Study of Protein Conjugation with Different Types of CdTe Quantum Dots

Jana Chomoucka, Jana Drbohlavova, Petra Businova, Jan Prasek, Jan Pekarek, Jaromir Hubalek
Department of Microelectronics
Brno University of Technology
Brno, Czech Republic
chomoucka@feec.vutbr.cz

Jana Chomoucka, Jana Drbohlavova, Marketa Vaculovicova, Petra Businova, Jan Prasek, Jan Pekarek, Jaromir Hubalek
Central European Institute of Technology
Brno University of Technology
Brno, Czech Republic

Abstract— **In this paper, the interaction between bovine serum albumin (BSA) and water soluble CdTe quantum dots (QDs) modified with different ligands (3-mercaptoacetic acid, thioglycolic acid and glutathione) was studied using the fluorescence spectroscopy. It was found that the presence of QDs led to a strong quenching effect of BSA, which could be explained by a covalent interaction between the protein and the quencher, demonstrating the formation of QDs-BSA bioconjugates.**

Keywords-quantum dots; conjugation; protein; bovine serum albumin; glutathione; 3-mercaptoacetic acid; thioglycolic acid.

I. INTRODUCTION

Semiconductor nanocrystals, also known as quantum dots (QDs), are nano-scaled inorganic particles in the size range of 1–10 nm [1]. Due to their quantum confinement, QDs show unique and fascinating optical properties, such as sharp and symmetrical emission spectra, high quantum yield (QY), good chemical and photo-stability and size dependent emission [2]. So far, QDs have been linked with bio-recognition molecules such as proteins, peptides and nucleic acids, and have been successfully used in biological and medical fields such as immunoassay, DNA hybridization, cell imaging and potential photodynamic therapy [3]. In general, reported QD bioconjugation approaches are mainly based on bifunctional linkage (such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydro-chloride, EDC hydro-chloride), electrostatic attraction, and biotin–avidin interaction. However, no matter what conjugation approach is used, QDs bioconjugates need to be purified and characterized. The adsorption of protein molecules on nanoparticles (NPs) surface changes their surface functionality, which influences their behavior in biological systems. Moreover, the formation of NP–protein conjugates provides NPs stability over the broad range of pH and ionic strengths. Smaller NPs favor native-like protein structure more strongly, whereas larger NPs provide larger surface area of contact for adsorbed proteins resulting in stronger interactions between proteins and NPs [4]. The efficiency of this interaction can be a decisive factor for the fate of a NP within the biological system. But at the same time, the interaction between QDs bioconjugation is of great importance in biological applications [5].

BSA has been one of the most extensively studied proteins, particularly because of its structural homology with human serum albumin. It was often used as coating reagent to modify the surface of NPs due to its strong affinity to the variety of NPs, such as gold NPs, silica NPs, and QDs. Serum albumins play an important role in the transport of many exogenous and endogenous ligands, binding covalently and reversibly to these ligands and increasing the tumor selectivity of the ligands by enhanced permeation and retention effect. Up to now, QDs modified by BSA have been applied as ion sensors, fluorescence resonance energy transfer, and chemiluminescence resonance energy transfer. Moreover, due to the increasing extension of nanotechnology in biological sciences, it is imperative to develop a detailed understanding how biological entities, especially proteins, may interact with nanoscale particles [6].

In this paper, the interaction between bovine serum albumin (BSA) and water soluble CdTe quantum dots modified with different ligands (3-mercatoacetic acid, thioglycolic acid and glutathione) was studied using fluorescence spectroscopy. It was found that the presence of QDs led to a strong quenching of fluorescence emission, which could be explained by covalent interaction between the protein and the quencher, demonstrating the formation of QDs-BSA bioconjugates [7].

This paper is structured as follows: Section I describes properties of QDs and their bioconjugation approach. Section II represents the synthesis of water soluble CdTe QDs modified with different ligands (3-mercatoacetic acid, thioglycolic acid and glutathione) and their bioconjugation with BSA. The interaction between BSA and CdTe QDs is discussed in Section III, Section IV concludes the paper.

II. MATERIAL AND METHOD

A. Chemicals

All chemicals were purchased by Sigma Aldrich (Czech Republic) in ACS purity unless otherwise stated. Aqueous solutions were prepared using MilliQ water.

B. Synthesis of CdTe QDs

The procedure for synthesis of glutathione (GSH)-capped CdTe QDs was adapted from the work of Duan et al. [8] with slight modifications. The synthesis of 3-mercaptopropionic acid (MPA)-capped CdTe QDs and thioglycolic acid (TGA)-capped CdTe QDs were adapted from the work of Wang et al. [9]. Sodium telluride was used as Te source. Due to the

 ISBN: 978-1-61208-303-2

fact that sodium telluride is air stable, all of the operations were performed in the air avoiding the need for inert atmosphere. The synthesis of CdTe QDs and their subsequent coating were as follows: 114 mg of the $CdCl_2$.2.5 H_2O was diluted with 25 mL of water. During the constant stirring, 65 µL MPA (56 µl TGA or 150 mg GSH), 25 mg of sodium citrate, 2 mL of Na_2TeO_3 solution (c = 0.01 mol/L), and 10 mg of $NaBH_4$ were added into cadmium(II) aqueous solution. 1 M NaOH was then used to adjust the pH to 10 under vigorous stirring. The mixture was kept at 95 °C under the reflux cooling for 3 hours.

C. Bioconjugation of CdTe QDs

1) Electrostatic attraction

BSA can easily conjugate with CdTe QDs by electrostatic attraction. 250 µL of QDs were added to a mixture of 137.5 µL BSA and 92.5 µL phosphate buffered saline (0.01 M, pH 7.4), and the solution stood at room temperature for 2 h. The reaction solution was stored in the refrigerator at 4 °C [10]. The final concentration of QDs was set to 0, 0.5, 1, 1.5, 2 and 5 mg/mL and final concentration of BSA was calculated to be 15 mg/mL.

2) Covalent conjugation

For the conjugation of BSA with CdTe QDs (final concentration 0; 0.5; 1; 1.5; 2 and 5 mg/mL), 100 µL of 0.05 M EDC and 100 µL of 5 mM N-hydroxysuccinimide (NHS) were added to 500 µL of QDs redispersed in 20 mM phosphate buffer (PB) of pH 7.4 and incubated at 32 ºC for 30 min under slight shaking conditions. 200 µL of BSA in 20 mM PB were added to the reaction mixture and further incubated at 32 ºC for 3 h under slight shaking conditions. The solution was kept at 4 ºC overnight to deactivate the remaining EDC-NHS. The unbound protein was removed by centrifugation at 10,000 rpm for 20 min [11]. The final concentration of QDs was set to 0, 0.5, 1, 1.5, 2 and 5 mg/mL and the final concentration of BSA was calculated to be 15 mg/mL.

D. Characterization of CdTe QDs

Photoluminescence spectra were measured at room temperature with Fluorolog, HORIBA Jobin Yvon and quantum yield was calculated with Quanta φ, HORIBA Jobin Yvon.

III. Results and Discussion

A. CdTe QDs characterization

To investigate the behavior of QDs in the presence of BSA, water soluble CdTe QDs were synthesized. We selected three kinds of mercaptan ligands such as MPA, TGA, GSH for the preparation of QDs. The emission spectra of typical CdTe QDs used in this study were measured at excitation wavelength of 380 nm. The emission spectrum is displayed by one emission peak at 620 nm in the case of MPA-capped CdTe QDs, one peak at 506 nm in the case of GSH-capped CdTe QDs and one peak at 540 nm in the case TGA-capped CdTe QDs. All peaks in characterized spectra showed a good symmetry and a narrow spectral width (see Figure 1). The quantum yield of CdTe QDs was evaluated to be 11.5 % in the case of CdTe-MPA, 16.5 % in the case of CdTe-GSH and 7% for CdTe-TGA.

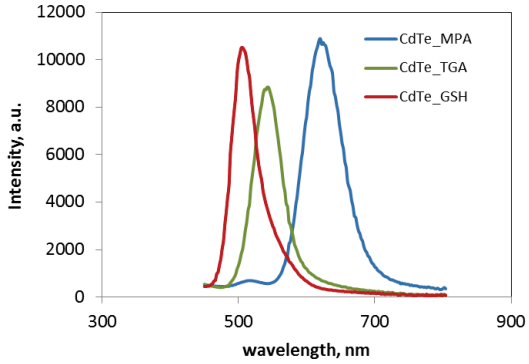

Figure 1. Fluorescence spectra of CdTe QDs capped with MPA, GSH and TGA.

B. The effect of QDs on BSA fluorescence spectra

Fluorescence (FL) quenching efficiency and the aspect of quenching mechanism of the BSA by QDs were studied by FL spectroscopy. CdTe QDs were prepared in aqueous phase using MPA, GSH or TGA as a stabilizer, resulting in the linkage of the thiol groups to the surface of CdTe QDs by SH-Cd coordination, while the functional carboxylic group is free, which can be easily coupled to biomolecules with amino groups, such as proteins, peptides or amino acids. BSA absorption spectrum shows absorption peak in UV region at 280 nm, and FL peak at 328 nm. It was found that the emission of CdTe QDs decreases progressively with increasing concentration of BSA.

1) Electrostatic attraction

The fluorescence intensity of BSA was quenched accompanied by a slight blue shift of the maximum emission wavelength with increasing concentration of CdTe QDs as can be seen in Figure 2 – Figure 4. These figures represent the emission spectra of MPA-QDs conjugation (GSH-QDs, TGA-QDs, respectively) with BSA via electrostatic interaction. The blue shift here indicated that tryptophan residue (BSA component) was in more hydrophobic environment due to the tertiary structural change of albumin. The intrinsic reason for this change might lie in the more flexible conformation of albumin adsorbed on the NPs surface, which favored the access of tryptophan residues to the bulk surface of QDs [12]. The FL quenching is known to occur due to excited state reactions, energy transfer, collisional quenching (dynamic quenching) and complex formation (static quenching). The last two processes are mainly considered. Both dynamic quenching and static quenching reveal the connection of linearity between relative FL intensity (F_0/F) and QDs concentration [13]. The quenching of BSA FL by QDs can be described by Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are FL intensity of BSA in the absence and presence of QDs, respectively, [Q] is QDs concentration and K_{SV} is the Stern-Volmer quenching constant. The F_0/F ratios were calculated and plotted against quencher concentration according to (1). After linear fit, K_{SV} were calculated from the slope of the plots [14]. The results show that the quenching constant K_{SV} is variant with different type of QDs and the higher K_{SV} is, the higher is the quenching effect [15]. The FL intensity decreased more significantly in the case of BSA-QDs than in the case of MPA-QDs or TGA-QDs. The ratios of MPA-QDs bonded with BSA exhibited a quite good linearity with determination coefficient R^2 of 0.9782 (see Figure 5). The Stern-Volmer plot of GSH-QDs quenching properties exhibited linear trend with coefficient of determination R^2 to be 0.9953 and in the case of TGA-QDs the coefficient of determination R^2 was 0.8717. The results suggest that QDs can effectively quench the FL of BSA in a ligand dependent manner.

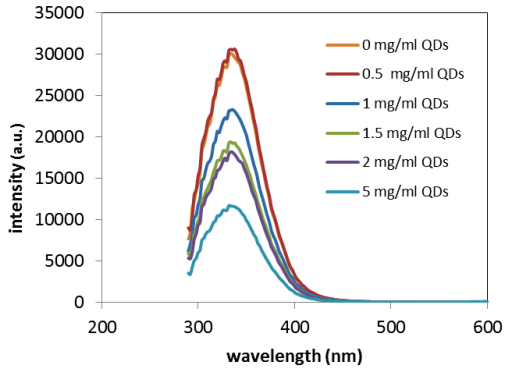

Figure 2. Emission spectra of BSA capped with MPA-CdTe QDs via electrostatic interaction at various QDs concentration.

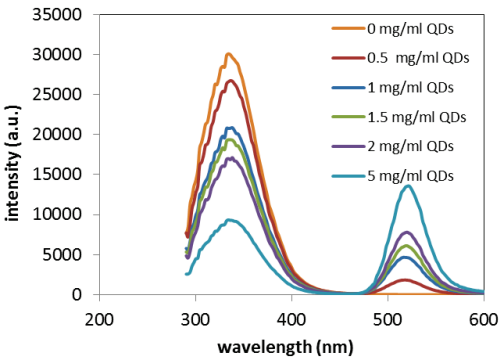

Figure 3. Emission spectra of BSA capped with GSH-CdTe QDs via electrostatic interaction at various QDs concentration.

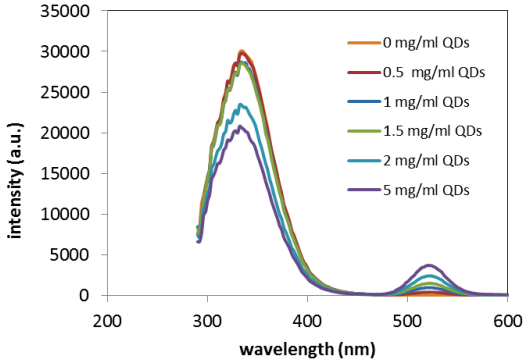

Figure 4. Emission spectra of BSA capped with TGA-CdTe QDs via electrostatic interaction at various QDs concentration.

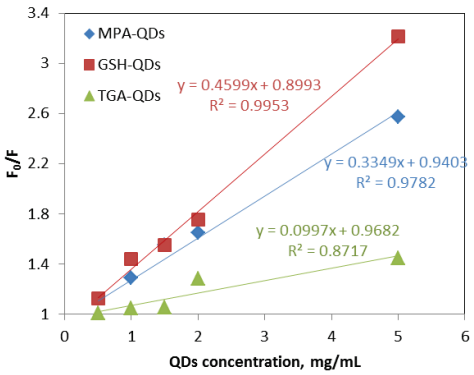

Figure 5. Stern-Volmer plot of BSA FL quenching effect caused by CdTe QDs electrostatic conjugation with BSA.

These results indicated that QDs can effectively quench the FL of BSA in a ligand-dependent manner. Structurally, this is due to the presence of NH_2 and COOH groups in the QDs capping agent, namely MPA (1 × COOH group); GSH (3 × NH_2 and 2 × COOH groups) and TGA (1 × COOH group). Therefore hydrogen bonds can be easily formed between GSH-QDs and BSA. In other words, the number of amino-groups can strongly influence the interactions between BSA and QDs capped with GSH. Therefore, the order of interactions between BSA and QDs is as follows: TGA-QDs < MPA-QDs < GSH-QDs.

2) Covalent conjugation

In the case of QDs covalently bonded with BSA, EDC and NHS were used as coupling agents. Figure 6 – Figure 8 represent the emission spectra of BSA with various concentrations of MPA-QDs, GSH-QDs and TGA-QDs. The FL intensity was quenched by the addition of various types of QDs (the most significantly in the case of GSH-QDs) with BSA concentration of 15 mg/mL.

 ISBN: 978-1-61208-303-2

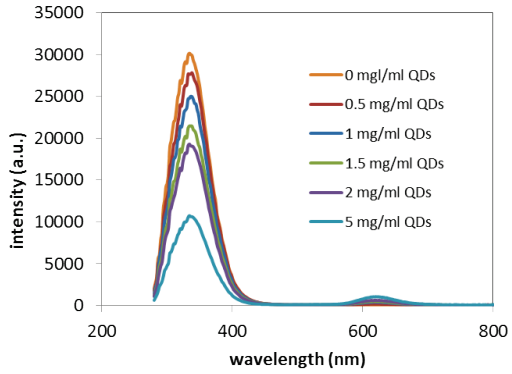

Figure 6. Emission spectra of BSA capped MPA-CdTe QDs via covalent interaction at various QDs concentration.

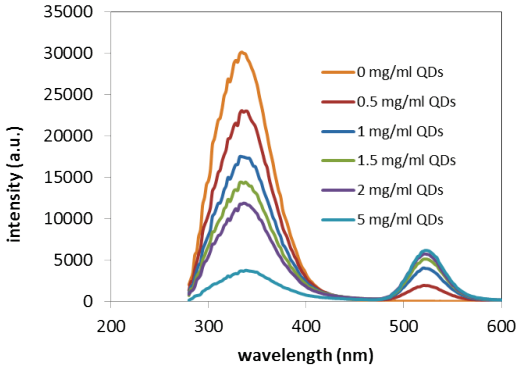

Figure 7. Emission spectra of BSA capped with GSH-CdTe QDs via covalent interaction at various QDs concentration.

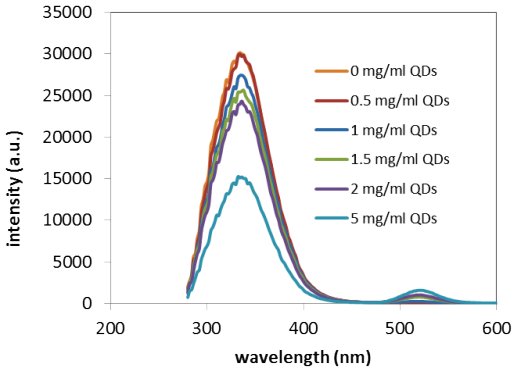

Figure 8. Emission spectra of BSA capped with TGA-CdTe QDs via covalent interaction at various QDs concentration.

The results suggest the interaction between BSA and QDs occurs and the quenching effect of QDs on the FL emission of BSA is found to be concentration dependent, thus QDs can bind to the BSA. In the case of MPA-QDs bonded with BSA, results exhibited a very good linearity with coefficient of determination R^2 of 0.9998 (see Figure 9). The Stern-Volmer plot of GSH-QDs quenching properties exhibited linear trend with coefficient of determination R^2 to be 0.9965 and in the case of TGA-QDs the coefficient of determination R^2 was 0.9904. This behavior suggests that only statistic quenching is taking place. The highest K constant was observed in the case of GSH-QDs, which is due to the presence of higher number of NH_2 groups in GSH (3 groups) compared to MPA and TGA. The K_{SV} constants of BSA covalently bonded to QDs are much higher compared to the electrostatic interactions between QDs and BSA.

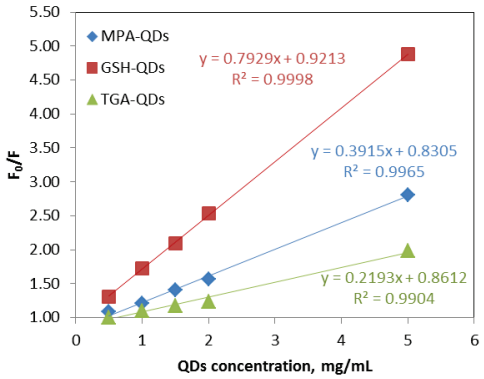

Figure 9. Stern-Volmer plot of BSA FL quenching effect caused by CdTe QDs covalently conjugated with BSA.

IV. CONCLUSION

Water soluble CdTe QDs modified with different ligands (MPA, TGA and GSH) were prepared by a simple one step method using Na_2TeO_3 and $CdCl_2$. The emission spectra display that the emission peak lies at 620 nm in the case of MPA-capped CdTe QDs, at 506 nm in the case of GSH-capped CdTe QDs and at 540 nm in the case of TGA-capped CdTe QDs. In the next stage, QDs were covalently and electrostatically conjugated to BSA. It was found that the presence of QDs led to a strong quenching of the FL emission, which could be explained by a covalent interaction between the protein and the quencher, demonstrating the formation of QDs-BSA bioconjugates.

ACKNOWLEDGMENT

This work has been supported by Grant Agency of the Czech Republic under the contract GACR 102/13-20303P, the operational program Research and Development for Innovation, by the project "CEITEC - Central European Institute of Technology" CZ.1.05/1.1.00/02.0068 from European Regional Development Fund and by the project NANOE CZ.1.07/2.3.00/20.0027 from European Social Fund.

 ISBN: 978-1-61208-303-2

REFERENCES

[1] J. Chomoucka, J. Drbohlavova, V. Adam, R. Kizek, and J. Hubalek, "Synthesis of Glutathione-coated Quantum Dots", 32nd International Spring Seminar on Electronics Technology, IEEE, 2009, pp 653-657.

[2] G. P. C. Drummen, "Quantum Dots-From Synthesis to Applications in Biomedicine and Life Sciences", Int. J. Mol. Sci., vol. 11 (1), Jan. 2010, pp. 154-163.

[3] M. Ryvolova, et al., "Glutathione modified CdTe quantum dots as a label for studying DNA interactions with platinum based cytostatics", Electrophoresis, vol. 34 (6), Mar. 2013, pp. 801-808.

[4] S. Singh, et al., "Conjugation of nano and quantum materials with bovine serum albumin (BSA) to study their biological potential", Journal of Luminescence, vol. 141, 2013, pp. 53-59.

[5] M. Ryvolova, et al., "Biotin-modified glutathione as a functionalized coating for bioconjugation of CdTe-based quantum dots", Electrophoresis, vol. 32 (13), Jun. 2011, pp. 1619-1622.

[6] J. Liang, Y. Cheng, and H. Han, "Study on the interaction between bovine serum albumin and CdTe quantum dots with spectroscopic techniques", Journal of Molecular Structure, vol. 892 (1–3), 2008, pp. 116-120.

[7] J. Drbohlavova, et al., "Effect of Nucleic Acid and Albumin on Luminescence Properties of Deposited TiO2 Quantum Dots", Int. J. Electrochem. Sci., vol. 7 (2), Feb. 2012, pp. 1424-1432.

[8] J. L. Duan, L. X. Song, and J. H. Zhan, "One-Pot Synthesis of Highly Luminescent CdTe Quantum Dots by Microwave Irradiation Reduction and Their Hg2+-Sensitive Properties", Nano Res., vol. 2 (1), Jan. 2009, pp. 61-68.

[9] J. Wang, and H. Han, "Hydrothermal synthesis of high-quality type-II CdTe/CdSe quantum dots with near-infrared fluorescence", Journal of Colloid and Interface Science, vol. 351 (1), 2010, pp. 83-87.

[10] X. Huang, et al., "Characterization of quantum dot bioconjugates by capillary electrophoresis with laser-induced fluorescent detection", Journal of Chromatography A, vol. 1113 (1–2), 2006, pp. 251-254.

[11] A. Chopra, et al., "CdTe nanobioprobe based optoelectrochemical immunodetection of diabetic marker HbA1c", Biosensors and Bioelectronics, vol. 44, 2013, pp. 132-135.

[12] P. Liu, Y. S. Liu, and Q. S. Wang, "Studies on the interaction of CdTe QDs with bovine serum albumin", J. Chem. Technol. Biotechnol., vol. 87 (12), Dec. 2012, pp. 1670-1675.

[13] Q. Wang, et al., "Interaction of different thiol-capped CdTe quantum dots with bovine serum albumin", Journal of Luminescence, vol. 132 (7), 2012, pp. 1695-1700.

[14] L. Ding, et al., "Spectroscopic Studies on the Thermodynamics of L-Cysteine Capped CdSe/CdS Quantum Dots-BSA Interactions", J. Fluoresc., vol. 21 (1), Jan. 2011, pp. 17-24.

[15] A. Baride, D. Engebretson, M. T. Berry, and P. Stanley May, "Quenching of coumarin emission by CdSe and CdSe/ZnS quantum dots: Implications for fluorescence reporting", Journal of Luminescence, vol. 141, 2013, pp. 99-105.

 ISBN: 978-1-61208-303-2