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# DESIGN AND DEVELOPMENT OF MEMBRANE CHIP SYSTEM FOR STRESS SENSOR

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## ABSTRACT

Protein-lipid membrane interaction is related to various biological phenomena including the amyloidosis caused by the conformational abnormality of certain proteins. In this paper, we presented the novel on-chip technology for the study on protein-lipid membrane interaction using liposomes which are a model cell membrane. First, nine kinds of liposomes entrapping the fluorescence marker (calcein) as a fluorescence marker were immobilized on the indium tin oxide (ITO) to make a membrane chip system. For the stability of liposome on ITO electrode, we could confirm by the experiment that the solubilization by Triton X-100 led to the calcein release. The four proteins were injected to each liposome arrayed chip to observe the calcein leakage under fluorescence microscopy. We successfully observed the protein-dependent calcein leakage from the liposome immobilized. By using the principle component analysis (PCA), we distinguished the amyloidgenic protein from others. This analysis is, to our knowledge, the first attempts to evaluate the individuals of lipid membrane by the membrane-immobilized chip.

## KEYWORDS

Membrane Stress Biotechnology, Membrane Chip, Liposome, Amyloidgenic Protein

## INTRODUCTION

Liposome-immobilized sensors, which have high sensitivity even at very low concentration of the analytes and can detect with real time, have recently been designed and developed (Morita et al., 2006, Anderluh et al., 2005, Jung et al., 2005). In the above sensor systems, a quartz crystal microbalance (QCM) (Morita et al., 2006), a surface plasmon resonance (SPR) (Anderluh et al., 2005) and a square-wave voltammograms (SWV) (Jung et al., 2005) have previously been utilized as a detection principle. Of course, in these micro-size sensor tools, the immobilized liposomes have to maintain the membrane fluidity and other physical property suitable. The use of immobilized liposome as a sensor element provides us any opportunity to acquire more useful information related to the biological phenomena, especially, relating to the biomembrane activities. However, in the above systems, it is difficult to immobilize different liposomes on a same chip. Micro-arrayed systems, which widely have been used for the analysis of interaction between DNAs or proteins (i.e. DNA chip or protein chip), can also be attractive to acquire the systematic and hierarchical data set. Therefore, in order to analyze the universal interaction between liposomes and proteins, the liposome-immobilized and micro-arrayed system could be an answer to overcome the difficulties in previous reports.

In our previous work, the dye release from liposome inside induced by the protein was shown to be an index for the strength of hydrophobic protein-lipid interaction (Kuboi et al., 2004). To develop the high-throughput analyzing system based on liposome, we supposed that the

immobilization of liposomes entrapping marker molecules were helpful for the direct observation with a microscopy and the detection with some electrochemical or spectroscopic methods.

In this study, we designed and developed a novel sensor chip, "membrane chip" with immobilizing liposomes, based on our previous findings on the protein-liposome interaction under stress condition. The data obtained by the membrane chip were analyzed by the principle component analysis (PCA) for the identification of both type of proteins.

## MATERIALS AND METHODS

### Materials

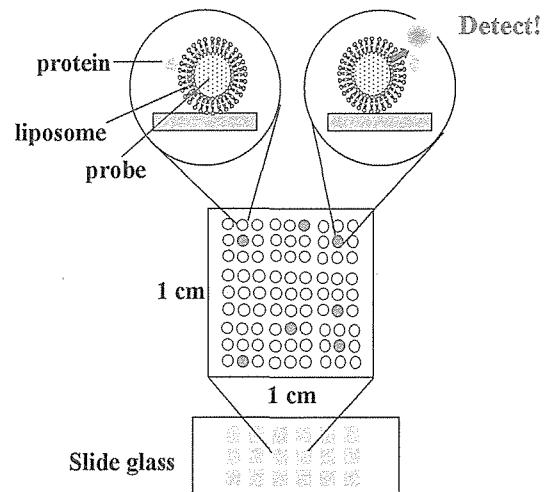
Dimyristoyl phosphatidylcholine (DMPC), dioleoyl phosphatidylcholine (DOPC), palmitoyl oleoyl phosphatidylcholine (POPC), oxidized stearoyl-arachidonyl-phosphatidylcholine (SAPC) and dipalmitoyl phosphatidylethanolamine (DPPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Calcein was obtained from Dojindo Co. Ltd. (Kumamoto, Japan). Nonionic detergent, Triton X-100 was purchased from Wako Pure Chemical Industries (Osaka, Japan). All the other chemicals were of analytical grade.

### Liposome preparation

Phospholipids (DMPC or DOPC) were mixed with GM or Oxidized SAPC in chloroform and the chloroformic solution of the lipid mixture was dried in a round-bottom flask by rotary evaporation. Oxidized SAPC was obtained by oxidizing SAPC in the presence of Hydroperoxide. The lipid film was kept under vacuum for at least 3 hr and the hydrated by 30 mM calcein solution to form multilamellar liposomes (MLVs). For the preparation of large unilamellar liposomes by extrusion (LUVs), the MLV suspension was frozen in dry ice/ethanol (-80 °C) for at least five cycles, and passed more than 11 times through two stacked polycarbonate filters with 100-nm pores (Nucleopore, Costar, Cambridge, MA) at room temperature by using an extrusion device (Liposofast; Avestin Inc.).

### Liposome immobilization

The outline of the design of membrane chip is shown in **Figure 1**. To fabricate the immobilized-liposome electrode, a self-assembled monolayer (SAM) using 16-mercaptophexadecanoic acid was formed on an ITO electrode by immersing in the acid solution of ethanol based on the previous method (Jung et al., 2003). To activate the SAM membrane, the above electrode was immersed in a solution of dioxane and distilled water (90/10, v/v) solution contained 17 mM NHS plus 17 mM WSC for 3-4 hr. The aliquot contained liposomes entrapping calcein was added by microinjection. And then the LUVs doped 1 mol% of DPPE were immobilized on the SAM layer by the formed on the SAM layer by the amino conjugate method. After 12 hr, the liposome-immobilized ITO electrode was rinsed with 50 mM Tris-HCl (pH 7.5).



**Figure 1 Schematic Illustration of membrane chip.**

### Calcein release experiment using LUVs

Calcein entrapped liposomes were also used to measure the extent of interaction between A $\beta$ (1-40) peptide and the membrane. Released calcein amounts were measured upon the externally addition of 5-15  $\mu$ M of A $\beta$ (1-40) peptide to the calcein entrapped liposome (0.1 mM) for 10 minutes.

## RESULTS AND DISCUSSION

### Preliminary study on protein-lipid membrane interaction by calcein release

For the development of such a membrane chip, partially oxidized liposomes were first used to study membrane-peptide interactions. Previous studies have been made on the interaction between amyloid- $\beta$  (1-40) and partially oxidized liposomes. For this case, the liposomes were composed of dimyristoyl phosphatidylcholine (DMPC) and oxidized stearoyl-arachidonyl-phosphatidylcholine (SAPC) lipids.

DMPC liposomes showed no significant change in the calcein release with the addition of A $\beta$ (1-40) while the liposomes containing 30% oxidized SAPC showed a considerable increase in the fluorescence intensity in the presence of A $\beta$ (1-40) at the concentration of more than 5  $\mu$ M (Figure 2). These results imply that the A $\beta$ (1-40) could detect the oxidized lipid within lipid membrane.

*Membrane chip tells the not only the property of protein but also that of the liposome.*

Based on these results, the micro-arrayed membrane chip was prepared. Liposomes were immobilized onto the surface of Indium-tin-oxide (ITO) electrodes through the covalent bonding between liposomes and thiol surface. The membrane chip contained nine kinds of liposomes, including DMPC and DMPC/SAPC oxidized 30% liposomes, that entrapped calcein inside. Calcein release was monitored by using the fluorescence microscope 60 minutes after the addition of different proteins, such as Carbonic anhydrase bovine (CAB), Lysozyme, Bovine serum albumin (BSA) and A $\beta$ (1-40), in order to compare the amount of released calcein. The fluorescence for each slot in the micro-arrayed membrane chip was evaluated by using the densitometric analysis as shown in Figure 3. Among the proteins, the addition of A $\beta$ (1-40) showed the highest release of calcein especially in the case of oxidized membrane slot. This results were consistent with the previous reports (Kakio et al., 2002). The above results show that the strong protein-membrane interaction led to large membrane destabilization effect. In other words, we can search which kinds of lipid composition is sensitive to amyloidogenic proteins from this measurement.

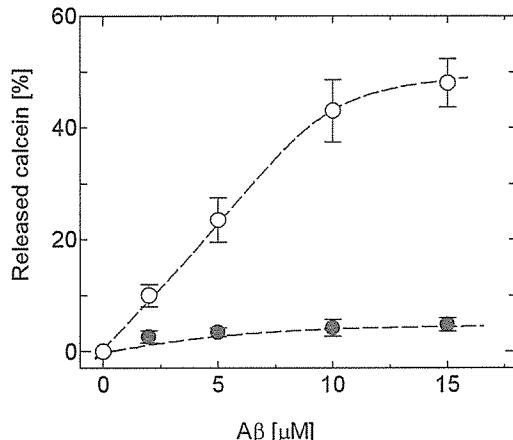


Figure 2 The interaction between amyloid  $\beta$ -peptide and oxidized liposome, evaluated by the calcein release from liposome suspension. Open symbol: DMPC/SAPC oxidized (30 mol%), Closed symbol: DMPC. Lipid concentration was 0.1 mM.

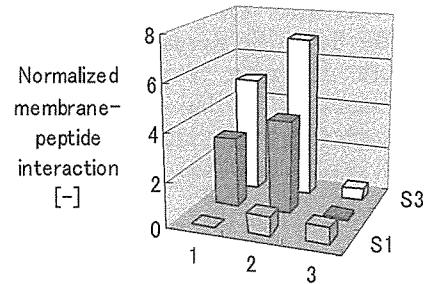


Figure 3 Profile of calcein released induced by proteins.  
Column; 1:DMPC,2:DOPC,3:POPC,  
S1:pure, S2: ganglioside modified,  
S3:SAPC oxidized (30 mol%) modified.

Based on these analysis, we tried to distinguish the A $\beta$ (1-40), from the other proteins using the principle component analysis, which is the proper mathematical methodology for displaying the distribution of each data based on the certain characteristics. In this study, the plot for A $\beta$ (1-40) was clearly separated from the other proteins (Figure 4), indicating the membrane chip system could detect the proteins with potentially conformational abnormality.

## CONCLUSIONS

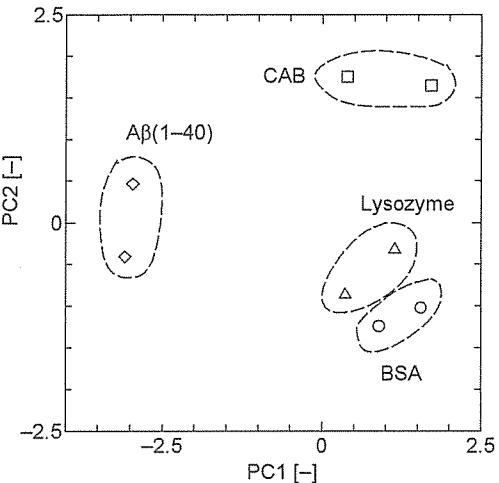
We developed the novel membrane chip system with the immobilized liposomes for the detection of the stress-induced structural change of proteins. This technique is based on the Membrane, and can be positioning to "Membrane-Based Analytical Method" as a frame of Membrane Stress Biotechnology since it permits us to obtain the possible quantitative information of not only proteins but also the liposomes interacting with the proteins having the conformational abnormality.

## ACKNOWLEDGEMENT

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**Figure 4 Examples of the principle component analysis for the detection of protein with conformational abnormality.**