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Structural effects and nanoparticle size are essential for quantum dots–metallothionein complex formation

Katerina Tmejova[a,b], David Hynek[a,b], Pavel Kopel[a,b], Jaromir Gumulec[b,c], Sona Krizkova[a,b], Roman Guran[a,b], Zbynek Heger[a,b], Michal Kalina[d], Marketa Vaculovicova[a,b], Vojtech Adam[a,b], Rene Kizek[a,b,*]

[a] Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, European Union, CZ-613 00 Brno, Czech Republic
[b] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, European Union, CZ-616 00 Brno, Czech Republic
[c] Department of Pathological Physiology, Faculty of Medicine, Masaryk University, European Union, Brno, Czech Republic
[d] Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkynova 118, European Union, CZ-612 00 Brno, Czech Republic

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ABSTRACT

Interaction between semiconductor nanocrystals, cadmium telluride quantum dots (CdTe QDs) capped with mercaptosuccinic acid (MSA) and metallothionein (MT) was investigated. MSA-capped CdTe QDs were synthesized in aqueous solution. Mixture of MT and MSA-capped CdTe QDs has been investigated by various analytical methods as follows: tris-tricine gel electrophoresis, fluorescence evaluation and electrochemical detection of catalysed hydrogen evolution. The obtained results demonstrate that MSA-capped CdTe QDs and MT do not create firmly bound stabile complex. However, weak electrostatic interactions contribute to the interaction of MT with MSA-capped CdTeQDs. It can be concluded that QDs size influences the QDs and MT interaction. The smallest QDs had the highest affinity to MT and vice versa.

1. Introduction

QDs are single crystals with nanometers in diameter (2–10 nm) [1], which exhibit excellent optical (high photoluminescence quantum yield, strong photostability, wide absorption yield coupled with narrow emission) [2], and semiconductor properties [3]. The size and shapes can be precisely controlled by temperature, duration and type of ligand molecules applied during the synthesis process [4]. Typically, high-quality luminescent QDs are prepared via an organic phase high temperature procedure and these QDs cannot be dispersed in aqueous media, but biomedical applications require water-soluble QDs. Aqueous dispersions of QDs often exhibit polydispersity [5]. But the aim is to synthesize monodispersed quantum dots soluble in water and involved actively in bioconjugate reactions. A number of solubilization and functionalization strategies have been proposed as follows: incorporation into a liposome phospholipid bilayer, loading into liposomes or binding to the liposome surface [5–7]. Usually, encapsulation of QDs into polymers or lipids considerably increases the particle size, which limits their biological applications. Another strategies are based on cross-linking of QDs with amphiphilic polymers, silica coating and ligand-exchange processes [5–7]. Very often water-soluble bifunctional molecules as mercaptocarbonic acids [HS-$(CH_2)n$-COOH, n = 1–15] are used as an exchanging ligand on QDs surface [6].

QDs can be utilized in a wide range of applications in numerous areas including chemistry, chemical biology and biomedicine [4]. However, their toxicity to living organism is well known and cited in many studies [8–10]. Toxicity of QDs probably occurs due to various mechanisms including release of free metal ions from the QDs core, their existance in cells and generation of reactive electron–hole pairs upon illumination leading to the generation of reactive oxygen species [1,11].

Proteins in the metallothionein family are small cysteine-rich proteins which are able to bind up to 20 monovalent or up to 7 divalent heavy metal ions [12]. The tertiary structure of metallothionein (MT) is based on the presence of two domains, that easily form cysteine (Cys) clusters to bind metal ions [13]. The main function of MTs in the organism is the regulation of zinc homeostasis [14], protection of cells against oxidative stress [15] and the role of MT in anticancer therapy is still discussed [12,16,17].

* Corresponding author at: Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, European Union, CZ-613 00 Brno, Czech Republic. Fax: +420 545 212 044.
E-mail address: kizek@sci.muni.cz (R. Kizek).

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Despite of wide range of applications of QDs in biology (*in-vivo* imaging), little effort has been made to understand the effect of QDs on proteins. Experiments presented by Huang et al. showed that various capping against of CdS QDs (mercaptoethanol (ME), L-cysteine and glutathione) provide different affinity to different proteins (bovine serum albumin (BSA) and lysozyme (LZY)) [18]. GSH-capped CdS QDs were shown to interact with BSA and LZY in the weakest manner, while 3-mercaptopropionic acid (MPA)-capped CdS QDs interaction with these proteins was significantly stronger. Moreover the secondary structure of proteins was changed dramatically, which was the second important observation highlighting biological significance of QDs capping agents [18]. Similar experiment using CdTe QDs capped with the same passivators showed the same results [19]. Investigating the interaction between CdTe QDs and proteins Lu et al. found that these interactions are due to the electrostatic attraction mainly [20]. This finding was supported by the work of Xia and Zhu, who found that interaction of MPA-capped CdTe QDs with Cys and homocysteine was strongly affected by different pH [21].

In general, many challenges need to be addressed for the practical bio-applications of QDs, including photo and colloidal instability, and cytotoxicity caused by the heavy metal ions in QDs. Because of the one of the main function of MTs is cells detoxification from heavy metals [17], it could be concluded that presence of QDs in cells leads to the expresion/presence of these proteins in a large quantities [22,23]. Mainly because of the release of free metal ions from the QDs core [9,24]. In this study, the interactions between MT and mercaptosuccinic acid (MSA)-capped CdTe QDs are determined by various methods as follows: tris-glycine gel electrophoresis, fluorescence determination, and electrochemical detection of catalyzed hydrogen evolution.

2. Materials and methods

2.1. Chemicals and material

All chemicals for preparation QDs were purchased from Sigma–Aldrich (St. Louis, MO, USA) in ACS purity. To pipette volumes down to microliters, pipettes purchased from Eppendorf Research (Eppendorf, Hamburg, Germany) with the highest certified deviation (±12%) were used. The deionised water was prepared using reverse osmosis equipment Aqual 25 (Brno, Czech Republic). The deionised water was further purified by using apparatus MiliQ Direct QUV equipped with the UV lamp. The resistance was 18 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany). Other chemicals for MT isolation and electrochemical measurements were also purchased from Sigma–Aldrich Chemical Corp. (Sigma–Aldrich, USA).

2.2. Preparation of QDs

CdTe QDs were prepared with a slightly modified method published in [25]. Cadmium acetate dihydrate $Cd(OAc)_2{\cdot}2H_2O$ (0.027 g) was dissolved in ACS water (43 mL) and 0.06 g of MSA and 1 M NH_3 (1.8 mL) was added. Solution of 0.0033 g Na_2TeO_3 in 0.75 mL of water was poured into the first solution. Afterwards, solid $NaBH_4$ (40 mg) was added with vigorous stirring and hydrogen evolution was observed, followed by color change of solution to slightly yellow. After 30 min of stirring 2 mL of solution was heated in glass vial in Multiwave 3000 Microwave Reaction System (Anton Paar, Graz, Austria) using rotor 64MG5. Reaction conditions were as follows – power 300 W, ramp 10 min, hold 10 min, temperature 50–130 °C.

2.3. Preparation of sample for MT isolation

2 g of defrosted rabbit liver was homogenized on ice [23] using Ultra-turrax T8 (Schoeller instruments, Prague, Czech Republic) in 8 mL of 10 mM Tris–HCl buffer (pH 8.6). The sample was subsequently vortexed (Vortex Genie, Scientific Industries, Inc., Bohemia, NY, USA) and centrifuged (Universal 320, Hettich Zentrifugen, Tuttlingen, Germany) at 5000 rpm, 30 min at 4 °C. The supernatant was again centrifuged (Eppendorf centrifuge 5417R) in micro test tube at 25000 rpm, 30 min at 4 °C and after that the supernatant was subsequently heated in thermomixer (Eppendorf thermomixer comfort, Germany) for 10 min at 99 °C and centrifuged (Eppendorf centrifuge 5417R, Germany) in micro test tube at 25000 rpm for 30 min at 4 °C. Sample prepared like this was used for isolation by using fast protein liquid chromatography.

2.4. Fast protein liquid chromatography for MT isolation

Fast protein liquid chromatography was purchased from Biologic DuoFlow system (Biorad, USA) and consists of two chromatographic pumps for the application of elution buffers, a gel-filtration column (HiLoad 26/60, 75 PG, GE Healthcare, Sweden), an injection valve with 2-mL sample loop, an UV–vis detector and an automated fraction collector [23]. The mobile phase was 150 mM NaCl in 10 mM Tris–HCl buffer (pH 8.6), while isocratic elution was employed for separation. Flow of the mobile phase was set to 4 mL min^{-1}. Column was washed for 60 min by mobile phase prior to every separation. Fraction containing metallothionein was collected in elution volume of 240 mL. Signal of metallothionein was well evident due to presence of Cd(II) ions in its structure, causing change in the absorbance measured at 254 nm [26]. Dialysis and lyophilisation of corresponding fraction were also carried out in deionised water. MT containing different fractions was performed by SDS-PAGE for MT Assay [23].

2.5. Tris-glycine SDS-PAGE [Tricine SDS-PAGE]

The electrophoresis was performed using a Mini Protean Tetra apparatus with gel dimension of 8.3 × 7.3 cm (Bio-Rad, Philadelphia, PA, USA). First 15% or 12.5% (*m*/*V*) running, then 5% (*m*/*V*) stacking gel was poured. The composition of running gel was 15% acrylamide, 0.5% bisacrylamide, 0.376 M Tris–HCl, 0.1% sodium dodecyl sulfate (SDS), 0.05% ammonium persulfate (APS), 0.083% tetramethylethylenediamine (TEMED), pH 8.3 [15% acrylamide, 0.5% bisacrylamide, 1 M Tris–HCl, 0.1% SDS, 10.5% glycerol, 0.1% APS, 0.03% TEMED, pH 8.45]. The composition of stacking gel was 4.5% acrylamide, 0.15% bisacrylamide, 0.125 M Tris–HCl, 0.1% SDS, 0.05% APS, 0.1% TEMED, pH 8.3 [7.8% acrylamide, 0.26% bisacrylamide,1.5 M Tris–HCl, 0.15% SDS, 0.2% APS, 0.1% TEMED, pH 8.45]. The polymerization of the running or stacking gels was carried out at room temperature for 45 min or 30 min, respectively. Prior to analysis the samples were mixed with non-reduction sample buffer in 2:1 ratio. The samples were incubated at 23 °C for 3 min, and the sample was loaded onto a gel. For determination of the molecular mass, the protein ladder “Precision plus protein standards” [“Polypeptide SDS-PAGE Molecular Weight Standards”] from Biorad was used. The electrophoresis was run at 150 V for 1 h [120 V for 2 h] at laboratory temperature (23 °C) (Power Basic, Biorad USA) in tris-glycine buffer (0.025 M Trizma-base, 0.19 M glycine and 3.5 mM SDS, pH 8.3) [in tris-tricine buffer (100 mM Tris, 100 mM Tricine, 0.1% SDS, pH 8.3)]. Then the gels were stained by Coomassie-blue and consequently with silver, if proteins concentration in the samples was below detection limit of Coomassie-blue staining. The procedure of rapid Coomassie-blue staining was adopted from Wong et al. [27], silver staining was performed according to Krizkova et al. [28] with omitting the fix-