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Biosensing of Molecular Behavior of Liposome and Target Protein, and Their Interaction by Dielectric Dispersion Analysis for 100-1000 MHz Range

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Abstract

We have examined biochemical interaction between liposome and target proteins by Dielectric Dispersion Analysis (DDA), especially for frequency range of 100-1000 MHz to consider molecular dynamics of the coexistence of the liposome membrane and target protein. Finally, we newly observed several new dielectric relaxations by the interaction in the low frequency range, thereafter we newly found and considered that some interaction manners between the each protein against liposome would exist and be different at least qualitatively for the two proteins because the manner of change in relaxation was different between each other.

1. Introduction

From the past, many of new biosensor and biosensing system to detect biomolecules such as protein have been actively developed and that biochemical phenomena have been significantly explored. And recently, the technique detecting these biomolecules has been greatly improved to sense slightly small amount of them and is applied for a medical aspect such as early detection of various disease [1].

In our previous work, we have developed some new biosensors and biosensing methods using liposome as biosensing molecule which has characteristics to interact with target biomolecules (e.g., proteins), and have investigated the one of interaction by Dielectric Dispersion Analysis (DDA) [2,3]. Since many DDA works based on impedance method was difficult to measure permittivity in the frequency range higher than 100 MHz [4], the dielectric dispersion properties in that range hardly evaluated for clarifying the phenomena. To solve this situation,
we have used and developed S-parameter method, which realizes the measurement in higher range especially over 1 GHz. In the past, using this method, we newly observed some dielectric relaxations derived from hydration water on the liposome surface in the range of more than 1 GHz and regarded them as an important signal to distinguish whether the liposome is interacting with the target protein or not [3]. Figure 1 shows an example of dielectric dispersion spectra of DPPC liposome suspension. Two dielectric relaxations were observed over 1 GHz. We have not focused so far, however, on the dielectric property especially in the range of 100-1000 MHz. It is expected, in that range, that both the motions of lipid molecules (translocation, flip-flop, and/or rotation etc.) and the hydration water molecules should exist and have influence on each other.

In this work, the range of 100-1000 MHz is featured to consider the coexistence of the molecular motions of lipid and bound water. We investigated this frequency range by DDA and evaluated the interaction between liposome membrane and target protein (lysozyme and carbonic anhydrase from bovine), and from this result, we considered their molecular dynamics.

![Fig1. A dielectric dispersion spectra of DPPC liposome suspension (30 mM).](image)

### 2. Dielectric Dispersion Analysis (DDA)

Dielectric measurement has already been used for nondestructive analysis of the structures of microbial cells and on-line monitoring of cultivation by analyzing the dielectric dispersion observed over 1-5 MHz. Dielectric measurement is applicable to the analysis of lipid bilayer membranes and liposomes. Generally, three main dispersions have been observed from the dielectric spectra of liposomes, and these three dispersions have been attributed to the rotational diffusion of water molecules (W region, 6 GHz<f<80 GHz), to the zwitterionic head groups of lipids (Lr region, 20 MHz<f<300 MHz), and to the limited translational diffusion of ionic lipid molecules and their counterions (Lt region, f<40 MHz)[4].

In this study, the dielectric dispersion spectra of liposomes were especially measured in the frequency range of 100-1000 MHz with an open-ended coaxial probe method. After confirming dielectric dispersion spectra of DPPC liposome suspension, either lysozyme or CAB protein was added as a target biomolecule to observe the interaction between the liposome and proteins.

### 3. Results and Discussions

#### 3.1. DDA of target protein

First, we observed the dielectric dispersion of target proteins (lysozyme and CAB) solutions to investigate the molecular motions of them. We prepared several solutions of lysozyme and CAB which were varied in the concentration and measured the relative permittivity of them in the frequency range of 100-1000 MHz. Figures 2 and 3 show dielectric dispersion spectra of both target protein solutions of lysozyme and CAB, respectively, as a
parameter of protein concentration, including the solvent of pure water. For the case of lysozyme, from Fig. 2, a dielectric relaxation occurs at frequency lower than 150-200 MHz, compared to the case of pure water, which shows simply a bulk water resonance known as at almost 20-25 GHz. In the previous DDA works of the protein solution with high lysozyme concentration (1-9 mM), a relatively large dielectric relaxation (almost around 5 to 10) was observed in the wide frequency range (about 1-1000 MHz)[5,6]. In our work, however, the change in relative permittivities are at most around 0.03 to 0.1 relatively in the narrow frequency range. Therefore, we think that we detect these small values by our open-ended coaxial probe and improved statistical technique in DDA, compared to those previously reported. For the case of CAB, although little has been reported on DDA of CAB protein, from Fig. 3, two types of dielectric relaxations occur for frequency at 200-500 MHz and that lower than 200 MHz, respectively. It is clear from Figs. 2 and 3 that their tendencies of dielectric dispersion are different from each other, depending on properties of the nature of protein.

3.2. DDA of interaction between DPPC liposome and target protein

To detect the interaction between these proteins and DPPC liposome and evaluate their molecular dynamics, we also investigated the dielectric dispersion of the solution in which one of these protein coexist with DPPC liposome. We added either lysozyme or CAB to DPPC liposome suspension (20 mM), and measured the dielectric dispersion spectra of them (frequency range is the same as in 3.1). Figures 4 and 5 show the spectra of DPPC liposome added with the each target protein solution as a parameter of protein concentration, respectively. As for the interaction between the liposome and lysozyme, by comparing Figs. 2 and 4, some plural dielectric relaxations newly occur at frequency more than 150 MHz and the change in the relative permittivity becomes decreased with increase in lysozyme concentration. These seem to be related to the behavior of liposome lipid membrane. Furthermore, as for the interaction between the liposome and CAB, by comparing Figs. 3 and 5, the spectra changed larger than the case of lysozyme addition (Figs. 2 and 4), especially at frequency between 150 and 400 MHz, where note that the vertical scale is different between Figs. 4 and 5.

We previously investigated the dielectric dispersion spectra for 1-5 GHz and observed two dielectric relaxations in the range. And it was found that the width of relaxation was decreased with increase in target protein concentration [3]. It was suggested that the relaxations found in 1-5 GHz is possibly derived from hydration water on the liposome surface and it was possible that the hydration water molecules on the liposome surface were excluded by the interaction with protein.

In this work, for the low frequency range less than 1 GHz (1000 MHz), we newly found and considered that some interaction manners between the each protein against liposome would exist and different at least qualitatively for the two proteins because the manner of change in relaxation was different between each other. At the present
stage, it is estimated as an origin that hydrophobic interaction of protein which is different according to its molecular structure is also play an important role for the detection of the target biomolecule [7,8].

4. Conclusions

It was found by our specific DDA especially for 100-1000 MHz range that the new type of interaction between liposome and target protein exists and its manners depend on a kind of protein. We newly observed several new dielectric relaxations by the interaction in the low frequency range, thereafter we newly found and considered that some interaction manners between the each protein against liposome would exist and different at least qualitatively for the two proteins because the manner of change in relaxation was different between each other. We also estimate that it should be related to their behaviors of hydrophobic interaction that are different due to their molecular bonding structures. On the other hand, we have detected successfully much smaller dielectric relaxation even in narrower frequency range, thus also able to detect the change with smaller concentration of protein than the previous works.

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