in which the DNA or other components are irrespectively damaged for various reasons, undergo apoptotic cell death. This phenomena acts as a self-destructive metabolism according to the genetically encoded cell death-signal. However, cancer cells, which are already irreversibly developed, obtain the capability to evade apoptosis in various ways. The aim of anticancer agents is to trigger the apoptosis signaling system in these cancer cells whilst disturbing their proliferation [11].

Metallic silver nanoparticles (Ag NPs) are among the up-and-coming nano-products that have gained increasing interest in the field of nano-medicine due to their structure and functional properties. In addition, their obvious therapeutic potential in treatment of several diseases, including retinal neovascularization [12,13] and acquired immunodeficiency syndrome due to human immunodeficiency virus (HIV) [14]. Ag NPs are also recognized for antimicrobial potential against several other viral diseases, including hepatitis B [15], respiratory syncytial virus, herpes simplex virus type 1, and monkey pox virus [16]. Silver oxide nanoparticles illustrated antitumor activity in transplanted Pliss lymph sarcoma cancer models while prescribed by intravenous injection in the form of aqueous dispersions [17]. Antimicrobial activity of silver is well recognized; however, there are scarce reports on its usage as an antitumor agent. Among these, there is a report on the cytotoxic effect of Ag NPs on human glioblastoma cells (U251) [18]. Furthermore, a variety of studies show cellular transformations resulting from oxidative stress were produced by the
generation of reactive oxygen species (ROS) in tumor cells which enhances the cytotoxicity activity of the chemotherapeutic drugs [19].

In this study, silver nanoparticles were coated with bovine serum albumin, and their antioxidant activity was compared with one natural antioxidant enzyme (superoxide dismutase) in human body. In addition, the anticancer activity of (Ag NPs coated with BSA NPs) against breast cancer cells of MCF-7 was also examined. As well, Dynamic Light Scattering analysis, UV visible spectroscopy, DPPH antioxidant and MTT anticancer assay were performed.

2. Materials and method

2-1. Materials

BSA (fraction V, minimum 98%), glutaraldehyde 8% solution was purchased from Sigma (Steinheim, Germany). DMEM and fetal bovine serum were purchased from Gibco (United States); 3-(4,5- dimethyl-thiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin from Sigma (United Kingdom); and silver nitrate (AgNO$_3$), dimethyl sulfoxide (DMSO) and 1,1-diphenyle-2-picrylhydrazyl (DPPH) from Merck (Germany).

2-2. Cell line and culture conditions

The MCF-7 human breast cancer cell line was purchased from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran, Iran). Cancer cell line was maintained in DMEM culture medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin. The resulting cells were incubated at a 5% carbon dioxide (CO$_2$) cell incubator and the temperature was kept constant at 37°C.

2-3. Synthesis of Ag NPs coated with BSA NPs

Preparation of Ag NPs coated with BSA NPs was done by a desolvation method. In principle, 100 mg BSA was dissolved in 2.0 mL of 10 mM NaCl solution and then titrated to pH 8.5. In another beaker, 1mg/mL Ag NPs was dissolved in 3.6 mL desolvating agent which was ethanol. In the next step, BSA solution was transformed into nanoparticles by the continuous addition of 3.6 mL of prepared ethanol under stirring (550 rpm) at room temperature at a defined rate of 1mL/min. During the process, solutions were mixed vigorously with hot-plate stirrer until change of color was evident (pale yellow). After the desolvation process, 100 µL of 8% glutaraldehyde was added to induce particle crosslinking. The crosslinking process was performed under stirring of the suspension over a time period of 24 h. The colloidal solution of Ag NPs coated with BSA NPs was separated by an ultracentrifugation and characterized by using UV-Visible spectroscopy [20].

2-4. Cytotoxicity assay

MTT-based assay was performed by preliminary seeding of $1 \times 10^4$ cancer cells in a 100 µl growth medium in the presence of increasing concentrations of Ag NPs (0, 5, 10, 15, 20, 30, 40, 50 µg/mL) in 96-well plate. Individual and subsequent incubation of cells was performed at 37°C in 5% CO$_2$ for 24 h. The cells were thereafter treated with 25 µl of
MTT (4 mg/mL) and incubated at 37°C for 4 h. Nontreated cells were used as control. After dissolving formazan crystals in DMSO, 96-well plates were read in a microplate reader at 570 nm. Each experiment was repeated three times and the MTT assay was performed in triplicate for each experiment. Cytotoxicity was calculated as the percentage of viable cells at different concentrations of samples relative to the control (untreated) cells. Also, the half maximal inhibitory concentration (LD_{50}) was calculated as the concentration required for inhibiting the growth of tumor cells in culture by 50% compared to the untreated cells [21,22].

2-5. Antioxidative assay

Superoxide was generated indirectly at alkaline pH by the action of oxygen on pyrogallol. SOD reacts with the superoxide and this slows down the formation rate of the o-hydroxy-o-benzoquinone and other polymer products. One unit of SOD is defined as the amount of enzyme that inhibits the rate of pyrogallol autoxidation by 50% . The assay was performed in 1 mL of 0.05 M Tris-HCl buffer at pH 8.2, containing 10^{-4} M EDTA at 27°C. The standard reaction mixture usually contains a certain concentration of pyrogallol, which produces an autoxidation rate of 0.07 (absorbance per minute) at 325 nm. Under this condition, the amount of SOD or its mimetic compounds required for decreasing the autoxidation rate of pyrogallol to 0.035 absorbance per min is defined as one unit of activity and is called half-maximal inhibitory concentration (IC_{50}) [23]. Native BSA and Ag^+ as controls in antioxidant assay were applied.

3. Results and discussion

3-1. Synthesis of silver nanoparticles and their characterization

Ag NPs coated with BSA NPs was prepared using the one-step desolvation technique of ethanol as the desolvating agent and glutaraldehyde as the crosslinking agent have been found to be highly stable in water and in cell medium. The synthesized Ag NPs coated with BSA NPs was characterized by UV-Vis spectroscopy (Fig. 1). The primary analysis of synthesized nanomaterials by UV–Vis spectroscopy has proven to be a very valuable technique for characterization of nanomaterials [24,25]. Synthesis of Ag NPs coated with BSA NPs was further confirmed by UV-Vis absorption spectrum as shown in Fig. 2 (solid line). Fig. 1 (dashed line) shows the absorbance of BSA. The strong absorption of BSA near 280 nm is the absorption peak of fluorophore tryptophan [26]. On the other side, Fig. 2 (dotted line) shows the absorbance of Ag NPs with the main absorption peak at 400 nm. The spectra of Ag NPs-loaded BSA nanoparticles showed main characteristic peaks of Ag NPs with negligible shift at 400 nm, which indicated no change in the chemical structure of Ag NPs in the formulation. A solid line in the main peaks of both Ag NPs and BSA can be observed in the spectrum of Ag NPs coated with BSA NPs in Fig. 2. This verifies the presence of both agents in the new nanoparticles.

The particle-size histogram of Ag NPs coated with BSA NPs (Fig. 3) indicates that Ag NPs coated with BSA NPs vary in size from 115nm to 190 nm. Most Ag NPs coated with BSA NPs (approximately 92%) obtained
by this method varied in their size from 115 nm to 150 nm. The mean diameter was 140.2 nm.

Figure 1. UV-Vis spectrum of Ag NPs coated with BSA NPs (solid line), Ag NPs (-----), BSA (-- - -).

Polydispersity of 0.337 verifies the non-uniformity that exists in the particle size distribution is narrow. Average of count rate which is a measure of signal intensity was 30.2 kilo counts per second.

3-2. Cytotoxicity assay

The cytotoxic effect of Ag NPs coated with BSA NPs was evaluated *in vitro* in MCF-7 human breast cancer cell lines at different concentrations of 0, 5, 10, 15, 20, 30, 40 and 50 µg/mL. As indicated in Fig. 4, cells show pattern of dose-dependent responses in presence of synthesized silver-albumin nanomaterials. In brief, at the low concentration of 10 µg/mL Ag NPs coated with BSA NPs, a significant cytotoxic effect was observed which reduced the percentage of viable cells from 100% to 20%, followed by a plateau at higher concentration.

Figure 2. Particle-size distribution histogram of Ag NPs coated with BSA NPs.

Figure 3. The effects of Ag NPs coated with BSA Nps on viability of $1\times10^4$ cell/mL MCF-7 cells

The LD$_{50}$ values associated with Ag NPs coated with BSA NPs are considered. The concentrations needed to produce 50% cell death were 5 µg/mL on the MCF-7 cell lines.

3-3. Antioxidative assay

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity. These analytical methods measure the radical scavenging-activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical ($O_2^{•-}$), the hydroxyl radical (OH$^•$), or the peroxyl radical.
(ROO•). The various methods used to measure antioxidant activity of food products can give different results depending on the specific free radical being used as a reactant. In this study we determined capability of nanoparticle to scavenge superoxide anion radical as superoxide dismutase (SOD) assay. The SOD activity of Ag NPs coated with BSA NPs is considered. As it was investigated in the presence of Ag NPs coated with BSA NPs, the autoxidation rate of pyrogallol decreases.

The $O_2^•$ scavenging activities (IC$_{50}$) of the Ag NPs coated with BSA NPs determined in a pyrogallol method was 8 $\mu$g/mL. Moreover, the IC$_{50}$ of BSA and Ag NPs were 40 and 18 $\mu$g/mL respectively. This shows that, by coating BSA on Ag NPs the SOD activity of resulting compound increases. IC$_{50}$ of native SOD was 0.1 $\mu$g/mL. It revealed that Ag NPs coated with BSA NPs have comparable $O_2^•$-scavenging activities with native SOD as natural defense in cellular system against oxidative stress.

4. Discussion

Ag NPs coated with BSA NPs was properly synthesized with desolvation technique. It was confirmed to exhibit antioxidant and anticancer activities. The superoxide radical scavenging assays suggest that the antioxidant activity is high enough and is comparable to the enzymatic antioxidants system of defense system against ROS in human body with superoxide dismutase. Their MTT assay revealed that Ag NPs coated with BSA NPs also contains a strong anticancer effect, which exhibited cyto toxicity against breast cancer cells of MCF-7. As the therapeutic characteristics such as anticancer effect are more important in comparison with antioxidant property (the assay of normal cell protection), the 5 $\mu$g/mL of Ag NPs coated with BSA NPs is suggested as therapeutic dose in vivo. However, further studies are needed to identify the exact molecular mechanisms of both the antioxidant and anticancer properties.

References

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