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Interactions between thioglycolic acid capped CdSe/ZnS nanoparticles and papain

Gurvir Kaur[1], Shivani Bharti, S.K. Tripathi*

Centre of Advanced Study in Physics, Department of Physics, Panjab University, Chandigarh 160014, India

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ABSTRACT

The paper presents the interactions among carboxyl surfaced Cadmium selenide/Zinc sulphide (CdSe/ZnS) core/shell nanoparticles (NPs) with papain, studied by various spectrometric methods. CdSe/ZnS NPs strongly quench the intrinsic fluorescence of papain and show contribution of both static and dynamic mechanisms. The binding constants for papain-CdSe/ZnS NPs conjugates were determined by studying the fluorescence quenching of papain at different temperatures. Using Vant-Hoff analysis, the change in enthalpy, entropy and Gibbs free energy of papain-CdSe/ZnS NPs interaction have been calculated. The results infer that electrostatic interactions exist in carboxyl surfaced NPs and papain provides two independent binding sites for the NPs. The distance between donor and acceptor for papain–CdSe/ZnS NPs conjugates is calculated to be 3.02 nm from Förster resonance energy transfer. The intrinsic fluorescence of CdSe/ZnS NPs has also been enhanced by the papain but only at low concentrations 1–20 μl. All the observations evidence the formation of papain-CdSe/ZnS NPs conjugates, where papain retains the enzymatic activity dependent on temperature and pH. The optimum range for activity of papain-CdSe/ZnS NPs conjugates is pH 6–7.6, which is same as that of free papain.

1. Introduction

Colloidal semiconductor nanocrystals, often called quantum dots (QDs), are centre of attention for the biologists, chemists, pharmacists as well as physicists due to their exclusive size-dependent optical and electronic properties. QDs have narrow, tuneable, symmetric emission spectrum, broad band excitation, high photobleaching threshold, and good chemical stability which make them potential fluorescent tag to replace the traditional organic fluorophores. Since 1998, several surface functionalizations have been adapted for successful use of NPs in the areas of in vitro diagnostics and imaging [1–3]. Cadmium-based NPs are being utilized for in vitro and in vivo studies; where the main constituent cadmium and selenium are severely toxic for vertebrates at high dosage. The toxicity of CdSe QDs depends on their physicochemical properties such as size, capping reagent etc. [4]. So the CdSe QDs are being worked to reduce the toxicity where one possibility is by developing a shell of higher band gap and less toxic material, for example, ZnS, silica etc. over the emissive semiconductor nanocrystal core (CdSe, CdTe, etc.) hence generating core/shell type NPs [5,6]. Here shell provides toxicity control as well prevents surface quenching of excitons in the emissive core which leads to high fluorescence quantum yield and photostability.

Organic ligands such as trioctylphosphine or trioctylphosphine oxide are capping reagents to produce high quality NPs however the biological applications demands the replacement of organic ligands by hydrophilic capping agents. A number of simple and quite feasible methods are there to synthesize water-soluble NPs directly [6–8], which is a great encouragement for employing NPs in medical science and pharmaceuticals. The biomolecules can be possibly linked to NPs by attractive forces such as covalent attachment, electrostatic attraction, hydrogen bonding interaction, and hydrophobic force [9,10].

Engineering bio-inorganic hybrid for detection of biological targets/imaging demands the retention of the biological function of the bio-entities immobilized. The enormously enhanced surface effects of nanocrystals affect the functionality of the biomolecules. So it is crucial to understand nanoparticles interactions with biological entities starting from the building blocks (proteins, nucleic acids etc.) and expanding the research to cells, tissues etc. [7,11,12]. To date so many proteins including bovine serum albumin [13], human serum albumin [14], trypsin [7,15,16] etc. have been worked for their immobilization on various supports. The immobilization allows the separation of enzyme from solvent for reutilization as well may enhance the enzymatic activity, activity range or lifetime of the enzyme.

In this manuscript a comprehensive study is made on luminescent

* Corresponding author.
E-mail addresses: surya@pu.ac.in, surya_tr@yahoo.com (S.K. Tripathi).
[1] Present address: Sri Guru Gobind Singh College, Sec-26, Chandigarh.

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thioglycolic acid (TGA) capped CdSe/ZnS NPs and their interactions with an important industrial protease papain. Papain consists of a single peptide chain containing 212 amino acids and is able to catalyze the hydrolysis of varieties of peptide, amide, and ester linkages. It has wide applicability in food, pharmaceutics, leather, cosmetic, and textile industries. At molecular level its applications includes protein structural studies, peptide mapping, preparation of Fab fragment from IgG, solubilization of integral membrane protein, and production of glycopeptides from purified proteoglycans. These applications have driven the research of immobilizing papain on inorganic surface. Till date, a number of methods have imputed the interaction of papain with different entities and its immobilization on different supports [17–22]. Papain provides the benefit as it has intrinsic fluorescence and its molecular structure have already been studied extensively. The interaction between NPs and papain affects the fluorescence of entities, through which the binding constants and various thermodynamic parameters of the interaction process have been obtained. The resonance energy transfer from papain to NPs is possible due to the overlap between emission spectra of papain and absorption spectra of NPs. This have been used to measure distance between NPs and sites in biological molecules. Very small conformational changes for the NP-papain conjugate have been depicted from the circular dichroism and the FT-IR studies and finally studied the effect of conjugation of NPs on the enzymatic activity of papain. Over all a thorough study have been made which is paradigm for NP research and applications in biology and medicines where biomolecule-inorganic interactions and interfacing is prerequisite.

2. Experimental

2.1. Materials

The chemicals were used without further purification and were of analytical grade. Papain from papaya latex (EC 3.4.22.2) was procured from Sigma Aldrich; α-benzoyal L-arginine ethyl ester hydrochloride (BAEE.HCl) from Across Organics, TGA [$HSCH_2CO_2H$] extra pure with concentration 80%, Sodium Sulphide [Na_2S] flakes Zinc acetate pure [$(CH_3COO)_2Zn{\cdot}2H_2O$)] and Potassium dihydrogen orthophosphate [KH_2PO_4] from Central Drug House Ltd.; Selenium [Se] powder from Loba Chemie Pvt. Ltd.; Cadmium acetate dihydrate [$(CH_3COO)_2Cd{\cdot}2H_2O$] and Potassium hydroxide (KOH) from Qualigen Fine Chemicals; Sodium hydroxide [NaOH] from Ranbaxy Laboratories Ltd.; Sodium sulphite anhydrous [Na_2SO_3] from Merck specialities Pvt. Ltd.; and di-Potassium hydrogen orthophosphate [K_2HPO_4] from Thermo Fisher Scientific India Pvt. Ltd. The synthesis was executed in standard glassware using deionised (DI) water. Stock solution of papain was made in phosphate buffer (50 mM, pH 6, 7.6 and 9) and stored in the dark at 0–4 °C.

2.2. Synthesis of CdSe/ZnS nanoparticles

TGA encapsulated CdSe/ZnS NPs were synthesized by the method adopted in Ref. [23,24]. 0.05 M solution of Cadmium acetate dihydrate was prepared in water and slowly added 0.6 M solution of TGA to it with continuous stirring. Adjusted the pH of the solution 9–10 and added Se source (Sodium selenosulphate) to it drop wise maintaining the ratio Cd^{2+}:Se^{2-}:: 4:3. This solution is refluxed at 60° C for 7–9 h which turns to an orange-yellow solution that infers formation of CdSe NPs. CdSe/ZnS core/shell NPs were prepared by controlled growth of ZnS over the surface of CdSe QDs. For this 10 ml of CdSe QDs is taken in round bottom flask and added 0.05 M Na_2S and 0.05 M $(CH_3COO)_2Zn{\cdot}2H_2O$ solutions alternatively in small aliquots with continuous stirring at room temperature. Further the NPs were separated from the non-reacted chemicals and bi products by adding acetone. The NPs precipitates were filtered and after several washings again dispersed in water at different concentrations to study the interactions with papain. The size of CdSe/ZnS NPs so obtained is 4.0 ± 1.2 nm from TEM images, which agrees with that calculated from the XRD and Absorption spectra as reported in Ref. [23]. However the hydrodynamic size of NPs is 100 nm, which is very large. Hydrodynamic size is usually higher because of the presence of the capping reagent TGA, but here the extremely large values indicate that NPs form clusters of small particles by aggregation of NPs in the solution. Addition of papain having hydrodynamic size nearly 5 nm have dispersed the particles giving the hydrodynamic size of NPs–Papain conjugates equal to 92 nm [25].

2.3. Interaction of nanoparticles with papain

The effect of papain on the steady state absorption in UV–Vis and IR region, the steady state and time resolved fluorescence of NPs have been studied to evaluate the nature of interactions between NPs and papain. FT-IR studies of powdered papain, CdSe/ZnS NPs and papain–NPs conjugates were made on Perkin Elmer (Spectrum 400-FT-IR) Spectrometer using KOH pallets ranging 4000–450 cm^{-1}.

Absorption spectra was acquired on Double Beam UV/VIS Spectrophotometer (Shimadzu) over the range 200–700 nm using 1 cm path length quartz cell for sample. The spectrometer was equipped with a water bath based temperature controller.

Fluorescence analysis was carried out on Shimadzu Spectrofluorophotometer (RF-5301PC) equipped with a thermostat bath. The samples were placed in a 1.0 cm quartz cell keeping the excitation and emission slit width 3 nm.

Emission lifetimes of the papain and the NP-papain conjugates were measured on Time Resolved Fluorescence Spectrometer (TRFS)-Edinburgh FL920 Fluorescence Life Time Spectrometer which works on the basis of the time-correlated-single-photon-counting (TCSPC) technique.

Circular Dichroism analysis was made on a Chirascan™ CD Spectrometer. Sample is placed in 0.1 cm and 0.2 cm path-length cells for far UV-CD and near UV-CD spectra, respectively. The measurements were made at 25° C, and scanned five times in the range 200–250 nm for far UV-CD and 250–350 nm for near UV-CD studies and finally averaged.

For pH measurements, used digital pH-meter (Max electronics, India) equipped with a combined glass electrode.

2.4. Determination of activity of papain

The activity of free papain and papain conjugated to the NPs is determined on the basis of hydrolysis of N-α-Benzoyl-L-Arginine Ethyl Ester (BAEE) hydrochloride as:

N-α-Benzoyl-L-arginine-ethyl ester hydrochloride + H_2O ↔ N-Benzoyl-L-arginine + Ethanol

The kinetic study of the hydrolysis process can be spectrophotometrically measured by monitoring the increase in absorbance of the reaction product at 253 nm [26]. The method was described by Worthington Chemical Corporation and here the enzymatic activity (1U) is defined as the change in the absorbance of 0.003 at 253 nm per minute and the specific activity (U/mg) is given by:

$$\text{Specific enzyme activity } (U/\text{mg}) = \frac{(A_1 - A_2)_{253nm}}{0.003TW} \quad (1)$$

where A_1 is the absorbance straight-line final reading, A_2 is absorbance straight-line initial reading, T is the elapsed time (minutes), between the initial and final readings, and W is the weight (mg) of the crystallized papain in the volume of solution used in determining the absorbance. 0.25 mM substrate solution was prepared by dissolving BAEE in 55 mM phosphate buffer solutions of different pH (6, 7.6 and 9 pH at 25 °C). Papain is activated before use to ensure maximum activity [26]. The enzyme is kept for 30 min by adding 1.1 mM EDTA, 0.067 mM mercaptoethanol and 5.5 mM cysteine-HCl to the normal buffer solution.