Extended Abstracts of the 2019 International Conference on Solid State Devices and Materials, Nagoya, 2019, pp29-30

DNA detection by Electrochemical Impedance Spectroscopy

Huanwen Han[1], Norhayati Binti Sabani[2*], Fumie Takei[2,3], Kazuyuki Nobusawa[1], Ichiro Yamashita[1]*
[1]Graduate School of Engineering, Osaka University 5-1 Yamadaoka Suita, Phone +81-6-6879-4137
Email:yamshita@pmdp.arl.eng.osaka-u.ac.jp
[2]The Institute of Scientific and Industrial Research, Osaka University (ISIR)
8-1 Mihogaoka Ibaraki, Japan, Phone+81-6-6170-1109
[3]National Defense Medical College (NDMC)
3-2 Namiki, Tokorozawa, Japan Phone: +81-4-2995-1693

Abstract

Electrochemical Impedance Spectroscopy (EIS) was applied for ligand-base DNA sensor. The sensor electrode was modified with ligands, *N,N'*-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine (DANPs). Probe-DNA composed of target specific anti-sense DNA sequence at one end and a cytosine-bulge (C-bulge) structure at the other end was used for finalization of the sensor. The EIS measurement showed the DANP on the electrode surface could anchor the probe-DNA through strong affinity between DANP and C-bulge. The finalized sensor could detect the target DNA.

1. Introduction

Electrochemical Impedance Spectroscopy (EIS) has been used as a technique to detect biomolecules. EIS uses three electrodes, working, counter and reference electrodes [1,2]. The electrodes were put into the solution which contains the target biomolecules to be measured and the Impedance spectroscopy is measured scanning frequency typically from 1MHz to 0.1Hz. The solutions are added with electroactive mediator, such as ferri/ferrocyanide and their concentration is usually several mM. Since EIS is not sensitive to the solution ionic strength, bio-sensing can be carried out with near physiological conditions, which is different from Field effect transistor (FET). We applied this EIS measurement to detect DNA.

DNA sensing EIS electrode was usually modified by the probe DNA, anti-sense DNA against the target. After modification, every electrode becomes target specific and the finalized electrode can be used for one target DNA sequence. To evade such lack of flexibility, we proposed ligand-DNA sensor. The EIS electrode was modified by the ligand, *N,N'*-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine (DANP) Our previous work showed that a cytosine-bulge (C-bulge) structure has strong affinity against the DANP [3,4]. Based on our previous work, we prepared a probe-DNA which was composed of target specific anti-sense DNA sequence at one end and a cytosine-bulge (C-bulge) structure at the other end. It was expected that putting the probe DNA solution onto the DANP modified electrode and pure water rinsing would finalize the EIS electrode.

The concept of this ligand-sensor was studied using EIS. measurement technique. The obtained results showed that the ligand-DANP electrode can be finalized by probe DNA with C-bulge structure and EIS measurement can be applicable to DNA sensing.

2. Surface modification

The DANP modification was carried out with three steps. Firstly, MUA/MCH mix self-assembly monolayer was produced. The cleaned gold electrode was immersed in a mixture solution of 11-mercaptoundecanoic acid (MUA) and 6-mercapto-1-hexanol (MCH) with the ratio of 1 : 3 for 24 hours. Secondly the carboxyl groups of MUA were activated by EDC-NHS. Thirdly, the DANP molecules were covalently attached to the MUA molecule top. The remaining unreacted carboxylic group was inactivated by ethanolamine.

3. Results and discussion

Figure **2.** shows the EIS measurement principle. The impedance is measured using working electrode and counter electrode. The frequency range is typically 1MHz ~ 0.1Hz and AC voltage (Vac), 10mV. From electric circuit point of

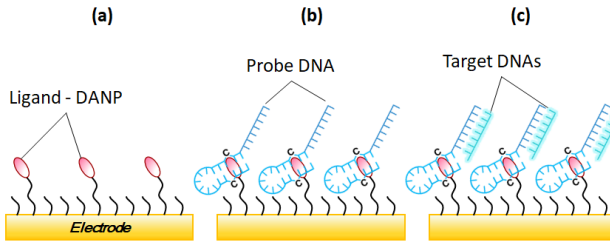

Figure1:Scheme. The concept of the flexible DNA-ligand sensor. The target specific DNA probes are anchored on the ligand modified surface using the specific binding between the DANP and cytosine bulge.

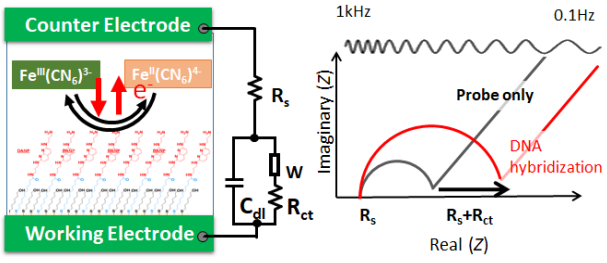

Figure2. Electrochemical Impedance Spectroscopy (EIS) measurement principle. The DANP immobilized working electrode could be represented by the Randle circuit

view, the working electrode surface could be represented by Randle circuit, active electrolyte resistance R_s in series with the parallel combination of the double-layer capacitance C_{dl} and charge transfer resistance R_{ct} accompanying by Warburg impedance. The Nyquist plot of the measured impedance would be a combination of a semicircle and a diagonal line with a slope of 45°. The diameter of the semicircle corresponds to the charge transfer resistance R_{ct}.The diagonal line at low frequencies, is the Warburg impedance. The reactants diffuse farther and the Warburg-impedance increases. When the probe-DNAs hybridize the target DNAs, the charge transfer is hindered and R_{ct} increases. The degree of R_{ct} increase should be in proportion to the degree of DNA hybridization, i.e. target DNA concentration.

To investigate the interaction between DANP fixed on an electrode and C-bulge structure, we carried out electrochemical impedance spectroscopy measurements (Gamry Interface 1000E potentiostat, Gamry Inc., USA). The commercial electrode was set as the 3-electrode system. A 3 mm diameter Au electrode modified with DANP was used as the working electrode, a 57 mm length 0.5 mm diameter platinum wire as the counter electrode, and an Ag/AgCl electrode was the reference electrode (RE) (ALS Co., Ltd. Japan).

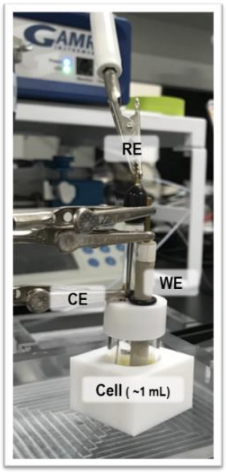

Figure 3. The set-up image of EIS measurement system.

Firstly, EIS measurement was carried out using nucleotide DNAs with and without C-bulge structure to investigate the DANP and C-bulge interaction. Buffer condition was10 mM Tris-HCl (pH 8.0), 50 mM KCl, and 1.5 mM $MgCl_2$ with 1 mM $[Fe(CN)6]^{3-}$and 1 mM $[Fe(CN)6]^{4-}$ as redox mediators. Frequency range was from 1MHz to 0.1Hz and Vac was 10mV. Figure 4(a) shows the EIS results before and after addition of 26 nucleotide DNA without C-Bulge. The R_{ct} increased roughly twice from 4kΩ to 8kΩ. On the other hand, when 28 nucleotide DNA with C-bulge structure was added, the R_{ct} increased over 6 times, reached aroung12 kΩ (Figure 4(b)) The result clearly shows that the DANP on the electrode can capture the DNA with C-bulge structure. The R_{ct} increase observed without C-bulge may come from the electrostatic attractive force between DANP and DNA. This non-specific binding DNA could be washed away.

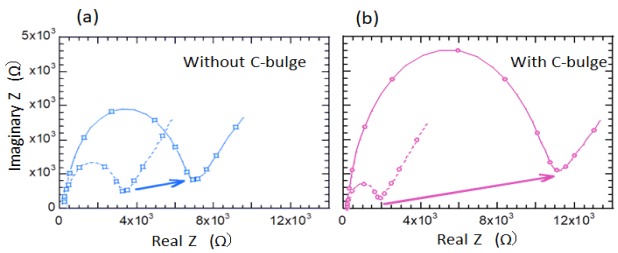

Figure 4. Nyquist plot of EIS using DANP modified electrode. The broken and real line represent before and after DNA addition, respectively. (a) DNA without and (b) with C-bugle structure.

Based on the results, probe-DNA fixation and target DNA detection by the DANP modified electrode was investigated. 20 nucleotide DNA which has an anti-sense sequence for the gene cytochrome *b* at 3'end was attached to 28 nucleotide DNA with the C-bulge (probe-DNA). Firstly, we measured EIS of the DANP surface. Secondly, we measured EIS after 1μM probe-DNA addition. Finally, we added 1μM target 20 nucleotide DNA and measured EIS. Figure 5 shows the result. The R_{ct} increased at each step and it was indicated that the DANP anchored the probe-DNA and the probe DNA hybridized the target DNA. The results demonstrated clearly the feasibility of the ligand-DNA sensor.

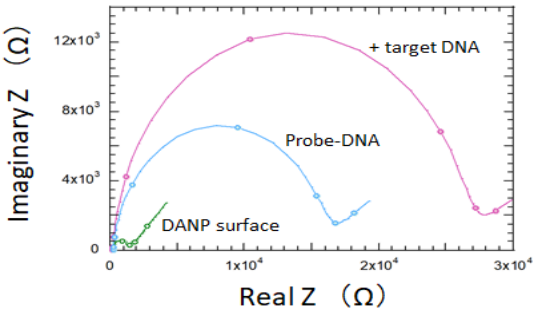

Figure 5. Nyquist plot of EIS of DAMP modified electrode, probe-DNA finalization, target DNA hybridization.

3. Conclusions

A ligand-base DNA sensor with EIS technique is proposed. EIS working electrode modified with DANP ligand successfully anchor the probe-DNA which is composed of target specific anti-sense DNA sequence at one end and a cytosine-bulge (C-bulge) structure at the other end. The finalized EIS electrode can hybridize the target DNA. It was also demonstrated the EIS sensitivity is high. However, the absolute R_{ct} is not so reproducible. The R_{ct} increase ratio could solve this issue.

Acknowledgements

This research is partially supported by the Center of Innovation Program from Japan Science and Technology Agency, JST.

References

[1] F. Lisdat, D. Schäfer, Anal Bioanal Chem **391** (2008) 1555.

[2] E. Souteyrand J.R.Martin C.Martelet, Sensors and Actuators B: Chemical, **20(1)** (1994) 63-69.

[3] F. Takei, M. Igarashi, M. Hagihara, Y. Oka, Y. Soya, K. Nakatani. Angew Chem Int Ed Engl **48 (42)**, (2009)7822-7824.

[4] F. Takei, H. Suda, M. Hagihara, J. Zhang, A. Kobori, K. Nakatani, **13 (16)** (2007) 4452-4457.