ELECTROCHEMICAL DETECTION OF DEOXYRIBONUCLEASE IN MICROLIQUID

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ABSTRACT

This paper presents a novel microdevice which detects deoxyribonuclease (DNase) electrochemically. The present device can be applied to diagnoses of diseases such as acute myocardial infarction and monitoring of DNase-free water used in genetic research. The device consists of a PDMS reservoir for storing a sample liquid and several microelectrodes including working electrodes where ferrocenyloligonucleotides (DNA strands carrying ferrocene molecules) are immobilized. DNase detection is achieved by the current reduction between before and after ferrocenyloligonucleotide cleavage by DNase. Experimental results showed a definite correlation between DNase concentration and the current reduction rate in the range of 10^{-4} to 10^{-1} unit/μl of DNase I.

KEYWORDS: DNase, DNA, Electrochemical measurement, Microelectrode

INTRODUCTION

DNase is an enzyme which nonspecifically cleaves phosphodiester linkages of DNA. In genetic researches such as DNA recombination, if the liquid solutions used include DNase, undesired DNA cleavage leads to failure of the experiments. Therefore, sensitive detection of DNase is essential for success in DNA researches. However, fluorometric analyses generally used for DNase detection have disadvantages such as the long time from preparation to detection and the relatively expensive cost of the fluorogenic oligonucleotide required. Therefore, development of a simple, rapid, inexpensive DNase detecting technique is strongly required to increase the operating efficiency and reduce cost in DNA researches.

In this paper, we present an electrochemically DNase-detecting device and show several experimental results using the fabricated device. This device allows more simple, rapid, efficient, and inexpensive DNase detection than fluorometric ones of other researches [1, 2].

THEORY OF DETECTION

Electrochemical DNase detection is given as follows; First, by sweeping voltage applied to a ferrocenyloligonucleotide-immobilized electrode (Fig. 1(a)), a current-voltage curve based on the redox reaction of ferrocene can be obtained (Fig. 1(b)). Next, when the electrode is treated with an aqueous solution containing DNase, the ferrocene-modified parts of the DNA strand will be removed from the electrode by a DNase-catalyzed reaction (Fig. 1(c)). This will cause a decrease in electrochemical current (Fig. 1(d)). The DNase detection can be achieved by this current change, and DNase concentration can be found from a current reduction rate. The device consists of a PDMS reservoir of 450μl in volume, an Ag/AgCl reference electrode (RE), an

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Au counter electrode (CE), and seven ferrocenyloligonucleotide-immobilized Au working electrodes (WE) which are patterned on a SiO_2-coated Si wafer (Fig. 2).

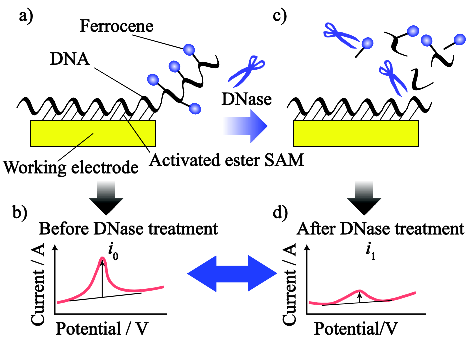

Figure 1. Principle of DNase detection.

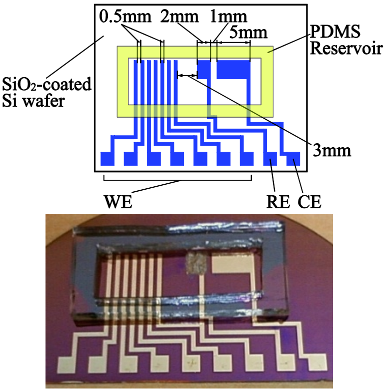

Figure 2. Fabricated DNase-detecting device.

EXPERIMENTAL PROCEDURE

Figure 3 shows experimental procedure. First, a SAM (self-assembled monolayer) having carboxyl groups was created on the working electrodes by treatment with dithiopropionic acid solution (Fig. 3(a)). Secondly, the SAM was activated by treatment with 1-Ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) (Fig.3 (b)). Then, the working electrodes were treated with solution of NaCl containing ferrocenyloligonucleotides for their immobilization (Fig. 3(c)). The peak current, i_0, was measured in a mixture of CH_3CO_2H/CH_3CO_2K buffer solution and KCl solution using square wave voltammetry (SWV) process (Fig. 3(d)). After the first measurement of i_0, the working electrodes were treated with a buffer solution including 10^{-4} to 10^{-1} unit/μl DNase I (Fig. 3(e)). Then, the second electrochemical measurement was carried out in the same manner as the first one, and it gave a peak current, i_1, that was decreased because of ferrocene removal (Fig. 3(f)).

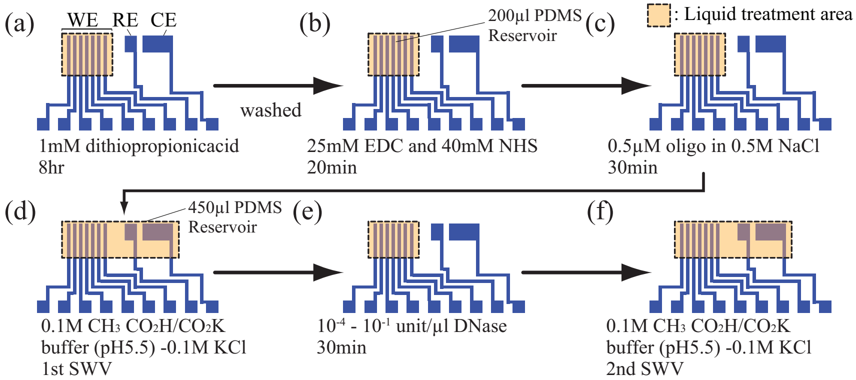

Figure 3. Experimental procedure.

EXPERIMENTAL RESULTES

The current-voltage curves as shown in Fig. 4 were obtained before and after DNase treatment. The peak currents based on the redox reaction of ferrocene molecules were found at the voltage of about 0.15 V. The peak current, i_0 or i_1, is indicated by a vertical arrow from a tangent line of the current-voltage curve to the peak current value. We defined a current reduction rate, Δi, as the following expressions: $\Delta i = (i_0 - i_1) / i_0$. In each DNase concentration, seven values of Δi were obtained from the seven working electrodes using one device. The average of the five values except the maximum and minimum were plotted against DNase concentration (Fig. 5). Because their definite correlation was obtained in the range of 10^{-4} to 10^{-1} unit/μl of DNase, the device can be used for quantitative analyses of DNase.

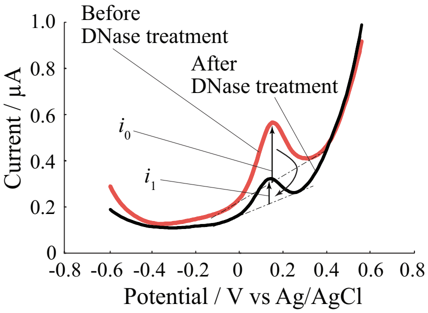

Figure 4. Reduction of the peak current from i_0 to i_1 (10^{-2} unit/μl of DNase).

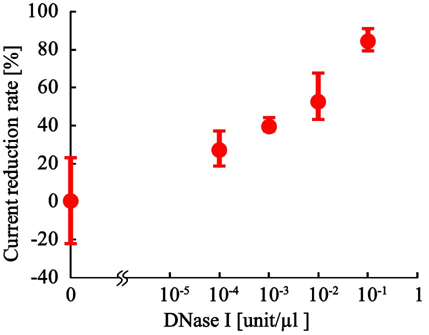

Figure 5. Relationship between DNase concentration and current reduction rate.

CONCLUSIONS

We designed, fabricated, and tested a microdevice for detecting DNase electrochemically with ferrocenyloligonucleotide-immobilized microelectrodes. When concentration of DNase I was 10^{-2} unit/μl, the current reduction rate of about 60 % was obtained after a treatment with DNase I. Moreover, a definite correlation between DNase concentration and the current reduction rate was obtained. This result can be used for quantitative analyses of DNase.

In the future, this device will be used as a simple DNase sensing kit prior to genetic research experiments, or will be mounted on ultrapure water production systems for genetic researches.

ACKNOWLEDGMENTS

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