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Direct observation of the enzymatically-released pyrophosphates using phenylboronic acid group-immobilized gold electrode by FET

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Abstract

Pyrophosphate (PPi) is ubiquitous in the living cells and often produced by the enzymatic reaction *e.g.* DNA synthesis by DNA polymerase. We have developed the novel detection system for the PPi molecule released from the enzyme by using the phenylboronic acid (PBA)-coated electrode connected to the field effect transistor (FET), since the PBA group is revealed to react with PPi molecule spontaneously and produce the voltage change which can be detected by FET. To capture the released PPi from the enzyme more efficiently, we also employed the enzyme-electrode conjugating method by using specific peptide sequence, spontaneously bound to the gold substrates. Combination of the enzyme-electrode conjugating method with the PPi detection by PBA enables the effective detection of the PPi released from the enzymatic activities.

1. Introduction

Pyrophosphate (PPi) is often released from nucleotide triphosphates by the enzymatic reactions in living cells. Especially, the enzymes involved in the DNA transaction, like as DNA polymerase and DNA ligase, release PPi as a product of their reactions. DNA polymerases are utilized in polymerase chain reaction (PCR) technique and indispensable for DNA sequencing, and DNA ligases are often applied to ligase chain reaction (LCR) technique for finding single nucleotide polymorphisms (SNPs) on the gene (1,2). Therefore the detection of the released-PPi from the enzyme must be an efficient means for analyzing the genetic information.

Phenylboronic acid (PBA) is a boronic acid containing a phenyl substituent and two hydroxyl groups attached to boron, is commonly used in organic synthesis, especially utilized in several cross-coupling reactions (3). PBA is known to form a dynamic covalent bond with divalent hydroxyl compounds (4). The fast and stable bond formation between aromatic boronic acids and diols to form boronate esters can be applied to build molecular assemblies. In spite of the stability of boronate esters covalent B-O bonds, their formation is reversible under certain conditions or external chemical stimuli. Using this characteristic of PBA to be spontaneously bound to diols, especially to sugars (5), we utilized the self-assembled monolayer (SAM) of PBA for the sialic acid detection at cell membrane (6). Among the several compounds possessing PBA group, 4-mercaptophenylboronic acid is known to easily form a self-assembled monolayer on gold surface (7). Here we discovered that PBA on gold chip could also be used as a probe for detecting pyrophosphates that possesses several hydroxyl groups, and applied the chip to observe the enzyme-released pyrophosphates combined with FET devices.

We have been investigating electrostatic detection of biomolecular recognition events using a biologically coupled field effect transistor (FET) (8), and also constructed the DNA detection system using FET, where detecting if the immobilized ss-DNA molecules on the chip hybridized or not (9).

Here we report the direct measurement of enzyme-released pyrophosphates by FET combining the enzyme-electrode conjugating method with the PPi detection by PBA.

2. Results

Construction of the Self-Assembled Monolayer on the gate electrode of FET

The gold electrode, to be connected to the gate of FET, was rinsed with absolute ethanol prior to adsorption of the thiols, then treated with 1mM 4-Mercaptophenylboronic

acid in ethanol for 24 h, as described previously (7).

FET measurement of the PPi-included solution

The FET measurement was done using PPi-included reaction buffer (10mM Tris-HCl pH7.7) solutions at final concentrations of 1, 6, 16, 66 and 166 μM, using three different gate electrodes. The three electrodes showed that the voltage increment according to the addition of higher concentration of PPi solution (Fig.1), while the gold electrodes without PBA showed almost no signals (not shown).

Preparation of DNA polymerase with the peptide tag sequence including cysteine residues

Cysteine residue is known to be spontaneously bound to gold substrates, and has been utilized in the protein immobilization on the gold or platinum surfaces. We constructed the series of the mutant DNA polymerases with the peptide tag sequence including more than three cysteine residues, and apply these mutants to the gold nano particle (ϕ100nm), in order to restrict the enzymatic reaction area within the short distance from the surface of an electrode, for the effective measurement by FET.

Measurement of the enzymatic reaction by FET

Nano gold particle-conjugated DNA polymerase was prepared by mixing the DNA polymerase with 1/10 molar amount of the gold particles. 1μL of 1mM particles was pipetted onto the PBA coated surface of the gate electrode and the voltage shift was monitored. When the substrate for the DNA synthesis (dNTP) was added, the sensorgrams for the primed-DNA substrate comprising channels showed the significant voltage increment, while the control channels exhibited the lower increment (Fig. 2).

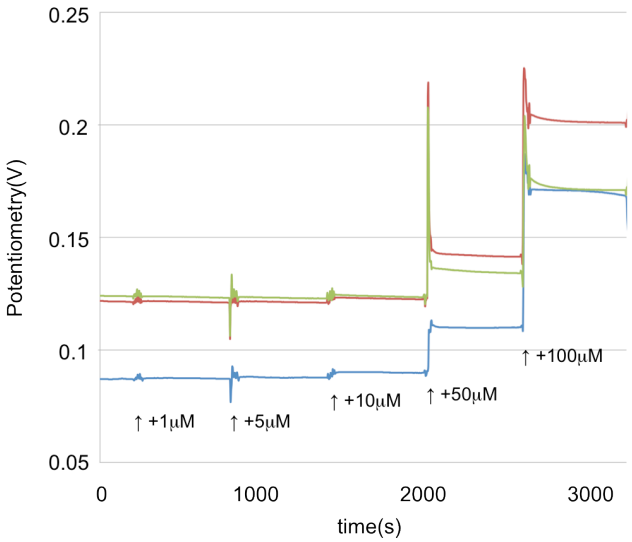

Fig. 1 Voltage shifts of the FET gate according to the increase of the PPi concentration.

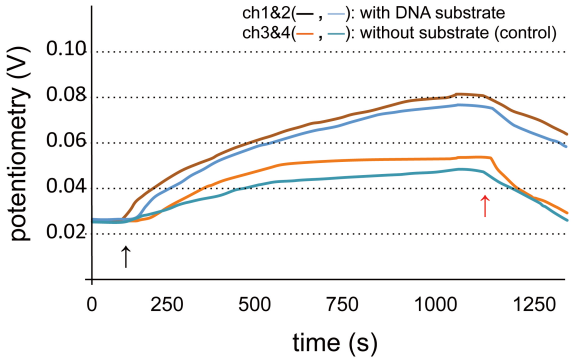

Fig. 2. FET measurement of the enzymatically released PPi with (ch1&2) or without (ch3&4) the DNA substrate. The reaction was induced by the addition of 10μM of dNTPs at 150s (↑), and washed by the reaction buffer at 1150s (↑).

3. Conclusion

The PBA-coated gold surface can be utilized in the detection of the PPi molecules often released from the DNA processing enzymes, such as DNA polymerase. The condensed enzymatic reaction on the surface of the electrode seemed to be also effective for FET measurement.

Acknowledgements

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