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Aptamer-Based Electrochemical Sensors with Aptamer−Complementary DNA Oligonucleotides as Probe

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This study describes a facile and general strategy for the development of aptamer-based electrochemical sensors with a high specificity toward the targets and a ready regeneration feature. Very different from the existing strategies for the development of electrochemical aptasensors with the aptamers as the probes, the strategy proposed here is essentially based on the utilization of the aptamer−complementary DNA (cDNA) oligonucleotides as the probes for electrochemical sensing. In this context, the sequences at both ends of the cDNA are tailor-made to be complementary and both the redox moiety (i.e., ferrocene in this study) and thiol group are labeled onto the cDNA. The labeled cDNA are hybridized with their respective aptamers (i.e., ATP- and thrombin-binding aptamers in this study) to form double-stranded DNA (ds-DNA) and the electrochemical aptasensors are prepared by self-assembling the labeled ds-DNA onto Au electrodes. Upon target binding, the aptamers confined onto electrode surface dissociate from their respective cDNA oligonucleotides into the solution and the single-stranded cDNA could thus tend to form a hairpin structure through the hybridization of the complementary sequences at both its ends. Such a conformational change of the cDNA resulting from the target binding-induced dissociation of the aptamers essentially leads to the change in the voltammetric signal of the redox moiety labeled onto the cDNA and thus constitutes the mechanism for the electrochemical aptasensors for specific target sensing. The aptasensors demonstrated here with the cDNA as the probe are readily regenerated and show good responses toward the targets. This study may offer a new and relatively general approach to electrochemical aptasensors with good analytical properties and potential applications.

Aptamers are one kind of single-stranded DNA and RNA sequences that could be in vitro synthesized with systematic evolution of ligands by exponential enrichment (SELEX).[1] The specific binding affinity of this kind of artificial oligonucleotide toward a variety of targets ranging from small molecules,[2] proteins,[3] and even to cells[4] substantially enable them to be used as recognition elements for biosensing applications.[5] To this end, the recent few years have witnessed substantial progress in the use of aptamers to develop aptasensors.[6] Particularly, the combination of the advantages of electrochemical methods with the specific recognition properties of the aptamers has enabled the investigations on electrochemical aptasensors, which are both theoretically interesting and practically useful.[7]

So far, most electrochemical aptasensors have been constructed mainly based on the conformational change of the aptamers induced by the specific target binding, as schematically shown in Scheme 1 A.[6,8] In this context, the aptamers that were used as the probes for electrochemical target sensing were normally functionalized with a thiol group at one end so that they could be anchored onto the electrode (e.g., Au electrode) to make the as-prepared aptasensors essentially reagentless. The other end of the aptamer probes was labeled with redox moieties such as ferrocene[8b,e] and methylene blue[8a,c,d] for electrochemical sensing.

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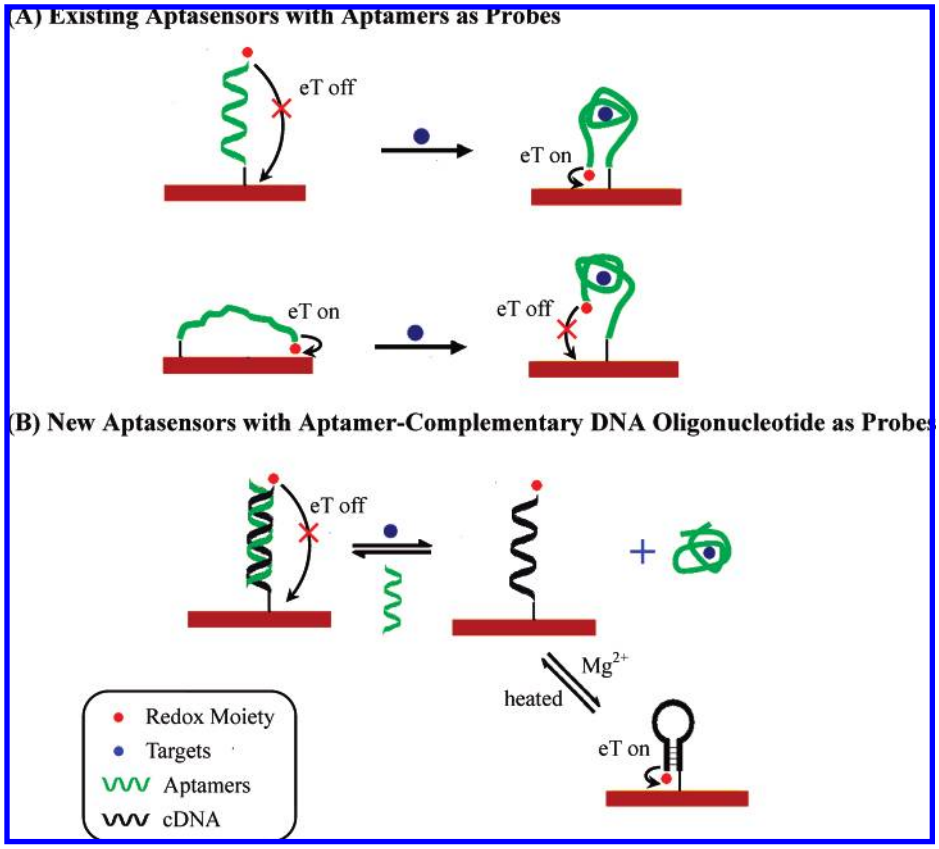
Scheme 1. Schematic Illustration of the Strategies for Electrochemical Aptasensors with (A) Labeled Aptamers and (B) the Aptamer−Complementary DNA Oligonucleotides as the Probes[a]



[a] The existing strategies with labeled aptamers as the probes are essentially based on the large-scale electrochemically utilizable conformational change of the aptamers induced by target binding, while the strategy proposed here with the aptamer−complementary DNA oligonucleotides as the probes is based on the formation of a hairpin structure of cDNA probes through the hybridization of the tailor-made complementary sequences at their both ends caused by the target binding-induced dissociation of the aptamers. ATP-binding aptamer, (5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′); complementary DNA, (5′-ACCTTCCTCCGCAATACTCCCCCAGGT-3′); thrombin-binding aptamer, (5′-GGTTGGTGTGGTTGG-3′); complementary DNA (I), (5′-CCAACCACACCAACCGGTTGG-3′).

The specific binding of the targets to the surface-confined and redox-labeled aptamers essentially induces the conformational change of the aptamer probes and, as such, leads to the change in the distance between the labeled redox moieties and the electrode, which eventually results in the large variation in the voltammetric signal that could be used for electrochemical sensing of the targets (Scheme 1A). Although this kind of electrochemical aptasensor with the aptamers as the probes has so far been employed for selective and sensitive electrochemical sensing of targets such as cocaine, ATP, and thrombin,[8b,c,e] those aptasensors generally required a large-scale conformational change of the aptamer probes induced by the specific target binding, which could be utilized electrochemically for target sensing.[8] Moreover, the electrochemical aptasensors with the surface-confined aptamers as the probes may suffer from limitations from the complex procedures for sensor regeneration presumably due to the strong binding affinity of the aptamers toward the targets.[8a,b,9b]

Different from the existing strategies with the surface-confined aptamers as the probes for electrochemical target sensing,[8,9] this study describes a new and relatively general approach to the electrochemical aptasensors by using the aptamer−complementary DNA (cDNA) oligonucleotides as the probes, as shown in Scheme 1B. For this purpose, the cDNA probes used here are tailor-made to have complementary bases at both ends, at which both the thiol group and redox moiety are labeled. The labeled cDNA probes are hybridized with their aptamers to form a Watson−Crick helix, i.e., double-strand oligonucleotides, and the electrochemical aptasensors are fabricated by confining the as-formed double-stranded oligonucleotides onto the Au electrode through a self-assembling approach.[10] Initially, the redox moiety labeled on the cDNA is kept relatively far from the electrode and thereby the electron transfer (eT) is essentially retarded. Upon the target binding, the label-free aptamers dissociate into solution from their cDNA oligonucleotides,[5d] leaving the single-stranded cDNA oligonucleotides onto the electrode surface. The single-stranded cDNA oligonucleotides with tailor-made complementary bases at both ends could form a stable hairpin structure with the presence of Mg^{2+},[11] and as a consequence, the electron

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transfer of the redox moiety is facilitated due to the shortened distance between the redox moiety and the electrode (Scheme 1B). Compared with the existing strategies for the development of electrochemical aptasensors with the aptamers as the probes (Scheme 1A),[8,9a] the approach demonstrated here with the aptamer−complementary DNA oligonucleotides as the probes could be advantageous because the sequences of the cDNA oligonucleotides may be rationally designed and readily tailored, and as a consequence, the conformational change of such cDNA probes resulting from the target binding-induced dissociation of the aptamers is envisaged to be readily utilized electrochemically for voltammetric sensing. Moreover, the approach described here may bear advantages in the simple procedures for the sensor regeneration and in the generality for the aptasensor development. To the best of our knowledge, the study undertaken here has not been reported and may offer a new and general approach to electrochemical aptasensors with good properties and potential applications.

EXPERIMENTAL SECTION

Chemicals and Reagents. The 27-mer ATP-binding aptamer (i.e., 5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′) and its complementary DNA (cDNA) oligonucleotide modified with −SH at the 5′ end and $-NH_2$ at the 3′ end (i.e., 5′-SH−$(CH_2)_6$-ACCTTCCTCCGCAATACTCCCCCAGGT−NH_2-3′) as well as the 15-mer thrombin-binding aptamer (i.e., 5′-GGTTGGTGTGGTTGG-3′) were synthesized and purified by Invitrogen Biotech Co. Ltd. (Shanghai, China). For the comparative studies, two complementary DNA oligonucleotides were synthesized and purified for the thrombin-binding aptamer, of which both were modified with −SH at the 5′ end and $-NH_2$ at the 3′ end, by the same company. One was synthesized by additionally introducing GGTTGG bases at the 3′ end of the cDNA oligonucleotide of the thrombin-binding aptamer (i.e., 5′-SH−$(CH_2)_6$−CCAACCACACCAACCGGTTGG−NH_2-3′ (cDNA (I)). The other was synthesized by additionally introducing AAAAAA bases at the 3′ end of the cDNA oligonucleotide of the thrombin-binding aptamer (i.e., 5′-SH−$(CH_2)_6$−CCAACCACACCAACCAAAAAA−NH_2-3′ (cDNA (II)). Under the present conditions, the melting temperature (T_m) values of the synthetic cDNA of the ATP-binding aptamer and the cDNA (I) and cDNA (II) of the thrombin-binding aptamer as well as the hairpin structure of the cDNA of the ATP-binding aptamer and thrombin-binding aptamer (i.e., cDNA (I)) were predicted to be 77.4, 73.3, 66.7, 30.4, and 64.4 °C, respectively, in accordance with the methods reported previously.[12]

Adenosine-5′-triphosphate (ATP) and cytidine-5′-triphosphate (CTP) were purchased from Merck (California) and Fermentas (Vilnius, Lithuania), respectively. Pure human thrombin was supplied by Newprobe Biotechnology Co. Ltd. (Beijing, China). Bovine serum albumin (BSA) was obtained from Proliant Co.. Ferrocene carboxylic acid and Immunoglobulin G (IgG) were purchased from Sigma. Tris(hydroxymethyl)aminomethane (Tris) was purchased from Amresco (Ohio). 1-Ethyl-3-[(3-dimethylamino) propyl] carbodiimide (EDC) and 3-mercaptopropionic acid (MPA) were obtained from Acros (Geel, Belgium). *N*-Hydroxysuccinimide (NHS) was purchased from Alfa Aesar (Texas). All other chemicals were of at least analytical reagent and used without further purification. Aqueous solutions were prepared with doubly distilled water.

Hybridization of Synthetic cDNA Oligonucleotides with Aptamers. The synthetic cDNA oligonucleotides were hybridized with their respective aptamers to form double-stranded DNA (ds-DNA) oligonucleotides by mixing 67 μM aptamers and 33 μM cDNA into 45 μL of distilled water, to which 5 μL of the annealing buffer (100 mM Tris + 1 M NaCl + 0.5 M EDTA, pH 7.4) was added. The resulting mixture was heated to 95 °C for 10 min, and the solution temperature was then gradually decreased from 95 °C to 20 °C at a rate of 1 °C/min to give ds-DNA solutions. The ds-DNA oligonucleotides were labeled with the ferrocene moiety by adding 100 μM ferrocene carboxylic acid, 5 mM EDC, and 25 mM NHS into the ds-DNA solutions, and the resulting mixture was incubated at room temperature for 2 h. The Fc-labeled ds-DNA were then desalted and purified by ultrafiltration.

Preparation and Regeneration of Electrochemical Aptasensors. The electrochemical aptasensors were prepared on Au electrodes (1.6 mm in diameter, Bioanalytical Systems Inc.). Au electrodes were polished with alumina powder (0.3 and 0.05 μm) and sonicated in acetone and doubly distilled water (each for 3−5 min). The electrodes were then electrochemically pretreated by consecutively cycling the potential between −0.2 and +1.6 V at 0.5 V s^{-1} in 0.5 M H_2SO_4 solution until a cyclic voltammogram characteristic of a clean Au electrode was obtained. An amount of 50 μL of SH− and Fc-labeled ds-DNA solution (3 μM) was dropped onto the electrodes, and the electrodes were covered with a plastic cap to avoid the solution evaporation. After being kept overnight at room temperature, the electrodes were immersed into a 0.1 M MPA solution in 20 mM Tris buffer for 10 min to further form a submonolayer at the unoccupied Au surface. As reported previously,[7b,8d] the formation of the MPA submonolayer could displace the unspecifically bound material at the electrode. The prepared aptasensors based on the Fc-labeled ds-DNA-modified Au electrodes were rinsed first with 20 mM Tris buffer containing 140 mM NaCl and 5 mM $MgCl_2$ (pH 7.6) and then with doubly distilled water. The surface coverage of the ds-DNA confined onto the Au electrode was estimated with the amount of charge consumed in the reductive desorption from Au electrode modified only with ds-DNA (i.e., without MPA submonolayer) in 0.5 M KOH aqueous solution[13] and was calculated to be 7.9×10^{-12} mol cm^{-2}.

The regeneration of the aptasensors was preformed by immersing the Fc-labeled ds-DNA-modified electrodes previously challenged to the targets into the annealing buffer diluted 10 times with distilled water containing 10 μM of the aptamers under 95 °C for 10 min, and the solution temperature was then gradually decreased from 95 to 20 °C at a rate of 1 °C/min. The regenerated aptasensors were finally rinsed with distilled water before reuse.

Apparatus and Measurements. Electrochemical measurements were performed with an AutoLab electrochemical system (PGSTAT 302, Ecochemie, The Netherlands) in 20 mM Tris buffer containing 140 mM NaCl and 5 mM $MgCl_2$ (pH 7.6). $MgCl_2$ was added into the electrolyte to induce the formation of the hairpin structure of cDNA assembled onto the electrode surface, as

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reported previously.[11] The Fc-labeled ds-DNA-modified Au electrodes were used as a working electrode, an Ag/AgCl (KCl-saturated) electrode as reference electrode, and Pt wire as an auxiliary electrode. Electrochemical impedance spectroscopy (EIS) was performed in 20 mM Tris buffer containing 140 mM NaCl and 20 mM $Na_3[Fe(CN)_6]$ within a frequency range from 2×10^4 to 0.1 Hz. Differential pulse voltammetry (DPV) was performed with an initial potential of 0.60 V, amplitude of 0. 05 V, step potential of 0.007 V, and scan rate of 0.03 V s^{-1}. Circular dichroism (CD) spectra were recorded on a J-815 CD spectrometer (JASCO, Japan) at a scan speed of 0.5 nm s^{-1}. Unless stated otherwise, all measurements were performed at ambient temperature.

RESULTS AND DISCUSSION

Design of the cDNA Probes. Because the strategy described here (Scheme 1B) is essentially based on the target binding-induced conformational change of cDNA oligonucleotides, i.e., from a double-stranded helix to a single-stranded oligonucleotide and finally to a hairpin structure, the presence of complementary bases at both ends of the cDNA oligonucleotides remains essential for the formation of the electrochemically utilizable hairpin structure of the cDNA. For this reason, we employed two different types of aptamers, i.e., ATP- and thrombin-binding aptamers, as the models to demonstrate the generality of our strategy for the development of electrochemical aptasensors. This is because the ATP-binding aptamer itself bears four pairs of complementary bases at its both ends (i.e., 5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′),[2,5b] and accordingly, its cDNA oligonucleotide inherently has four pairs of complementary bases (i.e., 5′-ACCTTCCTCCGCAATACTCCCCCAGGT-3′) at both of its ends. Thus, the strategy described here could be readily adopted for the development of ATP aptasensors without a further requirement of tailor-made design of the cDNA sequence for the formation of the hairpin structure for electrochemical sensing. Differently, the thrombin-binding aptamer does not have such complementary bases at both of its ends (i.e., 5′-GGTTGGTGTGGTTGG-3′).[3] For these kinds of aptamers, we may rationally design their complementary DNA sequences by simply introducing additional bases at one end that are complementary with the bases at the other end. For example, the sequence of the complementary DNA oligonucleotide of the thrombin-binding aptamer was designed as 5′-CCAACCACACCAACCGGTTGG-3′ by introducing six bases (i.e., GGTTGG) at the 3′ end of the complementary DNA of the thrombin-binding aptamer, which are complementary to the bases at the 5′ end (Scheme 1B). The cDNA oligonucleotides were labeled with the thiol group (−SH) at their 5′ end and ferrocene (Fc) at their 3′ end, and the labeled cDNA were then hybridized with their respective aptamers to give double-stranded oligonucleotides that were self-assembled onto Au electrodes to form the electrochemical aptasensors, as detailed in Experimental Section.

Formation of Watson−Crick Helix and Hairpin Structures of cDNA Probes. To confirm the formation of a Watson−Crick helix, i.e., double-strand oligonucleotides, of synthetic cDNA with their respective aptamers under the present conditions, we compared the CD spectra of the mixture of the cDNA and their respective aptamers before (− − −) and after (—) the annealing process by using the ATP-binding aptamer and its cDNA as an example, as displayed in Figure 1. The annealing treatment of

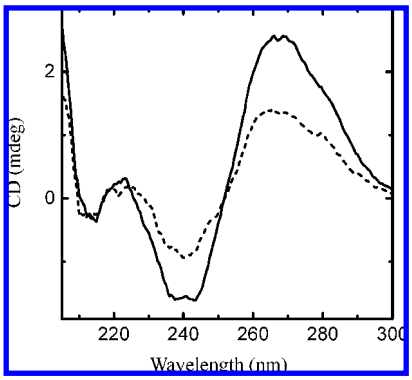

Figure 1. CD spectra of the mixture of 2.5 μM ATP-binding aptamer and 2.5 μM cDNA in 20 mM Tris buffer containing 140 mM NaCl before (− − −) and after (—) the annealing process.

the mixture essentially results in the increase in the molar ellipticity, suggesting the formation of the Watson−Crick helix between cDNA and the aptamer under the present conditions, as reported previously.[9c,14]

In order to verify the formation of the hairpin structures of cDNA probes with the presence of Mg^{2+}, two DNA oligonucleotides (i.e., cDNA (I) and cDNA (II)) were designed to be complementary to the thrombin-binding aptamer by introducing six bases at the 3′ end of the cDNA of the thrombin-binding aptamer, as described above. The cDNA (I) with tailor-made complementary sequences at both ends was anticipated to be able to form a hairpin structure,[15] while the cDNA (II) was not because it does not have the complementary sequences at both ends. The formation of a hairpin structure of cDNA (I) induced by Mg^{2+} could presumably be supported by the CD spectra of the cDNA (I) and cDNA (II) with the presence of Mg^{2+} in Tris buffer, as depicted in Figure 2. The addition of Mg^{2+} in Tris buffer essentially results in the amplification of the peak of cDNA (I) (Figure 2A), which was almost consistent with the previous report.[11b] Nevertheless, the CD spectrum of cDNA (II) remains almost unchanged with the addition of Mg^{2+}, as shown in Figure 2B. This comparison implies that Mg^{2+} could induce the structural transition of cDNA (I) with complementary sequences at both ends from a single-stranded oligonucleotide into a hairpin structure.

The formation of the Watson−Crick helix with the aptamer and the hairpin structure of cDNA could also be expected to occur on Au electrodes, which was preliminarily studied with electrochemical impedance spectrometry (EIS) at the cDNA-modified Au electrodes by using the cDNA of the ATP-binding aptamer as an example, as displayed in Figure 3. The cDNA confined onto Au electrodes was hybridized with ATP-binding aptamer by immersing the electrodes into annealing buffer diluted 10 times with distilled water containing 10 μM aptamer, and the hybridization was accomplished with the same procedure as those for DNA

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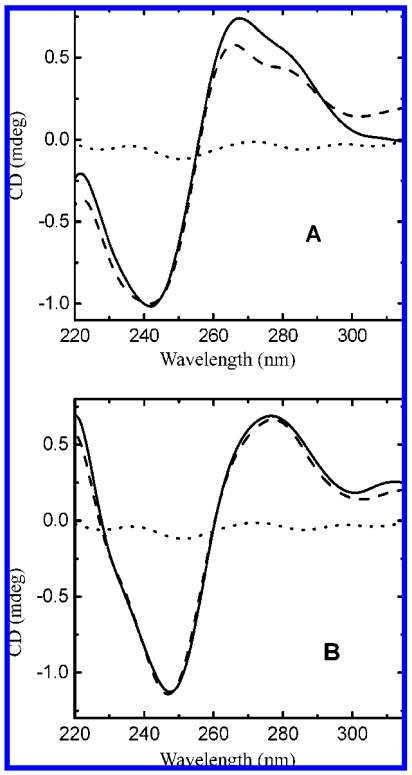

Figure 2. CD spectra of (A) 2.5 μM cDNA (I) (i.e., CCAACCACAC-CAACCGGTTGG) and (B) cDNA (II) (i.e., CCAACCACACCAAC-CAAAAAA) of the thrombin-binding aptamer in 20 mM Tris buffer containing 140 mM NaCl in the presence (—) and absence (– – –) of 5 mM Mg^{2+}. The dotted lines represent the CD spectrum of 5 mM Mg^{2+} in the same buffer.

hybridization in solution (i.e., heating the solution to 95 °C for 10 min and then gradually decreasing the solution temperature from 95 to 20 °C at a rate of 1 °C/min). At the bare Au electrode, very small charge-transfer resistance (R_{ct}), which was measured as the radius of the semicircle in the EIS, was recorded (black ■), suggesting a fast electron-transfer process at such electrode. Compared with the bare Au electrode, the cDNA-modified Au electrode shows a larger R_{ct} (pink ○) , mainly due to the enlarged charge-transfer distance caused by the electrostatic repelling between the $Fe(CN)_6^{3-/4-}$ redox couple and the negatively charged cDNA confined onto the Au electrode. The R_{ct} was further enlarged after the cDNA-modified electrode was treated in the diluted annealing buffer containing the ATP-binding aptamer with the procedures mentioned above (green ▲), suggesting the formation of the Watson–Crick helix of the cDNA with its aptamer on the electrode surface (i.e., ds-DNA-modified electrode). We reasoned this from the fact that the formation of the Watson–Crick helix on the Au electrode essentially increases the negative charge at the electrode surface and thereby enhances electrostatic repelling interaction between the ds-DNA duplex and the $Fe(CN)_6^{3-/4-}$ redox couple. The R_{ct} of the ds-DNA-modified electrode was almost restored to the value of the cDNA-modified electrode after the ds-DNA-modified electrode was challenged to the target (i.e., ATP) (blue ▼) , indicative of the dissociation of the aptamer from the electrode surface upon specific target binding. Also as shown in Figure 3, the subsequent addition of Mg^{2+} in solution results in the decrease in the R_{ct}, (red ♦) possibly

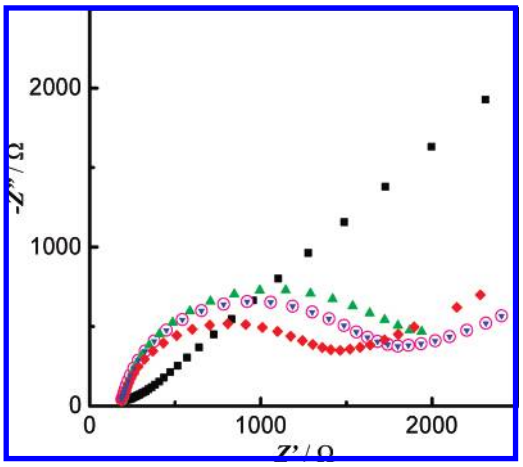

Figure 3. Nyquist plots obtained with bare (black ■), cDNA-modified (pink ○), and ds-DNA-modified (green ▲) Au electrodes in 20 mM Tris buffer containing 140 mM NaCl and 20 mM $Na_3[Fe(CN)_6]$. The ds-DNA-modified electrodes were prepared by immersing the cDNA-modified electrodes into diluted annealing buffer containing 10 μM ATP-binding aptamer, and the buffer was treated with the same procedures as those for DNA hybridization. The blue ▼ represents the Nyquist plot obtained with the ds-DNA-modified Au electrode after the electrode was immersed into Tris buffer containing 140 mM NaCl and 100 nM ATP for 30 min. The red ♦ represents the Nyquist plot obtained with the electrode in Tris buffer containing 140 mM NaCl and 5.0 mM Mg^{2+} after the ds-DNA-modified Au electrode was immersed into 20 mM Tris buffer containing 140 mM NaCl and 100 nM ATP for 30 min, taken out of solution, and rinsed with distilled water. EIS conditions: potential, open circuit potential; alternative voltage, 5 mV; frequency range, 2×10^4 to ~ 0.1 Hz.

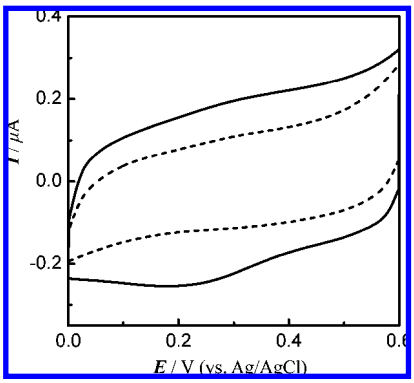

Figure 4. CVs at the Fc-labeled ds-DNA-modified electrode with ATP-binding aptamer in 20 mM Tris buffer containing 140 mM NaCl and 5 mM $MgCl_2$ (pH 7.6) before (– – –) and after (—) the electrode was incubated in 1 mM ATP in Tris buffer for 10 min. Scan rate, 0.1 V s^{-1}.

suggesting the formation of the hairpin structure of cDNA onto the Au electrode induced by Mg^{2+}.

The change in the R_{ct} described above essentially suggests that the cDNA probes assembled onto the electrode surface could form a Watson–Crick helix with their respective aptamers and a hairpin structure induced by Mg^{2+}. These properties substantially form a strong basis for the electrochemical sensing of the targets, as demonstrated below.

Aptasensor Responses to the Targets. Figure 4, with the ATP-binding aptamer as an example, depicts cyclic voltammograms (CVs) at the Fc-labeled ds-DNA-modified Au electrode in Tris buffer containing 140 mM NaCl and 5 mM $MgCl_2$. The

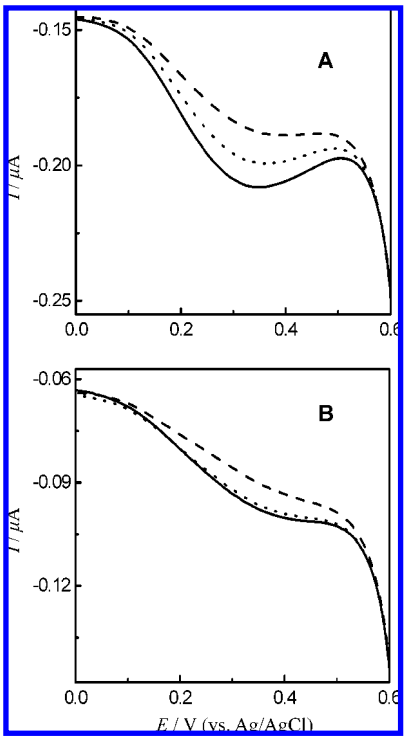

Figure 5. DPVs at the Fc-labeled ds-DNA-modified Au electrodes with thrombin-binding aptamer hybridized with its (A) cDNA (I) and (B) cDNA (II) in 20 mM Tris buffer containing 140 mM NaCl (pH 7.6) before (– – –) and after (•••) the electrodes were incubated in the aqueous solution of 2 nM thrombin in Tris buffer for 10 min. The solid curves represent the DPVs at the electrode under the same conditions after subsequent addition of 5 mM $MgCl_2$ into the electrolyte. DPV conditions: initial potential, 0.60 V; amplitude, 0.05 V; step potential, 0.007 V; and scan rate, 0.03 V s^{-1}. cDNA (I), CCAACCACACCAACCGGTTGG); cDNA (II), CCAACCACACCAACCAAAAAA.

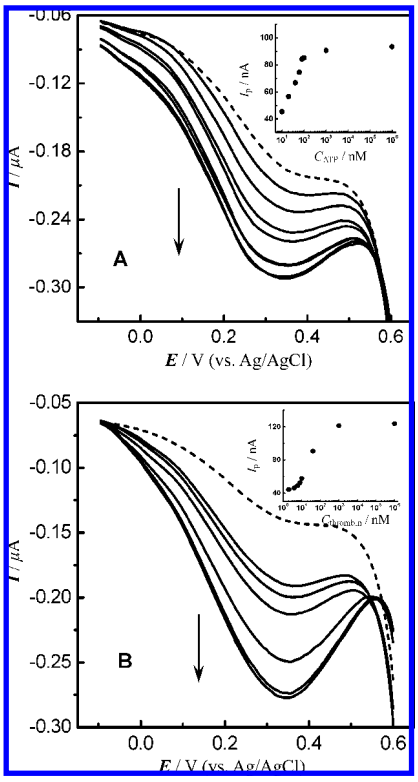

Figure 6. DPVs at the Fc-labeled ds-DNA-modified Au electrodes with (A) ATP- and (B) thrombin-binding aptamers in 20 mM Tris buffer containing 140 mM NaCl and 5 mM $MgCl_2$ (pH 7.6) before (– – –) and after (—) the electrodes were incubated in the aqueous solutions of (A) ATP and (B) thrombin in Tris buffer for 10 min. ATP concentrations were (from upper to lower) 10, 20, 40, 60, 80, 100, and 1000 nM and 1 mM. The curves for 80 and 100 nM and for 1000 nM and 1 mM ATP were almost overlapped in the figure. The concentrations of thrombin were (from upper to lower) 2, 4, 8, 10, 100, and 1000 nM. The curves for 100 and 1000 nM thrombin were almost overlapped. DPV conditions: initial potential, 0.60 V; amplitude, 0.05 V; step potential, 0.007 V; and scan rate, 0.03 V s^{-1}. Inset, plots of current responses versus target concentration.

electrode initially exhibits no voltammetric response in the potential window employed (– – –). After being incubated in 1 mM ATP in Tris buffer for 10 min, the electrode shows a pair of broad redox wave at the formal potential of approximately +0.30 V (—), corresponding to the redox process of the Fc moiety labeled on the cDNA.[8b,e] The change in the voltammetric response at the electrode with the exposure of ATP could be understood in terms of the conformational change of cDNA described above and shown in Scheme 1B: the stronger binding affinity of the aptamers with their targets than that with cDNA essentially destroyed the Watson–Crick helix structure of the ds-DNA assembled onto the electrodes, with the dissociation of the aptamers from their cDNA into solution. The conformation of the cDNA oligonucleotides assembled onto the electrode was then changed from helix to hairpin structures through the binding of the complementary bases at both of their ends. Such a conformational change eventually shortens the distance between the Fc moiety and the electrode and thereby produces the observed signal shown in Figure 4 (—). It should be noted that the observed voltammetric response of the aptasensors toward the targets may not be solely ascribed to the conformational change of the cDNA from a relatively rigid Watson–Crick helix to a single-stranded oligonucleotide, although the specific target binding could induce the dissociation of the aptamers from their helix structures with cDNA and such dissociation may result in a large-scale conformational change of cDNA, which could be used for electrochemical sensing. We deduced this from our control experiments on the effect of Mg^{2+} on the differential pulse voltammetric (DPV) responses of the electrochemical aptasensors with cDNA (I) and cDNA (II) as the probes toward thrombin, as displayed in Figure 5. We found that the aptasensors with both kinds of cDNA oligonucleotides as the probes are responsive toward thrombin even without addition of Mg^{2+} in solution, implying that the target binding-induced conformational change of the cDNA from a Watson–Crick helix to a single-stranded oligonucleotide could be utilized for electrochemical sensing, which was consistent with the previous reports.[8a,9b] However, after the addition of Mg^{2+} in solution, the DPV response of the aptasensor with cDNA (I) (i.e., with six complementary bases at both ends) as the probe toward thrombin became to be almost twice of that in the absence of Mg^{2+} in solution (Figure 5A), while the addition of the same amount of Mg^{2+} in solution did not result in an observable change in the DPV response of the aptasensor with cDNA (II) (i.e., no complementary bases at both ends) as the probe (Figure 5B). This comparison likely suggests that the presence of Mg^{2+} could induce the formation of the hairpin structure of the cDNA (I) probe and thus improve the aptasensor response toward the targets. The demonstrations described above essentially suggest

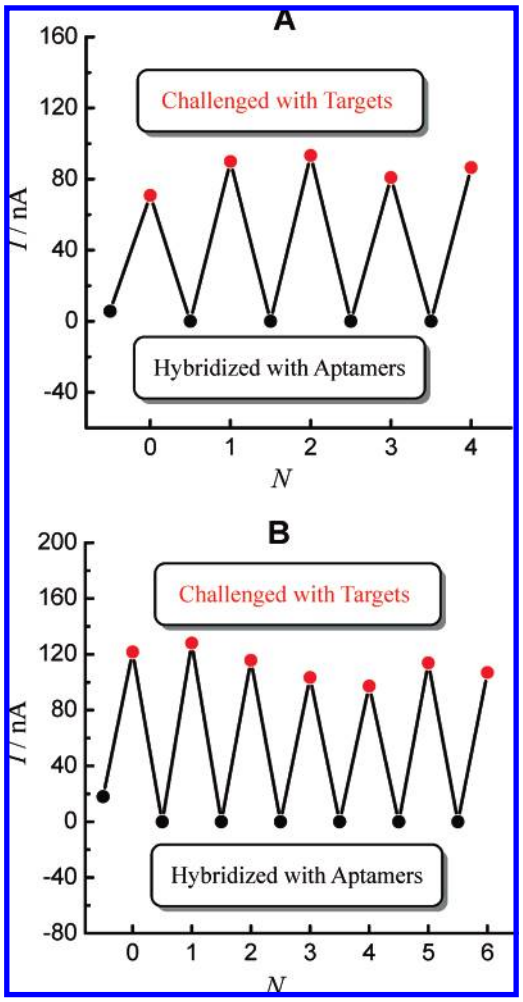

Figure 7. Graphics for the regeneration of the (A) prepared ATP and (B) thrombin aptasensors with the stepwise challenge with the targets of (A) ATP and (B) thrombin and then hybridized with the aptamers. The concentrations of the targets were 100 nM. The concentrations of the aptamers used for the hybridization were 10 μM. *N* represents the times for the sensor regeneration.

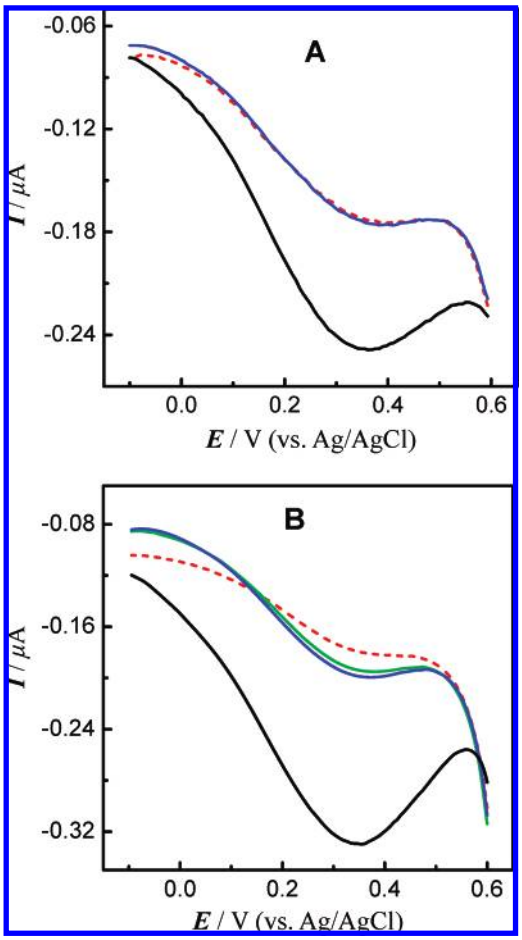

Figure 8. DPVs at the Fc-labeled ds-DNA-modified Au electrodes with (A) ATP- and (B) thrombin-binding aptamers in 20 mM Tris containing 140 mM NaCl and 5 mM $MgCl_2$ (pH 7.6) before (red curves) and after the electrodes were incubated in Tris buffer containing CTP (blue curve, part A), ATP (black curve, part A), BSA (green curve, part B), IgG (blue curve, part B), or thrombin (black curve, part B) for 10 min. The concentration of each species was 100 nM.

that the uses of cDNA as the probe could pave a new way to electrochemical aptasensors with a good response toward the targets.

Figure 6 displays typical DPV responses at the Fc-labeled ds-DNA-modified Au electrodes before and after the electrodes were incubated in the aqueous solutions of targets (i.e., ATP (Figure 6A) and thrombin (Figure 6B)) in 20 mM Tris buffer. The peak at approximately +0.35 V for the reduction of the Fc moiety became more evident upon the incubation of the electrodes in the aqueous solution of targets. The peak currents increased with increasing the target concentration in the incubation solution (Figure 6, inset) and were linear with the target concentration within a range from 10 to 80 nM (I_p (in nA) = $0.52C_{ATP}$ (in nM) + 44) for ATP and from 2 to 40 nM (I_p (in nA) = $1.2C_{thrombin}$ (in nM) + 43) for thrombin.

The responses of the prepared electrochemical aptasensors with two different kinds of aptamers may suggest the generality of the strategy demonstrated here. This is remarkable since the electrochemical aptasensors reported thus far with the aptamers as the probes have almost been based on the target binding-induced conformational change of the aptamers. Although it is known that the specific target binding could generally induce the conformational changes of the aptamers,[5a,c,16] not all kinds of induced conformational changes could be simply exploited for specific electrochemical sensing unless the conformational changes are large enough to be readily utilized electrochemically. In this sense, the strategy demonstrated here with the cDNA as the probe may be general because the conformational change of the cDNA caused by the target binding-induced dissociation of the aptamers from the ds-DNA oligonucleotides could be readily tailored to be electrochemically utilizable by, for example, rationally designing the sequences of the cDNA oligonucleotides, as described in this study.

Aptasensor Regeneration. The prepared electrochemical aptasensors with cDNA as the probe were more readily regenerated compared with the existing aptasensors with the aptamers as the probes, because the cDNA probes were covalently anchored to the sensing electrodes and the simple hybridization of the cDNA assembled onto the electrodes with the aptamers could regenerate the aptasensors. As shown in Figure 7, after being challenged with 100 nM targets and regenerated for at least four cycles, the sensor signal was almost recovered to the original

value, suggesting the prepared electrochemical aptasensors were reuseable. Of note, the procedures employed for the sensor regeneration in this work were relatively milder than those for the electrochemical aptasensors with the aptamers as the probes presumably because, in the latter cases, the aptamer probes were generally anchored onto the electrode and the strong affinity of the aptamers toward their targets essentially necessitated a strong force to dissociate the targets from their aptamers under severe conditions.[7b,8a,b,9b] Moreover, because the relatively expensive cDNA, caused by the labeling of thiol group and redox moiety, were anchored onto the electrode surface and could be reproducibly used upon hybridization with the relatively cheap aptamers, the regeneration of the electrochemical aptasensors demonstrated here is thus more cost-effective than those for the aptasensors with the aptamers as the probes. These demonstrations essentially suggest that the alternative utilization of cDNA, rather than aptamers, as the probes for electrochemical sensing could pave a new approach to electrochemical aptasensors with a ready and cost-effective regeneration property.

Aptasensor Selectivity. As the signaling of the electrochemical aptasensors with cDNA as the probes is essentially based on the conformational change of the cDNA caused by the target binding-induced dissociation of the aptamers, the aptasensors demonstrated here are specific for the target sensing. By using CTP as typical ATP analogues, we studied the specificity of the aptasensor to ATP over its analogues. Figure 8 A compares the DPV responses of the aptasensors after the aptasensors were incubated in the aqueous solutions of ATP and CTP. The incubation of the aptasensors into 100 nM of CTP solution did not produce voltammetric response (blue curve, Figure 8A) distinct from the background response of the fresh-prepared electrode (red curve, Figure 8A), while the incubation of the aptasensors into the same concentration of ATP produced a clear increase in the DPV signal (black curve, Figure 8A). This comparison essentially suggests that the aptasensors were very specific for ATP determination. Also, as displayed in Figure 8B, the aptasensors with the thrombin-binding aptamer produced approximately 15% DPV response toward the potential interferents such as bovine serum albumin (BSA) (green curve, Figure 8B) and immunoglobulin G (IgG) (blue curve, Figure 8B) relative to that toward thrombin (black curve, Figure 8B), indicating the aptasensors were almost specific for the determination of thrombin. The high specificity of the prepared electrochemical aptasensors, combined with their ready regeneration feature and good responses suggest that these sensors will be useful for determination of the targets in real samples.

CONCLUSIONS

In summary, we have demonstrated a new approach to preparation of electrochemical aptasensors by using aptamer−complementary DNA oligonucleotides, rather than aptamers, as the probes. The alternative utilization of the cDNA oligonucleotides, rather than the aptamers, as the probe essentially paves a relatively general and cost-effective way to electrochemical aptasensors with a ready regeneration feature and a good response as well as a high specificity toward the targets. These properties of the prepared electrochemical aptasensors with the cDNA oligonucleotides as the probes and with the aptamers as the recognition elements substantially enable them very striking for the determination of targets in real samples.

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