CHARACTERIZATION OF GOLD NANOPARTICLES-MODIFIED CDTE QUANTUM DOTS BY SCANNING ELECTROCHEMICAL MICROSCOPY

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Abstract

Gold-modified quantum dots (Au-QDs) are semiconductor nanocrystals, which serve as both optical and electrochemical tags. With these markers it is possible to label different types of molecules, which are complicated to detect otherwise. Scanning electrochemical microscope (SECM) is very suitable instrument for detection of surface electrochemical properties. Electrochemical detection of the redox label generates a specific tip current, which intensity depends on the local surface concentration of the redox macromolecules. Binding of various types of surface molecules to the QDs is accompanied by a change of detected current level. SECM provides detail characterization in pseudo 3D imaging with resolution in the order of micro to nanometre, depending on the size of the working electrode. Modification of CdTe quantum dots with gold was performed to enable specific interaction with selected oligonucleotide modified by thiol group. Two ways of synthesis were tested: 1) Conjugation with gold nanoparticles, 2) addition of HAuCl₄ with subsequent reduction by NaBH₄. The affinity between gold and SH-group of oligonucleotides creates the complex sterically convenient for hybridization of the complementary sequence. Moreover, the combination of optically and electrochemically active labels enables highly flexible multimodal detection.

Keywords: Quantum dots, scanning electrochemical microscope

1. INTRODUCTION

Quantum dots (QDs) are represented by nanometer-sized crystals or atomic clusters. QDs usually consist of few hundreds to a few millions of atoms, but only a small number of electrons (≤100) are free [5]. Typical QD sizes range between 2–20 nm. QDs are excellent candidates for biosensing given by their unique physical and optical properties [9]. Concerning the biological applications of QDs, two main groups may be cited: biosensors and labels. Wide application range in biosensing field is caused mainly by possibility of attaching various biomolecules to their surface. Because of this the core-shell structured QDs are more favourable due to their variations in shell structure (its easily possible modification). Recent advantages in using of QDs lead to a wide range of application area [1, 3, 7, 10, 11], mainly the optical properties are requisite. However, the electrochemical properties are very interesting and useful too [2]. QDs are mostly used as electrochemical tags [6, 8]. With these markers it is possible to label different types of molecules, which are complicated to detect otherwise. Electrochemical characterization of QDs is possible via the technique called scanning electrochemical microscopy (SECM). This technique is based on the scanning of the studying surface by ultramicroelectrode (UME) and electrochemical detection of surface options [12]. Such technique gives us the electrochemical picture of the surface. SECM employs an UME probe (tip) to induce chemical changes and collect electrochemical information while approaching or scanning the surface of interest (substrate). Application of SECM is very wide and allows study various structures and processes in micro and submicrometer-sized systems. The target of detection could be electron, ion, and molecule transfers, and other reactions at solid-liquid, liquid-liquid, and liquid-air interfaces. In recent years the study of nanoparticles and its application rapidly increases [4]. Our attention is focused on the gold nanoparticles...
modified quantum dots (Au-QDs) and theirs electrochemical properties. Combination of such particles with biocompounds suitable for biosensors construction (in our case oligonucleotides) is reflecting too.

2. MATERIALS AND METHODS

2.1 Au-QDs synthesis

2.1.1 Synthesis of MSA-CdTe QDs

General method for preparation of CdTe QDs was as follows: 5 ml of Cd(OAc)$_2$$
\cdot$$2\text{H}_2\text{O}$ (0.266 g/50 ml) was diluted with water (20 ml) and mercaptosuccinic acid (MSA) (30 mg in 1 ml of water) was added with stirring. pH of solution was adjusted to 8.13 by addition of 1 M NH$_3$ (0.9 ml). Afterwards, Na$_2$TeO$_3$ (0.0066 g) in water (23 ml) was added, stirred for 30 min and solid NaBH$_4$ (20 mg) was added. After 1 h of stirring, 2 ml of solution was pipetted into reaction vessel and heated in Multiwave 3000 Microwave Reaction System (Anton Paar, Graz, Austria) using rotor 64MG5. The reaction conditions were as follows: temperature 50-130°C, power 300 W and time of heating 10-18 minutes. Prepared CdTe QDs were stored in dark at 4°C.

2.1.2 Synthesis of gold nanoparticles (AuNPs)

The solution of 1mM auric acid was prepared by dissolving of 0.0197 g of HAuCl$_4$$
\cdot$$3\text{H}_2\text{O}$ in 50 ml of water and to the 10 ml of this solution 250 µl of sodium citrate was added (26.5 mg/1 ml). The reaction is completed after colour turns purple (1 hour).

2.1.3 Synthesis of Au QDs using AuNPs

500 µl of MSA-CdTe QDs (concentration of Cd 0.6 µg/ml, green and red light emitting) was mixed with 100 or 200 µl of AuNPs shaken for 2 hours and subsequently diluted up to the volume of 1 ml. Sample labelling:

1 – green QDs with addition of 100 µl of AuNPs, 2 – green QDs with addition of 200 µl of AuNPs, 5 – red QDs with addition of 100 µl of AuNPs, 6 – red QDs with addition of 200 µl of AuNPs

2.1.4 Synthesis of Au QDs using HAuCl$_4$

500 µl of MSA-CdTe QDs (green and red light emitting) was mixed with 100 or 200 µl of 1mM HAuCl$_4$ and 30 mg of NaBH$_4$ and shaken for 2 hours and subsequently diluted up to the volume of 1 ml. Sample labelling:

3 – green QDs with addition of 100 µl of HAuCl$_4$, 4 – green QDs with addition of 200 µl of HAuCl$_4$, 7 – red QDs with addition of 100 µl of HAuCl$_4$, 8 – red QDs with addition of 200 µl of HAuCl$_4$

2.2 X-ray fluorescence analysis

CdTe QDs and Au-QDs were measured on Spectro Xepos (Spectro Analytical Instruments, Kleve, Germany). The sample was measured on a Pd anode X-ray tube working at a voltage of 47.63 kV and a current of 0.5 mA and detected with Barkla scatter aluminium oxide. Measurement time was 300 s. For excitation a Mo secondary target was used. The excitation geometry was 90°. The QDs was measured through the PE bottle side wall 20 mm above the bottom. The Spectro Xepos software and TurboQuant method were applied to data analysis.
2.3 Scanning electrochemical microscopy

The scanning electrochemical microscope SECM 920C from CHInstruments (Austin, USA) was used for measurements. For measurement the four electrode setup was used. A platinum wire served as an auxiliary electrode and the reference electrode was Ag/AgCl. The substrate electrode was created by gold disk with a diameter of 3 mm. As a tip the platinum microelectrode with a diameter of 10 µm was used. The substrate electrode surface has been cleaned by sonication in ethanol, and then was treated for two minutes with a pyrana solution, which was made from 30% hydrogen peroxide and concentrated sulphuric acid in a ratio of 1:3 and scanned from -0.2 to +1.5 V until a reproducible cyclic voltamogram was obtained. Finally, the electrode was washed with ACS water and dried with nitrogen. After cleaning the measurement was carried out in 0.1 mM KCl (electrolyte) and 1 mM ferrocenemethanol (for amplification) in ratio 1:1 (total volume 1.5 ml). The scanning procedure was done with optimized parameters defined with previous determinations by cyclic voltammetry of mediator solution and approaches curves determination. The potential of the platinum tip was set to the 0.3 V and the potential of substrate electrode for the 0.35 V. The distance between tip and substrate electrode was set to the 10 µm. The area of 1000 x 1000 µm was scanned with the scan rate 1 µm for 0.2 s.

2.3.1 Au-QDs SCEM analysis

30 µl of prepared Au-QDs solution was dropped on the modified substrate electrode and was allowed to evaporate for 30 minutes. Subsequently 1.5 ml of the mediator (1 mM ferrocenemethanol in 0.1 mM KCl) was added and the surface scanning with optimized parameters was initiated.

2.3.2 DNA sensing

In the first step the bare electrode was scanned followed by the coating with 30 µl of avidin aqueous solution (100 µg/ml). After the evaporation of water (30 minutes), the mediator (1.5 ml) was added and the surface was scanned. The next step contained the avidin coating followed by coating by 30 µl of biotinylated oligonucleotide (200 µg/ml, biotin-5´-AGATGAGGCATAGCAGCAGGATG-3´) and after evaporation and mediator addition the measurement was carried out. Then, the layer of immobilized avidin coated by biotinylated oligonucleotide was covered by 30 µl of complementary SH-labelled oligonucleotide (200 µg/ml, SH-5´-CATCCTGCTGCTATGCCTCATCT-3´) and the result was measured. In the last step, the whole process was repeated and prepared Au-QDs were added. After evaporation (30 minutes between each layer) and mediator addition final measurement was carried out using the condition described above.

3. RESULTS AND DISCUSSION

Due to the great fluorescent properties of QDs, they are often used as fluorescent labels, however also electrochemical labelling by QDs has been described. Specific interaction between the targeted molecule and the QD can be realized by various manners. One of them is the utilization of the great affinity between thiol group and gold atoms. Therefore we proposed two ways of synthesis of gold modified QDs. The first method employs the incubation of MSA capped CdTe with gold nanoparticles and the second uses the addition of HAuCl₄ and its reduction by NaBH₄. To investigate these two approaches, two types of QDs were used – green and red light emitting QDs. Moreover two concentrations of gold sources were employed and therefore 8 types of QDs were obtained. One of the main purposes of the synthesis was to maintain the fluorescent properties of the QDs, which was successful as shown in the insets of Fig. 1A a B. The elemental characterization was performed by X-ray fluorescence analysis (data not shown).
Fig. 1 Dependence of the current levels of the Au-QDs A) Au-QDs prepared using different concentrations of AuNPs B) Au-QDs prepared using different concentrations of HAuCl\textsubscript{4} (insets: fluorescence photographs of the particular solutions under UV light illumination) Current level is the average value of detected current over the surface of 1000 x 1000 µm.

These Au-QDs were characterized by SECM and as shown in Fig. 1A and B the changes of detected current levels were detected. From the results it can be concluded that in the case of green QDs the increasing amount of applied gold led to the increasing coverage of the QD particles. This assumption is based on the better electron transfer between redox mediator and the surface of substrate electrode which is created just with the modified QDs. Moreover the same trend was observed for both ways of preparation of green QDs. On the other hand, in case of red QDs the current level did not changed significantly with increasing amount of applied gold which suggested saturation of the surface of the QD.

Prepared Au-QDs were next utilized for testing of the DNA sensing according to the scheme shown in the Fig. 2. Briefly, the gold electrode was coated by avidin, followed by biotinylated oligonucleotide. Subsequently the hybridization of the complementary oligonucleotide labelled by SH group took place followed by interaction with Au-QDs.

![Diagram](image)

Fig. 2 Scheme of the coating process. 1) Au electrode, 2) avidin coating, 3) immobilization of biotin-labelled oligonucleotide, 4) hybridization of the SH-labelled oligonucleotide, 5) interaction with Au QDs.

The current level changes were monitored in each step of the experiment and it was observed that interaction of each component of the reaction can be monitored by the SECM. Due to the main advantage of the SECM which is the recognition of the spatial distribution of the reagents we believe that the proposed method can be utilized as a DNA sensor. In the Fig. 3 the topological representation of the electrode partially coated according to the above described procedure is shown. In Fig. 3E the 2D-view is showing the borderline between the bare electrode (green colour) and the coted electrode (yellow colour). The significant...
decrease of the current level caused by the coating is obvious. In the Fig 3F the 3D-view of the same situation is shown.

Fig. 3 A) SECM scan B) SECM scan of electrode coated by avidin, C) SECM scan of electrode coated by avidin followed by biotinylated oligonucleotide, D) SECM scan of electrode coated by avidin followed by biotinylated oligonucleotide and hybridized SH-labelled complementary oligonucleotide E) SECM scan of the gold substrate electrode partially coated by avidin, biotinylated oligonucleotide, SH-oligonucleotide and Au-QD - 2D-view F) 3D-view of E.
CONCLUSION

Two approaches of synthesis of Au-QDs were proposed in this work and the resulting nanoparticles were characterized using SECM. By this method it was shown that both synthetic routes provided nanoparticles with similar electrochemical characteristics and moreover their fluorescent properties have been maintained. Additionally, the interaction of these nanoparticles with thiol tagged oligonucleotide was investigated utilizing avidin-biotin interaction as well as hybridization reaction of complementary DNA sequences.

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LITERATURE