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Study of metallothionein–quantum dots interactions

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ABSTRACT

Nanoparticles have gained increasing interest in medical and *in vivo* applications. Metallothionein (MT) is well known as a maintainer of metal ions balance in intracellular space. This is due to high affinity of this protein to any reactive species including metals and reactive oxygen species. The purpose of this study was to determine the metallothionein–quantum dots interactions that were investigated by spectral and electrochemical techniques. CuS, CdS, PbS, and CdTe quantum dots (QDs) were analysed. The highest intensity was shown for CdTe, than for CdS measured by fluorescence. These results were supported by statistical analysis and considered as significant. Further, these interactions were analysed using gel electrophoresis, where MT aggregates forming after interactions with QDs were detected. Using differential pulse voltammetry Brdicka reaction, QDs and MT were studied. This method allowed us to confirm spectral results and, moreover, to observe the changes in MT structure causing new voltammetric peaks called X and Y, which enhanced with the prolonged time of interaction up to 6 h.

1. Introduction

Metallothionein (MT) as a low-molecular protein with mass of 6–7 kDa has the tertiary structure based on the presence of two domains. These domains readily form cysteine clusters to bind metal ions [1]. The main functions of MT consist in the transport of metal ions, accumulation of Zn, and detoxification of metals [1]. Due to high affinity of MT to heavy metals, its interaction with quantum dots (QDs) is also possible [2]. An increased expression of MT after exposure of model organisms to Cd-based QDs was found in studies dealing with (eco)toxicity of QDs [3,4]. Further, the biosynthesis of QDs in rats and earthworms exposed to $CdCl_2$ was found [5,6]. In earthworms the QDs synthesis was co-localized with expression of MT.

In vitro interaction between MT and QDs was studied by fluorescence spectroscopy and differential pulse voltammetry (DPV) [7]. The authors determined the changes of electrochemical behaviour of rabbit liver MT during the interaction, especially decrease of catalytic peaks [8] and formation of X and Y peaks. In this study, we aimed at detailed investigating MT–QDs interaction by DPV Brdicka reaction, which is the catalytic reaction of protein based on the interaction of $[Co(NH_3)_6]Cl_3$ with —SH moiety of the protein [9].

2. Materials and methods

2.1. Preparation of QDs, MT and chemicals

CuS, CdS, and PbS QDs were prepared according to Krejcova et al. [10]; however, the procedure of preparation of PbS was slightly modified (mercaptosuccinic acid instead of 3-mercaptopropionic acid). CuS QDs were prepared by reaction of $Cu(OAc)_2{\cdot}H_2O$ (0.02 g, 0.1 mM) dissolved in ACS water (25 ml) with mercaptosuccinic acid (0.08 g, 0.53 mM). 0.5 ml of 1 M NH_4OH was added with stirring to yellow solution, followed by $Na_2S{\cdot}9H_2O$ (0.012 g, 0.05 mM) in 24.5 ml of ACS water. Colour of solution turned to light brown. CdS MPA QDs were prepared as follows: $Cd(NO_3)_2{\cdot}4H_2O$ (0.031 g, 0.1 mM) was dissolved in ACS water (25 ml). 3-Mercaptopropionic acid (35 μl, 0.4 mM) was slowly added to stirred solution. Afterwards, pH was adjusted to 6.8 with 1 M NH_3 (0.76 ml). $Na_2S{\cdot}9H_2O$ (0.024 g, 0.1 mM) in 24 ml of ACS water was poured into the first solution with vigorous stirring. Obtained yellow solution was stirred for 1 h. PbS QDs were prepared according this protocol: $Pb(OAc)_2{\cdot}3H_2O$ (0.038 g, 0.1 mM) was dissolved in ACS water (25 ml). Mercaptosuccinic acid (0.08 g, 0.53 mM) was slowly

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added to stirred solution. White precipitate was formed, which disappeared after addition of 1.8 ml of 1 M NH_4OH. Sodium sulfide nonahydrate $Na_2S{\cdot}9H_2O$ (0.012 g, 0.05 mM) in 23.2 ml of ACS water was added with vigorous stirring. Colour of solution was brown. CdTe QDs were done according to Skalickova et al. [8]. $Cd(OAc)_2{\cdot}2H_2O$ (0.027 g, 0.1 mM) was dissolved in ACS water (44 ml) and 100 mg of trisodium citrate dihydrate was added with stirring. Solution of 0.0055 g (0.025 mM) Na_2TeO_3 in 1.25 ml of water was poured into the first solution followed by 3-mercaptopropionic acid (100 μl, 1.14 mM), solid $NaBH_4$ (50 mg) was added with vigorous stirring and hydrogen evolution was observed, followed by colour 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, 120 °C and time 18 min. All chemicals for preparation QDs were purchased from Sigma–Aldrich (USA) in ACS purity. QDs were stored at 4 °C. MT was isolated from rabbit liver according to Skalickova et al. [8].

2.2. Electrochemical determination

Electrochemical determination was carried out by differential pulse voltammetry (DPV) in the Brdicka electrolyte [11] by using a standard cell with three electrodes and cooled sample holder and measurement cell to 4 °C (Julabo F25, JulaboDE). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3 M KCl electrode was the reference and platinum electrode was auxiliary. Sample consisting of 10 μl 0.8 μM MT and 10 μl 500 μM QDs was pipetted into an electrochemical cell (2 ml) and then the electrolyte (1 980 μl) was added. The MT–QD interaction was studied for 0, 240, 480, and 960 s, and 30, 60, and 90 min, and 2, 3, 4, 5 and, 6 h at 4 °C. After that, monitoring of the interaction was performed using the electrochemical detection (DPV). More details about electrochemical Brdicka experiments are shown in the paper Petrlova et al. [12].

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed using a Mini Protean Tetra apparatus in Tris–tricine gel system. Acrylamide concentration was 15% (m/V) in running gel and 5% (m/V) in the stacking gel. Prior to analysis samples were mixed with non-reducing sample buffer in a 1:1 ratio. The samples were incubated at 25 °C for 3 min. After the incubation, the samples were loaded onto a gel. More details can be found in the following paper [13]. Then, the gels were stained with Coomassie blue and consequently with silver.

2.4. Fluorescence camera Xtreme

To test the fluorescence properties of QDs, 50 μl of 500 μM solution of QDs was pipetted into a microtitration plate (Nunc Maxisorp, Thermo Fischer Scientific, USA). Fluorescence was measured using Xtreme Imaging System (Bruker, USA). The parameters were set as follows: excitation wavelength: 460 nm, emission wavelength: 700 nm, exposure time: 2 s, binning: 2 × 2, f-stop: 1.1, and field of view: 19.5 cm × 19.5 cm.

2.5. Statistical analyses

Factorial ANOVA following Bonferroni post hoc test were used to reveal differences between factors "QD" and "MT". p-Level < 0.05 was considered significant. Software Statistica 10 (Statsoft, USA) was used for analyses. Error bars were determined by standard deviation.

3. Results and discussion

3.1. Fluorescence detection

To study MT–QD interactions, MT-2 and four types of QDs as CdTe, CdS, CuS, and PbS were used. Firstly, the change of the fluorescence emission of QDs after interaction with MT was investigated. The colour changes of QDs after MT addition are shown in the upper part of Fig. 1A. After the addition of MT the intensity of colour became darker. These changes were studied by a transilluminator (312 nm), and changes in fluorescence intensity were measured by

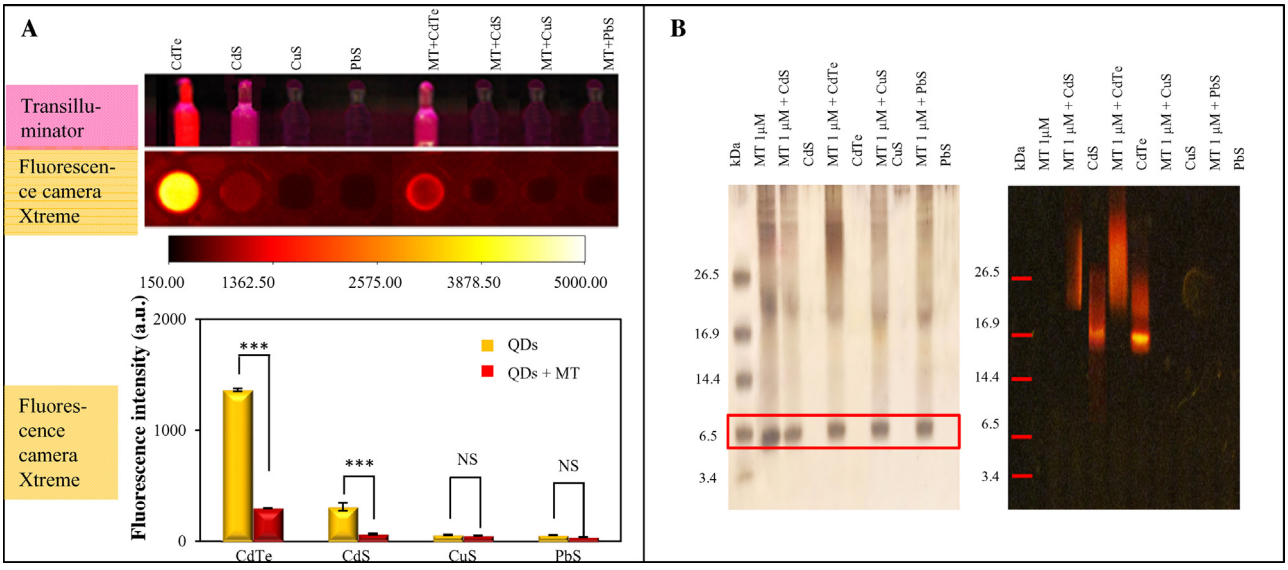

Fig. 1. Characterization of different QDs (CdTe, CdS, CuS and PbS). (A) The changes of colour intensity of QDs after MT addition measured by transilluminator and Xtreme Imaging System (final concentration of QDs 80 μM and MT 2 μM). All data represent mean ± s.d., NS, not significant; *p < 0.001 was significant. (B) SDS–PAGE of QDs and their complexes with MT (250 μM QDs and 0.5 μM MT).