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Bio-Thermochemical Sensor with Liposome Immobilized Intact for Protein Detection Using Their Interaction and Membrane Dynamics

Minoru Noda[1,2], Takeshi Asai[1], Kaoru Yamashita[1,2],
Toshinori Shimanouchi[2], Masanori Okuyama[2] and Ryouichi Kuboi[2]

[1]Grad. Sch. of Sci. & Tech., Kyoto Institute of Technology
Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
Phone: +81-75-724-7443 E-mail: noda@kit.ac.jp
[2]Grad. Sch. of Eng. Sci., Osaka University
1-3 Machikaneyama-cho, Toyonaka, Osaka 560-8531, Japan

1. Introduction

The application of cell membranes and model membranes onto sensing devices is essential for studies involving the detection, evaluation, and analysis of interactions between the cell membrane and biological materials. To utilize the functions of the phospholipid membrane that composes the liposome, it is important to maintain the fluidity or mobility of the membrane, which is deeply related to the expression of its functions, after immobilization onto sensing materials [1].

From viewpoints of realizing liposome biosensor that uses interaction between the membrane and biomolecules, it is important to make intact liposome on the sensor surface for realizing its intrinsic dynamics. We believe that this work is a first trial to realize a biomolecular device that uses intact liposome on a solid surface as a bio-detecting component.

Interactions between liposome and various proteins have been investigated from viewpoints of biochemistry using several chemical analyses as in [1]. Recently we have made a microbolometer with 1,2-dipalmitoyl- *sn*-glycero-3-phosphocholline (DPPC) liposome immobilized intact on a sensor solid surface and observed several phase transitions of DPPC itself [2]. Moreover, the interactions between them relating to hydration water and cholesterol have been evaluated using Dielectric Dispersion Analysis for frequency range of 0.1-20 GHz [3,4]. Based on the previous works, this time, we try to evaluate bio-thermochemical interactions especially between DPPC liposome and lysozyme with the microbolometer developed more in having a small droplet target solution and basic measurement conditions.

2. Pt Microbolometer with Immobilized DPPC Liposome

A surface-micromachined Pt microbolometer with immobilized DPPC liposome was designed and fabricated as shown in Fig. 1. Figure 1 shows a thermal isolation structure using surface bulk micromachining technology. The membrane comprises three-layer of $SiO_2/Si_3N_4/SiO_2$ and an upper electrode of Pt/Ti.

The fabrication process flow of the Pt bolometer is as follows. A thermal SiO_2 is grown up on a Si(100) substrate, and the silicon nitride (Si_3N_4) and another SiO_2 are deposited by Low Pressure Chemical Vapor Deposition (LPCVD) method. The Pt/Ti thin film is deposited by RF magnetron sputtering and the Pt film resistor pattern was formed by lift-off method. It is necessary to protect the surface membrane structure from Si etchant, which is Tetramethylammonium hydroxide (TMAH) solution this time, for surface cavity formation. Therefore, the structure was covered by a thick photoresist during the Si etching. When the thermal isolation film is the three-layer structure of $SiO_2/Si_3N_4/SiO_2$, the film stress originated from the difference in thermal expansion coefficient between each films cancels and becomes small, leading to good contact and little deformation between the $SiO_2/Si_3N_4/SiO_2$ membrane and upper Pt film.

3. Results and Discussions

Figure 1 also indicates that the Pt film resistance in the microbolometer becomes changed due to interaction between liposome and lysozyme on the film, where several thermochemical reactions will occur through liposome membrane dynamics [1]. Figure 2 shows a photomicrograph of a fixed droplet of DPPC liposome (about 450 μm in diameter) on a Pt microbolometer. We consider that little work has been done so far on a single minute droplet of target solution for biochemical sensing treatment. Interactions have been evaluated in thermochemical behavior of DPPC liposome solution (30 mM) and lysozyme (30 μM) in a small amount of droplet (about 65 nl). A droplet of the liposome was fixed on a detector part of Pt microbolometer composed in a

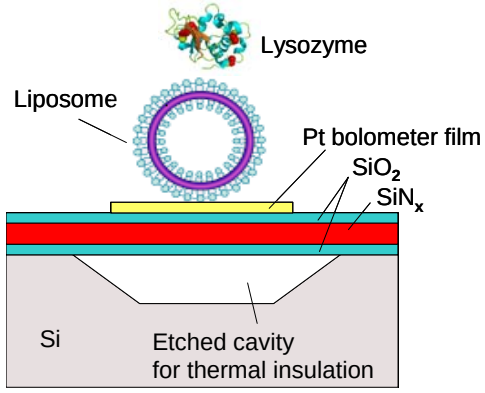

Fig. 1 An illustration of cross-sectional view of Pt film microbolometer with interaction of liposome and lysozyme.

Wheatstone bridge circuit [2], as in Fig. 3. Figures 4(a) and (b) show temperature dependence of output voltage difference in the bridge with the lysozyme droplet and the DPPC droplet added with the lysozyme, respectively. It is reported mainly from Differential Scanning Calorimetry (DSC) results that 1) a thermal phase transition temperature of lysozyme is around 25 deg, 2) endothermic reaction occur from 40 to 60 deg, and 3) lysozyme becomes heat-denatured from about 70 deg, respectively [5,6]. The peaks in Fig. 4(a) seem to be all corresponded to the temperatures above. We observe in Fig. 4(b) that unknown large peaks exist clearly between 43 and 46 deg, while those corresponding nearly for both DPPC and lysozyme are seen. The unknown is estimated to be from some endothermic reactions originated from the interaction between the DPPC and lysozyme. The other kind of evidence of the interaction has been also obtained by Dielectric Dispersion Analysis [3,4]. Finally, it is found that low concentration of lysozyme (30μM) is detected through the interaction with the DPPC liposome.

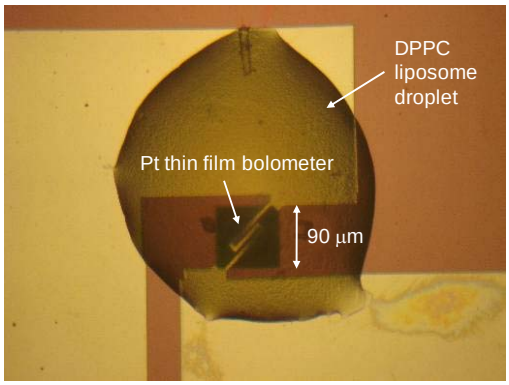

Fig. 2 A DPPC liposome droplet fixed on a Pt thin film bolometer.

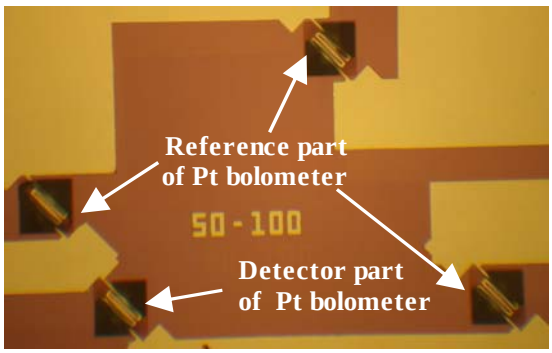

Fig. 3 A fabricated Pt microbolometer Wheatstone bridge circuit.

3. Conclusions

We have developed a new scheme of bio-thermochemical microbolometer with a small amount of droplet solution consisting from liposome and biochemical target molecules. And newly detected bio-thermochemical reaction of DPPC and protein of lysozyme (enzyme) in a droplet on a Pt thin film microbolometer, where they are dissolved with concentration of 30 mM and 30 μM, respectively, a droplet with volume of about 65 nl is immobilized on the surface of Pt film bolometer. This paper reports our first experimental results showing interaction between the DPPC liposome and lysozyme by the new scheme of bio-thermochemical sensor.

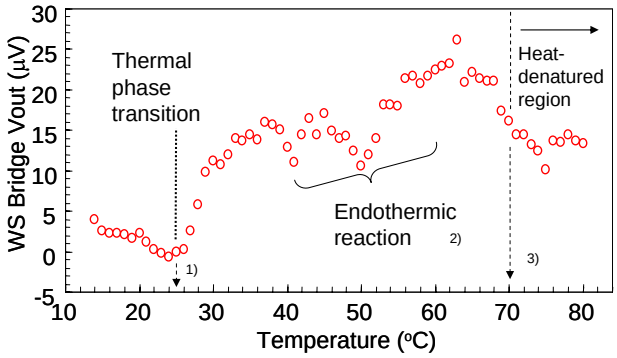

Fig. 4(a) Output voltage difference vs. temperature in the bridge between with and without Lysozyme droplet.

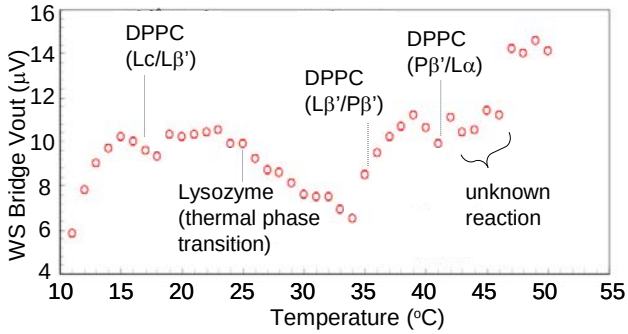

Fig. 4(b) Output voltage difference vs. temperature in the bridge between with and without the DPPC droplet added with Lysozyme.

Acknowledgements

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