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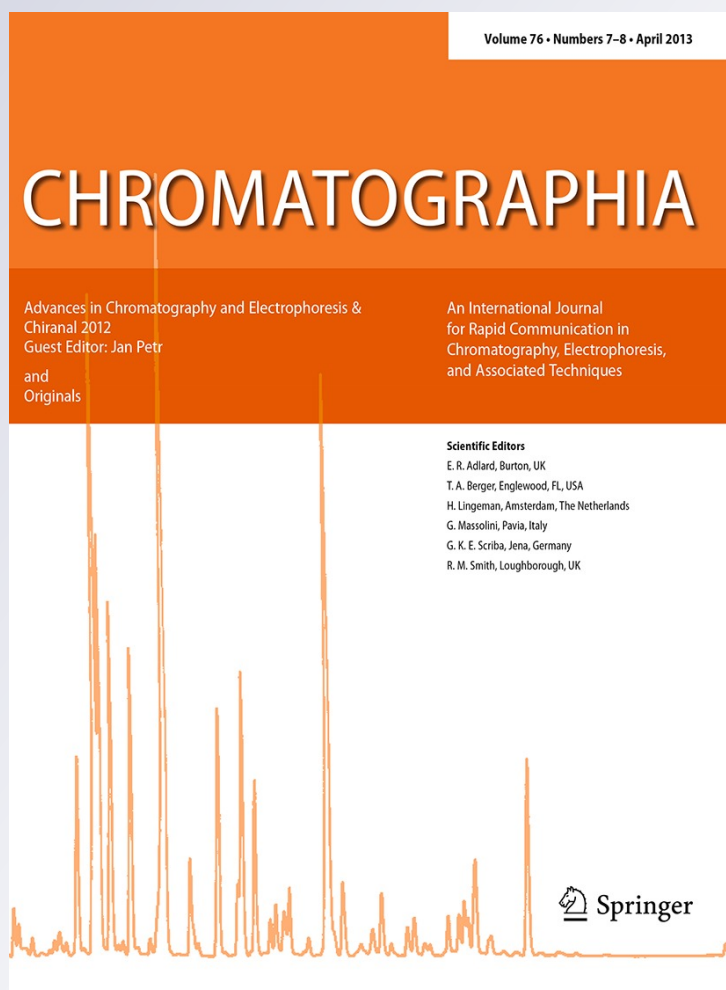
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Study of Interaction between Metallothionein and CdTe Quantum Dots

Sylvie Skalickova · Ondrej Zitka · Lukas Nejdil · Sona Krizkova · Jiri Sochor · Libor Janu · Marketa Ryvolova · David Hynek · Jarmila Zidkova · Vaclav Zidek · Vojtech Adam · Rene Kizek

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Abstract Quantum dots (QDs) belong to a new class of fluorescent agent for biochemical, medicinal or other purposes. However, QDs based on cadmium or other metals can be risky for an organism. As one of the mechanism how to detoxify cadmium-based QDs expression of metallothioneins (MT) can be considered. Due to high affinity of metallothionein to cadmium(II) ions, we attempted to develop an approach for studying of possible interaction with QDs. We prepared QDs with CdTe core and studied the interaction with MT, which we isolated from livers of Cd-administered rabbits. To study the interaction, we used the mixture of both components MT (3.6 μM): CdTe QDs (0, 0.34, 0.68, 1.02, 1.36, 1.7, 2.04 and 2.47 μM). The mixtures were studied by spectrophotometry within the range from 200 to 750 nm with detected maxima at 260 and 505 nm. Same mixtures were also

analysed by differential pulse voltammetry Brdicka reaction, which supported data from spectrophotometry. Subsequently, we used fast protein liquid chromatography for purification of protein–quantum dot conjugates. We obtained the different chromatograms for (1) Apo MT, (2) CdTe QDs and (3) MT–QD complex. We also collected the fractions and subsequently analysed them on the content of Cd and MT, which confirmed the formation of CdTe QDs–MT complex.

Keywords Fast protein liquid chromatography · Brdicka reaction · Differential pulse voltammetry · Fluorimetry · Spectrophotometry · Separation of quantum dots · ApoMT

Introduction

Quantum dots (QDs) light-emitting particles on the nanometre scale are emerging as a new class of fluorescent agent for in vivo imaging [1]. QDs often consist of cadmium(II) ions and/or ions of other metal such as selenium, tellurium or zinc [2] and can be used for fluorescent labelling of biomolecules [3, 4]. In addition, these particles can be modified by a recognition molecule such as an antibody and then, QD–antibody complex can be used for identification and visualisation of necrotic lesions or tumour cells [5]. Wang et al. [6] showed that the QDs could be bound by proteins in an organism very easily. However, toxicity of QDs must be considered. Their toxicity is predominantly caused by their disintegration to well-soluble inorganic ions, mostly cadmium(II) [7]. It has been demonstrated that the degree of QDs toxicity is closely connected with different parameters such as cell number, cell growth, apoptosis, cellular morphology or metabolic activity change of targeted tissue [8]. Thus, functionalisation of their surface

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by thiol group(s)-containing compounds, such as cysteine, mercaptopyropionic acid and glutathione is commonly applied [9]. In spite of this fact, studying of interactions of some protective proteins with QDs is of great interest.

Metallothioneins (MT) are a group of proteins rich in cysteine [10], which are able to bind metal ions especially essential zinc or toxic cadmium [11]. It is not surprising that metallothionein is biosynthesised due to stress caused by heavy metals, respectively, in the response to entering of a metal ion into the intracellular space. This process is realised via binding the metal ion on metal transcription factor-1 (MTF-1), which is zinc finger of the size of 70–80 kDa. MTF-1 subsequently binds to metal response element (MRE) localised in the promoter of gene for metallothionein [12–14], and this process activates transcription of this gene. The process itself is regulated by some factors such as metal-transcription inhibitor (MTI), which inhibits MT transcription by binding to metal responsive element (MRE). After the entry of metal ions into a cell, these ions bind to MTI, and this leads to the change in the conformation and dissociation of MTI from MRE. Therefore, binding site is free and ready for the interaction with MTF-1 [13]. Some studies show that the presence of other heavy-metal ions (not only zinc) may activate other redox-sensitive transcription factors such as NF- κ B, AP-1 and p53 [15].

From the structural point of view, metallothionein is a low-molecular protein of the size of 6–7 kDa of which tertiary structure is based on the presence of two domains, which form cysteine clusters for binding metal ions [16]. Due to the fact that MT contains almost no aromatic amino acids and due to its size, MT forms no secondary structures, thus, it is very difficult to apply analytical methods commonly used in proteomics as gel electrophoresis and mass spectrometry [17–19]. Metallothionein and similar cysteine rich proteins have been studied using the separation methods of affinity chromatography [20], reversed-phase chromatography [21], high-performance liquid chromatography with mass detection [22] and capillary electrophoresis [23–26]. Due to the high content of electrochemically active thiol groups in the structure of MT, electrochemical techniques represent the most sensitive analytical technique for MT quantification [10, 27–30].

Due to high affinity of metallothionein to cadmium(II) ions, in this study, we aim on developing an approach for studying of possible interactions between MT and cadmium-based quantum dots prepared according to [31]. Primarily, we isolated metallothionein from rabbit liver and used it as chelating agent for CdTe QDs. Further, we study the complex formation between MT and CdTe QDs. For that purpose, spectrophotometric, fluorimetric and differential pulse voltammetry were used. Moreover, fast protein liquid chromatography [32–34] was used for CdTe QDs–MT complex observation.

Experimental Section

Chemicals

Trizma base, HCl, NaCl, BSA, TCEP, EDTA, CdCl₂, Na₂TeO₃, trisodium citrate dihydrate, mercaptopyropionic acid, Co(NH₃)₆Cl₃, NH₃(aq) and NH₄Cl of ACS purity used were purchased from Sigma Aldrich Chemical Corp. (Sigma-Aldrich, USA), unless noted otherwise. Deionised water underwent demineralisation by reverse osmosis using the instrument Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) followed by further purification using Millipore RG (Millipore Corp., USA, 18 M Ω)—MiliQ water. The pH was measured using WTW inoLab pH meter (Weilheim, Germany).

QDs Synthesis

QDs were prepared according to Duan et al. [31]. Cadmium chloride solution (CdCl₂, 0.04 M, 4 mL) was diluted to 42 mL with ultrapure water, and then trisodium citrate dihydrate (100 mg), Na₂TeO₃ (0.01 M, 4 mL), MPA (119 mg), and NaBH₄ (50 mg) were added successively under magnetic stirring. The molar ratio of Cd²⁺/MPA/Te was 1:7:0.25. 10 mL of the resulting CdTe precursor was put into a Teflon vessel. CdTe QDs were prepared at 95 °C for 10 min under microwave irradiation (400 W, Multiwave 3000, Anton-Paar GmbH, Austria). After microwave irradiation, the mixture was cooled to 50 °C and the CdTe QDs sample was obtained. Re-purification of CdTe QDs was carried out using isopropanol condensing. The CdTe QDs was mixed with isopropanol in ratio 1:2 and then centrifuged for 10 min at 25,000 rpm (Eppendorf centrifuge 5417R). Pellet was dissolved into 500 μ L with Tris Buffer (pH 8.5).

Experimental Animals and Preparation of Samples for MT Isolation

The males of New Zealand rabbits weighing 3.0–3.5 kg were kept in separate cages on regular pelleted laboratory chow (MaK-Bergman, Kocanda, Prague, Czech Republic) and allowed free access to drinking water. Rabbits were given the intraperitoneal injection of 10 mg of CdCl₂/kg of weight (Sigma-Aldrich) in three equal doses (day 1, day 3 and day 5). In the aforementioned day intervals, animals were anaesthetised with Ketamine: 30 mg/kg and Xylazine: 3 mg/kg, (Vétoquinol Biovet, France). Animals were then bled out by heart puncture, individual livers were collected, weighed and immediately frozen on dry ice.

Preparation of Sample for MT Isolation

Amount of 2 g of defrosted rabbit liver was homogenised on ice using Ultra-turrax T8 (Scholler instruments,

Germany) in 8 mL of 10 mM Tris–HCl buffer (pH 8.6). The obtained sample was subsequently vortexed (Vortex Genuie, Germany) and centrifuged (Universal 320, Hettich Zentrifugen, Germany) at 5,000 rpm (30 min, 4 °C). Taken supernatant was again centrifuged (Eppendorf centrifuge 5417R) in 1.5-mL micro test tube at 4 °C (25,000 rpm, 30 min). The supernatant was subsequently heated in thermomixer (Eppendorf thermomixer comfort, Germany) at 99 °C (10 min) and centrifuged (Eppendorf centrifuge 5417R, Germany) in 1.5-mL micro test tube at 4 °C (25,000 rpm, 30 min). Sample prepared like this was used for isolation of MT.

Fast Protein Liquid Chromatography for Isolation of MT

Fast protein liquid chromatography (FPLC) was purchased from Biologic DuoFlow system (Biorad, USA), which consisted 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. Solution of 150 mM NaCl in 10 mM Tris–HCl buffer (pH 8.6) was used as a mobile phase. Flow of the mobile phase was set to 4 mL/min. Isocratic elution was used for metallothionein separation. Column was washed for 60 min by mobile phase prior to every separation.

Process of metallothionein isolation from rabbit liver after Cd(II) application is shown in (Fig. 1a). Fraction containing metallothionein was collected in elution volume of 240 mL. Signal of metallothionein was well evident due to binding of Cd(II) ions into protein structure, which caused change in the absorbance measured at 254 nm [35]. Dialysis and lyophilisation of corresponding fraction were also carried out in deionised water.

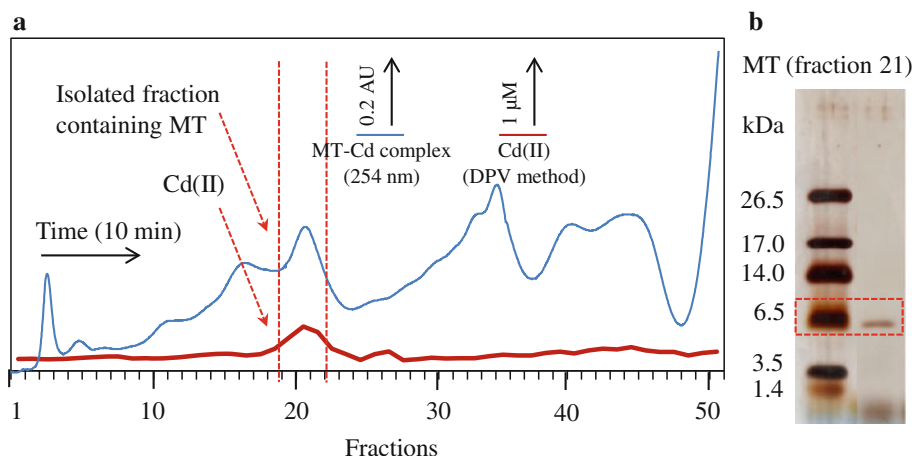
SDS PAGE for MT Assay

The electrophoresis of the fraction containing MT (Fig. 1b) was performed using Maxigel apparatus (Biometr, Germany). First 15 % (w/v) running, then 5 % (w/v) stacking gel was poured. The gels were prepared from 30 % (w/v) acrylamide stock solution with 1 % (w/v) bisacrylamide. The polymerisation of the running or stacking gels was carried out at room temperature for 45 min. Prior to analysis, the samples were mixed with non-reduction sample buffer in a 2:1 ratio. The samples were incubated at 93 °C for 3 min, and the sample was loaded onto a gel. For the determination of the molecular mass, the protein ladder “Precision plus protein standards” from Bio-Rad was used. The electrophoresis was run at 150 V for 1 h at room temperature (Power Basic, Bio-Rad) in Tris–glycine buffer (0.025 M Trizma-base, 0.19 M glycine, and 3.5 mM SDS, pH = 8.3). Then, the gels were stained with Coomassie blue and consequently with silver. The procedure of rapid Coomassie blue staining was adopted from Wong et al. [36], silver staining was performed according to Krizkova et al. [37] with omitting the fixation (1.1 % (v/v) acetic acid, 6.4 % (v/v) methanol, and 0.37 % (v/v) formaldehyde) and first two washing steps (50 % (v/v) methanol).

Differential Pulse Voltammetry for Cadmium(II) Ions Determination

Determination of cadmium(II) ions were performed with 797 VA Stand instrument connected to 889 IC Sample Center (Metrohm, Switzerland). The analyser (797 VA Computrace, Metrohm, Switzerland) employs a conventional three-electrode configuration with a hanging mercury drop electrode (HMDE) working electrode: 0.4 mm², Ag/AgCl/3MKCl as reference electrode, and a platinum auxiliary electrode. A sample changer (Metrohm 889 IC Sample

Fig. 1 **a** FPLC chromatogram of real sample of extract from rabbit liver treated with Cd(II) in overlay with determined concentration of cadmium in collected fractions. In the position of MT peak, cadmium(II) was 0.80 μ M (determined by differential pulse voltammetry). **b** Electrophoreogram from SDS PAGE analysis of fractions with MT



Center) performs the sequential analysis of 96 samples in plastic test tubes. Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon 120 s; start potential -0.9 V; end potential -0.3 V; deposition potential -0.9 V; accumulation time 800 s; pulse amplitude 0.025 V; pulse time 0.05 s; step potential 2 mV; time of step potential 0.2 s; volume of injected sample 20 μL ; cell was filled with 1,980 μL of electrolyte (0.2 M acetate buffer pH 5.0).

Differential Pulse Voltammetry Brdicka Reaction for MT Determination

Differential pulse voltammetric measurements were performed with 747 VA Stand instrument connected to 693 VA Processor and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder and measurement cell to 4 °C by Julabo F25 (JULABO, Germany). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm² was the working electrode. An Ag/AgCl/3 M KCl electrode was the reference and platinum electrode was auxiliary. For data processing, VA Database 2.2 by Metrohm CH was employed. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999 %) saturated with water for 120 s. Brdicka supporting electrolyte containing 1 mM Co(NH₃)₆Cl₃ and 1 M ammonia buffer (NH₃(aq) + NH₄Cl, pH = 9.6) was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, $E_{\text{ads}} = 0$ V, volume of injected sample: 25 μL , volume of measurement cell 2 mL (25 μL of sample + 1,975 μL Brdicka solution).

UV–VIS Spectrophotometry

An UV–VIS spectrophotometer Specord 210 (Analytik Jena, Germany) was used for spectrophotometric analyses. This apparatus was equipped by movable carousel with eight positions for cuvettes. Quartz cuvettes Microcuvette (1 cm, total volume of 1.5 mL, Kartell, Italy) were used for the analyses. Carousel was tempered to required temperature by a flow thermostat JULABO F12/ED (JULABO, Germany), where distilled water serves as a medium. All analyses were carried out at 25 °C. The range of wavelengths for the measurement was 200–750 nm. As a blank, we used Tris buffer (pH 7.5) with 150 mM NaCl.

Fluorescence Measurement

Fluorescence spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN,

Switzerland). 350 nm was used as an excitation wavelength and the fluorescence scan was measured within the range from 400 to 750 nm per 2-nm steps. Each intensity value is an average of three measurements. The detector gain was set to 100. The sample (50 μL) was placed in transparent 96 well microplate with flat bottom by Nunc (Thermo Scientific, USA). All measurements were performed at 25 °C controlled by Tecan Infinite 200 PRO (TECAN, Switzerland). As a blank, we used Tris buffer (pH = 7.5) with 150 mM NaCl.

Results and Discussion

Interaction between MT and CdTe QDs was investigated using the multi-instrumental approach. MT was isolated using fast protein liquid chromatography from the homogenate of liver of rabbits treated with cadmium(II) ions according to Demuynck et al. [35]. FPL chromatogram is shown in Fig. 1a. The presence of MT was verified by SDS-PAGE (Fig. 1b). CdTe QDs were prepared according to Duan et al. [31]. Prepared CdTe QDs were further characterised by differential pulse voltammetry (DPV), where concentration of cadmium(II) ions was determined [38–42]. Interaction between MT and CdTe QDs was primarily characterised using UV–VIS spectrophotometry according to [10] and subsequently by fluorimetry and differential pulse voltammetry Brdicka reaction. Finally, size exclusion separation of MT–CdTe QDs complex, where QDs, MT and Cd(II) were determined, was carried out.

Cd Content in the Prepared CdTe QDs

Quantum dots with CdTe core were prepared according to protocol mentioned in “[Experimental Section](#)” and the content of cadmium was determined by DPV to quantify prepared QDs. Redox signals of cadmium(II) ions were detected at -0.64 V. Parameters of calibration dependence were as follows: $y = 1.795x$, $R^2 = 0.9996$, $n = 5$ (R.S.D. 3.1 %). Using DPV, method it was determined that prepared stock solution of CdTe QDs contained 68 ± 2 μM ($n = 5$) of cadmium(II) ions.

Spectrophotometry of MT Interacted with CdTe QDs

For verification of the formation of MT–CdTe QDs complex the effect of addition of QDs to MT, which was isolated according to the protocol mentioned in “[Experimental Section](#)”, was investigated. Firstly, volume of 60 μL of MT (3.6 μM) was mixed with 120 μL of phosphate buffer (pH 7.5, 20 mM). Subsequently, addition of CdTe QDs (68 μM , individual step 1 μL) was carried out. Final concentration of MT in this mixture was 1.2 μM and final concentration of

CdTe QDs was 2.64 μM . Set of ten samples was prepared like this. One sample (1) represented only MT (without QDs addition) at a concentration of 1.2 μM , next sample (2) contained MT in combination with 0.34 μM CdTe QDs, and next samples had increasing concentrations of QDs—0.34, 0.68, 1.02, 1.36, 1.7, 2.04, and 2.47 μM . Total volume of sample, which was applied into cuvette, was always 180 μL . Spectrum within in the range from 200 to 350 nm in 5-min intervals for 30 min was analysed after the individual additions of QDs. It clearly follows from the obtained results that the applications of QDs to MT lead to the increase of absorbance at three wavelengths as 260, 310 and 510 nm (Fig. 2a). Maximum *I* detected at 260 nm was related to MT–QDs complex I, and the second maximum related to MT–QDs complex II was observed at 310 nm, which is in agreement with the previously published results [23], where the increase of absorbance at 240–260 nm as a result of origination of MT–Cd(II) was observed. Addition of CdTe QDs led to the almost linear increase in absorbance for both maxima corresponding to complex I and II, respectively, to complexes of MT (Fig. 2b). The increase of absorbance at 510 nm was caused by CdTe QDs themselves. This fact is well evident in the record of analysis of CdTe QDs at identical concentration without MT presence (Fig. 2a).

Electrochemical Study of CdTe QD–MT Complex

Interaction between CdTe QDs and MT was further monitored using DPV Brdicka reaction. This reaction belongs to the catalytic processes, where nascent signal is influenced by the formation of complexes between analyte with cobalt(III) ions [43]. CdTe QDs complexes with MT gave DP voltammograms, which are shown in Fig. 3a. The voltammograms contain five characteristic signals. RS_2Co , Cat1 and Cat2 are signals associated with MT itself, which have been described in our previous papers [10, 30, 44–46]. Signals X and Y can be related to the interaction between CdTe QDs with the electrolyte and MT. Addition of CdTe QDs leads to the vanishing of signal X and shift of signal Y towards more positive potentials (from -1.05 to -0.98 V). Moreover, signals X and Y formed one composed signal, which narrows with the increasing concentration of CdTe QDs. More detailed description of these processes will be published elsewhere. It follows from the results shown in Fig. 3b that the addition of CdTe QDs to MT causes decrease in catalytic signal Cat2. As it was described in the previous subchapter, the increasing amount of CdTe QDs (0.34, 0.68, 1.02, 1.36, 1.7, 2.04, 2.47, 2.72, 3.06 and 3.4 μM) was gradually added to the 3.4 μM solution of

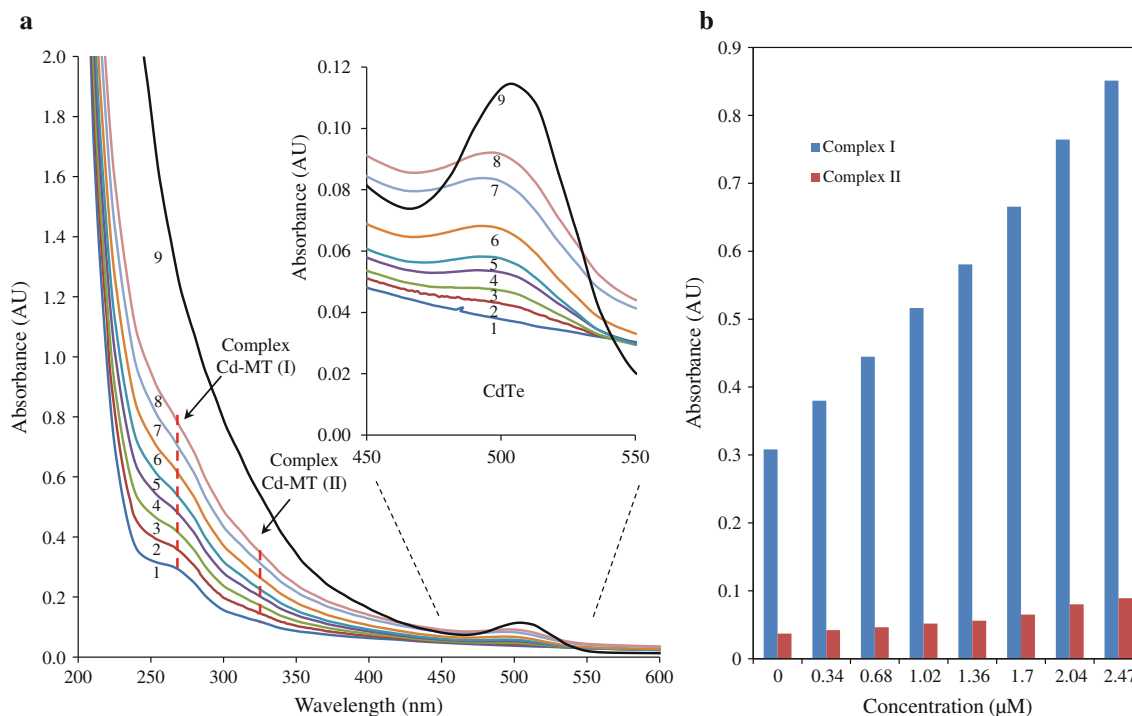


Fig. 2 a Overlay of absorption spectra obtained within the range from 200 to 600 nm for mixture of MT (1–1.2 μM), QDs (2–0.34; 3–0.68; 4–1.02; 5–1.36; 6–1.70; 7–2.04; 8–2.47;) and CdTe QDs (9–2.5 μM). Well observable complexes I and II were detected at

254 nm and 310 nm. Detail of spectra maxima of QDs (CdTe) is shown in inset (450–550 nm). **b** The increasing absorbance of complexes I and II after an addition of QDs into MT

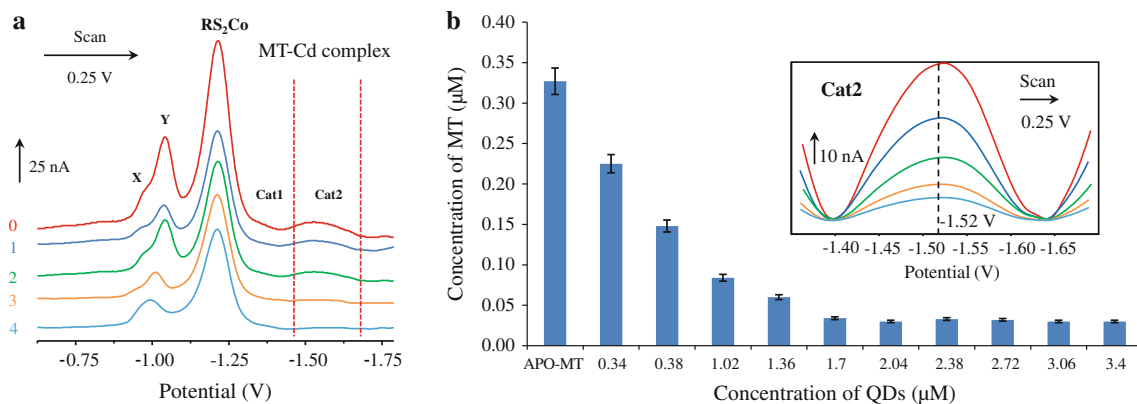
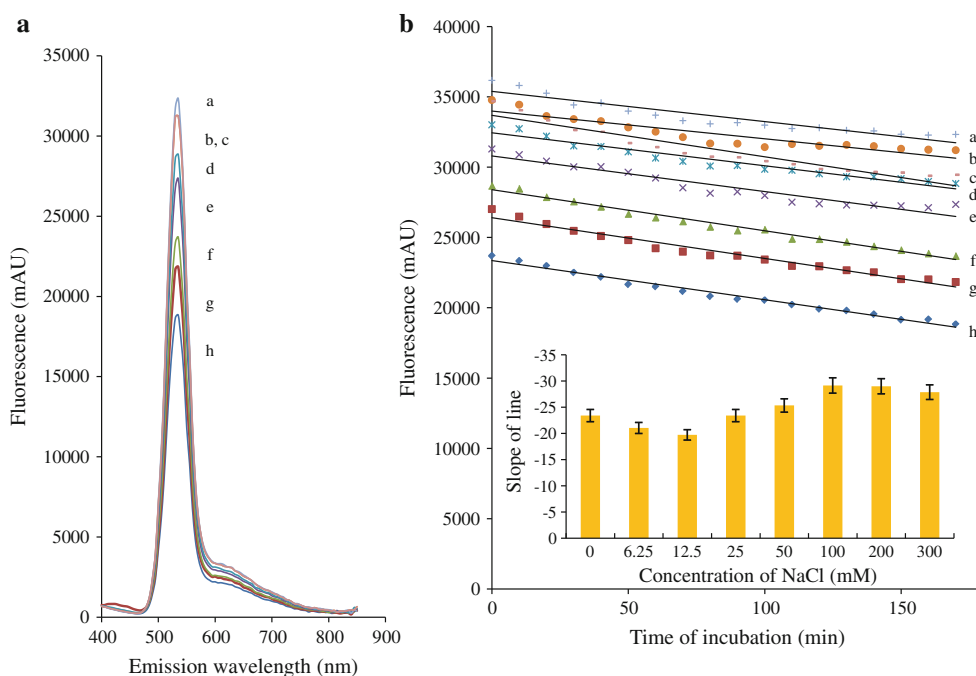


Fig. 3 **a** DP voltammogram of complex MT–CdTe measured in the presence of Brdicka solution. Five various additions of QDs (0.34–34 μM) was mixed with MT solution—voltammogram 0–4. **b** The influence of QDs addition to measured Cat2 MT signal. The parameters of the measurement were as follows: initial potential of

0.75 V, end potential of –1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude –250 mV, $E_{ads} = 0$ V, volume of injected sample: 25 μL, volume of measurement cell 2 mL (25 μL of sample + 1.975 μL Brdicka solution)

Fig. 4 **a** Emission spectra of QDs (CdTe 3.4 μM) after 3 h under applied concentrations of NaCl (a–0; b, c–12.5, 6.25; d–25; e–50; f–100; g–200; h–300 mM NaCl). **b** Influence of NaCl (0–300 mM) during time dependent measurement (0–1,800 min) on QDs (CdTe 17 μM) signal at 535 nm (excitation at 350 nm); in inset: the slope development owing to decrease of signal is shown



MT. Cat2 signal was decreased for more than 85 % compared to MT itself after the addition of 1.36 μM of CdTe QDs. However, further decrease of Cat2 signal was not observable with the increasing CdTe QDs concentrations (inset in Fig. 3b), which can be related to the saturation of MT moieties for metal interactions by QDs.

Quenching of QDs Emission by NaCl

Further, we aimed our attention on the isolation of QDs–MT complexes. Stability of QDs and their ability to emit radiation after excitation in the higher NaCl concentrations, which was necessary for effective separation of QD–MT

using FPLC, was investigated by fluorimetry. Solution of NaCl (0, 6, 12, 25, 50, 100, 200 and 300 mM) was added to 3.4 μM solution of CdTe QDs in Tris buffer. Then, we performed time-dependent fluorimetric analysis for 3 h with 10-min steps. As it is obvious from the obtained results, the increasing concentration of NaCl and time of interaction lead to the decrease of fluorescence of CdTe QDs (emission at 535 nm). The most significant reduction of the emission was detected at the highest applied NaCl concentration (300 mM) as 45 % after 3 h of incubation (Fig. 4a). For the evaluation of the trend of the reduction of the emission, the obtained dependences were plotted with linear lines (Fig. 4b). The least sharp decrease was detected

in the case of 6.25 and 12.5 mM NaCl. On the other hand, the sharpest increase was detected at 100 and 200 mM (in inset Fig. 4b). These results give evidence about the possible effect of ionic strength on CdTe QDs fluorescence quenching. It is necessary to apply higher ionic strength (100–200 mM NaCl) for the size exclusion separation, which was most suitable for the separation/characterisation of MT–QD complex. This fact is based on the necessity to eliminate possible non-specific interactions and other electrostatic and hydrophobic interactions. On the other hand, lower recovery rate must be carefully considered in the case like separated CdTe QDs. The decrease of emission after 2 h is for about 30 % in the case of 150 mM concentration, which must be taken into account as we discussed above.

Quenching of QDs Emission by MT Interaction

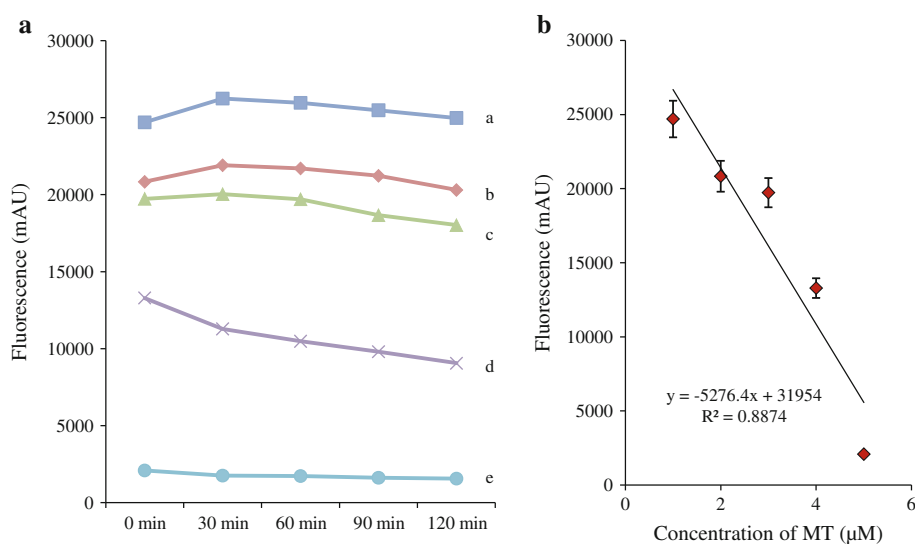
Due to the decrease of electrochemical signal of MT observed in QD–MT complex studied using the Brdicka reaction, we decided to determine the emission of CdTe QDs complex with MT. We carried out a time-dependent measurement of QDs emission within the range from 400 to 850 nm under the excitation of 350 nm for individual MT additions (0.43–1.7 μM) to CdTe QDs (1.7 μM) in the presence of Tris buffer (pH = 7.5). The results obtained are shown in Fig. 5a. It clearly follows from the obtained results that additions of 0.43 and 0.64 μM of MT did not influence the intensity of emission. However, addition of 0.85 μM of MT led to the decrease of the intensity of the emission by 20 % during 120 min of incubation. Higher concentrations as 1.28 and 1.7 μM of MT caused emission decrease almost immediately by 60 %, respectively, 90 % with only minimal progression during 120-min-long incubation. In general, change of the emission intensity during

incubation was minimal (up to 10 %) in all applied MT concentrations. These results indicate that the complex between MT and CdTe is formed rapidly (already in the time 0) and is stable for 120 min at least. The obtained slopes of linear regression characterise the decrease in emission with the increasing MT concentration with the linearity of $R^2 = 0.8874$ (Fig. 5b).

Separation of QD–MT Complex Using FPLC

For the verification of the formation of MT–QDs complex as well as for separation of this complex, we performed fast protein liquid chromatography with the application of Sephadex FPLC column (HiLoad 26/60, 75 PG, GE Healthcare, Sweden). For the monitoring of complex formation, mixture of 1.7 μM MT and 1.7 μM CdTe QDs was prepared. Volume of sample applied into system was 1 mL. Visible spectra detection of CdTe QDs was performed at 505 nm due to detection of CdTe QDs themselves. Fractions were collected during the whole separation (2 mL for 70 min). In addition to actual absorbance (505 nm) monitored behind the output of column, fractions were subjected to analysis of Cd(II) content and also MT content using the Brdicka's reaction (Fig. 6c). Obtained chromatogram (VIS detection) shows two distinct signals (signal *a* and signal *b*), where signal *a* represents probably a formed complex of QD and MT and signal *b* represents CdTe QDs. Application of only QDs leads to the formation of signal in the same position as signal *b* (Fig. 6b). Signal of metallothionein was observed in the same position as signal *a* (Fig. 6a). Application of Cd(II) ions did not affect the position of both signals as well as the formation of “new” signal. This presumption was supported by the determination of MT in very low concentration (0.2 μM) in the elution time of signal *a*. This fact

Fig. 5 **a** The influence of addition of apoMT (a–0.43; b–0.64; c–0.85; d–1.28; e–1.70 μM) on QDs (CdTe 1.7 μM) fluorescence at 535 nm (excitation at 350 nm) during time dependent measurement (0–120 min). **b** Calibration curve of addition of apoMT to QDs



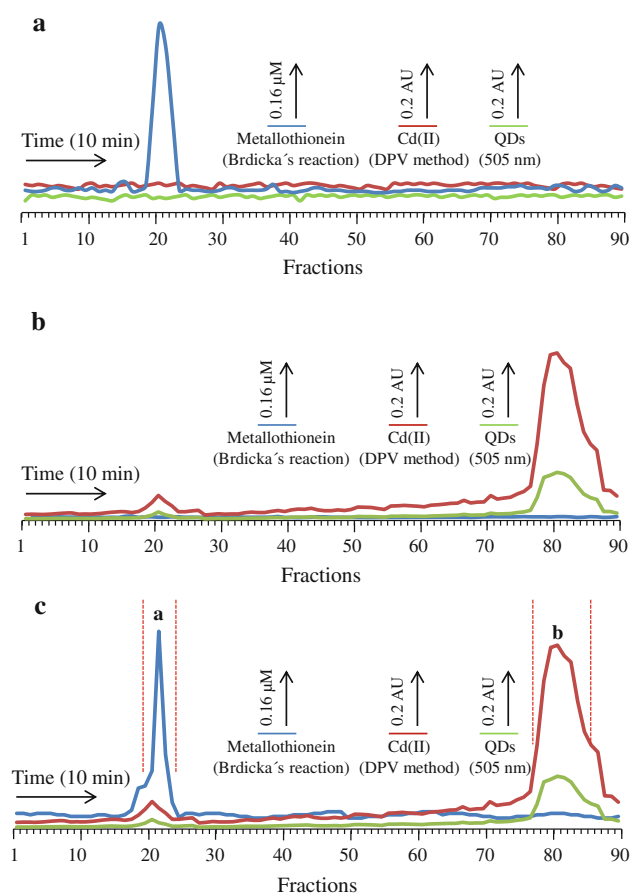


Fig. 6 Chromatogram of MT ($1.7 \mu\text{M}$) + QDs ($1.7 \mu\text{M}$) mixture separated by FPLC system and recorded at 505 nm (green line). Collected fractions (2 mL) were then analysed by DPV method for Cd content determination (red line) and by Brdicka's method for MT content determination (blue line) where **a** is analysis of apoMT, **b** is analysis of QDs, and **c** is analysis of the mixture. All measurements were carried out with Sephadex FPLC column (HiLoad 26/60, 75 PG, GE Healthcare, Sweden)

corresponds to the finding introduced in “[Experimental Animals and Preparation of Samples for MT Isolation](#)”, because, in the case of complex, the signal is reduced by almost 85 %. Observed signal *a* representing complex of MT and QD is eluted 40 min earlier than CdTe QDs represented by signal *b* at applied flow rate of 4 mL/min.

Conclusions

Interaction of inorganic-based nanoparticles with biomolecules including DNA and proteins can be used for diagnosis and treatment purposes in medicine [47–49]. Therefore, there is a need for hyphenating of analytical methods using QDs for this purpose. In this study, we verified the formation of MT–QDs complex by the use of spectrophotometric and fluorimetric methods with subsequent separation using FPLC method. Considering that

role of MT in cancerogenesis is discussed [19, 50–57], these results could be considered as a base for some imaging technologies for MT in vivo visualisation.

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