

Improvement of Electrochemical Response of Cocaine Sensors Based on DNA Aptamer by Heat Treatment

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We report on a biosensor for cocaine based on the conformation change of DNA aptamer by capturing the cocaine molecules. The oxidation current of ferrocene conjugated on the terminal end of aptamer immobilized on an Au electrode increased with increasing cocaine concentration. The sensor response has been improved by a simple heat treatment after immobilization, since the aggregates of DNA aptamer generated during the immobilization step could be dissociated and rearranged on the electrode.

Keywords Heat treatment, electrochemical measurements, cocaine detection, ferrocene conjugated DNA aptamer

(Received November 16, 2015; Accepted December 8, 2015; Published April 10, 2016)

Introduction

Aptamer-based biosensors have been utilized for selective detection of small molecules with several hundred molecular weight.¹⁻⁷ DNA aptamer modified with electroactive species, *i.e.* methylene blue (MB),^{2,3,8-10} ferrocene (Fc),¹¹⁻¹⁴ and their derivatives is immobilized on the electrode surface to develop the aptamer-based biosensors. The modification of electrodes with antibodies or oligonucleotides has been commonly used to develop the electrochemical^{2-6,15,16} and quartz crystal microbalance^{6,17,18} sensors based on the immunoreactions and DNA hybridizations. However, these sensors using antibodies and oligonucleotides as recognition elements require the separation steps between the captured and uncaptured target and labeled molecules for generating the sensing responses. For example, in the immunosensor using an enzyme as a label, the solution containing unreacted target and labeled enzyme must be removed from the surface captured with target molecules after the immunological recognition steps. Moreover, the substrate for the enzyme reaction should be added after removing the target.

Swensen *et al.* developed an electrochemical monitoring system for cocaine using cocaine binding DNA aptamer conjugated by MB³ which was immobilized on the Au electrode. MB conjugated on DNA aptamer is immobilized on the electrode with limited mobility, while the distance between MB and the electrode decreases with the conformation change of the aptamer by capturing the cocaine molecule. Therefore, the electron transfer was improved by adding cocaine molecules without any separation steps.

In this paper, we have developed the biosensors for cocaine based on DNA aptamer with Fc conjugated on the terminal end. Fc is useful as a modifier for obtaining an electrochemical signal because of the lack of an electrochemical reaction of interferences such as oxygen dissolved in aqueous solution with pH neutral at the redox potential of Fc. The present paper introduces the simple procedure to clarify the electrochemical signal derived from Fc on the terminal end of DNA aptamer, which was immobilized on the electrode. Cocaine was chosen as a target species because of its affordable molecular size. The sequence of DNA aptamer for cocaine sensing was referenced from some reports.¹⁻³

Experimental

The chamber with the through hole was mounted on a glass substrate coated with gold layer (100 nm thickness), which was used as a working electrode. The working electrode was oxygen plasma ashed in order to improve its hydrophilicity before use. The active area for immobilization and electrochemical reaction was defined by a silicone rubber O-ring placed between the chamber and gold layer and found to be 47.8 mm². Pt coil and Ag/AgCl (sat. KCl) electrode were inserted in the chamber from the top of the through hole to use as a counter electrode and a reference electrode, respectively. All electrochemical measurements were conducted with a potentiostat (ALS-440C, ALS Co., Ltd) in a faraday cage at 20°C.

The synthetic oligonucleotide (32 mer) modified both ends by thiol group and Fc (CocAp-Fc, Nihon Gene Research Laboratories Inc.) was used as a DNA aptamer for cocaine binding, which had the following sequence: 5'-HS-(CH₂)₆-AGACAAGGAAAATCCTTCAATGAAGTGGGTCG-(CH₂)₆-NHCO-Fc-3'. Thiol group on 5' terminal was protected with the

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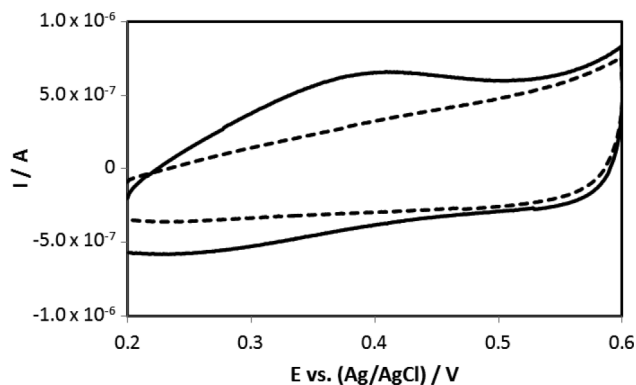


Fig. 1 Cyclic voltammograms of immobilized CocAp-Fc taken at 5 mV s^{-1} in HBS-EP⁺ buffer before (dashed line) and after (solid line) the heat treatment.

disulfide bond by 6-mercaptohexanol. The Au electrode was treated with 200 μL of HBS-N buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 150 mM sodium chloride, pH 7.4, GE Healthcare) containing 10 μM of CocAp-Fc for 12 h at 20°C . Electrodes modified with CocAp-Fc were immersed in HBS-EP⁺ buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 150 mM sodium chloride, 3.0 mM ethylenediaminetetraacetic acid and 0.05%(v/v) tween 20, pH 7.4, GE Healthcare) containing different concentrations of cocaine hydrochloride (Takeda Pharmaceutical Co., Ltd.). Heat treatment was then carried out by using a thermostat box at 70°C for 5 min, unless otherwise stated. The temperature returned gradually to 20°C for 15 min in order to promote cocaine binding reaction. Cyclic voltammetry (CV, scan rate of 5 mV s^{-1}) and differential pulse voltammetry (DPV) were performed in HBS-EP⁺ buffer containing cocaine molecules. The potential step, the amplitude and the pulse width for DPV were set at 5 mV, 25 mV and 50 ms, respectively. Methamphetamin (Sumitomo Dainippon Pharma Co., Ltd.), which is a representative example of stimulant drug was used to ensure the selectivity of DNA aptamer for cocaine.

Results and Discussion

The immobilization of CocAp-Fc with sulfur-gold interaction was investigated with surface plasmon resonance (SPR) spectroscopy (Biacore T200, GE Healthcare). The response increased and saturated within 4 h after injecting HBS-N buffer containing 10 μM CocAp-Fc (Fig. S1, Supporting Information). The constant response was obtained after the injection was switched to HBS-N buffer without CocAp-Fc, implying that immobilization of CocAp-Fc was saturated for approximately 4 h.

CV was used to investigate the effect of heat treatment to obtain the response from the CocAp-Fc modified on Au electrodes. Figure 1 shows CVs obtained before (dashed line) and after (solid line) the heat treatment (70°C , 5 min). Clear redox current peaks derived from immobilized Fc were observed at around 0.40 and 0.25 V after heat treatment, while no redox signal was observed before the treatment. We have obtained the similar results in other electrochemical techniques (*i.e.* DPV and square wave voltammetry), or in other DNA sequences having almost the same chain length (data not shown).

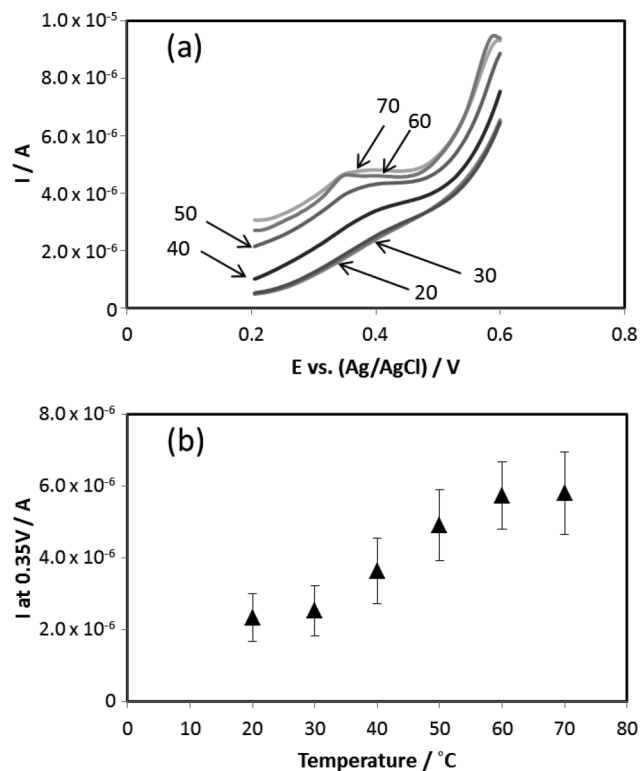


Fig. 2 (a) Differential pulse voltammograms of immobilized CocAp-Fc taken in HBS-EP⁺ buffer at each applied temperature and (b) plots of the anodic response at 0.35 V obtained in Fig. 2(a) as a function of the applied temperature ($n = 3$).

Figure 2(a) shows DPVs obtained by using the CocAp-Fc modified electrode treated at different temperatures. The inserted numbers mean the applied temperature. The anodic responses were observed at around 0.35 V by the electrode treated over 40°C . Figure 2(b) shows the relationship between the applied temperature and the anodic current at 0.35 V. The anodic current increased with increasing temperature for heat treatment and became saturated in the higher temperature region over 60°C . These results imply that Fc conjugated at 3' terminal come into contact with the electrode surface, since the aggregates of DNA aptamer generated during immobilization could be dissociated by the heat treatment, leading to an increase in mobility of Fc. Moreover, DNA molecules adsorbed on the electrode non-specifically could be removed to reduce the inhibition of the contact of Fc molecules to the electrode surface. Therefore, an improvement of sensitivity for cocaine responses can also be expected with the heat treatment.

The amount of the immobilized CocAp-Fc on the electrode was estimated by reductive desorption (Fig. S2, Supporting Information). The sharp peak was observed from the voltammogram obtained by the electrode after the heat treatment. The voltammogram obtained before the heat treatment, however, became broad and showed the negative shift of the peak potential. The results may indicate that CocAp-Fc molecules aggregated and adsorbed on the electrode were rearranged by the heat treatment to allow the well-ordered monolayer of CocAp-Fc. The amount of the immobilized CocAp-Fc was calculated from the cathodic charge ($1.2 \times 10^{-5} \text{ C}$) by considering the immobilization of CocAp-Fc with disulfide bond, and was found to be $1.3 \times 10^{-10} \text{ mol cm}^{-2}$. This value was slightly smaller than the theoretical value of closely packed

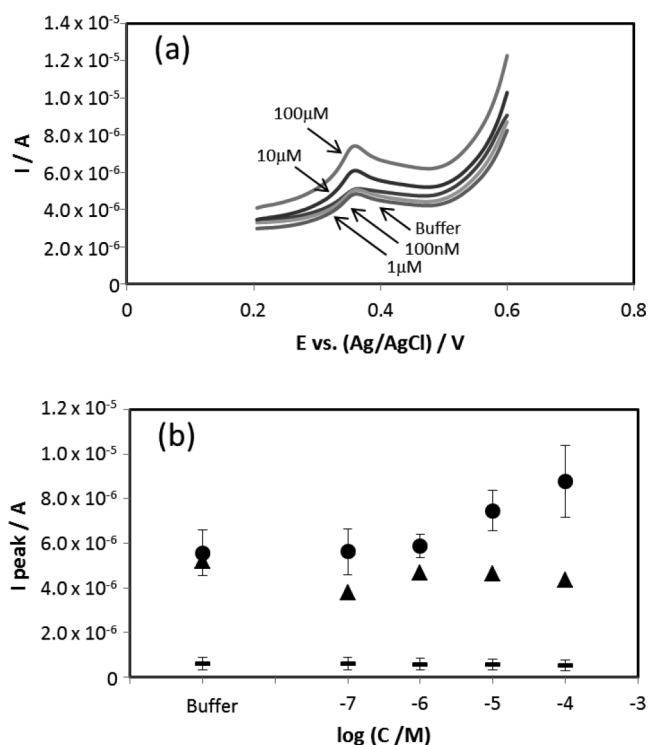


Fig. 3 (a) Differential pulse voltammograms of CocAp-Fc in HBS-EP⁺ buffer containing different concentrations of cocaine after the heat treatment and (b) plots of the anodic peak current obtained from DPV for cocaine detection by CocAp-Fc modified electrodes with (●) and without (○) the heat treatment ($n = 6$). Plots obtained for methamphetamine with the heat treatment (▲). C in the abscissa label indicates the concentration of target molecule.

alkanethiol on Au¹⁹ because of the steric crowding of DNA moieties. Electrochemical activity of CocAp-Fc modified electrode was also investigated by CV in the solution containing ferricyanide/ferrocyanide (Fig. S3, Supporting Information). The broad wave with relatively large peak separation was observed with CocAp-Fc modified electrode, while the wave obtained with the electrode after the reductive desorption of CocAp-Fc was identical to that obtained by the bare Au electrode. Therefore, almost all immobilized CocAp-Fc molecules could be removed by the reductive desorption in Fig. S2.

Figure 3(a) shows DPVs obtained by using the CocAp-Fc modified electrode after the heat treatment in HBS-EP⁺ buffer containing different concentrations of cocaine. Clear anodic currents for Fc oxidation were observed from each DPV. Plots in Fig. 3(b) (●) shows anodic peak current obtained in Fig. 3(a) as a function of the concentration of cocaine. The anodic current increased with an increase of the concentration of cocaine when heat treatment (70°C for 5 min) was performed to the electrode modified with CocAp-Fc. Cocaine molecules were recognized and trapped to CocAp-Fc immobilized on the electrode surface to cause the conformation change of CocAp-Fc,¹⁻³ resulting in the decrease in the distance between the electrode surface and Fc molecules conjugated to the 3' terminal of CocAp-Fc. In addition, no anodic signal appeared regardless of the concentration of cocaine without heat treatment (○). The binding site of CocAp-Fc could be regenerated by the heat treatment. Fc could be approached to the electrode surface caused by the conformation change of CocAp-Fc.

Methamphetamine was also measured in the same manner as cocaine with heat treatment (▲) and no signal was observed, suggesting that the selectivity of the CocAp-Fc was maintained.

Conclusions

A biosensing system without any separation steps has been developed to determine cocaine based on DNA aptamer conjugated with Fc. DNA aptamer for cocaine with Fc on 3' terminal was immobilized on Au electrodes by the thiol group on 5' terminal. The oxidation current for Fc increased with increasing cocaine concentration due to the decrease of the distance between Fc molecules and electrode, which was caused by the conformation change of immobilized aptamer with the recognition of the cocaine molecule. No separation or addition steps are required for the present method because the response was induced by the target recognition and the conformation change. The electrochemical response derived from Fc was improved by the heat treatment. The aptamer aggregated on the electrode surface would be dissociated and rearranged to form the well-ordered monolayer. The full effect of the heat treatment was observed above 60°C for 5 min for 32 mer of DNA aptamer, although the effect on the current response could depend on the chain length. This procedure could be applied in many fields using aptamer-based biosensors. Further investigations to improve sensitivity and background noise are currently in progress.

Supporting Information

Supporting Information includes SPR response of the Au electrode during CocAp-Fc immobilization (Fig. S1) and the changes in the reductive desorption of CocAp-Fc (Fig. S2) and in the electrochemical activities (Fig. S3). These materials are available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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