Membrane-Bound Conformations of Magainin 2 Depending on the Inherent Characteristics of Membrane Revealed by Synchrotron-Radiation Circular-Dichroism Spectroscopy

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Magainin 2 (MG2) is an antimicrobial peptide, and its activity occurs due to the interaction with membranes followed by the formation of transmembrane pores. The antimicrobial activity closely relates to the membrane-bound conformation of MG2, which largely depends on the constitutions of membrane or lipid molecules. Recently, it was found that the characteristics such as the spontaneous curvature of lipid molecule and the fluidity of membrane contributed to the membrane interaction of proteins [1, 2]. Hence, to clarify the contribution of these membrane characteristics to the MG2-membrane interaction, the membrane-bound conformations of MG2 in the presence of four types of lipid membranes (DLPC: dilauroyl phosphatidylcholine, DMPC: dimyristoyl phosphatidylcholine, DPPC: dipalmitoyl phosphatidylcholine, or DSPC: distearoyl phosphatidylcholine) were analyzed by a synchrotron-radiation circular-dichroism (SRCD) spectroscopy at various peptide-lipid concentration ratios and temperatures.

The results showed that MG2 formed a random coil structure in native state (without membrane) but changed to a helical structure in the presence of DLPC and DMPC lipid membranes at 25°C, showing that MG2 interacted with the both membranes (**FIGURE1**). On the other hand, MG2 retained its random coil structure in the presence of DPPC and DSPC lipid membranes, implying no membrane interaction (**FIGURE2**). Since the phase transition temperature (T_m) of these lipid molecules are -2°C for DLPC, 24°C for DMPC, 41°C for DPPC, and 55°C for DSPC, it was suggested that MG2 could interact with the membrane of liquid phase. To enhance the fluidity of DPPC and DSPC lipid membranes or to see the M2 conformation at their liquid phases, the temperature increased above T_m of the lipid molecules and decreased

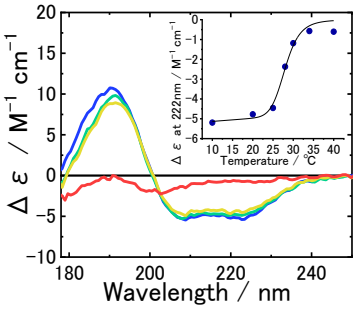

FIGURE1 SRCD spectra of MG2 in the presence of DMPC lipid membranes at 10°C (blue), 20°C (green), 25°C (yellow), 40°C (red).

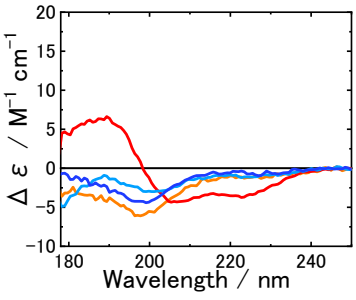

FIGURE2 SRCD spectra of MG2 in the presence of DPPC at 25°C (orange) and after annealing (red), and in the presence of DSPC (light blue) at 25°C and after annealing (blue).

until 25°C. After the annealing, MG2 formed helix structure in the DPPC lipid membrane but retained the random coil structure in the DSPC lipid membrane (**FIGURE2**). The DPPC and DSPC lipid membranes have the positive and negative spontaneous curvatures, respectively. Hence, these results suggest that in the membrane with the positive spontaneous curvature, MG2 could adsorb onto the membrane surface when its fluidity was enhanced, and penetrate when the temperature decreased. On the other hand, in the membrane with the negative spontaneous curvature, MG2 could not access the membrane surface even in the high temperature and retain its random coil structure in the low temperature. These differences mean that the lipid characteristics such as the spontaneous curvature and the fluidity would affect the membrane-bound conformation of MG2.

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