Dehydrogenase-based Enzyme Switches as Novel Biosensing Devices

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INTRODUCTION

Enzyme switch is a novel biosensing device based on the conductometric measurements of poly(aniline) (PANi) film covered with enzymes. We have already reported the oxidase-peroxidase based enzyme switches utilizing redox reaction between HRP and PANi.

The advantages of an enzyme switch are as follows: 1) Sensing chip itself has a memory function, 2) Response might be independent from the electrode size, 3) Miniaturization and integration are easy because of its simple structure. 4) Easy connection with actuators, since the resistance change is index.

In this study, the dehydrogenase-based enzyme switch was developed utilizing a redox reaction between NADH and PANi. As shown in Fig.1, NADH produced by the dehydrogenase reaction, reduces the insulative PANi (the oxidized form). Since the reduced form of PANi has electro-conductivity, the substrate concentration can be measured by the increase of drain current.

Dehydrogenase-based enzyme switch has several advantages over conventional enzyme switch: 1) Effect of sample stirring might be negligible since the reaction requires no oxygen, 2) OFF to ON type switch, 3) Wide application fields such as diagnostic tests and food analysis.

EXPERIMENTAL

Integrated miniature gold electrodes for enzyme switches were prepared on a poly(imide) film by a photolithography method. A PANi film was electro-chemically polymerized in the mixture of aniline and poly(vinylsulfate), over the two micro-patterned gold electrodes. The dehydrogenase enzymes were physically adsorbed into PANi film.

Measurements were done under the application of drain voltage (20mV) in 1.0M Tris-HCl buffer (pH7.1) containing 30mM NAD^+ , by monitoring the changes of drain current.

RESULTS AND DISCUSSION

First, we developed lactate, glucose and alcohol switches by using lactate dehydrogenase, glucose dehydrogenase and alcohol dehydrogenase, respectively. A calibration curve for ethanol was shown in Fig.2. The enzyme switches showed the responses depending on the substrate concentration, and electrochemical reset of the switch was also possible.

Since the electrochemical reset requires the three electrodes system and a potentiostat, the non-electro-chemical method was investigated. The peroxidase-H_2O_2 system was used for the oxidation of PANi. As shown in Fig.3, GDH-HRP co-immobilized enzyme switch could be reset by the addition of H_2O_2.

The measurements were also possible by only putting a drop of substrate solution directly onto the enzyme switch, and the obtained response was almost the same as a beaker test with sample stirring. This measurement method was also possible with the developed integrated 4-channel enzyme switch.

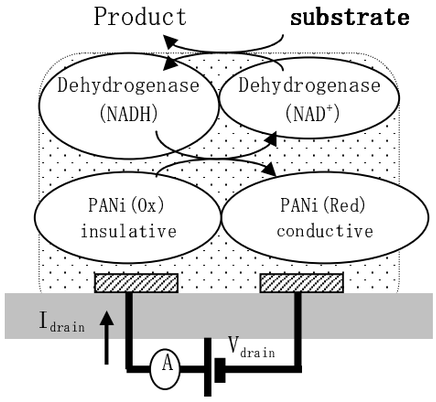

Fig.1 Dehydrogenase-based enzyme switch

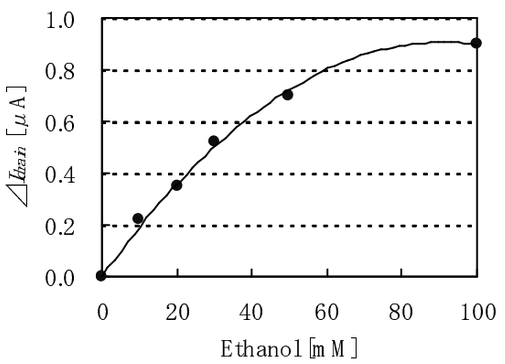

Fig.2 Calibration curve for ethanol

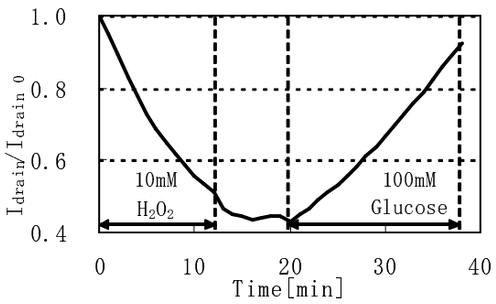

Fig.3 Reset by using HRP-H_2O_2 system