Incorporation of hydrophobic helix-bundle peptides into lipid bilayer membranes facilitated by a peptide-umbrella structure

Hiroki Ueda,[a] Shunsaku Kimura*[a] and Yukio Imanishi[b]

[a] *Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto, Japan 606-01*

[b] *Graduate School of Material Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara, Japan 630-01*

Hydrophobic helical peptides, which are covered by hydrophilic peptides, are transferred into a phospholipid bilayer to form helix-bundle structures as a consequence of their peptide-umbrella structure.

The hydrophobic helix-bundle structure is one of the common structures for membrane proteins found in nature, for example, in receptors of peptide hormones[1] and ion channels.[2] Therefore, phospholipid bilayer membranes embedded with helix-bundle peptides are of research interest for developing information-processing systems. However, a difficulty in the preparation of the molecular system exists in the insolubility of hydrophobic peptides in water, which restricts distribution of peptides in water to a phospholipid bilayer membrane. For this reason, water-soluble amphiphilic helical peptides have been synthesized[3] which form helix-bundle structures by association in water, and which have their hydrophilic surface facing outward and the hydrophobic surface facing inward. Generally, however, transfer of charged amino acid residues from the aqueous phase to the hydrophobic core region of the membrane is energetically unfavourable,[4] and most of amphiphilic peptides tend to stay at the membrane surface.[5] To realize a transmembrane helix-bundle in high yield, hydrophobic helices may be more favourable than amphiphilic helices,[6] and thus the design of hydrophobic helices able to be solubilized in water is presented here.

We have conceived a novel class of peptide molecules that mimic the structure of umbrellas, *i.e.* peptide molecules that are composed of hydrophobic helices in a 'handle' structure with hydrophilic segments shielding the helices. Upon binding to phospholipid bilayer membranes, the hydrophobic helices are inserted into the membrane vertically, with the hydrophilic segments staying at the membrane surface or in the aqueous phase just like an open umbrella. Regen[7] also presented a 'molecular umbrella' concept, but their molecules are designed for drug delivery and not for a functional membrane peptide.

In the present study, hydrophobic helix-bundle peptides were designed and synthesized with an umbrella structure, and their interaction with a phospholipid bilayer membrane was investigated. The molecular structures of the peptides are shown in Fig. 1. Two or three hydrophobic helices were connected together through pentaoxyethylenebis(amine) or tris(3-aminopropyl)amine in a parallel arrangement of helices. The hexadecapeptide –(Ala-Aib)$_8$– was chosen as the hydrophobic helix peptide because Boc–(Ala-Aib)$_8$–OMe has been found to have an α-helix conformation by X-ray diffraction[8] and to form a bundle structure for a voltage-dependent ion channel in a lipid membrane.[9] A naphthyl group was connected as a fluorescent probe. Oligosarcosine chains were connected to the hydrophobic peptides (**2αZ** and **3αZ**) to make them soluble in water (**2αS** and **3αS**). Nap–(Ala-Aib)$_8$–OBz **1α** (Nap represents a β-naphthaleneacetic group) was used as a reference hydrophobic helix peptide. The peptides were synthesized *via* conventional liquid phase methods.

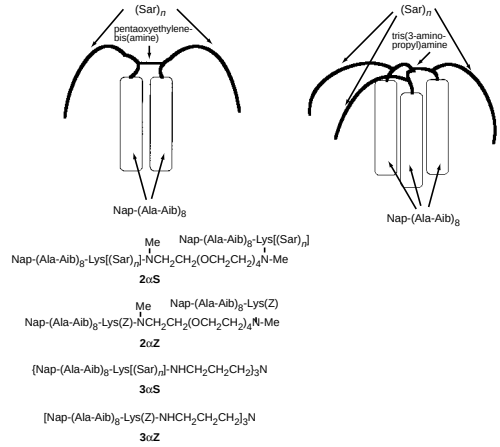

Fig. 1 Molecular structures of the hydrophobic helical peptides and peptide umbrellas. The average degree of polymerization of Sar is 11 for **2αS** and 9 for **3αS**.

The conformations of the peptides were investigated by circular dichroism (CD) spectroscopy. The CD spectra of the peptides showed a double-minimum pattern, indicating formation of an α-helical conformation in MeOH solution (Fig. 2). The helix content increases in the order **1α** < **2αZ** ≈ **2αS** < **3αZ** ≈ **3αS**. It is considered that the presence of helix chains in close proximity favours the formation of a helical conformation due to Van der Waals interactions. On the other hand, the introduction of oligosarcosine chains does not influence the conformation of the peptides in MeOH (**2αZ** ≈ **2αS** and **3αZ** ≈ **3αS**), suggesting the absence of any interactions between the oligosarcosine chains and the helix chains. However, in water

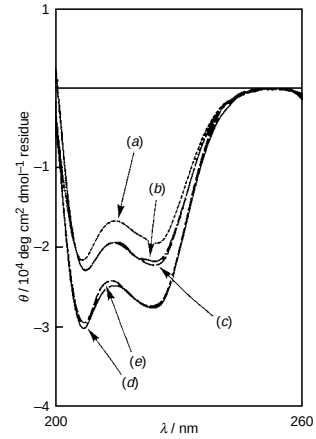

Fig. 2 CD spectra of the peptides in MeOH (**3αZ** and **3αS**) or MeOH–EtOH [2 : 1 (v/v), **1α**, **2αZ** and **2αS**]: (*a*) **1α**, (*b*) **2αS**, (*c*) **2αZ**, (*d*) **3αS** and (*e*) **3αZ**. Total concentration of Ala and Aib residues = 0.5 mM. The molar ellipticity stems from the amino acid residues of the helix segments.

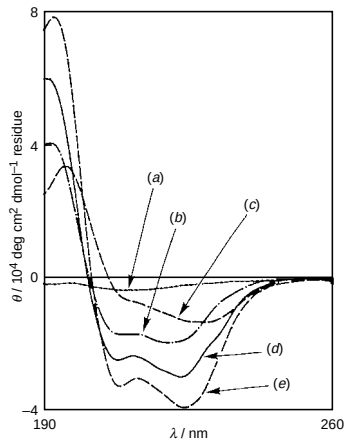

Fig. 3 CD spectra of the peptides in water: (*a*) **1α**, (*b*) **2αS**, (*c*) **2αZ**, (*d*) **3αS** and (*e*) **3αZ**. Total concentration of Ala and Aib residues = 0.5 mM.

the peptides show different CD spectra (Fig. 3). Compound **1α** showed very weak ellipticity due to low solubility in water. Compound **2αZ** showed a shoulder at 208 nm and a broad peak centred at 228 nm, suggesting the occurrence of severely distorted helices by strong aggregation in water. On the other hand, **2αS** shows the double-minimum pattern with increasing negative intensity at 222 nm, suggesting a weak association of helix chains in water.[10] It is evident in these CD patterns that the oligosarcosine chains of **2αS** suppresses the strong aggregation seen for **2αZ** by increasing water solubility. Compounds **3αZ** and **3αS** showed increasing negative intensity at 224 nm and their CD patterns are similar to those of **2αS** and **2αZ**. The oligosarcosine chains of **3αS** should stabilize the helical conformation of the peptide in water by avoiding strong aggregation. Unexpectedly, **3αZ** showed evidence of stable helix-bundle structures in water. It is considered that the tertiary amino group of the peptide increases the hydrophilicity of the molecule to form a micelle structure *via* self-assembly, which remains to be investigated.

The interactions of the peptides with DMPC liposomes was investigated by CD spectroscopy (Fig. 4). Compound **1α** showed a double-minimum-type CD spectrum with increasing negative intensity at 225 nm, indicating spontaneous distribution of the peptides to the membrane and formation of a helix-bundle structure. On the other hand, CD spectra of **2αZ** and **3αZ** showed a weak Cotton effect in the presence of DMPC liposomes. The liposome suspension became turbid upon addition of the peptides, indicating aggregation of liposomes triggered by enhanced hydrophobic contact of liposomes and/or

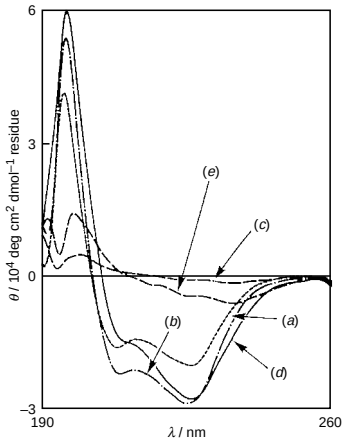

Fig. 4 CD spectra of the peptides in the presence of DMPC liposomes: (*a*) **1α**, (*b*) **2αS**, (*c*) **2αZ**, (*d*) **3αS** and (*e*) **3αZ**. [DMPC] = 1.0 mM. DMPC liposomes were prepared by the sonication method using Tris buffer (0.01 M, pH 7.4) containing NaCl (0.1 M) and EDTA (0.1 mM). Total concentration of Ala and Aib residues = 0.5 mM.

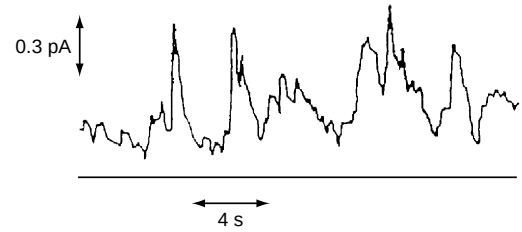

Fig. 5 Current fluctuation of the bilayer lipid membrane (BLM) in the presence of **3αS**. The membrane is made of soybean lectin and formed on an aperture of 0.2 mm diameter. Aqueous solution contains 1 M KCl. A membrane potential of 190 mV was applied using a salt bridge. [**3αS**] = 0.026 μM.

membrane disorder caused by the hydrophobic peptides. Notably, aggregation of liposomes was not induced by the addition of **2αS** or **3αS**, and the peptides were taken in spontaneously by the phospholipid membrane. Oligosarcosine chains should suppress liposomal aggregation by making the liposome surface hydrophilic. With **2αS** and **3αS**, the relative molar ellipticity at 208 nm to that at 225 nm was smaller in the presence of DMPC liposomes than in pure water, suggesting a stronger aggregation of the helix chains in the lipid membrane than in water.[10]

Distribution of the peptides into the phospholipid membrane was also investigated by fluorescence quenching of the naphthyl group with acrylmaide, which is a water-soluble quencher. The Stern–Volmer constants[11] for the collisional quenching process K_{SV} (mM^{-1}) were 8.3 for **2αS** in buffer, 1.8 for **2αS** with liposome, 6.5 for **3αS** in buffer and 2.1 for **3**α**S** with liposome. The lower K_{SV} values in the presence of liposomes indicate that the naphthyl groups are buried in the hydrophobic core region of the membrane due to vertical insertion of the helices.

It is considered that the helix-bundle structure, in which the peptide chains take a parallel arrangement, should function as ion channels in the lipid membrane. Compound **3αS** (0.026 μM) was added to the bilayer lipid membrane (BLM) to measure the current–voltage response. Current fluctuation at an applied voltage of 190 mV was observed (Fig. 5). Current peaks of the same level were observed frequently, but were not detected with **1α** at the same concentraton. This indicates an important contribution from the umbrella-like structure of **3αS** in the formation of the ion channel under a transmembrane electric potential. An unusual observation was that the current fluctuation was not a stepwise response typical of ion channels. Probably, a part of the current response may be due to disorder of the membrane produced by the peptide insertion.

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Notes and References

* E-mail: h54519@sakura.kudpc.kyoto-u.ac.jp

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