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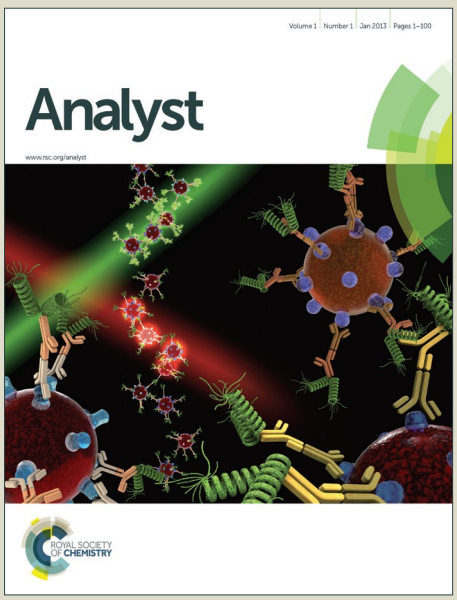

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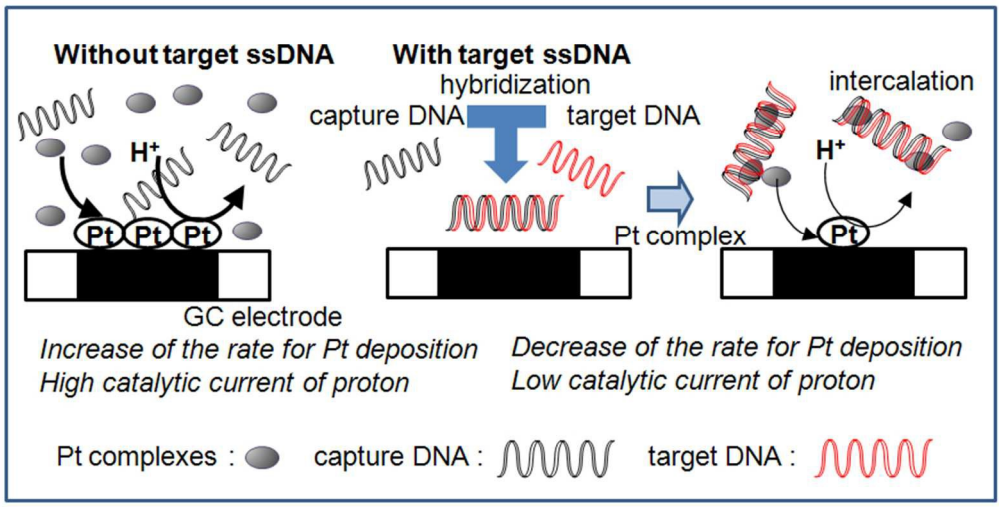

Text: A novel electrochemical system based on the catalytic reduction of protons with deposited platinum is proposed for the sensing of DNA with a specific sequence

A novel electrochemical system based on the catalytic reduction of protons with deposited platinum is proposed for the sensing of DNA with a specific sequence
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DNA hybridization sensor based on catalytic response by platinum deposition

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Abstract

We report a novel electrochemical sensing system for single-stranded DNA (ssDNA) with a specific sequence based on the catalytic reduction of protons with platinum deposited by the electrochemical reduction of chloro-2,2':6',2''-terpyridine platinum (II) chloride dihydrate (Pt complex) on a glassy carbon (GC) electrode. There was no catalytic property for proton reduction at the GC electrode, while the platinum deposited by the reduction of Pt complex shown the catalytic activity of proton reduction. The intercalation of Pt complex with double-stranded DNA (dsDNA) decreased the concentration of free Pt complex with a concomitant diminution in electrochemical catalytic current due to steric hindrance and a decrease in the diffusion coefficient of the intercalated Pt complex. Thus, the catalytic current of proton reduction by platinum deposited on a GC electrode decreased with an increase in the concentrations of target ssDNA, when capture DNA with complementary sequence was presented in the solution to form the hybrid dsDNA. A detectable concentration range was estimated and found to be 0.1–1.0 μM. The catalytic current was significantly larger than the reduction current of Pt complex, resulting in the sensitive detection of ssDNA. Furthermore, the present method is simply due to the immobilization of capture DNA being unnecessary.

Keywords: platinum deposition, hybridization, intercalation, proton reduction, current amplification

1. Introduction

Analytical technologies for DNA with specific sequences are of considerable and practical interest in the areas of gene identification for human genetic diseases, early diagnosis of infectious diseases promoted by pathogens, and development of novel drugs. Major challenges for the development of DNA biosensors involve the improvement of selectivity, sensitivity, rapidity and simplicity when performing an assay. The electrochemical behavior of the direct electron transfer of nucleobases was well studied by using mercury,[1] as well as boron-doped diamond[2, 3] and diamond-like carbon electrodes.[4] A DNA hybridization phenomenon contributes to the determination of specific sequences and the discrimination of single, mismatched base pairs.

Redox active species, which can selectively interact with double-stranded DNA (dsDNA) by minor groove[5-7] or intercalative[8-11] binding, have been used to convert the hybridization phenomenon to the electrochemical signal. Electrochemically active indicators for hybridization, such as bis-intercalaters[8] and threading intercalators[9-11], were strongly inserted into the planar moiety between complementary duplex bases and then detected in the solution without indicators to indentify the hybridized target single-stranded DNA (ssDNA). Metal nanoparticles are frequently used to improve sensitivity with low detection limits. Metal nanoparticles modified with single-stranded DNA (ssDNA) were captured by a DNA hybridization process and used as the preconcentrated signal sources. Dissolved metal particles can easily be detected as large faradic currents by stripping methods[12-14]. Enzyme labels have also been used to produce signal molecules for highly sensitive electrochemical detection[15-17]. The sensitive measurements were accomplished by modifying a large number of enzymes per hybridization events, respectively.

In the usual DNA-sensing methods, capture ssDNA has to be immobilized on an electrode or solid surface to capture the target ssDNA and/or labels for the signal conversion. Moreover, complex multiple-step processes were required for complementary recognition events and washing. The use of stem-loop structures with redox species at the free end of capture DNA allow for simple determination strategies[18, 19]. The accessibility of the tethered redox species to the electrode was altered by the conformation change of capture ssDNA with the hybridization of target ssDNA.

Recently, we have reported on the electrolytic deposition of cis-diamminedichloro-platinum(II) (cisplatin) and the application of the platinum deposition for the highly sensitive determination of DNA[20]. The suppression of the platinum deposition with the complexation between cisplatin and DNA was used to develop the sensitive DNA sensors, though the strategy does not support the determination of DNA with specific sequences. We therefore proposed a simple electrochemical technique for the determination of a DNA sequence and presented preliminary results as the conference proceedings[21]. The technique based on the combination of the intercalation of chloro-2,2':6',2''-terpyridine platinum (II) chloride dihydrate (Pt complex) with dsDNA formed by hybridization and the catalytic reduction of protons with the platinum deposited on glassy carbon (GC) electrodes. Square-planar platinum (II) complex derived from 2,2':6',2''-terpyridine can be bound to dsDNA by intercalative interaction[22-24]. In this paper, we investigated a reproducibility of the measurements and determined a detectable concentration range of target ssDNA with a specific sequence (20-mer oligonucleotide). Figure 1 shows the principle of the electrochemical determination of target DNA with a specific sequence. In the absence of target DNA, the Pt complex was reduced to deposit metal platinum on GC electrodes,

which follows an increase in cathodic current corresponding to the electrochemical reduction of protons on the deposited platinum surface (Fig. 1A). The formation of dsDNA by adding the target ssDNA with a specific sequence causes a decrease in the concentration of the free Pt complex by the intercalation, and thereby decreases the catalytic current of protons due to the decrease in the platinum deposition rate (Fig. 1B). The present procedure is very simple, as it does not require the immobilization of capture DNA.

2. Experimental

Cyclic voltammetry (CV) was usually performed by sweeping the potential of the GC electrode (diameter: 1.0 mm) between 0.4 and −1.5 V vs. Ag/AgCl with a scan rate of 50 mV/s in buffer solution containing 0.1 mM of Pt complex to determine the potential for the deposition of the Pt complex.

The increasing rates of the catalytic current for protons by deposited platinum were measured by amperometry with stirring in the presence of the different concentrations of target DNA. Pt complexes were synthesized according to published procedures[25]. Three synthetic oligonucleotides (20-mer) were used in this work and had the following sequences: 5'-TAT GGC TGG CTG GCT GGC AC-3' used as the target DNA, 5'-GTG CCA GCC AGC CAG CCA TA-3' used as the capture DNA, and 5'-TAT GAC TGA CTG ACT GAC AC-3' used as the negative control DNA with a non-complementary sequence, respectively. The different concentrations of target DNA were added to 0.1 M phosphate buffer (pH 7.0) containing 1.0 μg mL^{-1} of capture DNA to allow for hybridization. The solution containing Pt complex (5.0 μM) was added to perform the

intercalation of the Pt complex with the dsDNA formed by capture and target ssDNAs. After incubation for 90 min, the potential for the electrode was then stepped up to -1.1 V from 0.4 V to deposit the platinum and measure the increasing rate of the reduction current for protons catalyzed by the deposited platinum. We calculated each reduction current as an average of five data points.

3. Results and discussion

3.1 Electrolytic reduction of platinum complex

We investigated the reduction potential for Pt complex on the GC electrode. Figure 2 shows the cyclic voltammograms for 0.1 mM Pt complex in 0.1 M phosphate buffer (pH 7.0). For the first scan, the reduction current was slightly observed around -1.0 V (Fig. 2A, inset) and dramatically increased in the negative potential region beyond -1.0 V in buffer solution containing Pt complex (Fig. 2Aa), while no reduction response was obtained without Pt complex (Fig. 2Ab). Figure 2B shows cyclic voltammograms obtained from the second, fourth and sixth scans in the presence of 0.1 mM Pt complex. For the repeated scans, a discernible increase in the reduction current was given in the negative potential region beyond -1.0 V. A voltammetric measurement was performed in 0.1 M phosphate buffer in the absence of Pt complex using the deposited electrode. The voltammogram obtained in the absence of Pt complex was similar to that obtained in the presence of Pt complex. These results strongly suggest that Pt complex was reduced to deposit platinum on the GC electrodes by applying the potential < -1.0 V, which follows the appearance of a large reduction current corresponding to the electrochemical reaction of protons on the deposited platinum surface. Thus, the measurement of the

reduction current for protons is proven to be highly suitable for monitoring the deposition of Pt complex, since the catalytic current for reducing protons is much larger than the current for platinum deposition.

3.2 Platinum deposition by amperometry

Figure 3 shows the variations of reduction currents obtained by amperometry in the presence of different concentrations of Pt complex. The potential was stepped up from 0.4 to -1.1 V at zero seconds to reduce the complex. After step-like responses were observed, reduction currents gradually increased due to the platinum deposition. Unstable current responses were observed after 50 and 100 s in the presence of 10 and 5 μM Pt complex, respectively (Fig. 3a,b), because of the formation of hydrogen gas on GC electrodes by the electrochemical reduction of protons. The reduction current rate decreased when the concentration of Pt complex was decreased. A low concentration of Pt complex during deposition should decrease the deposition rate of platinum, hence decreasing the catalytic reduction rate of protons. Thus, monitoring the increasing rate of the reduction current for protons with a concomitant deposition of platinum is proved to be highly suitable for determining the concentration of Pt complex.

3.3 Effect of the addition of dsDNA from herring sperm

The increasing rates of the catalytic currents were investigated by amperometry in the presence of different concentrations of dsDNA from herring sperm. The dsDNA was added to 0.1-M buffer containing 5.0 μM Pt complex. After incubation for 90 min, the potential of the GC electrode was stepped up to -1.1 V to deposit the platinum and measure the catalytic currents. Figure 4 shows the current-time curves obtained in

buffer containing the different concentrations of dsDNA. The reduction currents gradually increase because of an increase in the formation of platinum particles on the GC surface. The increasing current rate is significantly reduced by increasing the dsDNA concentration up to 20 μg mL^{-1} (Fig. 4e). A dsDNA concentration as low as at least 20 ng mL^{-1} can be detected in the present system (Fig. 4a,b). Pt complexes were intercalated with added dsDNA, leading to a decrease in the free Pt complex concentration. A decrease in the platinum deposition rate is caused by steric hindrance and an increase in the Pt complex diffusion coefficient by intercalation with dsDNA. No current increase was observed from the amperograms obtained in solution without Pt complex. In addition, we investigated the effect of the adsorption of DNA on a GC electrode by CV in phosphate buffer containing 0.1 mM $Fe(CN)_6^{4-}$. A GC electrode was immersed in solution containing 50 μM dsDNA without Pt complex at -1.1 V for 120 s. The oxidation currents of $Fe(CN)_6^{4-}$ were slightly decreased by immersing the GC electrode into the solution containing dsDNA, indicating that DNAs with negative charge are little adsorbed on the electrode surface because of the application of negative potential.

3.4 Detection of target DNA

The increase in reduction current was investigated to determine the concentration of target DNA. Figure 5A shows the amperometric current responses in 0.1 M buffer solution containing 1.0 μg mL^{-1} capture DNA and different concentrations of target DNA. In the absence of target DNA, the reduction current drastically increased after the potential was stepped up to −1.1 V because of an increase in proton reduction by increasing the platinum surface (Fig. 5Aa). The current-time curve obtained with

capture DNA and Pt complex is similar to that obtained with only Pt complex shown in Figures 3b and 5Aa. The result provably indicated that Pt complex does not interact with ssDNA.

The addition of target DNA actually brought about a delay for an increase in reduction current (Fig. 5Ab – 5Af). Pt complexes were intercalated into the dsDNA formed by the complementary recognition event between the capture DNA and the target DNA added in the solution, and thereby decreased the concentration of free Pt complex. However, the increasing rate obtained in the solution containing added DNA with a non-complementary sequence is comparable with that in the solution without target DNA (Fig. 5Ag), because of the lack of dsDNA for intercalation. Thus, the presence of ssDNA with a non-complementary sequence does not inhibit platinum deposition by decreasing the concentration of free Pt complex. These results suggest that the DNA concentration can be determined from the current-time curve for the reduction of protons.

Figure 5B shows the relationship between the target DNA concentration and the reduction current obtained 90 s after applying the potential. The reduction current decreased with an increase in DNA concentration and became saturated in the higher concentration. Current densities were measured in lower concentration range of model target sequence compared to that obtained in our previous report[21], resulting in the detectable concentration range was estimated and found to be 0.1−1.0 μM. A significant difference in the increasing rate was observed in 0.1 μM target DNA. The coefficient of variation for five replicate measurements of 0.1 μM was 9.6%. Although the reproducibility is relatively poor probably due to the difference of the initial properties of GC electrode surface for the reduction of Pt complex, the procedure for the detection

is significantly simple. From the value of the binding constant between dsDNA and Pt complex (3.9×10^5 M^{-1})[26] and our assumption that all target ssDNA forms duplexes with capture DNA, a binding ratio was roughly estimated to 0.25 Pt complex per nucleotide, which is in agreement with the value of 0.2 reported previously[27]. However, the detectable concentration range obtained in this work is significantly higher than that obtained in our previous study.[20] This is due to the hydrogen bubble formation by applying relatively higher negative potential. However, the measurement of the catalytic reduction current for protons actually resulted in the simple determination of target DNA with a complementary sequence. We will optimize the concentration of Pt complex and duration for the electrochemical reduction of Pt complex to improve the sensitivity and detectable concentration range to lower level.

4. Conclusion

We have demonstrated that DNA with a target sequence can be electrochemically detected on the basis of the intercalation of Pt complexes with dsDNA which is formed by the hybridization between the target and capture DNAs. The electrocatalytic current for proton reduction, which is catalyzed by the platinum deposition, decreased with a decrease in free Pt complex and suppression of the reduction of Pt complex intercalated due to steric hindrance. The present procedure yields a simple determination of DNA with a specific sequence without the needing to immobilize capture DNA. The use of lower concentration of Pt complex is advantageous to improve the limit of detection of target DNA. We will investigate the increasing rate of the reduction current for proton depended on the electrode materials, size and surface roughness to optimize the initial

concentration of Pt complex.

Acknowledogement

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Figure Captions

Figure 1. Principle of the electrochemical determination of target DNA with a specific sequence by the intercalation of Pt complexes to the hybridized dsDNA in (A) the absence and (B) the presence of target DNA.

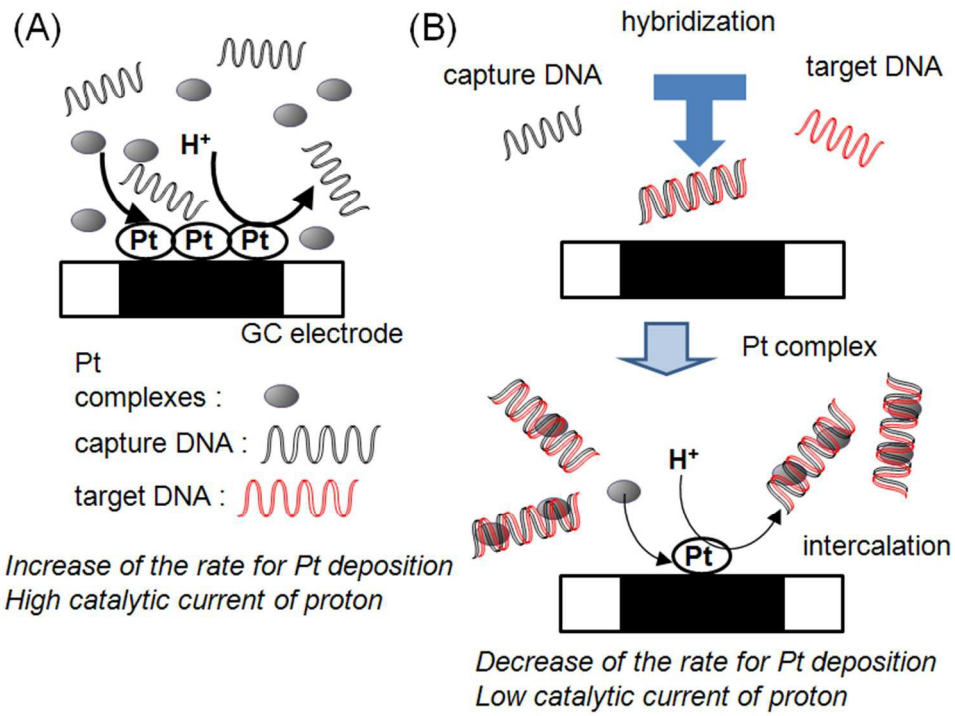
Figure 2. Electrochemical properties for Pt complex on a glassy carbon (GC) electrode by cyclic voltammetry. (A): Cyclic voltammograms of the first cycle on the GC electrode (a) in the presence and (b) in the absence of 0.1 mM Pt complexes. (B): Cyclic voltammograms of (a) second, (b) fourth and (c) sixth scan.

Figure 3. Variations of reduction currents on GC electrodes in 0.1 M phosphate buffer containing Pt complexes at -1.1 V. Concentrations of complexes are (a) 10, (b) 5.0, (c) 2.5, (d) 0.5 and (e) 0 μM.

Figure 4. Variations of reduction currents in 0.1 M phosphate buffer containing 5.0 μM Pt complexes and the different concentrations of dsDNA. Concentration of dsDNA: (a) 0, (b) 0.02, (c) 0.2, (d) 2.0 and (e) 20 μg mL^{-1}.

Figure 5. (A) Current-time curves in 0.1 M phosphate buffer containing 5.0 μM Pt complex, 1.0 μM capture DNA and the different concentrations of target DNA. Concentrations of target DNA: (a) 0, (b) 0.1, (c) 0.2, (d) 0.4, (e) 1.0 and (f) 2.0 μM. (g) Response obtained in the presence of 1.0 μM ssDNA with a non-complementary sequence to the capture DNA. (B) Relationship between the target DNA concentration

and the reduction currents obtained 90 s after applying the potential.



Principle of the electrochemical determination of target DNA with a specific sequence by the intercalation of Pt complexes to the hybridized dsDNA in (A) the absence and (B) the presence of target DNA.
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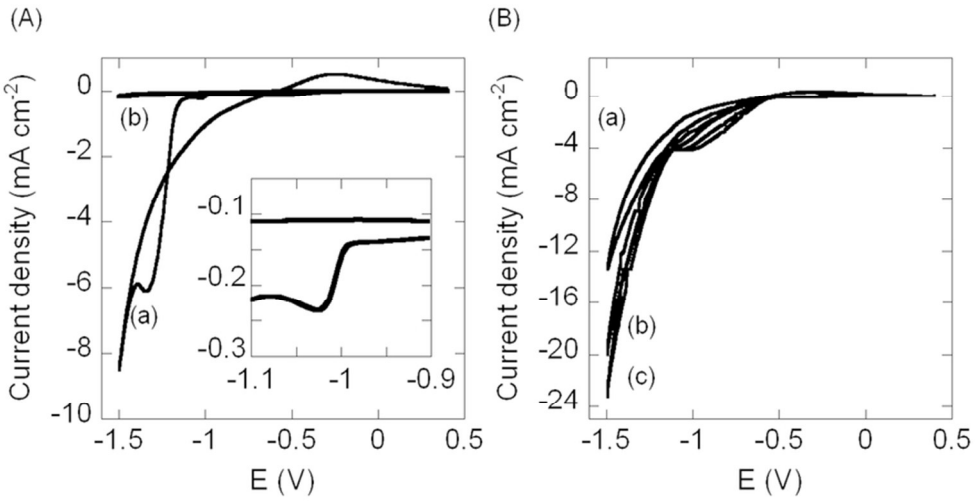

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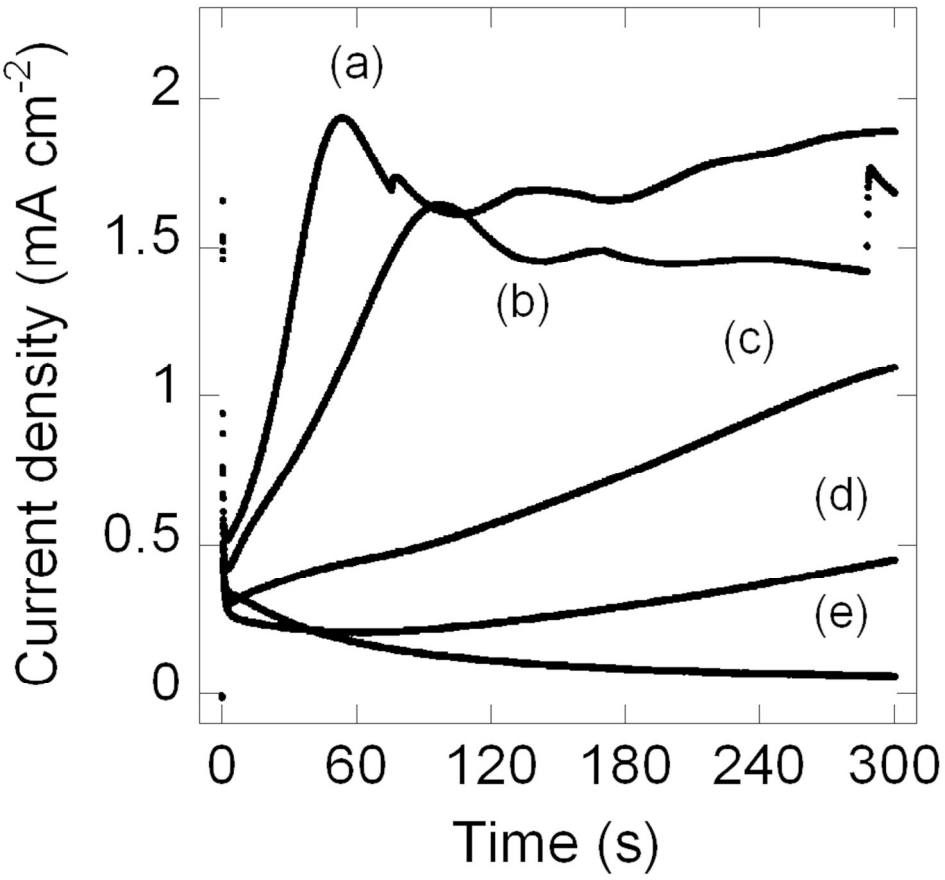

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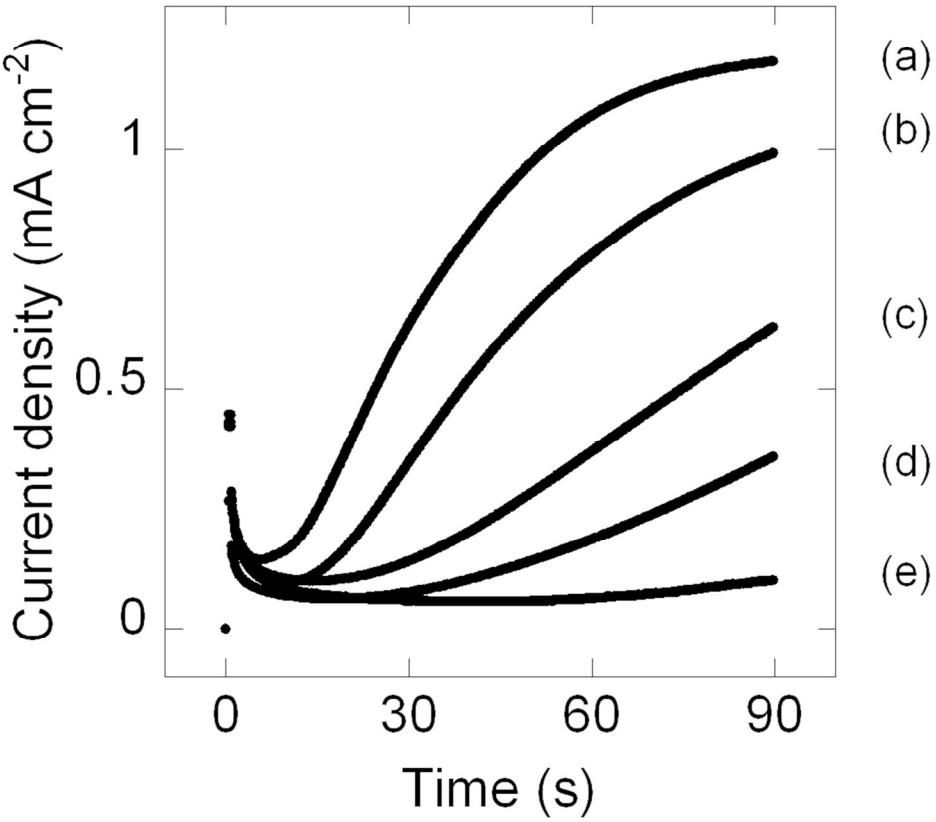

Figure 4. Variations of reduction currents in 0.1 M phosphate buffer containing 5.0 μM Pt complexes and the different concentrations of dsDNA. Concentration of dsDNA: (a) 0, (b) 0.02, (c) 0.2, (d) 2.0 and (e) 20 μg mL-1.
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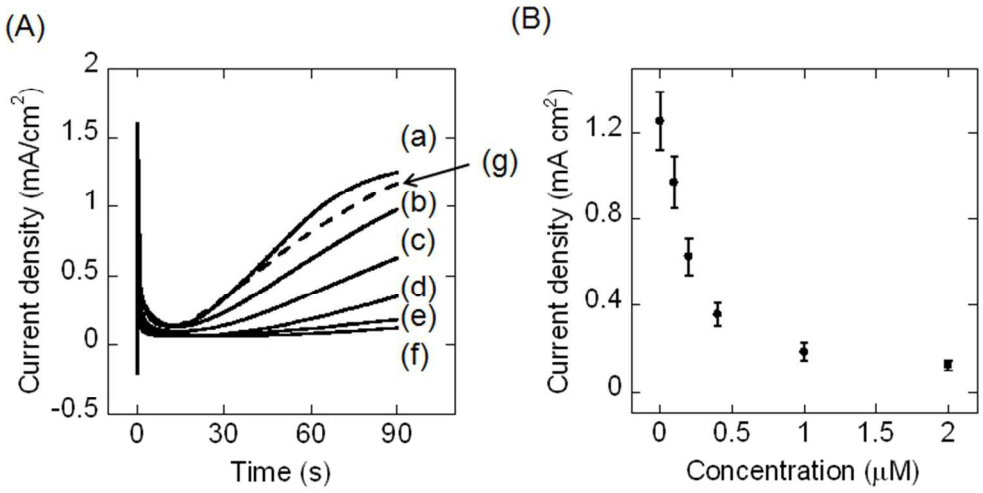

(A) Current-time curves in 0.1 M phosphate buffer containing 5.0 μM Pt complex, 1.0 μM capture DNA and the different concentrations of target DNA. Concentrations of target DNA: (a) 0, (b) 0.1, (c) 0.2, (d) 0.4, (e) 1.0 and (f) 2.0 μM. (g) Response obtained in the presence of 1.0 μM ssDNA with a non-complementary sequence to the capture DNA. (B) Relationship between the target DNA concentration and the reduction currents obtained 90 s after applying the potential.μ
66x34mm (300 x 300 DPI)