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Circular Dichroism Study of Magainin 2-Membrane Interaction: Evidence for β-Strand Formation upon Membrane Association

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The biological function of antimicrobial peptide magainin 2 (MG2) largely depends on the lipid binding properties of the peptide but one conclusion from a number of reports on the lipid membrane association of MG2 is that this peptide interacts with membrane, inducing the conformational change from random coil to α-helix, and forms the membrane pore [1, 2]. However, the molecular mechanism of the interaction is still a matter of debate. In this study, to gain further insight into the interaction on a molecular level, a synchrotron radiation circular dichroism (SRCD) spectroscopy was used to characterize the conformation of MG2 associated with dipalmitoyl-phosphatidylglycerol (DPPG) liposome membrane.

MG2 peptide (amino acid sequence: GIGKFLHSAKKFGKAFVGEIMNS) was synthesized in GenScript Biotech Corp. (New Jersey, USA). DPPG lipid molecule (T_m = 41 °C) was purchased from Avanti Polar Lipids, Inc. Each sample was dissolved with 10 mM phosphate buffer (pH 7.0). DPPG liposome suspension with 100 nm diameter was prepared by an extrusion technique, and then mixed with MG2 solution at lipid-to-peptide (L/P) molar ratio from 0 to 26. The SRCD spectra of MG2 in the presence of DPPG liposome were measured at BL-12 beamline in HiSOR. Temperature was controlled with a Peltier device, and varied between 25 °C and 55 °C. A sufficient number of SRCD spectra covered the whole ranges of L/P molar ratio was analyzed using the singular value decomposition (SVD) method. The L/P-dependent data were fitted using adsorption model based on scaled particle theory (SPT), whose procedure is briefly described below. The final solution of SPT for adsorption of a large self-associating ligand is described by

$$Kc_{\mathrm{f}} = \Phi_1\gamma_1(\Phi_1, \Phi_{\mathrm{z}}), \qquad \Phi_z = zK_{1z}\frac{\gamma_1(\Phi_1, \Phi_{\mathrm{z}})^{\mathrm{z}}}{\gamma_{\mathrm{z}}(\Phi_1, \Phi_{\mathrm{z}})}\Phi_1^{\mathrm{z}}, \tag{1}$$

where K is the association constant, z is the number of subunits of adsorbed aggregation, c_{f} is the concentration of the peptide free in solution (or native state), $\Phi_1 = nc_1/c_{\mathrm{L}}$ and $\Phi_{\mathrm{z}} = nc_z/c_{\mathrm{L}}$, c_1 and c_z are the concentrations of bound monomer and z-mer, respectively, c_{L} is the total lipid concentration, n is the number of lipid molecules covered by a single peptide, γ_1 and γ_z are the activity coefficient of adsorbed monomer and z-mer, respectively, K_{1z} is the equilibrium constant for the formation of z-mer [3]. Initial values of K, K_{1z}, n, and z were set, and then c_{f}, c_1, and c_z at each L/P were calculated according to Eq. (1), outputting them as matrix C. The matrix of component spectra S was calculated by $S = (C^{\mathrm{T}}C)^{-1}CD$ (D: data matrix) [4], and then the 2-norm for the error matrix $E = CS - D$ was calculated. This process was repeated to minimize the 2-norm. The secondary-structure contents of MG2 in native state, membrane-binding monomer state, and z-mer state were determined from respective SRCD spectra using SELCON3 program [5, 6].

Figure 1 shows SRCD spectra of MG2 in the presence of DPPG liposome at L/P molar ratio from 0 to 26. The SRCD spectra of MG2 at 55 °C clearly exhibited an iso-dichroic point around 204 nm, while the spectra of MG2 at 25 °C did not show such a point. SVD analyses for the SRCD spectra at 25 °C and 55 °C indicated that the SRCD spectra at entire ranges of L/P molar ratio can be explained by three and two components, respectively, within experimental errors (data not shown). These results suggested that MG2 involved three-state and two-state conformational transitions at 25 °C and 55 °C, respectively, when interacting with DPPG membrane.

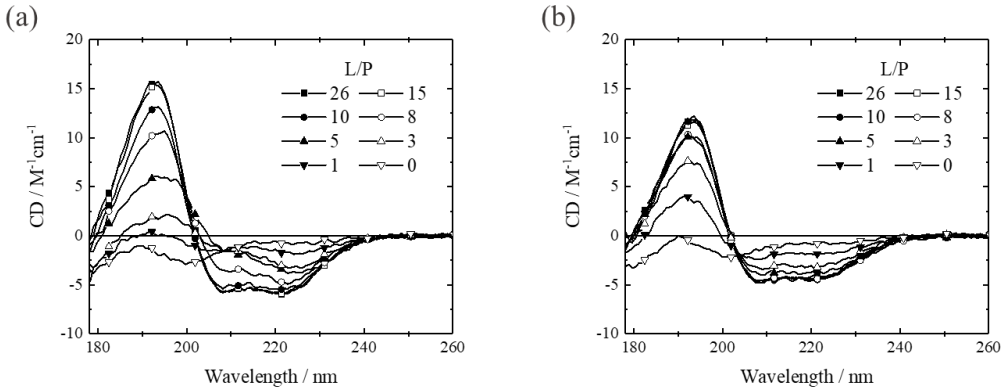

FIGURE 1. SRCD spectra of MG2 in the presence of DPPG liposome at L/P from 0 to 26 at 25 °C (a) and 55°C (b).

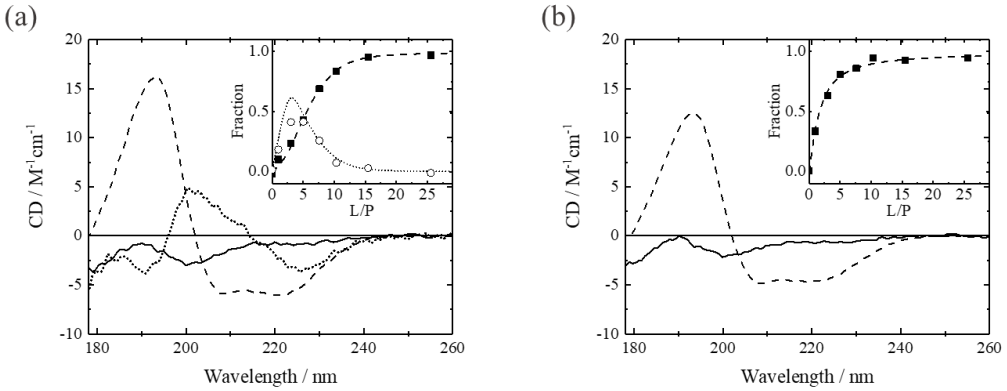

FIGURE 2. Component spectra of MG2 at 25 °C (solid line: native state; dashed line: membrane-binding monomer state; dotted line: z-mer state) (a) and 55°C (solid line: native state; dashed line: membrane-binding monomer state) (b). The inset of (a) shows the dependence of fractional population of the membrane-binding monomer state (closed square: experimental plot; dashed line: fitting curve) and z-mer state (open circle: experimental plot; dotted line: fitting curve) on the L/P molar ratio. The inset of (b) shows the dependence of fractional population of the membrane-binding monomer state (closed square: experimental plot; dashed line: fitting curve) on the L/P molar ratio.

Figure 2 shows component SRCD spectra of MG2. The L/P-dependent data could be fitted when *z* is greater than 3. The component spectra obtained under *z* = 5 is shown in figure 2 as a sample case. Native MG2 exhibited a negative CD peak at 200 nm, which is a characteristic peak of random coil structure. MG2 in the membrane-binding monomer state showed two negative peaks at 208 and 222 nm, and a positive peak around 193 nm, which are characteristic of α-helix structure. These results are corresponding with previous research [2]. On the other hand, MG2 in z-mer state showed two negative peaks around 220 nm and 190 nm, and a positive peak at 200 nm, which is a characteristic peak of β-strand structure. The secondary-structure analyses by SELCON3 showed that MG2 in native state, membrane-binding monomer state, and z-mer state at 25 °C included 8 %, 70 %, and 1 % α-helix, respectively, and 28 %, 11 %, and 46 % β-strand, respectively. These results suggested that MG2 self-associated to form β-strand structure when interacting with DPPG membrane, and also indicated that membrane fluidity is an important factor for the interaction of MG2 with membrane because T_m of DPPG is 41 °C. The result of β-strand formation of MG2 upon membrane association is supported by experiments of fourier transform infrared spectroscopy and solid-state nuclear magnetic resonance spectroscopy as well [7].

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