CHIRAL SEMICONDUCTOR NANOCRYSTALS: PHOTOLUMINESCENCE PROPERTIES WITHIN LIVING CELLS

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Abstract

Semiconductor nanocrystals, in particular, quantum dots (QDs) have been widely investigated as a new type of therapy and diagnostic agents in many fields of biomedical applications, including live cell imaging. It has therefore become critical for full understanding the interactions between QDs and living cells for long-term, multiplexed, and quantitative photoluminescence detection. Chirality is an important phenomenon of living systems and nearly all biological polymers are homochiral. In this work the chiral semiconductor CdSe/ZnS quantum dots have been prepared with the D- and L- enantiomeric forms of cysteine used as the stabilizers. D- and L-cysteine stabilized quantum dots produced corresponding mirror image circular dichroism spectra. We have used confocal photoluminescence and time-resolved photoluminescence microscopy to perform *in-vitro* study of photoluminescence properties of D- and L-cysteine stabilized quantum dots within living Ehrlich Ascite carcinoma cells. We have demonstrated that intracellular photoluminescence intensities of enantiomeric L- form of quantum dots were significantly higher than that of D- form. QD photoluminescence decay times were recorded within living cells that open a possibility to understand photophysics of intracellular behavior of different enantiomeric forms of chiral quantum dots.

Keywords:

chirality, quantum dots, cancer cells, photoluminescence

1. INTRODUCTION

Chiral molecules are the type of molecules that have no rotoreflection axis and have non-superposable mirror image. Such substances are optically active and reveal circular dichroism (CD)[1]. Chirality is an important property in both chemical and biological aspects. For example, all amino acids in the human body are levorotatory and all carbohydrates that make up RNA and DNA are dextrorotatory. Many properties such as the ability to penetrate cells, enzymatic activity and toxicity are strongly dependent on chirality of substances.

The concept of chirality is widely addressed in organic chemistry and catalysis as well as in studies of inorganic complexes, but it is less familiar in the field of inorganic colloidal nanocrystals. Recent studies demonstrated for the first time the induction of chiroptical activity in semiconductor quantum dots (QDs)[2-5]. QDs are able to obtain chirality as the result of attaching the chiral molecules to the QD surface[2]. Chiral QDs can be obtained by different methods, including adding chiral molecules to the synthesis medium as stabilizers[3, 4] or post-synthesis phase transfer using chiral molecules as capping ligands[5].

QDs possess unique optical properties such as wide absorption region, high photostability, high photoluminescence (PL) quantum yield, and the ability to control the photoluminescence wavelength by changing nanocrystall size [6, 7]. Owing to these properties, QDs can be widely used in biological studies as fluorescent probes [8]. Another promising field of QD application is photodynamic therapy, a traditional method of cancer cure. QDs can be used as energy donors in complexes with photosensitizers [9, 10]. The chirality of QDs can possibly enhance their efficiency when used in these fields.

Though QD chirality is being quite actively studied, the impact on living organisms of the chirality of QDs has been largely neglected. In[11] the cytotoxicity and autophagy of CdTe QDs, covered with D- and L-glutathione (GSH) molecules were investigated. In that study D- and L-GSH QDs did not produce CD spectra in the spectral region of QD intrinsic absorption therefore D- and L-GSH QDs cores were achiral. It was shown that cytotoxicity and autophagy of L-GSH QDs was higher than those of D-GSH QDs and that the efficiency of the QD cell uptake did not depend on chirality of the QD ligands.

PL properties of QDs are extremely sensitive to the QD microenvironment and conformational changes of QD surface ligands that occurring during interactions between QDs and living cells. Therefore it is a spectral-photoluminescence study which open a possibility to fully understand photophysics of intracellular behavior of different enantiomeric forms of chiral quantum dots.

In present research chiral semiconductor CdSe/ZnS QDs were obtained by the method of post-synthesis ligand exchange using D- and L-cysteine (Cys) as chiral ligands. Obtained D- and L-Cys QDs demonstrated antiphasic CD signal in the region of the QD intrinsic absorption and practically the same PL quantum yields. The *in-vitro* study of photoluminescence properties of different enantiomeric QD forms in living cells of Ehrlich's ascite carcinoma was done using confocal luminescent microspectroscopy and time-resolved photoluminescence microscopy. The data of intracellular QD photoluminescence intensity and QD photoluminescence decay times were obtained.

2. RESULTS AND DISSCUTION

2.1 Experimental

Chiroptical water-soluble QDs were prepared using post-synthesis ligand exchange method employing L- or D-cysteine as chiral ligands[5, 12]. For this solution of L- or D-cysteine hydrochloride in methanol (2 mol/L) was added to the chloroform solution of CdSe/ZnS/TOPO QDs with core size 2.5 nm (0.05 mmol/L) synthetized according to[13]. The volume of the addition did not exceed 10% of the initial volume of QD solution. The reaction mixture was shaken for 1 minute and then the water was added. The pH of the aqueous phase was adjusted to 10 by adding NaOH solution. The cysteine-capped CdSe/ZnS QDs transferred to the upper aqueous phase (CdSe/ZnS/TOPO QDs were insoluble in water). Finally cysteine-capped CdSe/ZnS QDs were purified 3 times using 10 kDa Amicon Ultra centrifugal filter units.

CD spectra of the L- or D-cys QDs were collected using Jasco J-815 spectropolarimeter. The UV-Vis absorbance and photoluminescence spectra were recorded using UV-Probe 3600 spectrophotometer (Shimadzu) and Cary Eclipse spectrofluorometer (Varian) respectively. Time-resolved PL spectroscopy was performed using a time-correlated single photon counting (TCSPC) spectrometer (PicoQuant, Inc.). A pulse laser (405 nm) with an average power of 1 mW operating at 40 MHz with pulse duration of 70 ps was used for PL excitation of the samples. The passband filter (480-560 nm) was used to collect QD PL signal.

The Ehrlich ascites carcinoma (EAC) cell culture was obtained from the Petrov Research Institute of Oncology from white laboratory mice with an average weight of 20 g on 8-9 days of ascite growth. The mice were killed by cervical dislocation. Cell suspension was purified from the erythrocytes by 5 min treatment with a lysis buffer (0.01 mol tris HCl, 0.87% NH_4Cl, pH=7.4). Then cells were washed 2 times with Hank's Balanced Salt Solution (HBSS) and resuspended in RPMI-1640 medium with 10% of bovine embryonic serum and 100 U/ml penicillin. The viability of cells was estimated by trypan blue assay.

To study influence of the chirality of QDs on their photoluminescence properties within living cells incubation of EAC cells with QDs was carried out in 6-cells plates («ThermoFisher», USA) in RPMI-1640 medium at 37°C, 5% CO_2, 90% relative humidity for 24 h. QD concentration was 1 μmol/L. Cell density was $1.2x10^3$ 1/mm^2. The viability of cells was comprised more then 95% during the whole experiment. After

incubation cells were washed and resuspended in HBSS. Then confocal images of living cells were recorded under a LSM 710 (Zeiss) laser scanning confocal microscope with 405 nm laser excitation. The emission was collected from spectral region corresponding to the QD emission band (450-650 nm). Then an average PL intensity per one cell was qualitatively estimated using LSM software.

2.2 Photoluminescence properties of chiral QDs within living cells

UV-Vis absorption and PL spectra of D- and L- Cys QDs and TOPO- quantum dots are presented in Fig. 1. L- and D- Cys CdSe QDs displayed narrow absorption maximum at 512 nm similar to TOPO-QDs that corresponds to the exitonic transition. The TOPO to cysteine ligand exchange thus had little effect on UV-Vis absorption characteristics of the exitonic peak.

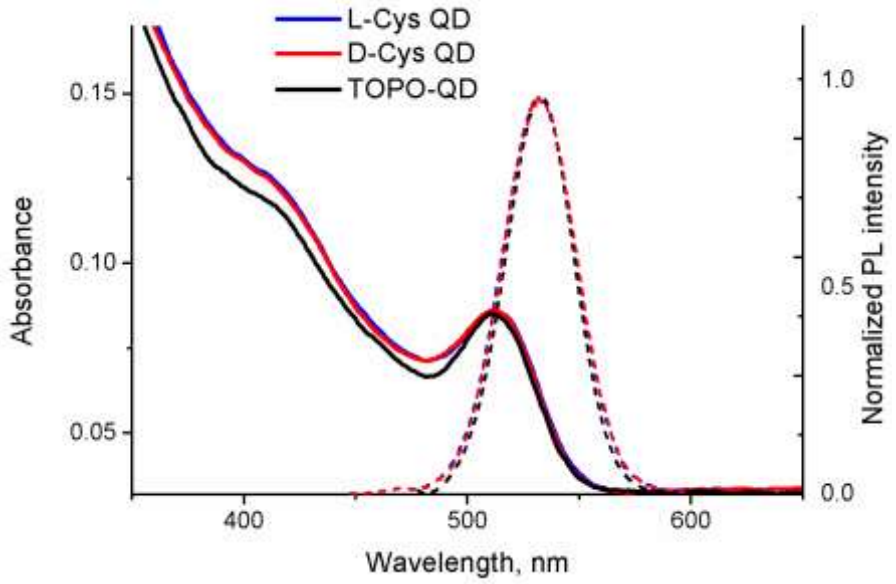

Fig. 1. The UV-vis absorption (solid lines) and normalized photoluminescence (dashed lines) spectra of CdSe/ZnS QDs solubilized with D- and L- Cys (red and blue dotted lines), as well as CdSe/ZnS-TOPO QDs (black curve). The concentration of QDs was C_{QD} = 0.2 μmol/L.

D- and L-Cys QDs demonstrated intensive exitonic photoluminescence, as it can be seen from Fig. 1 (dashed curves). No signs of D- and L-Cys QDs aggregation such as broadening of the PL band were discovered. The PL quantum yield and PL decay times of the D- and L-Cys QDs in an aqueous solution as well as of TOPO-QDs in chloroform, are given in Table 1. The values of PL quantum yield and PL decay times of TOPO-QDs were significantly higher than that of D- and L-Cys QDs indicating that the process of ligand exchange was accompanied with QD PL quenching. This quenching is commonly explained by the formation of the trap states on the QD surface by thiol ligands [14].

D- and L-Cys QDs revealed almost identical PL quantum yields and PL decay times testifying that amounts of cysteine ligands on QD surface are possibly the same.

Table 1. Photoluminescense properties of QDs in solutions and within cells

	TOPO-QD	L-Cys QD	D-Cys QD
PL quantum yield φ, %	11.6	4.9	4.8
PL decay time in solution τ_{sol}, ns	15.3	8.5	8.5
PL decay time in EAC cells τ_{cells}, ns		5.8	5.9
Mean PL intensity within cells, *I*		340	190

Circular dichroism spectra of D- and L-Cys QDs, demonstrated in Fig. 2, show that QD produced an mirror image circular dichroism spectra in the spectral region of QD intrinsic absorption (350-550 nm). The most intense CD signal was observed in the spectral region corresponding to the position of the QD exiton absorption. The data obtained approved preparation of D- and L-forms of chiral quantum dots with substantially the same PL quantum yields from achiral QDs by post-synthetic ligand exchange.

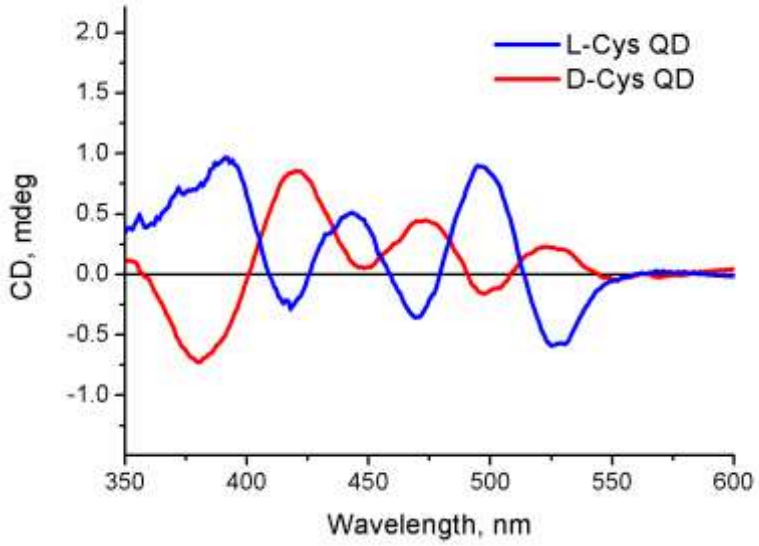

Fig. 2. Smoothed circular dichroism spectra of QDs solubilized D- and L-Cys. The concentration of QDs was C_{QD} = 10 μmol/L.

In-vitro study of D- and L-Cys QDs intracellular photoluminescence intensities done with a confocal luminescent microscope suggested that QDs were localized inside cellular cytoplasm during the incubation process. Confocal luminescent images of cells incubated with D- and L-Cys QD are shown in Fig. 3. Quantitative values of QD PL intensities (average QD PL intensity per cell) listed in Table 1 demonstrated that PL intensity of L- Cys QDs was higher than that of D- Cys QDs. Differences in intracellular PL intensities of D- and L- Cys QDs could be caused by a different QD cell uptake efficiency and/or differences in intracellular PL quantum yields of D- and L- Cys QDs, i.e. D- Cys QDs could be quenched by the intracellular medium stronger than L- Cys QDs.

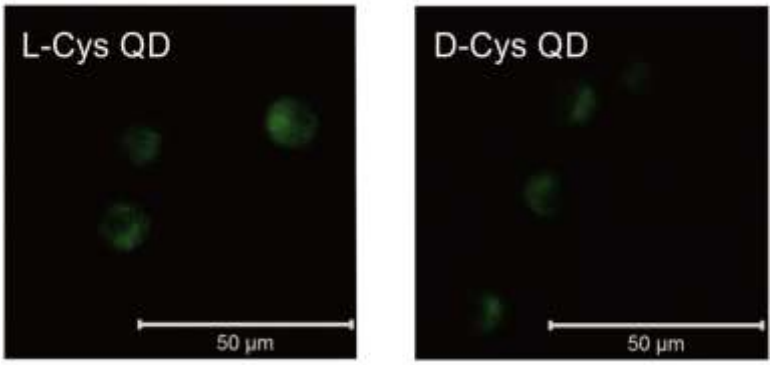

Fig. 3. Confocal luminecsence microscopy images of EAC cells incubated with L-Cys QDs (left) and D-Cys QDs (right). The concentration of QDs was C_{QD} = 1 µmol/L.

Accurate estimation of intracellular photoluminescence quantum yield is a difficult experiment challenge. Since quantum yield depends on photoluminescence decay time, we have registered the intracellular photoluminescence decay times of D- and L-Cys QDs (see Table 1). As follows from Table 1, D- and L-Cys QDs had very close photoluminescence decay times. It could be concluded that intracellular photoluminescence quantum yields of D- and L-Cys QDs were close to each other. Consequently higher values of intracellular photoluminescence intensity of L-Cys QDs could be explained by higher cellular uptake of L- Cys QDs in comparison with that of D-Cys QDs.

3. CONCLUSIONS

Chiral semiconductor quantum dots with optical activity in spectral region of QD intrinsic absorption were obtained by post-synthesis ligand exchange method using D- and L-cysteine as chiral ligands. We performed a spectral-photoluminescence study to clarify photophysics of intracellular behavior of D- and L- forms of chiral quantum dots. Our results show that average QD photoluminescence intensity per cell of L-Cys QDs was higher than that of D-Cys QDs while photoluminescence decay times were practically the same. Therefore observed higher values of intracellular photoluminescence intensity of L-Cys QDs compared with that of D-Cys QDs indicated higher L-Cys QD concentration within cells.

ACKNOWLEDGEMENTS

This work was supported by the RFBR (Grants 12-02-01263 and 12-02-00938), the Government of Russian Federation (Grant 074-U01), and the Ministry of Education and Science of the Russian Federation (Projects 14.B25.31.0002).

LITERATURE

[1] CINTAS, P. On Chirality and the Universal Asymmetry. Reflections on Image and Mirror Image. By Georges H. Wagnière. *Angewandte Chemie International Edition* 2007, 46, 9143-9144.

[2] XIA, Y.; ZHOU, Y.; TANG, Z. Chiral inorganic nanoparticles: origin, optical properties and bioapplications. *Nanoscale* 2011, 3, 1374-1382.

[3] MOLONEY, M. P.; GUN'KO, Y. K.; KELLY, J. M. Chiral highly luminescent CdS quantum dots. *Chemical Communications* 2007, 3900-3902.

[4] ELLIOTT, S. D.; MOLONEY, M. P.; GUN'KO, Y. K. Chiral Shells and Achiral Cores in CdS Quantum Dots. *Nano Lett.* 2008, 8, 2452-2457.

[5] TOHGHA, U.; VARGA, K.; BALAZ, M. Achiral CdSe quantum dots exhibit optical activity in the visible region upon post-synthetic ligand exchange with d- or l-cysteine. *Chemical Communications* 2013, 49, 1844-1846.

[6] FEDOROV, A. V.; RUKHLENKO, I. D.; BARANOV, A. V.; KRUCHININ, S. Y. *Optical Properties of Semiconductor Quantum Dots*. Nauka: St. Petersburg, 2011.

[7] EFROS, A. L.; LOCKWOOD, D. J.; TSYBESKOV, L. *Semiconductor Nanocrystals: From Basic Principles to Applications*. Springer: 2003.

[8] MEDINTZ, I. L.; UYEDA, H. T.; GOLDMAN, E. R.; MATTOUSSI, H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nature materials* 2005, 4, 435-446.

[9] MASLOV, V.; ORLOVA, A.; BARANOV, A. Combination Therapy: Complexing of QDs with tetrapyrrols and other dyes. In *Photosensitizers in Medicine, Environment, and Security*, Springer-Verlag, Ed. Springer: USA, 2012.

[10] LI, L.; ZHAO, J. F.; WON, N.; JIN, H.; KIM, S.; CHEN, J. Y. Quantum dot-aluminum phthalocyanine conjugates perform photodynamic reactions to kill cancer cells via fluorescence resonance energy transfer. *Nanoscale research letters* 2012, 7, 386.

[11] LI, Y.; ZHOU, Y.; WANG, H.-Y.; PERRETT, S.; ZHAO, Y.; TANG, Z.; Nie, G. Chirality of Glutathione Surface Coating Affects the Cytotoxicity of Quantum Dots. *Angewandte Chemie International Edition* 2011, 50, 5860-5864.

[12] ZHOU, Y.; ZHU, Z.; HUANG, W.; LIU, W.; WU, S.; LIU, X.; GAO, Y.; ZHANG, W.; TANG, Z. Optical Coupling Between Chiral Biomolecules and Semiconductor Nanoparticles: Size-Dependent Circular Dichroism Absorption. *Angewandte Chemie International Edition* 2011, 50, 11456-11459.

[13] TALAPIN, D. V.; ROGACH, A. L.; KORNOWSKI, A.; HAASE, M.; WELLER, H. Highly Luminescent Monodisperse CdSe and CdSe/ZnS Nanocrystals Synthesized in a Hexadecylamine−Trioctylphosphine Oxide−Trioctylphospine Mixture. *Nano Lett.* 2001, 1, 207-211.

[14] BAKER, D. R.; KAMAT, P. V. Tuning the Emission of CdSe Quantum Dots by Controlled Trap Enhancement. *Langmuir* 2010, 26, 11272-11276.