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Electrochemical Biosensor for Detection of Adenosine Based on Structure-Switching Aptamer and Amplification with Reporter Probe DNA Modified Au Nanoparticles

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In the present study, an electrochemical sensing strategy for highly sensitive detection of small molecules was developed based on switching structures of aptamers from DNA/DNA duplex to DNA/target complex. A gold electrode was first modified with gold nanoparticles (AuNPs), and thiolated capture probe was immobilized onto the electrode via sulfur−gold affinity. Then, a "sandwich-type" strategy was employed, which involved a linker DNA containing antiadenosine aptamer sequence and reporter DNA loaded on AuNPs. In the presence of adenosine, the aptamer part bound with adenosine and folded to the complex structure. As a result, the reporter probes together with AuNPs were released into solution and reduced a decrease in peak current. With the enhancement effect of AuNPs, a detection limit as low as 1.8×10^{-10} M for adenosine was achieved. The sensor exhibited excellent selectivity against other nucleosides and could be used to detect adenosine from real human serum samples.

The purine nucleoside adenosine is consensually identified as a major local regulator of tissue function especially when energy supply fails to meet cellular energy demand.[1] Due to its ability to equalize energy intake to metabolic demand in the 1980s it earned the reputation of a "retaliatory metabolite".[2] Adenosine performs extremely important signaling functions in both the peripheral and central nervous system. Peripherally, it is involved in the control of smooth muscle contraction and is a powerful vasodilator.[3] In the central nervous system, the diverse roles of adenosine include neuroprotection during ischemia,[4] regulation of spinal motor pattern generation,[5] and induction of sleep.[6] Because adenosine is a product of ATP degradation, its release from cells can also be a sign of a high metabolic rate or metabolic stress.[7] Direct monitoring of adenosine fluctuations under physiological conditions would be of utility in further characterizing the role of this purine in brain function and behavior.

The most popular assays for adenosine in microdialysis samples are based on reversed-phase high-performance liquid chromatography (HPLC) coupled with UV-absorbance or fluorescence detection.[8−10] However, the method suffers from low temporal resolution and tissue damage from implantation of the relatively large probes.[11] Biosensors are powerful analytical tools capable of detecting biological macromolecules and oligonucleotides using electrical or optical readout protocols.[12−14] An enzyme-based electrochemical sensor has also been developed for adenosine that requires three enzymes to break down adenosine and produce hydrogen peroxide, which is detected by amperometry. But it suffers from interferences of adenosine metabolites, has a slower temporal response, and is complicated to fabricate due to the necessity of three enzymes.[15]

Aptamers are single-stranded DNA or RNA oligonucleotides, which are selected from large combinatorial libraries by an in vitro evolution process of systematic evolution of ligands by exponential enrichment (SELEX).[16,17] The aptamers can bind with high affinity and specificity to a wide range of target molecules, including drugs, proteins, and other organic or inorganic molecules.[18] Because of their specific binding abilities and many advantages over antibodies such as simple synthesis, easy labeling, good stability, and high sensitivity, aptamers are starting to appear in biosensor applications.[19−23] Although the presence of potassium

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ions might have an effect on the structure of a G-rich aptamer,[24] it can be used in the absence of K^+ ions or with low concentrations. Efforts have been placed toward adenosine recently. Nutiu and Li designed an aptamer-based fluorescent reporter that functioned by switching structures from DNA/DNA duplex to DNA/target complex.[25] The duplex is formed between a fluorophore-labeled DNA (FDNA) aptamer and a small oligonucleotide modified with a quenching moiety (QDNA). When the target was absent, the aptamer binds to QDNA with maximum fluorescence quenching. When the target was introduced, the aptamer preferred to form the aptamer−target complex, resulted in a strong fluorescence signal owing to the dissociation of QDNA. This is the first time to detect low molecular weight substrate through structure-switching. Recently, Wu et al. have developed a reusable electrochemical sensing platform for detection of adenosine, where ferrocene labeling was involved.[26] Willner's group introduced a method for label-free and reagentless analysis of adenosine by the separation of an aptamer/nucleic acid duplex associated with ion-selective field-effect transistor (ISFET).[27] Notable publications are from Lu and co-workers for the development of analysis of adenosine through aptamer-linked nanostructures with optical method.[28−30] However, the sensitivities for adenosine with optical biosensors were quite low with millimolar or micromolar levels.

In this work, based on a similar concept, we fabricated an electrochemical sensor using adenosine as a model analyte based on a structure-switching aptamer and reporter DNA loaded on gold nanoparticles (AuNPs). The electroactive complex, $[Ru(NH_3)_6]^{3+}$, which can bind to anionic phosphates of DNA strands completely through electrostatic interactions, serves as a signaling transducer. Since a single AuNP is loaded with hundreds of reporter DNA strands, this offers a significant amplification for the detection of adenosine. With this strategy, the adenosine could be specifically detected with a relatively low detection limit of 1.8×10^{-10} M, which was 6, 4, and 2 orders of magnitude lower than the literature values, respectively.[26−28]

EXPERIMENTAL SECTION

Chemicals. All oligonucletides designed according to the literature[30] in the present study were purchased from SBS Genetech Co., Ltd. (Beijing, China), and sequences of all oligonucleotides are listed as follows.

Linker DNA: 5′-**ACT-CAT-CTG-TGA**-*AGA-GA*A-CCT-GGG-GGA-GTA-TTG-CGG-AGG-AAG-GT-3′.

Capture probe (probe 1): 3′-HS−$(CH_2)_6$−**TGA-GTA-GAC-ACT**-5′.

Reporter probe (probe 2): 3′-*TCT-CTT-GGA-CCC*−$(CH_2)_6$−SH-5′.

Probes 1 and 2 were thiolated with a −$(CH_2)_6$− spacer at either the 3′ or 5′ end, respectively. Linker DNA is a 44-base sequence that contains complementary sequences to both 1 (bold) and 2 (italic), as well as adenosine aptamer (normal). Adenosine, cytidine, and uridine from Sigma (St. Louis, MO) were used without further purification. 6-Mercapto-1-hexanol (MCH), hexaammineruthenium(III) chloride (98%), erythro-9-2(2-hydroxy-3-nonyl) adenine (EHNA, adenosine deaminase inhibitor), dipyridamole, α,β-methylene adenosine-5′-diphosphate, and disodium ethylenediaminetetraacetic acid (Na_2EDTA) were also purchased from sigma. Tri(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) was purchased from Alfa Aesar (Massachusetts). Other chemicals employed were all of analytical grade, and double-distilled water (DDW) was used throughout. The human serum samples from three nonpregnant healthy women, three singleton pregnant women, and three twin pregnancies were provided by the Medical School Hospital of Qingdao University (Qingdao, China).

Synthesis and Functionalization of Gold Nanoparticles with Reporter Probe DNA. Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ according to the literature.[31] Briefly, 10 mL of 38.8 mM sodium citrate was immediately added to 100 mL of 1.0 mM $HAuCl_4$ refluxing solution under stirring, and the mixture was kept boiling for another 15 min. The solution color turned to a wine red, indicating the formation of AuNPs. The solution was cooled to room temperature with continuous stirring. The sizes of the AuNPs were verified by scanning electron micrograph (SEM) using a JEOLJSM-6700F microscope (Hitachi, Japan) operated at 160 kV, and atomic force microscopy (AFM) using a Nanoscope IIIa Picoforce instrument (Veeco, U.S.A.).

The process of probe DNA labeling was performed as follows:[31] 3.0 nmol of thiolated DNA was activated with acetate buffer (pH 5.2) and 1.5 μL of 10 mM TCEP for 1 h and then added to 1 mL of freshly prepared AuNPs and shaken gently overnight. Over the course of 16 h, the DNA−AuNP conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for 24 h. Excess reagents were removed by centrifuging at 15 000 rpm for 30 min. The red precipitate was washed, recentrifuged, and dispersed in 1 mL of buffer containing 300 mM NaCl, 25 mM tris(hydroxymethyl)aminomethane (Tris) acetate, pH 8.2.

Gold Nanoparticle Deposition. The gold electrode was polished with a 0.05 μm alumina powder and soaked in an ultrasonic bath successively with distilled water, absolute alcohol, and distilled water for 5 min each. Then, the gold electrode was dipped in piranha solution (H_2SO_4/H_2O_2, 7:3 v/v) for 5 min at 90 °C and electrochemically treated by cycling the potential between +0.1 and +1.5 V in 0.1 M H_2SO_4 until a stable gold oxide cyclic voltammogram was obtained. The pretreated electrodes were immersed into the $HAuCl_4$ (6.0 mM) solution containing 0.1 M KNO_3, where electrochemical deposition was conducted at −400 mV by single-potential mode.

DNA Self-Assembly and Hybridization at Gold Electrodes. Gold electrodes coated with AuNPs were immersed into a immobilization buffer (IB: 20 mM Tris−HCl + 0.1 M NaCl + 5 mM $MgCl_2$ at pH 7.4) containing 1.0×10^{-8} M capture probe for 12 h at 100% humidity. The DNA-modified electrodes were further

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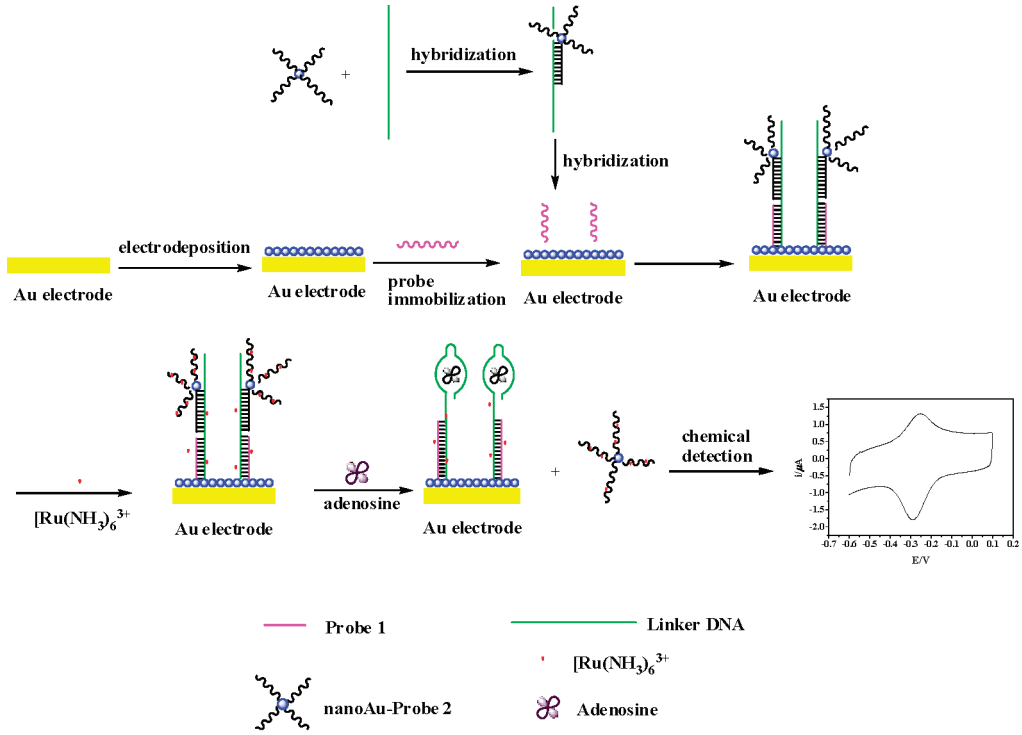
Scheme 1. Schematic Representation of the Adenosine Sensing[a]



[a] The aptamer bound to adenosine folded to the complex structure, and probe 2 together with AuNP was released into solution, which resulted in a decrease in the reduction peak in the cyclic voltammogram.

treated with 1 mM MCH for 2 h to obtain well-aligned DNA monolayers.

Linker DNA was preannealed with reporter probes loaded on AuNPs at 37 °C for 30 min in the hybridization buffer, and then 4 μL of the solution was placed on gold electrodes with DNA self-assembly monolayers (SAMs) for 1 h at room temperature. After hybridization, electrodes were extensively rinsed with washing buffer (10 mM Tris−HCl, pH 7.4) and dried under a stream of nitrogen prior to electrochemical characterization.

Electrochemical Detection of Adenosine. For the detection procedure, a 20 μL droplet of adenosine of various concentrations in IB buffer was deposited onto the sensing interface and kept for 90 min followed by a 20 min washing in wash buffer (20 mM Tris−HCl + 0.1 M NaCl + 5 mM $MgCl_2$ + 1.0% (v/v) Tween-20 at pH 7.4) to remove nonspecifically bound adenosine.[32] Cyclic voltammetry (CV) was performed at a scan rate of 500 mV/s in 2 mL of 10 mM Tris−HCl solution (pH 7.4) containing 5.0 μM $[Ru(NH_3)_6]^{3+}$, which was degassed with nitrogen for 15 min.

All electrochemical measurements were carried out at room temperature in a single-compartment, three-electrode glass cell using a potentiostat/galvanostat model 263A electrochemical workstation (Princeton, U.S.A.). The three-electrode system used consisted of the working electrode of interest, a Ag/AgCl reference electrode, and a platinum wire auxiliary electrode.

Serum Samples Treatment. Each serum sample (1.0 mL) was transferred to tubes containing an equal volume of ice-cold stop solution (120 μM EHNA, 20 mM dipyridamole, 60 mM α,β-methylene adenosine-5′-diphosphate, and 4.2 mM Na_2EDTA). The blood and stop solution mixture was then immediately centrifuged for 5 min at 10 000 rpm at 4 °C. The plasma was removed and frozen at −70 °C until used. Adenosine was measured after appropriate dilution and was calculated from the measured concentrations after correction for dilution. For comparison, adenosine levels were assayed with a modified HPLC method as described previously[33,34] (see the Supporting Information for details).

RESULTS AND DISCUSSION

Adenosine-Responsive Sensing System and Experimental Principle. To investigate an electrochemical sensing platform for highly sensitive detection of small molecules, the adenosine aptamer was studied as a model system. The system contained a thiolated DNA sequence as capture probe, linker DNA as detection probe, AuNP-linked DNA sequence as reporter probe, and adenosine as the model analyte, respectively. The linker DNA contained an adenosine aptamer fragment (normal font) and an extension (italic and bold). The bold part of the linker hybridized to the thiolated DNA immobilized on the electrode, whereas the italic part and a small fraction of the normal part of the linker hybridized to the reporter probe functionalized with AuNPs. The fabrication of the sensing design is shown in Scheme 1. The surface of a gold electrode was first modified with directly electrodeposited AuNPs, which could largely increase the surface area of electrode and enhance the immobilization amount and ability of probe DNA. Then, a 3′-thiol-modified DNA strand (capture sequence) was immobilized on the resulting electrode surface via sulfur−gold affinity. Subsequently, the 12-base segment close to the 5′ end of the linker DNA was hybridized with capture

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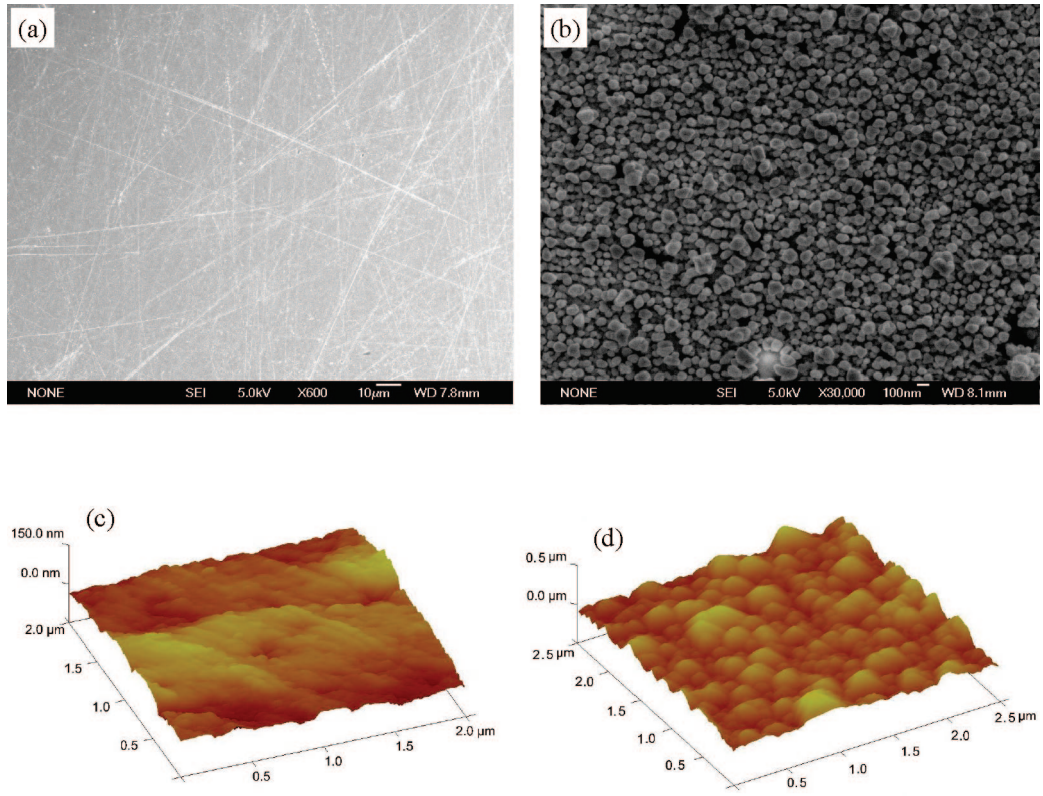

Figure 1. SEM and AFM images of Au electrodes before and after electrodeposition of AuNPs: (a) SEM of the bare Au electrode, (b) SEM of the AuNP-modified Au electrode, (c) AFM of the bare Au electrode, and (d) AFM of the AuNP-modified Au electrode. The electrodeposition solution: 0.1 M KNO_3 containing 6 mM $HAuCl_4$. The potential and time of electrodeposition: −0.4 V and 300 s.

DNA sequence and the next 12-base was hybridized to probe 2. The sensing interface was ready for adenosine assay. An aptamer can bind tightly and specifically to a variety of small molecules to form a tertiary complex with a binding constant greater than that of an ordinary DNA duplex. In the presence of adenosine, the aptamer part bound to adenosine and folded to the complex structure. As a result, only the italic part on the linker DNA was left to bind to probe 2, which was unstable at room temperature, and probe 2 together with AuNP was released into the solution. When the electrode was immersed into a Tris−HCl solution containing $[Ru(NH_3)_6]^{3+}$, the peak current was decreased. Since a single AuNP is loaded with hundreds of reporter DNA strands, the introduction of the adenosine removed a large fraction of probe from the electrode, offering a significant amplification for the detection of adenosine.[35]

The design relies on the structure-switching properties of aptamers binding to their target molecules.[25,36−40] Because there are no special requirements on the aptamer part, the design is generally applicable to many aptamers.

Characterization of the Modified Electrode. The bare Au electrode and the AuNP-modified Au electrode with the same geometric surface area were characterized by SEM and AFM, which is shown in Figure 1, from which one can observe that the AuNPs have deposited on the surface of the Au electrode. The active surface areas of the bare Au electrode and AuNP-modified Au electrode were measured by CV in 0.5 M H_2SO_4 (see Figure S1 in the Supporting Information). Assuming that a specific charge of 386 $\mu C/cm^2$ was required for gold oxide reduction,[41] the AuNP-modified Au electrode had a total active surface of 30.9 mm^2, whereas that of the corresponding bare Au electrode was 9.8 mm^2.

Previous studies have revealed that the assembly of nucleic acids on electrodes and the formation of double-stranded DNA on the support can be followed by faradaic impedance spectroscopy, FIS.[27] Figure 2 showed a Nyquist plot of impedance for Au electrodes (Figure 2a), modified with AuNPs (Figure 2b), after immobilization of probe 1 (Figure 2c), hybridization with linker DNA and probe 2 conjugated AuNPs (Figure 2d), and after treatment with 4.0 nM adenosine (Figure 2e). In the Nyquist plot of impedance spectra, the semicircle portion at higher frequencies corresponds to the electron-transfer-limited process and the linear portion seen at lower frequencies may be ascribed to the diffusion. The increase in the diameter of the semicircle reflects the increase in the interfacial charge-transfer resistance (R_{ct}). Numerical values of R_{ct} were derived from experimental impedance spectra by fitting an equivalent circuit model based on a modified Randles and Ershler model to the data. The R_{ct} of the bare Au electrode was 167 Ω; the value decreased to 9.8 Ω with the deposition of AuNPs. After immobilization of probe 1, the value of R_{ct} increased to 1494

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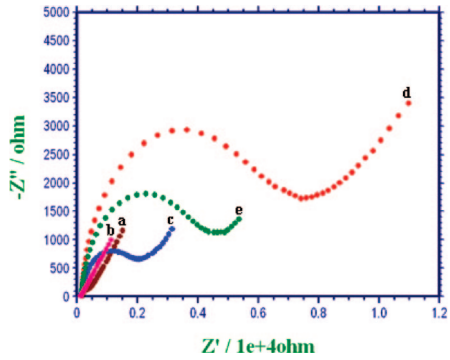

Figure 2. Faradaic impedance spectra (Nyquist plots) corresponding to Au electrodes (a) consisting of (b) the AuNPs, (c) after immobilization of probe 1, (d) hybridization with linker DNA and probe 2 conjugated AuNPs, and (e) after treatment with 4.0 nM adenosine. The data were recorded in the presence of $[Fe(CN)_6]^{3-/4-}$, 2.5 mM, as a redox label, and upon application of the biasing potential of 0.21 V, applying 5 mV alternative voltage in the frequency range of 50 mHz to 10 kHz. Data were recorded in a PBS solution (10 mM) that included KCl (100 mM); pH 7.4. The electrode areas before and after AuNP deposition are of 9.8 and 30.9 mm^2, respectively.

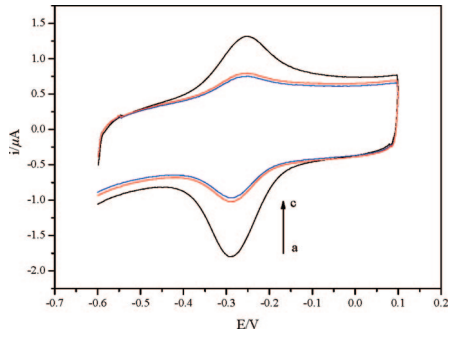

Figure 3. Cyclic voltammograms of 5.0 μM $[Ru(NH_3)_6]^{3+}$ on the tertiary DNA-modified gold surface in 10 mM Tris−HCl solution (pH 7.4) with (a) and without (b) probe 2 labeled AuNPs amplification, and after the treatment of (a) with adenosine (c). Scan rate = 500 mV/s. The electrode areas are 30.9 mm^2.

Ω. The increase in R_{ct} is due to the immobilization of negatively charged ODN probes on the electrode surface resulting in a negatively charged interface that electrostatically repels the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$ and inhibits interfacial charge-transfer.[42] After hybridization with linker DNA and probe 2, the value of R_{ct} was increased to 6050 Ω, due to the large amount of DNA linked on the AuNPs. After treatment of adenosine, the interfacial electron resistance decreased, consistent with the removal of the negative charge from the electrode by the removal of probe 2.

Cyclic Voltammetric Detection of Adenosine Using $[Ru(NH_3)_6]^{3+}$ as a Redox Marker. In comparing with other detection protocols, CV of electroactive species in a conducting aqueous solution is a valuable mean of probing the electrochemical characterization of the modified gold electrode, which is beneficial to potential miniaturization in terms of instrumentation particularly for a nonspecialist.[43] As shown in Figure 3a, a pair of well-defined peaks can be observed using CV when the tertiary DNA with (Figure 3, curve a) and without (Figure 3, curve b) probe 2 labeled AuNPs amplification was immersed in a solution containing $[Ru(NH_3)_6]^{3+}$, characteristic of a surface-confined redox process which reflects the amount of DNA strands localized at the electrode surface.[44] The similar voltammograms in Figure 3 indicated that the duplex has definitely been modified onto the gold electrode surface and a sensing interface was obtained. A current decrease (Figure 3c) appeared after treatment with adenosine, ascribed to the removal of the AuNP-labeled reporter probe. In the absence of AuNPs amplification, only a slight decrease in the peak current was observed. In contrast, with AuNPs amplification, significant decrease of peak current was found. These CV curves demonstrated an obvious impression of the amplification effect of AuNPs, implying that one could use AuNPs to realize adenosine detection with high sensitivity.

When the aptamer-modified surface was exposed to a solution containing $[Ru(NH_3)_6]^{3+}$, the redox cations bound electrostatically to the negatively charged phosphate backbone by replacing the native charge compensation ions (Na^+) and reached an ion-exchange equilibrium.[45] The concentration of $[Ru(NH_3)_6]^{3+}$ for the CV measurements should not be too high as this would result in the detection of solution $[Ru(NH_3)_6]^{3+}$ (which occurs at more negative reduction potentials). Previous study shown that 5.0 μM $[Ru(NH_3)_6]^{3+}$ was suitable for the experiments.[32] In the present study, 5.0 μM $[Ru(NH_3)_6]^{3+}$ was performed, which ensured saturation of the DNA-modified surface with the redox-active complex.

Effects of pH and Ionic Strength for Adenosine Detection. To reach a full understanding on the aptamer−AuNPs for sensing applications, the effects of pH and ionic strength were further investigated. At very low (pH < 4.0) or very high pH (pH > 10.0), AuNP aggregates were not stable and disassembled very quickly even in the absence of adenosine. This could be attributed to the denaturation of DNA at extreme pH conditions.[30] In the range of 5.0−9.0, the peak current was stable, suggesting that binding of the aptamer fragment to adenosine can occur over a wide pH range. Taking account of the efficiency of DNA hybridization, pH 7.4 was chosen as the reaction value.

It has been demonstrated that the presence of potassium ions can induce the folding of a G-rich aptamer from a loose random coil into a compact G-quadruplex in a manner similar to its target.[46] The structure of the aptamer for adenosine, composed of two stacked G-quartets, appears to be remarkably similar to that of the aptamer for thrombin. When the tertiary DNA-modified electrode was exposed to a solution of K^+, the signals decrease significantly.[47] When the concentration of K^+ was lower than 4.0×10^{-4} M, it had little effect on the CV signals (see Figure S2 in the Supporting Information). The aptasensor was used to analyze adenosine in the absence or concentration lower than 4.0×10^{-4} M K^+.

DNA duplexes were stabilized at high NaCl or low temperature, whereas the binding interaction between the aptamer and adenosine changed relatively less by these factors. As a result, it

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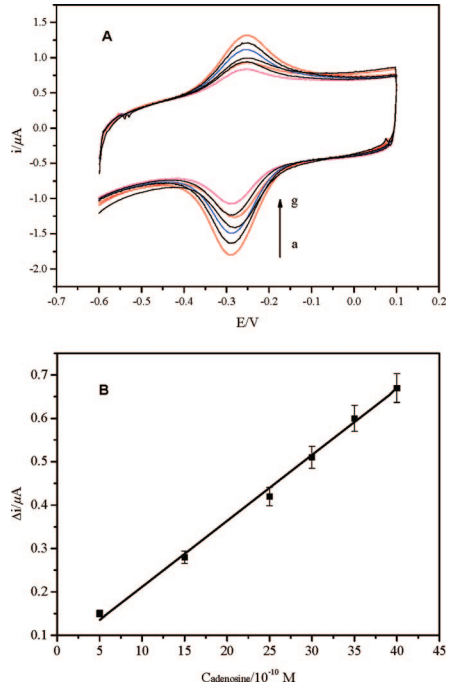

Figure 4. (A) Current response of sensing system to different concentrations of adenosine: (a) 0, (b) 5.0×10^{-10}, (c) 1.5×10^{-9}, (d) 2.5×10^{-9}, (e) 3.0×10^{-9}, (f) 3.5×10^{-9}, and (g) 4.0×10^{-9} M. (B) The linear relationship between the difference of peak current and adenosine concentration. The conditions are the same as in Figure 3.

was relatively more difficult for an aptamer to compete with DNA base-pairing interactions at higher ionic strength or lower temperature.[30] While the sensor relies on the electrostatic binding of $[Ru(NH_3)_6]^{3+}$ to the DNA phosphate sites, so the ionic strength of the $[Ru(NH_3)_6]^{3+}$ solutions should play a key role in the performance of the sensor. When NaCl is added to the $[Ru(NH_3)_6]^{3+}$ solution, the cations in the solution can compete with $[Ru(NH_3)_6]^{3+}$ for binding to the anionic DNA backbone. The increase in ionic strength will weaken the interactions between $[Ru(NH_3)_6]^{3+}$ and DNA, leading to a decrease in the amount of adsorbed $[Ru(NH_3)_6]^{3+}$ at the DNA-modified electrode. Thus, low ionic strength conditions were necessary for the detection of adenosine with $[Ru(NH_3)_6]^{3+}$ as redox indicator.

Detection of Adenosine. Comparing with the aptamer-based fluorescence-signaling system reported previously,[25] in which FDNA and QDNA were used, we used an alternative electrochemical detection system with high sensitivity, simple instrumentation, and low cost. Moreover, the application of DNA-loading AuNPs further enhanced the sensitivity of the present strategy. The introduction of adenosine at different concentrations to the sensing interface induced different decreases in peak current associated with the removal of the probe 2 linked AuNPs. The sensor using $[Ru(NH_3)_6]^{3+}$ as electrochemical hybridization indicator for the detection of adenosine was developed. The sensitivity of the sensor was investigated by monitoring the response to a range of concentrations of adenosine target. Figure 4A shows the CV for the signal of $[Ru(NH_3)_6]^{3+}$ at DNA-modified probe after different concentrations of adenosine were introduced. Figure 4B represents the relationship between the change in reduction current, Δi, and adenosine concentration. The graph

Table 1. Comparisons of the Aptamer-Based Method with the HPLC Method for the Detection of Adenosine in Human Sera[a]

sample	aptamer-based method (μM)	RSD (%)	HPLC method (μM)	RSD (%)
1	0.05	4.2	0.06	3.7
2	0.12	6.7	0.10	4.6
3	0.17	4.5	0.16	5.5
4	0.31	3.8	0.33	3.6
5	0.28	5.1	0.26	4.2
6	0.38	4.3	0.41	3.3
7	0.40	5.0	0.42	3.7
8	0.45	4.7	0.44	3.2
9	0.52	3.4	0.53	3.9

[a] Samples 1−3 were from nonpregnant, 4−6 for singleton pregnant, and 7−9 for twin pregnant women. Each sample was analyzed in triplicate, and the results are the average values.

can be also considered as a calibration graph because it gives the relationship between the sensor signal and the concentration of the target adenosine. The electrode current decreased continuously as a function of adenosine concentration in the range of 5.0×10^{-10} to 4.0×10^{-9} M. The regression equation was $y = 0.0152x + 0.06$ (shown in Figure 4B, y was the Δi, μA; x was the concentration of adenosine, 10^{-10} M; $n = 6$), and the regression coefficient of the linear curve was 0.9982. A detection limit of 1.8×10^{-10} M adenosine can be estimated using 3σ. The detection limit obtained using the present sensing system is 10 000-fold lower than the literature value achieved using label-free and reagentless aptamer-based sensor,[27] more than 6 orders of magnitude lower than the detection limit reported using a colorimetric method based on AuNP aggregation,[28] and 100 times lower than that of the ferrocene-labeled aptamer probe.[26] The lower limit of detection under 40 nM was sufficient to monitor changes in adenosine from basal levels,[48] which was estimated to be 50−200 nM in the brain.[49]

Although the experiments reported above were carried out in the standard buffer, the performance of the adenosine aptasensor in the presence of blood serum was also examined. Literature survey revealed that adenosine concentration in normal human blood ranged between 0.05 and 0.1 μM.[50,51] Given the very high sensitivity of the aptasensor, the serum samples were diluted 1000 times before analysis with the present method. It was reported that the average concentration of K^+ in serum was around 5.13 mM.[52] After dilution of 1000 times as performed in the present measurement, the concentration of K^+ was 5.1×10^{-6} M, which was lower than 4.0×10^{-4} M. So the interference of K^+ in the serum sample could be ignored. Blood samples prepared from nonpregnant healthy woman, singleton pregnant woman, and twin pregnancies were analyzed by this assay. For the introduction of serum of nonpregnant healthy woman, there was a slight decrease in the signal, whereas the reduction in peak current for samples of singleton pregnant woman and twin pregnancies was large. The results of the aptasensor determination were validated with HPLC

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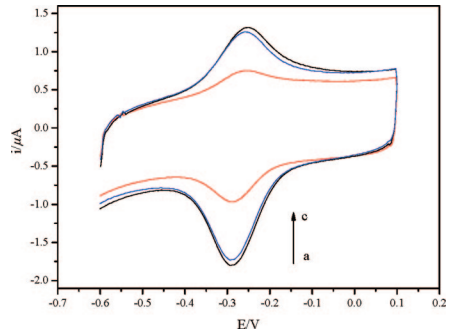

Figure 5. Cyclic voltammograms of the sensing system after being exposed to three nucleosides in 10 mM Tris−HCl solution: (a) cytidine; (b) uridine; (c) adenosine. All the concentrations were 4.0 nM. Other conditions are the same as in Figure 3.

analysis (see the Supporting Information for details). The results, in comparison with those of the HPLC method, are listed in Table 1, which show good agreement with each other. The results of the aptamer-based system are linearly proportional to those of the HPLC method, and the equation of linear regression is $y = 1.0409x - 0.0089$ (x is the result of the present method; y is the result of HPLC method; $n = 9$, $r = 0.9943$). The proposed assay shows a potential for detecting adenosine in clinical examination with satisfactory results.

Selectivity of the Sensing Structure. Not only does a biosensor have to be sensitive to different concentrations of the analyte, it must also be specific. Experiments were thus conducted on cytidine and uridine, which belong to the nucleosides family, to serve as control to assess the specificity of the aptamer-modified gold electrodes for the detection of adenosine. Guanosine was not tested because of its poor solubility at room temperature. Figure 5 exhibits different current response signals of the proposed sensing system after the addition of 4.0 nM adenosine, cytidine, or uridine under the same experimental conditions. Incubations of the aptamer-modified gold electrodes with cytidine (Figure 5a) or uridine (Figure 5b) did not produce any significant changes in the CV response as compared to the case of adenosine (Figure 5c). These results demonstrated that the developed strategy had a sufficient specificity to detect the interaction between adenosine and the antiadenosine aptamers immobilized on gold surface.

CONCLUSIONS

A novel electrochemical sensing strategy for highly sensitive adenosine detection was constructed. The design relies on the structure-switching properties of aptamers upon binding to their target molecules and signal enhancement of nanotechnology. This sensor exhibited very high sensitivity and selectivity, making it a promising alternative to conventional fluorescent adenosine detection. If the $[Ru(NH_3)_6]^{3+}$ removed into solution together with the reporter probe DNA can be used for electrochemical detection with preconcentration, the sensitivity can be further improved. Because there are no special requirements on the aptamer part, the design is generally applicable to many aptamers. The simultaneous detection and quantification of multiple small substrates will be discussed in future manuscripts.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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