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Spectroscopic studies on the interaction between novel polyvinylthiol-functionalized silver nanoparticles with lysozyme

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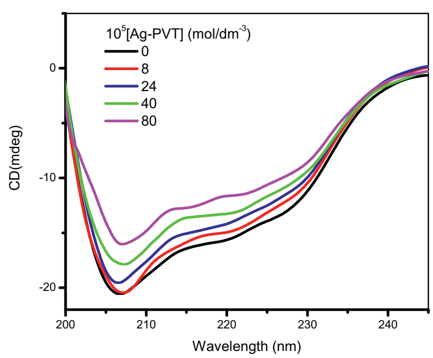
HIGHLIGHTS

- Ag-PVT nanoparticle quenched the fluorescence of lysozyme as a result of complex formation.
- Binding of Ag-PVT nanoparticles also induces secondary structural changes of the lysozyme.
- Appearance of blue shift in case of synchronous spectra signifies the involvement of hydrophobic forces in the interaction.

GRAPHICAL ABSTRACT



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ABSTRACT

Silver nanoparticles were functionalized with polyvinylthiol (Ag-PVT) and their effect on the conformation of hen-egg white lysozyme was seen by means of spectroscopic techniques, *viz.*, UV visible, fluorescence (intrinsic and synchronous), resonance Rayleigh scattering and circular dichroism. UV absorption spectra of lysozyme show a hyperchromic shift on the addition of Ag-PVT nanoparticles indicating the complex formation between the two. The interaction between lysozyme and Ag-PVT nanoparticles was takes place via static quenching with 1:1 binding ratio as revealed by the analysis of fluorescence measurements. Circular dichroism spectroscopic data show a decrease in α-helical content of lysozyme on interaction with Ag-PVT nanoparticles which was due to the partial unfolding of the protein. Synchronous fluorescence spectroscopy disclosed that the microenvironments of both tryptophan and tyrosine residues were perturbed in the presence of Ag-PVT nanoparticles and perturbation in the tryptophan environment was more prominent. Rayleigh scattering (RRS) intensity increases on increasing the Ag-PVT nanoparticles concentration till it reaches to the saturation. The RRS intensity increases four times as compared to the native protein indicating the possibility of protein aggregation at higher concentrations of nanoparticles.

Introduction

Functionalization of the nanoparticles is necessary for their implementation because of several requirements which include (i) good dispersibility and stability, not affected by changes in pH, ionic strength, polarity or temperature; (ii) high selectivity for tissue targeting, a long circulation time in the bloodstream, and limited nonspecific binding to albumins; and (iii) low renal toxicity [1]. Several reports on the nanoparticles functionalization for biomedical applications have been reported in the literature [2–5]. A variety of substances are being used in the surface functionalization of nanoparticles which include silica, synthetic

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polymers, biopolymers, dendrimers and small molecules. Surface coating by using synthetic polymers is exceptionally useful to solubilize hydrophobic inorganic nanoparticles and make a positive impact on the physicochemical properties of nanoparticles. Polymers, such as, poly (ethylene glycol), polyvinylpyrrolidone, poly (vinyl alcohol) can be used to modulate the surface charge, biocompatibility and sustained-release properties on nanoparticles [6].

Silver and its derivatives are very well known for their antimicrobial properties. Silver, when present in small amount, is lethal to most of microbes and safe to human, therefore, its use as water-disinfectant is widespread. Silver nanoparticles exhibited excellent anti-fungal activity on *Candida albicans* [7] and anti-fungal effect of these against *Trichophyton mentagrophytes* and *Candida* species was similar as compared with amphotericin B, but greater than fluconazole toward all the fungal strains examined [8]. Like other molecules Ag nanoparticles also need surface modifications in order to get the anticipated properties [9]. The surface of AgNPs was modified by phosphoryl disulfides which provides them improved biocompatibility and intracellular uptake [10]. Other functional agents such as carbonate, citrate and polyvinylpyrrolidone, have also been used for surface modifications of AgNPs to enhance their stability [11,12]. Correspondingly, thiol functionalization has been extensively used to alter the properties of metallic nanoparticles. Several properties, such as, biomedical functions [10,13], optical properties [14], shapes and size can be controlled by using thiol functionalization [15,16].

Recently, studies have been performed on the interaction of proteins with various nanoparticles which were either in pure form or functionalized with a suitable group [17–20]. In the present work we have synthesized novel polyvinylthiol-capped nanoparticles (Ag-PVT) and studied the interaction of these with lysozyme. Chicken egg white lysozyme is a small monomeric globular protein, having molecular weight of about 14.6 kDa and consisting of 129 amino acid residues with four disulfide bonds. It has 6 tryptophan and 3 tyrosine residues. It is used to carry drugs, such as, antibiotics to treat inflammation, abscess, stomatitis and rheum, [21]. Therefore, interaction of lysozyme with biologically important nanoparticles is of great interests. We have used several spectroscopic techniques to depict the interaction between lysozyme and Ag-PVT nanoparticles *viz.*, UV–visible, intrinsic and synchronous fluorescence, Rayleigh scattering, and circular dichroism.

Materials and methods

Materials

Lysozyme from chicken egg white (⩾98%) was procured from Sigma, USA. Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) was of analytical grade and also obtained from Sigma.

Synthesis of poly (vinyl alcohol) (PVA-SH)

Partially hydrolyzed (0.25 mol) poly(vinyl alcohol), PVA, having different molecular weights ranged from 15, 000 to 100, 000 g/mol was dissolved in a mixture of DMSO and DMF (50 mL each) at 65 °C. The reaction mixture then cooled at room temperature and triethylamine (18.35 mL) was added to it with continuous stirring. p-Toluene sulfonyl chloride (0.25 mol) dissolved in 100 mL of DMF added drop-wise to the reaction mixture at 10 °C in ice bath for 30 min. The mixture was then stirred overnight at room temperature. The reaction mixture poured in methanol to precipitate poly(-vinyl tosylate). The precipitate then washed with methanol and dried at room temperature.

Poly(vinyl tosylate), 0.1 mol, dissolved in 100 mL water and mixed with solution of 0.11 mol thiourea in 30 mL water and refluxed for 24 h at 90–100 °C. The reaction mixture cooled and 50 mL of (2 M, NaOH) was added to the reaction mixture. The reaction refluxed for 3 h under nitrogen atmosphere. The pH of solution was adjusted at 7 with neutralization the reaction mixture with 0.1 M of HCl aqueous solution after cooling the reaction mixture. The PVA-SH product was dried after precipitation of reaction mixture into methanol. The reaction yield ranged from 80% to 95%.

Synthesis of polyvinylthiol functionalized silver nanoparticles

PVA-SH coated silver nanoparticles were synthesized by adding (0.1–3 g) of PVA-SH with silver nitrate solution. The solution was kept at temperature of 90 °C for 1 h, to produce golden yellow color and then allowed to cool to room temperature. The silver nanoparticles were purified by ultracentrifugation (30 min at 30,000 rpm), followed by re-dispersion in water. The typical yield of PVA-SH-stabilized silver nanoparticles was around 75% (with respect to silver). Rest of the details of synthesis and characterization of Ag-PVT nanoparticles have been given elsewhere [22].

Characterization of the prepared TGA-coated CdTe nanoparticles

The morphology of the Ag-PVT nanoparticles was studied by using transmission electron microscopy (TEM). The TEM image (Fig. 1) illustrates that the Ag-PVT nanoparticles were spherical and homogeneously distributed in the solution.

Sample preparation

A stock solution of lysozyme was made in 20 mM of pH 7.4 tris HCl buffer and protein concentration of 10 μM was used throughout. Tris–HCl buffer was filtered through a 0.45 μm Millipore Millex-HV PVDF filter and pH was measured by using Mettler-Toledo pH meter (model S20).

UV–Vis absorption measurements

UV–Vis spectra were recorded between 240 and 320 on Perkin–Elmer Lambda 650 Spectrophotometer equipped with autosampler and water-bath with temperature controller. Quartz cuvettes of 1 cm path length were used for the measurements.

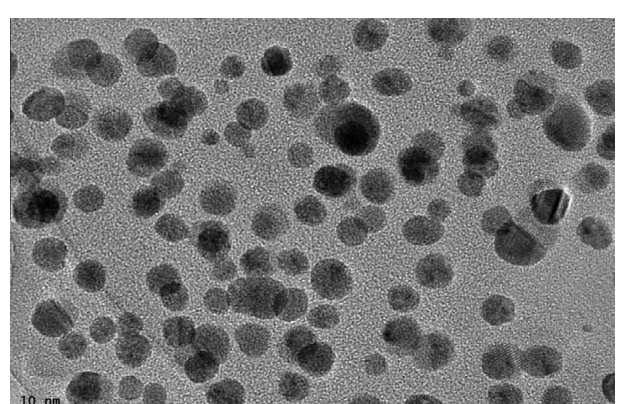

Fig. 1. TEM image of polyvinylthiol-functionalized silver nanoparticles.