Colloids and Surfaces A: Physicochem. Eng. Aspects 520 (2017) 131–137

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Interaction of plasma proteins with ZnSe and ZnSe@ZnS core-shell quantum dots

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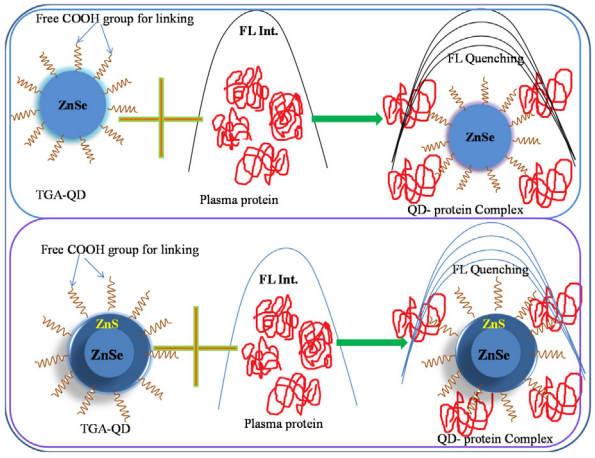
HIGHLIGHTS

- Direct aqueous synthesis of (cadmium-free) core-shell quantum dots (QDs).
- Plasma protein binding affinity and hydrophobic index followed the trend β-Lg > HSA > BSA.
- Binding of proteins with core-shell QDs compared to core only QDs shows 3-fold increment.
- The secondary and tertiary structure of proteins remained unchanged after interactions.

GRAPHICAL ABSTRACT

Interaction of Plasma Proteins with ZnSe and ZnSe@ZnSCore-ShellQuantumDots.(*)



ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 10 January 2017
Accepted 13 January 2017
Available online 15 January 2017

Keywords:
Core-shell quantum dots
Quantum dot-protein interaction
Secondary structure

ABSTRACT

Herein, we report, interaction of water-soluble ZnSe core only (size = 3.60 ± 0.12 nm, zeta potential = −45 mV), and ZnSe@ZnS core-shell (size = 4.80 ± 0.20 nm, zeta potential = −38 mV) quantum dots (QDs) with three globular plasma proteins (Human Serum Albumin (HSA), Bovine Serum Albumin (BSA) and β-lactoglobulin (β-Lg)). It was found that QDs effectively quenched the fluorescence of proteins that was size-dependent. The nature of quenching was static which resulted from the generation of QDs-Protein complexes. It was found that the binding affinity followed: BSA > HSA > β-Lg for both ZnSe and ZnSe@ZnS samples. Interestingly, the secondary and tertiary structure of proteins remained unaffected after interactions.

1. Introduction

Colloidal luminescent semiconductor quantum dots (QDs) are of considerable interest in the biological and medical disciplines, due to their promising applications in bio-imaging and bio-sensing among others [1–3]. For example, highly fluorescent QDs could be

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http://dx.doi.org/10.1016/j.colsurfa.2017.01.032
0927-7757/

prepared by pyrolyzing organo-metallic reagents at high synthesis temperature in organic solvents [4,5]. This utilizes high boiling point solvents and high temperature (typically 200–350 °C). On the other hand, organo-metallic compounds are not only harmful and toxic, but also are more expensive. Direct synthesis of II–VI semiconductor quantum dots, such as CdSe, CdTe, CdHgTe, and ZnSe, in aqueous medium using thiols as stabilizing agents can provide an important alternative to the previously described high temperature protocols [5–7]. Due to their high specific surface area and the deficiency in surface atomic coordination, the luminous efficiency of these QDs is significantly lower than other kinds of nanocrystals [8–11]. There are some recent reports on the morphology of the complex film (skin like) and the formation of multilayer structures at the liquid/liquid (chloroform/water) interface of pure β-lactoglobulin as well as the mixture of dipalmitoylphosphatidylcholine (DPPC)/β-lactoglobulin confirmed by Li et al. [12] with AFM studies. Phase transition and properties of DPPC monolayers penetrated by bovine β-lactoglobulin dissolved in a buffered aqueous sub-phase were experimentally studied by Brewster angle microscopy (BAM) and grazing incidence X-ray diffraction (GIXD). Further, conformational changes and sequestration out of proteins from the penetrated monolayer was studied by Zhao et al. [13]. The dynamic adsorption and penetration of human serum albumin (HSA) into the monolayers of five biologically important surfactants-DSPC, DPPC, DMPC, DMPE and DMPA-were systematically studied using Brewster angle microscopy, film balance and pendent drop techniques by Wang et al. [14].

Some recent studies have shown possible threats to human health with increasing exposure and uncontrolled application of nanomaterials [15–20]. Therefore, it is necessary to comprehend the effects of all nanoparticles on different cells and their possible interaction mechanisms. Fluorescence quenching spectroscopy is an appropriate method to obtain information about the cell-QD interactions. Regardless of the remarkable development in the field of nanoscience, little is known quantitatively about the interaction of low-dimensional nanocrystals with cells. For instance, no results in the literature is available on the synthesis of ZnSe QDs with tailored ZnS shell, and their interactions with plasma proteins like Human Serum Albumin (HSA), Bovine Serum Albumin (BSA) and β-lactoglobulin (β-Lg). This has motivated the present work, we have synthesized ZnSe and ZnSe@ZnS QDs directly in aqueous solution and studied their interactions with abovementioned model plasma proteins.

2. Materials and methods

Zinc acetate ($Zn(OAc)_2$, 99%) and thioglycolic acid (TGA,80%) ware purchased from CDH india, sodium borohydride ($NaBH_4$, 99%), selenium powder (−100 mesh, 99.999%) were purchased from SD-fine chemicals Ltd. India, thiourea (99%), was purchased from Sigma-Aldrich, USA. BSA, HSA and β-Lg were purchased from Sigma-Aldrich (U.S.A.). These were used without further purification. PBS buffer (pH = 7.2) was used as a solvent. Deionized water was used as base solvent. All procedures were performed at room temperature 25 °C.

ZnSe quantum dots were synthesized via an aqueous route in a reaction flask at room temperature following the protocol described in ref. [11]. We replaced glutathione (GSH) by thioglycolic acid (TGA),and used thiourea as sulphur source. This method could produce core ZnSe QDs of mean size of 3.60 ± 0.12 nm (TEM data).

For synthesis of core-shell structures, 20 ml of ZnSe reaction solution that contained about 0.08 g of ZnSe was loaded into a 100 ml three-neck flask to which 10 ml of mixture solution containing 0.18 mmol of TGA, 0.12 mmol of $Zn(OAc)_2$, and 0.12 mmol thiourea was added. Then the pH was adjusted to 10.2 using NaOH solution. The reaction flask was then heated to 90 °C. Samples of the reacted mixture were drawn at different time intervals to record their UV–vis absorption, and fluorescence emission spectra. The reaction was stopped by cooling the reaction mixture to room temperature. ZnSe@ZnS material was purified by repeated centrifugation, and decantation in ethanol. The excess residual precursors and ligand were removed by multiple purification steps prior to their characterization. This method produced core-shell structure of average size of 4.8 ± 0.20 nm (TEM data).

All the protein solutions were prepared in PBS (pH ≈ 7.2) at a fixed concentration of 20 μM (BSA), 20 μM (HSA) and 60 μM (β-Lg). All experiments were performed at room temperature 25 °C.

Due to the inherent effects of morphology and size on the optical properties of nanocrystals, it was imperative to first determine the size and shape of synthesized nanocrystals. A JEOL 2100F transmission electron microscope equipped with a tungsten filament and operated under accelerating voltage of 200 kV with maximum magnification = 150, 000X was employed.

Zeta potential experiment was performed on a ZEECOM instrument (USA) [21]. Electrophoretic mobility μ of a particle in dispersion can be defined in the Smoluchowski formalism given by

$$\mu = \frac{2\varepsilon\zeta}{3\eta_0} H(\kappa R) \quad (1)$$

Where R,ε, and κ are the particle radius, the solvent dielectric constant, and the Debye screening parameter respectively. The screening effect is accounted for by the Henry function $H(\kappa R)$ ($1 \le H(\kappa R) \ge 1.5$ for $0 \le \kappa R \le \infty$). Due to the high electrolyte concentration in buffer solution, and in the Smoluchowski approximation $H \approx 1.5$ is valid (for large κ). General discussion on zeta potential can be found in ref. [22]. The hydrodynamic sizes of these QDs were measured with (Photocor Instruments, USA) dynamic light scattering (DLS) instrument. For more details ref [23].

UV–vis absorption spectra of QD samples in aqueous solution were obtained at room temperature using Cecil model CE-7200 (Cecil Instrument, UK) spectrophotometer. Fluorescence and quantum yield (QY_0 = 95% for standard Rhodamine 6G in water) measurements (all diluted with 0.1 M PBS solution) were performed using a Varian Cary Eclipse fluorescence spectrophotometer (See Ref. [23]. for details).

Crystalline structure of quantum dots samples were determined using powder X-ray diffraction (XRD) techniques. XRD spectra were acquired using a Rigaku D/Max 2200 powder X-ray diffractometer with a Cu Kα target (λ = 1.54056Å) and a Peltier-cooled solid-state detector.

Circular dichroism (CD) spectra were recorded on an Applied Photophysics Chirascan instrument (USA) to estimate the secondary structure of proteins (See ref. [23] for details). Here, for a typical CD experiment, the concentration of BSA, HSA and β-Lg were kept as low as ≈2.2 μM, 2.0 μM and 8.4 μM, respectively in 0.01 M phosphate buffer solution (pH 7.2 ± 0.1).

3. Results and discussion

3.1. Structure and morphology

The crystal structure of the ZnSe core and ZnSe@ZnS core-shell NCs were analyzed. The XRD profile of ZnSe quantum dots comprised of the typical signature of cubic zinc blende (JCPDS File No: 32-0483), which normally broadens due to finite crystallite size, Fig. S1. The diffractive peaks were located at 27.4^0, 45.95^0, and 54.3^0 corresponding to the crystallographic planes (111), (220), and (311) of ZnSe. Moreover, from the XRD pattern, it was demonstrated that