Journal of New Materials for Electrochemical Systems 16, 293-296 (2013)

An Electrochemical Biosensor for Detection of Thrombin based on Displacement Mode

XiaoYun Wang, Han Feng Cui, Lin Cheng and Hao Fan*

Department of Pharmacy, JiangXi University of Traditional Chinese Medicine, JiangXi 330004, China

Received: April 30, 2013, Accepted: July 30, 2013, Available online: August 12, 2013

Abstract: *A sensitive electrochemical biosensor for detection of thrombin based on target protein-induced strand displacement is presented. For this proposed biosensor, dsDNA which was prepared by the hybridization reaction of the immobilized probe ssDNA (IP) containing thiol group and thrombin aptamer base sequence was initially immobilized on the Au electrode by self-assemble via Au-S bind, and a DNA labeled with PdS nanoparticles (DP-PdS) was used as a detection probe. When the so prepared dsDNA modified Au electrode was immersed into a solution containing target protein and DP-PdS, the aptamer in the dsDNA give priority to to form G-quarter structure with the present target protein and the dsDNA sequence was released one single strand and returned to IP strand which consequently hybridized with DP-PdS. After dissolving the captured PdS particles from the electrode, a mercury-film electrode was used for electrochemical detection of these Pd^{2+} ions which offered sensitive electrochemical signal transduction. The peak current of Pd^{2+} ions had a good linear relationship with the thrombin concentration in the range of 7.3×10^{-8}–7.3×10^{-11} mol/L and the detection limit was 2.3×10^{-11} mol/L of thrombin. The detection was also specific for thrombin without being affected by the coexistence of other proteins, such as BSA and lysozyme.*

Keywords: Electrochemical biosensor, Displacement, DNA hybridization, PdS nanoparticles, Thrombin

1. INTRODUCTION

Proteins play a key role in biochemical systems and their rapid, sensitive, and specific identification attracts a lot of attention worldwide. To date, the antibody-based immunological assays are commonly used to detect proteins for disease diagnosis, drug screening and bio-defense [1]. But the methods used for protein detection are not as sensitive and specific as those for detection of specific nucleic acids sequences.

DNA aptamers are the single stranded nucleic acids with high affinity to proteins or other macromolecular compounds, which is comparable with antibodies to antigens. In biosensor studies, aptamers attract more attention as more robust capture molecules compared to current sensitive antibodies or enzymes, with their features such as long-term, thermal stability which is very useful for realizing easy-to-stock, easy-to-use biosensors. Aptamer-based protein detections have been illustrated in connected to colorimetric [2], fluorescence [3], quartz crystal microbalance and electrochemical detection [4-8].

Recently, electrochemical detection of DNA and protein has been reported by using the interesting DNA structure transformation in a single-stranded DNA, such as molecular hairpin [9-11]. This kind of electrochemical biosensors are based on an electrochemical response accompanied with the target-induced structure change of a probe strand containing a redox-active reporter group immobilized on the electrode surface. Attributed to that only one electron transfer difference happens for one binding event, the detection limit was low sensitive when compared to the biosensors based on using nanoparticle labeling. Quantum-dot (QD) semiconductor nanocrystals owing to their unique properties have been applied for optical DNA detection [12] and nanoparticle labels [13-14]. Recent researches have demonstrated that inorganic QDs could be used for electrochemical detection of DNA [15], electrical coding of single nucleotide polymorphisms [16], and electrochemical sandwich immunoassays of proteins [17].

In this paper, a sensitive electrochemical biosensor for detection of thrombin based on target protein-induced strand displacement and QDs detection is presented (Figure 1). A 24-bases SH-DNA as firstly immobilized probe ssDNA (IP) was hybridized with a 15-bases DNA sequence as thrombin aptamer (Apt) by 15 complementary bases to form dsDNA. Such-prepared dsDNA was then self-assembled onto a gold electrode via Au-S binds to prepare

*To whom correspondence should be addressed: Email:
Phone:

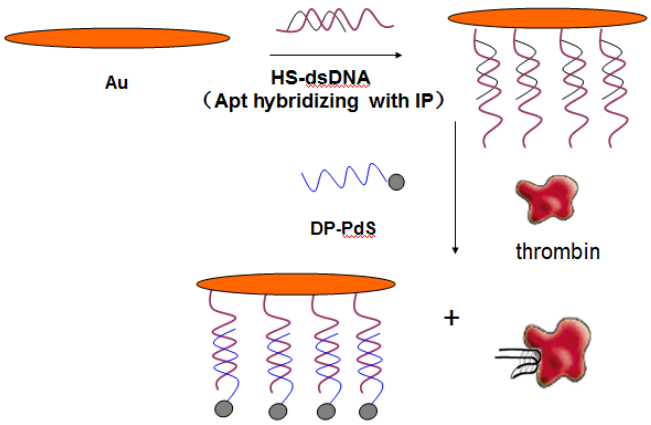

Figure 1. Schematic representation of the procedure to prepare electrochemical biosensor for the determine of thrombin via target protein-induced strand displacement and hybridization.

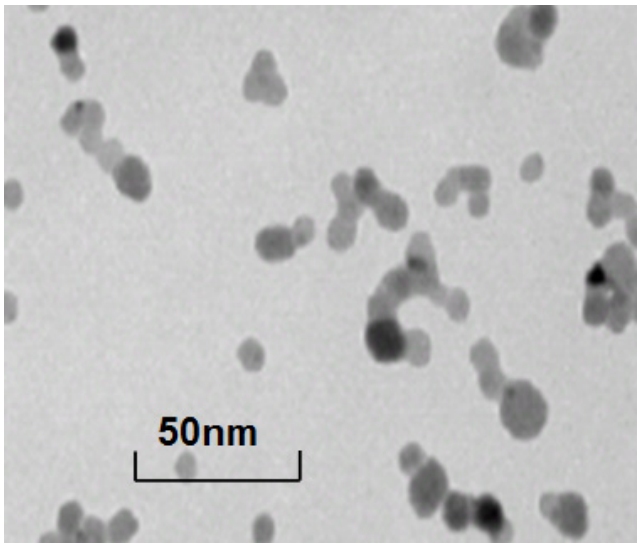

Figure 2. TEM image of the synthesized PdS nanoparticles

dsDNA modified electrode. When the dsDNA modified electrode was incubated in a solution containing thrombin and 18-bases detection probe ssDNA labeled PdS nanoparticles (DP-PdS) which has a partial complementary sequence with IP, Apt was separated from dsDNA because it tended to bind with the target thrombin, and the released IP single strand resulted to hybridize with DP-PdS. The amount of PdS captured on the electrode was proportional with the amount of thrombin in the solution. In the experiment, the PdS on the gold electrode was dissolved by HNO_3, and was then determined by differential pulse voltammetry using a mercury-film electrode.

This present method shares the merits of electrochemistry-based genesensor for DNA detection, such as high sensitivity, facile operation and low cost [18-19]. Compared to those methods based on the protein target inducing structure transformation, which used redox-active label and permitted about one electron transfer per binding event [10-11], this proposed biosensor takes the advantage of the signal amplification effect of the labeled PdS nanoparticle, resulting in a better detection sensitivity. Therefore, this electrochemical biosensor is expected to have wide applications in protein monitoring and disease diagnosis.

2. EXPERIMENTAL

2.1. Reagents

The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Shen energy Biocolor Biological Science and Technology Inc. (Shanghai, China). Bovine serum albumin (BSA) and thrombin were purchased from Dingguo Biotechnology Inc. (Shanghai, China). The nitric acid, cadmium chloride, sodium hydroxide, PBS (0.1 mol/L, PH=7.3), sodium acetate buffer (1 mol/L, pH=5.3) and other reagents were commercially available and of analytical reagent grade.

The DNA were obtained from Sangon Biotechology Inc. (Shanghai, China) with the following sequences:

Immobilized Probe ssDNA (IP):

5’-HS-C6-ATA CCA ACC ACA CCA ACC ATG CTC

Detection Probe ssDNA (DP):

5’-NH_2-C6-ATA GAG CAT GGT TGG TGT

Aptamer (Apt): 5’-GGT TGG TGT GGT TGG

2.2. Apparatus

Differential pulse voltammetry (DPV) measurements were performed using a CHI 660 Electrochemical Analyzer (CHI Instrument Inc., USA). The JB-1 stirring machine (Branson, Shanghai, China) and a TDL-16B centrifuge (Anting Science Instrument Inc., Shanghai, China) were used. The three-electrode electrochemical detection system consisted of a Au working electrode with sensing area of 3.14 mm^2, a Ag/AgCl reference electrode (saturated KCl) and a platinum wire counter electrode. The detection was carried out in a 5 ml electrochemical cell containing a mercury-coated glassy carbon working electrode (2 mm diameter), an Ag/AgCl reference electrode, and a platinum wire counter electrode.

2.3. Preparation of nano PdS

$Pb(NO_3)_2$ and Na_2S solutions were filtered through a 22 μm microporous membrane filter prior to use. PbS nanoparticles were prepared according to the literature [7] by using mercaptoacetic acid as the stabilizer. In brief, 9.22 μl mercaptoacetic acid was added to 50 ml 0.4 mM $Pb(NO_3)_2$ solution, and then the pH was adjusted to 7 with 0.5 M NaOH. The solution was bubbled with nitrogen for 30 min, followed by the slow addition of 1.34 mM Na_2S to the mixture solution. The molar ratio of Na_2S to $Pb(NO_3)_2$ was kept at 2.5. The reaction was carried out for 24 h under nitrogen protection and then gradually a brown colloid which is the PdS nanoparticles covered with a carboxyl group was obtained. As TEM images show, the diameter of PdS nanopatricles was about 7 nm (Figure 2).

2.4. Preparation of DNA–PdS conjugate

200 μl of 0.1 mol/L imidazole was added to 2OD of 5-amido-capped detection probe ssDNA (DP). After stirred for 30 min, 100 ml of 0.1 mol/L EDC and 5 ml of PdS colloids were added to the

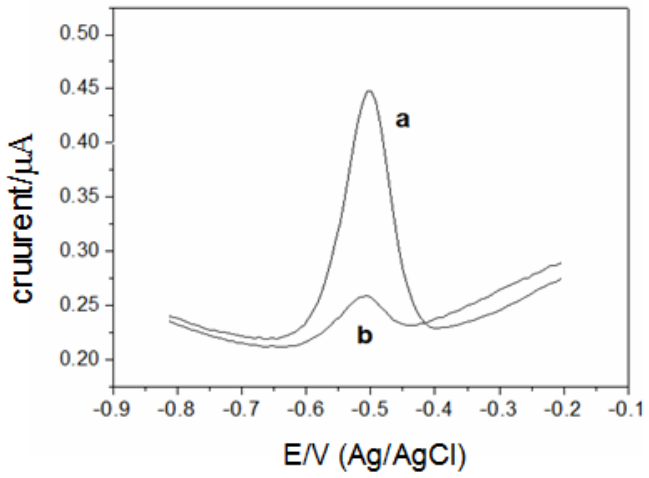

Figure 3. DPV response of the aptasensor in the prepared solution containing 0.6 mL of 10^{-6} mol/L P1 (b) in the presence of thrombin (c =2.6×10^{-9} M) (b) without thrombin.

mixture. The resulting mixture was stirred for 12 h at room temperature and then continued to centrifugate for at least 25 min at 14,000 rpm to remove the excessive DP. The yellow DP-PdS precipitate was washed with 0.1 mol/L PBS and re-dispersed in 0.1 mol/L PBS. Then, the resulting solution was stored in the refrigerator for further use.

2.5. Preparation of the dsDNA hybridized between IP and Apt

To obtain the dsDNA hybridized between IP and Apt, 100 μl 2×10^{-6} mol/L IP was added to 100 μl of 6×10^{-5} mol/L Apt, and the mixture was stirred to hybridize IP with Apt for 5 h at 37°C. The resulting dsDNA solution was stored in refrigerator for further use.

2.6. Immobilization of the dsDNA on Au electrode

The immobilization of dsDNA on Au electrode via Au-S binds was accomplished by first cleaning gold slides with a piranha solution (70% sulfuric acid, 30% H_2O_2) (Caution: Piranha solution reacts violently with many organic materials and should be handled with great care), followed by treatment of the electrodes with nitric acid, then with water and drying under air. 2 μl of appropriate dsDNA solution was dropped onto the electrode surface and the interaction was remained for 16 h. After that, the electrode was rinsed three times with phosphate buffer (0.1 mol/L, pH = 7.3) before next program.

2.7. Displacement and hybridization reaction

Fig.1 represents the procedure of preparing electrochemical biosensor for thrombin determination via target protein-induced strand displacement and hybridization. The displacement and hybridization reactions were done by immersing the dsDNA-modified Au electrode in the prepared solution containing appropriate thrombin and 600 μl of 10^{-6} mol/L DP-PdS, and then incubated at a certain temperature for 120 min with stirring. During this time, Apt that initially hybridized with IP preferred to form G-quarter structure with the target protein and dsDNA changed into IP. Subsequently, IP was hybridized with DP-PdS in prepared solution. The amount of thrombin could be indicated by the signal of DP-PdS hybridized with IP which came from thrombin-induced dsDNA dissociation.

2.8. Electrochemical detection

After a thorough washing procedure, the PdS nanoparticles on the gold substrate were dissolved by adding 200 μl of 0.10 mol/L HNO_3. Then 1.8 ml acetate buffer (0.1 mol/L, pH=5.3) was added into 200 ml of HNO_3 solution (containing dissolved Pd^{2+}). Electrochemical detection of the dissolved Pd^{2+} were performed at a mercury-film electrode using a 5 min deposition at -1.0 V in an acetate buffer solution (0.1 mol/L, pH=5.3). After the electrochemical deoxidation, DPV was immediately performed with the scan range from -0.8 to -0.2 V (Incr E 0.004V, amplitude 0.05 V, pulse width 0.05 s, pulse period 0.2 s), resulting in an analytical signal due to the oxidation of Pd, which relates to the amount of the PdS nanoparticles for the hybridization format. The DPV peak height at a potential of -0.52 V of the oxidation of Pd was used in all of the measurements. The mercury-film electrode was prepared on a polished glassy carbon electrode by applying a potential of -1.10 V for 10 min in a 0.1 mol/L HCl solution containing 100 mg /L Hg^{2+}.

3. RESULTS AND DISCUSSION

3.1. Principle of detection of thrombin based on protein-induced strand displacement

IP was firstly hybridized with Apt to form the dsDNA sequences for their 15 bases were complementary, and then the dsDNA were immobilized onto the Au electrode. DP was an 18-bases amino oligonucleotide with 60% DNA sequence identical with IP, and it was pre-modified with PdS nanoparticles. According to reference [21], as the base sequence identicalness less than 80% of the thrombin-aptamer would not react with thrombin, DP could be co-existed with thrombin in the same solution without binding. When the dsDNA-modified Au electrode was immersed into the solution containing thrombin and DP-PdS, thrombin molecules would capture IP from dsDNA since a thrombin molecule included specific aptamer binding sites. Then DP-PdS would have access to hybridize with IP. Because the quantity of IP on the Au electrode was directly proportional to that of thrombin in solution, the quantity of thrombin could be indicated by the amount of DP-PdS. The electrochemical detection of DP-PdS was performed following section 2.8. As shown in Figure 2b, the Pd stripping peak (Ep= -0.52 V) from the $MgCl_2$-phosphorate buffer solution containing DP-PdS only was resulted from the remained IP unhybridized with DP-PdS on the dsDNA immobilized Au electrode. When 2.3×10^{-9} mol/L thrombin was added in the solution mentioned above, a 5-fold signal increase was obtained as shown in Figure 2a. It means that more thrombin molecules lead to more dsDNA returned to IP and consequently an enhanced PdS signal due to the hybridization of IP and DP-PdS. According to the formula of Marmur-Doty [22], Apt has higher affinity than DP to IP sequence as it comprises more complementary GC bases, and additionally the DP should have taken several hours to hybridize with IP [23], thus DP could hardly displace Apt from dsDNA in the experiments.

3.2. Detecting specificity

In order to confirm the specificity of the aptasensor to thrombin, control experiments were performed using BSA and of lysozyme

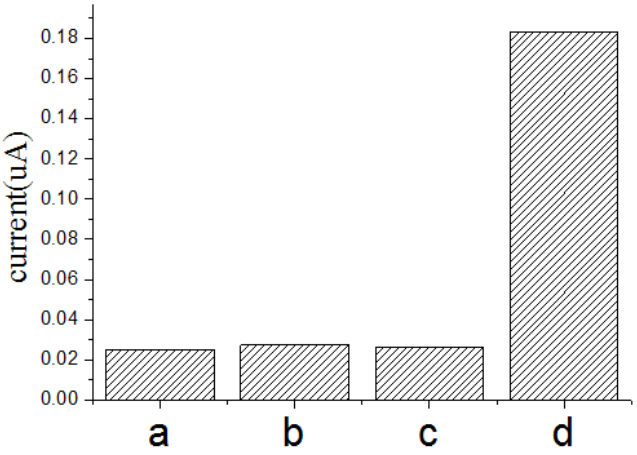

Figure 4. Comparison of the signal of dsDNA modified electrode when incubated in prepared solution contain 10^{-6} mol/L PdS nanoparticle-labeled DNA probe with (a) without thrombin,(b) with 3.0×10^{-7} M of lysozyme, (c) with 2.5×10^{-8} M of BSA, (d) with 2.15×10^{-10} M of thrombin.

respectively to replace thrombin. As shown in Figure 4 (a–d), only thrombin induced a marked response, while the other two proteins had almost negligible responses. These results showed that an extraordinary specificity of the detection system to the target protein could be obtained.

3.5. Thrombin determination

In the experiments, the Au electrode modified with dsDNA was incubated with the target protein of different concentrations in the presence of DP-PdS.The peak currents increased with the thrombin concentration increasing and a good linear relationship was displayed in the range of 7.3×10^{-8}–7.3×10^{-11} mol/L thrombin with a correlation coefficient of 0.995 and the detection limit was estimated at 2.3×10^{-11} M by using 3σ.

4. CONCLUSION

The present study has introduced a new electrochemical biosensor based on target protein-induced strand displacement for thrombin detection. The sensitivity of the method for thrombin determination could be comparable to the present PdS nanoparticle-based DNA detection schemes. As low as 2.3×10^{-11} mol/L of thrombin has been specifically recognized, and other proteins such as BSA and lysozyme didn't affect the target detection. It is expected that other proteins could be detected using this protocol at high sensitivities if choosing appropriate aptamers.

5. ACKNOWLEDGMENT

This work was financially supported by the NSF of China (Grant No. 21265007), and JiangXi Science and Technology Committee (Grant No. 20122BAB215024).

REFERENCES

[1] Ferrari M., Nat. ReV. Cancer., 5, 161 (2005).
[2] Stojanovic M.N., Landry D.W., J. Am. Chem. Soc., 124, 9678 (2002).
[3] Merino E.J., Weeks K.M., J. Am. Chem. Soc., 125, 12370 (2003).
[4] Liss M., Petersen B., Wolf H., Prohaska E., Anal. Chem., 74, 4488 (2002).
[5] Xu D., Yu X., Liu Z., He W., Ma Z., Anal. Chem., 77, 5107 (2005).
[6] Kazunori I., Chiharu K., Koji S., Biosensors and Bioelectronics., 20, 2168 (2005).
[7] Radi A.E., J. Sanchez L.A., Baldrich E., O'Sullivan C.K., Anal. Chem., 77, 6320 (2005).
[8] Kawde A.N., Rodriguez M.C., Lee T.M.H., Wang J., Electrochem. Commun., 7, 537 (2005).
[9] Xiao Y., Qu X.G., Plaxco K.W., Heeger A.J., J. Am. Chem. Soc., 129, 11896 (2007).
[10]Xiao Y., Lubin A.A., Heeger A.J., Plaxco K.W., Angew. Chem., 117, 5592 (2005).
[11]XiaoY., Piorek B.D., Plaxco K.W., Heeger A.J., J. Am. Chem. Soc., 127, 17990 (2005).
[12]Han M., Gao X., Su J.Z., Nie S., Nat. Biotechnol., 19, 631 (2001).
[13]Pavlov V., Xiao Y., Shlyahovsky B., Willner I., J. Am. Chem. Soc., 126, 11768 (2004).
[14]Levy M., Cater S.F., Ellington A.D., Chem. Bio. Chem., 6, 2163 (2005).
[15]Wang J., Liu G., Merkoçi A., J. Am. Chem. Soc., 125, 3214 (2003).
[16]Liu G., Lee T.M.H., Wang J., J. Am. Chem. Soc., 127, 38 (2005).
[17]Karin Y., Dai Z., Xiang Y., Wang J., J. Am. Chem. Soc., 128, 13676 (2006).
[18]Cai H., Shang C., Hsing I.M., Anal. Chim. Acta., 523, 61 (2004).
[19]Cai H., Lee T.M.H., Hsing I.M., Electroanalysis., 16, 81 (2004).
[20]Liu G., Lee T.M.H., Wang J., J. Am. Chem. Soc., 127, 38 (2005).
[21]Ellington A.D., Szostak J., Nature., 346, 818 (1990).
[22]Long D.D., Grosse I., Mark K.A., Biophysical chemistry., 110, 25 (2004).
[23]Hansen J.A., Mukhopadhyay R., Hansen J., Gothelf K..V., J. Am. Chem. Soc., , 128, 3860 (2006).