Journal of Photochemistry and Photobiology A: Chemistry 215 (2010) 118–122

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Study of the interaction between CdSe/ZnS core-shell quantum dots and bovine serum albumin by spectroscopic techniques

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ARTICLE INFO

Article history:
Received 13 May 2010
Received in revised form 2 August 2010
Accepted 4 August 2010
Available online 11 August 2010

Keywords:
Quantum dots
Bovine serum albumin
Quenching
Fluorescence
Biosensing

ABSTRACT

In this work we studied the interaction between CdSe/ZnS core-shell quantum dots (QDs) and bovine serum albumin (BSA) protein, and the temperature effects on the structural and spectroscopic properties of both, individual QDs and protein and their bioconjugates (QDs@BSA), by fluorescence and UV–vis spectroscopy. The recorded UV–vis data and the calculated rate of BSA fluorescence quenching by the QDs demonstrated that the interaction between them leads to the formation of QDs@BSA complex. Moreover we show that, compared to the monotonically decrease of the non-conjugated QDs fluorescence intensity, the temperature dependence of the QDs@BSA emission has a much more complex behavior, highly sensitive to the conformational changes of the protein.

1. Introduction

Determination of trace of biomolecular concentrations is of foremost importance for the discovery and development of biomolecular mechanisms and medical diagnosis. Most common detection methods are based on the design of sensors that can selectively recognize and signal the presence of a specific analyte. Classical staining agents like organic dyes, fluorescent proteins or lanthanide chelates, have several limitations, such as broad spectrum profile, low photobleaching threshold or poor photochemical stability [1]. Quantum dots (QDs) represent a new class of fluorescent labels that have attracted much interest for biosensing and bioimaging due to their unique optical properties [2–4]. In comparison to classical staining agents, the fluorescence emission spectra of QDs can be continuously tuned by changing the particle size, and a single wavelength can be used for simultaneous excitation of different-sized QDs. Also, surface-passivated QDs are highly stable against photobleaching and have narrow, symmetric emission peaks about 25–30 nm full width at half maximum.

However, due to the increasing extension of nanotechnology in biological sciences, it is imperative to develop a detailed understanding of how biological entities, and at the most basic level, proteins, may interact with nanoscale particles. In addition, relatively little is known about the effects of temperature on the QDs–biological system interfaces. Since QDs are intensely used as a fluorescent marker in biological applications, such effect can play a major role in their use. For instance, the interesting phenomenon of QDs blinking, i.e., intermittent fluorescence under excitation, is known to depend on several experimental parameters, including the biological environment or temperature. As result, the blinking behavior could represent a critical issue for the development of biological applications based on monitoring the photoluminescence of QDs as a function of time. A number of previous studies have been devoted to better understanding the influence of luminiscence blinking in fluorescence spectroscopy of bioconjugated core/shell QDs [5,6].

In this work we demonstrate the applicability of CdSe/ZnS core-shell QDs as probes to monitor the thermal behavior of bovine serum albumin (BSA) protein. BSA is one of the most extensively studied proteins, particularly because of its structural homology with human serum albumin [7,8]. Compared to non-conjugated QDs, which exhibit a linear dependence of the fluorescence intensity as a function of temperature, the bioconjugated QDs exhibit a more complex behavior, highly sensitive to the structural changes of the protein. Increasing the temperature up to 51 °C, the fluorescence intensity of bioconjugated QDs exhibits a decrease, due to the generation on the surface of the particles of alternative non-

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doi:10.1016/j.jphotochem.2010.08.008

radiative decay channels. At 51 °C, temperature very close to the first transition point of the BSA, at which conformational changes of the protein occurs, the fluorescence intensity of bioconjugated QDs exhibits a minimum. Between 51 and 69 °C, we observed an increase of the QDs fluorescence intensity, revealing the conformational changes of the protein. Increasing further the temperature, the recorded fluorescence intensity exhibits a plateau after 69 °C, temperature very close to the second transition point of the BSA, at which the denaturation of the protein occurs.

2. Materials and methods

2.1. Materials

CdSe/ZnS QDs of 3.3 nm dispersed in toluene were purchased from Evident Technologies (Evidot test Kit-AB 016DCP; Product EDC11Tol0520), BSA and Mercaptoacetic acid (MAA) from Aldrich and Phosphate Buffer Solution (PBS, pH 7.01) from Merk. All chemicals in this experiment were of analytical grade or the highest purity available.

2.2. Preparation and characterization of QDs@BSA bioconjugates

The typical high-temperature organometallic conditions employed for the synthesis of high-quality QDs, yield particles insoluble in water and non-biocompatible [9–11], whereas for fluorescent tagging or labeling, water-soluble QDs (w-QDs) with stable optical properties are required. In this work w-QDs were prepared through the direct adsorption of MAA on the nanocrystals surface, as previously described [12]. In this procedure, the mercapto group of MAA binds to Zn atoms from the QDs shell, while the polar carboxylic group of MAA yields w-QDs. Moreover the carboxylic group of MAA allows the coupling of QDs with various biomolecules (proteins, peptides, etc.). Briefly, the QDs solution, diluted 10 times before used, was mixed with MAA in volumetric ratio 10:1 and slowly stirred for 2 h at room temperature. Subsequently, PBS in 1:1 ratio was added and the mixture was rapidly stirred for 30 min, leading to the separation of water and toluene layers. The aqueous layer, containing the solubilized QDs was extracted and washed to remove the MAA excess. The BSA solution was prepared freshly by dissolving solid BSA in PBS to a concentration of 1 mg/ml. The w-QDs were mixed with the BSA solution and incubated for 2–5 days in order to obtain the QDs@BSA bioconjugates.

The optical absorbance spectra of the prepared solutions were recorded using a Jasco V-670 UV–vis-NIR spectrophotometer. The fluorescence spectra were recorded using a Jasco LP-6500 spectrofluorimeter coupled with a Jasco ADP-303T temperature controller (−10 to 110 °C). The fluorescence spectra were recorded using an excitation wavelength of 290 nm. Slit widths of 3 nm and quartz cells with 1.0 cm path length were used for all measurements.

3. Results and discussion

Typical absorption spectra of BSA, w-QDs and their bioconjugates QDs@BSA solutions are shown in Fig. 1. While the absorption spectrum of BSA solution exhibits the well-known absorbance maximum at 279 nm, due to the $\pi \rightarrow \pi^*$ transition of the aromatic amino acids residues, the diluted QDs solution exhibit a maximum absorption at 512 nm. However, when albumin protein was added to the QDs solution, a sensitive shift of the characteristic absorption band of BSA is measured, which clearly indicates the formation of bioconjugates. Moreover, the formation of bioconjugates is also

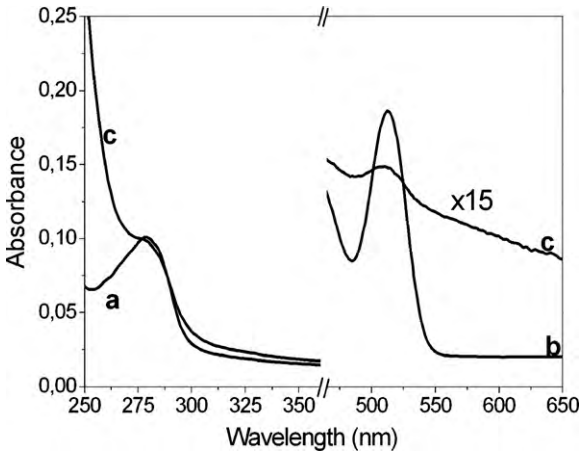

Fig. 1. Absorption spectra of (a) BSA, (b) w-QDs and (c) QDs@BSA solution.

confirmed by a decrease in intensity and a blue shift of 3 nm of the maximum absorption band of QDs.

The interaction between BSA and QDs was also studied by fluorescence spectroscopy, a technique which can provide valuable information concerning the binding of protein on the surface of QDs, such as binding constant and number of binding sites [13]. The fluorescence measurements presented in Fig. 2 were recorded within the spectral region between 300 and 600 nm, using an excitation wavelength of 290 nm, where both BSA and QDs absorb. The pure BSA aqueous solution at room temperature exhibits a strong intrinsic fluorescence emission band centered at 341 nm, which originates from the emission of tryptophan residues (see Fig. 2, spectrum a). Contrarily, QDs exhibits a narrow, symmetric band located at 530 nm with a full width at half maximum (FWHM) of 32 nm (see Fig. 2, spectrum b). After adding QDs to BSA solution a small blue shift and a decrease in intensity of the BSA characteristic band were observed (Fig. 2, spectrum c). This result indicates that tryptophan residues were in a more hydrophobic environment due to the tertiary structural change of albumin [14–17]. We point out that the recorded fluorescence spectra of BSA were not corrected to the absorption of QDs. Since the absorption of the QDs increases as wavelength decreases, the actual blue shift in the emission of BSA may be larger than the measured shift. However, a much more pronounced effect is observed for the QDs characteristic emission band. In this case, a drastic decrease of the emission intensity can be observed after the addition of albumin solution, together with a

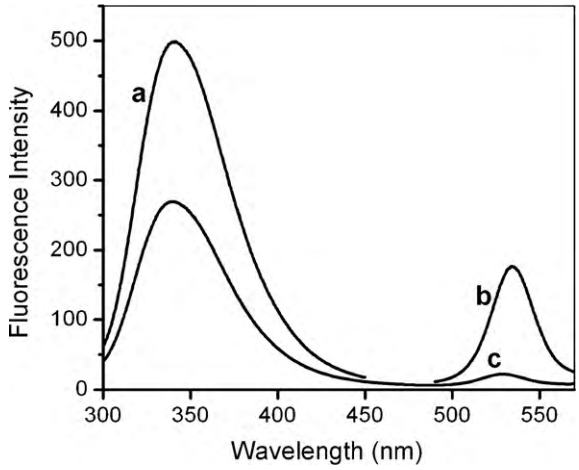

Fig. 2. The fluorescence spectra of (a) tryptophan residues in BSA, (b) w-QDs and (c) QDs@BSA bioconjugates.

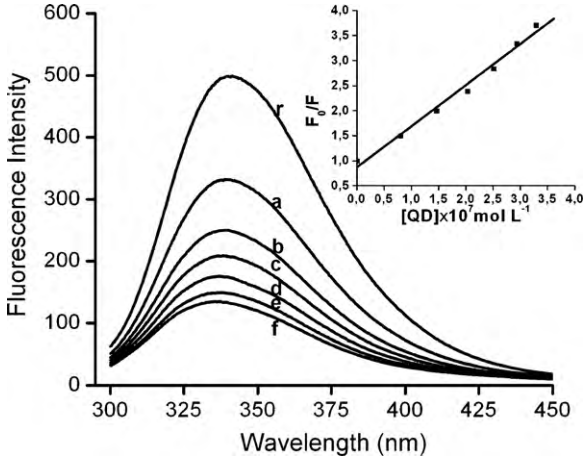

Fig. 3. Fluorescence intensity of BSA at various concentrations of QDs: $r = 0$ M; $a = 7.99 \times 10^{-8}$ M; $b = 1.46 \times 10^{-7}$ M; $c = 2.03 \times 10^{-7}$ M; $d = 2.51 \times 10^{-7}$ M; $e = 2.93 \times 10^{-7}$ M; $f = 3.29 \times 10^{-7}$ M. The inset shows the plot F/F_0 against QDs concentration.

broadening of the band and a small blue shift, similar to the results reported by Idowu et al. [18].

3.1. Fluorescence quenching mechanism

Generally, the quenching of the fluorescence emission originates either from static or dynamic interaction of the quencher with the fluorophore. In order to assess the quenching mechanism of BSA fluorescence due to QDs, we monitored the emission of tryptophan residues in the protein solution at different concentrations of the quencher. As shown in Fig. 3, the emission of BSA solution was found to decrease progressively with increasing concentration of QDs. Moreover, a slight blue shift (from 341 to 336 nm) of the maximum emission wavelength was observed, confirming the local interaction between albumin and QDs [13].

The inset in Fig. 3 demonstrates the linear dependence of the ration between tryptophan emission intensity with and without the quencher as a function of concentration of the QDs, in good agreement with the classical Stern-Volmer equation [19].

$$\frac{F_0}{F} = 1 + K_{SV}[\mathrm{QDs}]$$

where F_0 and F are the fluorescence intensities of BSA in the absence and presence of the quencher, respectively. K_{SV} is the Stern–Volmer constant and [QDs] is the concentration of the quencher. From the linear regression we calculated the Stern–Volmer constant, yielding $K_{SV} = 8.2 \times 10^6\ \mathrm{M}^{-1}$. Using the dimolecular quenching equation [19]

$$K_{SV} = K_q \tau_0$$

where K_{SV} represents the calculated Stern–Volmer constant, K_q the quenching rate constant and τ_0 (10^{-8} s) is the average lifetime of photoluminiscence without the quencher [14], we calculated the quenching constant, yielding $K_q = 8.2 \times 10^{14}\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. The calculated quenching constant is greater compared with the value obtained for biological macromolecules due to the collision mechanism ($2.0 \times 10^{10}\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$) [20,21] implying that the quenching is not initiated by dynamic collision but by the formation of a complex resulted from static quenching [22].

3.2. Determination of the binding constant between QDs and BSA

For a static quenching process, the binding constant, K_b, and the numbers of binding sites between QDs and BSA, n, were determined from the above fluorescence spectra, using the method described by Tedesco et al. [23]

$$\frac{F_0 - F}{F - F_{sat}} = \left(\frac{[\mathrm{QDs}]}{K_{diss}}\right)^n$$

where F_0 denotes the fluorescence intensity of pure BSA, F_{sat} the fluorescence intensity of BSA saturated with QDs in solution and F the fluorescence intensity of intermediate concentrations between F_0 and F_{sat}, [QDs] denotes the QDs concentration, n the number of binding sites per particle and K_{diss} is the reciprocal value of the binding constant K_b. From the slope and intercept of the best fitted line of the plot of $\log[(F_0 - F)/(F - F_{sat})]$ against $\log([QDs])$, we calculated the number of binding sites to be 2.32, whereas for the binding constant we obtained 1.05×10^7. It is known that for pH values higher than 5, as was in our case, the carboxylic group of MAA is deprotonated, yielding a negative charge shell at the surface of the QDs [24]. Subsequently the bioconjugation of w-QDs with BSA results from the interaction between the negatively charged QDs surfaces with positively charged proteins or protein subdomains.

However, besides electrostatic interactions, hydrophobic interactions or coordination binding can influence the conjugation of proteins with nanoparticles [16,20]. Moreover, the binding constant and the number of binding sites between proteins and nanoparticles are influenced by the size of the protein and nanoparticle [25] as well as the temperature of the environment [26].

3.3. The effects of the temperature on the fluorescence emission of QDs@BSA conjugate

The use of QDs in biological applications necessitates a very good understanding of the effect of the body temperature upon the optical response of the nanoparticles. As such we investigated the effect of the temperature on the fluorescence emission of QDs@BSA bioconjugate. Besides offering us valuable information regarding the effect of the temperature on the emission of QDs, we assess the possibility of using QDs fluorescence emission as sensing platform to study the effect of the temperature on the optical response and conformational properties of BSA adsorbed on the surface of QDs.

In order to investigate the temperature effect on QDs@BSA we monitored the changes in fluorescence emission of both tryptophan residues in albumin and QDs solution by varying the temperature between 25 and 75 °C.

It is well known that intrinsic fluorescence of tryptophan residue is highly sensitive to the microenvironment and, therefore, is widely used to investigate changes in protein structure [27]. Fig. 4 shows the influence of the environment temperature on the emission of tryptophan residues from QDs@BSA conjugates. We found that by increasing the temperature, the intensity of the emission decreases linearly. This behavior can be attributed to decreases of the quantum yield of the complex as a function of temperature [28]. The second remarkable effect was the blue shift of the BSA emission peak position as a function of temperature (the inset in Fig. 4).

For pure BSA solution we observed that at room temperature the emission peak position is at 341 nm, corresponding to native state of protein, while at 75 °C, the peak position blue shifted at 330 nm, consistent with protein unfolding. The denaturation curve of pure BSA exhibits a drop-off at 54 °C, which reflects proteins conformation changes. For the QDs@BSA conjugates we regain the same drop-off in the denaturation curve at 59 °C. This temperature difference implies that the presence of QDs has a minor effect on the thermal behavior of BSA and therefore we employed the fluorescence emission of QDs as probes to monitor the thermal behavior of BSA.

The temperature dependence of the fluorescence emission of bioconjugated QDs is shown in Fig. 5.

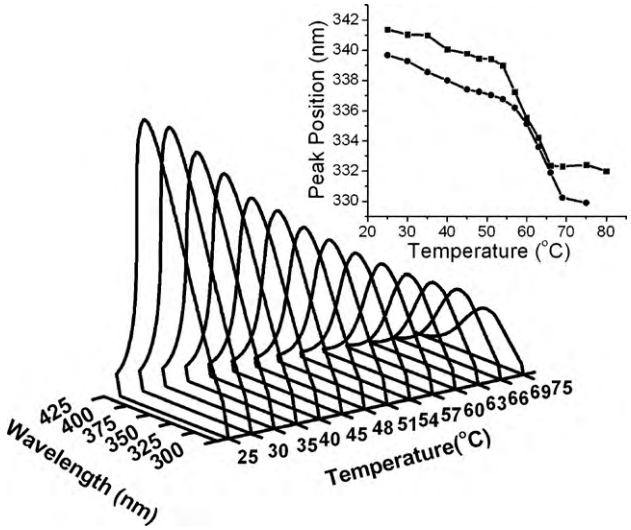

Fig. 4. The fluorescence quenching spectra of tryptophan residues in BSA as a function of temperature. The inset shows the temperature-induced variation of fluorescence peak positions of QDs@BSA (line-circle) and pure BSA (line-square).

The increasing temperature has three effects on the emission spectra of bioconjugated QDs. The first effect is related to a red shift of the emission peak spectral position [29]. Valerini and coworkers demonstrated that the shift towards lower energies of the band gap as the temperature increases is due to the temperature-dependent band-gap shrinkage [30]. According to Dai et al. the decrease of the band gap with increase temperature can be attributed to a mix contribution of thermal expansion and electron-phonon coupling [31]. The second effect is the continuously broadening of the emission band. The total broadening of the emission band contains contribution of both inhomogeneous and homogenous broadening. The inhomogeneous broadening results from fluctuations of the size, shape or composition of the nanocrystals and is temperature independent, whereas the homogenous broadening, which is related to exciton-phonon coupling, is responsible to the temperature-dependent broadening of the exciton peak [32]. The third effect of the temperature on the emission spectra of bioconjugated QDs is the fluorescence intensity modification. The inset in Fig. 5 shows the temperature dependence of the recorded emission intensity of the

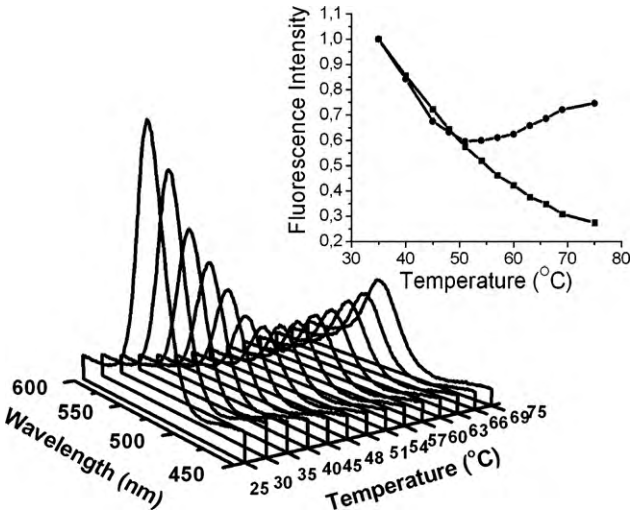

Fig. 5. Temperature dependence of the bioconjugated QDs emission spectra. The inset shows the temperature-induced variation of the fluorescence intensity of QDs (line-square) and bioconjugated QDs (line-circle).

non-conjugated and BSA conjugated QDs. The fluorescence intensity of the non-conjugated QDs exhibits a monotonically decrease, which is caused by the thermal activation of surface traps leading to non-radiative recombination of excitons [33]. In addition, Robelek et al. demonstrated that the continuous recording of the emission of QDs could lead to a gradual loss in fluorescence intensity, as a consequence of the blinking, which might contribute to the observed overall fluorescence intensity decrease [34].

However the temperature dependence of the QDs@BSA emission has a much more complex behavior, exhibiting a minimum value around 51 °C. This value is very close the first transition point of the BSA, at which the BSA exhibits the drop-off in the denaturation curve. On the other hand the intensity of the emission of QDs increases with temperature up to around 69 °C, which is comparable with the second transition point of the BSA. During the denaturation, BSA undergoes an unfolding of its structure, increasing thus the number of binding sites with the QDs, significantly inhibiting the non-radiative recombination of the surface vacancies [18]. This would account for the increase of the intensity of the emission of conjugated QDs, which occurs until the BSA molecule is completely unfolded, leading to a saturation of the number of binding sites, which would explain the plateau observed at temperatures above 69 °C.

4. Conclusions

In this work we monitored the interaction between CdSe/ZnS core-shell quantum dots (QDs) and bovine serum albumin (BSA) protein. We found that the presence of QDs leads to a strong quenching of the intrinsic Tryptophan residues fluorescence emission, which can be explained by a static interaction between the protein and the quencher, demonstrating the formation of QDs@BSA bioconjugates. Moreover, we studied the temperature effects on the structural and spectroscopic properties of both, individual QDs and protein and their bioconjugates (QDs@BSA), by fluorescence spectroscopy. We demonstrated that after bioconjugation, QDs exhibit a more complex temperature-dependent fluorescence emission, which corroborated with the thermal induced structural changes of the protein, suggests the possibility of employing QDs as probes to monitor the thermal denaturation of proteins. This study contributes to a better understanding of the temperature effects on QDs–proteins interfaces, which is a critical issue for applications in site-directed targeted imaging or medical therapy.

Acknowledgements

This work was supported by CNCSIS–UEFISCSU, project number PNII-ID_PCCE_129/2008. M. M. Dzagli gratefully acknowledges the Agence Universitaire Francophone (AUF) for a doctoral fellowship.

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