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CdTe nanocrystal-based electrochemical biosensor for the recognition of neutravidin by anodic stripping voltammetry at electrodeposited bismuth film

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ABSTRACT

CdTe quantum dots (QDs)-based electrochemical sensor for recognition of neutravidin, as a model protein, using anodic stripping voltammetry at electrodeposited bismuth film is presented. This biosensor involves the immobilization of the captured QDs conjugates which was dissolved with 1 M HCl solution to release cadmium ions and metal components were quantified by anodic stripping voltammetry after a 3-min accumulation at −1.2 V on bismuth-film electrode (BiFE) of the biotin, served as recognition element, onto the gold surface in connection with a cysteamine self-assembled monolayer. The modification procedure was characterized by electrochemical impedance spectroscopy and atomic force microscopy. We exploit QDs as labels for amplifying signal output and monitoring the extent of competition process between CdTe-labeled neutravidin and the target neutravidin for the limited binding sites on biotin. As expected for the competitive mechanism, the recognition event thus yields distinct cadmium stripping voltammetric current peak, whose response decreases upon increasing the level of target neutravidin concentrations. Under optimal conditions, the voltammetric response is highly linear over the range of 0.5–100 ng L^{-1} neutravidin and the limit of detection is estimated to be 0.3 ng L^{-1} (5 nM). Unlike earlier two-step sandwich bioassays, the present protocol relies on a one-step competitive assay, which is more accurate and sensitive, showing great promise for rapid, simple and cost-effective analysis of protein.

1. Introduction

As research moves into the era of proteomics, scientists are faced with challenge of developing highly specific, rapid and effective method for identifying and quantitating trace amount of biological substances (Zhu et al., 2003; Jie et al., 2007). Avidin is a glycoprotein (molecular weight, 68,000) found in egg white and is known to contain four identical binding sites to biotin. Biotin–avidin interactions play crucial roles in a variety of biological processes. The detection of avidin and related biotin–avidin interaction has thus received considerable attention (Dong et al., 2004; Salant et al., 2006; Rauf et al., 2006; Cui et al., 2003). Owing to the high affinity and specific interaction (the binding constant is 10^{15} M^{-1}) (Dontha et al., 1997), several methods including optical (Song and Swanson, 2001), electrochemical (Ding et al., 2005) and photoelectrochemical methods are available for quantitating biotin/avidin interaction. However, separation procedures are necessary to separate the label-free biotin from a bound biotin before measuring. Additional efforts are thus needed to create more accurate, sensitive, rapid and low-cost quantification.

In recent years, several inventive designs for sensors based on an electrochemical readout have appeared (Katz and Willner, 2004; Merkocüi et al., 2005). The combination of electrochemistry and nanotechnology provides very powerful tools for the development of highly sensitive and specific biosensors (Katz et al., 2001; Niazov et al., 2004; Weizmann et al., 2006). Especially the nanoparticle-based amplification processes have been reported to dramatically enhance the intensity of the electrochemical signal and lead to ultrasensitive bioassays (Wang, 2005). Quantum dots (QDs), also called semiconductor nanocrystals, have gained great interest for biosensing, bioanalytical assays, cell imaging, drug delivery and in vivo animal targeting due to their unique size-dependent optical and electronic properties (Michalet et al., 2005; Wu et al., 2006; Cui et al., 2007; Jiang and Ju, 2007; Liu et al., 2007; Hansen et al., 2006; Ge et al., 2008). In particular, electrochemical bioassays based on QDs provide an elegant way to simultaneously detect multiple protein analytes in connection with highly sensitive stripping

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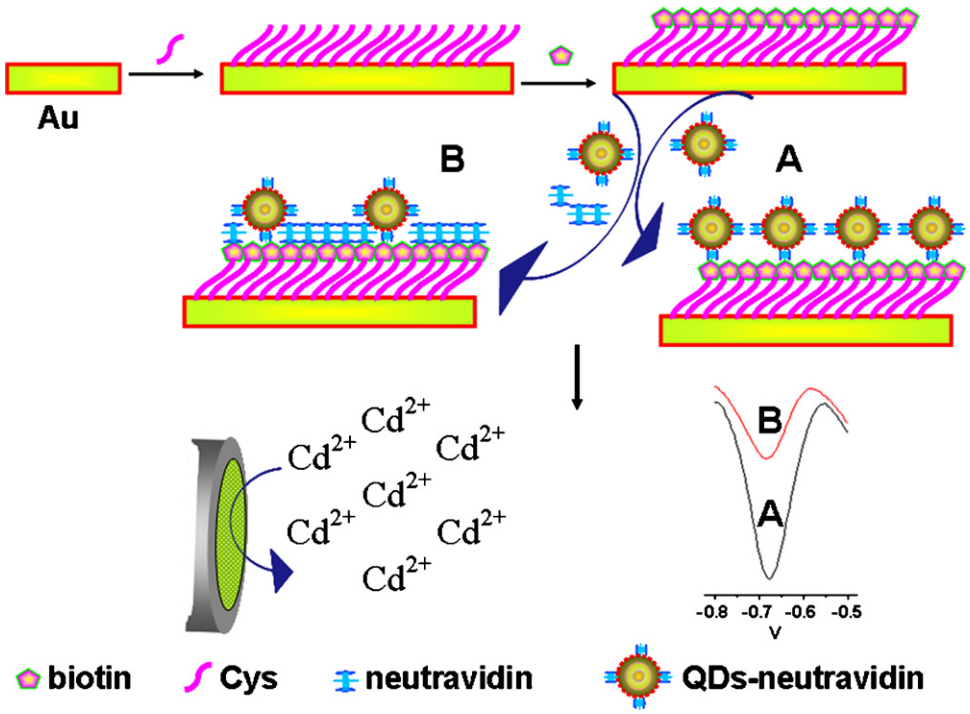

Fig. 1. Schematic diagram for one-step competition between a CdTe QDs-labeled neutravidin and the target neutravidin for the binding sites of biotin modified electrode.

analysis of the metal components. Wang et al. reported on electrochemical assays based on QD nanocrystals as tracers for enhanced electrochemical detection of DNA hybridization (Wang et al., 2003), electrical coding of single nucleotide polymorphisms (SNPs) (Liu et al., 2005), electrochemical sandwich immunoassays of proteins (Liu et al., 2004), monitoring of lectin–sugar interactions and bioassays of disease-related glycan markers (Dai et al., 2006). QDs exhibit sharp and well-resolved stripping voltammetric signals due to the well-defined oxidation potentials of the metal components. Recently, highly selective assay for binding and detection of multiple metal sulfide nanoparticles on a solid substrate has also been developed. Four encoding nanoparticles (CdS, ZnS, CoS and PbS) were used to differentiate the signals of four DNA targets, SNPs or antigens in connection with stripping voltammetric measurements of the corresponding metals (Hansen et al., 2006).

Although stripping analysis has proved a powerful technique for the determination of trace metals, a major disadvantage is the use, manipulation and disposal of metallic mercury or mercury salts. Since the year 2000, bismuth-film electrode (BiFE) has become an attractive new subject of electrochemical stripping analysis as a potential replacement for mercury-film electrode due to their environmental friendly nature (Wang et al., 2000; Hu et al., 2007).

Here we present the first report on CdTe QDs-based biosensing of neutravidin based on their recognition with surface-functionalized biotin. This novel bioassay involves: (1) the immobilization of the biotin, served as recognition element, onto the gold surface in connection with a cysteamine self-assembled monolayer; (2) competition between CdTe QDs-labeled neutravidin and the target neutravidin for the limited binding sites on biotin and (3) monitoring the extent of competition through highly sensitive electrochemical stripping detection of the captured CdTe nanocrystal using electrodeposited BiFE, as shown in Fig. 1. The recognition event thus yields distinct cadmium stripping voltammetric current peak, whose response decreases upon increasing the level. Unlike these earlier two-step sandwich bioassays, the present protocol relies on a one-step competitive assay.

2. Experimental

2.1. Reagents and materials

Biotin, neutravidin, *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), bovine serum albumin (BSA) and cysteamine were purchased from Sigma–Aldrich (St. Louis, USA) and used as received. Te powder and thioglycolic acid (TGA) were purchased from Acros Organics (Geel, Belgium). The bismuth stock solutions (1000 mg L^{-1} in 5 wt.% nitric acid) were obtained from TreeChem.co (Shanghai, China). $CdCl_2$, $NaHB_4$, acetate buffer, phosphate buffer saline (PBS) and other reagents used were of analytical reagent grade. All aqueous solutions were prepared with double distilled water.

2.2. Apparatus

Electrochemical measurements were performed on CHI-660C workstation (CH Instruments Co., Shanghai, China) with a conventional three-electrode system comprising platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference and 3-mm-diameter Au disk or bismuth film as working electrode. The electrochemical impedance spectra (EIS) were recorded in 5 mM $Fe(CN)_6^{4-/3-}$ with frequencies ranging from 100 kHz to 0.1 Hz with an amplitude of 5 mV. Atomic force microscopy (AFM) experiments were performed on SPA-300 HV atomic force microscopy with a SPI 3800 controller (Seiko, Japan). They were carried out using tapping mode. The SI-DF-3 cantilevers (Seiko Instruments Inc.) were used.

2.3. Synthesis of CdTe QDs

CdTe QDs were prepared according to the method described elsewhere (Li and Qu, 2007). The molar ratio of Cd^{2+}:TGA:HTe^- was fixed at 1:2.5:0.5. Briefly, 0.095 g $CdCl_2{\cdot}2.5H_2O$ was dissolved in 5 mL of water, and 0.092 mL of TGA was added. Next, the solution

was adjusted to pH 11 with 1 M NaOH and deaerated with N_2 for 30 min. Next, oxygen-free NaHTe solution, which was freshly prepared from tellurium powder and $NaBH_4$ in water, was injected into the above solution under vigorous stirring. The solution was then heated at 60 °C for 2 h. Finally, CdTe QDs were obtained in aqueous media.

2.4. Preparation of QD–neutravidin conjugates

The QDs functionalized with carboxyl groups were dispersed in PBS and its final concentration was 5 mM. First, QD nanocrystals were activated with 10 mM EDC and NHS, followed by stirring for 30 min. The resulting mixture was centrifuged for 1 min at 5000 rpm to remove the supernatant solution, and then re-dispersed in 250 μL PBS. At room temperature, 160 μL neutravidin with a concentration of 1 mg mL^{-1} was added into the above activated QD solution and mixed for 1 h. The resulting QD–neutravidin conjugate was collected by centrifugation at 15,000 rpm for 5 min. After removal of the supernatant solution, the purified QDs–neutravidin conjugates were achieved and re-suspended in 500 μL PBS containing 0.04% BSA. For further experiment, the solution was diluted to different concentrations.

2.5. Ex situ bismuth plating

Bismuth film was formed by ex situ deposition on glassy carbon electrode (GCE) at constant applied potential. Prior to plating, a GCE was polished carefully to a mirrorlike with 0.3-, 0.1- and 0.05-μm alumina slurry and sequentially sonicated for 3 min in methanol and water. The bismuth film was deposited from a separate acetate buffer solution (pH 4.5; ex situ deposition), containing 5 mg mL^{-1} Bi(III) by electrolysis at −1.2 V for 80 s while stirring the solution.

2.6. Fabrication of the biosensor

A gold disk electrode with a diameter of 3 mm was polished carefully with 1.0-, 0.3- and 0.05-μm Al_2O_3 paste and washed ultrasonically with water and absolute ethanol. Before modification, the bare gold electrode was soaked in piranha solution for 2 min and scanned in 0.5 M H_2SO_4 between −0.2 and +1.5 V until a reproducible cyclic voltammogram was obtained. After cleaning, the electrode was first immersed in 50 mM deoxygenated cysteamine aqueous solution for 8 h at room temperature in darkness to form cysteamine self-assembled monolayers (Cys SAMs/Au). Then the resulting electrode was washed thoroughly with double distilled water to remove physically adsorbed cysteamine and soaked in 0.225 mg mL^{-1} NHS–LC–biotin for 4 h. After rinsing thoroughly with water and dried in the air, a biosensor (biotin–Cys SAMs/Au) was obtained, which was finally used in QDs–neutravidin conjugates and target neutravidin mixed solution for the following competition assay.

2.7. Sample assay procedure

In one-step competitive assay, the resulting biotin–Cys SAMs/Au was incubated in mixed solution containing QDs–neutravidin conjugates, neutravidin and 0.04% BSA for 60 min. Before the electrochemical measurement, the captured QDs conjugates were dissolved with 1 M HCl solution for 5 min to release cadmium ions and metal components were quantified by anodic stripping voltammetry after a 3-min accumulation at −1.2 V on BiFE. The potential scanning range was from −1.4 to −0.2 V.

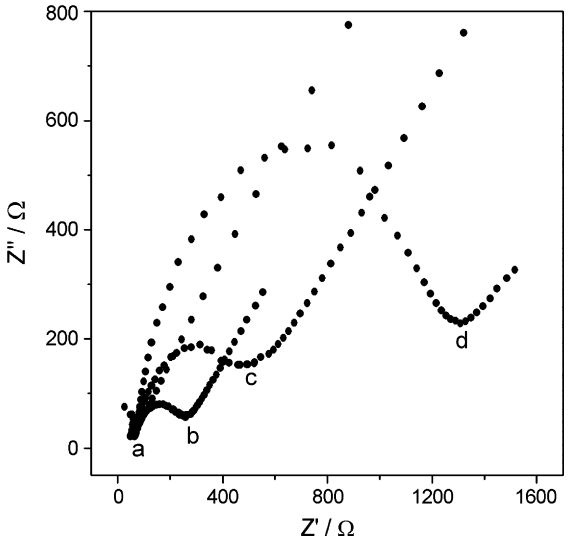

Fig. 2. EIS of (a) bare Au, (b) Cys SAMs/Au, (c) biotin–Cys SAMs/Au and (d) QDs–neutravidin–biotin–Cys SAMs/Au in 10 mM PBS containing 5.0 mM $Fe(CN)_6^{4-/3-}$ and 0.1 M KCl.

3. Results and discussion

3.1. Impedance characterization of biosensor fabrication

Fig. 2 illustrated typical Nyquist plots obtained from bare Au, Cys SAMs/Au, biotin–Cys SAMs/Au and QDs–neutravidin–biotin–Cys SAMs/Au using $Fe(CN)_6^{3-/4-}$ as the redox probe. An almost straight line on bare Au electrode (curve a) was exhibited, which was characteristic of a mass diffusion limiting electron-transfer process. After assembly of the cysteamine monolayer on the electrode surface, the EIS of the Cys SAMs/Au generated an apparent interfacial resistance with R_{ct} of 258 Ω (curve b). This was reflected by the increase of the semicircle part on the spectrum. Subsequently, biotin was assembled onto the Cys SAMs/Au and a further increase in the interfacial resistance to 460 Ω was observed (curve c) due to the resistance of protein. After an immobilization of QDs–neutravidin on the electrode surface, the interfacial resistance increased remarkably to 1325 Ω (curve d) due to the increase of the thickness of the interface. The phenomenon was consistent with the fact that the protein layer insulated the conductive support and blocked the interfacial electron transfer. This was the direct evidence of successful binding of QDs–neutravidin on the electrode surface.

3.2. AFM characterization of biosensor fabrication

In addition, AFM measurement was also employed to observe the surface topography and confirm the fabrication process. As seen from Fig. 3a, the pretreated bare Au slice displayed an even surface with height less than 1 nm. After cysteamine was assembled onto the Au electrode surface, the Cys SAMs/Au showed a uniform distribution. The thickness of the layer increased to ~7 nm (Fig. 3b). Upon incubation of biotin, Fig. 3c clearly illustrated that the formation of the biotin monolayer was accomplished on Cys SAMs/Au due to the interaction between –COOH of activated biotin and $–NH_2$ of cysteamine. The thickness of all the layers increased to ~15 nm. After QDs–neutravidin molecules were further immobilized onto the biotin–Cys SAMs, the image showed the presence of the large agglomerate pattern on the modified gold surface due to the strong affinity between biotin and avidin, and the thickness of all the layers increased to ~30 nm (Fig. 3d).

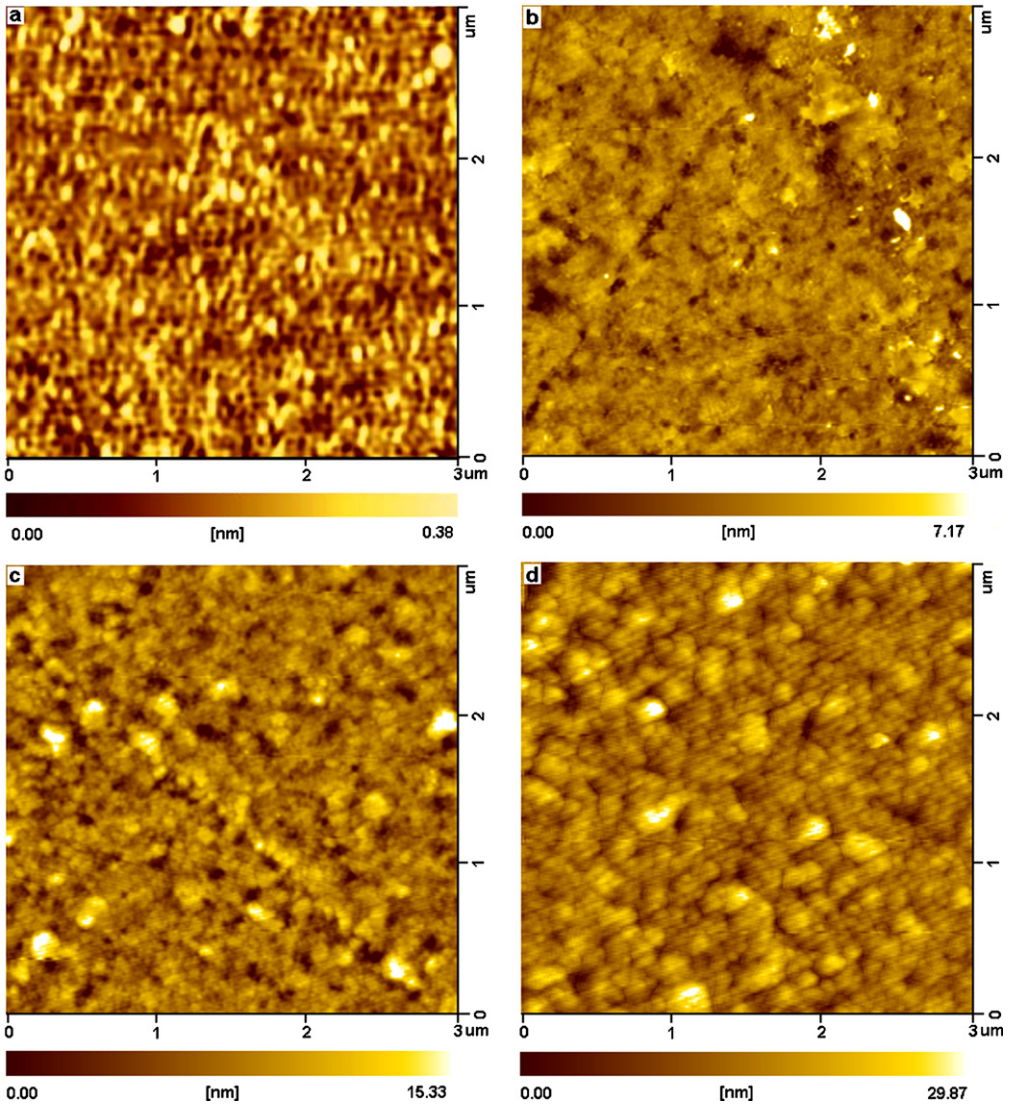

Fig. 3. AFM images of (a) bare Au slice, (b) Cys SAMs/Au, (c) biotin–Cys SAMs/Au and (d) QDs–neutravidin–biotin–Cys SAMs/Au. The AFM experiments were carried out using tapping mode with the scan speed of 1 Hz, and the each scan area was 3 μm × 3 μm.

The surface morphology could be clearly distinguished from that in Fig. 3c. On the base of EIS and AFM results, we conclude that biotin and QDs–neutravidin have been successfully immobilized on the electrode surface and thus the biosensor has been fabricated.

3.3. Electrochemical measurements of QDs

Before the electrochemical measurement, the captured QDs conjugates were dissolved with 1 M HCl solution for 5 min to release cadmium ions and metal components were quantified by anodic stripping voltammetry on BiFE. In order to achieve stable and sensitive BiFE by ex situ electrodeposition, several selected parameters such as plating solution composition, plating potential and time were studied. The optimum parameters were −1.2 V, 80 s and 5 mg mL^{-1} Bi(III), respectively, applying 0.05 M acetate buffer (pH 4.5) as supporting electrolyte with stirring. We examined the performance of the ex situ BiFE by recording adsorptive cathodic stripping current. A stripping peak at −0.2 V indicated that the bismuth film was stripped off. Then the electrochemical signal of the QDs conjugates was studied, measuring its metal components after the acid-dissolution step with a BiFE in 0.05 M acetate buffer (pH 4.5). Fig. 4 displayed a typical voltammogram of the CdTe QDs after dissolution with HCl. A well-defined peak was observed with peak potentials at −0.71 V (curve a) while no peak appeared in control experiments without QDs (curve c). When the concentration of QDs decreased from 5×10^{-6} to 5×10^{-8} mol L^{-1}, the peak current decreased 32.7% (curve b). Upon decreasing concentration of QDs conjugates, the cadmium stripping peak decreased continuously, which was used for the following determination based on competition procedure.

3.4. Optimization of incubation conditions

The stripping response mainly depended on the surface binding QDs–neutravidin conjugates. The concentration of QDs–neutravidin conjugates in the incubation solution was an important parameter. The effect of dilution of QDs–neutravidin conjugates on the ASV peak current was studied. With increasing volume of QDs–neutravidin conjugates added into the incubation solution, the stripping peak current increased and then tended

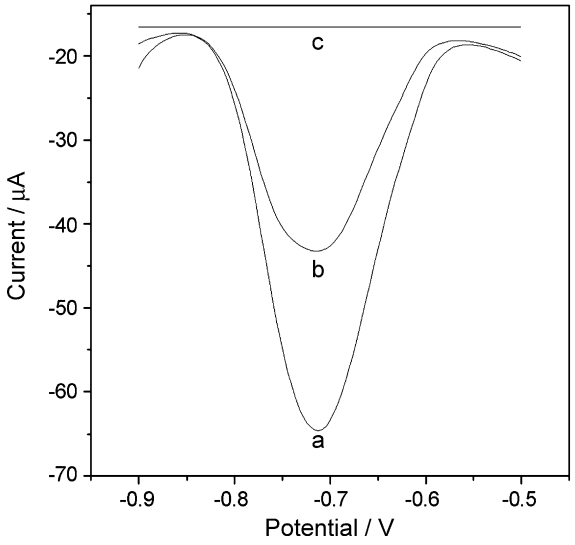

Fig. 4. Anodic stripping voltammetric signals in the presence of (a) 5×10^{-6} mol L^{-1} QDs, (b) 5×10^{-8} mol L^{-1} QDs and (c) without QDs in 0.05 M acetate buffer (pH 4.5). The captured QDs were dissolved with 1 M HCl solution for 5 min. The released metal ions were measured with ASV on BiFE, following a 3-min accumulation at −1.2 V.

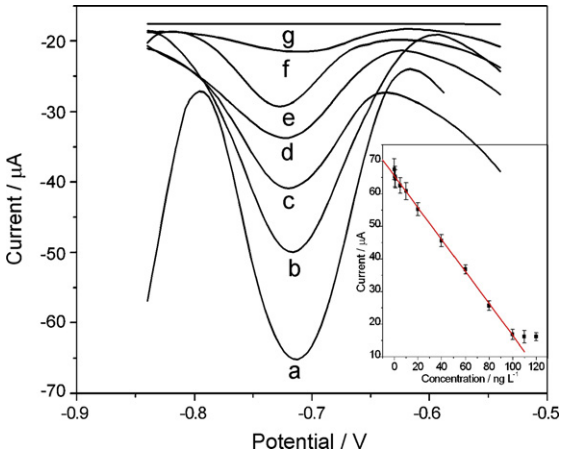

Fig. 5. Anodic stripping voltammogram of the captured QDs in the presence of (a) 0, (b) 10, (c) 40, (d) 60, (e) 80 and (f) 100 ng L^{-1} neutravidin and (g) the control experiment without capture QDs. Inset: calibration curves in the range of 0.5–100 ng L^{-1}. The conditions of ASV were the same as in Fig. 4, with 60 min incubation time.

to level off at a constant value. At a dilution of 50:100, the current reached a maximum value, indicating that the amount of the QDs–neutravidin conjugates in the incubation solution was enough to match the amount of the immobilized biotin on the surface. Thus, 1.0 mL of incubation solution containing 500 μL QDs–neutravidin conjugates was used for the reaction. Another factor which influenced the response was related to the incubation time. With increasing incubation time the stripping peak increased since more and more QDs–neutravidin has combined to biotin immobilized on the electrode surface. The stripping current reached a maximum at 60 min and longer incubation times did not improve the response. Thus, the optimal incubation time was 60 min. Although optical sensors are available, they involve several incubation and washing steps followed by spectrophotometric detection using a chromogenic substrate. Electrochemical method based on separation-free system avoids these disadvantages.

3.5. Competitive assay for neutravidin detection

Neutravidin, which has a near neutral isoelectric point and lowered nonspecific adsorption was used as a model protein. One-step competitive assay in connection with QDs–neutravidin conjugates was used for determination of neutravidin. The monitoring target neutravidin in the solution competed with the QDs–neutravidin conjugates to bind to the limited binding sites of the immobilized biotin. This was verified by the decrease of the cadmium stripping voltammetric response recorded after washing the substrate and dissolving the QDs at the surface. As expected for a competitive mechanism, the decrease of cadmium stripping voltammetric response was highly linear with the increasing concentration of neutravidin over the range of 0.5–100 ng L^{-1} (R = 0.994; Fig. 5). The detection limit was 5 nM (0.3 ng L^{-1}), which was significantly lower than previous reported of 120 nM based on a carbon paste electrode (Masarik et al., 2003). Since biotin–neutravidin is a kind of biospecific pair, it has high affinity and specific interaction (the binding constant is 10^{15} M^{-1}). Other proteins such as horseradish peroxidase (HRP), hemoglobin (Hb) and myoglobin (Mb) did not lead to a significant change in current (Fig. 6). Thus, the method had a good selectivity to neutravidin.

3.6. Reproducibility and stability of the sensor

The inter-assay precision was estimated by determining the response of 20 ng L^{-1} neutravidin at five different electrodes. The coefficient of variation was calculated to be 4.2% indicating acceptable inter-electrode reproducibility. The intra-assay precision of the sensors was evaluated by assaying one electrode for five replicate determinations, and the R.S.D. was 3.9%.

No obvious changes in fluorescence intensity and wavelength of both QDs and QDs–neutravidin conjugates were observed after a month storage period, indicating their highly stable size and photochemical stability. Thus, there is no degradation of QDs and no Cd ions. It does not affect the interaction between

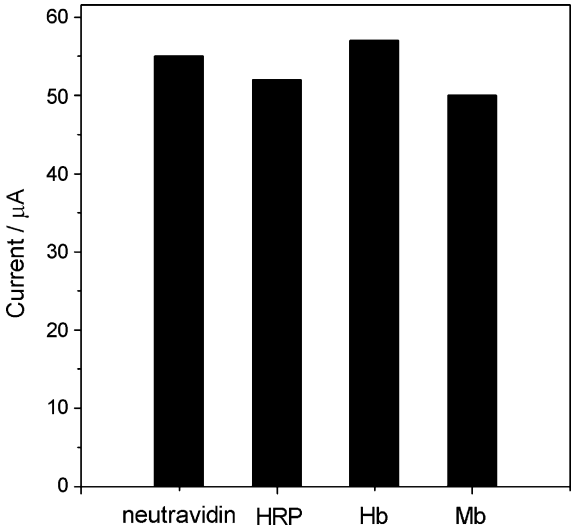

Fig. 6. Anodic stripping voltammetric signals of the captured QDs containing 20 ng L^{-1} neutravidin in the absence and presence of 100 ng L^{-1} HRP, 100 ng L^{-1} Hb, 100 ng L^{-1} Mb, respectively. The conditions of ASV were the same as in Fig. 4, with 60 min incubation time.

biotin and neutravidin in the detection. When the electrode was not in use, it was stored at 4 °C in dry condition. No obvious decrease in the response to neutravidin was observed in the first 5-day storage. After a 30-day storage period, the sensor retained 90% of its initial current response, indicating acceptable stability.

4. Conclusions

In this work, we have successfully developed a QDs-based electrochemical sensor for recognition and bioassay of protein. CdTe QDs were served as labels for amplifying signal output and monitoring the extent of competition process, which enabled a fast, sensitive and cost-effective measurement of neutravidin. Several advantages of the proposed method should be highlighted. First, CdTe QDs were functioned as an effective conjugate to provide a sufficient increase of stripping voltammetric response. Second, one-step competitive assay for monitoring recognition was more convenient, accurate and sensitive compared to earlier two-step sandwich bioassays. Third, the absence of nonspecific adsorption effects might be attributed to the high-density monolayer on the gold surface. Fourth, the biotin–avidin system reported here for electrochemical biosensing of free target could be readily expanded for other analogous measurements such as antigen–antibody and for other QDs-based transductions (e.g., fluorescence) of biotin–avidin recognition.

Acknowledgments

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