ELECTROCHEMICAL DETECTION OF THE INTERACTION QUANTUM DOTS WITH METALLOTHIONEIN

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ABSTRACT

In the last period the interest of scientists is focused on nanoparticles (quantum dots, nanotubes, nanowires). Quantum dots (QDs) are widely studied. They can emit light radiation and from this reason they could be used like a fluorescence label for *in vivo* imaging. QDs can also bind proteins by unspecific binding. One from the most important protein in human body is metallothionein (MT), small cysteine-rich protein, which is responsible for binding of heavy metals, for accumulation of Zn, protection of cells to oxidative stress and it participates in the regulation of expression a number of major genes and enters to oxidative-reductive balance in a cell. From these reasons the interaction of MT with QD could play the important role at using QD in living organism. The study of this interaction is possible due to the electroactivity of both integrated components by electrochemical methods.

The aim of this experiment was the study complexes MT-QDs created during the interaction of metallothionein with CuS QDs. Complexes determined by peaks Cat1, Cat2, RS2Co, Y and X were investigated in Brdicka´s solution by the differential pulse voltammetry on mercury electrode. The used interaction time was: 0 and 480 s; 30, 60, 90 min, and 2, 3, 4, 5 and 6 hrs. Brdicka´s solution was used as an electrolyte.

Key words: electrochemical detection; DPV, Brdicka´s reaction, metallothionein, quantum dot, MT-QD interaction

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INTRODUCTION

Metallothionein, small cysteine-rich protein, has the molecular weight of 6–7 kDa. This protein has the tertiary structure based on the presence of two domains, which are easily forming cysteine clusters to bind metal ions (Skutkova, Babula et al. 2012). Metallothionein is able to bind up to 20 monovalent and up to 7 divalent heavy metal ions (Krizkova, Fabrik et al. 2009). The considerable function of MT is the protection of cells to oxidative stress (Lee, Park et al. 2008), metal ions transportation and detoxification of heavy metals (Krzeslak, Forma et al. 2012). Metallothionein´s role in anticancer therapy has been discussed (Grabellus, Sheu et al. 2010).

Materials with nano dimensions became very important in many applications, such a optoelectronic applications (Lee, Park et al. 2008), chemical sensors (Susha, Javier et al. 2006) and/or in the gene technology (Jamieson, Bakhshi et al. 2007). Such wide spread use is caused due to their physical and chemical properties (Talapin, Poznyak et al. 2002; Michalet, Pinaud et al. 2005; Chen, He et al. 2012) also optical properties (high photoluminescence quantum yield, strong photostability, wide absorption yield coupled with narrow emission [3]); their size is well controlled by temperature, duration and ligand molecules during the synthetic processes.

The interaction between MT and CuS is usually studied by optical and electrochemical methods (Krejcova, Dospivova et al. 2012). In this study the changes in the electrochemical signal during the interaction QDs with rabbit liver MT were studied and especially formation of peaks X and Y were investigated (and the other peaks as RS2Co, Cat1 and Cat2 peak) by different pulse voltammetry.

MATERIAL AND METHODS

Preparation of sample for isolation of MT

Two grams of defrosted rabbit liver was homogenized on ice using Ultra-turrax T8 in 8 mL of 10 mM Tris–HCl buffer (pH 8.6). The sample was subsequently vortexed and centrifuged at 5 000 rpm, 30 min at 4 °C. The supernatant was again centrifuged in micro test tube at 25 000 rpm, 30 min at 4 °C and after that the supernatant was subsequently heated in thermomixer for 10 min at 99° and centrifuged in micro test-tube at 25 000 rpm for 30 min at 4 °C. Sample prepared like this was used for isolation of MT. The next step of preparation was fast protein liquid chromatography for MT isolation. More details about isolation are described in (Skalickova, Zitka et al. 2013). MT contains different fractions was performed by SDS PAGE for MT Assay. For the interaction experiment MT-2 was used.

Preparation of CuS quantum dot

CuS QDs were prepared by reaction of copper acetate monohydrate $Cu(OAc)_2 \cdot H_2O$ (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 1M NH_4OH was added with stirring to yellow solution, followed by sodium sulfide nonahydrate $Na_2S \cdot 9H_2O$ (0.012 g, 0.05 mM) in 24.5 ml of ACS water. Color of solution turned to light brown.

Electrochemical detection

Electrochemical detection was done in the Brdicka´s solution by differential pulse voltammetry (DPV) (Heyrovsky and Norrish 1963). 20 μl of mixed sample (10 μl 0.8μM MT and 10 μl 500μM PbS QD) was injected into an electrochemical cell and then the electrolyte (1 980 μl) was added (total volume 2 ml). The interaction of MT-PbS was studied in the interaction time from 0 s to 6 hours at 4 °C. After the expiration of the interaction time the interaction was monitored and the voltammogram performed by using the electrochemical detection. Experiments in Brdicka´s solution are more detail described in (Petrlova, Potesil et al. 2006).

RESULT AND DISCUSSION

Fig. 1 shows the voltammograms of the interaction MT-2 with CuS QD at various interaction times as follows: 0, 480 s, 30, 60, 90 min, 2, 3, 4, 5 and 6 hrs. There were peaks X (-0.94 V), Y (-0.99 V), RS2Co (-1.24 V), Cat1 (-1.35 V) and Cat2 (-1.52 V). Fig. 1A performs the voltammogram for lower interaction times and inserts show details for peaks X, Y, Cat1 and Cat2. Fig. 1B demonstrates measured records for longer interaction time (2-6 hours) and details records for interesting peaks. From these pictures is clear, that the height of X and Y peaks with increasing interaction time increased and Cat2 and Cat1 decreased. Height of peaks is evaluated in the Fig. 1C and 1D, where the trend of dependence of individual peaks according interaction time is more visible. Peak X (blue cross) increased twenty times in the range of studied interaction time (from 2.3 nA at 180 min to 49.5 nA at 360 min). Till 180 min, the time of interaction influenced the peak height only insignificantly. Peak Y (red cross) in the lower interaction time decreased, this effect is probably caused by the main role of MT in this interaction. After 16 min the signal increased from 41 nA to 95 nA. In contrast to this increase of these peaks Cat2 peak decreased in the whole range. Cat2 is connected with catalytic reaction of protein with Brdicka´s solution (Petrlova, Potesil et al. 2006) and X, Y is probably linked with the interaction process of protein with QD (Petrlova, Potesil et al. 2006). Fig. 1D describes the changes of peak RS2Co and Cat1. RS2Co (green cross) decreases in 4 min and after that the signal has approximately similar value. In contrast this Cat1 decreased to loss of signal.

CONCLUSIONS

Interaction of CuS QD with MT was studied in our work in Brdicka´s solution by Brdicka catalytic reaction. New peaks X and Y are associated with creation of MT-QD complexes. This study presents new information about the interaction of QD with MT and brings basic electrochemical information about QD and its ability to interact with metallothionein.

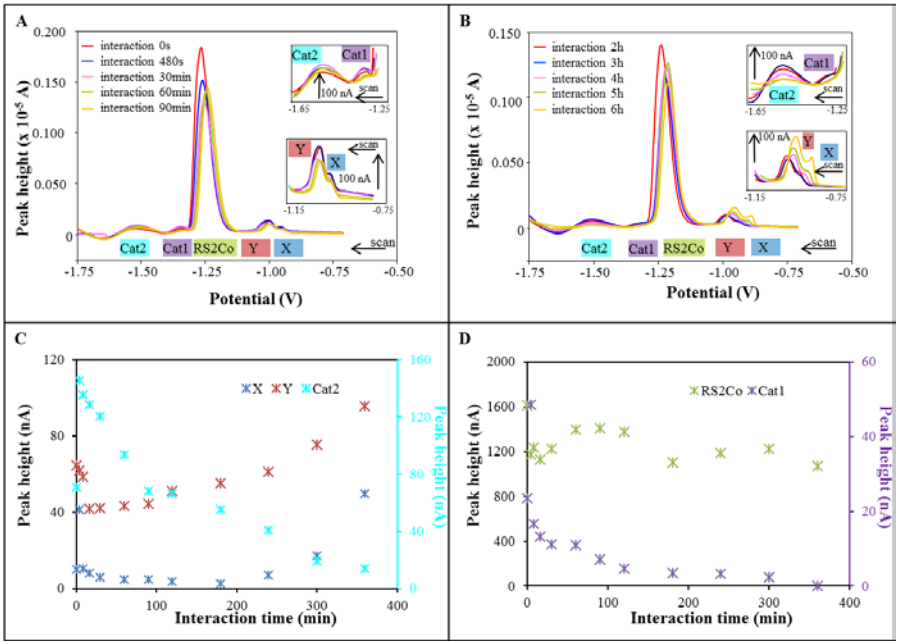

Fig. 1. Interaction MT with CuS QD. (A) Interaction of CuS with MT 0s, 480s, 30min, 60min and 90min. (B) Interaction of CuS with MT 2h, 3h, 4h, 5h and 6h. (C) Signal for tree peaks X (-0.94 V), Y (-0.99 V), Cat2 (-1.52 V). (D) Signal for more two peaks RS2Co (-1.24 V) and Cat1 (-1.35 V).

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