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Spectroscopic Studies of Interaction of Protein with Cerium Oxide Nanoparticles

SUJA ABRAHAM

Department of Physics, Hindustan Institute of Technology and Science, Chennai-603 103, India

Corresponding author: E-mail: sujaabrahamthuthiyil@gmail.com

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In anticancer therapy research, metal oxide nanoparticles are used experimentally to directly kill tumor cells *in vitro* and *in vivo*. Cerium oxide nanoparticles can be used to treat cancer and several other diseases. Structural changes of adsorbed protein are necessary for cellular uptake of nanoparticles. Therefore, in order to find structural changes of bovine serum albumin (BSA) when it interacts with cerium oxide nanoparticles spectroscopic studies were conducted.The changes in fluorescence emission parameters of bovine serum albumin with increasing concentrations of cerium oxide nanoparticles indicated minor conformational changes in the structure of bovine serum albumin.

Keywords: Proteins, Bovine serum albumin, Cerium oxide nanoparticles, Fluorescence, Structural changes.

INTRODUCTION

The use of nanoparticles in medicine for diagnosis and treatment of diseases is becoming a key technology nowadays. Due to its nano-scaled size, nanomaterials exhibit significant novel and improved physical, chemical, biological properties. Since the size of nanomaterials is similar to that of most biological molecules, nanomaterials can be used for both *in vivo* and *in vitro* biomedical research and applications. Nanoparticles are widely used to prevent, diagnose and treat cancer because it can destroy cancer cells without damaging normal cells [1]. The larger surface area, low molecular weight and high affinity of nano metal oxides made it as one of the most promising nanoparticles in biomedical applications. In anticancer therapy research metal oxide nanoparticles are used experimentally to kill directly tumor cells *in vitro* and *in vivo* [2]. Metal oxide nanoparticles are rapidly internalized by cells indicating that they can interact with intracellular proteins. Cerium oxide nanoparticles, also known as nanoceria is a metal oxide nanoparticle exhibit antioxidant, antiinflammatory and antibacterial properties both *in vitro* and *in vivo* hence can be used to treat cancer or neurologic diseases [3,4]. Nanoceria can eliminate cancer cells without damaging other normal tissues [5]. Cerium oxide nanoparticles treatment can prevent macular degeneration and progression of diabetes [6,7], showing protection of primary cells from the detrimental effects of radiation therapy [8], prevention of retinal degeneration induced by intracellular peroxides [9] and neuro protection to spinal cord neurons [10]. Nanoceria is biocompatible, non-toxic, can selectively target cancerous cells, used for treatment of Alzheimer's disease [11], neurodegenerative disorders and as an anticancer agent [12]. Thus, CeO_2 nanoparticles have extensive potential as a therapeutic agent for the treatment of cancer, as well as other diseases [13]. Serum albumin, the most abundant protein found in human blood functions as a carrier for various endogenous and exogenous ligands [14]. Owing to its physiological properties, purification and stability in biochemical reactions, bovine serum albumin is widely used as a model globular protein and is an ideal protein for intrinsic fluorescence measurements due to the presence of two intrinsic tryptophan residues. Since tryptophan is highly sensitive to its local environment it can be used to observe changes in fluorescence emission parameters due to protein conformational changes [15].

Protein adsorption is used in various disciplines particularly in medicine and biotechnology. Nanomedicine requires in-depth knowledge of nanoparticle-protein interactions. Proteins are commonly the first biomolecules that nanoparticles encounter when they interact with biological systems either *in vitro* or *in vivo* [16]. The nanoparticle-protein interactions gives rise to the formation of protein corona which has a major impact on nanoparticles cellular uptake. The protein corona ultimately determines the cell surface receptors used by the nanoparticle -protein complex and the subsequent cellular internalization of nanoparticles [17].

Several *in vitro* studies have explored cellular uptake of nanoparticles in the presence of serum proteins. When protein structure of an adsorbed protein is lost uptake of nanoparticles by cell will get inhibited whereas unfolding of an adsorbed protein facilitates cellular uptake of nanoparticles due to access

receptors on cell surface. Thus structural changes of adsorbed protein are necessary for cellular uptake of nanoparticles [18]. However, it is not very clear about the changes in the structure of protein after conjugation with nanoparticles therefore it is important to investigate how adsorption of blood proteins on nanoparticles will affect the protein's secondary structure. Fluorescence spectroscopy is an important tool to measure structural fluctuations in proteins [19].Therefore present work is focused to know about structural changes of serum protein (BSA) when it interacts with CeO_2 nanoparticles by spectroscopic techniques. To the best of our knowledge there is no prior report about the time resolved fluorescence measurements of BSA-CeO_2 nanoparticles interaction.

EXPERIMENTAL

Bovine serum albumin and cerium oxide nanoparticles (< 25 nm) were purchased from Sigma-Aldrich, USA.

JASCO FP-8600 spectrofluorometer was used for fluorescence measurements. The excitation slit width 2.5 nm, emission slit width 2 nm and scan rate 500 nm/min were maintained constant for all measurements.

Picosecond time correlated single photon counting (TCSPC) spectrometer was used for fluorescence lifetime measurements. The excitation source is the tunable Ti-sapphire laser (Tsunami, Spectra Physics, USA).

Stock preparation of BSA and CeO_2 nanoparticles: BSA and CeO_2 nanoparticles stock solutions were prepared and CeO_2 nanoparticles then subjected to ultrasonic vibration for 20 min.

Interaction of BSA with CeO_2 nanoparticles: The mixture of BSA with various concentrations of CeO_2 nanoparticles were homogenized and kept for 30 min for incubation.The emission spectra were taken in the range 310-420 nm at an excitation wavelength of 290 nm. Fouble distilled water was used for the interaction studies. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

Steady state fluorescence analysis: Fig. 1 shows the intrinsic fluorescence spectra for BSA and BSA-CeO_2 nanoparticles complex when excited at a wavelength of 290 nm. The protein emission is generally dominated by tryptophan fluorescence at this excitation wavelength [20]. Fig. 1 clearly shows the emission maximum of BSA is at 343 nm and fluorescence spectrum of BSA is different than that of BSA-CeO_2 nanoparticle complex. With increasing concentrations of CeO_2 nanoparticles in BSA a gradual decrease in fluorescence intensity without any shift in emission maximum of BSA was observed. This result is consistent with the studies in which it was reported that the binding abilities of TiO_2 nanoparticles and Ag doped TiO_2 nanoparticles with serum albumins showed fluorescence quenching without any shift in emission maxima [21,22]. The quenching in the fluorescence of BSA occurred at different concentrations of CeO_2 nanoparticles indicates possible role of CeO_2 nanoparticles for quenching process. The decrease in fluorescence intensity is related to quenching [23]. The lower fluorescence intensities of CeO_2 nanoparticle-BSA conjugates than native BSA may be due to interaction of BSA with CeO_2 nanoparticles. This result conveys the possibilities for complex formation between CeO_2 nanoparticles and BSA. Changes in fluorescence intensity revealed that the accessibility of CeO_2 nanoparticles to BSA. The concentration-dependent quenching of intrinsic fluorescence intensity of tryptophan residues in BSA suggested that CeO_2 nanoparticles bind to BSA. Fluorescence quenching without any shift in emission maxima were observed during interaction of BSA with TiO_2 nanoparticles [21] and silver doped TiO_2 nanoparticles [22]. A concentration dependent fluorescence quenching occurred during the interaction of human serum albumin (HSA) with CeO_2 nanoparticles [24], captopril [25] and BSA with Al_2O_3 nanoparticles [26] and ZnO nanoparticles [27].

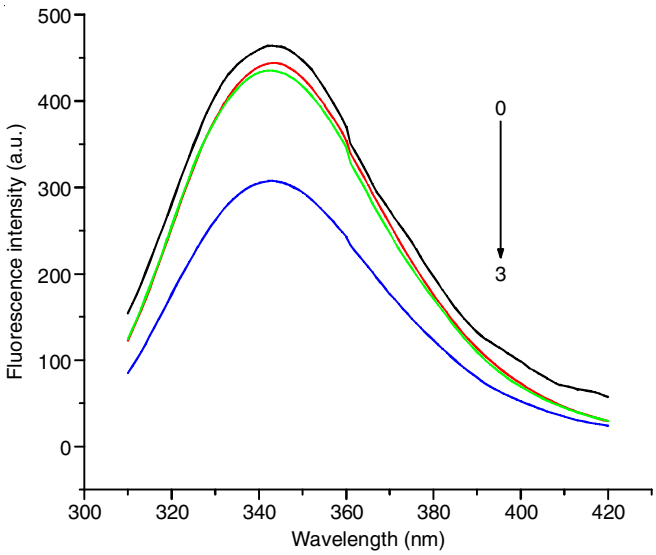

Fig.1. Fluorescence spectra of BSA at different concentrations of CeO_2 NPs (0, 6, 12 and 18 × 10^{-8} M)

Time resolved fluorescence analysis: The exponential decay curves of BSA and BSA with different concentrations of CeO_2 nanoparticles are shown in Fig. 2. The fluorescence decays of BSA were fitted with two exponentials, $T_1 = 6.50$ ns and $T_2 = 2.46$ ns and is consistent with the studies that lifetimes of tryptophan fluorescence are often multi exponential [28]. The longer and shorter lifetimes indicated that BSA contained two tryptophan residues that fluoresced in two different environments [29] and one of the tryptophan residues in the protein may be buried inside the hydrophobic interior of protein whereas the other tryptophan residue may be close to quencher [30]. This is in good agreement with the reports that BSA has two tryptophan residues, Trp-134 in the first domain located on the surface of molecule and Trp-212 in the second domain located within a hydrophobic binding pocket [14].

The fluorescence lifetime of both tryptophan residues in BSA decreased when interacted with first two lowest concentrations of CeO_2 nanoparticles. But for highest concentration of CeO_2 nanoparticles, both tryptophan residues fluorescence lifetimes increased compared to that of native BSA fluorescence lifetimes indicates tryptophan is shielded from quencher. While increasing concentrations of CeO_2 nanoparticles in BSA, lifetime of BSA-CeO_2 nanoparticle complex gradually increased for lifetime T_1 whereas decreased for initial two concentrations of CeO_2 nanoparticles and then increased for third concentration of

TABLE-1
MAXIMUMEMISSION WAVELENGTH, CORRESPONDING FLUORESCENCE INTENSITIES, LIFETIMES OF BSA AND BSA WITH DIFFERENT CONCENTRATIONS OF CeO_2 NPs

Sample	Emission maximum (nm)	Fluorescence intensity (a.u)	Lifetime (T_1) (ns)	Lifetime (T_2) (ns)
BSA	343	464	6.50	2.46
BSA + 6×10^{-8} M CeO_2 NPs	343	444	6.35	2.34
BSA + 12×10^{-8} M CeO_2 NPs	343	435	6.36	2.30
BSA + 18×10^{-8} M CeO_2 NPs	343	307	6.58	2.93

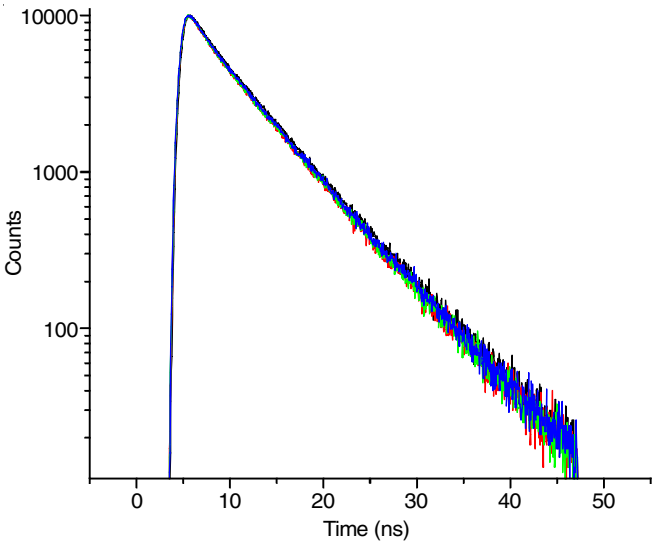

Fig. 2. Time resolved fluorescence decay of BSA at different concentrations of CeO_2 NPs (0, 6, 12 and 18×10^{-8} M)

CeO_2 nanoparticles (Fig. 2, Table-1). In effect lifetimes of both tryptophan residues did not show any significant change compared to lifetimes of native BSA. For static quenching, complex formation will not disturb fluorescence lifetime of tryptophan residues in BSA [19]. Static quenching refers to the formation of fluorophore-quencher complex in the ground state and it is well in agreement with fluorescence steady state analysis of this study. Hence, it is concluded that static quenching was consistent in this reaction process. This result is consistent with fluorescence quenching rate constant studies in which static quenching mechanism was observed when HSA interacted with CeO_2 nanoparticles [24], BSA interacted with Cu nanoparticles [31,32] and CuO nanoparticles [33]. Static quenching mechanism was confirmed by time resolved measurements when BSA interacted with colloidal ZnO nanoparticles [34], SnO_2 nanoparticles [35] and TiO_2 nanoparticles [36].

Monitoring conformational changes of CeO_2 nanoparticles bound BSA: The interaction of BSA with different concentrations of CeO_2 nanoparticles resulted in gradual reduction of the fluorescence intensity of BSA. The decrease in fluorescence intensity of tryptophan residues when nanoparticles bind with protein molecule indicates conformational change of protein molecule. Therefore, this behaviour means that the conformation around tryptophan residues of BSA-CeO_2 nanoparticle complex changes as compared to that of native BSA. Thus, it is concluded the possibility of binding induced conformational changes when CeO_2 nanoparticles interacted with BSA. In the present study, decrease in fluorescence intensity and small change in lifetimes (Figs. 1-2 and Table-1) implies minor conformational changes induced by CeO_2 nanoparticles on the structure of BSA. Conformational changes were observed when CeO_2 nanoparticles interacted with BSA [37], HSA [38], heme protein [39], BSA interacted with cerium [40], copper(I) oxide nanoparticles [41], Al_2O_3 nanoparticles [26], ZnO nanoparticles [20], CdO nanoparticles [42], TiO_2 nanoparticles [36] and tin oxide nanoparticles [35].

Conclusion

Spectroscopic analysis of BSA-CeO_2 nanoparticles interaction revealed gradual fluorescence intensity decrease of BSA due to fluorescence quenching in the conjugation process. Fluorescence data revealed fluorescence quenching of BSA by CeO_2 nanoparticles was the result of complex formation. The two lifetimes indicated that BSA containing two tryptophan residues that fluoresced in two different environments. The consistent fluorescence lifetimes of both tryptophan residues of BSA after binding with CeO_2 nanoparticles suggested that the quenching was static.The changes in fluorescence emission parameters of BSA due to BSA-CeO_2 nanoparticles interactions confirmed binding induced conformational changes in the structure of BSA, which may have further applications in biomedical field.

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