DNA 3D structure detection by Electrochemical Impedance Spectroscopy (EIS)

◦Huanwen Han[1], Kazuyuki Nobusawa[1], Fumie Takei[2], Ichiro Yamashita[1]

Grad. Sch. of Eng., Osaka Univ.[1], National Defense Medical College (NDMC)[2]

E-mail: huanwen@pmdp.arl.eng.osaka-u.ac.jp

Introduction: Easy, fast and trusty DNA sequencing and detection has been a central issue in clinical research, like pathogen's identification, drug resistance sensitivity. Many sensing principles have been studied, such as Field Effect Transistor (FET), Surface Plasmon Resonance (SPR), Quartz crystal Micro valance (QCM) and Electrochemical impedance spectroscopy (EIS). Among them, EIS has high sensitivity under a wide variety of solution conditions and is non-destructive and label-free measurement method[1]. EIS detects impedance of the modified electrode[2]. The interface is represented as an equivalent circuit, Randles equivalent circuit with Warburg. Randles circuit is a parallel capacitance and resistance, which represent charge bilayer capacitance (Cbl) and charges transfer resistance (Rct) respectively. Rct changesdepending on the molecular absorption[3] and accumulation on the electrode surface. In this study, we compare the performance of two DNA probes with EIS.

Experiment: We used a ligand sensor to detect target DNAs. The electrode surface was modified with ligands, 2, 7-diamino-1, 8-naphthyridine (DANP)[4] and blocked with 6-mercapto-1- hexanol (MCH). Two types of cytosine bulge involved detection probes were synthesized. Those had hairpin DNA (Hp) with cytosine bulge at the one end and, at the other ends, two types of 20 nucleotides (nt) antisense DNA for pUC18 multiple cloning sites (Hp-Pr) and Cytochrome *b* (HP-Cy). EIS measurements were carried out under10 mM Tris (pH8), 50 mM KCl and 1.5 mM MgCl2 named PCRi with the mediator - 1 mM $K_3[Fe(CN)_6]$ and 1 mM $K_4[Fe(CN)_6]$. The frequency range was 1M Hz to 0.1 Hz, and voltage amplitude was 5 mVms of EIS.

Results and Discussion: We fixed the solution pH and ionic strength to avoid unnecessary interference. After the detection probes HP-Pr and HP-Cy were anchored, EIS was measured. To analyses the EIS results, we introduced a new parameter, Rct increase ratio, Rir, wihch overcomes the discrepancy situation. Rir of HP-Pr was larger than HP-Cy, after target DNA addition, the Rir become nearly the same again. Compare the average Rir and SDS DNA gel, we suspect the probe ssDNA part -Pr, was extending in the solution and -Cy displayed a compact form. Therefore the Rir of HP-Pr was larger than HP-Cy. Since the target DNA hybridized with the probe, the dsDNA portion exhibits a stiff and extended rod-like shape, Rir became similar between -Pr and -Cy probes. There are few reports about that the DNA 3D structure difference change the Rct changes and our results showed EIS sensor could detect the DNA 2nd structure.

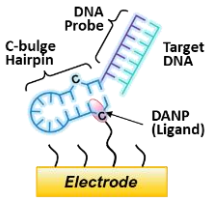

Fig 1. The concept of DNA-ligand sensor.

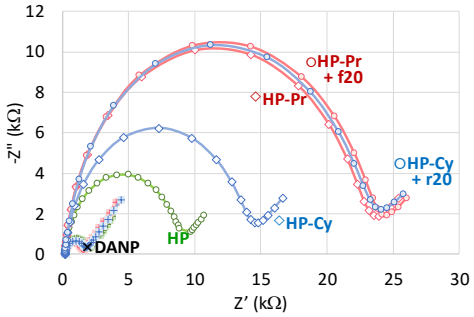

Fig 2. The Nyquest of HP-Cy, HP-Pr and after target DNA addition.

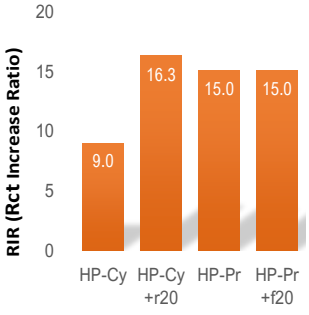

Fig 3. The Rir of HP-Cy and HP-Pr.

Reference

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