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Ultrasensitive Detection of DNA in Diluted Serum Using $NaBH_4$ Electrooxidation Mediated by $[Ru(NH_3)_6]^{3+}$ at Indium−Tin Oxide Electrodes

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There is a crucial need for simple and highly sensitive techniques to detect DNA in complicated biological samples such as serum. Here we present an ultrasensitive electrochemical DNA sensor using (i) single DNA hybridization with peptide nucleic acid (PNA), (ii) selective binding of $[Ru(NH_3)_6]^{3+}$ to hybridized DNA, (iii) fast $NaBH_4$ electrooxidation mediated by $[Ru(NH_3)_6]^{3+}$, and (iv) low background currents of $NaBH_4$ at indium−tin oxide (ITO) electrodes. The $[Ru^{III}(NH_3)_5NH_2]^{2+}$ formed from $[Ru^{III}(NH_3)_6]^{3+}$ in borate buffer (pH 11.0) is readily electrooxidized to both $[Ru^{IV}(NH_3)_5NH_2]^{3+}$ and Ru complex with a higher oxidation state. In the absence of $[Ru(NH_3)_6]^{3+}$ bound to the DNA-sensing ITO electrodes, the oxidation currents of $NaBH_4$ are very low. However, in the presence of $[Ru(NH_3)_6]^{3+}$, the oxidation currents of $NaBH_4$ are highly enhanced due to electron mediation of the oxidized Ru complexes. The significant enhancement in the electrocatalytic activity of sensing electrodes after $[Ru(NH_3)_6]^{3+}$ binding facilitates to obtain high signal-to-background ratios. PNA and ethylenediamine on DNA-sensing electrodes significantly decrease $[Ru(NH_3)_6]^{3+}$ binding, also allowing for high signal-to-background ratios. The oxidation charges of $NaBH_4$ obtained from chronocoulometry are highly reproducible. All combined effects enable the detection of DNA with a detection limit of 1 fM in ten-fold diluted human serum. The simple and fast detection procedure and the ultrasensitivity make this approach highly promising for practical DNA detection.

There is a crucial need for simple and highly sensitive techniques to detect DNA in complicated biological samples such as serum.[1−5] Among the many developed DNA sensors,[6,7] label-free DNA sensors using only single DNA hybridization provide simple detection procedures.[8−10] Generally, their detection limits are high, and they are highly susceptible to nonspecific binding of various proteins and nontarget DNA in complicated biological samples. On the other hand, sandwich-type DNA sensors using double hybridization offer much lower detection limits along with less susceptibility to nonspecific binding, but their detection procedures are more complex than those of the label-free DNA sensors.[11,12]

In recent years, the electrostatic binding of redox-active transition-metal cations (e.g., $[Ru(NH_3)_6]^{3+}$) to negatively charged DNA has been widely used to detect DNA and measure its surface density on electrodes.[13−26] The amount of electrostatically bound $[Ru(NH_3)_6]^{3+}$ increases with the surface concentration of negatively charged phosphate groups of hybridized or immobilized DNA. Generally, the amount of $[Ru(NH_3)_6]^{3+}$ is calculated by measuring the current or charge associated with the reduction of $[Ru^{III}(NH_3)_6]^{3+}$ to $[Ru^{II}(NH_3)_6]^{2+}$. In $[Ru(NH_3)_6]^{3+}$-based DNA sensors, single hybridization and strong $[Ru(NH_3)_6]^{3+}$ binding facilitate simple and fast DNA detection. However, the amounts of bound $[Ru(NH_3)_6]^{3+}$ at low surface densities of DNA are not sufficiently high to allow high signal levels, and facile electrostatic binding of $[Ru(NH_3)_6]^{3+}$ to any negatively charged surface causes high background levels. Accordingly, it is not easy to achieve high signal-to-background ratios, resulting in high detection limits. As an effort to increase the signal level, $[Ru(NH_3)_6]^{3+}$ was used as an electron mediator for the

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electrocatalytic reduction of $[Fe(CN)_6]^{3-}$.[18,22,24,26] As an approach to reduce the unwanted electrostatic binding, neutral peptide nucleic acids (PNAs) were employed as capture probes for target DNA.[18,27−30] Nevertheless, the detection limits in $[Ru(NH_3)_6]^{3+}$-based DNA sensors are much higher than those in sandwich-type DNA sensors. To increase the amount of bound $[Ru(NH_3)_6]^{3+}$, a DNA−Au nanoparticle conjugate capable of binding many $[Ru(NH_3)_6]^{3+}$ ions[21,23] and a liposome containing many $[Ru(NH_3)_6]^{3+}$ ions[31] have been employed as labels in sandwich-type formats. These approaches allow much lower detection limits but require double hybridization. Therefore, a sensitive $[Ru(NH_3)_6]^{3+}$-based DNA sensor using only single hybridization is more desirable for simple and fast DNA detection than sandwich-type sensors.

$NaBH_4$ undergoes multielectron (maximum $8e^-$) oxidation on catalytic or electrocatalytic surfaces.[32,33] When $NaBH_4$ is used as an electrochemically signaling species, high signal levels can be obtained.[34] However, self-hydrolysis of $NaBH_4$ and the resultant hydrogen-bubble generation could be a significant problem.[32] When high pH conditions are maintained, self-hydrolysis can be minimized.[35] Interestingly, the electrooxidation of $[Ru(NH_3)_6]^{3+}$ ($[Ru(NH_3)_5NH_2]^{2+}$) readily occurs at high pHs, although it does not at neutral and low pHs.[36] Generally, redox reactions of transition-metal complexes occur well, even at poorly electrocatalytic indium−tin oxide (ITO) electrodes.[37−40] Moreover, many transition-metal complexes show good electron-mediating behaviors.[36,39−41] Therefore, there is a possibility that $NaBH_4$ is oxidized at high pHs *via* electron mediation of $[Ru(NH_3)_6]^{3+}$, although $NaBH_4$ is not readily electrooxidized at ITO electrodes.[40,42,43]

When an anodic current or charge of electroactive species is measured at ITO electrodes, background levels are low and reproducible.[40,42,43] On the other hand, when the cathodic current or charge of $[Ru(NH_3)_6]^{3+}$ is measured in an electrochemical detection, electroreduction of dissolved oxygen can increase background levels. To minimize oxygen electroreduction, solutions should be purged with inert gas prior to electrochemical measurements.[13,17,23] Accordingly, signal acquisition using electrochemical oxidation of $[Ru(NH_3)_6]^{3+}$ would be better for simple detection and low background levels than that using electrochemical reduction of $[Ru(NH_3)_6]^{3+}$.

Herein, we present an ultrasensitive $[Ru(NH_3)_6]^{3+}$-based DNA sensor using $NaBH_4$ electrooxidation mediated by $[Ru(NH_3)_6]^{3+}$. First, we show the facile electrooxidation of $[Ru(NH_3)_6]^{3+}$ and the slow electrooxidation of $NaBH_4$ in a basic solution (pH 11.0) at bare ITO electrodes, along with the highly enhanced electrooxidation of $NaBH_4$ in the presence of $[Ru(NH_3)_6]^{3+}$. Second, we reveal that PNA- and ethylenediamine-modified DNA-sensing electrodes exhibit low electrostatic binding of $[Ru(NH_3)_6]^{3+}$. Finally, we present a detection limit of the DNA sensor in 10-fold diluted human serum by using chronocoulometry.

Experimental Section

Materials. 3-Phosphonopropionic acid (PPA), $[Ru(NH_3)_6]Cl_3$, $NaBH_4$, H_2O_2, and NH_4OH were purchased from Aldrich. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), human serum, and ethylenediamine were purchased from Sigma. *N*-hydroxysuccinimide (NHS) was obtained from Fluka. Acetonitrile was obtained from J. T. Baker. All buffer reagents and other inorganic chemicals were supplied by Sigma, Aldrich, or Fluka, unless otherwise stated. All chemicals were used as received. All aqueous solutions were prepared in doubly distilled water. Tris buffer (10.0 mM, pH 7.4) was prepared with tris(hydroxymethyl)aminomethane (tris) and HCl, borate buffer (50.0 mM, pH 11.0) was prepared with H_3BO_3 and NaOH, and phosphate-buffered saline (PBS) (pH 7.4) was prepared with 0.01 M sodium phosphate, 0.138 M NaCl, and 2.7 mM KCl.

PNA was obtained from Panagene (Daejeon, Korea), and the sequence of the capture-probe PNA was (from *N*-terminus to *C*-terminus) ATA *TTT AAA GAA GCC AGC*. The first three bases (ATA) at the *N*-terminus were used as a spacer. Fifteen of 18 bases were used for hybridization. DNA was obtained from Genotech (Daejeon, Korea). The DNA sensor was designed for the detection of the encoding residue 1038 of exon 11 of the *BRCA1* gene.[44] The DNA had the following sequences: complementary target DNA, 5′-TGA *GCT GGC TTC TTT AAA*-3′. The concentrations of DNA and PNA refer to those of strands.

Preparation of DNA-Sensing Electrodes. ITO electrodes were obtained from Geomatec (Yokohama, Japan). ITO electrodes were washed, pretreated, and modified with PPA as previously described.[40,43] The carboxylic acid groups of the PPA-modified ITO electrodes were activated by immersing the electrodes in a mixture of 50.0 mM EDC and 25.0 mM NHS for 2 h, followed by washing with water. Immediately, a 20.0% aqueous solution of acetonitrile containing 1.0 μM capture probe PNA was dropped onto the activated ITO electrode and left for 4 h at room temperature. The electrodes were washed with a 20.0% aqueous solution of acetonitrile and subsequently water. The unmodified carboxylic acid groups were activated again by immersing the electrodes in a mixture of 50.0 mM EDC and 25 mM NHS for 2 h. Afterward, a 20.0% aqueous solution of acetonitrile containing 1.0% ethylenediamine was dropped onto the activated ITO electrode for 2 h at room temperature, and the electrodes were washed with a 20.0% aqueous acetonitrile solution and subsequently water. The calculated minimal surface density of the capture probe PNA was $1.0 \pm 0.1 \times 10^{12}$ molecules/cm^2 (see Supporting Information). Afterward, the electrodes were immersed at room temperature for 30 min in tris buffer or 10-fold diluted human serum (diluted with tris buffer) containing different concentrations of target DNA. The electrodes were washed by dipping the electrodes in tris buffer for 5 min, followed by rinsing with tris buffer. Finally, the electrodes were incubated at room temperature for 15 min in tris buffer containing 10.0 μM

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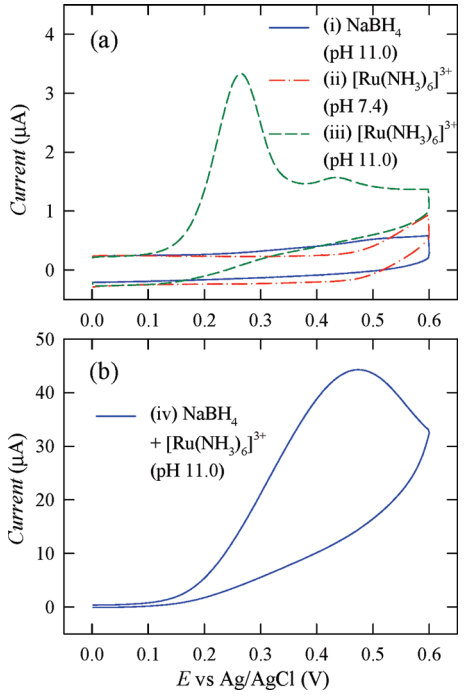

Figure 1. Cyclic voltammograms obtained at bare ITO electrodes (at a scan rate of 50 mV/s) in borate buffer (pH 11.0) containing (i) 5 mM $NaBH_4$, (iii) 10 μM $[Ru(NH_3)_6]^{3+}$, or (iv) 10 μM $[Ru(NH_3)_6]^{3+}$ and 5 mM $NaBH_4$ or in (ii) PBS buffer (pH 7.4) containing 10 μM $[Ru(NH_3)_6]^{3+}$.

$[Ru(NH_3)_6]Cl_3$, followed by rinsing with distilled water twice. This step was very important in obtaining reproducible results.

Electrochemical Experiments. Electrochemical experiments were performed using a CHI405a device (CH Instruments). The electrochemical cell consisted of a DNA-sensing working electrode, a platinum wire counter electrode, and an Ag/AgCl (3.0 M KCl) reference electrode. The cell was filled with a borate buffer solution containing 5.0 mM $NaBH_4$. The $NaBH_4$ solution was used within 30 min after its preparation. The electrode area of ITO was 0.28 cm^2.

Results and Discussion

Electrocatalytic Oxidation of $NaBH_4$ by $[Ru(NH_3)_6]^{3+}$. To achieve a high signal-to-background ratio in DNA detection based on $NaBH_4$ electrooxidation, the electrodes should be poorly electrocatalytic for $NaBH_4$ electrooxidation in the absence of $[Ru(NH_3)_6]^{3+}$, but highly electrocatalytic in the presence of $[Ru(NH_3)_6]^{3+}$. The formal potential of $NaBH_4$ (−1.24 V vs standard hydrogen electrode (SHE)) is very low,[32] and $NaBH_4$ electrooxidation readily occurs at electrocatalytic noble metal and carbon electrodes.[34,40] However, $NaBH_4$ electrooxidation is very slow at ITO electrodes.[34,40,42,43] Curve i of Figure 1a shows the cyclic voltammogram obtained at bare ITO electrodes in borate buffer (pH 11.0) containing 5.0 mM $NaBH_4$. The oxidation current of $NaBH_4$ at bare ITO electrodes was negligible, and the current behavior was similar to that in the absence of $NaBH_4$,[42] clearly indicating that the ITO electrodes were poorly electrocatalytic for $NaBH_4$ electrooxidation.

Curves ii and iii of Figure 1a show the cyclic voltammograms obtained at bare ITO electrodes in $[Ru(NH_3)_6]^{3+}$-containing solutions. No considerable oxidation currents were observed up to 0.5 V in PBS buffer (pH 7.4) (curve ii of Figure 1a), indicating that $[Ru(NH_3)_6]^{3+}$ electrooxidation does not readily occur at this neutral pH.[36] However, two anodic peaks were observed in borate buffer (pH 11.0) (curve iii of Figure 1a). It was reported that a significant amount of $[Ru^{III}(NH_3)_6]^{3+}$ is converted to $[Ru^{III}(NH_3)_5NH_2]^{2+}$ in basic pH solutions and that the formed $[Ru^{III}(NH_3)_5NH_2]^{2+}$ is readily electrooxidized to $[Ru^{IV}(NH_3)_5NH_2]^{3+}$ and Ru complex with a higher oxidation state.[36] Accordingly, the first anodic peak in curve iii of Figure 1a is related to the electrooxidation of $[Ru^{III}(NH_3)_5NH_2]^{2+}$ to $[Ru^{IV}(NH_3)_5NH_2]^{3+}$, and the second peak is related to further electrooxidation of $[Ru^{IV}(NH_3)_5NH_2]^{3+}$.

When two consecutive cyclic scans were performed between −0.5 and 0.2 V in borate buffer (pH 11.0), there was no noticeable difference between the two cyclic voltammograms of the first and second scans (Figure S2a in the Supporting Information). The result indicates that $[Ru^{II}(NH_3)_6]^{2+}$ is stable during the measurement, considering that the redox currents are mainly due to the redox reaction between $[Ru^{III}(NH_3)_6]^{3+}$ and $[Ru^{II}(NH_3)_6]^{2+}$. However, when two consecutive cyclic scans were performed between −0.5 and 0.9 V, the anodic peak current at −0.05 V at the second scan was much lower than that at the first scan (Figure S2b in the Supporting Information). Moreover, in curve iii of Figure 1a, no reduction peak was observed, and the second anodic peak was much smaller than the first. These results clearly show that $[Ru^{IV}(NH_3)_5NH_2]^{3+}$ and the Ru complex with a higher oxidation state are unstable.[36] Unwantedly, the instability of the oxidized Ru complexes may make them unsuitable for electron-mediated $NaBH_4$ oxidation.

When both $NaBH_4$ and $[Ru(NH_3)_6]^{3+}$ were present in borate buffer (pH 11.0), high oxidation currents were observed (Figure 1b). The anodic peak current was much higher than that in a solution containing only $[Ru(NH_3)_6]^{3+}$ (curve iii of Figure 1a) or only $NaBH_4$ (curve i of Figure 1a). The increased oxidation currents are due to $NaBH_4$ electrooxidation mediated by both $[Ru(NH_3)_5NH_2]^{2+}$ and the further oxidized Ru complex. Considering the previous study,[36] the reaction mechanism seems to be represented as follows:

$$[Ru^{III}(NH_3)_5NH_2]^{2+} \rightarrow [Ru^{IV}(NH_3)_5NH_2]^{3+} + e^- \quad (1)$$

$$n[Ru^{IV}(NH_3)_5NH_2]^{3+} + NaBH_4 \rightarrow n[Ru^{III}(NH_3)_5NH_2]^{2+} + \text{product} \quad (2)$$

Further oxidized Ru complex would also take part in electron mediation. Interestingly, although the oxidized Ru complexes ($[Ru^{IV}(NH_3)_5NH_2]^{3+}$ and the further oxidized Ru complex) are unstable as shown in curve iii of Figure 1a, the oxidation currents of $NaBH_4$ were highly enhanced in the presence of $[Ru(NH_3)_6]^{3+}$ (Figure 1b), indicating that the electron-transfer reaction between the oxidized Ru complexes and $NaBH_4$ is very fast. Accordingly, high oxidation currents of $NaBH_4$ can be achieved in the presence of $[Ru(NH_3)_6]^{3+}$, although $NaBH_4$ electrooxidation is negligible at ITO electrodes.

Low Electrostatic Binding of $[Ru(NH_3)_6]^{3+}$ to DNA-Sensing Electrodes. Figure 2 illustrates a DNA-sensing electrode and a DNA-detection scheme. A carboxylated monolayer was formed on an ITO electrode by using PPA. Capture-probe PNA was covalently bound to carboxylic acid groups of a PPA-modified ITO electrode. The remaining carboxylic acid groups were modified with ethylenediamine, resulting in the formation of a DNA-sensing electrode. In the detection process, target DNA was hybridized with PNA of the DNA-sensing electrode. After the target DNA-bound electrode was incubated in a $[Ru(NH_3)_6]^{3+}$-containing solution, electrocatalytic currents or charges

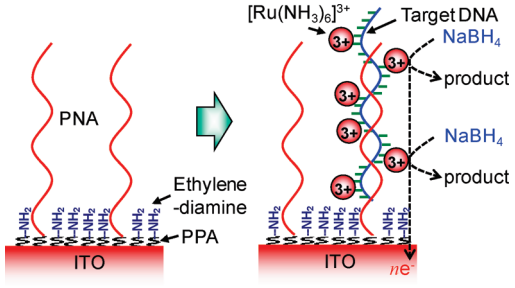

Figure 2. Schematic representation of a DNA-sensing electrode and an electrochemical DNA sensor using $NaBH_4$ oxidation mediated by $[Ru(NH_3)_6]^{3+}$.

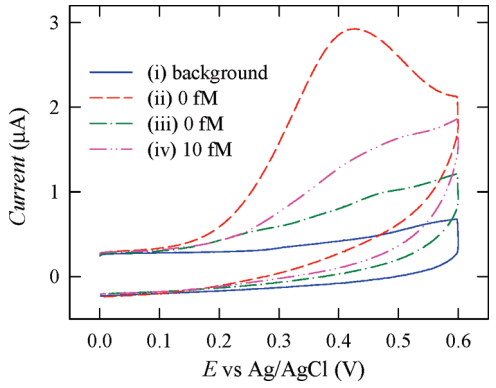

Figure 3. Cyclic voltammograms obtained at (i, iii, iv) ethylenediamine-modified and (ii) ethylenediamine-unmodified DNA-sensing electrodes at a scan rate of 50 mV/s in borate buffer (pH 11.0) containing 5 mM $NaBH_4$. (iv) DNA hybridization was performed in tris buffer (pH 7.4) containing 10 fM target DNA. The background data were obtained without treating a DNA-sensing electrode with target DNA and $[Ru(NH_3)_6]^{3+}$.

of $NaBH_4$ were measured. In the absence of bound $[Ru(NH_3)_6]^{3+}$, the oxidation currents of $NaBH_4$ were very low. However, in the presence of $[Ru(NH_3)_6]^{3+}$, the oxidation currents of $NaBH_4$ were highly enhanced due to electron mediation of the oxidized Ru complexes. The significant change in the electrocatalytic activity of the sensing electrodes after $[Ru(NH_3)_6]^{3+}$ binding facilitates a high signal-to-background ratio.

Multivalent cations readily adsorb onto anionic or even neutral surfaces. $[Ru(NH_3)_6]^{3+}$ can easily bind to DNA-sensing electrodes in the absence of hybridized target DNA. Selective $[Ru(NH_3)_6]^{3+}$ binding to target DNA is required to obtain a low detection limit. Therefore, it is essential to reduce $[Ru(NH_3)_6]^{3+}$ binding to DNA-sensing electrodes. To obtain low $[Ru(NH_3)_6]^{3+}$ binding, PNA was used as a capture probe (Figure 2), because the $[Ru(NH_3)_6]^{3+}$ binding to neutral PNA is very low.[18,27−30] Curve i of Figure 3 represents the cyclic voltammogram obtained in a $NaBH_4$ solution after a PPA-, PNA-, and ethylenediamine-modified ITO electrode was untreated with target DNA and $[Ru(NH_3)_6]^{3+}$. As expected, the oxidation currents of $NaBH_4$ were negligible in the absence of $[Ru(NH_3)_6]^{3+}$.

The oxidation currents of $NaBH_4$ obtained at an ethylenediamine-unmodified ITO electrode, after the electrode was treated with $[Ru(NH_3)_6]^{3+}$ (curve ii of Figure 3), were much higher than those at an ethylenediamine-modified ITO electrode (curve iii of Figure 3). Ethylenediamine treatment can block all negatively charged carboxylate groups. The higher oxidation currents at an ethylenediamine-unmodified ITO electrode are due to higher

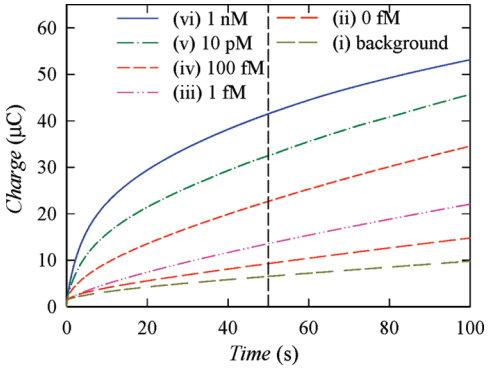

Figure 4. Chronocoulograms obtained by applying a potential step from 0.0 to +0.35 V to DNA- and $[Ru(NH_3)_6]^{3+}$-bound sensing electrodes in borate buffer (pH 11.0) containing 5 mM $NaBH_4$. DNA hybridization was performed in 10-fold-diluted human serum containing 1 fM, 100 fM, 10 pM, and 1 nM target DNA. The background data were obtained without treating a DNA-sensing electrode with target DNA and $[Ru(NH_3)_6]^{3+}$.

$[Ru(NH_3)_6]^{3+}$ binding to negatively charged carboxylate groups of the electrode. Curve iv of Figure 3 represents the cyclic voltammogram obtained after a PPA-, PNA-, and ethylenediamine-modified ITO electrode was treated with 10 fM target DNA and then with $[Ru(NH_3)_6]^{3+}$. The oxidation currents were clearly higher than those in curve iii of Figure 3. These results indicate that the $[Ru(NH_3)_6]^{3+}$ binding to the PPA-, PNA-, and ethylenediamine-modified ITO electrode was low enough to detect 10 fM target DNA. When a detection limit for target DNA in tris buffer was determined with cyclic voltammetry, a detection limit of 10 fM was obtained (see Supporting Information).

DNA Detection in 10-Fold Diluted Serum. Because serum is a complicated biological fluid, it is not easy to measure DNA concentration without dilution.[2] If a high amount of biomolecules in serum is nonspecifically adsorbed onto the sensing electrode, the adsorbed biomolecules would interfere with the specific binding of target DNA to the sensing electrode and/or increase the nonspecific binding of $[Ru(NH_3)_6]^{3+}$. Such results could make it difficult to achieve low detection limits. The cyclic voltammogram at the DNA-sensing electrode, incubated in 10-fold-diluted human serum containing 1 nM target DNA, was similar to that in tris buffer containing 1 nM target DNA (pH 7.4) (Figure S5a in the Supporting Information). Moreover, the cyclic voltammogram at the sensing electrode incubated in 10-fold-diluted human serum containing no target DNA was similar to that in tris buffer containing no target DNA (Figure S5b in the Supporting Information). These results show that the signal and background levels in 10-fold-diluted human serum are not significantly different from those in tris buffer.

To determine the detection limit of the proposed DNA sensor, concentration-dependent data were obtained with chronocoulometry instead of cyclic voltammetry. Figure 4 shows the chronocoulograms obtained in borate buffer (pH 11.0), in which a potential step from 0.00 to 0.35 V was applied. Curve i of Figure 4 represents the chronocoulogram obtained after a DNA-sensing electrode was untreated with target DNA and $[Ru(NH_3)_6]^{3+}$. Curve ii of Figure 4 was obtained after a DNA-sensing electrode was untreated with target DNA but treated with $[Ru(NH_3)_6]^{3+}$. The difference between curves i and ii of Figure 4 results from unwanted $[Ru(NH_3)_6]^{3+}$ binding to the DNA-sensing electrode, but it is not significant. Preferably, the charges increased over a wide range of concentrations, as the concentration of target DNA increased (Figure 4).

Figure 5 shows the dependence of the charge at 50 s on the concentration of target DNA. All data were subtracted by the mean

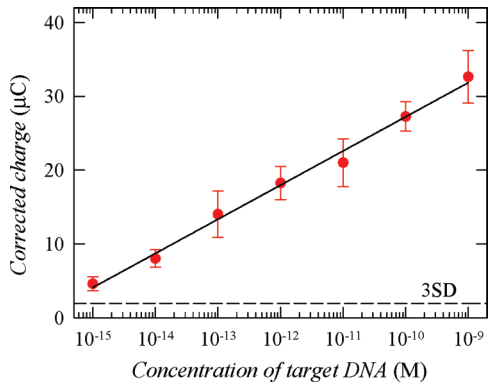

Figure 5. Dependence of the charges at 50 s in chronocoulograms of Figure 4 on the concentration of target DNA. All data were subtracted by the mean charge at a concentration of zero target DNA. The dashed line corresponds to 3 times the standard deviation (SD) of the charge at a concentration of zero target DNA. The error bars represent SD of at least four measurements.

charge at a concentration of zero target DNA. The charge at 1 fM target DNA, 14.5 ± 1.0 μC (mean ± standard deviation), was higher than the charge at zero target DNA, 9.8 ± 0.7 μC. Accordingly, the detection limit of the sensor was 1 fM. Importantly, DNA could be detected over a wide range of concentrations from 1 fM to 1 nM (i.e., over 7 orders of magnitude). The detection limit obtained with chronocoulometry (1 fM) was lower than that with cyclic voltammetry (10 fM). The reason for this is that chronocoulometry provides more reproducible data and lower background levels than cyclic voltammetry. The high pH condition minimized self-hydrolysis of $NaBH_4$, facilitating reproducible data.

Conclusions

An ultrasensitive electrochemical DNA sensor has been developed, based on (i) fast $NaBH_4$ electrooxidation mediated by $[Ru(NH_3)_6]^{3+}$ and (ii) low $[Ru(NH_3)_6]^{3+}$ binding to PNA- and ethylenediamine-modified DNA-sensing electrodes. PNA and ethylenediamine on DNA-sensing electrodes significantly decreased $[Ru(NH_3)_6]^{3+}$ binding. The $[Ru^{III}(NH_3)_5NH_2]^{2+}$ formed from $[Ru^{III}(NH_3)_6]^{3+}$ in basic solutions acted as a fast and effective electron mediator for $NaBH_4$ electrooxidation. There was a significant enhancement in the electrocatalytic activity of DNA-sensing ITO electrodes after $[Ru(NH_3)_6]^{3+}$ binding. All combined effects enabled the detection of DNA with a detection limit of 1 fM in 10-fold-diluted human serum. This DNA-detection method is highly promising for practical DNA detection.

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Supporting Information Available: Surface density of PNA on ITO electrodes; more cyclic voltammograms of $[Ru(NH_3)_6]^{3+}$; cyclic voltammograms at DNA-sensing electrodes in tris buffer containing target DNA; calibration plot obtained in tris buffer; comparison between cyclic voltammograms in tris buffer and serum. This material is available free of charge via the Internet at http://pubs.acs.org.