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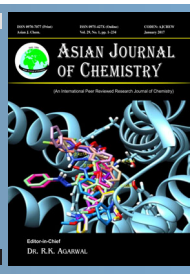

Effect of Al_2O_3 Nanoparticles on Bovine Serum Albumin: A Spectrofluorometric Study

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As metal oxide nanoparticles are widely used in biomedical field the interaction between bovine serum albumin (BSA) and Al_2O_3 nanoparticles and the effect of Al_2O_3 nanoparticles on the conformation of bovine serum albumin has been analyzed by various spectroscopic techniques. With increasing concentrations of Al_2O_3 nanoparticles in bovine serum albumin a shift in emission maximum, change in fluorescence intensity and change in lifetimes were observed compared to the emission maximum, fluorescence intensity and lifetimes of bovine serum albumin. Fluorescence emission spectra showed a blue shift with increasing concentrations of Al_2O_3 nanoparticles in bovine serum albumin which is indicative of increasing hydrophobicity. These studies showed that Al_2O_3 nanoparticles induced conformational changes in the structure of bovine serum albumin.

Keywords: Biomolecules, Metal oxide nanoparticles, Fluorescence, Conformational changes.

INTRODUCTION

Nowadays there is a considerable increase in the usage of nanoparticles and other nanomaterials in biomedical applications. Before development of any therapeutics and diagnostic applications it is important to understand the properties of biomolecule bound nanoparticles [1,2]. In biomedical applications, Al_2O_3 nanoparticles are used as the load bearing hip prostheses, dental implants, vertebrae spacers and extensors [3]. Also it has shown its capacity to neutralize free radicals in cells, preventing inflammation and demonstrating neuroprotective effects [4]. Bovine serum albumin has a wide range of physiological functions and is an ideal protein for intrinsic fluorescence measurements due to the presence of two intrinsic tryptophan (trp) residues [5]. Since tryptophan is highly sensitive to its local environment, it can be used to observe changes in the fluorescence emission spectra due to protein conformational changes [6].

When biological molecules are integrated with nanoparticles the native conformation and therefore the protein function can be easily changed by these interactions with the surface [7]. Thus stabilization of the system as well as introduction of biocompatible functionalities into these nanoparticles for further biological interactions or coupling takes place by conjugation of proteins with nanoparticles. However it is not very clear about the changes in the structure of protein after conjugation with nanoparticles hence some basic understanding is required before such complexes are used for biomedical applications. The interaction of bovine serum albumin with metal oxide nanoparticles was reported by some workers such as ZnO [8] and TiO_2 [9]. Previous studies reported conformational changes of protein molecules adsorbed on ZnO [10] and Ag TiO_2 nanoparticles [11].

Due to its major physiological significance, unusual ligand binding properties, high purification, stability in biochemical reactions and its soluble nature in water, bovine serum albumin has been taken as a model protein in this study [12-14]. To the best of my knowledge there is no prior report about the time resolved fluorescence measurements of bovine serum albumin-Al_2O_3 nanoparticle interaction. Therefore the present work is focused to study the interaction between bovine serum albumin protein and Al_2O_3 nanoparticles by using various spectroscopic techniques.

EXPERIMENTAL

Bovine serum albumin and Al_2O_3 nanoparticles (< 50 nm) were purchased from Sigma-Aldrich, USA. The samples were used as received without any further purification. Double distilled water was used for the interaction between bovine serum albumin and Al_2O_3 nanoparticles.

Stock preparation of bovine serum albumin and Al_2O_3 nanoparticles: Bovine serum albumin stock solution was prepared by dissolving 100 mg of bovine serum albumin in 10 mL double distilled water and it was used for further studies. Similarly stock solution of Al_2O_3 nanoparticles was prepared by dissolving 10 mg of Al_2O_3 nanoparticles in 10 mL double

distilled water and then subjected to ultrasonic vibration for 15 min. This dispersion was used further for the interaction studies.

Interaction of bovine serum albumin with Al_2O_3 nanoparticles: Bovine serum albumin was interacted with various concentrations of Al_2O_3 nanoparticles. This mixture was homogenized and kept for 30 min for incubation. All measurements were recorded after 30 min of incubation required for the interaction between Al_2O_3 nanoparticles and bovine serum albumin. 3 mL of the sample was subjected to analysis at the excitation wavelength of 290 nm and emission spectra in the range 310-470 nm. All measurements were performed at room temperature.

Characterization

Steady state fluorescence measurements: The fluorescence measurements were carried out with JASCO FP-8600 spectrofluorometer. The excitation wavelength was 290 nm. The excitation slit width 2.5 nm, emission slit width 2 nm and scan rate 500 nm/min were maintained constant for all measurements. The quartz cuvette (4 cm × 1 cm × 1 cm) with path length of 1 cm was used.

Time resolved fluorescence measurements: Fluorescence lifetime measurements were carried out in a Picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source is the tunable Ti-sapphire laser (Tsunami, Spectra Physics, USA).

RESULTS AND DISCUSSION

Steady state fluorescence analysis: The intrinsic fluorescence spectra for native bovine serum albumin and bovine serum albumin-Al_2O_3 nanoparticle complex at the excitation wavelength of 290 nm are shown in Fig. 1. When excited at 290-295 nm the emission of proteins is generally dominated by the tryptophan fluorescence [15-17]. When excited at 290 nm wavelength bovine serum albumin has a strong emission band at 345 nm (Fig. 1), supported by several previous works [9,14]. Fluorescence spectra of Al_2O_3 nanoparticles attached with bovine serum albumin were found to be different than in the native bovine serum albumin (Fig. 1). After the addition of Al_2O_3 nanoparticles fluorescence intensity of bovine serum albumin gradually decreased for all the three concentrations of Al_2O_3 nanoparticles. At different concentrations of Al_2O_3 nanoparticles a quenching in the fluorescence of bovine serum albumin was found which indicates that Al_2O_3 nanoparticles are responsible for quenching the fluo-rescence of bovine serum albumin. The decrease in fluorescence intensity is due to change in local environment around tryptophan residues. Also the decrease in fluorescence intensity is related to quenching [18]. Fluorescence intensities of Al_2O_3 nanoparticle-bovine serum albumin conjugates were lower than native bovine serum albumin that could be due to the interaction of bovine serum albumin with the Al_2O_3 nanoparticles. This suggests that Al_2O_3 nanoparticles destabilize the native state of bovine serum albumin and altering the microenvironment around the tryptophan residues. Increased fluorescence quenching with increasing Al_2O_3 nanoparticle concentrations indicate changes in microenvironment of bovine serum albumin due to Al_2O_3 nanoparticles-bovine serum albumin interactions.

After the addition of Al_2O_3 nanoparticles the maximum emission wavelength of bovine serum albumin showed a blue shift. It was observed that the maximum of the peak shifted by 4 nm with increasing concentrations of Al_2O_3 nanoparticles indicates a change of the microenvironment polarity around the tryptophan residues and they were exposed to a hydrophobic environment. Al_2O_3 nanoparticles modified the polarity in the vicinity of tryptophan residues. When the interacted molecule quenches the fluorescence with a blue shift in the spectral maximum it suggests a decrease in the polarity or an increase in the hydro-phobicity of the microenvironment surrounding the fluorophore site [19]. The significant blue shift in emission maximum indicates that polarity near tryptophan residues is decreased or hydrophobicity is strengthened as a result of binding of Al_2O_3 nanoparticles to bovine serum albumin.

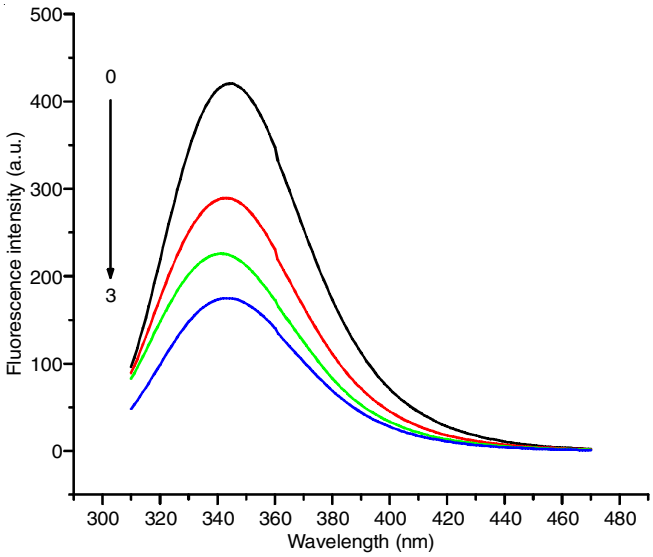

Fig. 1. Fluorescence spectra of bovine serum albumin at different concentrations of Al_2O_3 nanoparticles (0, 6, 12 and 18 × 10^{-8} M)

The interaction studies of metal oxide nanoparticles such as ZnO nanoparticles [8,10], TiO_2 nanoparticles and Ag doped TiO_2 nanoparticles [11,20] with bovine serum albumin confirmed the occurrence of fluorescence quenching process with change (blue/red/no shift) in fluorescence emission maximum. The interaction of bovine serum albumin with Al_2O_3 nanoparticles did not show any concentration dependent fluorescence quenching probably due to the presence of excess Al_2O_3 nanoparticles in the system [21]. No change in fluorescence was observed when Al_2O_3 nanoparticles interacted with human plasma proteins [22].

Time resolved fluorescence analysis: The fluorescence effect of bovine serum albumin in the absence and presence of Al_2O_3 nanoparticles were measured. The exponential decay curves of bovine serum albumin and bovine serum albumin with different concentrations of Al_2O_3 nanoparticles are shown in Fig. 2. The fluorescence decays of bovine serum albumin were fitted with two exponentials, T_1 = 7.0 ns and T_2 = 3.56 ns. The two lifetimes with significant changes between them indicated that bovine serum albumin contained two tryptophan residues that fluoresced in two different environments [23,24] and one of the tryptophan residues in the protein may be

relatively exposed whereas the other tryptophan residue appears to be deeply buried inside the protein [5].

The lifetime of both tryptophan residues in bovine serum albumin decreased gradually with increasing Al_2O_3 nanoparticle concentrations and the reduction was more prominent in lifetime T_2 compared to that of lifetime T_1. The fluorescence lifetime of bovine serum albumin gradually decreased for lifetime T_1 from 7.0 ns to 6.56 ns, 6.46 ns and 6.40 ns and for lifetime T_2 from 3.56 ns to 3.37 ns, 3.03 ns and 2.77 ns upon interaction with different concentrations of Al_2O_3 nanoparticles respectively (Fig. 2 and Table-1). For static quenching the complex formation will not disturb the fluorescence lifetime of bovine serum albumin but in dynamic quenching it will be cut down due to the collision between the excited protein fluorophore and nanoparticles [6]. Therefore in this case the decrease in fluorescence lifetimes of both tryptophan residues of bovine serum albumin indicated consistent dynamic quenching process in the bovine serum albumin-Al_2O_3 nanoparticle complex.

Time resolved fluorescence measurements showed that consistent dynamic quenching occurred when bovine serum albumin interacted with TiO_2 nanoparticles [9], silver nanoparticles [15] and static quenching mechanism was confirmed when bovine serum albumin interacted with SnO_2 nanoparticles [9] and colloidal capped CdS nanoparticles [25]. Time resolved studies showed that the average lifetime of tryptophan in the bovine serum albumin-gold nanoparticle conjugate does not undergo any significant change with respect to that of native bovine serum albumin [26,27].

Monitoring conformational changes of Al_2O_3 nanoparticle-bound bovine serum albumin: The interaction of proteins and other macromolecules with nanoparticles has been shown to result in marked changes to the physical properties of nanoparticles as well as the structure and function of the proteins [28,29]. The interaction of bovine serum albumin with different concentrations of Al_2O_3 nanoparticles resulted in significant gradual reduction in the fluorescence intensity of bovine serum albumin. Also, a significant blue shift in the fluorescence emission maximum was observed. When the proteins bind to other substances or the conformation of protein is changed the corresponding fluorescence intensity and/or the wavelength of the maximum peak of the protein will generate some alterations [30,31]. Therefore this behaviour means that the conformation around the tryptophan residues of the bovine serum albumin-Al_2O_3 nanoparticle complex changes as compared to that of the native bovine serum albumin. The lifetime of both the tryptophan residues in bovine serum albumin decreased gradually with increasing Al_2O_3 nanoparticle concentrations and the reduction was more prominent in the lifetime T_2 compared to that of lifetime T_1. Only the dynamic quenching process reduces the fluorescence lifetimes compared to that of static quenching process [6]. Therefore, the decrease in fluorescence lifetimes of both tryptophan residues of bovine serum albumin indicated consistent dynamic quenching process in the bovine serum albumin - Al_2O_3 nanoparticle complex. In the present study the significant blue shifts in the fluorescence emission maximum, significant decrease in fluorescence intensity and change in lifetimes (Figs. 1 & 2 and Table-1) implies the probable con-formational changes induced by Al_2O_3 nanoparticles on bovine serum albumin. Spectroscopic investigations showed con-formational changes when bovine serum albumin interacted with ZnO nanoparticles [8,10], TiO_2 nanoparticles [9] and colloidal ZnO nanoparticles [14].

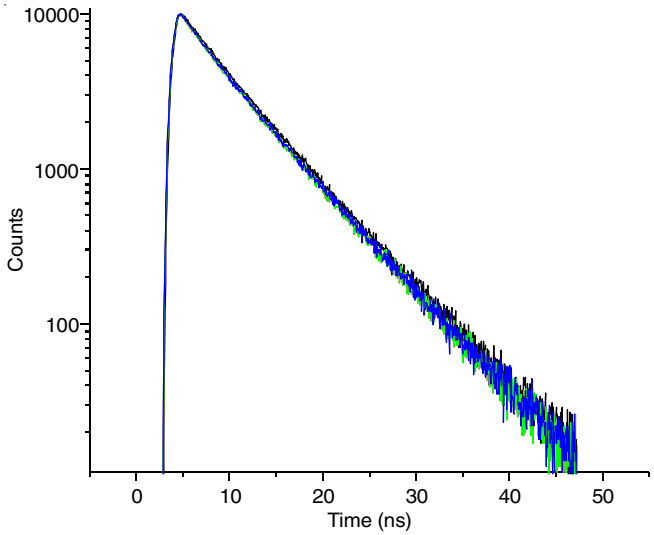

Fig. 2. Time resolved fluorescence decay of bovine serum albumin at different concentrations of Al_2O_3 nanoparticles (0, 6, 12 and 18×10^{-8} M)

Conclusion

In the present study, the interaction between Al_2O_3 nanoparticles and bovine serum albumin has been studied by various spectroscopic techniques. The decrease in intensity and blue shifts of fluorescence emission maximum of Al_2O_3 nanoparticles-bovine serum albumin conjugated system confirms the occurrence of fluorescence quenching in the conjugation process. The analysis of fluorescence spectra indicated that the microenvironment close to tryptophan residues of bovine serum albumin is perturbed. The analysis of lifetime measurements indicated that the lifetime of both the tryptophan residues in bovine serum albumin decreased gradually with increasing Al_2O_3 nanoparticles concentrations confirmed the dynamic quenching process. The two lifetimes indicated that bovine serum albumin contained two tryptophan residues that fluoresced in two different environments. The bovine serum albumin-

TABLE-1
WAVELENGTH OF EMISSION MAXIMUM, CORRESPONDING FLUORESCENCE INTENSITY AND LIFETIMES OF BOVINE SERUM ALBUMIN AND BOVINE SERUM ALBUMIN WITH DIFFERENT CONCENTRATIONS OF Al_2O_3 NANOPARTICLES

Sample	Emission maximum (nm)	Fluorescence intensity (A.U.)	Lifetime T_1 (ns)	Lifetime T_2 (ns)
Bovine serum albumin	345	420.32	7.00	3.56
Bovine serum albumin + 6×10^{-8} M Al_2O_3 nanoparticles	343	289.29	6.56	3.37
Bovine serum albumin + 12×10^{-8} M Al_2O_3 nanoparticles	341	225.79	6.46	3.03
Bovine serum albumin + 18×10^{-8} M Al_2O_3 nanoparticles	343	174.85	6.40	2.77

Al_2O_3 nanoparticle conjugate made a significant change in bovine serum albumin fluorescence emission parameters confirmed that Al_2O_3 nanoparticles induced conformational changes in the structure of bovine serum albumin.

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