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Interaction of E6 Gene from Human Papilloma Virus 16 (HPV-16) with CdS Quantum Dots

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Abstract The aim of this study was to analyze the interactions of blue and yellow fluorescent CdS quantum dots (CdS-QDs) with human papillomavirus 16 (HPV-16) oncogene E6. The interactions were investigated using chip capillary electrophoresis, spectrophotometry and square wave voltammetry (SWV). Using chip capillary electrophoresis we proved that blue fluorescent CdS-QDs (0.5 mM) caused an increase of the migration time of the E6 HPV-16 DNA–CdS-QDs complex by 42 s compared to control DNA (E6 HPV-16). The same concentration of yellow fluorescent CdS-QDs caused an increase in the migration time of the DNA–CdS-QDs complex by 108 s compared to the control DNA (E6 HPV-16). The difference in the migration times between both complexes was 66 s. Using square wave voltammetry (SWV), the reduction signal of cytosine and adenine (peak CA) was observed, after the complex with 2.5 µg mL⁻¹ DNA was formed. A decrease of the peak CA reduction signal of the complex DNA–CdS-QDs by 90 % was caused when yellow fluorescent CdS-QDs (0.03 mM) were used. The same concentration of blue fluorescent CdS-QDs caused only a 50 % decrease of the C and A reduction signal of the DNA–CdS-QDs complex. The difference between both CdS-QDs was 40 %. Electrochemical measurements and chip electrophoresis analyses confirmed that the yellow fluorescent CdS-QDs show higher affinity to the DNA (E6 HPV-16) compared to blue ones.

Keywords Chip capillary electrophoresis · Square wave voltammetry · DNA interaction, human papilloma virus · E6 HPV-16 gene · Cadmium sulphide quantum dots

Introduction

Human papillomaviruses (HPVs) are small circular, double-stranded DNA viruses infecting epithelial tissues [1]. The principal agent in the etiology of cervical cancer, i.e., human papillomavirus type 16 (HPV-16), encodes two oncoproteins as E6 and E7 [2]. These viral oncoproteins play important roles in regulation of viral functions during the viral life cycle and also contribute to the development of cancers [3]. Due to numerous unclear mechanisms, some methods enabling us to monitor changes in vivo are searched for.

The quantum dots (QDs) belong to the new tool for fluorescence imaging of biological tissues, cancer targeting and diagnostic purposes [4–6]. The functionalized surface of QDs could be coupled with various biomolecules as aromatic heterocyclic compounds (naphthyridine) [7], proteins (metallothionein) [8] and DNA probes [9, 10]. Especially the nucleic acid can serve as robust ligand for aqueous synthesis of semiconductor nanocrystals or...
quantum dots [11]. It was shown that amino acid-modified QDs could be used as useful siRNA carriers to effectively silence a target HPV E6 gene as well as fluorescence probes for intracellular imaging in vivo [12]. QDs could also be used for detection of HPV viral particles in the cervical swamp samples using the biological separation and total time for detection is no more than 1 h [13]. Interaction of DNA with QDs can also be used to create biosensors utilizing fluorescence, [14] electrochemiluminescence or resonance energy transfer [15, 16].

The QDs fluorescence can be quenched by platinum anticancer drugs via photo-induced electron transfer process, rendering the system into “turn off” status, and the system can then be “turned on” when fluorescence is restored due to covalent conjugation between DNA [17]. These mechanisms may be used for studying the interactions between DNA and cytostatic drugs [18]. The binding of the QDs with dsDNA could be investigated by indirect electrochemical [19, 20] or spectrophotometric methods [20, 21].

The most widely used method for the separation of samples in a chip is capillary electrophoresis (CE) [22, 23]. Laser-induced fluorescence is the most generally used detection method in CE separations [24]. The main advantages of micro-chips include mobility, speed of analysis, low sample or reagent consumption and control of reaction conditions [25]. The binding between QDs and DNA could be investigated by CE, due to the shift in the electrophoretic mobility of QDs as a consequence of DNA binding [26].

In this paper the interactions of both blue and yellow fluorescent CdS-QDs with the gene encoding the HPV-16 protein E6 were observed. For this purpose the chip capillary electrophoresis, UV/vis spectrophotometry, and square wave voltammetry (SWV) were used. The main aim of this work was to investigate if different sizes of quantum dots affects their affinity for DNA. In this study, we used methods that have not yet been used in publications for this purpose such as square wave voltammetry and chip capillary electrophoresis.

**Experimental Section**

**Chemicals**

Working solutions like buffers and standard solutions were prepared daily by diluting the stock solutions. Standards and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity unless noted otherwise.

**Preparation of CdS-QDs**

Blue fluorescent CdS quantum dots (QDs) were prepared with a slightly modified method published in [27]. Mercaptosuccinic acid (45 mg) was added slowly to the stirred solution of Cd (OAc)\(_2\)·2H\(_2\)O (26.6 mg) in water (25 ml). Afterwards, pH was adjusted to 7.14 with 1 M NH\(_3\) (1.3 ml) and Na\(_2\)S·9H\(_2\)O (4.8 mg) in 23.7 ml of water and was poured into the first solution with vigorous stirring (1 h). Yellow fluorescent CdS-QDs were prepared from original blue fluorescent CdS-QDs, which were dried at 80 °C for 24 h and subsequently scraped and resuspended in distilled water. CdS-QDs were stored in dark at 4 °C.

Cloning and Amplification of E6 Human Papillomavirus 16 Gene using Polymerase Chain Reaction

The gene E6 of human papillomavirus 16 (GenBank accession number: BAN15931) was synthesized and cloned into the plasmid pUC57-Amp (GENEWIZ Gene Synthesis, Sigma-Aldrich, St. Louis, MO, USA) resulting in pUC57 vector containing the E6 HPV-16. The chemical transformation protocol was performed following the instructions of Invitrogen, using TOP10 chemically competent E. coli strain as host. Bacteria transformed with pUC57-HPV-E6 plasmid were selected by ampicillin resistance. The positive transformants were confirmed by polymerase chain reaction (PCR) screening. The plasmid was purified using the Qiagen Miniprep Kit (Qiagen, Maryland, USA) and the amplification of gene was done using a set of primers flanking the complete open reading frame 5-ATGCACCAAAAGAGAACTGC (HPV-E6fw) and 5-TTACAGCTGGTGTTCTCTAC (HPV-E6re) by PCR. The amplified product was analyzed by agarose gel electrophoresis and the conditions were as follows: 2 % agarose gel (High melt/Medium fragment, Mercury, Sand Diego, CA, USA) with 1 x TAE buffer, 60 V and 160 min (Bio-Rad, Hercules, CA, USA). The 100 bp DNA ladder (New England Biolabs, Ipswich, MA, USA) was used as a molecule size marker. Bands were visualized using UV transilluminator at 312 nm (Vilber-Lourmat, Marne-la-Vallée Cedex, France). Prepared E6 HPV-16 gene was characterized by MALDI-TOF, electrochemical method and UV/vis spectrophotometry, see S1.

**Chip Electrophoresis**

Analyses were carried out in DNA chip using automated microfluidic electrophoresis system Experion (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions with supplied components: Experion priming station, Vortex station and Spin filters (Bio-Rad), and chemicals Experion DNA 1 K reagents and supplied kit containing: DNA gel, DNA stain, DNA loading buffer, and DNA ladder (Bio-Rad). Experion Software v. 3.2 (Bio-Rad) was used for processing of the data obtained. Briefly
to the protocol, the priming was performed as follows: 9 μL of GS (gel mixed with fluorescent stain) was pipetted into the well labelled GS (gel priming well) and primed using the priming station. Loading of the chip was done according following procedure. First, 9 μL of GS was pipetted into the three other wells labelled GS. Then, 5 μL of loading buffer was placed into each sample well and the ladder well. Subsequently, 1 μL of DNA ladder was pipetted into the well labelled L and 1 μL of each sample was pipetted into sample wells. Before the CE analysis, the wells were carefully inspected to avoid air bubbles. The chip was vortexed for 60 s to ensure the mixing and immediately analyzed on Experion electrophoresis station.

For optimisation of chip capillary electrophoresis, see S2.

UV/vis Spectrophotometry

Spectra of CdS-QDs were recorded within the range 200–800 nm using quartz cuvettes (1 cm, Hellma, Essex, UK) on a spectrophotometer SPECORD 210 (Analytik Jena, Jena, Germany) at 25 °C Julabo (Labortechnik, Wasserburg, Germany). Denaturation of 2.5 μg mL⁻¹ DNA (E6 HPV) was monitored spectrophotometrically using a spectrophotometer SPECORD S600 with a diode detector (Analytik Jena, Jena, Germany). The sample was incubated for 3 min at the increasing temperatures within the range 25–99 °C and the absorbance was measured within the range 200–800 nm. Changes in absorbance spectra samples were recorded during denaturation and evaluation of the program WinASPECT version 2.2.7.0.

Fluorescence Measurement

Fluorescence spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Männedorf, Switzerland). 350 and 400 nm were used as an excitation wavelength and the fluorescence scan was measured within the range 400–750 nm per 2 nm. Each intensity value was 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 (ThermoFisher Scientific, Waltham, MA, USA). All measurements were performed at 25 °C controlled by Tecan Infinite 200 PRO (TECAN, Männedorf, Switzerland).

Electrochemical Determination of Cadmium in CdS-QDs

Determination of cadmium in quantum dots was performed with 746 VA Stand instrument connected to 693 VA Processor and 695 Autosampler (Metrohm AG, Herisau, Switzerland), using a standard cell with three electrodes by differential pulse voltammetry. 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 glassy carbon electrode was auxiliary. For data processing GPES 4.9 software was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999 %). Acetate buffer (0.2 M CH₃COONa and CH₃COOH, pH 5) was used as a supporting electrolyte. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: purging time 90 s, deposition potential −0.8 V, accumulation time 240 s, equilibration time 5 s, modulation time 0.057 s, interval time 0.2 s, initial potential −0.8 V, end potential 0 V, step potential 0.004 V, modulation amplitude 0.025 V, volume of injected sample: 10 μL, volume of measurement cell 2 mL (10 μL of sample and 1,990 μL acetate buffer).

Similarly, the calibration curve of Cd²⁺ standard solution (Cd(NO₃)₂·4H₂O) was prepared and measured. The concentration range of Cd²⁺ was 0.125–2 mM.

Electrochemical Measurements of DNA

Determination of DNA was performed with 797 VA Stand instrument connected to 889 IC Sample Center (Metrohm, AG, Herisau, Switzerland). The analyser (797 VA CompuTrace, Metrohm, Switzerland) employs a conventional three-electrode configuration with a hanging mercury drop electrode (HMDE), with a drop area of 0.4 mm² as the working electrode. An Ag/AgCl/3 M KCl electrode was the reference and glassy carbon electrode was auxiliary. For data processing GPES 4.9 software was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999 %). Acetate buffer (0.2 M CH₃COONa and CH₃COOH, pH 5) was used as a supporting electrolyte. The supporting electrolyte was exchanged after each analysis. The parameters of the square wave voltammetry were as follows: purging time 120 s, frequency 280 Hz, accumulation time 2 min, initial potential 0 V, end potential −1.8 V, step potential 0.005 V, modulation amplitude 0.025 V.

MALDI-TOF MS

The interaction of DNA and QDs was characterized by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). The mass spectra of the compounds of interest were measured on a MALDI-TOF/TOF mass spectrometer Bruker ultrafleXtreme (Bruker Daltonik GmbH, Bremen, Germany) equipped with a laser operating at wavelength of 355 nm with an accelerating voltage of 25 kV, cooled with nitrogen and with a maximum energy of 43.2 μJ. The matrix used in the
MALDI method was 3-hydroxypicolinic acid (3-HPA; Sigma-Aldrich, St. Louis, MO, USA). The saturated solution of 3-HPA was prepared in 50% acetonitrile with 10 mg/mL diammonium hydrogen citrate. Matrix mixtures were vortexed and ultrasonicated using Bandelin 152 Sonorex Digital 10P ultrasonic bath (Bandelin electronic GmbH, Berlin, Germany) for 2 min at 50% intensity at room temperature. A dried-droplet method was used for sample preparation. Briefly, the sample solution was mixed with matrix solution in volume ratio 1:1. After obtaining a homogeneous solution, 1 µL of mixture was applied on the target plate and dried under atmospheric pressure at room temperature. A mixture of protein and peptide calibration standards was used to externally calibrate the instrument. All measurements were performed in the linear positive mode in the m/z range 1–20 kDa. The mass spectra were acquired by averaging 2,500 subspectra from a total of 2,500 shots of the laser. Laser power was set 5–10% above the threshold.

Results and Discussion

The Characterization of CdS-QDs

Quantum dots (QDs) are fluorescent nanoparticles, which receive the increasing attention as a viable alternative to conventional organic fluorophores for molecular labelling [28, 29]. The size of the CdS-QDs can vary between 2 and 10 nm according to their preparation [27]. In our experiment two types of cadmium sulphate quantum dots (CdS-QDs) were used.

The both CdS-QDs differed in their size. The sizes of CdS-QDs were determined according to the emission spectra; 3 nm = yellow fluorescent CdS-QDs (Fig. 1aa) and 1.5 nm = blue fluorescent QDs (Fig. 1bb) [29]. Simultaneously the control sample (distilled water) was tested with negative fluorescence (Fig. 1cc). CdS-QDs were visualized using UV transilluminator. The stock solutions of CdS–QDs were quantified using the

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The obtained Cd²⁺ reduction signal was consistent within the tolerance 5% (RSD) and corresponded with the concentration of 1 mM Cd²⁺ (Fig. 1b, c). The signal of Cd²⁺ was not obtained for the control sample as distilled water (Fig. 1d).

The highest fluorescence recorded by Tecan Infinite 200 PRO was obtained for the yellow fluorescent 1 mM CdS-QDs (\( \lambda_{\text{Ex}} = 350 \) nm, Fig. 1e–aa). At the blue fluorescent CdS-QDs, a twice less fluorescence intensity was obtained in comparison to yellow ones \( \lambda_{\text{Ex}} = 350 \) nm (Fig. 1e–bb). The control sample (distilled water) did not show the fluorescence activity (Fig. 1e–cc). In the case of yellow fluorescent CdS-QDs, the increase of excitation wavelength by 50 nm (\( \lambda_{\text{Ex}} = 400 \) nm) caused the decrease in fluorescence intensity by 400% (Fig. 1f–aa). On the other hand, the fluorescence intensity of blue fluorescent 1 mM CdS-QDs increased by 25% (Fig. 1f–bb). The fluorescence intensity of the control was not changed (Fig. 1f–cc).

The yellow fluorescent 1 mM CdS-QDs had the absorbance maximum at \( \lambda = 355 \) nm (Fig. 1g) and the blue fluorescent 1 mM CdS-QDs at \( \lambda = 378 \) nm (Fig. 1h). The control sample did not absorb in the mentioned wavelengths (Fig. 1i).

The Interactions of CdS-QDs with DNA (E6 HPV-16)

As mentioned above, the blue fluorescent CdS-QDs (1.5 nm) and yellow fluorescent CdS-QDs (3 nm) were prepared and characterized. Using the chip electrophoresis proved that yellow fluorescent particles had a higher affinity to the DNA (E6 HPV-16), compared with the blue fluorescent particles (1.5 nm). The CdS-QDs interactions with DNA were investigated based on the change of the migration time of the complex 2.5 mM DNA (E6 HPV-16) with 0.03–0.5 mM CdS-QDs. The biggest migration time changes showed the yellow fluorescent CdS-QDs (Fig. 2a). The migration time of 2.5 µg mL⁻¹ DNA (E6 HPV-16) corresponded to 84.7 min (Fig. 2a–aa). The addition of 0.5 mM of the yellow fluorescent CdS-QDs to E6 HPV 16 DNA caused an increase in the

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**Fig. 2** The electrophoreogram of 2.5 µg mL⁻¹ DNA (E6 HPV-16) in the complex with: a yellow and b blue fluorescent CdS-QDs. c The comparison of the migration times of 2.5 µg mL⁻¹ DNA (E6 HPV-16) in the complex with 0–0.5 mM yellow and blue fluorescent CdS-QDs.
migration time of the complex DNA–CdS-QDs to 1.8 min compared to the control DNA (E6 HPV–16, Fig. 2a–ff). The lower concentrations (0.03–0.25 mM) of CdS-QDs caused the linear decrease in the migration time from 86.3 to 85.6 min (Fig. 2a–bb, cc, dd, ee). The less noticeable decrease of the reduction CA peak approximately by 2.5 % (Fig. 2d–ff). The lower concentrations (0.03–0.25 mM) caused the linear decrease in the migration time from 86.3 to 85.6 min (Fig. 2a–bb, cc, dd, ee). The concentration 0.5 mM of the blue fluorescent CdS-QDs caused the migration time increase of the complex DNA–CdS-QDs by 0.7 min compared to the control DNA (E6 HPV–16), which is by 46 % less than 0.5 mM yellow fluorescent CdS-QDs (Fig. 2b–ff). The higher concentrations (0.03–0.25 mM) caused the linear decrease in the migration times (85.2–84.8 min) similarly as in the previous case (Fig. 2a–bb, cc, dd, ee). Migration time comparison of the examined complexes DNA–CdS-QDs is summarized in the Fig. 2c. The DNA–CdS-QDs interaction was proved using square wave voltammetry (SWV). First reports about electrochemical reduction and oxidation signal of nucleic acids were published by the end of the 1950s [31]. It was pointed out that these signals are due to residues of bases in DNA. Adenine and cytosine in DNA yielded reduction signals (CA peak) [32]. The cytosine (C) and adenine (A) reduction signal (peak CA) of 2.5 µg mL⁻¹ DNA (E6 HPV–16) after the CdS-QDs application was observed. 0.03 mM yellow fluorescent CdS-QD caused the 89 % decrease (Fig. 2d–bb) of the reduction CA peak in comparison to control (2.5 µg mL⁻¹ DNA, Fig. 2d–aa). Higher concentrations caused the decrease of the reduction CA peak approximately by 2.5 % (Fig. 2d–cc, dd, ee, ff). The yellow fluorescent CdS-QDs highest concentration (0.5 mM) had no reduction CA peak (Fig. 2d–ff). The interactions of the blue fluorescent CdS-QDs and DNA were performed in the same way. The 0.03 mM blue fluorescent CdS-QDs caused approximately the 49 % decrease of reduction CA peak (Fig. 2d–bb) in comparison to the control DNA (Fig. 2d–aa). This decrease is by 40 % less than in the case of yellow fluorescent CdS-QD. Higher concentrations (0.06–0.5 mM) caused the decrease of reduction CA peak approximately by 5 % (Fig. 2e–cc, dd, ee, ff). The comparison of the DNA–CdS-QDs reduction CA peaks is summarized in Fig. 2f. The most likely explanation of DNA interactions with CdS-QDs lie in the fact that nanoparticles that could interact with DNA via various types of the interactions discussed previously and ds DNA interacts stronger with QDs than ssDNA [20, 33]. Further, we found that the interactions between DNA and CdS-QDs were dependent on their size. We assume that the smallest particles (QDs) could bind to the DNA with hydrogen bonds, whereas the bigger particles use the van der Waals interaction in the major groove [34].

Conclusions

In this work the E6 oncogene of the E6 HPV–16 protein was cloned, amplified and analyzed. The target gene (E6 HPV–16) was mixed with various concentrations of yellow and blue fluorescent CdS-QDs. Using the chip electrophoresis and SWV was found that bigger CdS-QDs (yellow fluorescent ones) had a stronger affinity to the DNA (E6 HPV–16) compared with smallest blue fluorescent CdS-QDs. Based on the change of the complex DNA–CdS-QDs migration times and the change of the reduction CA peaks, DNA interactions were investigated, which can be used for diagnosis and treatment purposes [6].

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Conflict of interest The authors have declared no conflict of interest.

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