

Morphological Behavior of Acidic and Neutral Liposomes Induced by Basic Amphiphilic α -Helical Peptides with Systematically Varied Hydrophobic-Hydrophilic Balance

Akiko Kitamura,* Taira Kiyota,* Mitsunori Tomohiro,* Akiko Umeda,[#] Sannamu Lee,* Tohru Inoue,* and Gohsuke Sugihara*

*Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-ku, Fukuoka 814-0180, and [#]Department of Bacteriology, Faculty of Medicine, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

ABSTRACT Lipid-peptide interaction has been investigated using cationic amphiphilic α -helical peptides and systematically varying their hydrophobic-hydrophilic balance (HHB). The influence of the peptides on neutral and acidic liposomes was examined by 1) Trp fluorescence quenched by brominated phospholipid, 2) membrane-clearing ability, 3) size determination of liposomes by dynamic light scattering, 4) morphological observation by electron microscopy, and 5) ability to form planar lipid bilayers from channels. The peptides examined consist of hydrophobic Leu and hydrophilic Lys residues with ratios 13:5, 11:7, 9:9, 7:11, and 5:13 (abbreviated as Hels 13–5, 11–7, 9–9, 7–11, and 5–13, respectively; Kiyota, T., S. Lee, and G. Sugihara. 1996. *Biochemistry*. 35:13196–13204). The most hydrophobic peptide (Hel 13–5) induced a twisted ribbon-like fibril structure for egg PC liposomes. In a 3/1 (egg PC/egg PG) lipid mixture, Hel 13–5 addition caused fusion of the liposomes. Hel 13–5 formed ion channels in neutral lipid bilayer (egg PE/egg PC = 7/3) at low peptide concentrations, but not in an acidic bilayer (egg PE/brain PS = 7/3). The peptides with hydrophobicity less than Hel 13–5 (Hels 11–7 and Hel 9–9) were able to partially immerse their hydrophobic part of the amphiphilic helix in lipid bilayers and fragment liposome to small bicelles or micelles, and then the bicelles aggregated to form a larger assembly. Peptides Hel 11–7 and Hel 9–9 each formed strong ion channels. Peptides (Hel 7–11 and Hel 5–13) with a more hydrophilic HHB interacted with an acidic lipid bilayer by charge interaction, in which the former immerses the hydrophobic part in lipid bilayer, and the latter did not immerse, and formed large assemblies by aggregation of original liposomes. The present study clearly showed that hydrophobic-hydrophilic balance of a peptide is a crucial factor in understanding lipid-peptide interactions.

INTRODUCTION

The amphiphilic α -helix is defined as an α -helical structure in which the amino acid residues are distributed in the secondary structural form of opposite polar (hydrophilic) and nonpolar (hydrophobic) faces. It is one of the common structural features in biologically active peptides and proteins, such as polypeptide hormones, polypeptide antibiotics, and polypeptide venoms. The structure has been shown to play an important role in cell-lytic and antimicrobial peptides that act by perturbing the barrier function of membranes (Kini and Evans, 1989; Segrest et al., 1990; Saberwal and Nagaraj, 1994). In particular, the cationic peptide antibiotics have recently been suspected as drugs against microorganisms that have resistance to well-known antibiotics, penicillin, ampicillin, etc., because the mechanism of the peptide antibiotics is entirely different from that of other ones, which may not face bacterial resistance (Maloy and Kari, 1995; Hancock, 1997). A specific feature of cationic peptides is that they have both a hydrophobic face, comprising nonpolar amino acid residues, and a hydrophilic face of polar positively charged residues.

Because amphiphilic peptides have hydrophobic and hydrophilic moieties, like detergents, their relative amphiphilic potential should bring about a difference in biological activity. The structural motif of a helix is determined by the manner in which an amphiphilic helical peptide is induced with the lipid. The peptide either stabilized or lysed the membrane (Segrest et al., 1990; Epand et al., 1995). To analyze the relationship between the relative magnitude of the hydrophobic-hydrophilic moiety and membrane-binding property, we 1) obtained a hydrophobic plot related to the hydrophobic moment and hydrophobicity of the segment (Eisenberg, 1984); 2) classified the lipid-associating helices according to their molecular hydrophobic potential (Brasseur, 1991; Brasseur et al., 1997); and 3) applied the reciprocal wedge hypothesis, considering the reciprocal effect of phospholipid shapes on membrane structures (Tytler et al., 1993). The interaction of peptides with membrane mainly involves two binding properties: 1) hydrophobic interaction between the lipid acyl chain and the hydrophobic residues of the peptide; and 2) electrostatic interactions among the polar residues of the peptides, the phospholipid headgroup, and the solvent molecules. Recent lipid-peptide binding studies show that insertion of the peptides into lipid bilayers, membrane perturbation, and the fusion event largely depend on their hydrophobic interaction (Yoshimura et al., 1992; Reynaud et al., 1993; Polozov et al., 1997), whereas electrostatic interactions generated mainly by anionic phospholipids in the membrane and positively charged residues

Received for publication 22 June 1998 and in final form 4 November 1998.

Address reprint requests to Dr. Sannamu Lee, Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-ku, Fukuoka 814-0180, Japan. Tel.: +81-92-871-6631, ext. 6248; Fax: +81-92-865-6030; E-mail: leesan@ssat.fukuoka-u.ac.jp.

© 1999 by the Biophysical Society

0006-3495/99/03/1457/12 \$2.00

in peptides promote membrane perturbation, resulting in membrane lysis, or fusion (Suenaga et al., 1989; Park et al., 1995; Dathe et al., 1996; Ghosh et al., 1997; Silverstro et al., 1997; Wieprecht et al., 1997). In fact, the hydrophobicity, hydrophobic moment, and the angle subtended by the positively charged helix face of a set of model peptides have been shown to be effective modulators of antimicrobial and/or hemolytic activity (Dathe et al., 1996; Blondelle and Houghton, 1992; Cornut et al., 1994; Dathe et al., 1997). A recent study on the structure-activity of antimicrobial peptides has suggested that the combination of hydrophobicity and net positive charge may be sufficient to exhibit antibacterial activity (Shai and Oren, 1996; Oren and Shai, 1997).

In a previous study, we have shown that *de novo* designed amphiphilic α -helical model peptides that have a systematically varied hydrophobic-hydrophilic balance (HHB, relative amphiphilic potential) as a result of different hydrophobicities but a similar hydrophobic moment determined peptide-membrane interaction and even biological function (Kiyota et al., 1996). The model peptides (the Hel series), consisting of 18 residues, are made from three kinds of amino acids, hydrophilic Lys, hydrophobic Leu, and fluorescent Trp, in which the ratio of hydrophobic-hydrophilic residues is varied as follows: 13:5, 11:7, 9:9, 7:11, and 5:13 (abbreviated as Hels 13-5, 11-7, 9-9, 7-11, and 5-13, respectively). Helical wheel representations of the Hel series of peptides are given in Fig. 1. These peptides generate ideal amphiphilic α -helical structure, which have systematically varied HHB as a result of different hydrophobicities (H, 0.07–0.66) but almost the same hydrophobic moments (μ H, 0.39–0.51). Their HHB is estimated theoretically from the calculated hydrophobicity values and shown to agree with experimental reverse-phase high-performance liquid chromatography (RP-HPLC) retention times.

Circular dichroism, liposome-lytic, and Trp fluorescent studies and encapsulated fluorescence leakage and hemolytic measurements have demonstrated that helix-forming

ability and the model and biomembrane perturbation abilities are completely parallel to the magnitude of the hydrophobic face area in α -helical structure. In the lipid-binding study in guanidine-HCl solution, it has been shown that peptides with a higher hydrophobicity (the hydrophobic face is wider than the hydrophilic face) such as Hels 13-5 and 11-7 immerse their hydrophobic regions in lipid bilayers. Conversely, more hydrophilic peptides (Hels 7-11 and 5-13) interact only with anionic lipid headgroups and cationic peptide residues on liposome surfaces. The peptide Hel 9-9, which has exactly the same hydrophobic and hydrophilic regions, was found to be at a critical boundary among these peptides.

Based on our results, we have introduced the concept of hydrophobic-hydrophilic balance, namely, that the proportions of hydrophilic and hydrophobic moieties is directly related to their physicochemical and biological properties. Our hypothesis may explain the mode of lipid-peptide interaction (pathways to *a-h* in Fig. 8). However, the working hypothesis was mainly considered from lipid-peptide interaction and the standpoint of peptide behavior in the presence of lipid bilayers. In the present study, to establish the hypothesis more precisely, we examined how changes in the lipid bilayer are induced by the addition of peptides to neutral and acidic liposomes. We account for membrane solubility by turbidity measurements, the size of liposomes by dynamic light scattering measurements, and morphology by electron microscopy. In addition, we have measured Trp quenching by liposomes containing brominated phospholipid (1,2-bis(9,10-dibromostearoyl)phosphatidylcholine, DBRPC) and the channel-forming ability of each peptide in neutral and acidic lipid bilayers. We make a slight revision to our previous working hypothesis by presenting a new model for interaction of the cationic amphiphilic α -helical peptides with neutral and acidic lipid bilayers.

MATERIALS AND METHODS

Materials

Peptides were synthesized as described previously (Kiyota et al., 1996). The stock solutions of Hel peptides in buffer (5 mM TES/100 mM NaCl, pH 7.4), except for Hel 13-5, were kept in a refrigerator. In the case of Hel 13-5, the powder was dampened with only a little 30% acetic acid and then diluted in buffer solution. The peptide concentration in the buffer solution were determined from UV absorbance of Trp at 280 nm ($\epsilon = 5500$). The peptide concentrations in methanol on channel measurements were estimated by weight percent. Egg PC, egg PE, and egg PG were purchased from Sigma Chemical Co. (St. Louis, MO). 1,2-Bis(9,10-dibromostearoyl)phosphatidylcholine (9,10-DBRPC) was prepared from 1,2-bis-oleoyl-phosphatidylcholine (Sigma Chemical Co.) by bromination according to the literature (Dawidowicz and Rothman, 1976). All other reagents were of analytical grade.

Liposome-lytic experiment

A lipid (egg yolk phosphatidylcholine (egg PC) or egg PC/egg yolk phosphatidylglycerol (egg PG) (= 3/1) mixture) solution in chloroform was placed in a round-bottomed flask. After drying in a stream of N_2 gas, the residual film was further dried under vacuum over night. The lipid film

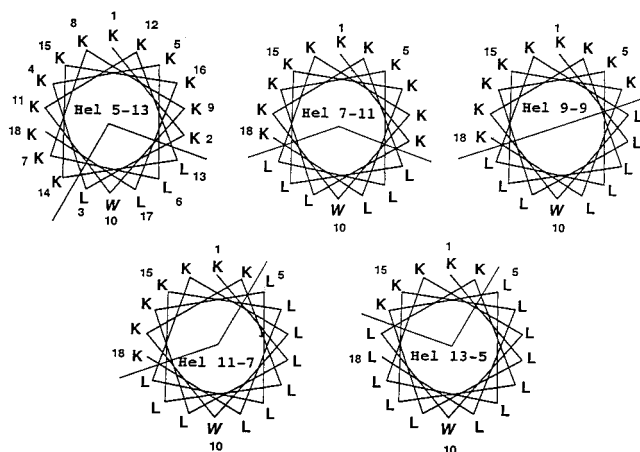


FIGURE 1 Helical wheel representations of the Hel-series peptides with systematically varied hydrophobic-hydrophilic balance (HHB).

was hydrated with a *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (5 mM TES/100 mM NaCl, pH 7.4), and then the suspension was vortexed for 20 min. The turbid liposome solution obtained was diluted to a concentration of $\sim 100 \mu\text{M}$ with the same buffer (5 mM TES/100 mM NaCl, pH 7.4). The peptide solutions were then added to the solution to attain a given mole ratio of peptide to lipid and then incubated at 25°C . The transmittance of the sample solution was recorded at 400 nm using a JASCO spectrometer (Tokyo, Japan) after vigorous vortexing.

Trp-quenching measurements by egg PC liposomes containing DBRPC

Small unilamellar vesicles with and without 9,10-DBRPC were prepared as follows. The turbid liposome solution described above was sonicated for 10 min ($\times 3$) by titanium tip, in an ice bath under nitrogen flow. Then the liposome solution was centrifuged at 5000 rpm for 20 min.

Peptide (4 μM) in a buffer solution (5 mM TES/100 mM NaCl, pH 7.4) was titrated with the liposome solution (200 μM) prepared from a mixture of the appropriate ratio of egg PC and DBRPC as described previously (Lee et al., 1997). Fluorescence spectra were recorded at 280 nm, and emission and excitation band passes were kept at 5 nm. Quenching data were collected 5 min after the peptide was mixed with liposome solution and analyzed by the Stern-Volmer plot, using the equation

$$F_0/F = 1 + K_{SV}[Q],$$

where F_0 , F , K_{SV} , and $[Q]$ are fluorescence intensities at maximum wavelength in the absence and presence of liposomes, the Stern-Volmer quenching constant, and the quencher concentration, respectively.

Liposome size determination

Unilamellar liposomes in a controlled size distribution ~ 80 – 90 nm in diameter were prepared by the extrusion method (Mayer et al., 1986). The lipid films obtained by the procedure described above were dispersed in buffer solution by ultrasonic irradiation in the cup horn of a Branson model 185 sonifier at room temperature. The resultant liposome suspension was transferred to an extruder (Lipex Biomembranes, Vancouver, BC, Canada), extruded through two stacked polycarbonate filters with 100 nm and 200 nm pore size by applying nitrogen pressure, collected, and reextruded. The extrusion was repeated 10 times.

The size of liposomes was measured with a NICOMP submicron particle sizer (model 370) with an argon ion laser ($\lambda = 488.0$ nm) with a maximum power of 75 mW and evaluated as described previously (Minami et al., 1996). The peptide and buffer solutions were filtered through the membrane filters with pores of $0.45 \mu\text{m}$ and $0.1 \mu\text{m}$ diameter (Cosmonice Filter W, Japan Milipore, Osaka, Japan, and Millex-VV, Milipore, Bedford, MA). The liposome solution (0.2 mM, 1 ml) was added to peptide solutions (1 ml) of appropriate concentrations, and the liposome size was monitored at 25°C .

Electron microscopy

Liposomes were prepared in 5 mM TES buffer (pH 7.4) containing 100 mM NaCl and 1 mM EDTA by the extrusion method described above and adjusted to a concentration 10 times higher than that in other experiments to easily obtain electron microscopic images. The peptide concentration was 200 μM (lipid/peptide molar ratio, $L/p = 5/1$). The peptide and buffer solutions were filtered through pores of $0.45 \mu\text{m}$ and $0.1 \mu\text{m}$ diameter, respectively, before the sample was prepared. Electron microscopy was examined by the negative-staining method with and without the addition of peptides. All of the peptides were incubated for ~ 24 h with liposomes before staining. The sample solutions were placed on formvar, carbon-coated grids and stained with 0.5% phosphotungstic acid adjusted to pH 7.4 with NaOH at room temperature. Liposomes were observed through a JEM-2000 EX electron microscope.

Measurements of membrane current

Planar lipid bilayers were formed by the folding method (Montal and Mueller, 1972) as described previously (Iwata et al., 1994). To a 10 mM tris(hydroxymethyl)aminomethane (Tris)-HEPES buffer solution (0.5 ml, pH 7.4) in a Teflon chamber with two compartments (each ~ 1.5 ml in internal volume) separated by a Teflon septum (25 μm thick) with a 200- μm -diameter aperture, a small amount of a lipid solution ($\sim 15 \mu\text{l}$) in hexane (10 mg/ml) was spread. A lipid monolayer was formed at the air/water interface of each compartment by spontaneous evaporation of the solvent. The water level in each compartment was then raised over the aperture, resulting in formation of the lipid bilayer in the aperture.

To stabilize the membrane, the solution was left to stand for 5 min, and then a small amount of a peptide solution in methanol (1 mg/ml) was added to one compartment of the chamber, which was defined as the *cis* side. The *cis* solution was stirred for ~ 10 s with a magnetic stirrer, and then a potential was applied to measure conductance. The potential was expressed as that of the *cis* compartment relative to the *trans* side, which was earthed. The current across the membrane was measured with an amplifier (Patch/Whole Cell Clamp Amplifier CE Z-2400; Nihon Kohden, Tokyo, Japan) and was displayed on a digital storage oscilloscope (COR5521; Kikusui Electronics, Kawasaki, Japan). The data were recorded and stored with a Hioki 8840 Memory Hicorder (Hioki, Nagano, Japan).

RESULTS

Trp-quenching measurements by neutral and acidic liposomes containing DBRPC

To examine the translocation of peptides to lipid bilayers, Trp fluorescence was measured in the presence of egg PC liposomes or egg PC/egg PG (3/1 or 7/1) liposomes and those containing 9,10-DBRPC. When titrated with neutral liposomes, Hels 13–5 and 11–7 (highly hydrophobic) were strongly quenched with increasing liposome concentration, and moderately hydrophobic Hel 9–9 and less hydrophobic Hel 7–11 and Hel 5–13 were quenched to a lesser extent, as shown in Fig. 2. Because Trp fluorescence is quenched by the bromine moiety, the Trp's in Hels 13–5, 11–7, and 9–9 are located in the phospholipid acyl chain of lipid bilayers. The dissociation constants of Hels 13–5, 11–7, and 9–9 to egg PC liposomes were 9.7 μM , 52 μM , and 0.50 mM, respectively, when calculated from the data of a previous peptide-lipid titration study by the method of Surewicz and

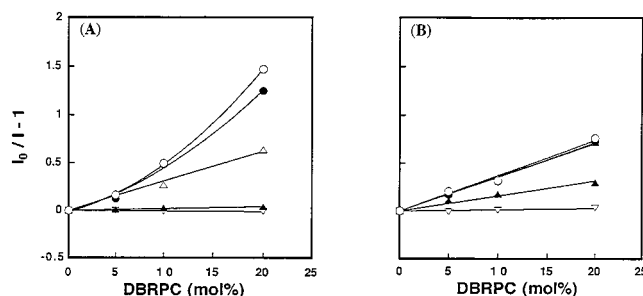


FIGURE 2 Quenching of Trp fluorescence by egg PC (A) and by egg PC-egg PG (B) containing different concentrations of DBRPC at 25°C . Hels 13–5 (\circ), 11–7 (\bullet), 9–9 (\triangle), 7–11 (\blacktriangle), and 5–13 (∇). Peptide and lipid concentrations are 4.5 μM and 180 μM ($L/p = 40$). (Egg PC + DBRPC)/egg PG was 3:1 for Hels 13–5, 11–7, and 9–9 and 7:1 for Hels 7–11 and 5–13.

Epand (1984). Because Hels 13-5, 11-7, and 9-9 adopt an α -helical structure in the presence of egg PC liposomes (75%, 50%, 22% in helical contents, respectively) as reported previously (Kiyota et al., 1996), these results reveal that the hydrophobic parts of the amphiphilic helical structure were immersed in lipid bilayers. It should be mentioned that the Trp of Hel 7-11 is not quenched by DBRPC. In this connection, Hel 7-11 exhibited no wavelength shift of Trp fluorescence on lipid-titration study and contained no α -helical structure in the presence of neutral liposomes (Kiyota et al., 1996).

Interestingly, when titrated with acidic liposomes, Hels 13-5, 11-7, and 9-9 were quenched strongly in a parallel manner with increasing DBRPC concentrations, Hel 7-11 was only moderately quenched, and Hel 5-13 only slightly quenched. Because in acidic liposomes all of the Hel-series peptides can take an α -helical structure to varying degrees (Kiyota et al., 1996), Hel 7-11 as well as Hels 13-5, 11-7, and 9-9 can also immerse the hydrophobic part of their amphiphilic structure in the lipid bilayer with the assistance of the charge interactions between peptide and lipid.

Liposome-lytic activity of peptides

We have previously shown the change in the turbidity of acidic and neutral liposomes with time after the incubation of peptides at 25°C (Kiyota et al., 1996). In Fig. 3, the turbid liposome-clearing ability is shown as a function of concentration of the Hel-series peptides in egg PC and egg PC/egg PG (= 3/1) liposomes 24 h after incubation of the peptides.

The peptides were added to a neutral liposome solution exhibiting a 60% transmittance. As shown in Fig. 3A, the transmittance of Hels 11-7 and 13-5 increased gradually with increasing peptide concentrations and exceeded 90% at 20 μ M (L/p = 5/1), suggesting that the size of vesicles became smaller than that of liposomes in the absence of peptides (hereafter referred to as "original liposomes"). It

should be mentioned that the clearing ability of Hel 11-7 is slightly greater than that of Hel 13-5 in the range of concentrations examined, as observed in previous time-dependent experiments (Kiyota et al., 1996). This phenomenon was previously not understood because the magnitude of hydrophobicity is usually parallel to solubilizing ability. However, this was clearly solved; that is, the mode of action of both peptides with lipid bilayer is completely different as described later. Hel 9-9 decreased its turbidity slightly. No change in turbidity, on the other hand, was observed for Hels 7-11 and 5-13, indicating no interaction of the peptides with neutral liposomes.

The turbidity change of acidic liposomes induced by Hel-series peptides exhibited some characteristic features differing from that of neutral liposomes. The transmittances of Hels 11-7 and 9-9 decreased at 5 μ M (L/p = 20) and then increased sharply with increasing peptide concentration. Above 20 μ M, the clearing ability of Hel 11-7 was stronger than that of Hel 9-9. The transmittance of Hel 13-5 also decreased to a minimum below 10 μ M and then increased gradually with increasing peptide concentration, but was not over that of original liposomes, suggesting that the liposomes became larger than the original liposomes. In contrast, no change in turbidity for Hels 5-13 and Hel 7-11 was observed with increasing peptide concentration, suggesting that these peptides do not interact with acidic lipid bilayers. However, as described later, the size of Hels 7-11 and 5-13 became larger immediately even at low peptide concentrations, meaning that no change in turbidity may come from the increase in size due to the liposome aggregation.

Change in liposome size induced by peptides

To obtain more precise information on the interaction of the peptides with neutral and acidic liposomes, the effect of the peptides on the size distribution of liposomes was examined by dynamic light scattering measurements. Fig. 4 shows the

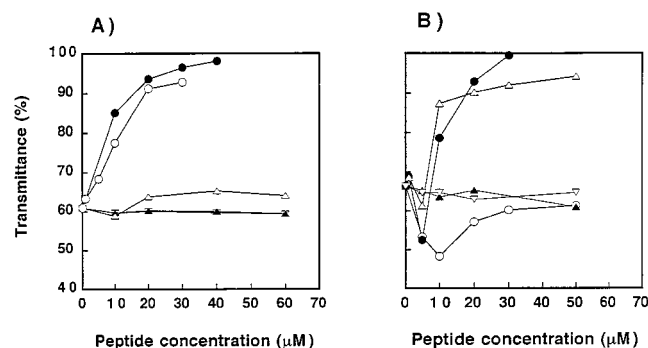


FIGURE 3 The turbid liposome-clearing ability of the Hel-series peptides as a function of concentration in egg PC (A) and egg PC-egg PG (3:1) (B) liposomes. The peptides were incubated in turbid liposomes (100 μ M) prepared by vortexing of lipid films in buffer solution at pH 7.4. Data were collected 24 h after incubation of the peptide. The transmittance was recorded at 400 nm. Hels 13-5 (○), 11-7 (●), 9-9 (△), 7-11 (▲), and 5-13 (▽).

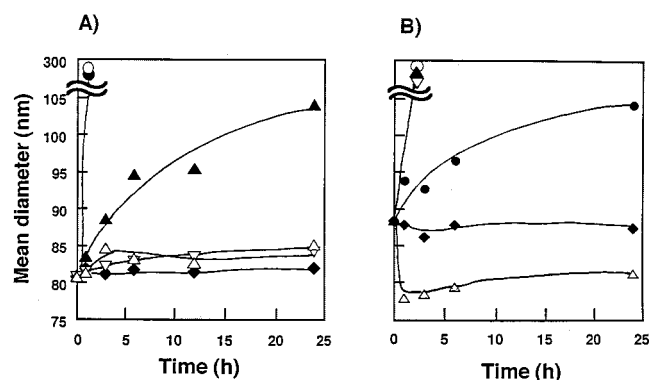


FIGURE 4 Change in mean hydrodynamic diameter of egg PC (A) and egg PC-egg PG (3:1) (B) liposomes as a function of time after incubation of the Hel-series peptides. Peptide and lipid concentrations are 20 μ M and 100 μ M, respectively. No peptide addition (◆), Hels 13-5 (○), 11-7 (●), 9-9 (△), 7-11 (▲), and 5-13 (▽).

change in liposome size with time after the addition of the peptides to liposome solution at peptide/lipid concentrations of 20 μM /100 μM . Fig. 5 depicts the dependence of liposome size on the peptide concentration. The size of liposomes was measured after a 24-h incubation.

As shown in Fig. 4 *A*, the addition of Hels 11–7 and 13–5 to egg PC liposomes resulted in an immediate and drastic increase in liposome size. These peptides led to an increase in the liposome size over 300 nm compared with the original size (~ 80 nm) at a peptide concentration of 20 μM ($L/p = 5/1$) (Fig. 5 *A*). Hel 7–11 exhibited a gradual increase in liposome size at 20 μM (Fig. 4 *A*) but drastically increased at 40 μM (more than 300 nm) (Fig. 5 *A*). In contrast, Hels 9–9 and 5–13 increased the liposome size only slightly within 3 h after their addition. Some of the results obtained by the size determination of liposomes seem to be inconsistent with those of liposome-lytic experiments monitored by turbidity measurements. For instance, 1) for incubation with Hel 13–5, the turbidity of the liposome solution is decreased, but the liposome size increased; 2) for Hel 7–11, the turbidity did not change, but the liposome size increased. We will discuss this problem later with the aid of electron microscopy.

Contrary to the case of neutral liposomes, the changes in size distribution of acidic liposomes composed of egg PC/egg PG (= 3/1) resulting from the addition of the peptides are more apparently consistent with the results of turbidity experiments (Figs. 3 *B*, 4 *B*, and 5 *B*). The peptides Hels 5–13, 7–11, and 13–5 all induced an increase in turbidity and led to a rapid and drastic increase in the size of liposomes (more than 300 nm), even at low peptide concentrations. On the other hand, Hel 9–9, which, remarkably, decreased turbidity, led to a decrease in the size of liposomes (a mean diameter of ~ 75 –80 nm) at a peptide concentration below 10 μM ; $L/p = 10/1$). After that, no size change was observed with increasing peptide concentration (80 μM ; $L/p \approx 1$). Interestingly, Hel 11–7, which decreased

the turbidity of the liposome solution, led to a gradual increase in the liposome size with time at a peptide concentration of 20 μM ($L/p = 5/1$); the size increase became more pronounced at high peptide concentration ($L/p = 5/2$).

Electron microscopy

To investigate how the changes in turbidity and the size of liposomes induced by the Hel-series peptides are related to morphological changes of liposomes, an electron microscopy study was carried out. Egg PC and egg PC/egg PG (= 3/1) liposomes (1 mM) prepared by the extrusion method (Mayer et al., 1986) in the presence of EDTA were visualized through an electron microscope before and after the addition of peptide (200 μM). Fig. 6, *A-n* and *A-a*, shows representative micrographs of egg PC and egg PC/egg PG (= 3/1), respectively, taken at pH 7.4 without peptide. The particle diameter of liposomes of neutral or acidic liposomes was in the range of 30–100 nm. The mean diameter was ~ 60 nm.

Micrographs taken 24 h after the addition of the Hel-series peptides to neutral and acidic liposomes are shown in Fig. 6 *B-F*. In the presence of Hel 5–13, the shape and size of neutral liposomes did not change (Fig. 6 *B-n*), but for acidic liposomes, large aggregates of the liposomes (more than 1000 nm in diameter) were observed (Fig. 6 *B-a*), in which the size and shape of the respective constituent liposomes are almost the same as those of the original liposomes. It was noted that occasionally liposomes larger (but less than 150 nm) than the original liposomes were also observed for acidic liposomes in electron micrographs, suggesting that liposome fusion rarely took place (Fig. 6 *B-a*). The electron micrographs of Hel 7–11 in neutral liposomes showed the existence of small aggregates (maximum diameter: ~ 150 nm) as well as the original liposomes. In the case of acidic liposomes, however, in a manner similar to that of Hel 5–13, large aggregates were occasionally observed to contain the larger liposomes (Fig. 6 *C*).

Morphological changes to both acidic and neutral liposomes induced by Hel 9–9 were similar to those seen for the addition of Hel 11–7 (Fig. 6 *D-E*). This indicated that the two peptides interact with acidic and neutral lipid bilayers by a similar mechanism. In the case of neutral liposomes, the liposome became smaller in size (particle diameter distribution in 20–60 nm) and more rugged in shape after the addition of peptides. This indicates bicellar or micellar formation, resulting from the fragmentation of the original liposomes. Similar bicellar formation was observed as a flat sheet structure for the interaction of a lytic peptide, mellitin, with egg PC-dicetylphosphate-cholesterol (7:2:1) liposomes (Sessa et al., 1969) or egg PC (Dufourcq et al., 1986). Hel 11–7 occasionally forms large assemblies of the small particles (bicelles and/or micelles; diameter distribution in 20–40 nm), shown in Fig. 6 *E-n*. In acidic liposomes, the morphological change was more marked than that in neutral

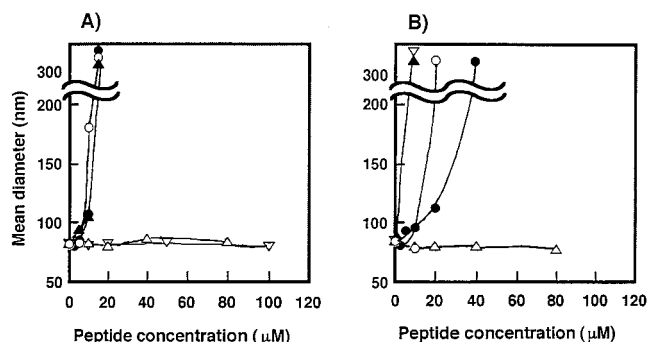


FIGURE 5 Change in mean hydrodynamic diameter of egg PC (*A*) and egg PC-egg PG (3:1) (*B*) liposomes as a function of peptide concentration at 24 h after incubation with the Hel-series peptides. Lipid concentration is 100 μM . No peptide addition (\blacklozenge), Hels 13–5 (\circ), 11–7 (\bullet), 9–9 (\triangle), 7–11 (\blacktriangle), and 5–13 (∇).

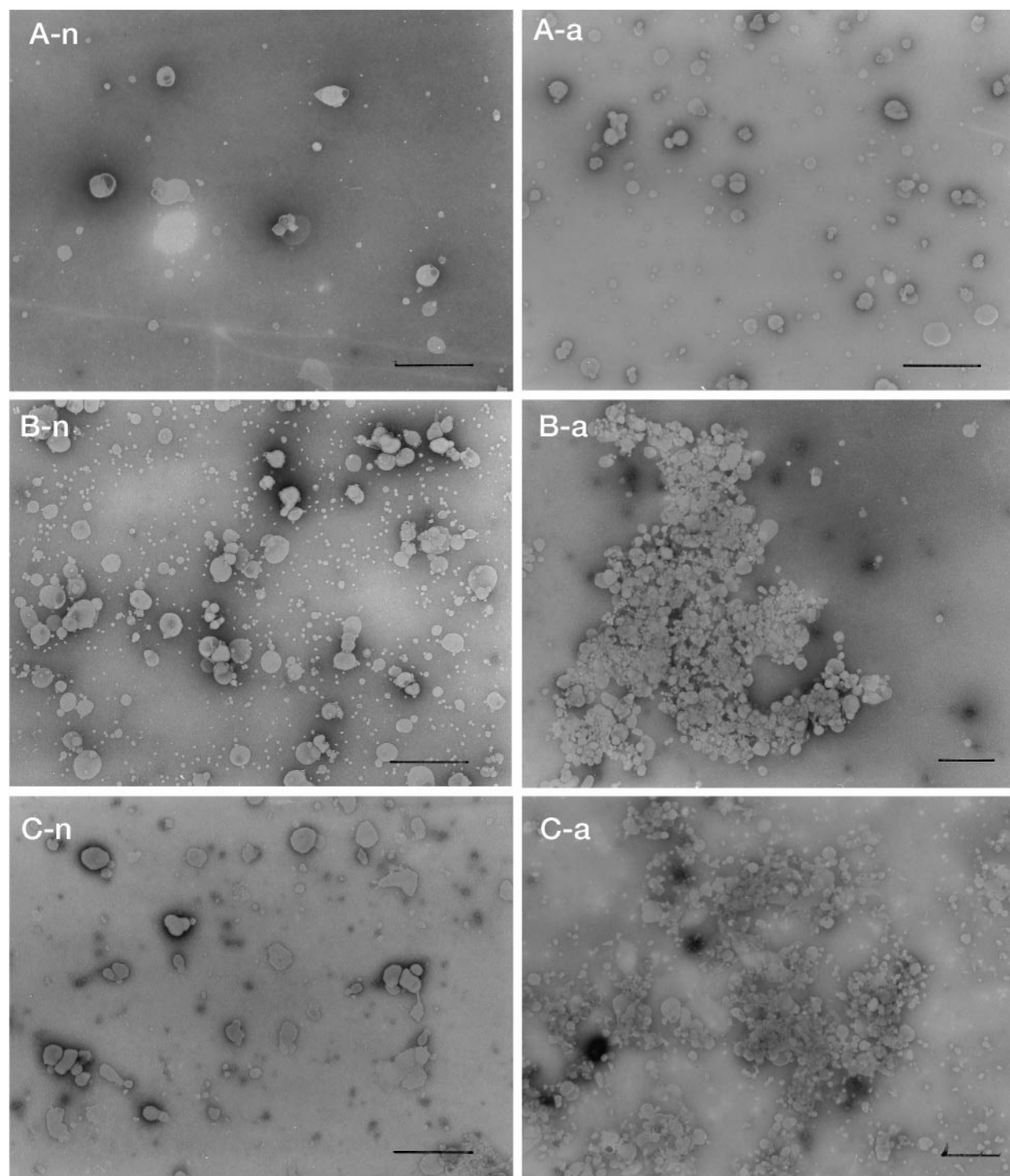


FIGURE 6 Electron micrographs of negatively stained liposomes. Egg PC alone (*A-n*) and egg PC-egg PG (3:1) liposomes alone (*A-a*); egg PC and egg PC-egg PG (3:1) liposomes incubated with Hels 5–13 (*B-n* and *B-a*), 7–11 (*C-n* and *C-a*), 9–9 (*D-n* and *D-a*), 11–7 (*E-n* and *E-a*), and 13–7 (*F-n* and *F-a*) for 24 hr. Peptide and lipid concentrations are 0.2 mM and 1 mM, respectively. The bar represents 200 nm.

liposomes; bicelles (or micelles) induced by both peptides were more abundant. The assemblies of the small particles induced by Hel 11–7 were much larger (more than 200 nm in diameter) than those induced by Hel 9–9 (~150 nm in diameter) (Fig. 6, *D-a* and *E-a*). The increase in the size of

liposomes was also observed by dynamic light scattering measurements. This rules out the possibility that the aggregation observed by electron microscopy was an artifact resulting from dehydration during preparation on the grid used for observation by electron microscopy.

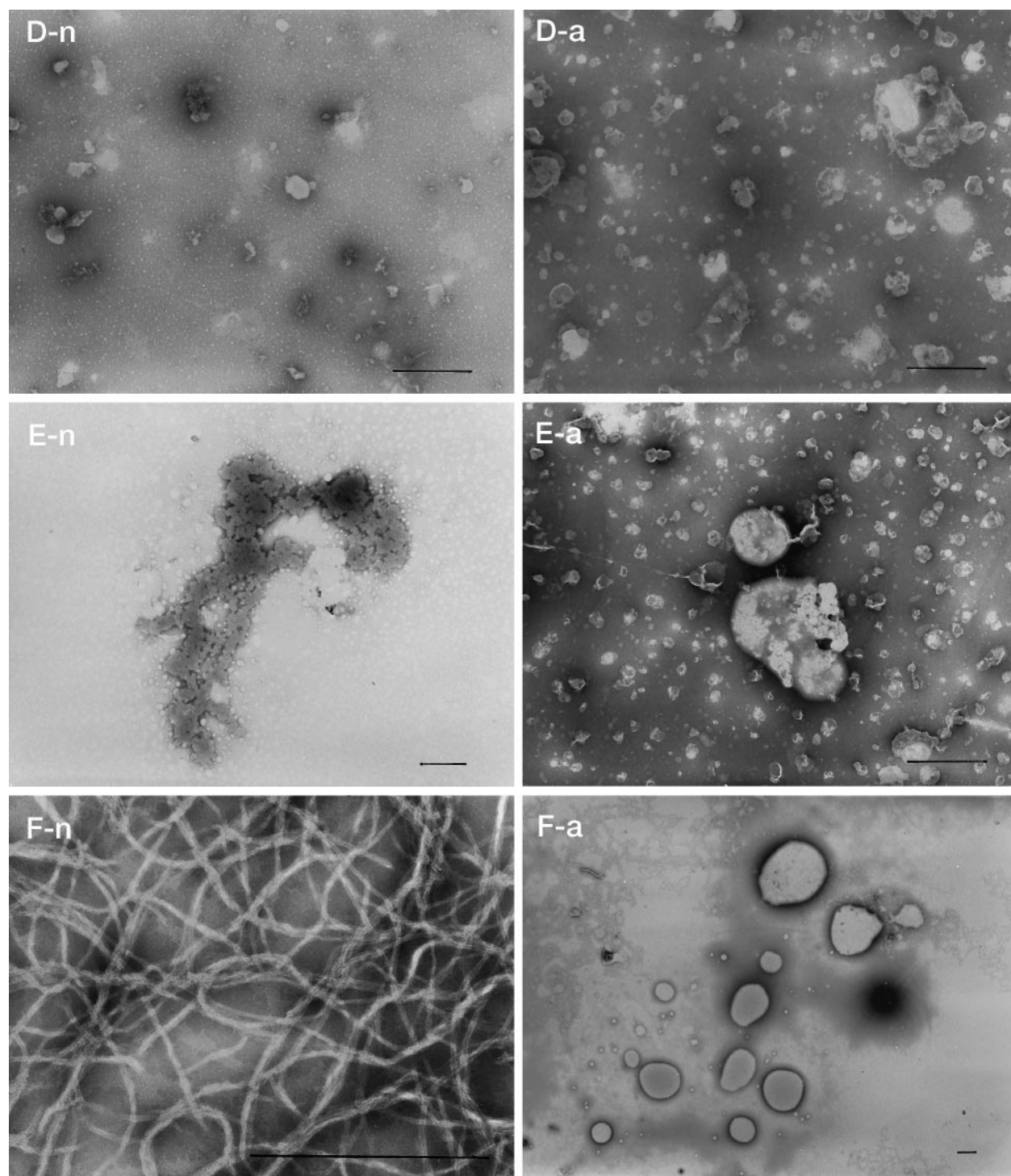


FIGURE 6—Continued.

Interestingly, Hel 13–5 interacted with neutral and acidic liposomes in a quite different manner, as shown in Fig. 6 *F*. When the peptide was added to neutral liposomes, the vesicle structure of the original liposomes disappeared completely, and lots of twisted ribbon-like fibers longer than 500 nm (mean diameter \sim 10 nm) were observed to lie on each other (Fig. 6 *F-n*). We have determined that the fiber structure does not result from a conformational change in

the peptide itself (from α -helical structure to β -structure). The circular dichroism (CD) spectrum of the peptide in the electron microscopy specimen showed α -helical structure in the liposome solution. There must be a new architecture formed at a certain composition of peptide and lipid. On the other hand, in acidic liposomes large assemblies, often more than 500 nm in diameter, were observed (Fig. 6 *F-a*). The assemblies were spherical, which indicated that they are

large unilamellar liposomes resulting from the fusion of several smaller liposomes.

Formation of ion channels

The planar bilayer method (Montal and Mueller, 1972; Iwata et al., 1994) was employed to examine whether the Hel-series peptides are able to form ion channels. Egg yolk phosphatidylethanolamine (egg PE)/egg PC and egg PE/bovine brain phosphatidylserine (brain PS) (7:3) were used as neutral and acidic phospholipid bilayers, respectively, because of the difficulty of forming stable planar bilayers with egg PC and egg PC/egg PG (= 3/1). As the current fluctuation between acidic and neutral planar bilayers induced by methanolic Hel-series peptides was quite different, the fraction of the number of runs in which membrane fluctuations were observed at an applied 50-mV membrane potential for 15 min after the addition of each peptide to the *cis* compartment was counted as listed in Table 1. No current change was observed when only methanol, the solvent, was added to the chamber. Hel 11-7 (3.3 μ M) exhibited constant membrane currents in both media. Hel 9-9 also caused moderate channel fluctuation, which occurred more frequently in acidic liposomes than in neutral liposomes. Interestingly, for Hel 13-5 a channel-like fluctuation occurred only at 6.6 μ M in neutral liposomes and was not observed in acidic liposomes. No current fluctuation was observed for Hels 7-11 and 5-13.

Representative channel traces formed by peptides at an applied potential of 50 mV at a low concentration of 3-7 μ M in neutral liposomes are shown in Fig. 7. After the addition of Hel 11-7 to the *cis* compartment of the chamber, a spike-like increase in current appeared after a time lag of ~10 min. By expanding the time scale (Fig. 7 *a*, inset), the current mode was found to consist of channel-type openings. The channel shows many different conductance levels. The lifetimes of the open states are very short, less than a second. By changing the membrane potential from positive to negative, the direction of the currents was reversed, but the channel events were slowed down.

The addition of Hel 13-5 also resulted in spike-like increases in current (Fig. 7 *b*); however, the open state had a much shorter lifetime, as shown in the channel trace of an

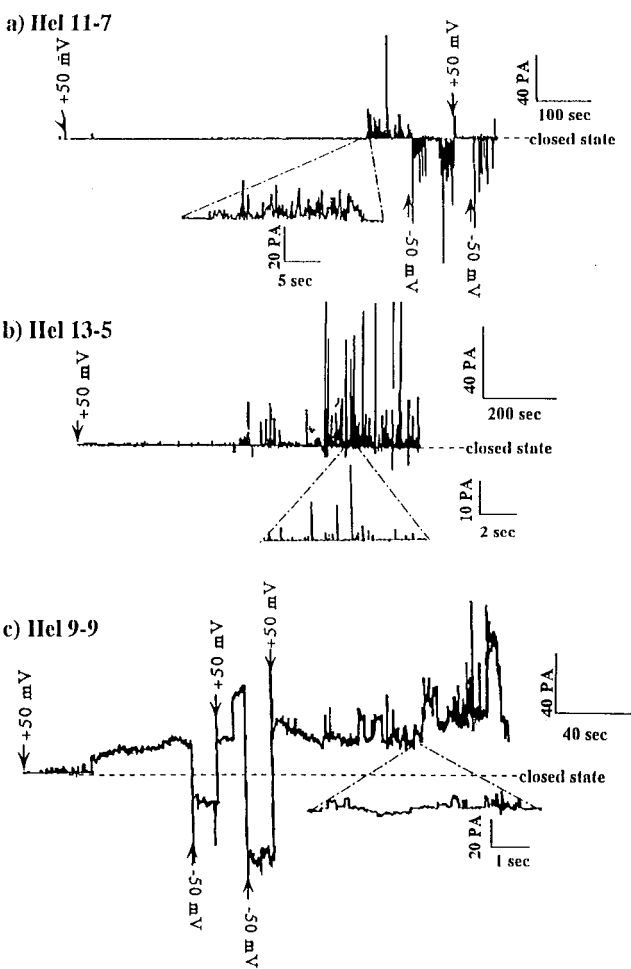


FIGURE 7 Current traces after the addition of Hels 11-7 (*a*), 13-5 (*b*), and 9-9 (*c*) in planar lipid bilayers (PE/PS = 7:1). Methanolic solutions of peptides were added to the *cis* compartment of the chamber with stirring, and then a membrane potential of +50 mV was applied. When necessary, the membrane potential was changed to -50 mV. The dashed line indicates the zero current level. A time expansion for part of the trace is also shown. Peptide concentrations of the *cis* compartment are ~3.3 μ M for Hels 11-7 and 9-9 and ~6.6 μ M for Hel 13-5.

expanded time scale. In Fig. 7 *c*, Hel 9-9 shows two current modes: one is a gradual increase in the conductance with time (mode 1 current), and the other is a spike-like increase

TABLE 1 Channel appearance probabilities of the Hel peptide series

	Channel formation probability					
	PE/PC			PE/PS		
	3.3 μ mol	6.6 μ mol	Total	3.3 μ mol	6.6 μ mol	Total
Hel 13-5	0/2	2/7	2/9	0/4	0/2	0/6
Hel 11-7	5/5	3/3	8/8	5/8	1/1	6/9
Hel 9-9	2/5	1/2	3/7	2/5	2/6	4/11
Hel 7-11	0/2	0/2	0/4	0/2	0/2	0/4
Hel 5-13	0/1	0/1	0/2		0/2	0/2

The probability is expressed as a function of the number of runs in which channel activity was observed at 50 mV within 30 min after addition of each peptide to the *cis* compartment of the chamber. Experiments were performed in 100 mM KCl solution in a symmetrical cell.

in the current (mode 2) appearing with a time lag and overlapping the mode 1 current. Mode 2 current also shows channel-like opening and closing with many different conductances. These results indicate that Hels 13–5, 11–7, and 9–9 are all able to aggregate to form a pore in lipid bilayers, although the channel properties of each are quite different. The channel traces of the Hel-series peptides in acidic lipid bilayers or their detailed channel properties in both media will be reported elsewhere.

Interestingly, in asymmetrical KCl solution (*cis*, 1 mM; *trans*, 100 mM) with no membrane potential (0 mV), the current flowed from *cis* to *trans*, indicating that the channel of Hel 11–7 is cation-selective (data not shown). These findings are consistent with those of amphiphilic peptides with repeating units of basic residues (Anzai et al., 1991; Iwata et al., 1994).

DISCUSSION

The structure of molecular assemblies of lipid-peptide mixture is mainly constructed with the following three interactions: the hydrophobic interaction between the apolar face of the peptide helices and lipid core, the polar interaction between the hydrophilic side of peptide and lipid head-group, and the interaction of hydrophilic residues of peptide with aqueous solvent (McLean et al., 1991). In a previous study, we have shown that the HHB of amphiphilic peptides largely dominates peptide-peptide and peptide-lipid interaction modes. Based on the experimental results, we have proposed a model to explain the interaction of the Hel-series peptides with lipid bilayers, which corresponds to the transformation processes *a–h* in Fig. 8 (Kiyota et al., 1996). All of the modes of interaction *a–h* of lipid-peptides in Fig. 8 were confirmed with minor revision by the present experiments, and furthermore, new molecular assemblies and transformation pathways (to *i–m*) were found. The experimental evidence for the present models will be described below.

Hel 13–5 has the largest hydrophobic face of the Hel series and binds most strongly to the lipid bilayers. It exhibited quite different interaction modes for acidic and neutral liposomes; fibril formation occurred after its addition to egg PC liposomes (see Fig. 8, pathway to *i*), and a fusion body resulted from its addition to egg PC/egg PG (= 3/1) (see pathway to *j* in Fig. 8). The channel-forming ability in planar lipid bilayers is different for neutral lipid bilayers (egg PE/egg PC = 7/3) and acidic bilayers (egg PE/brain PS = 7/3); Hel 13–5 forms an ion channel in neutral but not in acidic lipid bilayers (Table 1 and pathway to *h* in Fig. 8).

Dynamic light scattering measurements for neutral liposomes revealed that at an L/p ratio below 5 (peptide concentration, 20 μ M), liposome size increased gradually after the addition of Hel 13–5 (Fig. 5 *A*), indicating that at these concentrations, Hel 13–5 mainly forms ion channels (see Fig. 7 and pathway to *h* in Fig. 8). The liposome size

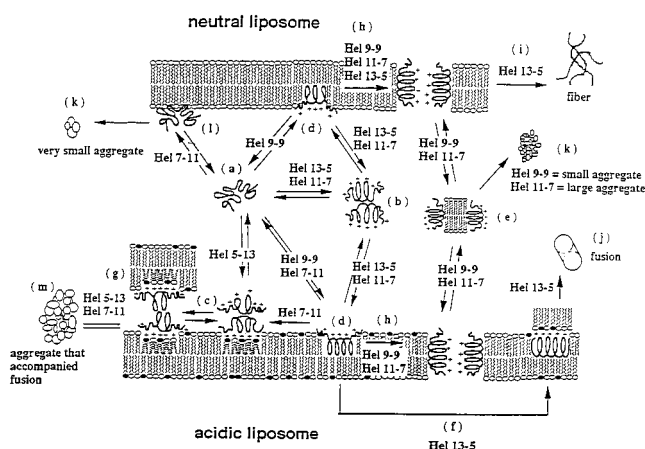


FIGURE 8 A representative scheme for the interaction mode of the Hel-series peptides with lipid bilayers. The captions of the pathways (*a–h*) have been given previously (Kiyota et al., 1996). New molecular assemblies and transformation pathways are as follows. (*i*) A twisted ribbon-like fibril structure resulting from the effect of Hel 13–5 on neutral lipids. (*j*) A fused form induced by Hel 13–5 in acidic liposomes. (*k*) An aggregated form of bicelles and/or micelles formed by peptides interacting with neutral or acidic liposomes (Hel 9–9 and 11–7). (*l*) A hydrophobic interaction between some hydrophobic residues of a random coil peptide and neutral lipid bilayers (Hel 7–11). (*m*) A large aggregate formed from intact and occasionally fused liposomes (Hels 5–13, 7–11). In the figure, the head-group of acidic phospholipid is marked in black.

increased from 80 nm to more than 300 nm at L/p = 5. However, the drastic decrease in turbidity after incubation of the peptide together with neutral liposome suggests that the liposome might have fragmented into bicelles or micelles. This discrepancy originated from different experimental techniques and was solved by electron microscopic observation; the electron microscopy clearly demonstrated that the fibril structure (~ 10 times more than 500 nm) is formed from a mixture of liposomes and Hel 13–5 (see Fig. 6 *F–n*).

The turbidity depends on the difference in the refractive indices between the scattering entity and the medium; the larger the difference is, the more turbid the solution becomes. It is likely that the lipid and peptide molecules are packed more loosely in the fibril assembly than in the liposomal assembly. In this case, the difference in the refractive indices between the molecular assembly and aqueous phase was smaller for the fibril structure than for the liposomal structure. The addition of Hel 13–5 to the neutral liposome solution results in a decrease in turbidity, accompanied by a transformation from liposome to fibril structure. In dynamic light scattering, on the other hand, the “size” of particles in solution is evaluated based on the translational diffusion coefficient of one particle, which is reflected in the fluctuation of scattered light intensity. The diffusion rate of the solutes with fibril structure should be quite low. Thus the size of the molecular assembly with fibril structure would apparently become very large, when derived from dynamic light scattering by a conventional analysis procedure.

On the other hand, the increase in turbidity (Fig. 3 *B*) and in liposome size (Figs. 4 and 5) caused by the addition of

Hel 13–5 to acidic liposomes and the appearance of relatively smooth spheres (Fig. 6 *F-a*) revealed that the large unilamellar liposomes are formed by the fusion of original small acidic liposomes (pathway to *j* in Fig. 8). The liposome size increase is not prominent below $L/p = 10/1$ at 24 h after the addition of the peptide, in which the liposome turbidity is at maximum. Above $L/p = 5$, the liposome size is drastically increased and the turbidity is slightly decreased. These results suggest that at high L/p , peptides may accumulate in the lipid bilayer surface, partially penetrating the hydrophobic group, which accompanies the reduction of the surface charge due to the electrostatic peptide-lipid interaction (Fig. 2 *B*). With lower L/p , the surface charge of the outer leaflet in bilayers is reduced or neutralized by the cationic groups of peptides. This facilitates the interaction with other liposomes through charge interaction (see pathway to *f* in Fig. 8). Furthermore, the reduction in surface charge may lead to a decrease in the anchoring ability of peptide molecules in the lipid bilayer. As a result, the predominant hydrophobic interaction between peptide and lipid favors the penetration of Hel 13–5 from the bilayer surface into the nonpolar acyl chain region, resulting in the disturbance of the membrane and fusion (see pathway to *j* in Fig. 8). A comparable interaction mode has recently been proposed on the basis that the membrane-disturbing effect of a peptide, KLAK, having almost the same hydrophobicity and hydrophilic angle as Hel 13–5, is enhanced with a decreasing amount of anionic phospholipid in the bilayer, despite the drastic reduction of binding affinity (Dathe et al., 1996). It is noted that Hel 13–5 did not form ion channels.

Hels 11–7 and 9–9 interact with lipid bilayers in a similar manner, although there are some differences. As shown in the previous and present fluorescence studies, both peptides can partially insert the hydrophobic part of their amphiphilic helix into a lipid bilayer. Both peptides solubilize liposomes to small bicelles or micelles (~20–60 nm in diameter), because many particles smaller than the original liposomes (30–100 nm) were observed in electron micrographs (Fig. 6, *D* and *E*). The reduction in turbidity by the addition of peptides to liposomes also supports such observations (Fig. 3). As compared with their solubilization ability, Hel 11–7 is much higher than Hel 9–9, especially for neutral lipid bilayers. However, according to the results of the dynamic light scattering analysis, the addition of Hel 11–7 to neutral and acidic liposomes led to an increase in particle size in comparison with the original liposomes. Electron micrographs of acidic and neutral liposomes 24 h after the addition of peptide showed the presence of large assemblies more than 500 nm in diameter, formed by the gathering of small particles produced by fragmentation of liposomes. These results indicate that Hel 11–7 first solubilizes liposomes to small particles (bicelle and/or micelle) (see pathway to *e* in Fig. 8), and then the bicelles formed produce large aggregate (see pathway to *k* in Fig. 8), resulting in an increase in mean diameter, as observed by the light scattering experiment. Hel 9–9, like Hel 11–7, also produces a small aggregate (no longer than 150 nm) and many small

bicelles (Fig. 6 *D*) with a mean diameter (see Fig. 4) smaller in acidic liposomes and slightly larger in neutral liposomes than the original liposomes. It should be mentioned that the difference between Hels 11–7 and 9–9 in acidic liposomes may come from a difference in charge as well as hydrophobicity. Hels 11–7 and 9–9 form ion channels in neutral and acidic lipid bilayers. Hel 11–7 formed channels more frequently than Hel 9–9 (Table 1), and its ion selectivity was cationic. The peptides with an HHB equivalent to Hels 11–7 and 9–9 are common in recent literature and are as follows: 1) naturally occurring antibacterial and toxic peptides, such as cecropin, magainin, mastoparan (Segrest et al., 1990; Saberwal and Nagaraj, 1994); 2) amphiphilic model peptides that dissolve membranes by forming bicelles (Reynaud et al., 1993); and 3) cation-selective ion channel-forming model peptides (Polozov et al., 1997; Iwata et al., 1994; Agawa et al., 1991; Anzai et al., 1991; Lee et al., 1993).

Hels 7–11 and 5–13 interact with neutral or acidic liposomes in a basically similar manner, as observed in present electron micrographs. In a previous study, we proposed that although both peptides interact with an acidic lipid bilayer by charge interactions between anionic lipid headgroups and cationic Lys residues, the hydrophobic parts of the peptides are not inserted into the lipid bilayers (see pathway to *c* in Fig. 8). But as described in the present Trp-fluorescent quenching studies (Fig. 2 *B*), Hel 7–11 is able to insert its hydrophobic parts into the lipid core with a weaker interaction than Hels 9–9, 11–7, and 13–5 (see pathway to *d* in Fig. 8). The charge interaction between the cationic residues in peptides and the anionic headgroups of acidic phospholipid may help such hydrophobic interactions. However, Hel 7–11 cannot fragment acidic liposomes, despite the insertion of its hydrophobic parts into lipid bilayers. This may be due to the fact that there is no deep transfer from the surface to the lipid core because of low hydrophobicity, resulting in no channel or pore formation.

In any case, the electron micrograph seen 24 h after the addition of both peptides demonstrates the presence of large assemblies produced by the aggregation of original liposomes (Fig. 6, *B-a* and *C-a*, and pathway to *m* in Fig. 8), which took place rapidly after the addition of the peptides, as shown in Fig. 4 *B*. These assemblies are easily dissociated to original liposomes by weak sonication, as reported in previous investigations (Kiyota et al., 1996). It should be mentioned that in electron micrographs, liposomes larger (less than 200 nm) than the original liposomes were occasionally observed, suggesting that liposome fusion rarely takes place.

As shown in the previous study, no interaction behavior was observed for Hels 7–11 and 5–13 in neutral liposome. However, the present light scattering study on Hel 7–11 shows that the size of liposomes is gradually increased with time after the addition of peptide (Fig. 4 *A*) and became 1.3 times larger than the original liposomes after 24 h (Fig. 5 *A*). Such results are supported by the formation of small aggregates, as shown by electron micrographs (Fig. 6 *C-n*). It should be noted that in such a situation, the peptide does not

take an α -helical structure (Kiyota et al., 1996) and does not immerse the Trp residue in the lipid core, as seen in Fig. 2 A. These results suggest that Hel 7–11 interacts with neutral lipids, probably by a hydrophobic interaction between hydrophobic Leu residues in the peptide and the lipid bilayer (pathway to *l* in Fig. 8).

It would be helpful to determine for any given peptide the value of its HHB at which it inserts into the hydrophobic region of the bilayer. The degree of hydrophobicity at which peptides will insert into the lipid bilayer has been extensively studied as a function of their amino acids, using model peptides and model membranes (Deber and Goto, 1996; Blondelle et al., 1997). Spontaneous insertion of peptides into lipid bilayers has also been studied for alteration of hydrophobicity of model peptides (Chung and Thompson, 1996; Liu and Deber, 1997). However, the boundary hydrophobicity partitioning peptides into lipid bilayers is not yet understood. In the present study, the boundaries of hydrophobic interaction are likely to present at a slightly less hydrophobic angle (between Hel 7–11 and Hel 9–9). Such peptides can bind to lipids through their hydrophobic residues or by the formation of a hydrophobic part of the helix generated by a helpful interaction with the charged groups of a lipid. In connection with this, Oren et al. (1997) have shown by antibacterial studies on Gram-negative bacteria that if the peptide possesses enough hydrophobic residues to inhibit the growth of bacteria, an α -helical structure is not needed.

Brasseur (1991) has attempted a classification of lipid-associating helices with different hydrophobic and hydrophilic angles. If the hydrophobic angle is greater than 180° , then the helix will pack as a transmembrane pore (class I); if the angle is equal to 180° , then the helix will lie parallel to the membrane surface (class II); and if the angle is less than 180° , the helix will solubilize the lipid bilayers by the formation of discoidal particles as the helices around the edge of the lipid bilayer (class III). Brasseur et al. (1997) have reviewed how peptides tip the balance of membrane stability (named as oblique peptides) by possessing a hydrophobicity between 0.2 and 0.6 and a high amphiphilicity as well as a hydrophobic moment around 0.4. Peptides play an important role in intra- and intercellular functions, such as the cellular process of vesicular fusion, protein transport across subcellular compartments, and remodeling of lipid cores (class IV).

The Hel-series peptides have relatively high hydrophobic moments ($\mu H = 0.35$ – 0.50) and different hydrophobicities ($H = 0.07, -0.11, -0.29, -0.48$, and -0.66) resulting from various hydrophobic angles ($260^\circ, 220^\circ, 180^\circ, 140^\circ$, and 100°). When applying the Brasseur classification of the Hel-series peptides, Hel 11–7 and 9–9 possess the character of class I, II, and III, namely channel formation, lying parallel to the lipid surface and bicellar and micellar formation. Most naturally occurring membrane-acting peptides, such as magainin, cecropin, and mastoparan, belong to these classes.

Only Hel 13–5 belongs to class IV. Most surprising is the fact that the mixing of Hel 13–5 with neutral liposomes resulted in fibril formation and mixing with acidic liposomes induced liposome fusion. Obliquely oriented peptides (class IV) often mediate the formation of neurotoxicity amyloid fibrils as well as a fusion event. This suggests that peptides occurring in membrane, such as amyloid peptide or prion disease peptide, may change the action mode by their existing surroundings as follows: 1) by affecting the remodeling of lipid cores to fibril forms with lipids or (2) by mediating cytotoxicity through a direct perturbation of the cellular plasma membrane or vesicle fusion.

In conclusion, we have shown that the HHB of a peptide is a crucial factor in understanding its lipid-peptide interaction. Furthermore, the composition of phospholipids within a lipid bilayer largely affects its morphological change. In this connection, there is a report that a membrane-lytic peptide that belongs to class II according to the Brasseur classification as described above can fragment acidic liposomes composed of dioleoylphosphatidylcholine (DOPC)-dioleoylphosphatidylglycerol; this is consistent with our experimental results. However, it induced fusion of neutral liposomes composed of DOPC and dioleoylphosphatidylethanolamine, which contradicts our results (Polozov et al., 1997). This indicates how a subtle difference in lipid composition can induce a different mode of interaction. A study of interactions of the Hel-series peptides with lipid bilayers composed of different phospholipids and various compositions is in progress.

We extend our great thanks Dr. Soji Yamashita, Kyushu University, Fukuoka, for his helpful discussion and to Dr. Eric Chamber, University of Bristol, Bristol, for his reading of this paper and helpful discussion.

This work was supported in part by a Grant in Aid for Scientific Research (C) from the Ministry of Education Culture and Science of Japan, and by grants from the Science Promotion Foundation of Kim Man-Yu and from the Central Institute of Fukuoka University.

REFERENCES

- Agawa, Y., S. Lee, S. Ono, H. Aoyagi, M. Ohno, T. Taniguchi, K. Anzai, & Y. Kirino. 1991. Interaction with phospholipid bilayers, ion channel formation, and antimicrobial activity of basic amphipathic α -helical model peptides of various chain lengths. *J. Biol. Chem.* 266: 20218–20222.
- Anzai, K., M. Hamasuna, H. Kadono, S. Lee, H. Aoyagi, and Y. Kirino. 1991. Formation of ion channels in planar lipid bilayer membranes by synthetic basic peptides. *Biochim. Biophys. Acta.* 1064:256–266.
- Blondelle, S. E., B. Forood, R. A. Houghton, and E. Pérez-Payá. 1997. Secondary structure induction in aqueous vs. membrane-like environments. *Biopolymers.* 42:489–498.
- Blondelle, S. E., and R. A. Houghton. 1992. Design of model amphipathic peptides having potent antimicrobial activities. *Biochemistry.* 31: 12688–12694.
- Brasseur, R. 1991. Differentiation of lipid-associating helices by use of three-dimensional molecular hydrophobicity potential calculations. *J. Biol. Chem.* 266:16120–16127.
- Brasseur, R., T. Pillot, L. Lins, J. Vandekerckhove, and M. Rosseneu. 1997. Peptides in membranes: tipping the balance of membrane stability. *Trends Biochem. Sci.* 22:167–171.

- Chung, L. A., and T. E. Thompson. 1996. Design of membrane-inserting peptides: spectroscopic characterization with and without lipid bilayers. *Biochemistry*. 35:11343–11354.
- Cornut, I., K. Büttner, J.-L. Dasseux, and J. Dufourcq. 1994. The amphipathic α -helix concept. Application to the de novo design of ideally amphipathic Leu, Lys peptides with hemolytic activity higher than that of melittin. *FEBS Lett.* 349:29–33.
- Dathe, M., M. Schümann, T. Wieprecht, A. Winkler, M. Beyermann, E. Krause, K. Matsuzaki, O. Murase, and M. Bienert. 1996. Peptide helicity and membrane surface charge modulate the balance of electrostatic and hydrophobic interactions with lipid bilayers and biological membranes. *Biochemistry*. 35:12612–12622.
- Dathe, M., T. Wieprecht, H. Nikolenko, L. Handel, W. L. Maloy, D. L. MacDonald, M. Beyermann, and M. Bienert. 1997. Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides. *FEBS Lett.* 403:208–212.
- Dawidowicz, E. A., and J. E. Rothman. 1976. Fusion and protein-mediated phospholipid exchange studied with single bilayer phosphatidylcholine vesicles of different density. *Biochim. Biophys. Acta.* 455:621–630.
- Deber, C. M., and N. K. Goto. 1996. Folding proteins into membranes. *Nature Struct. Biol.* 3:815–818.
- Dufourcq, J., J.-F. Faucon, G. Fourche, J.-L. Dasseux, M. Le Maire, and T. Gulik-Krzywicki. 1986. Morphological changes of phosphatidylcholine bilayers induced by melittin: vesicularization, fusion, discoidal particles. *Biophys. Biochim. Acta.* 859:33–48.
- Eisenberg, D. 1984. Three-dimensional structure of membrane and surface proteins. *Annu. Rev. Biochem.* 53:595–623.
- Epand, R. M., Y. Shai, J. P. Segrest, and G. M. Anantharamaiah. 1995. Mechanisms for the modulation of membrane bilayer properties by amphipathic helical peptides. *Biopolymers*. 37:319–338.
- Ghosh, A. K., R. Rukmini, and A. Chattopadhyay. 1997. Modulation of tryptophan environment in membrane-bound melittin by negatively charged phospholipids: implications in membrane organization and function. *Biochemistry*. 36:14291–14305.
- Hancock, R. E. W. 1997. Peptide antibiotics. *Lancet*. 349:418–422.
- Iwata, T., S. Lee, O. Oishi, H. Aoyagi, M. Ohno, K. Anzai, Y. Kirino, and G. Sugihara. 1994. Design and synthesis of amphipathic 3_{10} -helical peptides and their interactions with phospholipid bilayers and ion channel formation. *J. Biol. Chem.* 269:4928–4933.
- Kini, R. M., and H. J. Evans. 1989. A common cytolytic region in myotoxins, hemolysins, cardiotoxins and antibacterial peptides. *Int. J. Peptide Protein Res.* 34:277–286.
- Kiyota, T., S. Lee, and G. Sugihara. 1996. Design and synthesis of amphiphilic α -helical peptides with systematically varied hydrophobic-hydrophilic balance and their interaction with lipid- and bio-membrane. *Biochemistry*. 35:13196–13204.
- Lee, S., T. Iwata, H. Oyagi, H. Aoyagi, M. Ohno, K. Anzai, Y. Kirino, and G. Sugihara. 1993. Effect of salts on conformational change of basic amphipathic peptides from beta-structure to α -helix in the presence of phospholipid liposomes and their channel-forming ability. *Biochim. Biophys. Acta.* 1151:76–82.
- Lee, S., T. Kiyota, T. Kunitake, E. Matsumoto, S. Yamashita, K. Anzai, and G. Sugihara. 1997. De novo design, synthesis, and characterization of a pore-forming small globular protein and its insertion into lipid bilayers. *Biochemistry*. 36:3782–3791.
- Liu, L.-P., and C. M. Deber. 1997. Anionic phospholipids modulate peptide insertion into membranes. *Biochemistry*. 36:5476–5482.
- Maloy, W. L., and U. P. Kari. 1995. Structure-activity studies on magainins and other host defense peptides. *Biopolymers*. 37:105–122.
- Mayer, L. D., M. J. Hope, and P. R. Cullis. 1986. Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochim. Biophys. Acta.* 858:161–168.
- McLean, L. R., K. A. Hagaman, T. J. Owen, and J. L. Krstenansky. 1991. Minimal peptide length for interaction of amphipathic α -helical peptides with phosphatidylcholine liposomes. *Biochemistry*. 30:31–37.
- Minami, H., T. Inoue, and R. Shimozaawa. 1996. Beryllium ion can induce the aggregation of phosphatidylcholine vesicles. *Langmuir*. 12:3574–3579.
- Montal, M., and P. Mueller. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA.* 69:3561–3566.
- Oren, Z., J. Hong, and Y. Shai. 1997. A repertoire of novel antibacterial diastereomeric peptides with selective cytolytic activity. *J. Biol. Chem.* 272:14643–14649.
- Oren, Z., and Y. Shai. 1997. Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: structure-function study. *Biochemistry*. 36:1826–1835.
- Park, N. G., Y. Yamato, S. Lee, and G. Sugihara. 1995. Interaction of mastoparan-B from venom of a hornet in Taiwan with phospholipid bilayers and its antimicrobial activity. *Biopolymers*. 36:793–801.
- Polozov, I. V., A. I. Polozova, E. M. Tytler, G. M. Anantharamaiah, J. P. Segrest, G. A. Woolley, and R. M. Epand. 1997. Role of lipids in the permeabilization of membranes by class I amphipathic helical peptides. *Biochemistry*. 36:9237–9245.
- Reynaud, J. A., J. P. Grivet, D. Sy, and Y. Trudelle. 1993. Interactions of basic amphiphilic peptides with dimyristoylphosphatidylcholine small unilamellar vesicles: optical, NMR, and electron microscopy studies and conformational calculations. *Biochemistry*. 32:4997–5008.
- Saberwal, G., and R. Nagaraj. 1994. Cell-lytic and antibacterial peptides that act by perturbing the barrier function of membranes: facets of their conformational features, structure-function correlations and membrane-perturbing abilities. *Biochim. Biophys. Acta.* 1197:109–131.
- Segrest, J. P., H. De Loof, J. G. Dohlman, C. G. Brouillette, and G. M. Anantharamaiah. 1990. Amphipathic helix motif: classes and properties. *Proteins Struct. Funct. Genet.* 8:103–117.
- Sessa, G., H. J. Freer, G. Colacicco, and G. Weissmann. 1969. Interaction of alytic polypeptide, melittin, with lipid membrane systems. *J. Biol. Chem.* 244:3575–3582.
- Shai, Y., and Z. Oren. 1996. Diastereoisomers of cytolytic peptides, a novel class of potent antibacterial peptides. *J. Biol. Chem.* 271:7305–7308.
- Silverstro, L., K. Gupta, J. N. Weiser, and P. H. Axelsen. 1997. The concentration-dependent membrane activity of cecropin A. *Biochemistry*. 36:11452–11460.
- Suenaga, M., S. Lee, N. G. Park, H. Aoyagi, T. Kato, A. Umeda, and K. Amako. 1989. Basic amphipathic helical peptides induce destabilization and fusion of acidic and neutral liposomes. *Biochim. Biophys. Acta.* 981:143–150.
- Surewicz, W. K., and R. M. Epand. 1984. Role of peptide structure in lipid-peptide interaction; a fluorescence study of the binding of penta-gastrin-related pentapeptides to phospholipid vesicles. *Biochemistry*. 23:6072–6077.
- Tytler, E. M., J. P. Segrest, R. M. Epand, S.-Q. Nie, R. F. Epand, V. K. Mishra, Y. V. Venkatachalapathi, and G. M. Anantharamaiah. 1993. Reciprocal effects of apolipoprotein and lytic peptide analogs on membranes. Cross-sectional molecular shapes of amphipathic α -helices control membrane stability. *J. Biol. Chem.* 268:22112–22118.
- Wieprecht, T., M. Dathe, M. Beyermann, E. Krause, W. L. Maloy, D. L. MacDonald, and M. Bienert. 1997. Peptide hydrophobicity controls the activity and selectivity of magainin 2 amide in interaction with membrane. *Biochemistry*. 36:6124–6132.
- Yoshimura, T., Y. Goto, and S. Aimoto. 1992. Fusion of phospholipid vesicles induced by an amphiphilic model peptide: close correlation between fusogenicity and hydrophobicity of the peptide in an α -helix. *Biochemistry*. 31:6119–6126.