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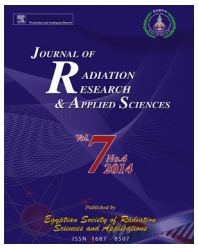

UV radiation sensitivity of bovine serum albumin bound to silver nanoparticles

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ABSTRACT

We report here the UV radiation sensitivity of Bovine Serum Albumin (BSA) bound silver nanoparticles (62 nm diameter) to various power density between 468 mJ/cm^2 to 1872 mJ/cm^2 under physiological conditions. The functional properties associated with BSA such as esterase activity, free thiols and copper ion binding have been studied. Decrease in free thiols, with increase in copper ion binding and P- nitrophenyl acetate (PNPA) turnover were observed in BSA bound silver nanoparticles (SNP) in the presence of UV radiation. Intrinsic fluorescence intensity of BSA bound SNP was decreased with UV radiation. Circular Dichroism results indicated a decrease in alpha helical content of BSA bound SNP. The overall results suggest modifications in structure–function properties of BSA bound to SNP in the presence of UV radiation. The possible mechanisms of interaction between BSA and SNP have been explained in presence of UV radiation.

1. Introduction

Understanding the mechanism of radiation induced damages either X-ray or UV radiation, in biological systems is of prime importance in radiation biology (Durchshlag, Hefferle, & Zipper, 2003). Radiation induced damage especially in presence of chemicals, are considered important due to the differential effects of radiation over them tumour and normal tissue. These differential effects can be achieved by effective chemical agents that specifically damage the tumour and spare normal tissue. Numerous investigators are taking efforts in this regard to develop chemical agents which could serve as efficient radiosensitizer or radioprotector. A large number of compounds have been identified as radiosensitizer or radioprotectos, and some of them have been under clinical trials. Proteins are important biomacromolecules that are sensitive to ionizing radiation as well as UV radiation (Boulton, Cleary, Papworth, & Plumb, 2001; Burke & Augenstein, 1969; Davies, 2003). Radiation induced alterations of molecular properties of proteins like breaking of hydrogen/covalent bonds, fragmentation, inactivation has been reported by several investigators (Cho & Song, 2000; Moon & Song, 2001). The hydroxyl and superoxide anion radicals generated by radiation which modify the primary structure of protein results in distortion of secondary and tertiary structure (Davis & Delsignore, 1987). Conformational changes in protein induced by UV, X-ray and γ- radiation have been reported in several studies (Durchshlag et al. 2003; Lee & Song, 2002).

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Recently nanoparticles have greatly attracted the attention of many investigators due to their unique physico-chemical properties and potential application in biology and medicine (Salata, 2004). Amongs these, silver nanoparticles (SNP) are being developed in the field of nanomedicine for treating various diseases (Couvreur & Vauthier, 2006). Expérimental studies had shown that SNP showed strong action against microbes (Ahmad, Wani, Manzoor, Ahmed, & Asiri, 2013; Dongre et al. 2010; Kim et al. 2007; Sukumaran & Poulose, 2012) viruses (Elechiguerra et al. 2005; Galdiero et al. 2011; Lara, Ayala-Nuñez, Ixtepan-Turrent, & Rodriguez-Padilla, 2010) and fungus (Jo, Kim, & Jung, 2009; Nasrollahi, Pourshamsian, Mansourkiaee, 2011; Panacek et al. 2009). SNPs are used in toothpaste, face creams, soaps and disinfectants because of their antimicrobial properties (Salata, 2004). A recent study had shown that SNP induced the antioxidants properties of some enzymes which reduced reactive oxygen species (Sharma et al. 2012). There are few reports are available on modification of radiation effect by SNP. Radiosensitizing effects of SNP have been reported on malignant glioma cells in vitro (Liu et al. 2013). Zheng et al. has used SNP alone for radiation therapy in cancer (Zheng, Yang, Wei, Tong, & Shu, 2013). The effectiveness in cancer due to shape of SNP have been studied (Boca et al. 2011). In contrast to this, radioprotecting property of SNP revealed when it is complexed with a phytoceutical Glyzyrrhizic acid (Chandrasekharan, Khanna, & Nair, 2011) and an antioxidant alpha-lipoic acid (Ramachandran and Nair 2011).

In the present study, attempts have been made to understand the UV radiation sensitivity of bovine serum albumin bound silver nanoparticles. BSA is the model of choice because it is the most abundant plasma protein (Carter, Chang, Ho, & Krishnaswami, 1994; Ziegler & Foegeding, 1990) has simple structure, small molecular weight and only one polypeptide chain. BSA is a carrier of fatty acids, amino acids, metals and drugs and has a great affinity for fatty acids, hematin and bilirubin etc. (Curry, Mandelkow, Brick, & Franks, 1998; Reed, 1997).

2. Material and methods

All the chemicals used were of an analytical grade, double distilled water used throughout the experiment. BSA and 5, 5 – Dithiobis (2- nitrobenzoic acid), silver nitrate, p-nitrophenol acetate (PNPA), EDTA, sodium ascorbate, copper sulphate and bathocuproine disulphonic acid (BCS) were used.

2.1. Synthesis and characterization of silver nanoparticles

SNP_S were synthesized by chemical reduction method. The method was slightly modified form of Lee and Meisel (Lee & Meisel, 1982). One millimole solution of silver nitrate heated on a hot plate with magnetic stirrer up to 80 °C, 1% sodium citrate solution was added drop by drop. The colourless solution turned pale yellow, which indicating the formation of SNP. SNPs were characterized by UV visible (Fig 1) and dynamic light scattering method. The average size of SNPs was approximate 62 nm.

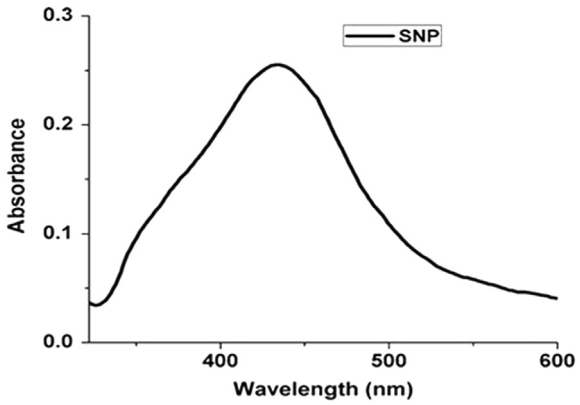

Fig. 1 – Absorption spectrum of silver nanopart.

2.2. UV radiation source

Fifteen Watts of UV radiation source was used and the energy of radiation measured by ILT 77 Germicidal radiometer (International Light Technologies, Inc.). The samples were exposed for 30 min (468 mJ/cm^2), 60 min (936 mJ/cm^2), 90 min (1404 mJ/cm^2) and 120 min (1872 mJ/cm^2).

2.3. Structural and functional assays of BSA

Fluorescence emission intensity of irradiated and non irradiated BSA (1 mg/ml) was monitored by using fluorescence spectrophotometer (VARIAN, Cary Eclipse, Netherland). The samples were excited at 280 nm. The emission spectra recorded at the range 290–500 nm in phosphate buffer (5 mM) at pH 7.4. A circular Dichroism (CD) spectrum of BSA was recorded at 190–250 nm (Jasco J-815 Spectropolarimeter). Samples were prepared in 5 mM solution of phosphate buffer at pH 7.4.

Esterase activity was measured by hydrolysis of P-nitrophenyl acetate (500 mM) catalyzed by BSA(1 mg/ml) in presence and absence of silver nanoparticles(1 mM) alone as well as after UV radiation by monitoring the formation of p-nitrophenol at 400 nm on nanophotometer.

Ellman's assay was used for the estimation of free sulfhydryl groups in bovine serum albumin. Ellman's reagent was dissolved (4 mg/ml) in sodium phosphate buffer (0.1 M) containing 1 mM EDTA at pH 8.0. Thiol groups were measured by making reaction system containing 2.5 ml of reaction buffer, 250 μl sample and 50 μl Ellman's reagent. The system was incubated at room temperature for 15 min. The absorption was measured at 412 nm. The Free thiol concentration was calculated interpolating the absorbance at 412 nm with the help of calibration curve. Results have been expressed as number of free thiol groups per mole of BSA.

Copper ion binding to BSA was measured spectrophotometrically by using bathocuproine disulphonic acid (BCS). BSA alone and with SNP was prepared in 0.15 M NaCl and incubated with 10 μM of $CuSO_4$ for 2 h. Both the samples were irradiated by UV radiation at 30, 60, 90, 120 min. After irradiation, protein was dialyzed overnight against 0.15 M NaCl. The reagent BCS was (400 μM) incubated at room temperature for