Biochimica et Biophysica Acta, 1151 (1993) 76–82

BBAMEM 76054

Effect of salts on conformational change of basic amphipathic peptides from β-structure to α-helix in the presence of phospholipid liposomes and their channel-forming ability

Sannamu Lee [a,c], Toshihide Iwata [a], Hatsuyo Oyagi [a], Haruhiko Aoyagi [a], Motonori Ohno [a], Kazunori Anzai [b], Yutaka Kirino [b] and Gohsuke Sugihara [c]

[a] *Laboratory of Biochemistry, Department of Chemistry, Faculty of Science, Kyushu University, Higashi-ku, Fukuoka (Japan),* [b] *Faculty of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka (Japan) and* [c] *Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-ku, Fukuoka (Japan)*

(Received 15 March 1993)

Key words: Conformational transition; Alpha helix; Lipid–peptide interaction; Beta structure; Ion channel forming peptide; Liposome

A synthetic model peptide, H-(Leu-Alα-Arg-Leu)$_3$-(Leu-Arg-Alα-Leu)$_3$-OH (4_6) can form ion channels in planar lipid bilayers by taking an amphipathic α-helix (Agawa, Y., Lee, S., Ono, S., Aoyagi, H., Ohno, M., Taniguchi, T., Anzai, K. and Kirino, Y. (1991) J. Biol. Chem. 266, 20218–20222). For further study of ion channels formed by this type of peptides, we planned to synthesize [Trp^1]-4_6(Ser) and [Trp^{12}]-4_6(Ser) in which a hydrophilic amino acid, Ser, was introduced in several positions of 4_6 instead of hydrophobic ones. This modification was expected to decrease the ability of membrane perturbation and to simplify various current levels of the channel observed for 4_6. Furthermore, additional Trp was introduced to the N-terminus or position 12 to monitor the lipid–peptide interaction. CD study showed that both peptides formed a random structure in buffer, but an α-helix in the presence of egg PC and a β-structure in egg PC/egg PG (3 : 1). Moreover, addition of NaCl to the acidic liposomes induced the conformational transition in the peptide from β-structure to α-helix. Salt-induced conformational transition in the presence of acidic liposomes was discussed in terms of membrane binding and ion-channel formation in planar lipid bilayer. Despite introduction of hydrophilic residues instead of hydrophobic residues in 4_6, the peptide showed nearly the same dye-release ability from egg PC- egg PG liposomes as 4_6. [Trp^{12}]-4_6(Ser) was able to form cation-selective ion channels with two levels of conductance (mainly 250 and occasionally 125 pS) in asolectin planar lipid bilayer, suggesting that appropriate orientation of hydrophobic and hydrophilic residues in amphipathic peptide can simplify channel current levels.

Introduction

Various ion channels have been found to have a function of controlling ion gradients between inner and outer of cell membranes, which are divided into voltage-gated and ligand-gated with types of their activating objects [1,2]. On the other hand, many amphipathic α-helical peptides, such as gramicidin A [3,4], alamethicin [5], *d*-toxin [6], melittin [7], mastoparan [8], cecropin [9] and magainin I [10], have also been found to form ion channels to show their antimicrobial and toxic activity. Despite the large number of investigations, there has scarcely been a systematically detailed study of amphipathic peptides on the relationship among their structure, channel formation and biological activity.

Therefore, we have developed the studies on the relationship between peptide structure and ion-channel-forming ability using several amphipathic model peptides [11,12]. Among those model peptides an amphipathic basic helical peptide 4_3 with 12 residues in the sequence was found to form cation-selective ion channels probably with head-to-tail structure [11]. A peptide named 4_6 with 24 amino-acid residues (Fig. 1), which can span lipid bilayers by itself by forming an

Correspondence to: S. Lee, Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814-01, Japan.

Abbreviations: Ac, acetyl; Boc, *t*-butyloxycarbonyl; Bzl, benzyl; DiphytPC, diphytanoyl-L-α-phosphatidylcholine; DPPC, dipalmitoyl-D,L-α-phosphatidylcholine; DPPG, dipalmitoyl-D,L-α-phosphatidylglycerol; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; egg PC, egg-yolk phosphatidylcholine; egg PG, egg-yolk phosphatidylglycerol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; Tos, *p*-toluenesulfonyl; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

Ac-(Leu-Ala-Arg-Leu)3-NHCH3	43
(Leu-Ala-Arg-Leu)3-(Leu-Arg-Ala-Leu)3	46
Leu-Ala-Arg-Ser-Leu-Ala-Arg-Ser-Leu-Ser-Arg-Trp-Ser-Arg-Ala-Leu-Ala-Arg-Ser-Leu-Ser-Arg-Ala-Leu	[Trp12]-46(Ser)
Trp-Ala-Arg-Ser-Leu-Ala-Arg-Ser-Leu-Ser-Arg-Leu-Ser-Arg-Ala-Leu-Ala-Arg-Ser-Leu-Ser-Arg-Ala-Leu	[Trp1]-46(Ser)

Fig. 1. Structure of model peptides.

amphipathic α-helix in the presence of lipid bilayers, formed ion channels in the planar bilayer [12]. Interestingly, the cationic peptides showed cation selectivity. Thus, we needed more detailed analysis of these channels. However, it was difficult because of the appearance of channels with various single-channel conductances and the very strong membrane disrupting abilities of 4_3 and 4_6.

In order to find a suitable model for studying the properties of ion channel formed by amphipathic basic α-helical peptide, [Trp^1]-4$_6$(Ser) and [Trp^{12}]-4$_6$(Ser) (Fig. 1) were designed to synthesize. We investigated the peptide conformation in the presence and absence of liposomes, membrane perturbation ability, ion-channel formation and antimicrobial activity.

Materials and Methods

Materials. Egg PC, egg PG, DPPC and DPPG were purchased from Sigma. Asolectin, Type II-S from Sigma, was purified by acetone washing. Carboxyfluorescein from Eastman Kodak was purified by recrystallization from ethanol/water mixture. All other reagents were of the highest analytical grade.

Synthesis of peptides. [Trp^{12}]-4$_6$(Ser) and [Trp^1]-4$_6$(Ser) were synthesized by the solution method. Synthetic route for [Trp^{12}]-4$_6$(Ser) is outlined in Fig. 2. Short fragments were prepared stepwise from the C-terminus, and the fragments were condensed to the desired sizes by the EDC-HOBT method [13]. During the condensation, the N-terminus was protected by the Boc group, and the C-terminus was protected by the phenacyl or benzyl ester. After treatment of the fully protected peptides with HF, the free peptide obtained was converted to the corresponding acetate by passing through a Sephadex G-15 column. The crude peptide obtained was purified by reverse-phase high performance liquid chromatography (TSK ODS-120T, 0.46 × 25 cm) using an acetonitrile gradient in the aqueous phase of 0.1% TFA. Data of amino-acid analysis after

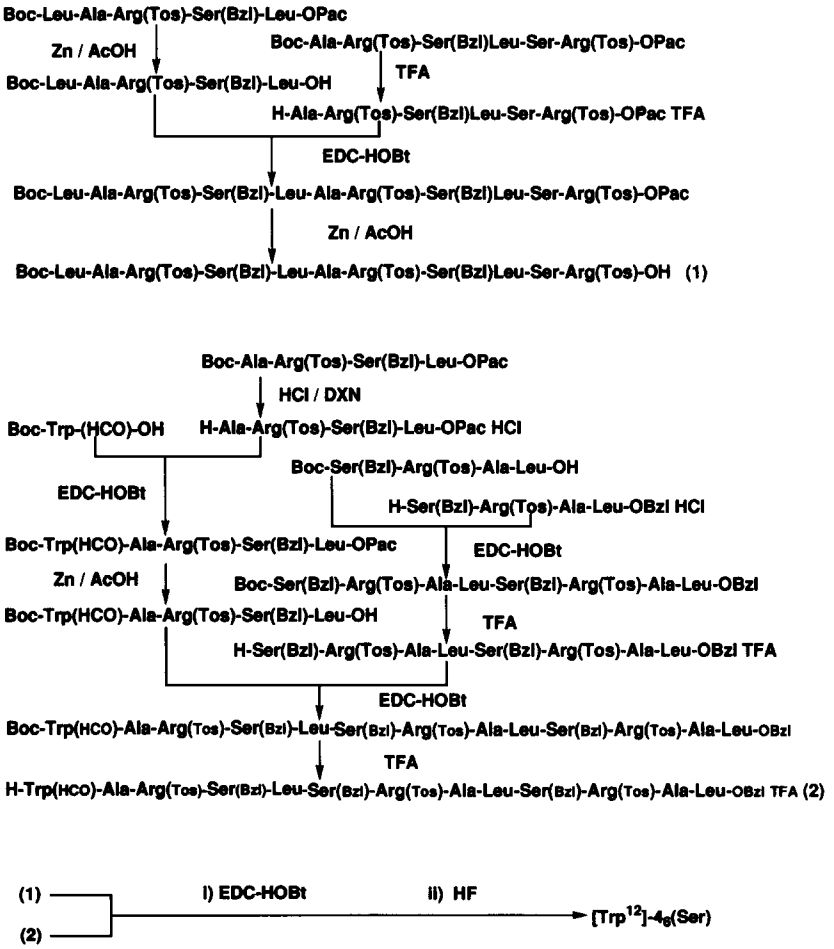

Fig. 2. Synthetic route for [Trp^{12}]-4$_6$(Ser).

hydrolysis in 6 M HCl were Ala 5.0, Ser 5.6, Leu 6.1 and Arg 5.5 in molar ratio.

Preparation of phospholipid liposomes. Phospholipid (20 mg) was dissolved in $CHCl_3$ (about 2 ml) and dried under a stream of N_2 gas in a conical glass tube and keeping in vacuo overnight. The dried lipid was hydrated in 2 ml of 5 mM Hepes buffer (pH 7.4) with repeated vortex-mixing at 25°C for egg PC and egg PG and 50°C for DPPC and DPPG for 30 min. The suspension was sonicated at the same temperature as hydration described above for 1 h using a Tomy Seiko ultrasonic disrupter model UR-200P and diluted to 25 ml with the same buffer, in which phospholipid concentration was approx. 1.1 mM. The mixture of uni- and multilamellar vesicles were used for the CD measurement without further purification. The unilamellar vesicles trapping carboxyfluorescein were prepared as described above, except that the dried lipid was hydrated in 2.0 ml of 5 mM Hepes buffer (pH 7.4) containing 100 mM carboxyfluorescein. The mixture of uni- and multilamellar vesicles trapping carboxyfluorescein was subjected to gel filtration through a Sepharose 4B column (1 × 20 cm) in 5 mM Hepes buffer (pH 7.4). The separated small unilamellar vesicles were used in carboxyfluorescein leakage measurement. The lipid concentration was determined by an assay using the phospholipids test reagent purchased from Wako (Osaka, Japan) and was expressed in terms of phosphorus concentration.

CD experiment. CD spectra were recorded on a JASCO J-600 spectropolarimeter using a quartz cell of 1 mm pathlength. Spectra in 5 mM Hepes buffer (pH 7.4) were measured at a peptide concentration of 10 μM and at a lipid concentration of 1 mM. Peptide concentration in solution was determined by amino-acid analysis using a Hitachi Model 835 high speed amino-acid analyzer. All the measurements were carried out at room temperature. The CD data were expressed as mean residue ellipticities.

Carboxyfluorescein leakage. The dye-leakage experiment was carried out as described previously [14]. To 2 ml of 5 mM Hepes buffer (pH 7.4) in a cuvette was added 50 μl of the vesicles containing 100 mM carboxyfluorescein which was prepared as described in above and then, several quantities of an appropriate dilution of peptides in 5 mM Hepes buffer were add to the solutions. The fluorescence intensity was recorded at 3 min after the addition of the peptides. Complete dye-release from the liposome was obtained by addition of 10 μl of Triton X-100 solution (20% in Hepes buffer) to the vesicles. The percentage of dye-release caused by peptides was evaluated by the equation, $100 \times (F - F_0)/(F_t - F_0)$, where F is the fluorescence intensity achieved by addition of peptides, F_0 and F_t are intensities observed without peptides and after Triton X-100 treatment, respectively.

Titration study. Titration of the peptides with phospholipids was examined to obtain the information of the peptide orientation in lipids, because the blue shift reflects the incorporation of a Trp residue into the lipid bilayer. Several quantities (5, 10, 20 and 30 μl) of lipid solution of a concentration of 1.1 mM were added to the solution of peptides (2.5 ml) at the concentration of 6.0 μM and mixed for 10 min until it reached equilibrium. The tryptophan fluorescence of the solution was recorded in the range of 300 nm to 400 nm with excitation at 280 nm.

Planar bilayer and current measurement. The planar lipid bilayer membranes were formed by the folding method as described previously [11,15]. Asolectin was used as a bilayer-forming lipid. Small quantity (usually 5–10 μl) of the peptide dissolved in methanol was added to one compartment (*cis* is side) of the chamber with stirring. Membrane current measurement was performed under voltage-clamp conditions (usually +40 mV). The membrane potential was defined as the potential of *cis* with respect to *trans* compartments. The current across the bilayer was measured with a hand-made current voltage converter (bandwidth 800 Hz) and displayed on both a digital storage oscilloscope (DSS5020A, Kikusui Electronics, Kawasaki) and a chart recorder (R61VL, Rikadenki, Tokyo). The data were recorded on videotape with a videotape recorder after A/D conversion with a modified digital audio processor (PCM-501ES, Sony).

Results

Peptides designed

In these peptides, a hydrophilic amino acid, Ser, was introduced in several positions of 4_6 in an attempt to decrease the ability of membrane perturbation. Tryptophan was also introduced in position 12 and the N-terminus, respectively, to monitor the lipid–peptide interaction (Fig. 1).

As shown in Fig. 3, 4_6, [Trp^1]-4_6(Ser) and [Trp^{12}]-4_6(Ser) have a hydrophilic region on one side and a hydrophobic region on the other side, and they are amphipathic on the whole. The hydrophilic region is increased from 1/4 in 4_6 to 1/2 in [Trp^1]-4_6(Ser) and [Trp^{12}]-4_6(Ser). Increase in the hydrophilic region may make the ability of membrane perturbation of [Trp^1]-4_6(Ser) and [Trp^{12}]-4_6(Ser) less than that of 4_6.

CD study

CD spectra of the synthetic peptide [Trp^{12}]-4_6(Ser) in Hepes buffer (pH 7.4) and in the presence of vesicles are shown in Fig. 4. The studies showed that the peptide has a marked conformational sensitivity to the environment. In the buffer, [Trp^{12}]-4_6(Ser) exhibited minimum below 200 nm, showing that it takes no significant secondary structure (Fig. 4a). In the pres-

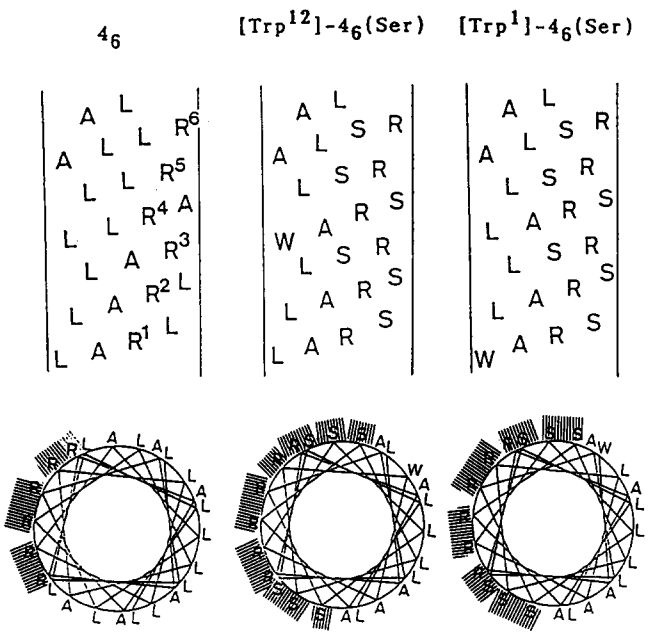

Fig. 3. α-Helical nets and wheels of peptides 4_6, [Trp^{12}]-4_6(Ser) and [Trp^{1}]-4_6(Ser). In helical wheels, hydrophilic amino-acid residues are shaded.

ence of neutral phospholipid vesicles, it had a double minimum at 208 and 222 nm characteristic of α-helix (Fig. 4b). Interestingly, an addition of acidic liposomes instead of neutral liposomes changed the spectrum to an one composed of mainly β-structure with a minimum near 220 nm (Fig. 4c). We have previously reported that a model peptide, Ac-(Ser-Val-Lys-Val)$_2$-$NHCH_3$, with 8 amino-acid residues changed its conformation from β-structure to α-helix with adding NaCl [17]. Therefore, CD spectra of [Trp^{12}]-4_6(Ser) were measured at the concentrations of 0, 50, 100 and 200 mM NaCl in buffer and in vesicles. In buffer and in neutral vesicles, the peptide remained in a mixture of random-like unordered structure and α-helix regardless the presence and absence of NaCl, respectively, whereas in acidic vesicles the β-structure changed to α-helix with increasing NaCl concentration. These results suggest that the charge interaction of the peptide with acidic phospholipids induces the β-structure, whereas the presence of NaCl diminishes such interaction, consequently, similarly to neutral phospholipid liposomes the α-helix is formed by the hydrophobic interaction between the peptide and phospholipid vesicles.

Leakage of carboxyfluorescein from liposomes

In order to study the interaction of [Trp^{12}]-4_6(Ser) with model membrane, the ability of membrane perturbation was examined by measuring its effect on release of carboxyfluorescein from phospholipid liposomes in comparison with 4_6 and 4_3 [18]. Profiles of the dye-leakage from egg PC/egg PG (3:1) liposomes caused by peptides are shown in Fig. 5. The ability of membrane perturbation of [Trp^{12}]-4_6(Ser) was slightly inferior to that of 4_6.

Titration study

To attain further information on the peptide-lipid interaction in the lipid bilayer, the interactions of [Trp^{12}]-4_6(Ser) and [Trp^{1}]-4_6(Ser) with egg PC and egg PC- egg PG (3:1) liposomes were monitored by measuring tryptophan fluorescence (Figs. 6 and 7). The emission spectrum of [Trp^{12}]-4_6(Ser) in the buffer solution where the peptide is present in random structure showed a maximum peak at 346 nm. Upon addition of egg PC vesicles in which [Trp^{12}]-4_6(Ser) formed α-helix, the fluorescence of the maximum increased strongly and shifted to 327 nm (Fig. 6). Such a fluorescence change induced by binding to neutral liposomes can be explained by the change in the state of Trp^{12} from a hydrophilic to a hydrophobic environment.

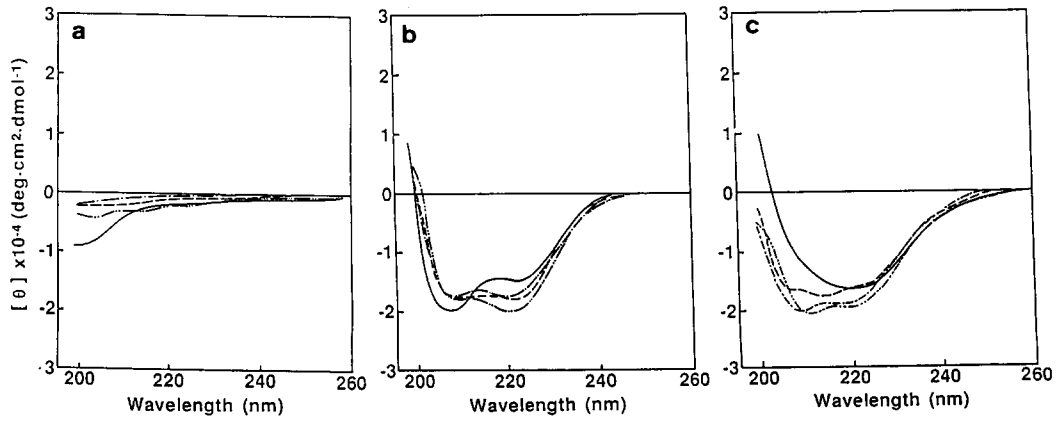

Fig. 4. CD spectra of [Trp^{12}]-4_6(Ser) in various media. Spectra were measured (a) in Hepes buffer (pH 7.4), (b) in the presence of DPPC liposomes and (c) in the presence of DPPC-DPPG (3:1) liposomes as a function of NaCl concentration. Peptide concentration was 10 μM and lipid concentration was about 1 mM. NaCl concentration is zero (—), 50 mM (---), 100 mM (-·-) and 200 mM (-···).

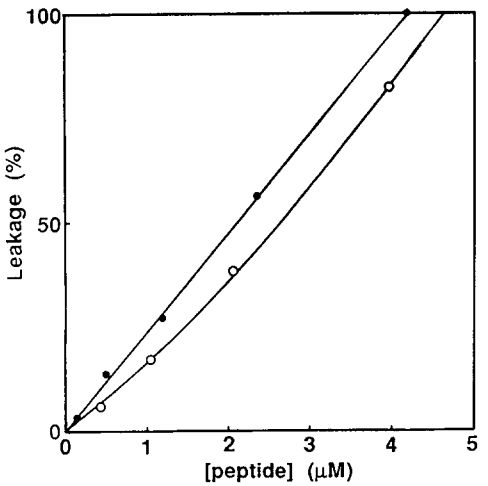

Fig. 5. Profiles of carboxyfluorescein leakage from egg PC/egg PG (3:1) liposomes induced by model peptides. Individual peptides were incubated at 25°C in 2 ml of 5 mM Hepes buffer (pH 7.4) containing 86 μM egg PC/egg PG (3:1) liposomes at various peptide concentrations. Peptide 4_6 (•) and [Trp^{12}]-4_6(Ser) (○) The fluorescence intensity was recorded at 515 nm with excitation at 470 nm.

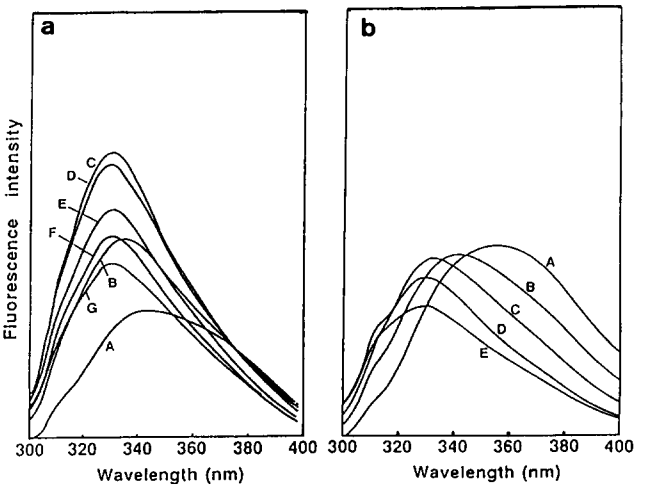

Fig. 7. Fluorescence emission spectra of [Trp^{12}]4_6(Ser) (a) and [Trp^1]-4_6(Ser) (b) in egg PC/egg PG (3:1) liposomes as a function of the lipid concentration. Peptide concentration was 6 μM. [Lipid]/[Peptide]; A (peptide only); B, 6; C, 12; D, 24; E, 36; F, 60 and G, 120.

The emission spectra of [Trp^1]-4_6(Ser) in the buffer solution gave a maximum peak at 354 nm. Upon addition of egg PC liposome, the blue shift to 330 nm was also observed, but the intensity of maximum peak of [Trp^1]-4_6(Ser) did not increase as much as that of [Trp^{12}]-4_6(Ser). In lipid bilayers, the Trp residue in [Trp^1]-4_6(Ser) is located to less hydrophobic environment than that in [Trp_6^{12}]$^{-4}$(Ser).

The fluorescence spectra of [Trp^{12}]-4_6(Ser) forming β-structure in the presence of egg PC/egg PG (3:1) vesicles shifted to shorter wavelength with increasing lipid concentration as observed in neutral liposomes (Fig. 7). On the other hand, the fluorescence intensity of maximum peak increased gradually at a concentration ratio below [L]/[P] = 24 and decreased at a lipid concentration above L/P = 24. Such fluorescence change may indicate conformational change of peptide from random structure to β-structure. A similar fluorescence spectrum change was observed for [Trp^1]-4_6(Ser) in the presence of the acidic lipids, although the intensity of maximum peak of [Trp^1]-4_6(Ser) was changed less than that of [Trp^{12}]-4_6(Ser).

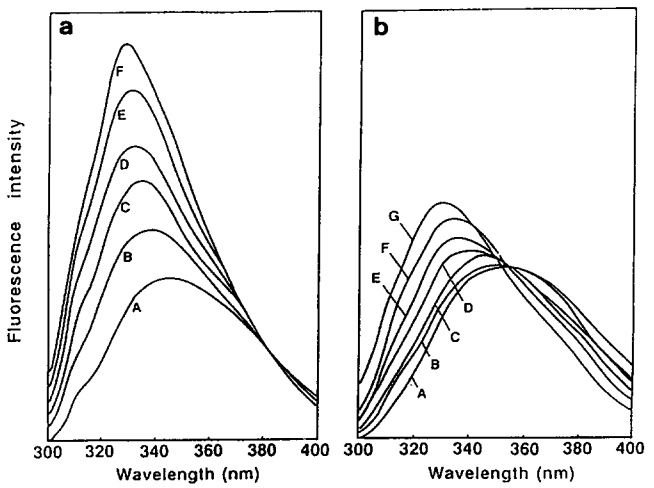

Fig. 6. Fluorescence emission spectra of [Trp^{12}]4_6-(Ser) (a) and [Trp^1]4_6-(Ser) (b) in egg PC liposomes as a function of the lipid concentration. Peptide concentration was 6 μM. [Lipid]/[Peptide]; A (peptide only); B, 6; C, 12; D, 24; E, 36; F, 60 and G, 120.

The difference in the magnitude of blue shifts of the wavelengths or increase in the fluorescence intensities at maximum peaks between [Trp^{12}]-4_6(Ser) and [Trp^1]-4_6(Ser) reveals that Trp residue in [Trp^{12}]-4_6(Ser) is present in more hydrophobic surroundings than that in [Trp^1]-4_6(Ser). These results suggest that Trp residue of [Trp^{12}]-4_6(Ser) is located at the core part of phospholipid bilayer and [Trp^1]-4_6(Ser) is located at a hydrophobic part near the head groups of phospholipids such as H-belt region. The reduction of maximum peak intensity, however, can not be explained clearly. It may be due to the quenching of the fluorescence according to an access of chromophore groups on the aggregates of peptide molecules by a conformational change from random to β-structure induced with increased charge interaction or according to access of anions in phospholipid head groups.

Measurement of ion channel activity

The planar bilayer method was used to determine whether [Trp^{12}]-4_6(Ser) was able to form ion channels in phospholipid bilayer membranes. We have used an acidic phospholipid, asolectin, as a membrane-forming lipid. Asolectin, soybean phospholipids, consist of 41% PC, 38% PE, 18%PA and 3% others (Nakagawa, S., personal communication). Records of current across the planar lipid bilayer doped with the peptide under

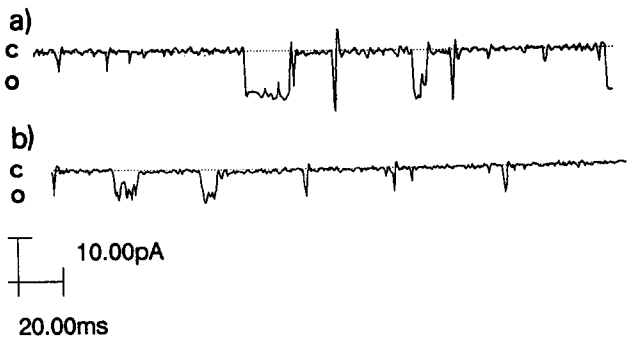

Fig. 8. Traces of current, induced by addition of [Trp^{12}]-4$_6$(Ser), across a planar bilayer membrane made of asolectin at a holding voltage of +40 mV. The peptide was added to cis compartment of the chamber. The solution was symmetrical 100 mM KCl and 10 mM Tris-Hepes (pH 7.4). The downward deflection corresponds to a current from the *cis* to the *trans* compartment. Symbols C and O indicate current levels corresponding to closed and open states of channel, respectively.

study observed at +40 mV in symmetric 100 mM KCl and 10 mM Tris-Hepes (pH 7.4) are shown in Fig. 8. The single-channel conductance was about 250 pS (Fig. 8a). A 125 pS channel with similar gating behavior was also observed in different experiments (Fig. 8b). Open-channel duration of these channels (about several tens of ms) was shorter than that observed for the 4$_6$ peptide (more than several hundreds of ms) [12]. These channels were cation-selective based on the direction of the current (*cis* to *trans*) in asymmetric KCl solutions (400 mM/100 mM for *cis*/*trans*) at zero membrane potential (data not shown). Asolectin was not available for the previous channel formation experiment with 4$_6$, since 4$_6$ disrupted its bilayers. [Trp^{12}]-4$_6$(Ser) was found to be able to form the ion channel even in asolectin, indicating that the ability of membrane perturbation of [Trp^{12}]-4$_6$(Ser) is weaker than that of 4$_6$.

Discussion

Peptides [Trp^1]-4$_6$(Ser) and [Trp^{12}]-4$_6$(Ser) were designed for facilitating the analysis of the character of channel formed by amphipathic α-helical peptides. We reported previously that 4$_6$ formed α-helix in the presence of neutral and acidic phospholipid. The present CD experiment showed that [Trp^{12}]-4$_6$(Ser) formed α-helix in neutral phospholipids vesicles as observed for 4$_6$ and interestingly β-structure in acidic phospholipid vesicles. It should be kept in mind that this peptide is impossible to take amphipathic structure when it adopts β-structure. Moreover, addition of NaCl to the acidic liposomes induced the conformational change from β-structure to α-helix. We have found such conformational change on the amphipathic β-structural peptides which took β-structure in acidic liposomes in the absence of NaCl [17]. These conformational changes may explain that in the absence of NaCl the electrostatic interaction between anionic head group in acidic phospholipid and cationic group in peptide induces the β-structure, but in neutral liposomes or in acidic liposomes in the presence of NaCl, some or complete reduction of the electrostatic interaction, which facilitates the hydrophobic interaction between hydrophobic part of peptide and phospholipid vesicles, leads it to amphipathic α-helical structure.

The ability of membrane perturbation of [Trp^{12}]-4$_6$(Ser) was similar to that of 4$_6$. The increase from a quarter to half in hydrophilic area of amphipathic α-helical peptide as shown in Fig. 4, could not make the ability of membrane perturbation weaker. However, [Trp^{12}]-4$_6$(Ser) was able to form ion channels in asolectin planar bilayer while 4$_6$ disrupted the bilayer. The leakage ability obtained using small unilamellar vesicles may not always be simply correlated to the disrupting ability on planner lipid bilayer. A similar finding has been described in our previous paper where the leakage ability of the amphipathic 3$_{10}$ helical peptides such as H-(Leu-Arg-Leu)$_8$-OH having a hydrophilic amino acid at every three residues in their sequences, in which their hydrophilic regions occupy 1/3 in helical wheel, is much stronger than that of 4$_6$, in which hydrophilic residues are 1/4 in its sequence [22]. Therefore, we assume that an ability of membrane perturbation by peptides may depend not only on an affinity for membrane determined by the ratio of hydrophilic and hydrophobic regions, but also on facility of penetration into the lipid bilayer. This is because leakage ability is correlated to the penetration of the hydrophobic part of the peptides into the hydrophobic core of liposomes [16].

Such a view is correlated to the result that both model peptides exhibited no or weak antimicrobial activity. The amphipathic 3$_{10}$-helical peptides described above also showed no antimicrobial activity. The peptide reacts with bacterial membrane to perturb it but cannot lyse it due to its weak hydrophobicity.

The CD study showed that [Trp^{12}]-4$_6$(Ser) can adopt several different conformations under specific environmental conditions. In particular the different lipids or different ion strengths induce the conformational change of [Trp^{12}]-4$_6$(Ser) from β-structure to α-helix. These conformational transitions may facilitate the initiation process of the channel formation in lipid bilayer. Amphipathic peptides in bulk aqueous solution would be an unordered structure. Upon approaching a lipid surface, the peptide may adopt a β-structure by the charge interaction between lipid–peptide. This form could be the conformation that initially inserts into the hydrophilic part of lipid bilayers. In this situation, such β-structure may be present with an aggregated form. Insertion should lead to a preferential amphipathic α-helical conformation and finally would

span the membrane to form an oligomer with ion-channel activity.

The current measurement with a planar asolectin membrane showed that [Trp^{12}]-4$_6$(Ser) peptide formed a stable ion channel at the concentration of 0.5 μM, the same as at that of 4$_6$ measured in DiphytPC [12]. The conductance of the channels mainly observed was 250 pS and 125 pS. The former is twice as large as the latter one. Since peptide 4$_6$ gave several conductance levels which could not be attributed to integral multiple of smaller ones, introduction of a hydrophilic residue into hydrophobic part of 4$_6$ appears to simplify the conductance levels. It is probable that the difference of the conductance is due to different conformation or different number of aggregated molecules in a bundle. It is quite reasonable that [Trp^{12}-4$_6$]-(Ser) takes an α-helical structure under the conductance measuring conditions, that is, in the presence of acidic liposomes in 100 mM KCl. The clear segregation of hydrophobic and hydrophobic parts in [Trp^{12}]-4$_6$(Ser) as compared 4$_6$ (see α-helical nets in Fig. 3) may prohibit various conformations and/or different oligomeric bundles, resulting in the reduction of different conductance levels.

It is of interest that the life-time of open-close states in the [Trp^{12}-4$_6$]-(Ser) channels is shorter than that observed for 4$_6$. Transmembrane ion channels must be composed of a bundle of amphipathic α-helices which assemble at the lateral sides in bilayer and have an ion-conducting pore at the center. The rate (or time constants) of the association and dissociation of the bundle formation seem to be determined by the shapes and area of the hydrophobic region in amphipathic peptides by substitution of Leu to Ser.

Fluorescence study of the model peptides indicates that Trp-1 and Trp-12 may be located at or near boundary of the ester link (H-belt) part and at lipid core in lipid bilayer, respectively. This result supports that these amphipathic peptides are able to span lipid bilayer by forming ion channels.

Basic amphipathic peptides, such as cecropin [19], magainin [20] dermaceptine [21], have been found to show a strong antimicrobial activity. We previously reported that peptide 4$_6$ was effective against Gram-positive and -negative bacteria, although relatively weak (50–100 μg/ml) [12]. [Trp^{12}]-4$_6$(Ser) exhibited a weak antibacterial activity only against the Gram-positive bacteria, *B. subtilis*. Minimum inhibitory concentration was 50 μg/ml. However, it had no activity against other microorganisms such as Gram-positive bacteria *S. aureus* and the Gram-negative bacteria *E. coli* and *S. flexneli*. [Trp^{1}]-4$_6$(Ser) showed no antimicrobial activity.

Recently, anion-dependent conformational transition of basic amphiphilic polypeptides has been reported [23,24]. It has been shown that anions bind preferentially to cations in peptides to induce the compact secondary structure. Such salt-induced conformational change may play an important role in the function of some globular proteins. As shown in the present study, conformational change of amphipathic peptide is firstly induced in coexistence of acidic phospholipid and salts. Such conformational change can also play an important role in folding or acting of the peptide and protein associated with membrane.

Acknowledgements

We thank Prof. H. Nishikawa and Dr. S. Ando (Fukuoka University) for performing CD measurements. We also thank Dr. N.G. Park (Kyushu University) for helpful discussion in peptide synthesis. This work was supported in part by a Grant-in-Aid for Scientific Research (c) and by the Asahi Glass Foundation for Industrial Technology.

References

1 Tsien, R.W., Hess, P., McClesfey, E.W., Rosenberg, R.L. (1987) Annu. Rev. Biophys. Biophys. Chem. 16, 265–290.
2 Hucho, F. and Hilgenfeld, R. (1989) FEBS Lett. 257, 17–23.
3 Wallace, B.A. and Ravikumar, K. (1988) Science 241, 182–187.
4 Langs, D.A. (1988) Science 241, 188–191.
5 Hall, J.E., Vodyanoy, I., Balasubramanian, T.M. and Marshall, G.R. (1984) Biophys. J. 45, 233–247.
6 Mellor, I.R., Thomas, D.H. and Sansom, M.S.P. (1988) Biochim. Biophys. Acta 942, 280–294.
7 Tosteson, M.T. and Tosteson, D.C. (1981) Biophys. J. 36, 109–116.
8 Okumura, K., Inui, K., Hirai, Y. and Nakajima, T. (1981) Biomed. Res. 2, 450–452.
9 Christensen, B., Fink, J., Merrifield, R.B. and Mauzerall, D. (1988) Proc. Natl. Acad. Sci. USA 85, 5072–5076.
10 Duclohier, H., Molle, G. and Sach, G. (1989) Biophys. J. 56, 1017–1021.
11 Anzai, K., Hamasuna, M., Kadono, H., Lee, S., Aoyagi, H. and Kirino, Y. (1991) Biochim. Biophys. Acta 1064, 256–266.
12 Agawa, Y., Lee, S., Ono, S., Aoyagi, H., Ohno, M., Taniguchi, T., Anzai, K. and Kirino, Y. (1991) J. Biol. Chem. 266, 20218–20222.
13 König, W. and Geiger, R. (1970) Chem. Ber. 103, 788–798.
14 Suenaga, M., Lee, S., Park, N.G., Aoyagi, H., Kato, T., Umeda, A. and Amako, K. (1989) Biochim. Biophys. Acta 981, 143–150.
15 Montal, M. and Mueller, P. (1972) Proc. Natl. Acad. Sci. USA 69, 3561–3566.
16 Papahadjopoulos, D., Moscarello, H., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335.
17 Ono, S., Lee, S., Mihara, H., Aoyagi, H., Kato, T. and Yamasaki, N. (1990) Biochim. Biophys. Acta 1022, 237–244.
18 Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A. (1977) Science 195, 487–492.
19 Merrifield, R.B., Vizioli, L.D. and Boman, H.G. (1982) Biochemistry 21, 5020–5031.
20 Zasloff, M. (1987) Proc. Natl. Acad. Sci. USA 84, 5449–5453.
21 Mor, A., Nguyen, V.H., Delfour, A., Migliore-Samour, D. and Nicolas, P. (1991) Biochemistry 30, 8824–8830.
22 Iwata, T., Lee, S., Oishi, O., Aoyagi, H., Ohno, M., Shinozaki, K., Anzai, K. and Kirino, Y. (1991) in Peptide Chemistry 1991 (Suzuki, N., ed.), pp. 175–178, Protein Research Foundation, Osaka.
23 Goto, Y. and Aimoto, S. (1991) J. Mol. Biol. 218, 387–396.
24 Goto, Y. and Hagihara, Y. (1992) Biochemistry 31, 732–738.