Dynamic Structural Study of the Antimicrobial Peptide Magainin 2 Interacting with Membranes by Time-Resolved Circular Dichroism Spectroscopy

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Antimicrobial peptides (AMPs) have attracted considerable attention as next-generation therapeutic agents to combat antimicrobial resistance. Magainin 2 (M2), originally isolated from the African clawed frog (*Xenopus laevis*)(1), is one of important AMPs and exhibits antimicrobial activity by interacting with bacterial membranes, assembling and forming pores on the membrane surface, which induces the disruption of membrane (2). However, the membrane interaction and pore-formation mechanisms remain controversial, and it is therefore necessary to elucidate the structural changes of M2 upon interaction with the membrane. In this study, we investigated the structural dynamics of M2 interacting with model lipid membranes (composed of phospholipid molecules with anionic or neutral head group) using time-resolved vacuum-ultraviolet circular dichroism (TR-VUVCD) spectroscopy.

M2 peptide and membrane (anionic and neutral) solutions were respectively prepared and the both were rapidly mixed with a microfluidic mixer (3). The CD spectra after mixing were measured in the time range from sub-seconds to several tens of seconds, and exhibited that CD spectra commonly showed that the conformation of M2 changed from a random coil (native, N state) to an α-helix–rich structure (membrane-bound, MB state). However, in the case of anionic membranes, the spectral transition from N to M states completed within 0.2 seconds, while in neutral membranes, the transition to the M state required approximately 70 seconds. Kinetic analysis of the TR data set for the M2-neutral membrane interaction was performed using global fitting, revealing the presence of an intermediate state (I state). Further, the secondary structure analysis showed a stepwise increase in α-helical content in the order of N, I, and M states. To obtain the structural insights and membrane interaction sites at the amino-acid residue level, molecular dynamics (MD) simulations were conducted for the helical structure of M2 in the presence of neutral or anionic membranes (4). The simulation results on the membrane affinity and structural stability of M2, depending on membrane type, were consistent with the TR-VUVCD experimental results. Further, MD simulations disclosed that the electrostatic interactions played crucial roles in the early stage of membrane interaction and the hydrophobic interactions contributed to the formation of stable helical structure of M2 in the membrane. Thus, the integrated TR-VUVCD and MD analyses showed that M2 undergoes distinct membrane interaction mechanisms depending on the charge conditions of lipid headgroups prior to pore formation.

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