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Research Article

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Binding of Lysozyme on the Surface of ZnS Nanoparticles: Spectroscopic, Microscopic Study and Interaction Phenomenon

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ABSTRACT

Here we used wet chemical method to grow zinc sulphide nanoparticles (ZnS NPs). HRTEM and XRD pattern were used for structural characterization. The Phase of the ZnS NP unit cell was cubic and the average crystal size is found to be ≈15 nm. The defect state related photoluminescence spectrum showed visible emission of ZnS NPs. We used optical spectroscopy like UV–VIS and fluorescence spectra, HRTEM images and FESEM images to study the interaction and the formation of bioconjugate of Lysozyme and ZnS NPs. Our result showed occurance of spontaneous binding process between Trp of the Lysozyme and ZnS NPs. Formation of ground state complex with small red shift of the absorption peak of Lysozyme is observed due to binding of the Lysozyme with ZnS NPs. Zinc Sulphide nanoparticles quench the fluorescence emission of tryptophan in the Lysozyme. We calculate the Stern–Volmer quenching constant and the number of binding sites.

Keywords: ZnS nanoparticles; Lysozyme; Absorption spectra; Photoluminescence spectra; High resolution transmission electron microscopy

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INTRODUCTION

Nanoscience is a powerful field of Pharmaceutical and medical diagnostics. There is a gradual interest in the study of interaction between nanoparticles with protein. Sulfide nano-materials have great potential as the antineoplastic drugs based on the fact that they can slowly release the heavy metal ion sources. Zinc sulfide is an important II–VI semiconductor with wide bandgap energy of 3.68 eV at 300 K and has been widely applied in the field of optoelectronics and biomedicine [1,2]. From last decade, one of the important issue is the identification of the the nature of protein-nanoparticle interactions and favored binding sites in functional characterization of biomolecules regarding their physiological responses. Herein, interaction of ZnS NPs with lysozyme (Lyz) has been monitored via absorption spectroscopy, fluorescence spectroscopy, and microscopic measurements (FESEM and HRTEM). Here we used Lyz as a model protein. Semiconductor nanomaterials have wide ranging implications in a variety of areas and have received considerable attention. Semiconductor nanoparticles have several advantages such as. The unique properties of semiconductor nanoparticles like broad excitation spectra, narrow and symmetric bandwidth emission spectra, precise tunability emission peak have shown great potentials in photonic crystals, light-emitting devices, and especially biological labels [3-7]. ZnS NPs are important semiconductor labelling materials for biomedical applications. However, there are still many concerned problems about how to apply them safely in biological systems. The nanoscale effects about nanoparticles and protein molecules have not been well understood. Lysozyme (Lyz) is a small monomeric globular protein. It contains protein structural elements like α -helix, β-sheet, turns and disorder. Its structure is formed by 129 tactic amino residues containing 6 tryptophanes, 3 tyrosines and 4 disulfide bonds [8,9]. The interactions between proteins and non-metal ions, metal ions have been extensively investigated [10-16]. However, the detailed studies about the interaction between different semiconductor nanomaterials and

proteins are very rare [17]. In the peptidoglycan of certain microorganisms Lysozyme hydrolyzes the β(1→4) glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine [18]. Recent study showed that the biomolecule's have potential ability in killing HIV virus, different antibacterial activity, and delivery of drug molecules [19]. In this work, the water-soluble ZnS NPs were synthesized directly in a cost effective chemical method. The optical properties like absorption and fluorescence spectroscopy have been used to investigate the interaction of ZnS NPs and Lyz. Here we try to study the binding mechanism of ZnS NPs to Lyz with respect to the binding sites. Our aims to gain insight into lysozyme- ZnS NPs interaction and binding mechanism. In this study, the effects of ZnS nanoparticles on the lysozyme (a model protein) is studied in detail. Formation of complex between the lysozyme and ZnS NPs induced a steady state reduction in the emission intensity of Trp of Lyz at different concentrations of nanoparticles. Lyz emission quenching spectra suggested that ZnS NPs act as a foreign quencher. The quenching of lysozyme showed that lysozyme has undergone slide structural perturbations in the ZnS-Lyz composite system compared to the bare Lyz. The quenching constant was studied by analysis of the Stern-Volmer plot. Our results of this paper will help biomedical safety of the the ZnS NPs in biomedical applications.

EXPERIMENTAL SECTION

Anhydrous $ZnCl_2$ (1362.8 mg), Sulfur powder (320.6 mg) and a stoichiometric amount of sodium borohydride (378.3 mg) were taken to prepare ZnS nanoparticles. Sodium borohydride was taken to initiate the reaction at room temperature. The stirring was continued for three hours at a fixed speed. The final power sample was collected for characterization and interaction with protein. The Transmission Electron Micrograph of the prepared nano sample was acquired using JEOL-JEM-200 operating at 200 kV. The SAED pattern of the said nano sample was also carried out. The surface topography and composition was acquired using ZESIS Gemini2 FESEM instrument. The XRD patterns of the said samples are obtained by using Rigaku MiniFlex-II X-ray diffractometer. The optical absorption spectrum of the samples was taken by using Shimadzu-Pharmaspec-1700 UV-VIS. The photoluminescence spectrum of the as-prepared samples was obtained by using a Hitachi-F7000-FL spectrophotometer.

RESULTS AND DISCUSSION

Absorption Spectra

Synthesised ZnS Nanoparticles shows a strong absorption around 315 nm with band gap of 4.3 eV (Figure 1). The tryptophan (Trp) in the Lyz exhibits absorption peak at ~278 nm (Figure 2) due to the π-π* transition of aromatic amino acid residues. The UV visible spectra presented in Figure 2 illustrate the effect of binding of ZnS NPs with Lyz. In Figure 2A (b-f) our results showed that the absorbance at 278 nm increases in increases with C_{ZnS}. The increase in intensity of absorbance of Trp in the presence of ZnS may be due to binding of Trp with ZnS NPs and the formation of the ground state complex [20]. The increase in the absorbance of the tryptophan with the increase of the ZnS nanoparticle concentration is linear and shown in Figure 2B.

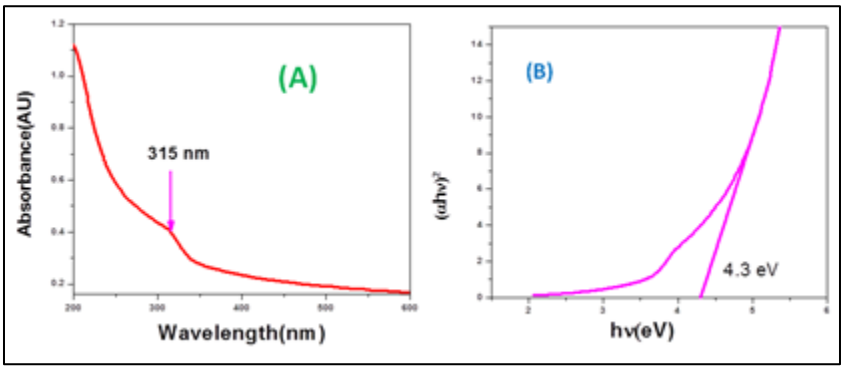

Figure 1: (A) Absorption spectra of ZnS nanoparticles; (B) band-gap determination plot of the ZnS nanoparticles

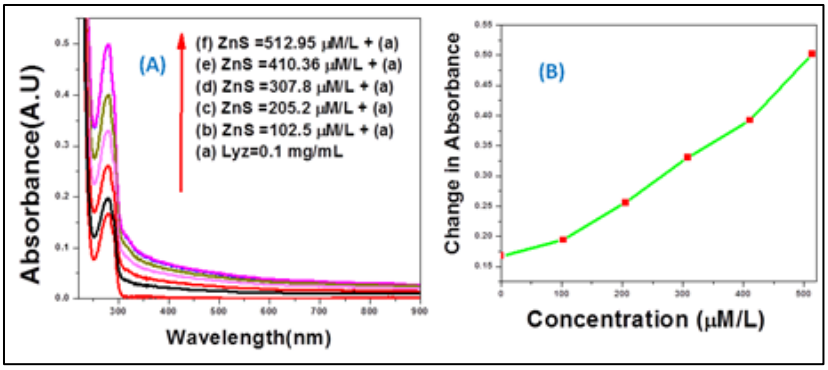

Figure 2: (A) Absorption spectra of (a) pure Lysozyme (0.1 mg/mL), (b) Lysozyme with 102.5 µM ZnS NPs, (c) Lysozyme with 205.2 µM ZnS NPs, (d) Lysozyme with 307.8 µM ZnS NPs, (e) Lysozyme with 410.36 µM ZnS NPs, (f) Lysozyme with 512.95 µM ZnS NPs; (B) Variation of the change in absorbance of the tryptophan (Trp) in the Lyz with the different concentration of ZnS nanoparticles

X-Ray Diffraction (XRD)

The measured XRD data's are plotted in the Figure 3. The pattern shows the peaks (111), (220), (311) corresponding to simple cubic phase of the crystal unit cell [21]. Here, the intensities of different diffraction peaks are different. The different intensity indicates that the formation mechanism of various planes is different.

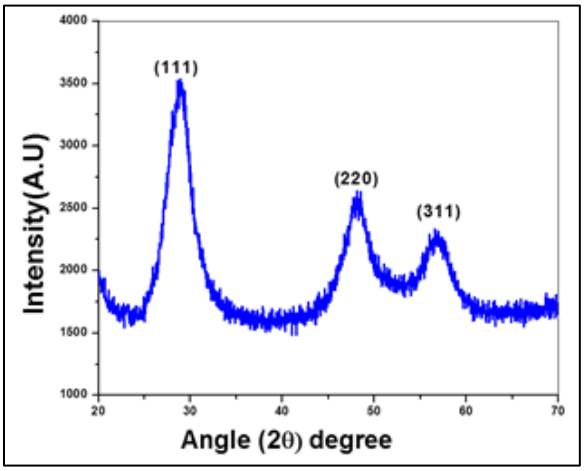

Figure 3: XRD spectrum of the ZnS nanoparticles

Fluorescence Quenching Study

Fluorescence spectroscopy is an important tool to study of information about the deformation/ conformational changes of protein molecules. The emission spectra of pure ZnS NPs are shown in Figure 4A. This photoluminescence spectrum shows strong visible emission around 424 nm. The fluorescence quenching measurements has been analyzed to study the the binding of ZnS NPs-Lyz bioconjugates. The addition of ZnS NPs of different concentrations (C_{ZnS}) with Lysozyme results a change in the maximum fluorescence emission spectrum intensity (I_{max}) of the the Lyz, suggesting the occurrence of quenching process (Figure 4B) [22]. The quenching occurs via the adsorption and interaction of the Tryptophan residues accessible to the metallic surface of the ZnS NPs signifies the unfolding as well as denaturation of Trp in the presence of ZnS NPs. To disclose the mechanism and quenching, the stern-volmer equation was used [23]. The graphical plot of the stern-volmer equation and corresponding binding sites determination plots are shown in Figures 4C and 4D respectively. The binding constant *K* along with the number of binding sites (*n*) between Lyz and ZnS NPs are 2.06 $(mM/L)^{-1}$ and 0.71 respectively. This indicates that a negative cooperative take place [23].

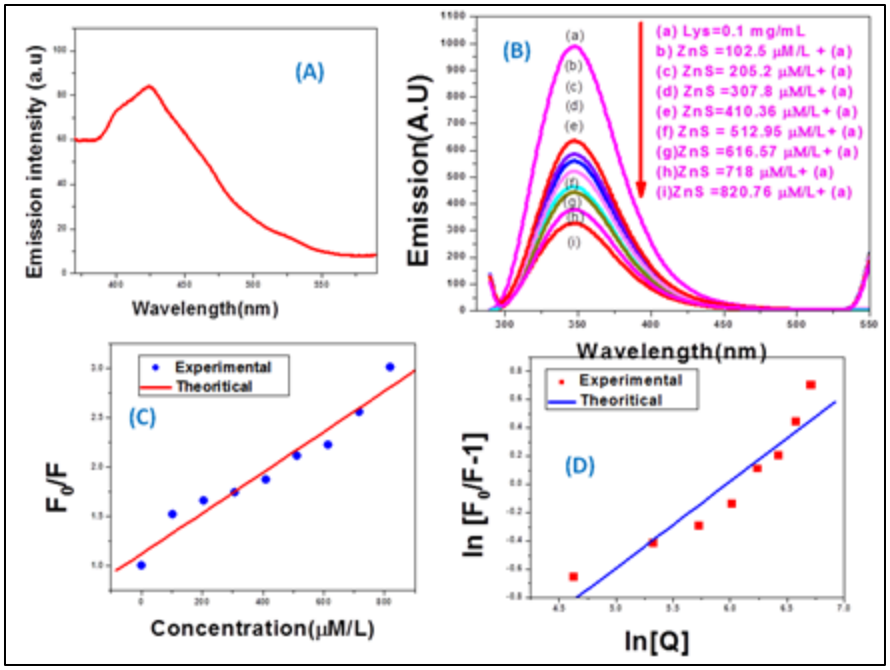

Figure 4: (A) Emission spectra of pure ZnS NPs, (B(a) pure Lysozyme (0.1 mg/mL), (b) Lysozyme with 102.5 µM ZnS NPs, (c) Lysozyme with 205.2 µM ZnS NPs, (d) Lysozyme with 307.8 µM ZnS NPs, (e) Lysozyme with 410.36 µM ZnS NPs, (f) Lysozyme with 512.95 µM ZnS NPs; (g) Lysozyme with 616.57 µM ZnS NPs (h) Lysozyme with 718 µM ZnS NPs (i) Lysozyme with 820.75 µM ZnS NPs; (C) F0/F vs Q(µM); (D) ln [(F0-F)/F] vs ln[Q]

TEM and HRTEM Study

Figure 5 shows the TEM images of the synthesized ZnS nanostructure. The average diameter of the nanostructure is approximately diameter 15 nm with spherical in shape. The SAED pattern of pure ZnS nanoparticles is crystalline (Figure 5) in nature with presence of the rings (111), (220), (311). Figures 6a-6c shows the behavior of the ZnS nanoparticles inside Lysozyme molecules. HRTEM of Figures 6a and 6b clearly indicate that core ZnS nanoparticles are coated by shell Lysozyme. Due to interaction a fibrillar like structure is found in the ZnS-lysozyme composite system [24]. Schematic of the Trp-ZnS NPs conjugation is shown in Figure 6d.

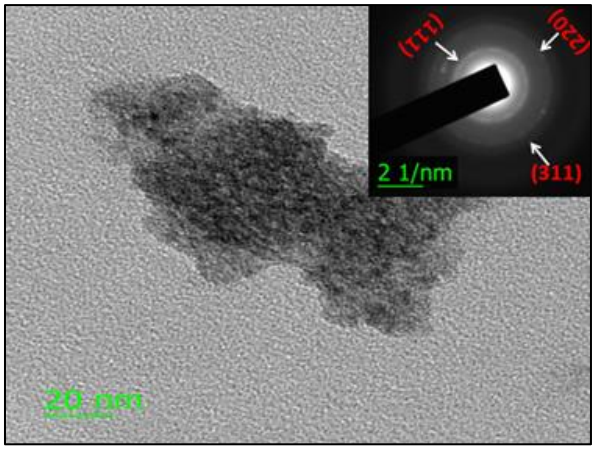

Figure 5: TEM image of the ZnS nanoparticles; Inset shows corresponding SAED pattern of the ZnS nanoparticles

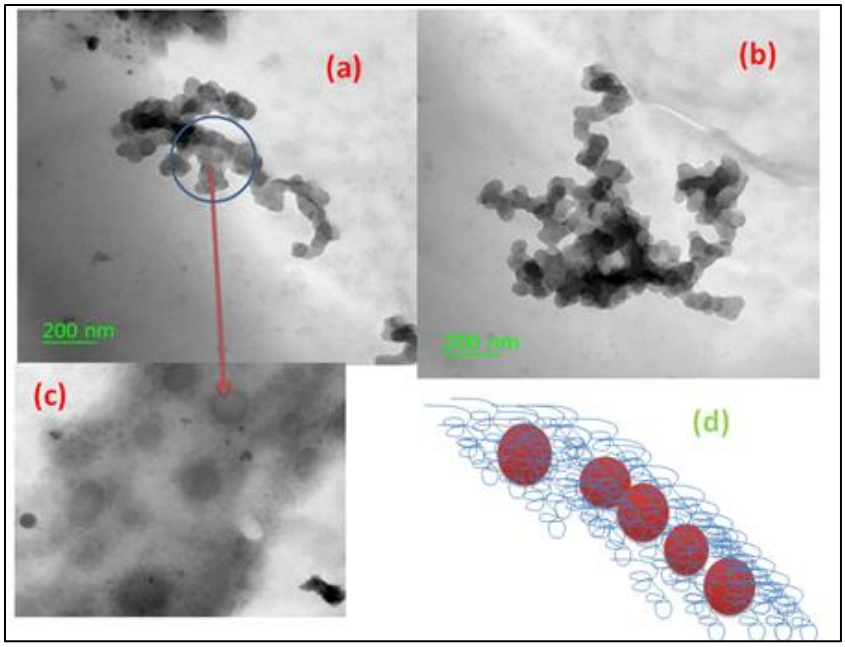

Figure 6: HRTEM of (a) ZnS-Lysozyme conjugate; (b) ZnS-Lysozyme conjugate; (c) zoom of the circular portion indicated in figure (a); (d) schematic representation for interaction and conjugate formation of ZnS NPs and Lysozyme

FESEM

The morphology of the ZnS-Lysozyme bioconjugate was observed in a ZEISS Field emission scanning electron microscope (FESEM) operated at 5 kV. Typical FESEM images of the bioconjugated material is shown in Figure 7b. There areglobular and modular structure formation represented by the picture. The easy acess of of ZnS Nanoparticle into the cavity of the lysogyme is by diffusion process [25]. The formation of bioconjugate and well protein coated ZnS nanoparticles are clear from FESEM images (Figure 7).

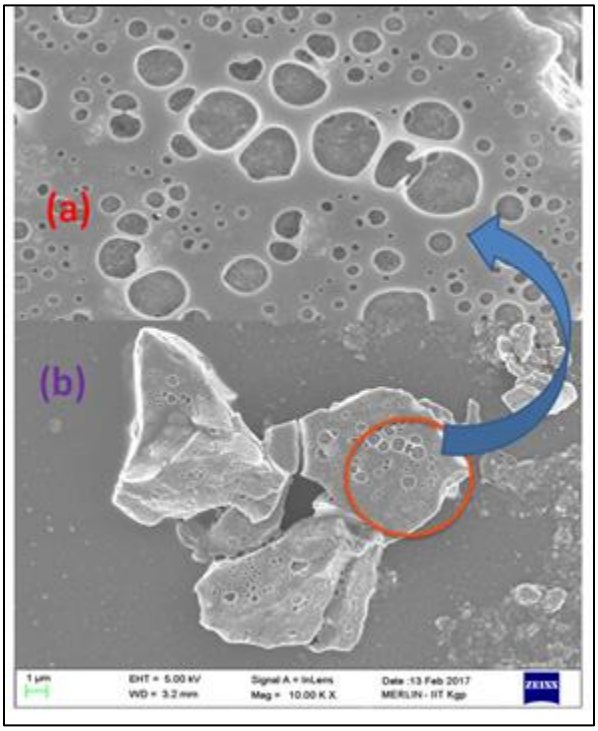

Figure 7: FESEM of (a) ZnS-Lysozyme conjugate (b) Zoom of the circular portion of figure (b); (b) ZnS-Lysozyme conjugate

CONCLUSION

In conclusion, we have synthesized Zinc Sulphide nanoparticles of average diameter ~15 nm using a simple wet chemical method. The XRD pattern of the as synthesized samples shows a cubic phase. The emission quenching of the nanoparticle-lysozyme system showed a strong denaturation phenomenon of protein. The interaction between ZnS nanoparticles with Lyz showed negative cooperative reaction phenomenon. The image in the HRTEM picture and SEM pattern of the ZnS NPs-Lyz bioconjugate system indicates that the NPs are completely covered by Lyz protein molecules and formed fibrillar structure. The present investigation provides details about the binding mechanism and interaction of the physiologically important protein Lysozyme with optically important semiconductor ZnS NPs. This study possesses potential applications in biomedical sciences and nano-bio interface sciences including biotechnology.

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