View Online / Journal Homepage / Table of Contents for this issue

Quantum dot-based DNA hybridization by electrochemiluminescence and anodic stripping voltammetry

Haiping Huang, Jingjing Li, Yanglan Tan, Jinjun Zhou and Jun-Jie Zhu*

Received 23rd February 2010, Accepted 14th April 2010
First published as an Advance Article on the web 17th May 2010
DOI: 10.1039/c0an00108b

Simple and convenient assays with quantum dots (QDs) as the labels for DNA detection are developed. The probe DNA modified with thiol was first immobilized on a pretreated Au electrode, and then the complementary DNA (cDNA) oligonucleotides were hybridized with the immobilized probes by immersing the probe-modified Au electrode into the cDNA oligonucleotide solution. Finally, the avidin-modified QDs were bound to the biosensor in the presence of biotin-modified cDNA. The fabrication process for the biosensor was monitored by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Different from the traditional sandwich-structure strategy, the QDs bind to the target DNA directly *via* the biotin–avidin-system. By observing the ECL signal and determination of the cadmium component in QDs, the DNA hybridization event was detected by ECL and square wave anodic stripping voltammetric technique (SWASV) respectively. For SWASV detection, the signal linearly increased with the increase of the logarithm of the cDNA concentration over the range of 50 nM $\sim$ 5 μM. The minimum detectable concentration is 50 pM. For ECL, it showed wider linearity range over 5 nM $\sim$ 5 μM and lower detectable concentration of 10 pM. This indicated that the ECL assay could be comparable to the conventional electrochemical assay. Furthermore, this biosensor possesses high selectivity over different sequences of target DNA oligonucleotides.

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

1. Introduction

Since the discovery of the double helix structure by Watson and Crick in 1953,[1] especially accompanying the accomplishment of the Human Genome Project, sequence-specific DNA detection has attracted more and more interest from chemists. Sensitive, rapid and cost-effective approaches for DNA analysis are associated tightly with tissue matching, genetic diseases and forensic applications in molecular diagnostics.[2–4] Sensitive detection of specific nucleic acid sequences on the basis of the hybridization reaction can be improved by target or signal amplification strategies. Most of recent developments in ultrasensitive detection of DNA are based on nanotechnology. Many assays are developed for DNA detection such as electrochemistry,[5–8] fluorescence,[9–11] colorimetry,[12,13] surface plasmon resonance (SPR)[14–16] *etc*, and the overwhelming majority use the strategy of sandwich-type structure. This strategy involves two DNA probes (capture and labeled reporter probes) that hybridize partially with the target DNA sequence. For example, Fan *et al.* developed a novel nanoparticle-based electrochemical DNA detection approach based on a "sandwich" detection strategy, which involved capture probe DNA immobilized on a gold electrode and signal DNA probe labeled with gold nanoparticles that flanked the target DNA sequence.[17] Such dual hybridization processes may improve the signal-to-noise ratio while making the experimental processes complicated and time-consuming.

Newly developed inorganic fluorescent materials, semiconductor nanocrystals (NCs) or quantum dots (QDs) have been extensively studied because of their unique size-dependent electronic, magnetic, optical, and electrochemical properties.[18] They have been widely used in cell imaging, bacteria detection, and immunoassay.[19] Particularly, since Bard's group found that QDs could generate efficient and stable ECL during the potential cycling or pulsing,[20] highly luminescent QDs have gained increasing attention for applications in bioconjugates and optical biosensors, especially in the fields of immunoanalysis and aptasensor.[21–24] An alternative approach to generate QDs ECL is through the use of a coreactant. After the reaction with the coreactant, an efficient and stable ECL signal in aqueous solution can be obtained by applying a cathodic potential to the QDs,[25] and this phenomenon has also aroused the widespread interest of chemists.[26]

Nowadays, the electrochemical method is widely used for DNA detection. A typical electrochemical DNA biosensor involves a solid electrode for the immobilization of the capture probe DNA; upon hybridization, the ds-DNA structure can form between the immobilized probe DNA and the sequence-specific target DNA. The redox labels that bind to the hybridized ds-DNA generate corresponding electrochemical signals.[27] As a conventional electrochemical assay, square wave anodic stripping voltammetry (SWASV) has the advantages of having a simple, short cycle of detection, and is usually used for metal ion detection, such as lead, thallium, copper and cadmium *etc.* [28] It also plays an important role in the fields of environmental

Key Lab of Analytical Chemistry for Life Science (MOE), School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, P. R. China. E-mail: jjzhu@nju.edu.cn; Fax: +86-25-83317761; Tel: +86-25-83594976

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

monitoring, food safety and medical diagnoses.[29] Furthermore, SWASV is used for the indirect detection of DNA and biomolecules, it has been reported in the fabrication of DNA biosensors and aptasensors.[30] By indirect determination of lead ions with SWASV, an electrochemical stripping assay for ultrasensitive detection of target DNA was developed.[31] It has also been used in the aptasensor field for the detection of thrombin based on a biobarcode amplification assay.[32]

Herein, different from the common sandwich-type strategy, the target DNA oligonucleotides are directly hybridized with the probe DNA oligonucleotides for DNA detection, which reduces the hybridization process, thus it is relatively simple and time-saving. After the QDs bind to the target DNA *via* the biotin–avidin system, the DNA hybridization event can be detected by ECL signal. On the other hand, by employing the SWASV assay, cadmium content in the bound QDs can be used to detect the target DNA indirectly. Thus, It provides a relatively simple, time-saving and multi-approach for DNA detection. As a comparison of the two methods found, although the two methods are both beneficial for DNA detention, the ECL assay can provide a wider linear range and lower detectable concentration.

2. Experimental

2.1 Reagent and apparatus

Labeled DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). All the sequences are given below:

The probe ss-DNA oligonucleotides (probe):
5′-HS-$(CH_2)_6$-GGTCCGCTTGCTCTCGC-3′
The complementary ss-DNA oligonucleotides (cDNA):
5′-biotin-GCGAGAGCAAGCGGACC-3′
The single base-mismatched complementary
ss-DNA oligonucleotides (sDNA):
5′-biotin-GCGAGAG*A*AAGCGGACC-3′
The non-complementary ss-DNA oligonucleotides (non-DNA):
5′-biotin-CTAGTCGTATAGTAGGC-3′

Bovine serum albumin (BSA) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich. Avidin–QDs were provided by Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). All other reagents were of analytical reagent grade and used without further purification. The buffer solution for oligonucleotides contained 100 mmol L^{-1} Na_2HPO_4 and NaH_2PO_4, 5 mmol L^{-1} $MgCl_2$, and pH 7.4, abbr. PBS^+. Electrochemical characterization was recorded in the solution of 0.1 mol L^{-1} KCl and 2 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. The electrolyte for ECL measurement was 5 mL PBS solution containing 0.1 mol L^{-1} $K_2S_2O_8$ and 0.1 mol L^{-1} KCl. Millipore ultrapure water (resistivity $\geq$ 18.2MΩ) was used throughout the experiment.

The electrochemical impedance spectroscopy (EIS) analyses were performed on an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) with the frequency range of 0.1–1.0 × 10^5 Hz. The ECL emission was detected by model MPI-A electrochemiluminescence analyzer (Xi'an Remex Analysis Instrument Co., Ltd., Xi'an, China) with a photomultiplier tube voltage of 600 V. SWASV measurements were carried out on a CHI660B electrochemical workstation (Shanghai CH Instruments Co., China). In the experiments of CV, EIS and ECL, the conventional three-electrode system was employed with a modified gold electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt wire as the counter electrode. While in the SWASV measurements, a pre-polished glassy carbon electrode (GCE) was used as the working electrode.

Prior to use, the gold electrode was cleaned with freshly made piranha solution (98% H_2SO_4 : 30% H_2O_2 = 7 : 3, v/v) for 10 min twice (*CAUTION*: *piranha solution should be handled with great care*), all the oligonucleotides solutions were heat-treated at 90 °C for 3 min and then cooled in ice for 10 min.

2.2 Preparation of the DNA biosensor

20 μL 5 μmol L^{-1} probe solution was first spread on the pre-cleaned Au electrode surface for 12 h at 37 °C in 100% humidity, followed by immersing in 1 mmol L^{-1} MCH for 2 h to remove nonspecific DNA adsorption. Subsequently, 20 μL 5′-biotin-modified cDNA oligonucleotides solution was dropped onto the above electrode for 1 h incubation to form double-stranded DNA (ds-DNA) structure. Afterwards, 20 μL of diluted avidin–QDs liquid was dropped onto the electrode surface for 1 h. Before use, 1 μL obtained avidin–QDs solution was dissolved in 100 μL bovine serum albumin (BSA) solution (1 mg mL^{-1} in PBS^+) under shock conditions for 1 h for the purpose of blocking the active sites of the QDs. All these above incubation steps were carried out at 37 °C. The whole preparation process is outlined in Fig. 1. Before measurement, the biosensor was washed with wash buffer to remove physical absorption of the QDs.

2.3 Measurement of the DNA biosensor

The modified electrode is immersed in 5 mL of the electrolyte for ECL measurement. For the SWASV measurement, the electrode was first dipped in 400 μL of 0.1 M HNO_3 solution for 4 h to dissolve the bound QDs. The above solution was subsequently poured into 3.6 mL of acetate buffer (0.1 mol L^{-1}, pH 4.6) containing 10 ppm mercury(II). Next, a pro-polished GCE was used as the working electrode for pretreatment at +0.6 V for 1 min in acetate buffer solution, followed by electrodeposition at −1.1 V for 4 min. Finally, SWASV measurement was carried out at potential range from −1.1 V to −0.2 V with 4 mV potential steps, 25 Hz frequency and 25 mV amplitude.

3. Results and discussion

3.1 Electrochemical characteristics of the biosensor

Electrochemical impedance spectroscopy (EIS) has been reported as an effective assay to inspect the features of the electrode surface and to understand the chemical transformations associated with the conductive electrode surface.[33] The EIS spectrum contains a semicircle portion and a linear portion. The semicircle portion at higher frequencies corresponds to the electron transfer limited process, and the semicircle diameter equals the electron-transfer resistance (*Ret*). *Ret* controls the electron-transfer kinetics of the redox probe at the electrode interface. The linear part at lower frequencies corresponds to the diffusion process.

This journal is © The Royal Society of Chemistry 2010

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

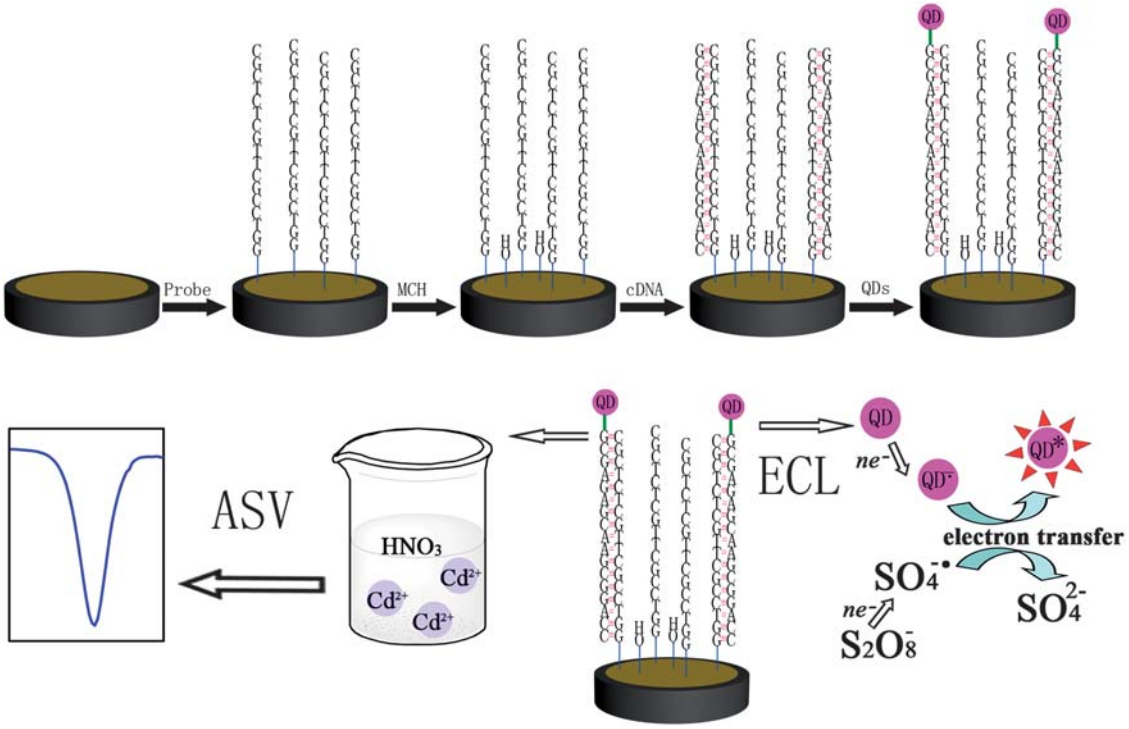

Fig. 1 Schematic diagram of biosensor fabrication.

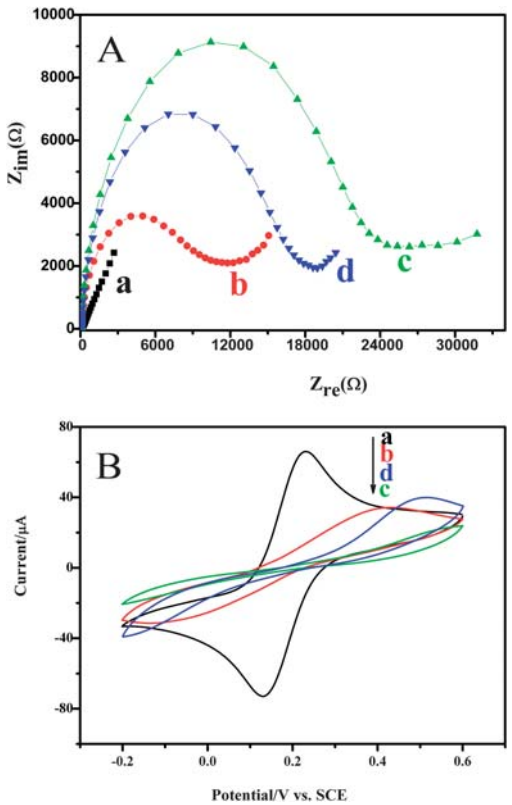

Fig. 2 (A) EIS and (B) CV of the electrode at different stages in 0.1 mol L^{-1} KCl and 2 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. (a) bare Au electrode, (b) probe/Au electrode, (c) cDNA/probe/Au electrode, (d) QDs/cDNA/probe/Au electrode. The frequency range: 0.1~1.0 × 10^5 Hz. The CV scan rate: 100 mV s^{-1}.

Fig. 2A shows the EIS changes upon each experimental step. Compared with the bare Au electrode (curve a), the probe modified at Au electrode shows a larger eT resistance (curve b), mainly due to the electrostatic repulsion between negative charges of the DNA oligonucleotides backbone and the electroactive probe $[Fe(CN)_6]^{3-/4-}$. After the cDNA solution was dropped onto the electrode, the largest eT resistance (curve c) was observed. When cDNA hybridized with probe DNA to form the ds-DNA structure, this essentially increased the negative charge at the electrode surface, and enlarged the eT resistance.

As a traditional electrochemical technique, cyclic voltammogram (CV) of ferricyanide is a valuable tool to monitor the barrier of the modified electrode, and to investigate the changes to the electrode behavior after each assembly. Shown in Fig. 2B is the CVs of $[Fe(CN)_6]^{3-/4-}$ on the modified electrode at different stages. As can be seen, stepwise modifications of the Au electrode were accompanied by changes in the amperometric response, as well as separation between the cathodic and anodic peak of the redox probe $[Fe(CN)_6]^{3-/4-}$. On the bare Au electrode, a well-defined redox peak pair was observed (curve a), showing the excellent electron-transfer kinetics of $[Fe(CN)_6]^{3-/4-}$. The amperometric response decreased and the peak-to-peak separation was enlarged after the probes were immobilized on the Au electrode (curve b). The CV response was further decreased after the formation of the Watson–Crick helix. The CV changes are consistent with EIS changes, and again confirm the success of each assembly step.

3.2 ECL characteristics of the biosensor

QDs can be electrochemically reduced during the potential scan and react with the coreactant $S_2O_8^{2-}$ to generate strong ECL

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

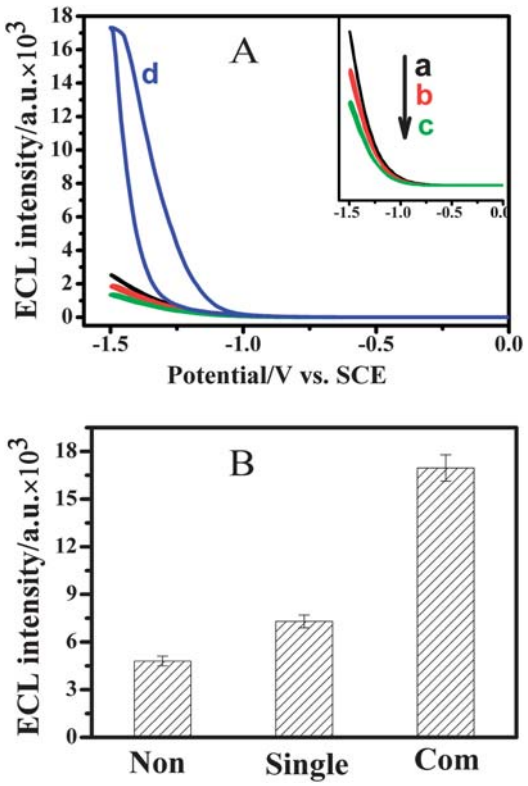

Fig. 3 (A) The ECL potential curves of the DNA biosensor with different modified electrodes. (a) bare Au electrode, (b) probe/Au electrode, (c) cDNA/probe/Au electrode. (B) ECL signals of the probe DNA modified electrodes that hybridized with different target DNA, (Non) noncomplementary sequences; (Single) single-base mismatch sequences; (Com) complementary sequences. The electrolyte: 5 mL pH 7.4 PBS^+ solution containing 0.1 mol L^{-1} $K_2S_2O_8$ and 0.1 mol L^{-1} KCl. DNA oligonucleotide concentration: 5 μmol L^{-1}. The voltage of the photomultiplier tube: 600 V. Scan rate: 100 mV s^{-1}.

signal in aqueous solution.[21] The ECL intensity is proportional to the amount of QDs. In order to confirm that the ECL signal was generated by the QDs, the signals of the different modified electrodes were also examined as shown in Fig. 3A. In the absence of QDs, the signals of bare Au electrode (curve a), probe/Au electrode (curve b), cDNA/probe/Au (curve c) were very low, even negligible. The signal enlarged significantly after the QDs were bound to the electrode (curve d). This proved that the signal was generated by the QDs. The pH influence of the electrolyte on the ECL response was also examined in the range of 5.0∼10.0. The ECL intensity increased first and reached a maximum ECL response at pH 7.0∼8.0, it then decreased when pH was larger than 8.0. Because the physiological pH for the biological systems is about 7.4; pH 7.4 was chosen in the subsequent experiments. The influence of $K_2S_2O_8$ concentration on the ECL response was also studied. Three different $K_2S_2O_8$ concentrations of 0.05 M, 0.1 M and 0.2 M were employed in the measurement. As the coreactant concentration became higher, the time needed to reach the intensity peak value was shorter, and no intensity peak value change was observed. Therefore, 0.1M was chosen for the coreactant $K_2S_2O_8$ concentration.

3.3 ECL detection of the biosensor

In this system, the ECL signal was proportional to the amount of QDs that bind to the modified electrode. After the cDNA was hybridized with the probes to form the ds-DNA structure, the QDs bind to the modified electrode *via* the biotin–avidin system, and this leads directly to the increased ECL signal. Shown in Fig. 4A are the ECL signals of the biosensor that are responsive to the different concentrations of cDNA. The ECL signal increased with the cDNA concentration in the range of 0 ∼ 5 μM. Fig. 4B is the plot of ECL intensity *versus* the logarithm of the cDNA concentration. It could be seen in Fig. 4B that the ECL signal linearly increased with the increase of the logarithm of the cDNA concentration over the 5 ∼ 5000 nM range. The regression equation is $y = 1765 + 4115\ \lg x$ (x: nmol L^{-1}) with $R^2 = 0.9943$. The minimum detectable concentration is 1.5 nM. This detection limit was lower that the SPRi (10 nM) and QCM (250 nM),[34] and could be comparable with the tris (2,2′-bipyridyl) ruthenium(II) label.[35]

Furthermore, different kinds of target DNA oligonucleotide were used to study the selectivity of the biosensor. Under the same concentration 5 μM, single-base mismatched, noncomplementary and complementary DNA oligonucleotide solutions

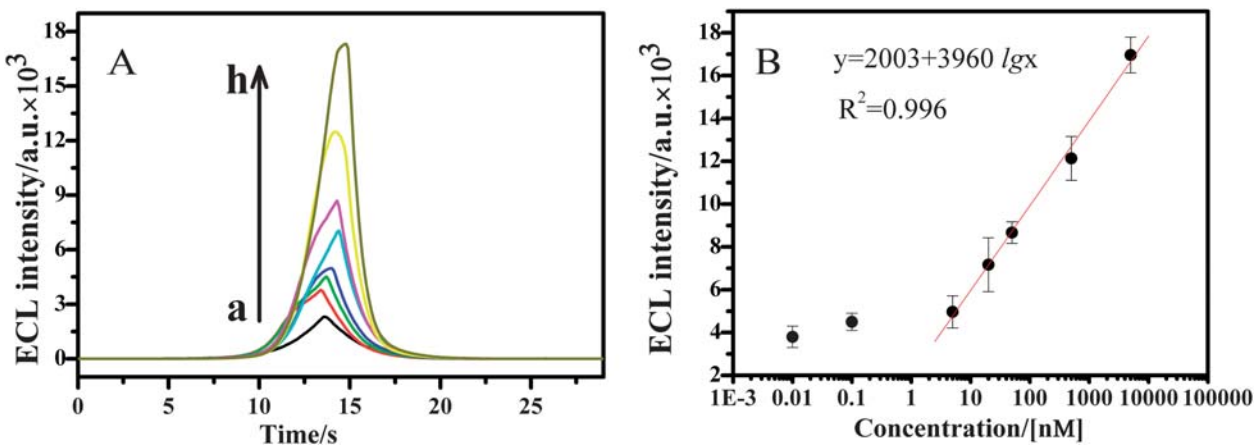

Fig. 4 (A) ECL intensity of the DNA biosensor with different target DNA concentrations (nM) *versus* time. From a to h: 0, 0.01, 0.1, 5.0, 20.0, 50.0, 500.0 and 5000.0 nM, respectively. (B) The ECL intensity-concentration curve drawn from (A). The ECL detection conditions are the same as in Fig. 3.

This journal is © The Royal Society of Chemistry 2010

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

were dropped for hybridization and the ECL signal was measured. As shown in Fig. 3B, compared with the complementary DNA, the signals from the single-base mismatched and noncomplementary DNA weaken to a large extent. This comparison demonstrated that this biosensor had high sensitivity for the sequence specific detection over the target DNA.

3.4 SWASV characteristics of the biosensor

Owing to the advantage of being simple, reliable, cheap and sensitive, electrochemical sensors based on impedance[36] and voltammetry[37] have been reported for genetic detection. In recent years, QDs have been applied for electrochemical DNA sensors.[38] The quantum dots exhibit sharp and well resolved stripping voltammetric signals for the corresponding targets due to the well-defined oxidation potentials of the metal components. SWASV is a widely used electrochemical assay for metal detection in aqueous media at mercury-plated electrodes.[39] In our system, the DNA hybridization event was detected by measuring the cadmium ion content in the bound QDs.

The QDs used here were composed of CdSe/ZnS with core–shell structure, and were bound to the biosensor *via* the avidin–biotin affinity. As different concentrations of biotin-modified cDNA were hybridized with the probes, different amount of QDs bind to the biosensor. This resulted directly in the different amounts of cadmium ion component on the biosensor, and gives

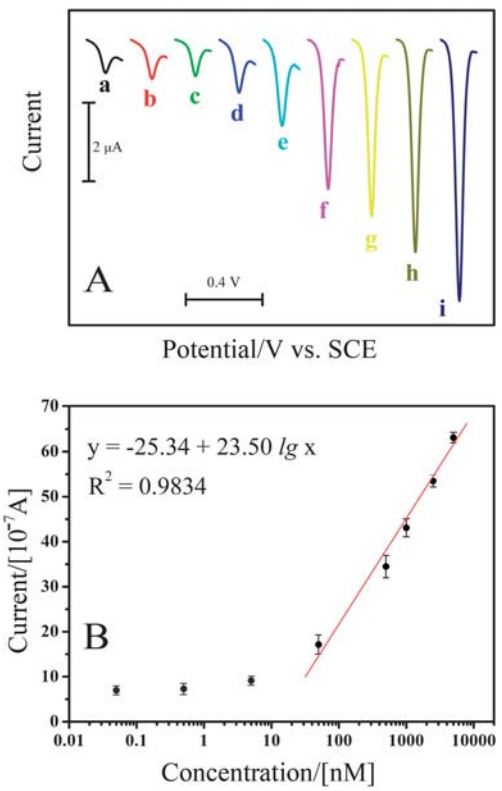

Fig. 5 (A) The ASV current–potential curve with different cDNA concentrations and (B) the corresponding current–concentration curve. The cDNA concentrations from a to i are 0, 0.05 nM, 0.5 nM, 5 nM, 50 nM, 500 nM, 1 μM, 2.5 μM, 5 μM. The ASV electrolyte: 4.5 mL 0.1 M HAc/NaAc + 4.5 μL 40 mg mL^{-1} $HgCl_2$, pH 4.6.

the corresponding ASV signal of Cd^{2+}. Shown in Fig. 5 is the SWASV response of cadmium ion concentration after the DNA biosensor hybridized with different concentrations of cDNA. The DNA biosensor was first dipped into HNO_3 solution for 4 h in order to dissolve the bound QDs and to change the cadmium component into Cd^{2+}, then a mercury-plated glassy carbon electrode was used as the working electrode for SWASV detection. The SWASV signal for Cd^{2+} that responded to the cDNA oligonucleotides was measured in the concentration range of 0∼5 μM. As can be seen in Fig. 5A, because of the high cDNA concentrations, the corresponding electrochemical signal becomes strong. More double-stranded DNA (ds-DNA) structure could be formed between probe DNA oligonucleotides and cDNA oligonucleotides. This resulted directly in more avidin-modified QDs binding to the 5′-biotin modified cDNA oligonucleotides *via* the avidin–biotin system. Fig. 5B shows the concentration–current curve drawn in Fig. 5A. It reveals that the SWASV signal increased linearly with the logarithm of the cDNA concentration in the range of 50 nM ∼ 5μM. The regression equation is $y = -24.17 + 23.94 \lg x$ (x: nmol L^{-1}) with $R^2 = 0.9739$. The minimum detectable concentration is 15 nM. This result was better than the reported electrochemical impedance DNA biosensor,[40] which indicates that the SWASV assay can greatly improve the performance of the biosensor. What is more, by comparing the linear range and the minimum detectable concentration, it is found that although the ECL and SWASV methods are both beneficial for DNA detection, the ECL assay provides a wider linear range and a lower detectable concentration.

The reproducibility of the biosensor was investigated by measuring cDNA concentration of 500 nM with three biosensors prepared independently under the same experimental conditions. The variation coefficients obtained were 8.46% for ECL and 7.25% for ASV, respectively, indicating acceptable precision and fabrication reproducibility.

4. Conclusion

QDs ECL in aqueous solution and SWASV were employed for DNA detection. Through the biotin–avidin-system, avidin–QDs bind tightly to the biotin-modified cDNA oligonucleotides. By utilizing the ECL property of QDs, the DNA hybridization event was converted into a detectable ECL signal. On the other hand, by detecting cadmium content in the bound QDs, the target DNA was indirectly detected through the SWASV assay. It provided a relatively simple, time-saving and multi-approach for DNA detection.

Acknowledgements

We greatly appreciate the support of the National Natural Science Foundation of China for the Key Program (20635020), Creative Research Group (20821063). This work is also supported by National Basic Research Program of China (2006CB933201)

References

1 J. D. Watson and F. H. Crick, *Nature*, 1953, **171**, 737.
2 R. F. Service, *Science*, 1998, **282**, 399.

View Online

Published on 17 May 2010 on http://pubs.rsc.org | doi:10.1039/C0AN00108B
Downloaded by NANJING UNIVERSITY on 26 April 2012

3 L. M. Staudt, *Trends Immunol.*, 2001, **22**, 35.
4 J. M. Butler, *J. Forensic Sci.*, 2006, **51**, 253.
5 M. R. Gore, V. A. Szalai, P. A. Ropp, I. V. Yang, J. S. Silverman and H. H. Thorp, *Anal. Chem.*, 2003, **75**, 6586.
6 W. Zhang, T. Yang, X. Li, D. B. Wang and K. Jiao, *Biosens. Bioelectron.*, 2009, **25**, 428.
7 S. Cosnier and P. Mailley, *Analyst*, 2008, **133**, 984.
8 H. X. Chang, Y. Yuan, N. L. Shi and Y. F. Guan, *Anal. Chem.*, 2007, **79**, 5111.
9 J. Zhang and J. R. Lakowicz, *J. Phys. Chem. B*, 2006, **110**, 2387.
10 Z. S. Wu, J. H. Jiang, L. Fu, G. L. Shen and R. Q. Yu, *Anal. Biochem.*, 2006, **353**, 22.
11 H. Y. Kim, M. D. Kane, S. Kim, W. Dominguez, B. M. Applegate and S. Savikhin, *Biosens. Bioelectron.*, 2007, **22**, 1041.
12 R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, *Science*, 1997, **277**, 1078.
13 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959.
14 C. Ananthanawat, T. Vilaivan, W. Mekboonsonglarp and V. P. Hoven, *Biosens. Bioelectron.*, 2009, **24**, 3544.
15 B. P. Nelson, T. E. Grimsrud, M. R. Liles, R. M. Goodman and R. M. Corn, *Anal. Chem.*, 2001, **73**, 1.
16 F. Nakamura, E. Ito, Y. Sakao, N. Ueno, I. N. Gatuna, F. S. Ohuchi and M. Hara, *Nano Lett.*, 2003, **3**, 1083.
17 J. Zhang, S. P. Song, L. H. Wang, H. P. Wu, D. Pan and C. H. Fan, *J. Am. Chem. Soc.*, 2006, **128**, 8575.
18 Y. D. Yin and A. P. Alivisatos, *Nature*, 2005, **437**, 664; A. P. Alivisatos, *Science*, 1996, **271**, 933; C. Burda, X. B. Chen, R. Narayanan and M. A. El-Sayed, *Chem. Rev.*, 2005, **105**, 1025.
19 R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, C. Merril, K. Nagashima and S. Adhya, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4841–4845; R. Bakalova, Z. Zhelev, H. Ohba and Y. Baba, *J. Am. Chem. Soc.*, 2005, **127**, 9328–9329; X. Y. Wu, H. J. Liu, J. Q. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. F. Ge, F. Peale and M. P. Bruchez, *Nat. Biotechnol.*, 2003, **21**, 41–46.
20 Z. Ding, B. M. Quinn, S. K. Haram, L. E. Pell, B. A. Korgel and A. J. Bard, *Science*, 2002, **296**, 1293.
21 G. F. Jie, H. P. Huang, X. L. Sun and J. J. Zhu, *Biosens. Bioelectron.*, 2008, **23**, 1896; G. F. Jie, B. Liu, H. C. Pan, J. J. Zhu and H. Y. Chen, *Anal. Chem.*, 2007, **79**, 5574; J. J. Miao, G. F. Jie, Y. P. Chen, L. Zhang, L. P. Jiang and J. J. Zhu, *Chem. Commun.*, 2008, 3762; Q. M. Shen, L. P. Jiang, J. J. Miao, W. H. Hou and J. J. Zhu, *Chem. Commun.*, 2008, 1683; G. X. Liang, L. L. Li, H. Y. Liu, J. R. Zhang, C. Burda and J. J. Zhu, *Chem. Commun.*, 2010, **46**, 2974.
22 J. H. Choi, K. H. Chen and M. S. Strano, *J. Am. Chem. Soc.*, 2006, **128**, 15584.
23 H. P. Huang, G. F. Jie, R. J. Cui and J. J. Zhu, *Electrochem. Commun.*, 2009, **11**, 816.
24 C. L. Feng, X. H. Zhong, M. Steinhart, A. M. Caminade, J. P. Majoral and W. Knoll, *Adv. Mater.*, 2007, **19**, 1933.
25 S. K. Poznyak, D. V. Talapin, E. V. Shevchenko and H. Weller, *Nano Lett.*, 2004, **4**, 693.
26 D. Wang, A. L. Rogach and F. Caruso, *Nano Lett.*, 2002, **2**, 857; S. Y. Ding, M. Jones, M. P. Tucker, J. M. Nedeljkovic, J. Wall, M. N. Simon, G. Rumbles and M. E. Himmel, *Nano Lett.*, 2003, **3**, 1581.
27 R. J. Lao, S. P. Song, H. P. Wu, L. H. Wang, Z. Z. Zhang, L. He and C. H. Fan, *Anal. Chem.*, 2005, **77**, 6475.
28 S. Laschi, I. Palchetti and M. Mascini, *Sens. Actuators, B*, 2006, **114**, 460; N. Spano, A. Panzanelli, P. C. Piu, M. I. Pilo, G. Sanna, R. Seeber and A. Tapparo, *Anal. Chim. Acta*, 2005, **553**, 201; C. Locatelli and G. Torsi, *Microchem. J.*, 2004, **78**, 175; G. J. Lee, H. M. Lee and C. K. Rhee, *Electrochem. Commun.*, 2007, **9**, 2514; M. Wojciechowski and J. Balcerzak, *Anal. Chim. Acta*, 1991, **249**, 433.
29 H. J. Kim, K. S. Yun, E. Yoon and J. Kwak, *Electrochim. Acta*, 2004, **50**, 205; D. E. Mays and A. Hussam, *Anal. Chim. Acta*, 2009, **646**, 6; S. Y. Ly, Y. H. Kim, I. K. Han, I. G. Moon, W. W. Jung, S. Y. Jung, H. J. Sin, T. K. Hong and M. H. Kim, *Microchem. J.*, 2006, **82**, 113.
30 J. Wang, G. D. Liu and A. Merkoçüi, *J. Am. Chem. Soc.*, 2003, **125**, 3214; J. A. Hansen, J. Wang, A. N. Kawde, Y. Xiang, K. V. Gothelf and G. Collins, *J. Am. Chem. Soc.*, 2006, **128**, 2228.
31 K. C. Hu, P. Liu, S. J. Ye and S. S. Zhang, *Biosens. Bioelectron.*, 2009, **24**, 3113.
32 X. R. Zhang, B. P. Qi, Y. Li and S. S. Zhang, *Biosens. Bioelectron.*, 2009, **25**, 259.
33 A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*; Wiley: New York, 1980 .
34 T. Livache, E. Maillart, N. Lassalle, P. Mailley, B. Corso, P. Guedon, A. Roget and Y. Levy, *J. Pharm. Biomed. Anal.*, 2003, **32**, 687.
35 Z. Chang, J. M. Zhou, K. Zhao, N. N. Zhu, P. G. He and Y. Z. Fang, *Electrochim. Acta*, 2006, **52**, 575.
36 E. Katz and I. Willner, *Electroanalysis*, 2003, **15**, 913; S. Pan and L. Rothberg, *Langmuir*, 2005, **21**, 1022.
37 A. Anne, A. Bouchardon and J. Moiroux, *J. Am. Chem. Soc.*, 2003, **125**, 1112; L. Alfonta, A. Singh and I. Willner, *Anal. Chem.*, 2001, **73**, 91.
38 L. Authier, C. Grossiord and P. Brossier, *Anal. Chem.*, 2001, **73**, 4450; H. Cai, N. Zhu, Y. Jiang, P. He and Y. Fang, *Biosens. Bioelectron.*, 2003, **18**, 1311.
39 T. M. Florence, *J. Electroanal. Chem.*, 1974, **49**, 255; J. M. Zen and M. J. Chung, *Anal. Chim. Acta*, 1996, **320**, 43.
40 H. Peng, C. Soeller, M. B. Cannell, G. A. Bowmaker, R. P. Cooney and J. Travas-Sejdic, *Biosens. Bioelectron.*, 2006, **21**, 1727.

 This journal is © The Royal Society of Chemistry 2010