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Orientation and aggregation of hydrophobic helical peptides in phospholipid bilayer membrane

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Hydrophobic sequential peptides with various chain lengths, Boc-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8) and Boc-Ser(CH$_2$Ant)-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8, 10, Ant represents *O*-anthrylmethyl; abbreviated as A2–A10), were synthesized and their orientation and aggregation in a lipid bilayer membrane were investigated. Circular dichroism (CD) measurements revealed that the peptides took a partially helical structure, and that the helix content increased with increasing chain length and upon distribution to phospholipid vesicles. When long-chain peptides, A8 and A10, were incorporated into lipid bilayer membranes, the membrane fluidity was reduced, while 5/6-carboxyfluorescein (CF) leakage through the bilayer membranes was enhanced. Fluorescence quenching of the anthracene group with 12-doxylstearic acid suggested that these peptides took a perpendicular orientation in the membrane. Detection of excimer emission and large fluorescence depolarization of the peptides indicated an aggregation in the membrane. In addition, Boc-(Ala-Aib)$_n$-OMe (n = 4, 8) showed a channel-like activity in a bilayer lipid membrane (BLM). The channel-forming ability of the hexadecapeptide was higher than that of the octapeptide. Taken together, the long-chain hydrophobic helical peptides tend to aggregate in lipid bilayer membranes with a transmembrane orientation.

Introduction

Alamethicin forms a voltage-dependent ion channel in phospholipid membrane. It has been suggested that the ion channel is composed of a bundle of α-helices with a transmembrane orientation [1]. However, the on/off mechanism, for example, of a voltage-dependent ion channel by membrane potential is still controversial, and several explanations have been proposed [2–5]. Since alamethicin contains 8 Aib residues in a total of 20 residues, several model peptides composed of Aib residues have been synthesized and studied. Karle et al. [6] analyzed the conformation of Boc-(Aib-Ala-Leu)$_3$-Aib-OMe by X-ray diffraction, and found that the α-helix prevails from the middle to the carboxyl terminal of the peptide chain and that it is distorted in the other half of the chain by the presence of an inserted water molecule. Consequently, some carbonyl groups are directed outward to form a hydrophilic region. The decapeptide, therefore, possesses an amphiphilic character, though it is composed exclusively of hydrophobic amino acids [7]. The amphiphilic property is the key factor for the formation of an ion channel. On the other hand, Menestrina et al. [8] have synthesized another group of hydrophobic peptides, Boc-(Ala-Aib-Ala-Aib-Ala)$_n$-OMe (n = 1–4). The peptides formed a voltage-dependent ion channel in BLM, indicating that the hydrophobicity and α-helical structure are necessary conditions for ion-channel formation. However, the formation of a bundle structure by transmembrane helices in the membrane remains to be clarified.

We have synthesized hydrophobic helical peptides which would take a transmembrane orientation in phospholipid membrane. However, hydrophobic homopolypeptides such as polyalanine, polyleucine, poly(γ-benzyl glutamate), and poly(*N*-methylalanine) were not easily incorporated into phospholipid membrane, because of formation of a β-sheet structure, low solubility in lipid membrane, or serious damage on the membrane structure [9]. The Aib unit is effective in suppressing the formation of a β-sheet structure, because the torsion angles, ϕ and ψ, of the Aib residue are confined to the right- and left-handed helical (3_{10}

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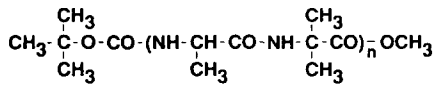
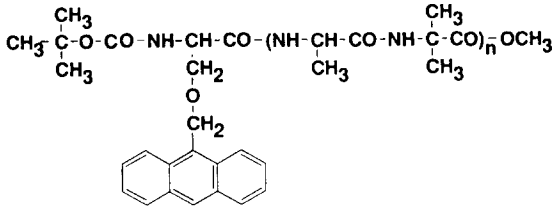
Abbreviations: Aib, 2-aminoisobutyric acid; An, Boc-Ser(CH$_2$Ant)-(Ala-Aib)$_n$-OMe; Ans, 1-anilinonaphthalene-8-sulfonate; Ant, *O*-anthrylmethyl; CD, circular dichroism; CF, 5/6-carboxyfluorescein; DMPC, dimyristoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoylphosphatidylcholine; 12-DS, 12-doxylstearic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.



Boc-(Ala-Aib)$_n$-OMe n = 2, 4, 6, 8



Boc-Ser(CH$_2$Ant)-(Ala-Aib)$_n$-OMe n = 2, 4, 6, 8, 10, A$_n$

Fig. 1. Molecular structure of Boc-(Ala-Aib)$_n$-OMe and Boc-Ser-(CH$_2$Ant)-(Ala-Aib)$_n$-OMe.

or α) regions of the conformational energy map [10]. α-Helical conformation is interesting because of the macrodipole, which may make the peptide susceptible to the electric field applied across the membrane. However, the terminally blocked (Aib)$_{10}$ is reported to take a 3_{10}-helix conformation [11], which has a lower dipole moment than the α-helical conformation. Therefore, we have chosen the alternating sequence of Ala and Aib, Boc-(Ala-Aib)$_n$-OMe (n = 1–10), for hydrophobic helical peptides, which are expected to form ion channels by taking a transmembrane orientation. Boc-(Ala-Aib)$_n$-OMe (n = 4, 6, 8) were shown to take an α-helix conformation in CD_3CN by ^{1}H-NMR analysis, while the octapeptide in a crystalline state was shown to take a 3_{10}-helix conformation by X-ray analysis [12].

In the present investigation, Boc-(Ala-Aib)$_n$-OMe and Boc-Ser(CH$_2$Ant)-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8, 10, Ant represents *O*-anthrylmethyl; abbreviated as A2–A10) were synthesized (Fig. 1) and their interactions with phospholipid bilayer membranes were investigated. In order to know the orientation and aggregation of the peptides in the membrane, an anthryl group was connected to the N-terminal unit of the peptides as a fluorescence probe. Ion-channel formation of the peptides in BLM was also examined, and discussed in terms of the peptide structure in the membrane.

Methods

Materials. Dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), 12-doxylstearic acid (12DS), and 5/6-carboxyfluorescein (CF) were purchased from Sigma and used without further purification. Azolectin (Associated Concent.) was purified by the method reported by Kagawa et al. [13].

Synthesis of peptides. Peptide synthesis was carried out by the conventional liquid phase method. Fragment condensation was repeated by using dicyclohexylcarbodiimide and N-hydroxybenzotriazole as coupling reagents to ultimately obtain Boc-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8). The synthesized peptides were purified by either recrystallization from a methanol/diethyl ether mixture or chromatography through a Sephadex LH-20 column with methanol as eluant. Each compound was identified by ^{1}H-NMR, and the purity was checked by thin-layer chromatography. The results of elemental analysis are summarized in Table I. An (n = 2, 4, 6, 8, 10) were synthesized similarly. For example, A8 was synthesized as follows: Boc-(Ala-Aib)$_8$-OMe was treated with trifluoroacetic acid. The solid product was dissolved in dimethylformamide and coupled with Boc-Ser(CH$_2$Ant) by using dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole. The product was purified by a Sephadex LH-20 column using methanol as eluant and a Cosmosil 5C$_{18}$P reversed-phase HPLC column using methanol/water (9:1, v/v) as eluant.

Preparation of liposome. Small unilamellar vesicles were prepared by sonication of DMPC or DPPC dispersion in a buffer solution (10 mM Hepes, 0.1 M NaCl, 0.1 mM EDTA, pH 7.4) and ultracentrifugation at 100 000 × g. Lipid concentration was determined by

TABLE I

Elemental analysis of synthetic peptides

Sample	Formula		C (%)	H (%)	N (%)
Boc-(Ala-Aib)$_2$-OMe	$C_{20}H_{36}N_4O_7$	calcd.	54.03	8.16	12.60
		found	54.21	8.36	12.60
Boc-(Ala-Aib)$_4$-OMe·2H$_2$O	$C_{34}H_{64}N_8O_{13}$	calcd.	51.50	8.14	14.13
		found	51.83	7.99	14.22
Boc-(Ala-Aib)$_6$-OMe·H$_2$O	$C_{48}H_{86}H_{12}O_{16}$	calcd.	53.02	7.97	15.46
		found	53.19	8.08	14.90
Boc-(Ala-Aib)$_8$-OMe·H$_2$O	$C_{62}H_{110}N_{16}O_{20}$	calcd.	53.20	7.92	16.01
		found	53.15	7.88	16.22

the colorimetric method using phospholipase D (Diacolor, Toyobo).

Measurements. Circular dichroism (CD) and fluorescence spectra were measured on a JASCO J-20 or J-600 spectropolarimeter and a Hitachi MPF-4 fluorophotometer, respectively. The CD data are presented as mean residue ellipticities. Fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-anilinonaphthalene-8-sulfonate (ANS), or the anthryl group of the peptides was measured by an equipment installed on the fluorophotometer reported previously [14].

CF leakage. CF-encapsulated DPPC vesicles were prepared by the method reported by Barbet et al. [15]. The excitation and monitor wavelengths of CF were 470 and 515 nm, respectively. Complete release of CF was attained by destruction of vesicles with Triton X-100.

BLM experiments. A thin Teflon film (0.25-mm thick) with a hole of 0.1-mm diameter was clamped between two compartments of a Teflon trough. The hole was coated with hexadecane/hexane (6:4, v/v) mixture in advance. The azolectin membrane was formed by the method reported by Montal et al. [16]. An unbuffered KCl solution (1 M) was used. Before the measurement, an AC voltage of 200 mV (peak-to-peak, 1 kHz) was applied to the membrane for 30 min, which made the planar bilayer membranes virtually solvent-free [8]. The peptide was added to the aqueous phase in both compartments.

Results and Discussion

Conformation in solution

CD measurements of Boc-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8) and A10 revealed that the intensity of negative Cotton effect at 208 and 222 nm increased as the peptide chain was elongated, probably with increasing helix content (Fig. 2). We have reported that the critical chain-length for 3_{10}- to α-helix transition is eight on the basis of the experimental fact that the preferred conformation of Boc-(Ala-Aib)$_4$-OMe was 3_{10}-helix in a solid state but α-helix in solution [12]. It is therefore considered that Boc-(Ala-Aib)$_n$-OMe (n = 6, 8) and A10 take an α-helix conformation, and that the helix content of A10 is about 50%, which is highest among these peptides.

Distribution to lipid bilayer membrane

Fluorescence spectra of An (n = 2, 4, 6, 8, 10) in a buffer solution are shown in Fig. 3. Monomer emission around 410 nm is predominant in the spectra of A2 and A4, indicating that these peptides are molecularly dispersed in a buffer solution. On the other hand, excimer emission around 470 nm was observed with longer peptides, and prevailing in the spectra of A8 and A10. Excimers of A8 and A10 should have resulted from aggregation of these peptides due to hydrophobic interactions.

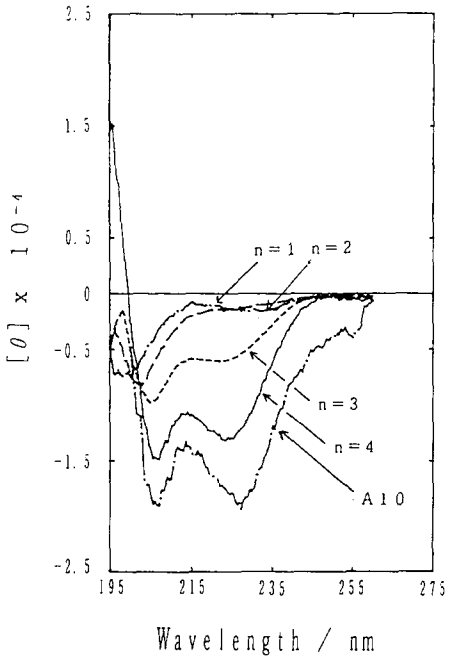


Fig. 2. CD spectra of Boc-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8, the residue concentration was 2 mM) and A10 (the residue concentration was 1.4 mM) in ethanol.

Addition of DMPC liposomes to the peptides in a buffer solution changed the fluorescence spectra, reflecting incorporation of the peptides into the phospholipid bilayer membranes. The monomer emission intensities of A2, A4, and A6 decreased in the

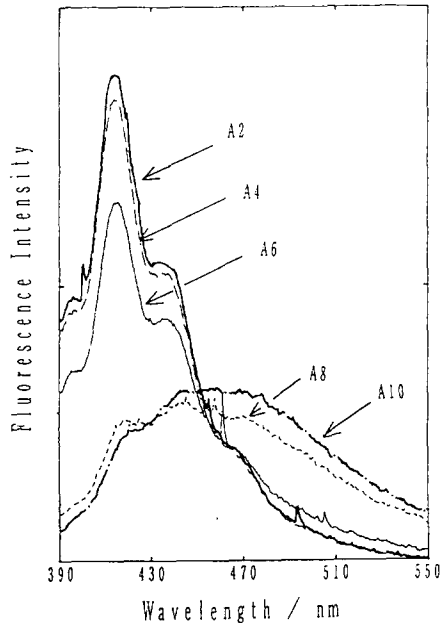


Fig. 3. Fluorescence spectra of An (n = 2, 4, 6, 8, 10) in a buffer solution. The peptide concentration was in the range of $0.95 \cdot 10^{-6}$ – $1.1 \cdot 10^{-6}$ M. Excitation wavelength was 367 nm.

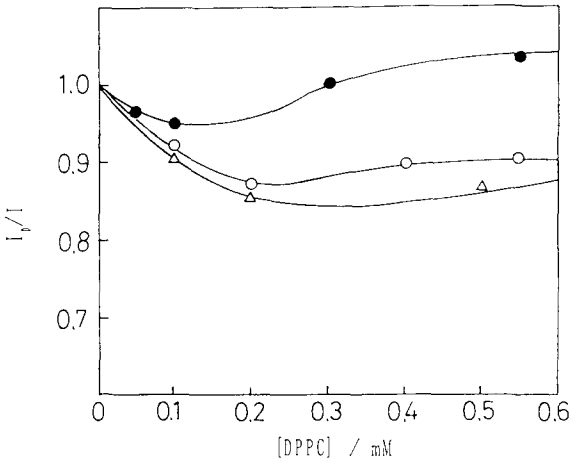


Fig. 4. Change of monomer emission intensity of A2 (△), A4 (○), and A6 (●) by the addition of DMPC liposome. The peptide concentration was in the range of $0.95 \cdot 10^{-6} - 0.99 \cdot 10^{-6}$ M. Excitation and monitor wavelengths were 367 and 414 nm, respectively.

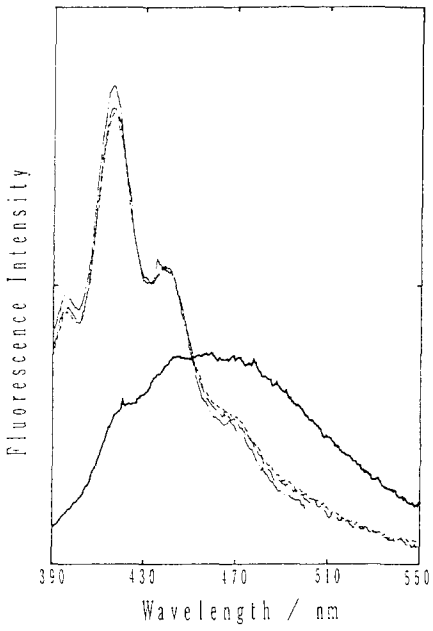


Fig. 5. Change of fluorescence spectra of A10 ($0.94 \cdot 10^{-6}$ M) by the addition of DMPC liposome, DMPC] = 0 mM; ———, 0.12 mM; - - - - - -, 0.29 mM; — — —, 0.57 mM; ·–·–·.

presence of a small amount of liposomes, but increased when the lipid concentration increased beyond 0.3–0.4 mM (Fig. 4). The decrease of monomer emission intensity is a result of excimer formation due to condensation of peptide molecules into a narrow region of a lipid bilayer membrane. However, dilution of peptides in large amounts of lipid membranes increased the intensity of monomer emission.

With A8 and A10, excimer emission prevailed in a buffer solution, but monomer emission became prevailing after addition of DMPC liposome, indicating dissociation of aggregates by distribution to the lipid membrane (Fig. 5). However, excimer emission remained in the presence of excess amounts of DMPC liposome. This situation occurred especially with A10 which tended to associate with themselves in the lipid membrane.

Conformation in lipid membrane

CD spectra of Boc-(Ala-Aib)$_n$-OMe ($n = 2$, 4, 8) were measured in the presence of DPPC liposome of varying concentrations (Fig. 6). The ellipticity at 220 nm of the tetrapeptide changed the sign from minus to plus in the presence of a small amount of liposomes, but decreased again to a negative value by further addition of lipid molecules. The spectrum showing a positive 220-nm ellipticity resembles those of Aib-containing oligopeptides taking a β-turn structure [17]. The β-turn structure of the tetrapeptide was also indicated by IR spectrum in chloroform, showing the amide I absorption at 1676 cm^{-1} and the N–H stretching

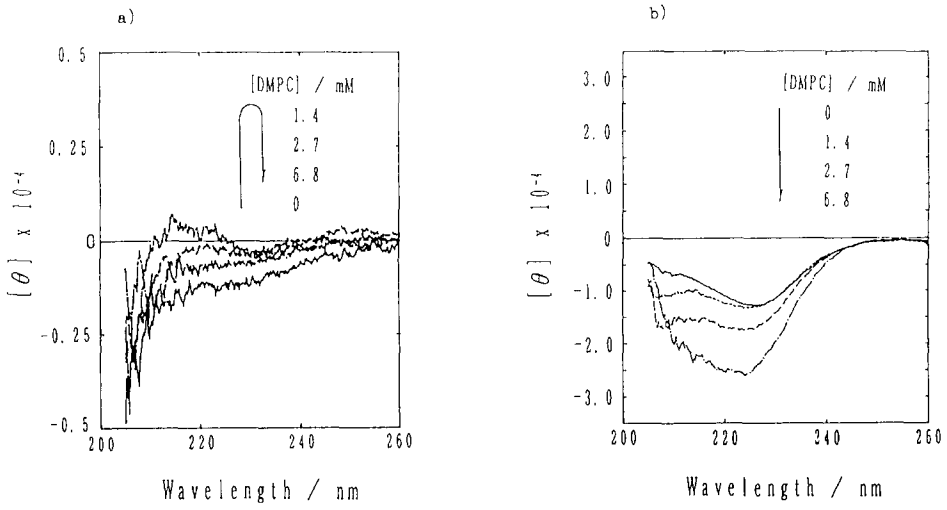


Fig. 6. Change of CD spectra of (a) Boc-(Ala-Aib)$_2$-OMe (0.23 mM) and (b) Boc-(Ala-Aib)$_8$-OMe (0.072 mM) by the addition of DMPC liposome, [DMPC] 0 mM; ———, 1.4 mM; - - - - - -, 2.7 mM; — — —, 6.8 mM; ·–·–·.

absorption at 3348 cm^{-1} [18]. Therefore, the tetrapeptide forms a β-turn structure under a condensed state in the lipid membrane. However, it takes a random conformation under diluted conditions.

Boc-(Ala-Aib)$_4$-OMe behaved similarly to the tetrapeptide in CD spectroscopy. In the presence of a small amount of liposomes, a small positive Cotton effect was observed around 220 nm, which decreased to a negative value with increasing addition of liposomes.

On the other hand, Boc-(Ala-Aib)$_8$-OMe in a buffer solution showed a negative ellipticity around 230 nm (Fig. 6), which is typical for α_{II}-helix, aggregated helices, as exemplified by rhodopsin [19]. Such aggregation in a buffer solution is consistent with the excimer formation as observed by the fluorescence measurement described above. However, by the addition of liposomes, CD spectrum turned to a double-minimum type that is typical for an α-helix conformation. Therefore, the hexadecapeptide takes an α-helix conformation in lipid membranes with weakened intermolecular interactions.

Membrane perturbations

The effect of peptide distribution on the membrane structure was evaluated by measuring the membrane fluidity and the leakage of encapsulated substance. Fluorescence depolarization of DPH and ANS represents the fluidity of the hydrophobic core and the surface of the membrane, respectively. As shown in Fig. 7, fluorescence depolarization of ANS, that is, the fluidity of the membrane surface, decreased upon distribution of Boc-(Ala-Aib)$_n$-OMe (n = 2, 4, 6, 8) into

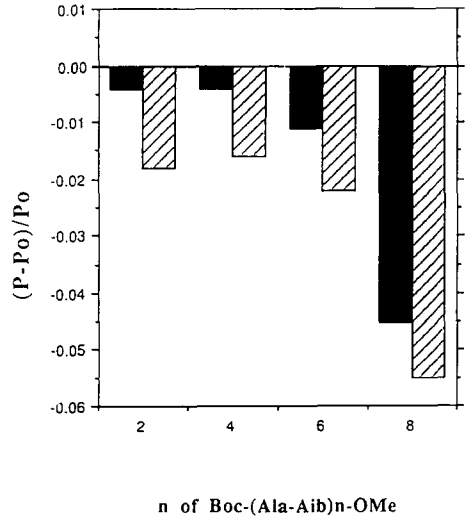


Fig. 7. Change of fluorescence depolarization of DPH, ■, and ANS, ▨, with the addition of Boc-(Ala-Aib)$_n$-OMe in the presence of DPPC liposome. ■: [DPH] = 7.5 μM, [DPPC] = 0.77 mM, and [peptide] = 12 μM. Excitation and monitore wavelengths are 357 and 457 nm, respectively. ▨: [ANS] = 0.51 mM, [DPPC] = 0.76 mM, and [peptide] = 20 μM. Excitation and monitor wavelengths are 395 and 480 nm, respectively. P_0 and P represent fluorescence depolarization without additive and with peptide, respectively.

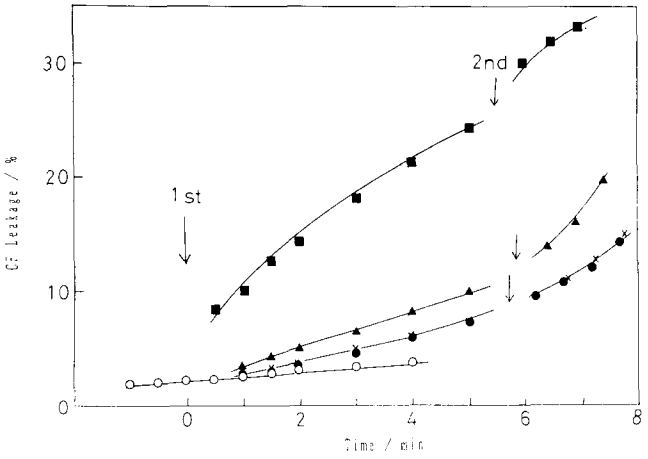


Fig. 8. CF leakage from DPPC liposome by the addition of Boc-(Ala-Aib)$_n$-OMe; n = 2, ● 4, ×; 6, ▲ 8, ■. ○, liposome without peptide. [DPPC] = $1.7 \cdot 10^{-5}$ M. [peptide] = $2.4 \cdot 10^{-6}$ M (at 1st arrow) and $4.8 \cdot 10^{-6}$ M (at 2nd arrow). Temperature was 31°C.

the membrane. The hexadecapeptide remarkably decreased the fluidity of the hydrophobic core as well as the surface of membrane, and enhanced the leakage of CF encapsulated in DPPC liposome (Fig. 8).

The reduced fluidity of the membrane reflects a tight packing of lipid molecules as a result of molecular motion suppressed by Boc-(Ala-Aib)$_8$-OMe. Since the tight membrane is a barrier to CF leakage, the enhanced CF leakage might have resulted from the channel formation by aggregation of Boc-(Ala-Aib)$_8$-OMe spanning across the membrane. This point will be discussed further subsequently.

Orientation of peptides in bilayer membrane

The position of an anthryl group of An (n = 2, 4, 6, 8, 10) in the bilayer membrane was determined by fluorescence quenching experiments with 12DS (Fig. 9). The doxyl group of 12DS was located in the middle part of the DMPC bilayer membrane. The emission of anthryl group of A6 and A8 was quenched most effectively by 12DS. When A6 and A8 take an α-helix conformation with a perpendicular orientation to the bilayer membrane, the molecular length becomes 20–25 Å and the emission from anthryl group located close to the doxyl group in the membrane is quenched effectively. On the other hand, the quenching rate of A10 by 12DS was very low. The molecular length of α-helical A10 is about 35 Å. This is somewhat too long to place the anthryl group in the middle of the bilayer membrane having the peptide oriented perpendicularly. The quenching experiment indicated that long peptides take an α-helix conformation with a perpendicular orientation to the bilayer membrane. The N-terminal portion of the peptides should be located in the depth of the membrane rather than on the surface of the membrane owing to the hydrophobic property of the anthryl group.

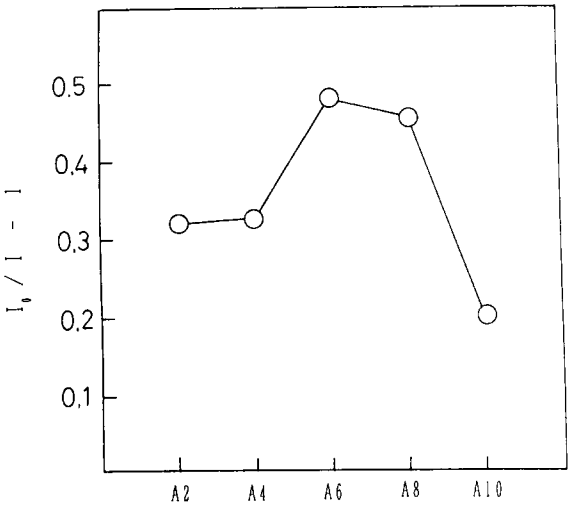


Fig. 9. Change of monomer emission intensity ($I_0/I-1$) of An by the addition of 12DS in DMPC liposome at 15°C. [DMPC] = $5.7 \cdot 10^{-4}$ M. [12DS] = $2.9 \cdot 10^{-5}$ M. The peptide concentration was in the range of $0.9 \cdot 10^{-6} - 1.0 \cdot 10^{-6}$ M. Excitation and monitor wavelengths were 367 and 414 nm, respectively.

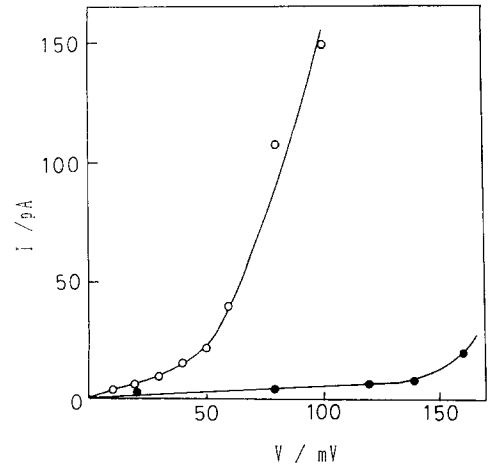


Fig. 11. Current–voltage response of Boc-(Ala-Aib)$_n$-OMe (n = 4, ● 8, ○) in a planar bilayer membrane. The diameter of a hole in Teflon film was 0.3 mm. The peptide concentration was $3 \cdot 10^{-6}$ g/ml.

Association of peptides in bilayer membrane

Fluorescence depolarization of anthryl groups of the peptides was examined in a mixture of glycerol and ethanol (1 : 4, v/v). As shown in Fig. 10a, fluorescence depolarization decreased monotonously with increasing length of peptide chains due to restricted motion of large molecules. On the other hand, in the presence of DMPC liposomes, it decreased with increasing peptide length below dodecapeptide, but increased beyond it in the order of A6 < A8 < A10 (Fig. 10b). The increasing fluorescence depolarization of longer peptides is ascribed partly to the increased fluidity around the anthryl group, which is located in the middle of the lipid bilayer membrane. However, the substantially high value of fluorescence depolarization of A10 should be due to energy migration between anthryl groups. The temperature dependence of fluorescence depolarization of A10 was studied at two different lipid concentrations (Fig. 10c). It was more extensive at lower lipid concentrations, in other words, at higher peptide concentrations which facilitate peptide aggregation. Taken together, it is suggested that A10 is incorporated into lipid bilayer membranes accompanying aggregation in which the helix axis is perpendicularly oriented to the membrane surface.

BLM measurement

A bundle of hydrophobic helical peptides should act as an ion channel in bilayer membranes. Current–voltage (I–V) response across BLM containing Boc-(Ala-Aib)$_n$-OMe (n = 4 or 8) is shown in Fig. 11. The I–V curve at higher voltages can be described by a hyperbolic function of voltage, where current increases expo-

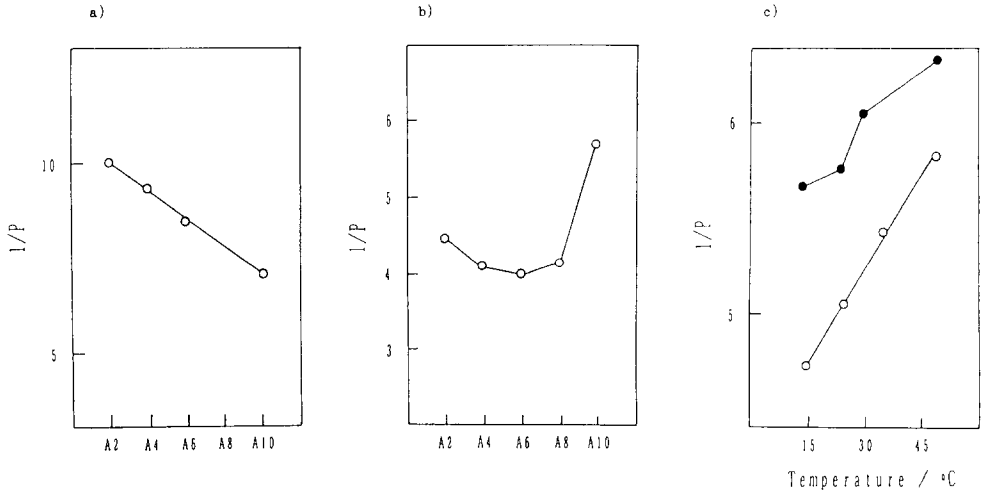


Fig. 10. Fluorescence depolarization of anthryl group of An (a) in a mixture of 20% glycerol and 80% ethanol and (b) in the presence of DMPC liposome at 15°C. (c) Temperature dependence of fluorescence depolarization of A10 at two different concentrations; ●, [DMPC] 1.7 mM and [A10] = $2.7 \cdot 10^{-6}$ M; ○, [DMPC] = 3.0 mM and [A10] = $2.4 \cdot 10^{-6}$M.

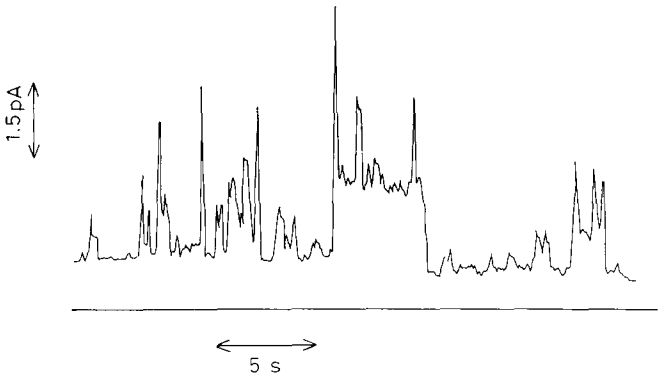


Fig. 12. Single-channel current fluctuations at a constant applied voltage of 120 mV, in the presence of Boc-(Ala-Aib)$_8$-OMe ($3.0 \cdot 10^{-7}$ g/ml). The diameter of the hole was 0.1 mm.

nentially beyond the critical voltage, V_c. V_c was about 150 mV for the octapeptide, but drasticaly decreased to about 50 mV for the hexadecapeptide.

When the membrane was kept under slightly higher voltage than V_c in the presence of peptides, fluctuation of membrane conductance was observed (Fig. 12). Although the current fluctuation was not stepwise changes between defined levels, the peptides are suggested to form ion channels with various conductance levels. This type of ion channel has been reported by Ghosh and Stroud [20] in the case of a model peptide of δ subunit of the nicotinic acetylcholine receptor, which is suggested to form a bundle of membrane-spanning helices with various sizes. Although the single-channel-like response was formed at the applied voltage of 80 mV in the case of hexadecapeptide, the octapeptide did not show such a response at the applied voltage of 200 mV. When the peptide concentration of the octapeptide was doubled, a single-channel-like response was detected at the applied voltage of 160 mV. The channel conductance and opening frequency of the octapeptide was much lower than those of the hexadecapeptide.

These peptides may be regarded as a voltage-dependent ion channel, and Boc-(Ala-Aib)$_8$-OMe is highly potent in forming this type of channel. The high ability of the hexadecapeptide in channel formation can be explained on the basis of its tendency for aggregation in bilayer membrane with a perpendicular orientation. These findings are consistent with the other report that the hydrophobic helical 21-peptide analogs span the lipid bilayer with their long axis normal to the membrane surface [21]. It should be pointed out, however, that the number of reports, which confirmed the orientation of helical peptides in the phospholipid bilayer membranes by using spectroscopic methods (e.g. Refs. 22–24), is relatively small. Although it is generally admitted that hydrophobic α-helical peptides with more than 20 amino acid residues traverse the lipid bilayer [25–27], the relation between the orientation and the peptide-chain length was not clarified clearly. In the present study, the significant differences were observed between A10 (21mer peptide) and A8 (17mer peptide) as shown in Fig. 9 and Fig. 10b. Thus, the chain length of the peptides is one of the critical factors for determining the peptide orientation in the membrane. The peptides with chain length enough to traverse the membrane predominantly take the perpendicular orientation, probably because amide protons and carbonyl groups at the N- and C-terminal regions of the helix, which don't participate in the hydrogen bonds, should be stabilized at the polar region of the membrane surface. However, A8 and Boc-(Ala-Aib)$_8$-OMe (16mer) also took the perpendicular orientation, though the fraction was less than A10, and formed an ion channel effectively.

Another important conclusion is the aggregation of the peptides in the lipid bilayer. Previously, we have reported that hydrophobic oligopeptides aggregated in the membrane of the gel state [28], suggesting that interactions between peptides are stronger than those between peptide and lipid. Although A10 is composed only of hydrophobic residues, the peptide still tends to aggregate in the membrane. Therefore, the formation of the helix bundle in the membrane is ascribed to the molecular property of the hydrophobic peptides in the membrane.

Amphiphilic helical peptides have been shown to form ion channels in the membrane [29–32]. However, amphiphilic helical peptides tend to stay at the membrane surface oriented parallel to the surface [23,33]. Therefore, further studies on the orientation of peptides in the membrane should be needed as described here.

Molecular design for voltage-dependent ion channel should be a preparation of hydrophobic helical peptide having a chain long enough to span across lipid bilayer membrane. However, the pore size, which determines the cation selectivity of the channel, is variable depending on the number of the helix rods in the bundle formed in the membrane.

References

1 Mueller, P. and Rudin, D.O. (1968) Nature 217, 713.
2 Fox, R.O., Jr. and Richards, F.M. (1982) Nature 300, 325.
3 Hall, J.E., Vodyanoy, I., Balasubramanian, T.M. and Marshall, G.M. (1984) Biophys. J. 45, 233.
4 Boheim, G., Hanke, W. and Jung, G. (1983) Biophys. Struct. Mech. 9, 181.
5 Kelsh, L.P., Ellena, J.F. and Cafiso, D.S. (1992) Biochemistry 31, 5136.
6 Karle, I.L., Flippen-Anderson, J., Uma, K. and Balaram, P. (1988) Proc. Natl. Acad. Sci. USA 85, 299.
7 Karle, I.L. and Flippen-Anderson, J.L. (1989) Biopolymers 28, 773.
8 Menestrina, G., Voges, K.-P., Jung, G. and Boheim, G. (1986) J. Membr. Biol. 93, 111.

9 Otoda, K., Kimura, S. and Imanishi, Y. (1990) Bull. Chem. Soc. Jpn. 63, 489.
10 Narita, M., Doi, M., Sugasawa, H. and Ishikawa, K. (1985) Bull. Chem. Soc. Jpn. 58, 1473.
11 Toniolo, C., Crisma, M., Bonora, G.M., Benedetti, E., Di Blasio, B., Pavone, V., Pedone, C. and Santini, A. (1991) Biopolymers 31, 129.
12 Otoda, K., Kitagawa, Y., Kimura, S. and Imanishi, Y. (1993) Biopolymers, in press.
13 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477.
14 Ohtoyo, T., Shimagakii, M., Otoda, K., Kimura, S. and Imanishi, Y. (1988) Biochemistry 27, 6458.
15 Barbet, J., Machy, P., Truneh, A. and Leserman, L.D. (1984) Biochim. Biophys. Acta 772, 347.
16 Montal, M. and Mueller, P. (1972) Proc. Natl. Acad. Sci. USA 69, 3561.
17 Vijayakumar, E.K.S., Sudha, T.S. and Balaram, P. (1984) Biopolymers 23, 877.
18 Surewicz, W.K. and Mantsch, H.H. (1988) Biochem. Biophys. Res. Commun. 150, 245.
19 Gibson, N.J. and Cassim, J.Y. (1988) Biochemistry 28, 2134.
20 Ghosh, P. and Stroud, R.M. (1991) Biochemistry 30, 3551.
21 Voges, K.-P., Jung, G. and Sawyer, W.H. (1987) Biochim. Biophys. Acta 896, 64.
22 Erne, D., Sargent, D.F. and Schwyzer, R. (1985) Biochemistry 24, 4261.
23 Vogel, H. (1987) Biochemistry 26, 4562.
24 Chung, L.A., Lear, J.D. and DeGrado, W.F. (1992) Biochemistry 31, 6608.
25 Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105.
26 Vogel, H. (1992) Quart. Rev. Biophys. 25, 433.
27 Langosch, D., Hartung, K., Grell, E., Bamberg, E. and Betz, H. (1991) Biochim. Biophys. Acta 1063, 36.
28 Uemura, A., Kimura, S. and Imanishi, Y. (1983) Biochim. Biophys. Acta 729, 28.
29 Oiki, S., Danho, W. and Montal, M. (1988) Proc. Natl. Acad. Sci. USA 85, 2393.
30 Christensen, B., Fink, J., Merrifield, R.B. and Mauzerall, D. (1988) Proc. Natl. Acad. Sci. USA 85, 5072.
31 Lear, J.D., Wasserman, Z.R. and DeGrado, W.F. (1988) Science 240, 1177.
32 Sansom, M.S.P. (1991) Prog. Biophys. Mol. Biol. 55, 139.
33 Kimura, S., Erne, D. and Schwyzer, R. (1992) Int. J. Peptide Protein Res. 39, 431.