Orientation Analysis of Antimicrobial Peptide Magainin 2 Bound to Phospholipid Membrane by Synchrotron-Radiation Linear Dichroism Spectroscopy

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Antimicrobial peptide (AMP) interacts with and induces the damages to the cell membrane of antimicrobial-resistant microorganisms via the complicated mechanisms that remain to be further explored. Recently, we have investigated the interaction mechanism between a model AMP or magainin 2 (M2) and dipalmitoyl-phosphatidylglycerol (DPPG) lipid membrane using a synchrotron-radiation circular dichroism spectroscopy and revealed that α-helix monomers of M2 assembled and transformed into β-strand oligomers with increasing peptide-to-lipid (L/P) molar ratio at 25 °C [1]. However, it is still unclear whether the β-strand oligomers insert into the membrane and are involved in the disruption of cell membrane. In this study, we measured synchrotron-radiation linear dichroism (LD) spectrum of M2 to characterize the orientation of the β-strand oligomers in the membrane.

M2 peptide was synthesized in GL Biochem (Shanghai, China), and DPPG phospholipid was purchased from Avanti Polar Lipids, Inc. Each sample was dissolved in 10 mM phosphate buffer (pH 7.0). DPPG liposome with 100 nm diameter was prepared by an extrusion technique, and then mixed with M2 solution at the L/P molar ratio of 4, which corresponds to the experimental condition mainly occupied by the β-strand oligomeric state [1]. The LD spectrum of M2 in DPPG liposome was measured at BL-12 beamline in HiSOR. The details of the flow LD measurement system are described elsewhere [2].

Figure 1 shows the LD spectrum of M2 in DPPG liposome at L/P = 4. The β-strand oligomers of M2 in the DPPG liposomes showed the peak around 200 nm with a small shoulder at 220 nm. According to previous research [3], the net electric or magnetic dipole moments of β-strand around 195 nm and 220 nm are vertical to the strand axis. The β-strand polarized parallel and perpendicular to the membrane normal shows positive and negative LDs at 195 and 220 nm, respectively [3]. Hence, the positive peak LD around 200 nm means that the axis of the β-strands of M2 was parallel to the membrane normal on average, suggesting that the β-strand oligomers of M2 may insert into the membrane and contribute to the formation of pores in the membrane to induce the membrane disruption. Fluorescence anisotropy measurements supported the discussion (data not shown).

In this presentation, we will discuss the antimicrobial mechanism of M2 based on our paper [4].

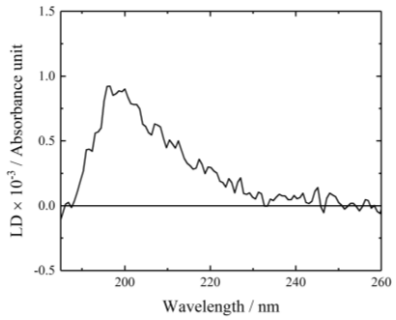

FIGURE 1. LD spectrum of M2 in DPPG liposome at L/P = 4. The LD was recorded at 25 °C with the flow velocity of 1.0 mL min^{-1}.

REFERENCES

1. M. Kumashiro and K. Matsuo, *The 25th Hiroshima International Symposium on Synchrotron Radiation*, Hiroshima, Japan, Mar 2021.
2. K. Matsuo, et al., *Proteins* **84**, 349-59 (2016).
3. A. Rodger, et al., *Phys. Chem. Chem. Phys.* **4**, 4051–7 (2002).
4. M. Kumashiro, et al., *Membranes* **12**, 131 (2022).