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## A Highly-Sensitive Leakage Current Microsensor

by Using Denaturant:

### Detection of Target Protein by DPPC Liposome

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#### Abstract

We have developed a highly-sensitive leakage current microsensor by using the DPPC liposome entrapping  $K_4[Fe(CN)_6]$  solution to detect existence of biomolecules especially proteins and their dynamic conditions. In this work, the addition of guanidinium hydrochloride (GuHCl) as protein denaturant successfully obtain a prominent improvement in sensitivity by 129-fold of magnitude, although target protein, which is carbonic anhydrase from bovine (CAB), weakly interacts with liposome under the normal condition. Moreover, we can use it to evaluate the conformation state of CAB protein such as 'Native', 'Molten-Globule' and 'Unfold' states.

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Keywords: Leakage current microsensor; Electrochemical; Liposome; Biomolecule; Protein; Denaturant

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#### 1. Introduction

The cell membrane has gained attention as one of the most important constituents in biological systems. It is not only a physical boundary against the environment. It is a functional unit that senses environmental conditions and recognizes other molecules (nucleic acids, proteins, etc.) or other self-assemblies (viruses, cells, etc.). It has been reported that liposome, artificial cell membrane, interacts with partly denatured proteins under an adequate stress condition [1]. For example, it has been known that liposome assists in the refolding of proteins and induces the translocation of proteins.

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The liposomes entrapping a fluorescence probe or electrolyte can release them by an interaction with specific biomolecules such as protein through a membrane perturbation effect [1]. This characteristic of the liposome is advantageous for the design of the biomolecule sensor as a leakage current sensor on a basis of DC amperometry, whereas the protein is generally insensitive to an electrochemical detection. The released ions can contribute to the current generation, which relates directly to the interaction between liposome membranes and external proteins.

In this paper, compared to our previous work [2], we have newly developed a highly-sensitive leakage current microsensors by using the entrapping DPPC liposome and a protein denaturant. The addition of denaturant successfully resulted in the prominent improvement in sensitivity up to 129-fold of magnitude, although target protein weakly interacts with liposome under the normal condition.

## 2. Prepared Biochemical Solution

This time,  $K_4[Fe(CN)_6]$  solution was entrapped inside of liposome of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). Carbonic Anhydrase from Bovine (CAB, Mw=28.8 kDa) was used as target protein for the interaction. Guanidinium hydrochloride (GuHCl) was used as a protein denaturant. The CAB and GuHCl are selected because they were conventionally used and evaluated in the experiments with chemical laboratory instruments such as chromatography by one of coauthors [3], so the experimental results are compared between this work and the conventional ones.

We evaluate the new approach of the microsensors with the figure-of-merits of target sensitivity, target volume and entrapping liposome concentration, as a protein sensor.

## 3. Leakage Current Microsensor

### 3.1 Device fabrication

The fabrication process of leakage current sensor is shown in Fig. 1. A 280  $\mu\text{m}$  thickness Si wafer with 1  $\mu\text{m}$  thickness  $\text{SiO}_2$  layer is used as an initial substrate. At first, a micro-well that will hold a droplet of entrapping DPPC liposome steadily is formed by BHF etching and TMAH anisotropic wet etching. After that, the surface of the well are thermally oxidized to eliminate external leakage current between the sensor electrodes and to have a hydrophilic surface for keeping intact molecular structure of the liposome. In the last process, Pt/Ti film electrodes are formed by rf-sputtering and lift-off method. The chip size, the depth of the well and the widths of electrode are about 14 mm, 100  $\mu\text{m}$  and 500 to 2000  $\mu\text{m}$ , respectively.

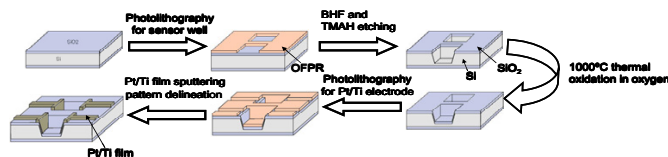


Fig. 1. Fabrication process flow of our leakage current microsensor.

### 3.2 Device structure with a droplet of liposome added with denatured protein

A droplet of the liposome suspension was immobilized in the sensor well shown in Fig. 2. Thereafter, the solution of CAB treated with GuHCl (0.5  $\mu\text{L}$ ) was manually supplied to the liposome droplet (0.5  $\mu\text{L}$ ) with a micropipette. Time course of the leakage current in the microsensor with 0.1 V bias between the electrodes was monitored before and after the dropping of CAB solution with a semiconductor parameter analyzer (Agilent 4156B) as shown in Fig.3.

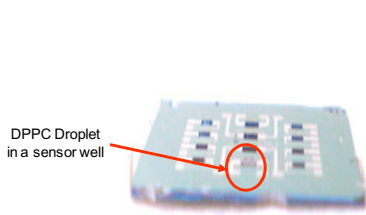


Fig. 2. A photomicrograph of bird's-eye view of the fabricated arrayed sensor with liposome droplet.

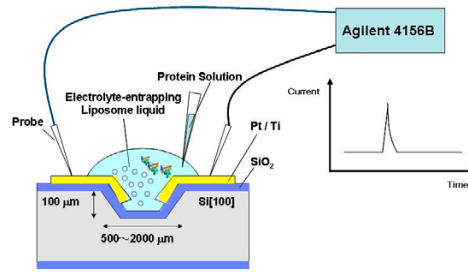


Fig. 3. A measurement setup of leakage current.

3.3 Consideration on droplet volume and liposome concentration.

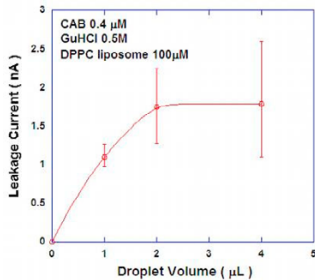


Fig. 4. Leakage current vs. droplet volume of liposome and protein solution.

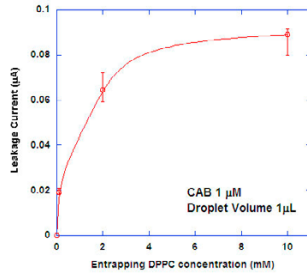


Fig. 5. Leakage current vs. DPPC concentration.

A consideration of the sensor system is done on the biochemical solution used in the leakage current microsensor such as droplet volume of liposome and protein and entrapping liposome concentration. By increasing droplet volume of biochemical solution, the leakage current increases non-linearly and have saturation point around 2 μL as shown in Fig. 4. After that, we increased the entrapping DPPC liposome concentration. In

Fig. 5 the leakage current increases non-linearly and have saturation point around 2 mM as a similar manner in Fig. 4. It is considered that the leakage current is sufficiently correlated with both the droplet volume and entrapping liposome concentration but has saturation points which limit the current. From these results, at this stage, we selected the droplet volume more than 1 μL and the entrapping DPPC liposome more than 2 mM to obtain the clear interaction peak of leakage current from nA to μA order in the sensor system.

4. Evaluation of Interaction between Liposome and Denatured Protein by Leakage Current Microsensor

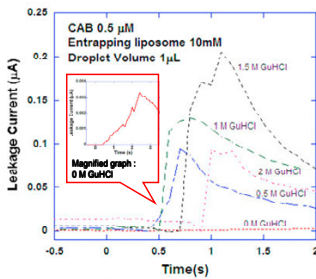


Fig. 6. Leakage current vs. time as a parameter of denaturant concentration.

In this experiment we used 10 mM entrapping DPPC liposome concentration and 1 μL of droplet volume. We obtained time-course of the leakage current as a parameter of GuHCl concentration in Fig. 6. The leakage current corresponds to the generated charge of Fe<sup>2+</sup> ions released by the denatured CAB-liposome interaction. The peak value of leakage current increased in a dependent manner of GuHCl concentration. When the solution including 0 M of GuHCl is used, the resulting leakage current (GuHCl 0 M) was not sufficiently large to detect the CAB molecule. Meanwhile, it was found that the addition of GuHCl (up to 2 M) made the prominent improvement of the leakage current possible (Fig. 7). The improvement of sensitivity by 129-fold was observed at 1.5 M of GuHCl (Fig. 8). This observation is considered to result from the interaction between liposomes and GuHCl-induced CAB at Molten-Globule (MG) state because the protein at MG state (around 1.5 M of GuHCl) can strongly interact with liposome membranes [1]. The above detection results are consistent with the previous report

with respect to the conformation of CAB using a conventional chromatography setup of laboratory instruments [3], as seen in Fig. 9.

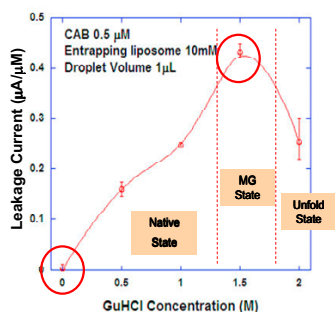


Fig. 7. Leakage current per CAB concentration vs. GuHCl concentration.

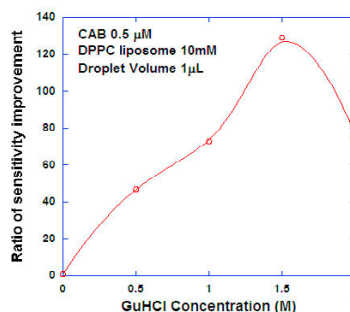


Fig. 8. Ratio of sensitivity improvement vs. GuHCl concentration.

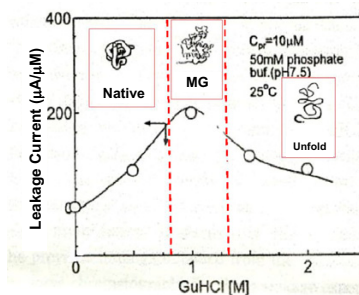


Fig. 9. Leakage current per protein concentration vs. GuHCl concentration results in the immobilized liposome chromatography method [1].

## 5. Conclusions

We have successfully fabricated and measured a specific biomolecule microsensor for a measurement of a leakage current in liposome solution. A clear leakage current in  $0.1 \mu\text{A}$  order was able to be detected from the denatured CAB-liposome interaction. The sensitivity of the leakage current microsensor could simply be improved by more than 100-fold of magnitude, due to the enhancement of protein-liposome interaction. Moreover, we evaluated the conformation state of denatured CAB such as ‘Native’, ‘Molten-Globule’ and ‘Unfolded’ state from the leakage current. The addition of denaturant is appropriate for this purpose.

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