— Note —

Analysis of Electrochemical Reaction of Ferrocenyl-naphthalene Diimide Captured by Double-Stranded DNA during the Electrochemical Detection of DNA Hybridization

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Recently, studies of electrochemical DNA sensors are attracting considerable attention, because they will be developed for an electrochemical DNA chip using integration, etc. The response current that originated from the formation of the DNA duplex was clearly observed using differential pulse voltammetry for a DNA-immobilized gold electrode in an electrolyte solution containing ferrocenyl-naphthalene diimide as the hybridization indicator. A mismatch-discrimination was also exhibited. The authors carried out fundamental electrochemical analyses to obtain each parameter controlling the electrochemical response of the electrode system and to more qualitatively characterize.

Key Words : Fundamental Electrochemical Analysis, Hybridization Intercalator, Ferrocenyl-naphthalene Diimide, DNA-immobilized Electrode

1 Introduction

In recent years, the studies of the electrochemical DNA sensors are attracting considerable attention, which is increasingly becoming important for developing gene diagnosis, gene inspection, and an electrochemical DNA chip using integration.¹⁾ The authors have developed highly sensitive DNA sensors using combinations of ferrocenyl-naphthalene diimide (FND, Scheme 1) as a hybridization indicator and DNA probe electrodes.¹⁾ Figure 1 shows a scheme for developing a DNA sensor using an electrochemical intercalator, FND. It mainly consists of (1) hybridization between the electrode surface-confined probe-DNA and sample-DNA, and (2) intercalation of FND between adjacent pairs of DNA duplex. Thus, FND is concentrated by hybridization with an increase in the amount of the double-stranded DNA (dsDNA) of a target DNA fragment at the electrode surface-confined probe-DNA.^{2, 3)} An electrochemical signal due to the target DNA fragment was obtained from the ferrocene moiety of the FND concentrated on DNA bound to the electrode.^{2, 3)}

At the DNA detection using FND as an intercalator, the complex of FND with ssDNA was less stable than that with dsDNA.¹⁾ Using this property, mismatch discrimination between complementary chain and uncomplementary chain was possible.¹⁾ However, no such a factor for the mismatch discrimination in electrochemical parameters was present. Using more stable complex of FND with dsDNA, DNA hybridization assay enabled us DNA detection at femtomol level.²⁾ However, reason for this much lower detection limit was not discussed from the standpoint of an electrode reaction. In addition, immobilized amount of complementary chain estimated using ferrocene oxidation response at a cyclic voltammogram³⁾ is not reliable, because ferrocene oxidation may not be dominated adsorbed or immobilized. In this paper, an electrode reaction at the DNA-immobilized electrode in an electrolyte solution containing FND is especially discussed.

On the basis of electrochemistry and electrochemical measurements, the authors carried out fundamental elec-

Scheme 1

Fig. 1 Scheme for hybridization of sample-DNA with electrode surface-confined probe-DNA, and intercalation of FND.

trochemical analyses to obtain detailed information about each parameter controlling the electrochemical response of the electrode reaction of FND on the electrode. In the present study, the authors describe the information obtained from the electrochemical studies of a fully-matched or mismatched DNA duplex-immobilized on a gold electrode in an electrolyte containing FND. In this paper, knowledge used to more qualitatively characterize the electrode reaction by electrochemical analysis using a DNA-immobilized gold electrode in an electrolyte containing FND is presented. Such a mechanism analysis is absolutely necessary to establish methodologies for DNA sensors with a higher sensitivity. Such information and knowledge should be important for not only the development of even more sensitive DNA sensors, but also fabrication into a multi-electrode array system (electrochemical DNA chip).

2 Experimental

Three oligonucleotides, 5'-HS (CH₂)₆-pd (AAA AAA AAA AAA AAA AA)-3', 5'-pd (TTT TTT TTT TTT TTT TTT TT)-3', and 5'-pd (TTT TTT TTT ATT TTT TTT TT)-3' were obtained from Hokkaido System Sciences Co., Ltd.. The bases for the probes mismatching the target are underlined. FND was synthesized according to a previous described method.^{2, 3)}

A gold disk electrode (ALS, Inc.) was polished to a mirror finish with a 6 μm diamond slurry, a 1 μm diamond slurry, and a 0.05 μm alumina slurry (MARUTO INSTRUMENT Co., Ltd.). The geometric surface area was 0.02 cm² for this gold electrode. This gold electrode was boiled in 2 M NaOH for 1 hr, and subsequently immersed in conc. nitric acid for 30 min. Soon after this pre-treatment, after rinsing this electrode with water and then argon dried, a 2 mM thiolated oligomer (1 μL) solution was placed on the electrode surface, and was left for 2 hrs at 37 °C. After rinsing this electrode with water and then argon blow drying, 10 mM 6-mercaptohexanol (1 μL) was then placed on the electrode surface, and left for 2 hrs at 45 °C (*preparation of a single-stranded DNA (ssDNA)-modified electrode*). Finally, after rinsing this electrode with water and then argon drying, a 10 mM complementary oligomer / 2 $\times$ SCC (1 μL) was placed on the electrode surface, and left for 1 hr at 25 °C (*preparation of a dsDNA-modified electrode*). The 2 $\times$ SCC denotes 30 mM sodium citrate buffer containing 0.3 M NaCl at pH 7.0.

The electrochemical measurements were made using the above-mentioned dsDNA- or ssDNA-modified gold electrode as the working electrode at 40 °C. The electrolyte solution used in this study was a 0.1 M AcOK-AcOH buffer (pH 5.6) containing 0.1 M KCl and 50 μM FND. Here, Ac denotes the acetyl group. KCl, AcOK, and AcOH (extra pure grade, Wako Chemical Co.) were used without further purification. All other chemicals were of reagent grade and used without purification. Water was purified with a Milli-Q system (Millipore Co.). Its resistivity was over 18.0 M Ω cm.

3 Results and Discussion

Differential pulse voltammograms (DPVs) of DNA-modified gold electrodes in a 50 μM FND solution are shown in Figure 2. Current responses originating from the oxidation of the ferrocene moiety of FND are observed at the potential of +374 mV. The change in the peak current with the formation of the DNA duplex (calculated versus the peak current at the ssDNA-modified electrode) is 82 % for the fully-matched DNA duplex-immobilized electrode. FND may not react on the surface of the prepared modified electrode, because 6-mercaptohexanol was used to inhibit non-specific adsorption of FND onto the electrode surface. Only in the presence of FND in the electrolyte solution, redox response of ferrocene moiety of FND is measured (data not shown). So it is explained that dissolved FND may react through DNA chain. Thus, the response current that originated from the formation of the DNA duplex is clearly observed in DPV. For the mismatched duplex-modified electrode, the change is 65 %. This value is lower than the one for the fully-matched duplex-modified electrode. This suggests a mismatch-discrimination for the present electrode system, because this voltammetric behavior is reasonable for the lack of a binding site of the intercalator into the DNA duplex followed by the formation of a mismatch site.

The slopes of the scan rate-dependence (log-log plot) of anodic peak current of cyclic voltammograms of DNA-immobilized gold electrodes in 50 μM FND solution are 0.71 for fully-matched dsDNA-, 0.71 for mismatched dsDNA-, and 0.75 for ssDNA-immobilized gold electrode. If the slope approaches 0.5, then the electrode reaction is dominated by diffusion of the FND. If the slope approaches 1, then the electrode reaction is more dominated by the reaction of the FND located in the vicinity of the electrode surface. These slopes are almost intermediate between 0.5 and 1, which suggests, respectively, the case of classical diffusion and the case of a monolayer without transport.⁴⁾ Here, these slope values indicate that the electrode process was simultaneously controlled by the heterogeneous electron transfer rate.^{5, 6)} It may be suggested that reacting FND under intercalation and FND reaction in DNA chain after diffused as dissolved FND are existed in this electrode sys-

Fig. 2 DPVs of DNA-immobilized gold electrodes in 50 mM FND solution. (a) fully-matched dsDNA-; (b) mismatched dsDNA-; and (c) ssDNA-immobilized gold electrode.

tem.

Complex-plane plots of the ac impedance (Z) of the DNA-immobilized gold electrode in 50 μM FND solution are shown in Figure 3. In these treatments, the definition of the impedance is taken as $Z = Z_{\text{Re}} + j Z_{\text{Im}}$, where Z_{Re} and Z_{Im} are the real and imaginary parts of the impedance, respectively. In the presence of FND in the electrolyte solution, the charge transfer resistance at the dsDNA-modified electrode is actually lower. That is to say, the conductivity of dsDNA may also be higher in the presence of FND in the electrolyte solution. The order of the conductivity, that is to say, inversion of the charge transfer resistance may correspond to the order of the peak current in DPV. The current response originated from oxidation of the ferrocene moiety of FND increases with a concentrated amount of FND for the DNA immobilized on the electrode surface,¹⁻³⁾ so the order of the conductivity may be related to the increase in this concentrated amount of FND. Thus, the measurement in this study may be applied to the evaluation of the DNA hybridization.

Fig. 3 Complex-plane plots of ac impedance (Z) of DNA-immobilized gold electrode in 50 μM FND solution. Centered dc potential: +406 mV vs. Ag/AgCl ($\approx$ formal potential of ferrocene moiety of FND); ac amplitude: 86 mV; ac frequency: 100 kHz — 1 mHz. (a) fully-matched dsDNA-; (b) mismatched dsDNA-; and (c) ssDNA-immobilized gold electrode.

4 Conclusion

The response current that originated from the formation of the DNA duplex was clearly observed using differential pulse voltammetry for the DNA-immobilized gold electrode in an electrolyte solution containing FND. A mismatch-discrimination was also exhibited. The authors carried out fundamental electrochemical analyses to obtain each parameter controlling the electrochemical response of the electrode system and to more qualitatively characterize.

On the basis of this obtained knowledge, using an approach for measuring the ac voltammogram at higher frequencies in which one may find region of mass transfer-controlled responses, an increase with a change in the peak current with the formation of the DNA duplex and more well-defined mismatch-discrimination may be expected. This may be reasonable for the analysis results of the ac impedance in Figure 3. For realizing an improved electrochemical DNA detection method using the above-suggested method, the results of the electrochemical study of FND on the DNA-modified gold electrode may provide important information.

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