24. Peptide Self-Assembly in Phospholipid Bilayer Membrane

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Abstract: A series of peptides has been synthesized and studied on the conformation, location, molecular orientation, and self-association in phospholipid bilayer membrane. Hydrophobic peptides tend to associate in the membrane of a crystalline state irrespective of the peptide chain length. Hydrophobic α -helical peptides of a chain length corresponding to the membrane thickness formed an aggregate in the membrane of a liquid-crystalline state with the helix axis oriented perpendicularly to the membrane surface. A hydrophobic octapeptide also took a perpendicular orientation predominantly in the membrane by connecting a hydrophilic group, lactose or crown ether, at the C-terminal of the peptide, which provides an amphiphilic property in the primary structure. The peptides taking transmembrane orientation formed a voltage-dependent ion channel. On the other hand, α -helical peptides with secondary amphiphilicity were incorporated at the membrane surface with the helix axis oriented parallel to the membrane. Some peptides with net charge associated at the membrane surface, though the size of aggregate was not large.

Key words: Peptide/membrane interaction; primary amphiphilicity; secondary amphiphilicity; peptide orientation in membrane; peptide self-association; voltage-dependent ion channel.

It has been proposed that protein folds into a defined tertiary structure via a molten-globule structure, in which the secondary structures are formed but those peptide fragments are not yet organized into a proper spatial arrangement.¹⁾ Recently, a series of proteins, so called molecular chaperones, has been found in a ribosomal system, where proteins are produced according to the information on mRNA.^{2),3)} The newly synthesized polypeptides can fold to the native structure with the help of chaperones, which are considered to recognize secondary structure elements and regulate the interactions between them. Though the primary structure, the amino-acid sequence, has been believed to contain the all information on the native structure, the folding process should be reconsidered especially in terms of the control of interactions of the peptide fragments. It is thus important to accumulate informations on the peptide interactions for understanding the protein folding. We have been studying on interactions between α -helical peptides, which were immobilized on a cyclic octapeptide.^{4),5)} The association of α -helices on a cyclic peptide in aqueous solution was found to be dependent on the amphiphilicity of the α -helical peptide and the conformation of the cyclic peptide.

It is well known that sophisticated membrane function owes much to the protein organization in the membrane as shown typically in the energy-transduction system and photo-synthetic system. However, the molecular mechanism for assembling proteins into a specific topology in the membrane remains to be solved. Membrane proteins may take a pathway of protein folding similar to that of secretory proteins as described above. It is therefore of interest to study on peptide interactions in phospholipid bilayer membranes. In the present study, several oligopeptides having a fluorescent group were synthesized and investigated on interactions with phospholipid bilayer membranes. Fluorescence

method was used for analysis because of low concentration (μM) required for measurements, which avoids a severe damage on lipid bilayer structure. This type of study will pave the way for construction of an artificial peptide assembly in phospholipid bilayer membrane with a newly designed function.

Methods. Peptides synthesis. Peptides were synthesized by a conventional liquid-phase method except mastoparan X analogs. Dicyclohexylcarbodiimide and N-hydroxysuccinimide or N-hydroxybenzotriazole were used as coupling reagent. Mastoparan X analogs were synthesized by combining both liquid- and solid-phase methods. Oxim resin was adopted for preparation of mastoparan X fragments, and BOP was used as coupling reagent.⁽⁶⁾ The products were purified by gel permeation chromatography, and HPLC using a reversed-phase column if necessary.

Results and discussion. Dipeptide in bilayer membrane. The distribution of a hydrophobic dipeptide in phospholipid bilayer membrane was studied by measuring the excited energy transfer from Boc-Trp-Phe-OEt to 12-(9-anthroyloxy)stearic acid in DPPC vesicle.⁽⁷⁾ The intermolecular energy-transfer efficiency was constant above the phase-transition temperature (42°C), but increased by lowering the temperature below the phase-transition temperature (Fig. 1). Therefore, the average distance between the dipeptide and the stearic acid decreased in the membrane of a gel state compared with that in a liquid-crystalline state. The condensation of peptide and stearic acid in the gel-state membrane is ascribed to formation of crystalline domain composed of pure DPPC. The peptide molecule and stearic acid are excluded from the crystalline domain, and are concentrated in the other domain. Occurrence of the phase separation in the gel-state membrane indicates that lipid-lipid interaction should be stronger than lipid-peptide interaction.

The energy acceptor molecule was changed from stearic acid to a dipeptide, Boc-Lys(Anth)-Phe-OEt (Anth represents 9-carboxyanthryl group). The intermolecular energy-transfer efficiency from Boc-Trp-Phe-OEt to Boc-Lys(Anth)-Phe-OEt was higher than that from Boc-Trp-Phe-OEt to 12-(9-anthroyloxy)stearic acid in the temperature range examined here (Fig. 1). The shorter average distance from Boc-Trp-Phe-OEt to Boc-Lys(Anth)-Phe-OEt than to 12-(9-anthroyloxy)stearic acid indicates that an attracting force such as formation of hydrogen bonding should act between peptides in the membrane. The effect of phase transition on the energy-transfer efficiency between peptides was not so clear as that observed between the peptide and stearic acid, which also

Fig. 1

Fig. 1. The temperature dependence of the excited energy transfer from Boc-Trp-Phe-OEt to 12-(9-anthroyloxy)stearic acid (○) or Boc-Lys(Anth)-Phe-OEt (●) in the presence of DPPC liposome.

Fig. 2

Fig. 2. Molecular structure of hydrophobic α -helical peptides.

supports the favorable interaction acting between peptides to stabilize their association.

Hydrophobic α -helical peptide in bilayer membrane. Hydrophobic peptides composed of α -helix-forming residues, Boc-(Ala-Aib)_n-OMe (n=1,2,4,6,8, Fig. 2), were synthesized and studied on the interaction with phospholipid bilayer membrane. The dipeptide and tetrapeptide were found by CD measurement to take an irregular structure in ethanol. On the other hand, octa-, dodeca- and hexadecapeptide took a partially helical conformation, and the helix content increased as the chain length was elongated. Aib-containing peptides have been shown to form either α - or 3_{10} -helical structure.⁸⁾ The octapeptide was shown to take a 3_{10} -helical conformation in a solid state by X-ray analysis, but an α -helical conformation in solution by NMR measurement. The dodeca- and hexadecapeptide also took an α -helical conformation in solution by NMR measurement. Generally, the peptides, which possess high content of Aib residue in the primary structure and short chain length, tend to take 3_{10} -helical structure. In case of Boc-(Ala-Aib)_n-OMe (n=2,4,6,8), the octapeptide should have a critical chain-length for 3_{10} - to α -helix transition.

The hydrophobic peptides were elongated to the N-terminal with Ser having a fluorescent probe at the side chain, Boc-Ser(Ant)-(Ala-Aib)_n-OMe (Ant represents 9-anthrylmethyl group, n=2,4,6,8,10, Fig. 2), and were studied on orientation and association in phospholipid bilayer membrane. The fluorescence quenching of Boc-Ser(Ant)-(Ala-Aib)_n-OMe by 12-doxylstearic acid (12DS) was examined in the presence of DMPC liposome (Fig. 3). The tridecapeptide was most intensively quenched by 12DS, indicating that the N-terminal region of the tridecapeptide locates at the middle of hydrophobic core of the lipid bilayer membrane. This result suggests that the peptides take an α -helical conformation with the helix axis oriented perpendicularly to the membrane surface. The chain length of the tridecapeptide with an α -helical conformation is calculated to be about 20 Å, and the N-terminal will be located at the middle of the membrane when the peptide is inserted to the membrane from the N-terminal region. Since the heptadeca- and heneicosapeptides possess longer peptide chain length, the anthryl group at the N-terminal region locates at the site close to the opposite surface of the membrane, resulted in less quenching by 12DS than the tridecapeptide.

Peptide association in the membrane was studied by measuring the fluorescence depolarization of anthryl group of the peptides. The fluorescence depolarization value decreased with increasing the chain length less than tridecapeptide, but increased again in the order of A6 < A8 < A10. A10 showed a substantially high value. In addition, the

Fig. 3

Fig. 4

Fig. 3. The fluorescence quenching of An by 12DS in the presence of DMPC liposome at 15°C. I_0 and I represent the fluorescence intensity in the absence and presence of 12DS, respectively. $[DMPC]=5.7 \times 10^{-4}$ M. $[12DS]=2.9 \times 10^{-5}$ M.

Fig. 4. Schematic picture of the incorporation of Boc-Ser(Ant)-(Ala-Aib)₁₀-OMe into phospholipid bilayer membrane.

fluorescence depolarization value became higher at the lower lipid concentration, where the peptide concentration in the membrane was high. The high value and concentration dependence of fluorescence depolarization of A10 are ascribed to energy migration between anthryl groups due to peptide association in the membrane. Taking these points into consideration, A10 is considered to be incorporated into lipid bilayer membrane with forming an aggregate in which the helical axis is perpendicularly oriented to the membrane surface (Fig. 4).

Voltage-dependent ion channel. An α -helical bundle structure with transmembranous orientation is expected to act as an ion channel.^{9),10)} Current/voltage (I/V) response across BLM in the presence of Boc-(Ala-Aib)_n-OMe (n=4,8) was studied. The current across the membrane increased dramatically beyond a critical voltage, V_c , indicating formation of a voltage-dependent ion channel. V_c was around 150 mV for the octapeptide, but drastically decreased to around 50 mV for the hexadecapeptide. The higher channel-forming ability of the hexadecapeptide than the octapeptide can be explained by easier formation of aggregates with perpendicular orientation in the membrane of the former peptide. However, the octapeptide can be modified to show a high ability for channel formation, which is described in the next section.

Primary amphiphilic peptide. It has been proposed that the primary amphiphilicity of the peptide is one of the factors which determine the peptide orientation in the membrane.¹¹⁾ For example, peptide molecule with a large value of the amphiphilic moment tends to take a perpendicular orientation in the membrane.¹²⁾ Based on this concept, the hydrophobic peptide was modified by two ways.

Firstly, the C-terminal ester group of Boc-(Ala-Aib)₄-OMe was replaced by lactose to provide amphiphilicity to the primary structure of the peptide (Boc-(Ala-Aib)₄-Lac, Fig. 5). Although Boc-(Ala-Aib)₄-Lac and Boc-(Ala-Aib)₄-OMe are composed of the same octapeptide, V_c of the former peptide in the I/V response was significantly lower than that of the octapeptide with methyl ester at the C-terminal, indicating high ability for ion-channel formation. The amphiphilic property in the primary sequence is considered to facilitate the peptide taking a perpendicular orientation in the membrane. Interestingly, lectin addition lowered V_c and increased opening frequency of the channel. Presumably, lectin crosslinks Boc-(Ala-Aib)₄-Lac molecules in the lipid bilayer membrane to promote the association of peptide molecules, resulting in stabilization of the single channel.

Secondly, crown ether was connected to the C-terminal of Boc-(Ala-Aib)₄-OMe (Boc-(Ala-Aib)₄-Ala-Cr, Fig. 5). The crown-ether part becomes hydrophilic upon complexation with cations. As expected, Boc-(Ala-Aib)₄-Ala-Cr showed a higher potency in formation of a voltage-dependent ion channel than Boc-(Ala-Aib)₄-OMe. It is notable that Boc-(Ala-Aib)₄-Ala-Cr showed cation specificity in the channel formation. The peptide showed single channel current fluctuation in the presence of Cs⁺ at 140 mV of voltage applied to the membrane, but failed in the presence of K⁺ under the same peptide

Fig. 5. Molecular structure of primary amphiphilic peptides.

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