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Electron-transfer quenching of nucleic acid-functionalized CdSe/ZnS quantum dots by doxorubicin: A versatile system for the optical detection of DNA, aptamer–substrate complexes and telomerase activity

Sara Raichlin[a,1], Etery Sharon[a,1], Ronit Freeman[a], Yehuda Tzfati[b], Itamar Willner[a,*]

[a] Institute of Chemistry, The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
[b] Department of Genetics, The Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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ABSTRACT

The optical detection of DNA or the sensing of low-molecular-weight substrates or proteins by aptamer nucleic acids is a long term challenge in the design of biosensors. Similarly, the detection of the telomerase activity, a versatile biomarker of cancer cells, is important for rapid cancer diagnostics. We implement the luminescence quenching of the CdSe/ZnS quantum dots (QDs) as a versatile process to develop DNA sensors and aptasensors, and to design an analytical platform for the detection of telomerase activity. The formation of nucleic acid duplexes on QDs, or the assembly of aptamer–substrate complexes on the QDs (substrate = cocaine or thrombin) is accompanied by the intercalation of doxorubicin (DB) into the duplex domains of the resulting recognition complexes. The intercalated DB quenches the luminescence of the QDs, thus leading to the detection readout signal. Similarly, the telomerase-induced formation of the telomere chains on the QDs is followed by the hybridization of nucleic-acid units complementary to the telomere repeat units, and the intercalation of DB into the resulting duplex structure. The resulting luminescence quenching of the QDs provides an indicating signal for the activity of telomerase.

1. Introduction

Substantial research efforts are directed to the development of optical DNA sensors (Peter et al., 2001; Wood, 1993) and aptasensors (Pavlov et al., 2005; Ho and Leclerc, 2004). Colorimetric DNA sensors have implemented the catalytic or biocatalytic generation of colored products, as a result of DNA recognition, or the formation of aptamer–substrate complexes. Chemiluminescent optical DNA sensors or Aptasensors that used enzyme- (Patolsky et al., 2003b), DNAzyme- (Pavlov et al., 2004; Li et al., 2007), or metal nanoparticle-(Niazov et al., 2004) mediated generation of chemiluminescence as a result of DNA analysis or the formation of aptamer–substrate complexes were developed. A further optical sensing platform for the sensing of DNA or aptamer–substrate complexes included surface plasmon resonance (SPR) spectroscopy (He et al., 2000; Golub et al., 2009).

Semiconductor quantum dots (QDs) exhibit unique luminescence properties (Alivisatos, 1996), High luminescence quantum yields, narrow luminescence bands (Willard et al., 2001), high stability against photodegradation, and most importantly size-controlled luminescence (Brus, 1991; Grieve et al., 2000; Alivisatos, 2004) represent attractive photophysical properties of QDs that enable their use as optical labels for sensing events. Indeed, many QDs-based biosensors were developed, and the subject was extensively reviewed (Gill et al., 2008). Specifically, QDs of variable sizes were used for the multiplexed analysis of different DNAs (Kim et al., 2009; Goldman et al., 2002). Also, fluorescence resonance energy transfer (FRET) processes were used to detect DNA hybridization events (Kim et al., 2004; Patolsky et al., 2003a), and the formation of the aptamer–substrate complexes (Freeman et al., 2009). Similarly, QDs modified with hemin/G-quadruplex labels were used for the optical detection of DNA (Sharon et al., 2010b). Previous studies have implemented the intercalation property of doxorubicin, DB, into duplex DNA (Liao et al., 2005) for the optical imaging of DNA hybridization in the presence of QDs (Freeman et al., 2007). In the present study, we develop a general sensing platform for the analysis of DNA and aptamer–substrate complexes by the intercalation of doxorubicin into the resulting duplex nucleic acids recognition complexes, associated with quantum dots. The electron-transfer quenching of the QDs provides the readout signal for the sensing events. We further implement the quenching of the luminescence of QDs by doxorubicin, incorporated into duplex DNA, to follow the activity of telomerase in cancer cells. During the cell life-cycle the telomeres are constantly shortened, and at a limiting length they signal the cells to stop proliferation and end the cells' life cycle

* Corresponding author. Tel.: +972 2 6585272; fax: +972 2 6527715.
E-mail address: willnea@vms.huji.ac.il (I. Willner).
[1] Equal contributors to the study.

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(Bryan and Cech, 1999; Olovnikov, 1996). Telomerase, a ribonucleoprotein, catalyzes the elongation of the telomeres by constant repeat units. As this biocatalytic process opposes the natural shortening of the telomeres, the cell is not signaled to terminate its life cycle and immortal malignant or cancerous cells are generated. Indeed, in over 85% of different cancer cells elevated amount of telomerase were detected (Shay and Bacchetti, 1997; Kim, 1997) and it is considered as a versatile biomarker for cancer cells. Different sensing platforms for the detection of telomerase were reported. The most frequently used method is the telomeric repeat amplification protocol (TRAP) (Kim et al., 1994), which is PCR-based. Electrochemical detection of the telomeric tetraplexes generated by telomerase was achieved using ferrocenyl naphthalene diimide as label (Sato et al., 2005). Optical methods for detection of telomerase activity include the fluorescence resonance energy transfer (FRET) process between quantum dots and an acceptor dye incorporated into telomeres synthesized by telomerase on the quantum dots (Patolsky et al., 2003a). Also, nanotechnology-based detection of telomeres by magnetomechanical deflection of cantilevers has been reported (Grimm et al., 2004). Although substantial progress has been achieved in the analysis of telomerase activity, the sensitivity of the various platforms is usually insufficient and requires a preceding PCR amplification step.

2. Materials and methods

2.1. Materials and reagents

Hops yellow core–shell EviDots, CdSe/ZnS quantum dots, 5.8 ± 0.3 nm, in toluene were purchased from Evident Technologies. The oligonucleotides, HPLC-purified, Sigma Life Science (U.K.) were used as provided and diluted in 10 mM phosphate buffer solution, pH 7.4, to give stock solutions of 100 μM. The deoxynucleotide solution mixture, 25 μM each, and M13φ ssDNA were purchased from New England BioLabs.

The oligonucleotides used in the study are:

(1) 5′- $HS(CH_2)_6$GGGAGTCAAGAACAA-3′
(2) 5′- TTCGTTCTTGACTCCC-3′
(3) 5′- $HS(CH_2)_6$ CCCCCACGTTGTAAAACGACGGCCAGT-3′
(4) 5′- $NH_2(CH_2)_6$ TTCGTTCTTCAATGAAGTGGGACGACA-3′
(5) 5′- GGGAGTCAAGAACGAA-3′
(6) 5′- $NH_2(CH_2)_6$CACTGTGGTTGGTGTGGTTGG-3′
(7) 5′- CCAACCACAGTG-3′
(8) 5′- $HS(CH_2)_6$TTTTAATCCGTCGAGCAGAGT-3′
(9) 5′- CCCTAACCCTAAAAAA-3′

2.2. Preparation of nucleic acid-capped QDs

The preparation of glutathione (GSH)-functionalized QDs is detailed in the Supplementary Material Section 1S. To the GSH-capped QDs (1 nmol) in HEPES buffer solution, 200 μL, were added 100 μL of an N-(ε-maleimidocaproyloxy)succinimide ester (EMCS) or bis(sulfosuccinimidyl) suberate (BS^3) stock solution (1 mg/mL), and the mixture was shaken for 15 min. The QDs were purified by precipitation by the addition of 1 mL of methanol and 3 mg of NaCl, to remove excess of EMCS or BS^3, and the QDs were re-dissolved in 10 mM HEPES buffer solution (pH 7.4). The thiol or amine modified DNA stock solution, 1×10^{-4} M, was added, and then the resulting solution was shaken for 2½ h. Finally, the excess DNA was removed by precipitation of the QDs, and the purified particles were dissolved in phosphate buffer solution (100 μL, pH 7.4, 10 mM). The resulting QDs were stable for at least four weeks with no noticeable precipitation or change in their luminescence properties. The determination of the loading of the nucleic acids on the modified QDs is described in the Supplementary Material Section 2S.

2.3. Analysis of nucleic acid (*2*) and cocaine

The experiments were performed in a phosphate buffer solution (10 mM, pH = 7.4, 50 mM NaCl). The nucleic acid (**2**) or cocaine and (**5**) were added to a solution of (**1**)- or (**4**)-modified QDs that included DB, 1×10^{-8} M. The fluorescence was followed at time intervals of 5 min, for 1 h at room temperature, $\lambda_{excitation} = 430$ nm, $\lambda_{emission} = 610$ nm.

2.4. Analysis of M13φ DNA

The (**3**)-modified CdSe/ZnS nanoparticles were incubated in a solution containing the dNTPs mixture (2 mM each) in the presence of different concentrations of the M13φ DNA, T4 DNA Polymerase (15 U mL^{-1}) and DB, 1×10^{-8} M, in a buffer consisting of 10 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$ and 1 mM dithiothreitol. The luminescence spectra of the sample were followed after 15 min of incubation, at time intervals of 6 min, for 1 h at 37 °C, $\lambda_{excitation} = 430$ nm, $\lambda_{emission} = 610$ nm.

2.5. Analysis of thrombin

The (**6**)-modified QDs were incubated in the presence of (**7**) and DB, 1×10^{-8} M, in 20 mM Tris buffer (pH = 8.2, 5 mM KCl, 1 mM $MgCl_2$). The luminescence spectra of the QDs were followed in the presence of variable concentrations of thrombin, at time intervals of 4 min, for 30 min at 37 °C, $\lambda_{excitation} = 430$ nm, $\lambda_{emission} = 610$ nm. It should be noted that in all experiments the concentration of DB was 1×10^{-8} M. This represents an optimized value, and higher concentrations led to electron transfer quenching of the QDs even in the absence of the analytes.

Error bars in the different calibration curves were extracted from $N = 5$ experiments.

2.6. Telomerization of the (*8*)-modified QDs

The extraction of telomerase from the 293-kidney cancer cells was performed as previously described (Sharon et al., 2010a), and it is detailed in the Supplementary Material Section 3S.

The telomerization reaction was performed by using telomerase primer (**8**)-modified QDs in the presence of dATP, dTTP, dGTP, dCTP (2 mM each), and telomerase solution (20 mM Tris–HCl buffer, pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween 20, 1 mM EGTA, BSA 0.1 mg/mL) at 38 °C for 1 h (Telomerase solution consisted of telomerase originating from the specified number of cancer cells). For control experiments, telomerase extracts were heat treated (90 °C for 4 min). For the analysis of telomerase DB, 1×10^{-7} M, was added to the telomerized (**8**)-modified QDs. The nucleic acid (**9**), complementary to the telomere repeat units, 1×10^{-5} M, was then added to the mixture, and the luminescence spectra of the QDs were recorded at time-intervals of 3 min for 1 h at room temperature, $\lambda_{excitation} = 430$ nm.

3. Results and discussion

3.1. Analysis of DNA

Fig. 1A outlines the method to detect DNA by the fluorescence-quenching assay. CdSe/ZnS QDs (620 nm) were functionalized with glutathione and the thiolated nucleic acid (**1**) was covalently coupled to the glutathione capping layer. Spectroscopic assay of the (**1**)-modified QDs revealed an average surface coverage of ca. 34 units of (**1**) per particle (see Supplementary Material Fig. 2S). The