

INTERACTION OF COPPER OXIDE NANOPARTICLES WITH BOVINE SERUM ALBUMIN BY SPECTROSCOPIC STUDIES

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ABSTRACT

Objective: Since structural changes of adsorbed protein are necessary for cellular uptake of nanoparticles (NPs) it is of prime importance to know about structural changes of bovine serum albumin (BSA) when it interacts with CuO NPs—a potential new antitumor drug.

Methods: CuO NPs prepared by sol-gel technique were characterized by x-ray diffraction (XRD) and tunneling electron microscope (TEM) techniques. The conformational changes induced by CuO NPs on BSA were studied by various spectroscopic techniques such as steady state and time-resolved fluorescence measurements. The changes in fluorescence emission parameters such as fluorescence intensity, fluorescence emission maximum and lifetimes of fluorescent residues in BSA were studied.

Results: XRD analysis showed the average particle size as 32 nm. The TEM micrograph showed particles of different size varying from 10 to 45 nm. Fluorescence quenching was confirmed due to a decrease in fluorescence intensity of CuO NPs–BSA complex. The analysis of lifetime measurements indicated BSA contained two tryptophan (trp) residues that fluoresced in different environments. Static quenching mechanism was confirmed by time-resolved measurements when BSA interacted with CuO NPs.

Conclusion: Minor structural changes of BSA protein were observed during the interaction studies.

Keywords: Protein, Copper oxide nanoparticles, Spectroscopy, Structural changes

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INTRODUCTION

Nanotechnology holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment and prevention [1-4]. NPs large functional surface area allows them to bind absorb and carry other compounds [5]. Copper compounds have been used to treat cancer and several diseases for thousands of years [6]. *In vitro* and *in vivo* studies showed that metal oxide NPs can directly kill tumor cells [7]. CuO NPs have many advantages such as simple preparation procedure, long-term stability, and anticancer properties and showed a strong affinity to bind blood carrier proteins [8, 9]. BSA is a good model to study protein conformational changes due to its wide range of physiological functions [10], an ideal protein for intrinsic fluorescence measurements [11], well-characterized structure and property and readily undergoes conformational changes [12, 13].

The NP-protein interactions give rise to the formation of protein corona which has a major impact on NPs cellular uptake. When protein structure of an adsorbed protein is lost uptake of NPs by the cell will get inhibited whereas unfolding of an adsorbed protein facilitates cellular uptake of NPs due to access receptors on cell surface. Thus structural changes of adsorbed protein are necessary for cellular uptake of NPs [14]. NPs exhibit unique behaviour in human body, but there is only a limited knowledge of how NPs interact with cells and proteins. Since blood circulatory system is the most probable treatment administration option for NPs into the human body, it is important to investigate how adsorption of blood proteins on NPs will affect the protein's secondary structure [15]. Spectroscopic techniques can be used as a powerful tool to accomplish this need. Therefore, in the present study interaction of CuO NPs with BSA was studied by spectroscopic techniques. To the best of our knowledge lifetime measurements by TCSPC technique of BSA–CuO complex is reported for the first time.

MATERIALS AND METHODS

Chemicals

BSA was purchased from Sigma-Aldrich, USA. Copper chloride and sodium hydroxide were purchased from SD fine chemicals, India.

Preparation of CuO NPs

0.5M CuCl₂·6H₂O was dissolved in de-ionized water in a 250 ml conical flask with constant stirring. A very little amount of citric acid was added to the above solution [16]. The solution mixture was heated at 80 °C with continuous stirring. Then a required quantity of separately prepared NaOH solution was added slowly into the above heated solution under vigorous stirring. The colour of the solution turned to black from blue which confirmed the formation of CuO. The addition of NaOH solution was stopped after the formation of large amount of black precipitate. The obtained black precipitate was collected by a centrifuge. The obtained mass was washed several times with ethanol and de-ionized water to remove impurities. The product was further dried at 80 °C for 12 h in oven. The dried product was grinded well using mortar and pestle and then it was annealed at 300 °C to obtain crystalline CuO Nano powder.

Stock preparation and interaction of BSA with CuO NPs

Among the prepared stock solutions of BSA and CuO NPs, CuO NPs were subjected to ultrasonic vibration for 20 min. The mixture of BSA with various concentrations of CuO NPs was 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 [17]. Double distilled water was used for interaction studies. All measurements were performed at room temperature.

Characterization

XRD

The structure of CuO was confirmed using Bruker K 8600 X-ray diffractometer in the 2θ range of 20 ° to 80 °.

TEM

The particle size of the prepared nano-sized CuO was estimated with the help of JEOL JSM 5610 LV tunnelling electron microscope.

Steady-state fluorescence measurements

JASCO FP-8600 spectrofluorometers was used for fluorescence measurements with excitation wavelength 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.

Time-resolved fluorescence 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).

RESULTS AND DISCUSSION

XRD analysis

The structure of CuO has been investigated using XRD profile. Fig. 1 shows XRD pattern of CuO NPs. All the existing diffraction peaks in XRD profile are in good agreement with standard JCPDS data (File no. 05-661). The diffraction peaks also confirmed the monoclinic structure of CuO. The peaks are broad due to nano size effect. The average particle size was calculated as 32 nm using Scherrer formula.

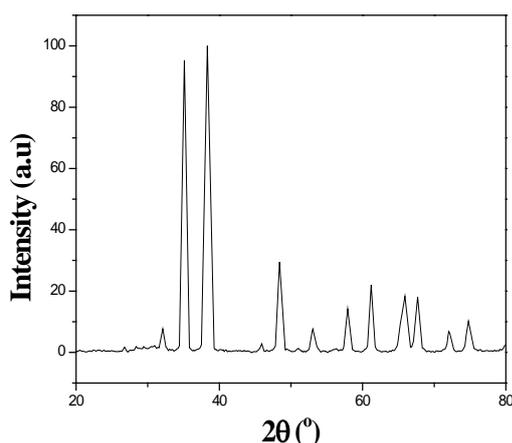


Fig. 1: XRD pattern of CuO NPs

TEM study

The size and morphology of CuO NPs have been examined using TEM. The nanoparticles are spherical in shape as shown in fig. 2. The TEM micrograph has shown particles of different size approximately from 10 nm to 45 nm. The TEM result also coincides with the estimated particle size in XRD analysis.

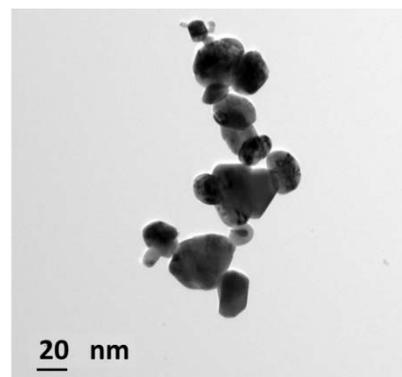


Fig. 2: TEM micrograph of CuO NPs

Steady-state fluorescence analysis

The intrinsic fluorescence spectra of BSA and BSA-CuO NP complex at excitation wavelength 290 nm are shown in fig. 3. BSA-metal oxide NPs interaction was reported earlier at this same excitation wavelength [18, 19]. Fig. 3 clearly shows that the emission maximum of BSA is at 343 nm and fluorescence spectrum of native BSA is different than that of BSA-CuO NP complex. With increasing concentrations of CuO NPs 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 TiO₂ NPs and Ag-doped TiO₂ NPs with serum albumins showed that both TiO₂ NPs and Ag-doped TiO₂ NPs quench fluorescence without any shift in emission maxima [20, 21]. The fluorescence intensity of BSA gradually decreased for increasing concentrations of CuO NPs indicating CuO NPs are responsible for quenching the fluorescence of BSA. The decreased fluorescence intensities of CuO NP-BSA conjugates suggests interactions between CuO NPs and BSA. A concentration-dependent quenching of intrinsic fluorescence intensity of BSA, suggests that CuO NPs binds to BSA.

Increased concentrations of Copper (I) oxide NPs and gold NPs in BSA resulted in a decrease in fluorescence intensity due to quenching [22, 13]. Quenching of the intrinsic fluorescence of BSA with a blue shift in emission maximum was reported during the interaction studies of BSA with increasing concentrations of ZnO NPs [23], Cu NPs [24, 25], silver NPs [26] and colloidal capped CdS NPs [27]. The addition of ZnO NPs of different concentration with BSA resulted in a small red shift with a change in maximum emission intensity suggesting the occurrence of fluorescence quenching process [19]. The interaction of BSA with Al₂O₃ NPs did not show any concentration-dependent fluorescence quenching [28].

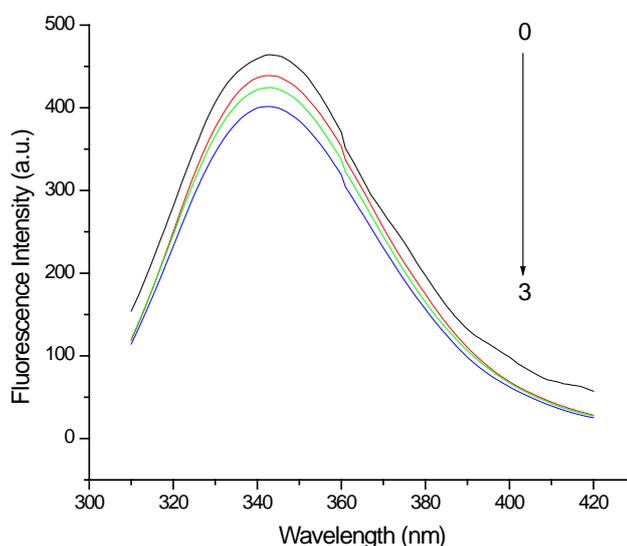


Fig. 3: Fluorescence spectra of BSA at different concentrations of CuO NPs (0, 6, 9 and 12 x 10⁻⁸ M)

Time-resolved fluorescence analysis

The exponential decay curves of BSA and BSA-CuO NP complex are shown in fig. 4. The fluorescence decay of BSA was fitted with two exponentials, $T_1 = 6.50$ ns and $T_2 = 2.46$ ns and is consistent with the studies that lifetimes of trp fluorescence are often multi-exponential [29]. The changes in a lifetime, give information about the local environment of the trp-residues [30]. The marked difference between two lifetimes indicated that one of the trp residues in a protein may be relatively exposed whereas other trp residue appears to be deeply buried inside the protein [31].

The fluorescence lifetime of both trp residues in BSA decreased when interacted with different concentrations of CuO NPs. The decrease was more prominent for lifetime T_2 . Similar results were observed with decrease in fluorescence lifetime when BSA interacted with TiO₂ NPs

and silver NPs [32, 33]. While increasing concentrations of CuO NPs in BSA, lifetime of BSA-CuO NP complex gradually increased for lifetime T_1 whereas an increase and then decrease in fluorescence lifetime was observed for lifetime T_2 (fig. 4 and table 1). Overall there was no significant change in lifetime of both trp residues compared to that of native BSA. For static quenching, complex formation will not disturb fluorescence lifetime of trp residues in BSA [12]. Therefore, present results clearly indicated 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 BSA interacted with Cu NPs [24, 25] and CuO NPs [34]. Static quenching mechanism was confirmed by time-resolved measurements when BSA interacted with colloidal ZnO nps [35], SnO₂ nps [32], TiO₂ nps [36] and no significant change in average lifetime of trp residues with gold NPs [37].

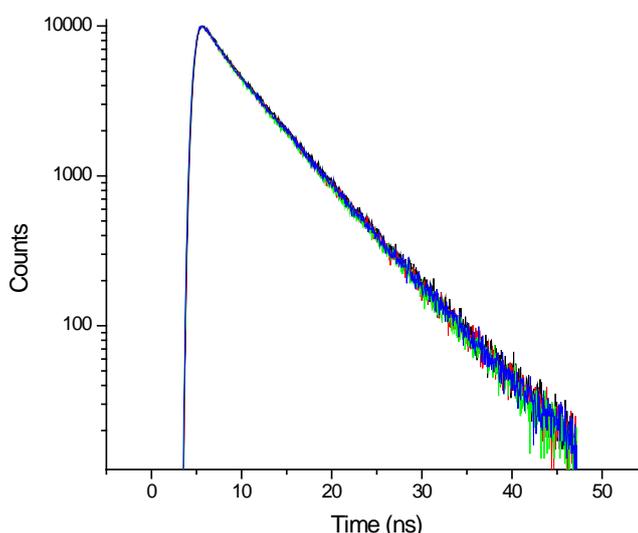


Fig. 4: Time-resolved fluorescence decay of BSA at different concentrations of CuO NPs (0, 6, 9 and 12 x 10⁻⁸ M)

Conformational changes of BSA-CuO NP complex

The protein NP interaction result in considerable changes of the structure and function of proteins [38, 39]. When different concentrations of CuO NPs interacted with BSA a gradual reduction of BSA fluorescence intensity was observed. Protein conformational changes will generate some alterations in fluorescence intensity and disturb microenvironment around trp residues [40, 41]. Thus it is concluded that conformational changes occurred during this

interaction studies. There was no significant change in fluorescence lifetime of both trp residues in BSA when interacted with CuO NPs indicating static quenching process. For static quenching, fluorescence lifetime will not get disturbed [18]. In the present study, a decrease in fluorescence intensity and a small change in lifetimes (fig. 3, fig. 4 and table 1) implies minor conformational changes induced by CuO NPs on BSA. Conformational changes were observed when BSA interacted with Copper I oxide NPs [15], Cu NPs [25], ZnO NPs [23], colloidal ZnO NPs [35], TiO₂ NPs [20] and tin oxide NPs [32].

Table 1: Emission wavelengths, corresponding fluorescence intensities, lifetime of BSA and BSA with different concentrations of CuO 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+6x10 ⁻⁸ M, CuO NPs	343	438	6.38	2.12
BSA+9x10 ⁻⁸ M CuO NPs	343	424	6.40	2.37
BSA+12x10 ⁻⁸ M CuO NPs	343	401	6.42	2.32

CONCLUSION

CuO NPs were synthesized by sol-gel technique. XRD analysis showed the average particle size as 32 nm. The TEM micrograph showed particles of different size varying from 10 to 45 nm. Based on fluorescence spectroscopic studies of BSA and BSA-CuO NP complex following conclusions were made. Fluorescence quenching was confirmed due to a decrease in fluorescence intensity of CuO NPs-BSA complex. The analysis of lifetime measurements indicated that no significant change in both lifetimes T_1 and T_2 of trp residues in BSA, when interacted with different concentrations of CuO NPs, confirmed static quenching was dominant in this reaction process.

The two lifetimes indicated that BSA contained two trp residues, longer lifetime indicated that trp residue is buried inside hydrophobic interior of protein and shorter lifetime indicated trp residue is closer to the quencher. BSA-CuO NP conjugate made changes in BSA fluorescence emission parameters probably confirmed minor conformational changes in the structure of BSA.

AUTHORS CONTRIBUTIONS

Suja Abraham-Principal Investigator-did steady state fluorescence analysis, time-resolved fluorescence analysis and prepared the manuscript.

Vellaichamy Parthasarathy–Co-investigator–Synthesized CuO NPs, did XRD analysis and TEM study.

CONFLICT OF INTERESTS

There is no conflict of interest between authors

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