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Design and synthesis of basic peptides having amphipathic β-structure and their interaction with phospholipid membranes

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Basic amphipathic β-structural peptides, Ac-(Ser-Val-Lys-Val)$_n$-NHCH$_3$ (1_n, $n = 1–3$) and Ac-(Lys-Val)$_n$-NHCH$_3$ (2_n, $n = 2–4$), were synthesized and their interaction with DPPC and DPPC-DPPG (3:1) bilayers was studied by CD, dye-leakage and fluorescence experiments. The CD data indicated that oligopeptides consisting of more than eight residues with alternating hydrophobic (Val) and hydrophilic amino acids (Ser and Lys) were able to form an amphipathic β-structure in acidic phospholipid bilayers, but not or weakly in aqueous solution and in neutral phospholipid bilayers. The dye-leakage experiment showed that the basic amphipathic β-structural peptides interact with acidic phospholipid bilayers to perturb them, but less effectively compared with basic amphipathic α-helical peptides. Fluorescent spectroscopic data suggest that hydrophobic side of the amphipathic peptides may immerse into membrane without deep penetration. Based on these results, we postulate that the formation of the basic amphipathic β-structure on acidic lipid bilayers may be due to the combined effect of electrostatic and hydrophobic interactions between basic peptides and acidic lipid bilayers.

Introduction

Currently, there is growing interest in the conformation on binding site of biologically active peptides since their activities acting on biomembranes are evoked by conformational changes induced by the interaction with biomembranes [1–3]. Since membrane surfaces provide an amphipathic environment, the affinity of the peptides to membranes would depend on an ability to form amphipathic structures in the amphipathic environment. There are several reports on the relationship between amphipathic nature of helical peptides and their biological activity (for reviews see Refs. 1 and 2) [4,13,14,19]. However, the structure-function relationship of β-structural amphipathic peptides has not been studied precisely.

The polypeptides composed of alternating hydrophobic and hydrophilic amino acid residues have been considered to favor an amphipathic β-structure, since the side chains of component residues project alternately up and down at the plane of the peptide backbone [5]. For example, sequential polypeptides with high molecular weight such as poly(Lys-X) (X is Ala, Leu or Ser) and poly(Glu-X) (X is Ala or Val) take a β-structure, e.g., in the salt solutions, at low and high pH region [5–7] or in surfactant solution [8]. There is, however, little information concerning oligopeptides with alternating hydrophobic-hydrophilic amino acid residues. It has been reported that a cooligopeptide, (Val-Glu-Val-Orn)$_3$-Val, in which alternating acidic and basic residues are aligned on the hydrophilic face, forms a β-structure in an aqueous solution and in the presence of low-density lipoproteins and a mixture of lipids [9,10]. Altmann et al. showed that oligopeptides (Leu-Ser)$_n$ and (Val-Thr)$_n$ with non-ionizable side chains took a β-structure in organic solvents [11]. Recently, De-

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Abbreviations according to IUPAC-IUB Commission (1984) European J. Biochem, 138, 9–37, are used throughout. Others: DPPC, dipalmitoyl-D,L-α-phosphatidylcholine; DPPG, dipalmitoyl-D,L-α-phosphatidylglycerol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EDC, 1-ethyl(3,3'-dimethylaminopropyl)-carbodiimide; HOBt, 1-hydroxybenzotriazole; Pac, phenacyl; TFA, trifluoroacetic acid.

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Ac-(Ser-Val-Lys-Val)$_n$-NHCH$_3$	1_n (n=1-3)
Ac-(Lys-Val)$_n$-NHCH$_3$	2_n (n=2-4)
Ac-Ser-Val-Lys-Val-Ser-Trp-Lys-Val-NHCH$_3$	3
Ac-(Leu-Ala-Arg-Leu)$_3$-NHCH$_3$	4_3

Fig. 1. Structures of model amphipathic β-structural peptides and α-helical peptide 4_3. All amino acids are of the L-configuration.

Grado and Lear reported that Fmoc-(Leu-Lys)$_3$-Leu took a β-structure in aqueous solutions (pH 7.3) and in a monolayer state of the peptide [12]. Such repeating peptides are thus implicated in taking a stable amphipathic β-structure in suitable environments, but their biological activities and the behavior in biomembranes remains unsolved.

To shed further light on the structure-function relationship of basic amphipathic β-structural peptides, we planned to synthesize several model peptides composed of alternating hydrophobic and hydrophilic residues as shown in Fig. 1. In the present work, we studied the interaction of basic amphipathic β-structural peptides with liposomes in the amphipathic environment such as a membrane/water interface. We also examined whether these peptides show antimicrobial activity similar to that observed for basic amphipathic α-helical model peptides [13,14].

For a preliminary communication on this subject see, Ono, S., Lee, S., Mihara, H., Aoyagi, H., and Kato, T. (1987) Peptide Chemistry 1986 (Miyazawa, T., Ed.), pp. 111–114, Peptide Research Foundation, Osaka.

Materials and Methods

Materials

Dipalmitoyl-D,L-α-phosphatidylcholine (DPPC) and dipalmitoyl-D,L-α-phosphatidylglycerol (DPPG) were purchased from Sigma Chemical Co. Carboxyfluorescein from Eastman Kodak Co. was further purified by recrystallization from ethanol. All other reagents were of analytical grade.

Synthesis of peptides

All peptides were synthesized by solution method. The synthetic routes for Ac-octapeptide-NHCH$_3$ (1_2) and Ac-dodecapeptide-NHCH$_3$ (1_3) are shown in Fig. 2. Three protected dipeptides (1, 2 and 10) were deprotected to give amine or acid components and then the components were coupled by the EDC-HOBt method [15] to give the tetrapeptides (5 and 12). Ac-tetrapeptide (8) derived from Boc-tetrapeptide (5) by consecutive deprotection of Boc, acetylation and deprotection of Pac, was coupled with tetrapeptide methylamide (13) to give Ac-octapeptide-NHCH$_3$ (15). Since protected octapeptide, Boc-(Ser-Val-Lys(Z)-Val)$_2$-NHCH$_3$, was insoluble in the usual solvents for peptide synthesis, we chose the following route to synthesize 1_3. Ac-tetrapeptide acid (8) and tetrapeptide methylamide (13) were elongated to hexapeptides (17) and (16) by condensation with dipeptides (9) and (14), respectively. The condensation of 18 and 19 gave Ac-dodecapeptide-NHCH$_3$ (20). Side chain-protecting groups of 15 and 20

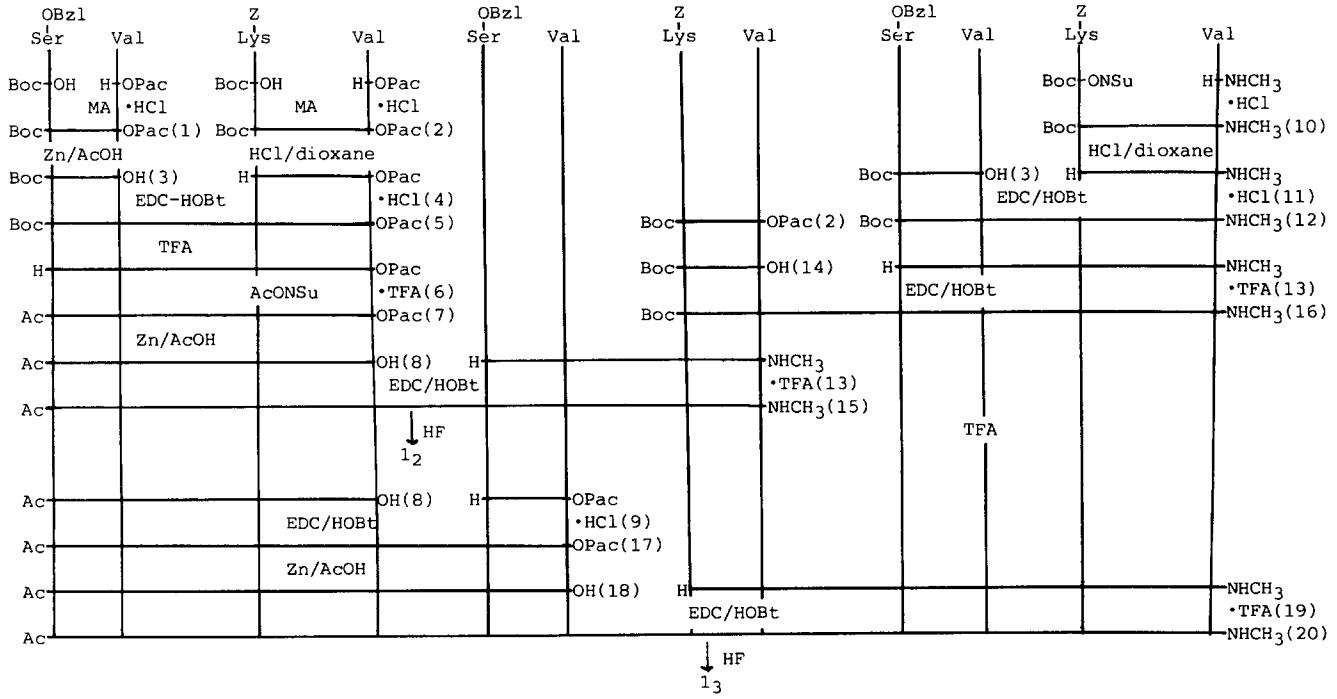

Fig. 2. Synthetic route for peptides 1_2 and 1_3. The longitudial lines denote each amino acid residue and the horizontal lines show the sequence of the peptides.

TABLE I

Yield and physical constants of model peptides

Compound	(M_r) [a] Formula	Yield [b]	R_{Lys} [c]	$[\alpha]_D^{28}$ [d]	Amino acid analysis [e]		
					Ser	Val	Lys
1_1	$C_{22}H_{42}O_6N_6 \cdot \frac{1}{2}H_2O$ (495.6)	82	0.60	−99.2	0.86	1.90	1.00
1_2·2AcOH	$C_{41}H_{77}O_{11}N_{11} \cdot 2C_2H_4O_2 \cdot 5H_2O$ (1110.3)	50	0.64	−101.8	0.82	2.14	1.00
1_3·3AcOH	$C_{60}H_{112}O_{16}N_{16} \cdot 3C_2H_4O_2 \cdot 6H_2O$ (1601.9)	13	0.62	−71.4	0.90	2.04	1.00
2_2·2AcOH	$C_{25}H_{49}O_5N_7 \cdot 2C_2H_4O_2 \cdot 2H_2O$ (683.8)	87	0.92	−133.8		0.95	1.00
2_3·3AcOH	$C_{36}H_{70}O_7N_{10} \cdot 3C_2H_4O_2 \cdot 3H_2O$ (989.2)	89	0.98	−109.6		1.05	1.00
2_4·4AcOH	$C_{47}H_{91}O_9N_{13} \cdot 4C_2H_4O_2 \cdot 4H_2O$ (1281.5)	37	1.04	−76.0		0.86	1.00
3·2AcOH	$C_{47}H_{78}O_{11}N_{12} \cdot 2C_2H_4O_2 \cdot 6H_2O$ (1215.4)	69	0.63	−46.0	1.68	2.74	2.00

[a] The reasonable compositions were considered from the results of elemental analysis.

[b] This was calculated from the step of HF treatment.

[c] Paper electrophoresis was carried out on a Toyo Roshi No. 52 paper with the solvent system of HCOOH/AcOH/MeOH/H_2O (1:3:6:10, v/v) for 2 h at 600 V. Lysine was used as a reference.

[d] Optical rotations in 30% acetic acid were measured on a JASCO DIP-370 digital polarimeter.

[e] Amino acid analyses were performed on a Hitachi 835 high speed amino acid analyzer after the hydrolysis in 6 M HCl in sealed tubes at 110 °C for 24 h.

were removed by HF treatment [16]. The final products 1_2 and 1_3 were purified on semipreparative reversed-phase high-performance liquid chromatography (2.0 × 25 cm, D-ODS-5, YMC packed column) with gradient elution using two solvents of 0.1% TFA in H_2O and 0.1% TFA in acetonitrile. The purity of the final products was established by paper electrophoresis and elemental and amino acid analyses. Peptide 3 was also synthesized similarly using N^{in}-formyltryptophan [17]. The product obtained after deprotection with HF showed a UV-absorption spectrum characteristic of Trp. The chemical and physical properties of the final products are summarized in Table I.

Spectroscopic experiments

CD spectra were recorded on a JASCO J-600 spectropolarimeter. Liposomes were prepared by sonication using a Tomy Seiko ultrasonic disrupter Model UR-200P as previously described [14]. All measurements were done in Hepes buffer. For preparation of the solution of the poorly soluble peptides in the buffer solution containing NaCl, the peptides were first dissolved in the buffer at high concentration and then diluted with the NaCl solution to appropriate concentration. No precipitation was observed on storing the peptide solution in a refrigerator for several months. For measurements of CD spectra in DPPC and DPPC-DPPG (3:1) liposomes, the peptides were dissolved at a concentration of 10–15 μM in 5 mM Hepes buffer (pH 7.4) containing 0.9 mM DPPC or 0.9 mM DPPC-DPPG (3:1) liposomes. The solution was left to stand for 10 min at room temperature before measuring the CD. All measurements were performed at 22°C and the data were expressed in terms of mean residue ellipticities. To eliminate a scattering due to liposomes, each CD spectrum of liposomes was subtracted from that of the peptides measured in the presence of liposomes. A cloud of liposomes by addition of peptides was not observed in the experimental conditions except for 1_3. The β-structure content in a sample composed of β-structure and random coil conformations was estimated by the method of Osterman and Kaiser [10] on the basis of the value given by Greenfield and Fasman for poly(L-lysine) at 218 nm [18] which are generally used for a rough estimation of β-structure content. To examine the effect of salt on the CD spectra of 1_2 in the presence and absence of liposomes, the peptide solution in 5 mM Hepes buffer (pH 7.4) was added to a mixed solution of liposomes and NaCl at a given concentration.

Fluorescence spectra were recorded on a JASCO FP-550A spectrofluorophotometer equipped with a thermostatted cell holder. The tryptophan fluorescence was recorded with excitation at 280 nm. Quenching of the tryptophan fluorescence of 3 by I^- was performed in 20 mM Tris-HCl buffer (pH 7.4) in the absence and presence of 210 μM DPPC-DPPG (3:1) liposomes as previously described [19].

Leakage of liposome contents was determined with the fluorescence dye carboxyfluorescein by the procedure of Weinstein et al. [22] with a minor modification [24]. Liposomes containing 100 mM 5(6)-carboxyfluorescein were prepared by the sonication described above. After being passed through a Sepharose 4B column to remove untrapped carboxyfluorescein, the liposomes were incubated at the concentration of 15 μM of peptides and at the temperature from 0°C to 42°C for the phase-transition release experiment. The liposomes were also incubated at the appropriate concentration of the peptides below (25°C) and above (45°C) the phase-transition temperature for the leakage ability experiment as a function of peptide concentration and data were collected at 5 min after the incubation. The change in the fluorescence of 5(6)-carboxyfluorescein due to its

dilution upon leaking out of the liposomes was monitored by fluorescence at 516 nm with excitation at 443 nm.

Results

Design and synthesis of peptides

On planning the model peptides, valine was chosen as a hydrophobic amino acid with the highest β-forming potential, and serine and lysine as hydrophilic amino acids with typical β-forming potential [20]. The N- and C-termini of the peptides were blocked with Ac and methylamide groups, respectively, to eliminate charge effects. We synthesized various peptides to study the effects of chain length on the peptide conformation. A series of the model peptides 1_n were composed of alternative serine and lysine residues on the hydrophilic side in amphipathic β-structure, and a series of 2_n composed of alternative lysine and valine were chosen to compare with 1_n in terms of the change in the electronic charges. The values of hydrophobic moment, which is a parameter estimating the amphipathy of peptide, are 0.96 for 1_n and 1.29 for 2_n, meaning that both are highly amphipathic and 2_n more amphipathic than 1_n. However, the hydrophobicity per residue is 0.12 for 1_n and −0.21 for 2_n, meaning that 1_n more hydrophobic than 2_n [26]. In addition, since fluorescence spectra of tryptophan offer the information concerning environment of Trp, we designed and synthesized peptide 3 in which Val of the position 6 in 1_2 was replaced by Trp.

CD study

Fig. 3 shows the CD spectra of 1_n and 2_n in the absence and presence of neutral and acidic liposomes. Octapeptide 1_2 had a minimum below 200 nm, which is characteristic of random structure in buffer and in the presence of neutral liposomes. In the presence of DPPC-DPPG (3 : 1) liposomes, however, 1_2 showed a maximum at 194 nm and a minimum at 217 nm, close to the value of 195 and 218 nm observed for poly(L-lysine) in the β-form in aqueous solution [13]. The magnitude of the mean residue ellipticity of 1_2 was identical to that given for poly(L-lysine) with 100% β-sheet conformation. Peptide 2_4 took a random structure in aqueous solution and in neutral liposomes similar to 1_2 and a β-structure with 80% in acidic liposomes. On the other hand, dodecapeptide 1_3 showed somewhat β-structural nature both in the buffer and neutral liposomes, but it took a complete β-structure in the presence of acidic liposomes like 1_2. Peptides 1_1 and 2_2 with chain length less than eight amino acid residues showed no restricted structure under the above conditions. It is noteworthy that the spectrum of 2_3 composed of six amino acid residues had shallow double troughs at the region of 205–225 nm which are characteristic of α-helical structure or type I β-turn [21] in

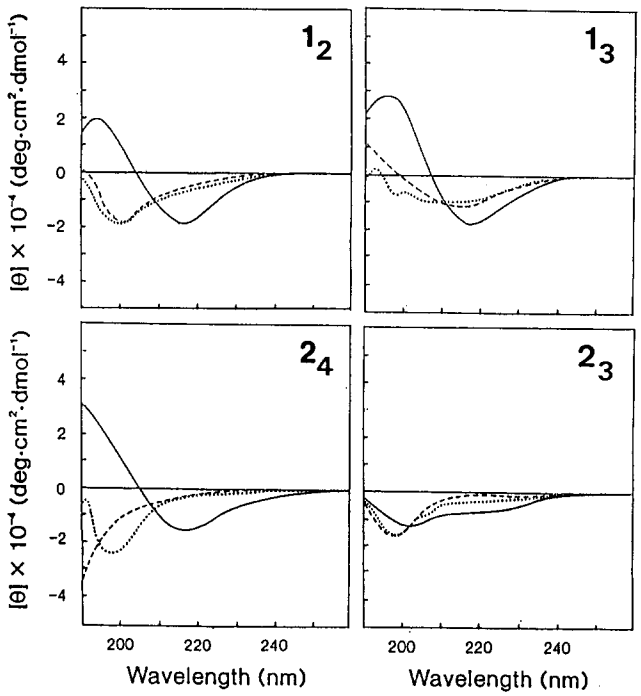

Fig. 3. CD spectra of peptides 1_2, 1_3, 2_3 and 2_4. The spectra were measured in 5 mM Hepes buffer (pH 7.4) (— — —), in the presence of 0.9 mM DPPC (·····) and DPPC-DPPC (3:1) (———) liposomes. Peptide concentration is 10–15 μM.

acidic liposomes, while it showed a random structure in buffer and neutral liposomes. These results indicate that β-structure is induced for the peptides having eight amino acid residues in acidic liposomes, and peptides with longer than eight residues could take a β-structure even both in buffer and in neutral liposomes as observed for 1_3. The CD spectrum of 3 also showed a minimum at 219 nm corresponding to 80% β-structure in the presence of acidic liposomes (data was not shown), indicating that the replacement of Val by Trp did not have appreciable influence on the formation of amphipathic β-structure.

Leakage of liposome contents

In order to study the effect of peptides on thermotropic phase transitions of phospholipid membranes, the time course of the peptide-mediated efflux of the dye from DPPC-DPPG (3 : 1) liposomes was examined. As shown in Fig. 4, in the range of the temperature from 0° to 42°C, a release of fluorescent dye was observed for 12 μM of 1_2 and 1_3 which take a β-structure in acidic liposomes. These peptides gradually released dye at about 10°C and reached the maximum after about 4 min near the phase transition temperature at 40°C in the acidic liposomes. Peptide 2_4, taking slightly less β-structure than 1_2 and 1_3 in acidic liposomes, leaked the dye near the phase transition temperature but the ability was much weaker than those of 1_2 and 1_3. Peptide 2_3 having somewhat ordered structure had a weak but distinct dye release. These results showed

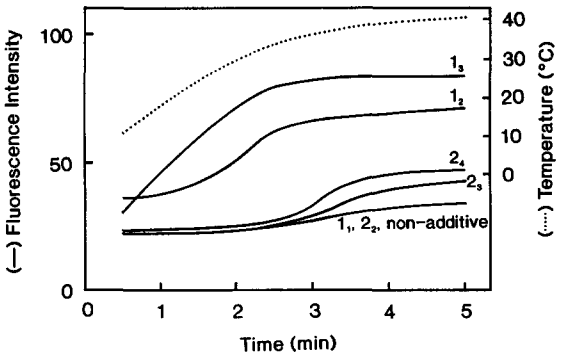

Fig. 4. Phase transition release of carboxyfluorescein from DPPC-DPPC (3 : 1) liposomes induced by model peptides. Individual peptides (12 μM) were incubated in 2 ml of 0.1 M NaCl/5 mM phosphate buffer (pH 7.4) containing 70 μM DPPC-DPPC (3 : 1) vesicles at temperatures from 0°C to 42° C for indicated scanning time.

that 1_n which have higher hydrophobicity than 2_n interact much strongly with liposomes to perturb them. However, 1_1 and 2_2 consisting of four amino acid residues failed to release the dye from the acidic liposomes. Peptide 4_3 which takes an amphipathic α-helical structure in acidic liposomes rapidly released the dye quantitatively at the the concentration of 1 μM [23]. These results indicate that the abilities of the peptides to release the dye are $4_3 \gg 1_3 > 1_2 \gg 2_4 > 2_3$.

The ability of the peptides to interact with liposomes was assayed as a function of peptide concentration. Above the phase transition temperature of DPPC and DPPG (45°C), the ability of 1_2 and 1_3 to leak the dye increased rapidly at the peptide concentrations below 20 μM but slowly above this concentration (Fig. 5a). The solution of 1_3 above the concentration of 20 μM in the presence of liposomes became turbid due to the aggregation of liposomes. Peptides 2_3 and 2_4 also leaked the dye weakly when compared to 1_2 and 1_3. The abilities of leakage decreased in order of 1_3, 1_2, 2_4 and 2_3 similar to that of phase transition release. The peptides 1_2 and 1_3 also leaked the dye at 25°C although the abilities were much weaker than those observed above the phase transition temperature. Such a leakage of the dye, however, was not observed for 2_2 and 2_3 (Fig. 5b). These findings implies that less amphipathic but higher hydrophobic peptides interact favorably with the acidic liposomes. Other peptides which have a shorter sequence than eight residues and take no β-structure in the presence of DPPC-DPPG (3 : 1) liposomes, failed to release carboxyfluorescein from the liposomes even at 45° C. All the peptides used here did not show any release of the dye from neutral liposomes (data not shown).

Fluorescence study of peptide 3

To attain better understanding on the location of the peptides in lipid bilayers, the interaction of 3 with DPPC-DPPG (3 : 1) liposomes was examined by fluorescence spectroscopy and quenching of tryptophan fluorescence by I^-. The fluorescence spectrum of 3 in buffer solution showed an emission maximum at 357 nm. Upon addition of DPPC-DPPG (3 : 1) liposomes, the emission maximum shifted to shorter wavelength by 12 nm (Fig. 6). Such a blue-shift of the fluorescence spectrum can be explained in terms of the translocation of the tryptophan residue from hydrophilic region to hydrophobic one. The magnitude of the blue shift indicated that the Trp residue was not deeply immersed in lipid bilayers [24]. Fluorescence intensity in acidic liposomes was somewhat lower than that in buffer, suggesting that the Trp residue is not located at the region of hydrophobic core in lipid bilayers [25].

Fig. 7 shows the Stern-Volmer plots for quenching of tryptophan in 3 by I^- in the absence and the presence of DPPC-DPPG (3 : 1) liposomes. The slope of the plot in the presence of the DPPG-DPPC liposomes (K_{SV} = 4.3 M^{-1}) was about one-half of that given in the buffer solution (K_{SV} = 9.1 M^{-1}). Since the I^- anion quenches the fluorescence of only surface-localized Trp residues, the result can implicate that in the liposomes the Trp residue in 3 was shielded from aqueous environment.

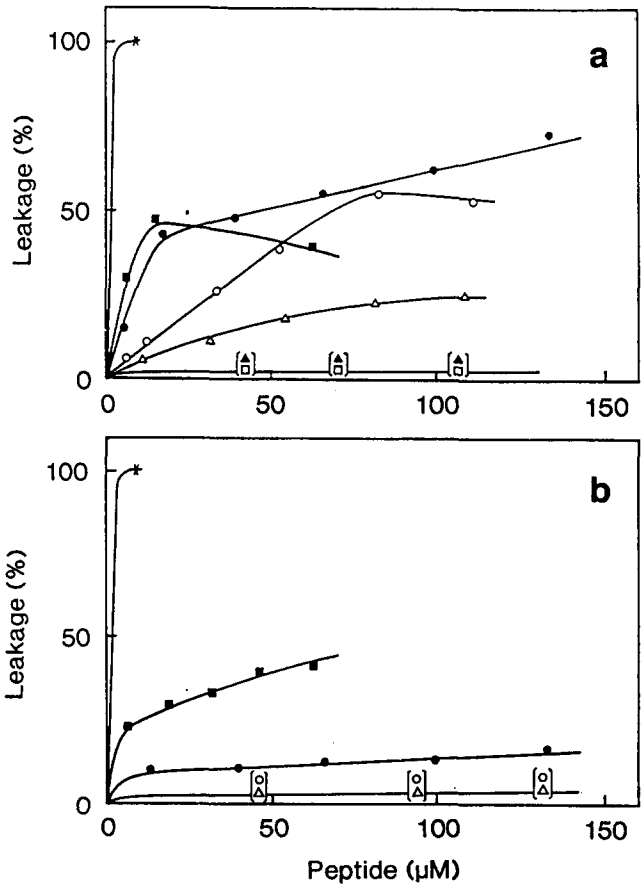

Fig. 5. Release of encapsulated carboxyfluorescein from liposomes as a function of the peptide concentration at 45° C (a) and at 25°C (b). The data were collected at 5 min after incubation of peptide in liposomes. 1_3 (■), 1_2 (●), 1_1 (▲), 2_4 (○), 2_3 (△), 2_2 (□), and 4_3 (×).

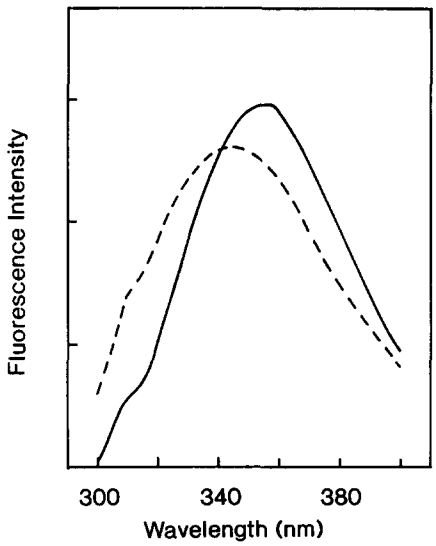

Fig. 6. Fluorescence spectra of peptide 3. The spectra were recorded in 20 mM Tris-HCl buffer (pH, 7.4) in the absence (———) and presence of DPPC-DPPC (3:1) liposomes (— — —). The concentrations of peptides and liposomes were 11 μM and 220 μM, respectively.

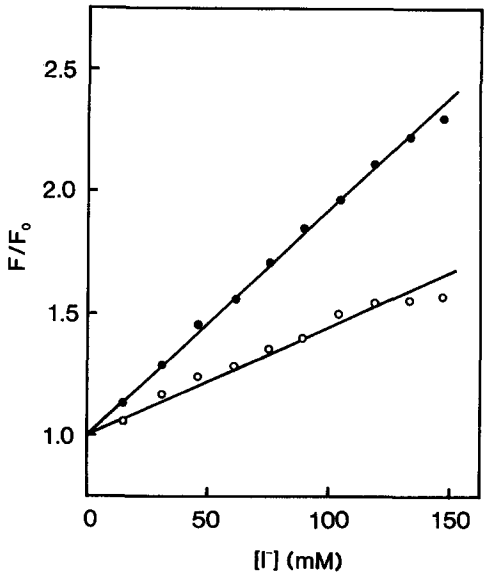

Fig. 7. Stern-Volmer plots of the quenching of tryptophan fluorescence in peptide 3 by KI. The fluorescence spectrum of peptide 3 was recorded in 20 mM Tris-HCl buffer (pH, 7.4) at 25°C in the absence (●) and presence (○) of 210 μM DPPC-DPPC (3:1) liposomes. The concentration of the peptides was 5 μM and the excitation wavelength was 280 nm.

Effect of salt on the peptide–lipid interaction

Salt-concentration dependency was examined in the absence and presence of DPPC-DPPG (3:1) liposomes as a function of NaCl concentration at 25°C. As seen in Fig. 8, the CD spectrum of 1_2 in the buffer solution had a trough at 200 nm. It is noticeable that the addition of NaCl induced the change in the CD spectrum of 1_2. The CD spectra of 1_2 are characterized by the positive maximum at 230 nm and a negative trough at 214 nm, and the intensities of these extrema increased with increasing salt concentration (Fig. 8a). This is due to conformational change from a random structure to an

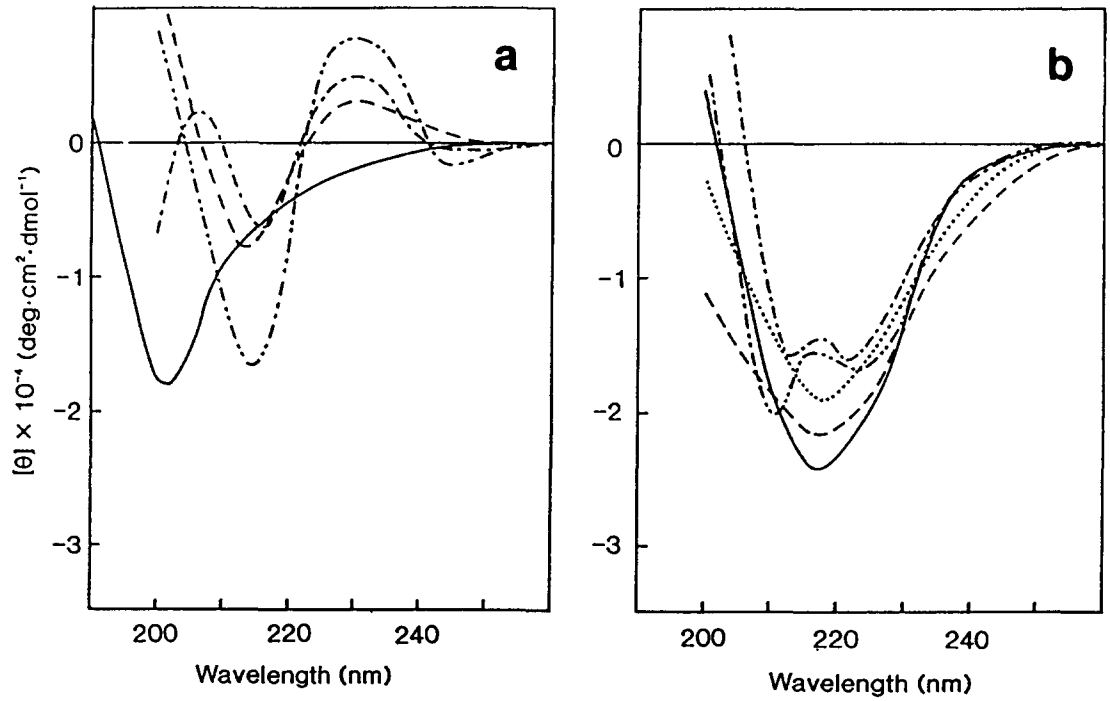

Fig. 8. CD spectra of peptide 1_2 as a function of NaCl concentration. The spectra were recorded in 5 mM Hepes buffer in the absence (a) and presence (b) in DPPC-DPPG (3:1) liposomes at 22°C. The concentrations of peptide and DPPC-DPPC were 12 μM and 0.9 mM, respectively. NaCl concentrations were 0 mM (———), 50 mM (— — —), 100 mM (· · · · · ·), 200 mM (·—·—·), and 400 mM (· · —·—·—· ·).

ordered one which may be caused by aggregation of the peptides. On the other hand, in the presence of liposomes, such a conformational change of 1_2 by NaCl was not observed below the salt concentration of 100 mM. Thus, β-structure of the peptide was maintained independently on the salt concentration although the content of the β-structure decreased slightly (Fig. 8b). Interestingly, the peptide showed double minima at 208 and 222 nm at the concentration above 200 mM of NaCl, suggesting that an α-helical structure was induced by the high concentration of NaCl.

Antimicrobial assay

The antimicrobial assay was carried out using same organisms as previously reported [14]. All the peptides showed no antimicrobial activity against both Gram-positive and -negative bacteria (minimum inhibitory concentration, > 100 μg/ml).

Discussion

The results presented here provide some insight into the conformation of the model peptides in lipid bilayers. The repeating basic oligopeptides consisting of alternating hydrophobic and hydrophilic amino acid residues can form an amphipathic β-structure on amphipathic interface. Spectroscopic data indicate that 1_2 and 2_4 having eight amino acid residues existed as a random structure in aqueous solutions or in the presence of neutral liposomes, while they took a β-structure in the presence of acidic liposomes. Peptide 1_3 consisting of more than eight amino acid residues exists as a β-structure in aqueous solution and in the presence of neutral or acidic liposomes. On the other hand, 2_2 and 2_3, each having four and six residues took no β-structure. The chain length of peptides seems to be an important factor in the formation of the amphiphilic β-structure. Altmann et al. [11] reported that the critical chain length for the formation of β-structure of the peptide with alternating Ser and Leu residues in organic solvents or water was at the level of the eight residues. Taking these results into account, it can be suggested that the tendency of formation of the stable β-structure in the amphipathic peptides principally depends on the intrinsic ability of the peptides to form β-structure, and hydrophobic environment like liposomes may assist the formation of the β-structure of the peptide which is not or less capable of forming a β-structure in aqueous solution.

The model peptides capable of interacting with acidic phospholipid liposomes specifically have a potency to release the fluorescent dye from the liposomes, but not from neutral phospholipid liposomes. This indicates that the electrostatic interaction of basic peptides with phospholipid membranes is an important factor for the induction of amphipathic β-structure conformation to perturb liposomes. An appreciable difference was observed in the ability to form β-structure between 1_2 and 2_4 which have the same chain length but different charges. The leakage ability of 1_2 also was stronger than that of 2_4. This may be explained by the fact that hydrophobicity of 1_2 is much higher than that of 2_4 [23,26].

There are two possible modes of action of basic peptides with amphipathic β-structure on lipid bilayers. One of them is that there may be electrostatic interaction between the basic groups of the peptide side chains and the acidic groups of lipid bilayers. In this case, the peptides can aggregate in buffer solution due to the interaction of their hydrophobic face. Another possibility is that the peptides penetrate into the hydrocarbon region of bilayers where the hydrophobic side chains can interact with the hydrophobic portion of membranes, while the hydrophilic residues face to interact with the aqueous environment. The tryptophan residue of 3 is present in the apolar environment in both action modes as described above. Such a view can be supported by the blue shift of the fluorescence spectrum of 3 induced by addition of acidic liposomes. These findings indicate that the hydrophobic face of amphipathic β-structure of 3 is in the apolar environment. It should be noticed that salt in aqueous solution alters the conformation of free peptide but it does not induce the conformational change of the peptide in the complex with liposomes when the NaCl concentration was increased to 100 mM. The peptide may interact with membranes in such a manner as to penetrate its hydrophobic region into the lipid bilayers, leading to the loss of the effect of NaCl on the conformation of the peptide immersed into the apolar face of the lipid bilayer. It is interesting that the conformational change from β-structure to α-helical one was observed at a higher NaCl concentration than 200 mM in the presence of acidic liposomes. Further study is in progress.

In the preceding paper, we reported that basic peptide 4_3 consisting of repeating units of Leu-Ala-Arg-Leu took an amphipathic α-helical structure in the presence of neutral liposomes and their α-helical contents increased slightly in acidic liposomes [13]. Contrary to this, the basic amphipathic β-structure was only induced in the presence of acidic liposomes, suggesting that peptides with amphipathic β-structure and those with α-helical structure interact with lipids in a different mode. It can be postulated that basic amphipathic α-helical peptides with a strong hydrophobicity interact with lipids mainly by the hydrophobic force, while basic amphipathic β-structural peptides having less hydrophobicity than the α-helical peptides bind to lipid by both electrostatic and hydrophobic interactions. This is not inconsistent with the fact that α-helical peptides induce a remarkable release fluorescence dye from both neutral and acidic phospholipid liposomes, while the

ability of 1_2 and 1_3 to release carboxyfluorescein from acidic phospholipid liposomes is about 1/100 less than that of 4_3. We postulate that the amphipathic β-structural peptides interact with lipid bilayers too weakly to perturb the biomembrane for exhibiting the antimicrobial activity.

It is known that the formation of β-structure depends on ionic strength, pH, temperature, organic solvents, and surfactants. As demonstrated here, acidic phospholipid bilayers induce and stabilize the amphipathic β-structure of the peptides. Including the fact that biological membranes contain a variety of phospholipids, we consider that β-structure might be formed, when proteins and peptides have segments possessing amphipathic secondary structure, on the biomembranes. The interaction of these segments with membranes may be affected by phospholipid composition.

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