Effects of CdTe quantum dots on protein structural changes

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ABSTRACT

Quantum dots (QDs) have been widely used in imaging, labeling and tracking because of their excellent optical properties. However, researchers found QDs` toxicity is the potential risk to human health and environment, which leads to their application limitation. According to discussing structural changes in human serum albumin and lysozme bound to CdTe quantum dots, the CD and three-dimensional fluorescence spectroscopy results indicated that the secondary structure has been significantly impacted and proteins reduce their stability with a more flexible folded structure. From fluorescence quenching studies, the quenching mechanism between CdTe and proteins is consistent with static quenching mechanism. These results give rise to the understanding and evaluation about the safety of QDs application in nanobiotechnology and nanomedicine.

Keywords: nanomaterials, quantum dots; proteins; safety; structural change

1 INTRODUCTION

The nano-scale material, quantum dots (QDs), have been widely used in biological labels [1], imaging of fixed cells and tissues [2] due to their excellent properties, such as small size, large surface area, good photostability, and negligible photobleaching in comparison with dyes. However, the toxicity [3] impedes QDs applying in biology, especially with respect to the potential risk to human health. Thus, the problem regarding nanotoxicity to human health is getting a concerned issue [4].

Proteins are crucial components in our body and play fundamental roles in sustaining life and closely realted to the origin, evolution and metabolism of life. The function and optical property of the organism will be changed if the structure perturbs. We have chosen human serum albumin (HSA) and Lysozyme (LYS) which have two different structural classes and molecular sizes.

In this study, the interaction between QDs and proteins were investigated under physiological conditions by circular dichroism (CD) and three-dimensional fluorescence spectroscopy. According to this work, the adverse effect on human health even at very low concentration levels was discussed, which one can get a better insight into the safety of QDs on biological implication.

2 EXPERIMENTAL

2.1 Materials

HSA and LYS (purity >90%) were purchased from Sigma and used without further purification. The HSA and LYS solutions were prepared by dissolving in Tris-NaCl solutions (0.05 mol·L^{-1} Tris, 0.15 mol·L^{-1}, NaCl, pH =7.4), with a final concentration of 1.0×10^{-6} mol·L^{-1}. The solution was stored under 4℃ without lighting. The CdTe QDs were synthesized by using a modified method according to previous reports [5]. All the chemicals were of analytical grade.

2.2 Spectral measurements

The fluorescence spectra data and three-dimensional fluorescence spectra were collected from fluorescence spectrofluorometer (Hitachi F-7000). And the circular dichroism (CD) data were recorded with JASCO-J810 (Application of optical physics company，England).

3. RESULTS AND DISCUSSION

3.1 Characterizations of CdTe (QDs)

The XRD pattern of CdTe was obtained from CdTe powders, which were precipitated from aqueous solution and isolated by centrifugation and dried at vacuum. As shown in Figure 1, the three diffraction peaks of CdTe could be indexed to the (111), (220), and (311) planes of cubic CdTe lattice, which corresponds to the plane of cubic CdTe (JCPDS NO. 15-0770).

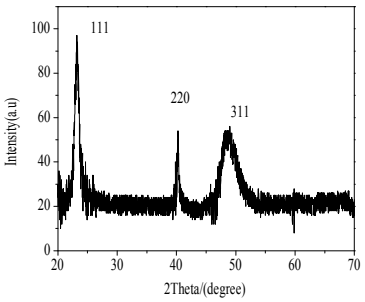

Figure 1: XRD patterns of the CdTe

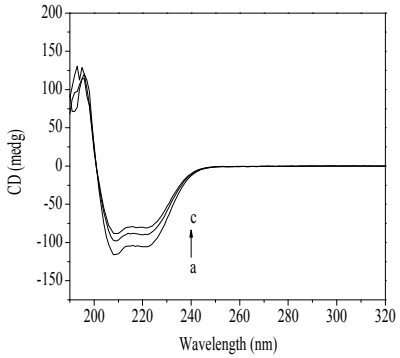

Figure 2: The CD spectra of black HSA (a) and HSA-CdTe system with different concentrations of CdTe at the room temperature, HSA-QDs: (b) 50:1, (c) 100:1.

3.2 Conformational investigations

Structure changes of HSA and HSA-CdTe system

In order to discuss whether the CdTe is security or not for biology, the CD spectra and three-dimensional fluorescence spectroscopy were utilized. As shown in Figure 2, with the addition of various amounts of CdTe QDs, the intensity of peaks at 208 and 222 nm were decreased, which clearly indicated the changes in secondary structure of HSA as evident from the decrease in α-helical content of protein. The decrease in α-helical content indicates the protein reduced its stability with a more flexible folded structure and CdTe bound with amino acid residues of the main polypeptide chain of protein and destroyed their hydrogen bonding networks [6] to make more HSA exposure to the hydrophobic cavities. Thus, a loss of α-helical content meant that the function of HSA would be changed.

Three-dimensional fluorescence spectroscopy has the advantage of making the investigation of the characteristic conformational change of protein more scientific and credible. Two typical fluorescence peaks could be easily found in Figure 3. Peak a ($\lambda_{ex}=\lambda_{em}$=280 nm) is the Rayleigh scattering peak and peak b (λ_{ex}=280 nm, λ_{em}=335 nm) mainly shows the spectral behavior of tryptophan (Trp) and tyrosine (Tyr) residues, because HSA was excited at 280 nm, it chiefly reveals the intrinsic fluorescence of Tyr and Trp residues, and the phenylalanine (Phe) residue fluorescence can be negligible [7]. It is clear that the intensity of peak a reveals dramatically enhancement with the addition of CdTe. The reasonable explanation is that a HSA-CdTe complex arouse after adding of CdTe and made the diameter to the macromolecule increased, which in return lead to the scattering effect enhanced. However, the peak b intensity has been strongly quenched by CdTe.

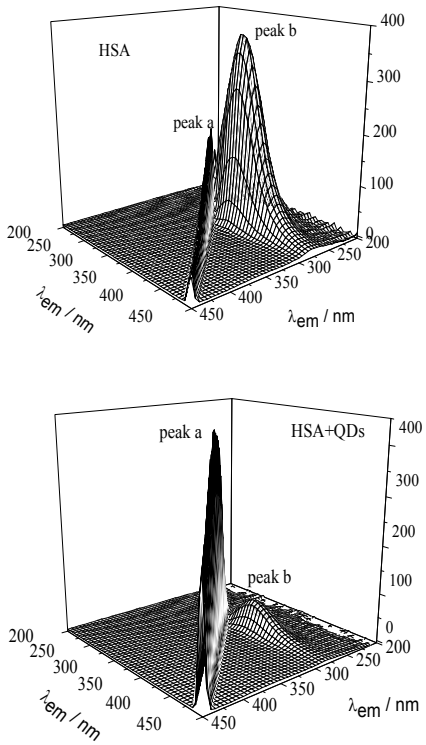

Figure 3: Three-dimensional fluorescence spectroscopy of free HSA and HSA-CdTe system at the room temperature

Structure changes of LYS and LYS-CdTe system

In all progress, LYS was at the high isoelectric point of around 9.5 and pH=7.0 [6]. LYS is positively charged protein thus has potential to interact with thioglycolic acid (TGA)–capped CdTe, which are negatively charged particles. Figure 4 revealed that the intensity of the CD spectrum decreases significantly compared with the free LYS, while retaining the original shape. This indicated that the protein interaction with QDs and led to unexpected biological function and toxicity [7]. From the fluorescence spectroscopy (Figure 5), the Rayleigh scattering peak (peak

a) exhibited dramatically the increase with almost 2-fold after adding CdTe, while the spectral characteristic of Trp and Tyr residues (peak b) is quenched by 90%. The fluorescence changes in combination with the CD spectral results suggested that the interaction between LYS and CdTe led to the loosening and unfolding of the LYS backbone and decreased the hydrophobicity of the microenvironment of LYS [8]. All of these phenomena revealed that the binding CdTe to proteins induced conformational and hydrophobic regions changes.

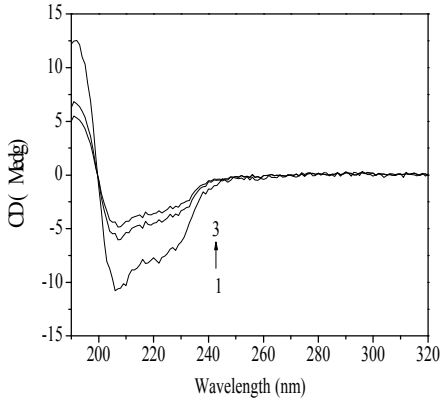

Figure 4: The CD spectra of LYS (1) and LYS-CdTe system with different concentrations of CdTe at room temperature, HSA-QDs: (b) 50:1; (c) 100:1.

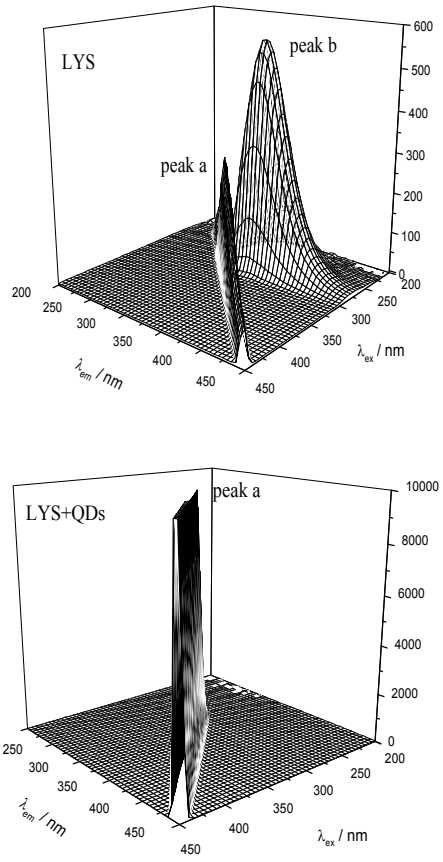

Figure 5: Three-dimensional fluorescence spectroscopy of free LYS and LYS-CdTe system at the room temperature

3.3 Fluorescence quenching studies of proteins upon adding CdTe

Fluorescence quenching can also be identified as either static quenching or dynamic quenching by their differences on temperature [9]. Figure 6 showed the fluorescence property of LYS (A) and HSA (B) with the different concentrations of CdTe. It is clear that the intensity of the proteins at around 340 nm was gradually decreased after adding CdTe, indicating that CdTe could interact with proteins and quench their intrinsic fluorescence. In Figure 6 (B-H), the CdTe fluorescence intensity could be neglected. We research the fluorescence quenching by the well-known Stern-Volmer equation [10]. Figure 7 and Figure 8 revealed the Stern-Volmer plots of F_0/F versus [Q] at the three different temperatures. The parameters were shown in Table 1. Obviously, the quenching constant is gradually decreased with the increase of the temperature, which is consistent with static quenching mechanism.

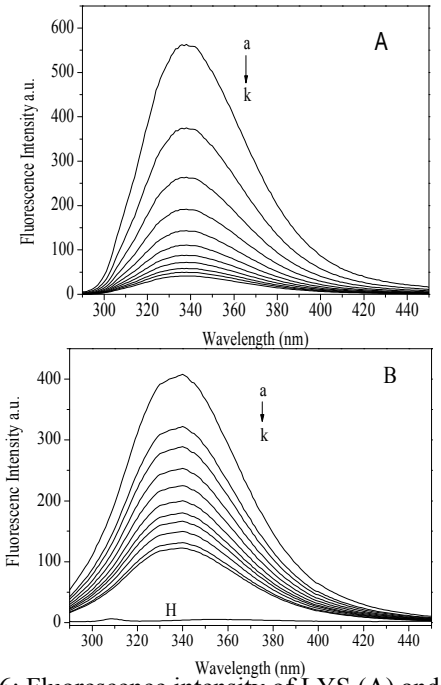

Figure 6: Fluorescence intensity of LYS (A) and HSA (B) with the different concentrations $C_{LYS} = C_{HSA} = 1.0\times10^{-6}$ mol·L^{-1}; $C_{QDs} = (10^{-6}$ mol·L$^{-1})$, a–k: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. at 298 K.

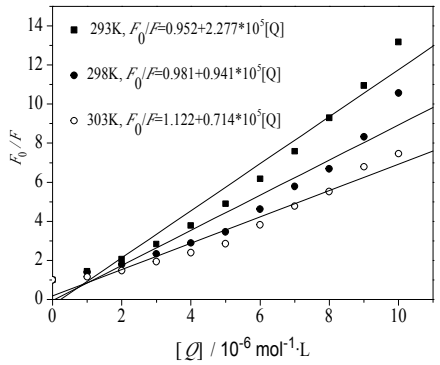

Figure 7: Stern-Volmer plots for the CdTe-LYS system at three different temperatures at pH =7.4

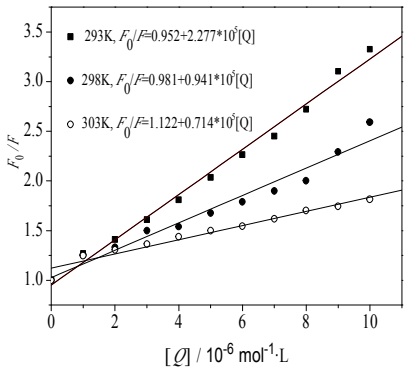

Figure 8: Stern-Volmer plots for the CdTe-HSA system at three different temperatures at pH=7.4

Table 1 Characteristic parameters of the fluorescence spectra

	T(K)	$K_{SV}\times10^5$ (L·mol^{-1})	R
HSA	293	2.277	0.995
	298	1.379	0.982
	303	0.713	0.981
LYS	293	12.035	0.980
	298	8.988	0.965
	303	6.731	0.978

4 Conclusion

When the QDs interact with proteins, the protein structure was changed and exposed new epitopes on the protein surface, or perturbed the normal protein function. The influence on LYS was stronger than HSA mainly due to the electrostatic force of positive and negative charges. The fluorescence intensity of the protein was greatly quenched by CdTe and quenching mechanism was consistent with static mechanism.

5 Aknowledge

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