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Two mode ion channels induced by interaction of acidic amphipathic α-helical peptides with lipid bilayers

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Abstract

In order to investigate the ion permeability and selectivity of ion channel formed by amphipathic α-helical peptides, we designed to synthesize an acidic peptide Ac(-Leu-Ala-Glu-Leu-)$_3$NHCH$_3$ (Glu-4$_3$) and its channel property was compared with a basic peptides Arg-4$_3$ in which Glu in Glu-4$_3$ was replaced by Arg. Two modes of the conductance change were observed by the interaction of Glu-4$_3$ with planar lipid bilayers; a steady increase of the conductance with the elapse of time (mode 1) and a spike-like increase of the current (mode 2) appearing with a lag time and overlapping the model current increase. The application of negative membrane potential induced the mode 1 current and the lowering pH decreased it, suggesting that the mode 1 current is caused by slow insertion of Glu-4$_3$ into the lipid bilayer and then by forming certain unknown bundles like semichannels. Mode 2 was found to be consisted of channel type opened-close current with several different conductances and relatively short opening lifetimes. There was no ion selectivity in the mode 1 current, whereas the mode 2 current was cation selective. The peptide Arg-4$_3$ has formed a cation-selective ion channel but not shown such two mode current changes. The membrane potential formation experiment in liposomes using DiSC$_3$(5) also showed the cation selectivity for Arg-4$_3$ and non-ion selectivity for Glu-4$_3$. The difference between Arg-4$_3$ and Glu-4$_3$ was also observed in conformational analysis by CD and in dye-release experiment from liposome. Such difference was discussed in terms of electrostatic interaction between peptides and lipid head groups.

Key words: Amphipathic α-helical peptide; Lipid–peptide interaction; Ion channel-forming peptide; Liposome

1. Introduction

Ion channels are an important target to the understanding of membrane biology. Recently, channels, integral membrane proteins spanning the membrane, have been shown to consist of α-helix [1,2] or β-structure [3]. The channel pore is postulated to be formed by self-aggregated oligomer of amphipathic peptide segments with hydrophilic residues facing inward and with hydrophobic residues facing outward in contact with the hydrophobic protein subunits or the hydrophobic regions of phospholipid molecules in the interior of the membranes. A number of naturally occurring amphipathic peptides have also been reported to form ion channels [4–7]. Recent studies using peptides covalently attached to a template molecule with a four or five helix bundle configuration make sure that oligomeric clusters of the amphiphilic α-helices are the structural feature in channel forming

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Abbreviations: Ac, acetyl; Boc, *t*-butyloxycarbonyl; Bzl, benzyl; DiSC$_3$(5), 3,3′-dipropylthiadicarbocyanine iodide; DiphytPC, diphytanoyl-L-α-phosphatidylcholine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; egg PC, egg-yolk phosphatidylcholine; egg PE, egg-yolk phosphatidylethanolamine; egg PG, egg-yolk phosphatidylglycerol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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Ac-(Leu-Ala-Arg-Leu)3-NHCH3 Arg-43
H-(Leu-Ala-Arg-Leu)3-(Leu-Arg-Ala-Leu)3-OH Arg-46
Ac-(Leu-Ala-Glu-Leu)3-NHCH3 Glu-43

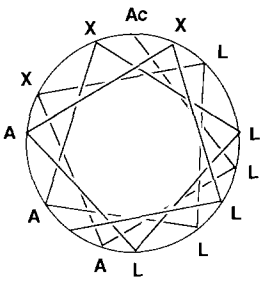


Fig. 1. Structures of the model amphipathic peptides and their helical wheels.

peptides [8,9]. Thus, studies of channel-forming amphipathic peptides will contribute to the understanding of general mechanism of structural and functional properties of ion channel proteins in biomembranes. However, the ion selectivity of the pore formed from such amphipathic peptides still remains undissolved.

We have shown previously that various cationic and amphipathic model peptides are able to form ion channels in artificial membranes taking α-helix or 3_{10}-helical structure in the presence of lipid bilayers [10–12]. For example, basic model peptides Arg-4_3 or -4_6 (Fig. 1) containing a repeat sequence of the tetrapeptide unit, (Leu-Ala-Arg-Leu), could form an amphipathic α-helical structure in the presence of phospholipid liposomes and form an ion channel in planar lipid bilayers. It is worth noting that these basic amphipathic model peptides unexpectedly showed a cation selectivity on ion transference across the membrane; since the peptides contain many cationic amino acid residues, the anion selectivity had been expected when they form a channel by aggregation in lipid bilayers. The cation selective ion channels were also found for the S4 segment in sodium channel proteins [13] and for the model peptides (Xxx-Arg-Leu)$_8$ (Xxx; Ala, Leu or Val) [12]. Note that the latter model peptides have basic residues at every third position in the sequence and showed amphipathic properties by taking 3_{10}-helical structure. In order to reveal the mechanism of exhibiting such unexpected cation selectivity of the channels formed with cationic amphipathic peptides, in the present study, we darely designed to synthesize an anionic amphipathic α-helical peptide, Glu-4_3, in which Arg in Arg-4_3 is replaced to Glu and the mode of the interaction of the peptide with lipid bilayer was investigated in terms of the secondary structure measured by CD, carboxyfluorescence leakage from liposomes and ion permeation through planar lipid bilayer membranes.

2. Materials and methods

Materials. Egg PC, egg PE and egg PG, were purchased from Sigma (St. Louis, MO). DiphytPC was purchased from Avanti Polar Lipids (Delham, AL). $DiSC_3(5)$ was obtained from Molecular Probes, (Eugene, OR) and used without further purification. Carboxyfluorescein from Eastman Kodak was purified by recrystallization from ethanol/water mixture. All other reagents were of the highest analytical grade.

Synthesis of peptide. Glu-4_3 was synthesized by the solution method. Synthetic route for Glu-4_3 is essentially same as that for Arg-4_3 described previously except that Arg (Tos) was replaced with Glu(OBzl). Tetrapeptide units were prepared stepwise from the C-terminus, and the units were condensed to the desired sizes by the EDC-HOBT method [14]. During the condensation, the N-terminus was protected by the Boc group, and the C-terminus was protected by the phenacyl or *N*-methylamide, the crude peptide obtained after hydrogenation of the fully protected peptide in the presence of Pd-black 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% ammonium acetate (pH, 7.0). Data of amino acid analysis after hydrolysis in 6 M HCl were Ala 2.08, Leu 1.00 and Glu 0.96 in molar ratio.

Preparation of phospholipid liposomes. Egg PC or egg PC/egg PG (3 : 1) (20 mg) in $CHCl_3$ (about 2 ml) was dried under a steam 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 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 approximately 1.1 mM. The mixture of uni- and multilamellar vesicles was 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 Wako reagent pur-

chased from Wako Pure Chemical Industries (Osaka, Japan) and was expressed in terms of phosphorus concentration.

CD experiment. CD spectra were recorded on a JASCO J-600 spectropolarimeter using 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 [15]. To a 2 ml of 5 mM Hepes buffer (pH 7.4) in a cuvette was added a 50 μl of the vesicles containing 100 mM carboxyfluorescein which was prepared as described in 'Liposome preparation' 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 equation, $100 \cdot (F - F_0)/(F_t - F_0)$, 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.

Planar bilayer formation and current measurement. Planar lipid bilayer membranes were formed by the folding method as described previously [11,16]. Egg PC/egg PE (7:3) or DiphytPC was used as a bilayer-forming lipid. A small quantity (usually 5–10 μl) of the peptide dissolved in methanol was added to one compartment (*cis*) of the chamber with stirring. Membrane current was measured under voltage clamp conditions. The membrane potential was defined as the potential of *cis* with respect to the *trans* compartment. 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).

Measurement of membrane potential formation in liposomes. Egg PC small unilamellar liposomes (200 mg lipid/ml) were prepared in a solution containing 158 mM sucrose/1 mM KCl/10 mM Tris-Hepes (pH 7.4). The liposomes (0.2 ml) were diluted to 2 ml with a medium containing 100 mM KCl/10 mM Tris-Hepes (pH 7.4) in a cuvette. The solution for the preparation of the liposomes and the one for the dilution of the liposomes had the same osmolarity as checked with an osmometer (OM 801S, Vogel). The cuvette was set in a fluorescence spectrophotometer (F2000, Hitachi) and to the cuvette 2 ml of ethanolic solution of $DiSC_3(5)$ [17] was added with stirring (final 1 mM). After waiting for a few minutes, 5 μl of a sample solution was added, and the change of the fluorescence intensity was monitored at 670 nm with excitation at 622 nm. The cuvette was thermostated at 25°C.

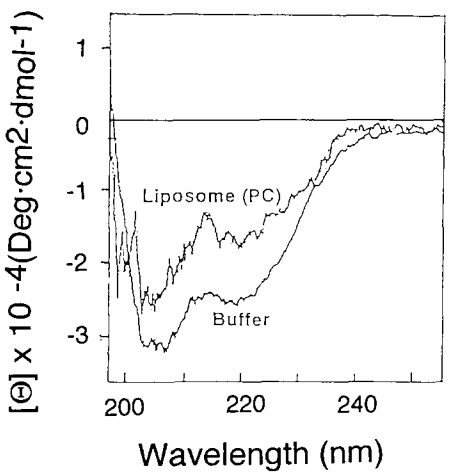


Fig. 2. CD spectra of the peptides in 5 mM Hepes buffer (pH 7.4) in the presence and absence of egg PC liposomes. Spectra in Hepes buffer were measured at a concentration of 100 μM. Multilamellar vesicles were prepared by sonication and the peptides were dissolved at a concentration of 10 μM in Hepes buffer solution containing 0.9 mM liposomes.

3. Results

As previously reported, Arg-4_3 forms a stable α-helical structure and has a strong channel forming-probability in neutral liposomes [11,18]. Therefore we chose it as parent peptide. Replacement of Arg in

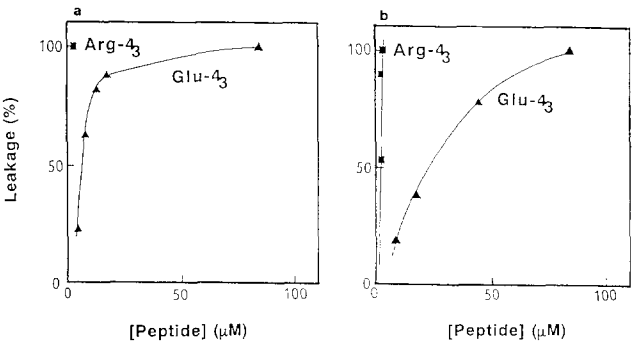
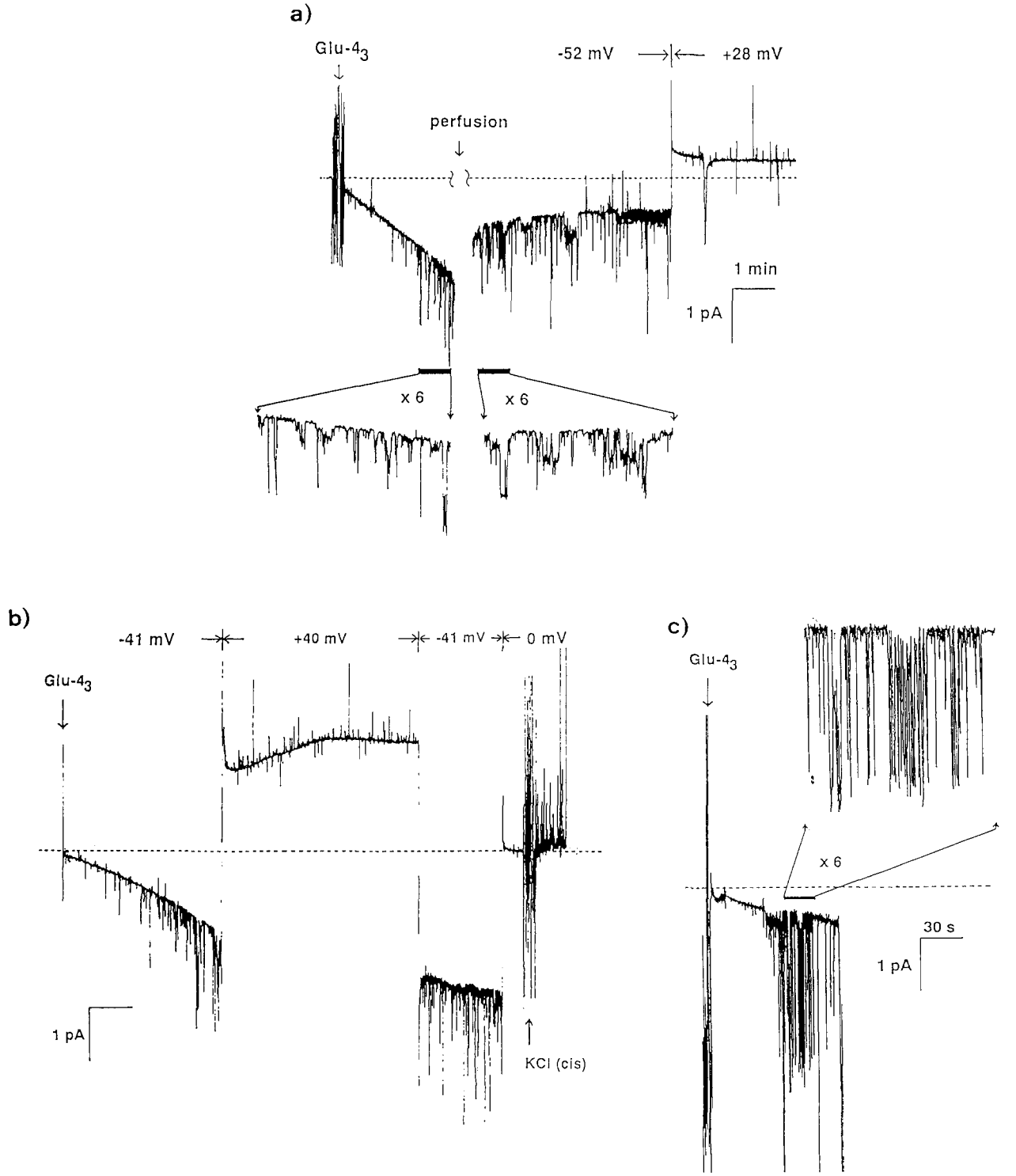


Fig. 3. Profiles of carboxyfluorescein leakage from egg PC (a) and egg PC/egg PG (3:1) (b) liposomes (60 μM) induced by the model peptides. The data were collected at 3 min after incubation of the peptides in liposomes. Arg-4_3 (■), Glu-4_3 (▲). The fluorescence intensity was recorded at 515 nm with excitation at 470 nm.

Arg-4_3 by Glu makes not only the peptide acidic but also would be expected to take amphiphilic structure in neutral liposomes. Fig. 1 illustrates Glu-4_3 in its helical wheel formats to show the distributions of the amino acids. The peptide was blocked at its N-and C-termini with an acetyl and *N*-methylamide groups, respectively, to minimize a charge effect at both termini. These blocking will make possible the peptide forming an oligomeric bundle of head to tail dimers which has been proposed as a channel model [11].

CD spectra of Glu-4_3 in Hepes buffer (pH 7.4) in the presence or absence of egg PC liposomes are



shown in Fig. 2. The peptide had double minima at 206 and 222 nm responsible for an α-helix in buffer solution. Interestingly, the addition of neutral liposomes resulted in a decrease in the ellipticities of both minima, indicating that Glu-4_3 takes a stable α-helical structure in aqueous medium but less stable one in the amphipathic surroundings like liposomes. These results obtained for this acidic peptide were opposite when compared with those for the basic peptide, Arg-4_3, that took a random-rich structure in buffer solution and α-helical structure first in the presence of neutral liposomes [18].

In order to attain a better understanding of the mode of interaction with model membranes, the ability of the membrane perturbation was examined by measuring the effect of the peptide on the release of carboxyfluorescein from phospholipid liposomes. The dye-release from neutral egg PC and acidic egg PC/egg PG (3 : 1) liposomes by Glu-4_3 is shown as a function of peptide concentration in comparison with Arg-4_3 in Fig. 3. In both neutral and acidic liposomes, Glu-4_3 leaked the dye more weekly than Arg-4_3 did. It is of interest that peptide Glu-4_3 leaked the dye more strongly from neutral liposomes than from acidic liposomes, suggesting that a charge repulsion between the anionic group in the peptide and anionic lipid head group in acidic lipid may reduce the penetration of the peptide in lipid bilayers.

The planar lipid bilayer technique was used to investigate the ability of Glu-4_3 to induce an ion-permeation through lipid bilayers. The effect of Glu-4_3 on the membrane current through the bilayer composed of egg PC/egg PE (7 : 3) is shown in Fig. 4a. Two distinctive current changes were observed by the addition of Glu-4_3 to the *cis* compartment of the chamber: one is a steady increase of the conductance with the elapse of time (mode 1) and the other is spike-like increase of the current (mode 2) appearing with a lag time and overlapping the mode 1 current. No current change was observed when only methanol, the solvent, was added to the chamber. By expanding the time scale (insert of Fig. 4a), the latter mode was found to be consisted of channel-type openings. The channel showed many different conductances ranging from 5 to 35 pS. The lifetime of the open states is relatively short with a value of about several seconds. It should be noted that the conductance and the open lifetime of the Glu-4_3 channel are much less than those of the Arg-4_3 channel reported previously [11].

The mode 1 current-increase was stopped and the current level was even decreased by the removal of Glu-4_3 in the solution by perfusion of the *cis* compartment (Fig. 4a). The current-increase was also slowed down and stopped by changing the membrane potential from negative to positive (Fig. 4b). These results suggest that the mode 1 current increase is caused by slow events which are voltage-dependent and are correlated with insertion of Glu-4_3 to the bilayer membrane.

Both the rate of the mode 1 current increase and the appearance of the mode 2 channel-type current were dependent on the peptide concentration. The mode 2 channel current consisted of several steps of opening was also observed at higher concentration of the peptide (Fig. 5c). Fig. 6 shows a double-logarithmic plot of the rate of the conductance increase vs. Glu-4_3 concentration. The plot can be fitted by a straight line with a slope of 3, suggesting that a unit structure formation comprising three molecules contributes to the mode 1 increase of the membrane current.

Glu-4_3 affected similarly to DiphytPC bilayer, although the rate of the mode 1 current increase was smaller but the conductance of the mode 2 channel current (50–75 pS) was larger than those in the egg PE-egg PC bilayer (Fig. 4c). Interestingly lowering the pH at the *cis* solution greatly enhanced the rate of the mode 1 current increase (data not shown), suggesting that protonation of carboxyl anions in the peptide may contribute to the model 1 type channel formation.

In order to determine the ion selectivity of the ion-conducting structure responsible to the each current mode, KCl concentration was made asymmetric and the membrane potential was clamped at 0 mV (Fig. 4b). The mode 1 current disappeared, while mode 2 current, the direction of which was from *cis* to *trans*, remained. The results indicate that the mode 1 current

Fig. 4. Current traces after the application of Glu-4_3. (a) Methanolic solution of Glu-4_3 (29 μM) was added to the *cis* compartment of the chamber with stirring at a membrane potential of μ52 mV. The planar lipid bilayer was made by folding method with egg PE/egg PC (1 : 1) in symmetrical 100 mM KCl and 10 mM Tris-Hepes (pH 7.4). About 3 min after the addition of the peptide, the *cis* compartment was perfused with 100 mM KCl and 10 mM Tris-Hepes (pH 7.4), which resulted in the decrease of the steady-state current (mode 1) but the channel current (mode 2) was not altered. The membrane potential was then changed to +28 mV. Six-fold time-expansion at two parts of the trace is also shown. The dashed line indicates the zero-current level. (b) Experimental conditions were similar to those in Fig. 4a except that the membrane potential initially applied was −41 mV. Several minutes after the addition of Glu-4_3, the membrane potential was changed to +40 mV, which resulted in slow-down and then stop of the mode 1 current-increase and also the decrease of the number of mode 2 events. The mode 1 current again increased and the mode 2 current events reappeared by changing back the membrane potential to −41 mV. Then, the membrane potential was set at 0 mV and the KCl concentration at the *cis* compartment was increased to 400 mM, which diminished the mode 1 current but not the mode 2 current. (c) The planar lipid bilayer was formed with DiphytPC in the same solution as Fig. 4a,b. Glu-4_3 (38.7 μM) was added to the *cis* compartment at a membrane potential of −37.8 mV. Six-fold time-expansion at a part of the trace is also shown.

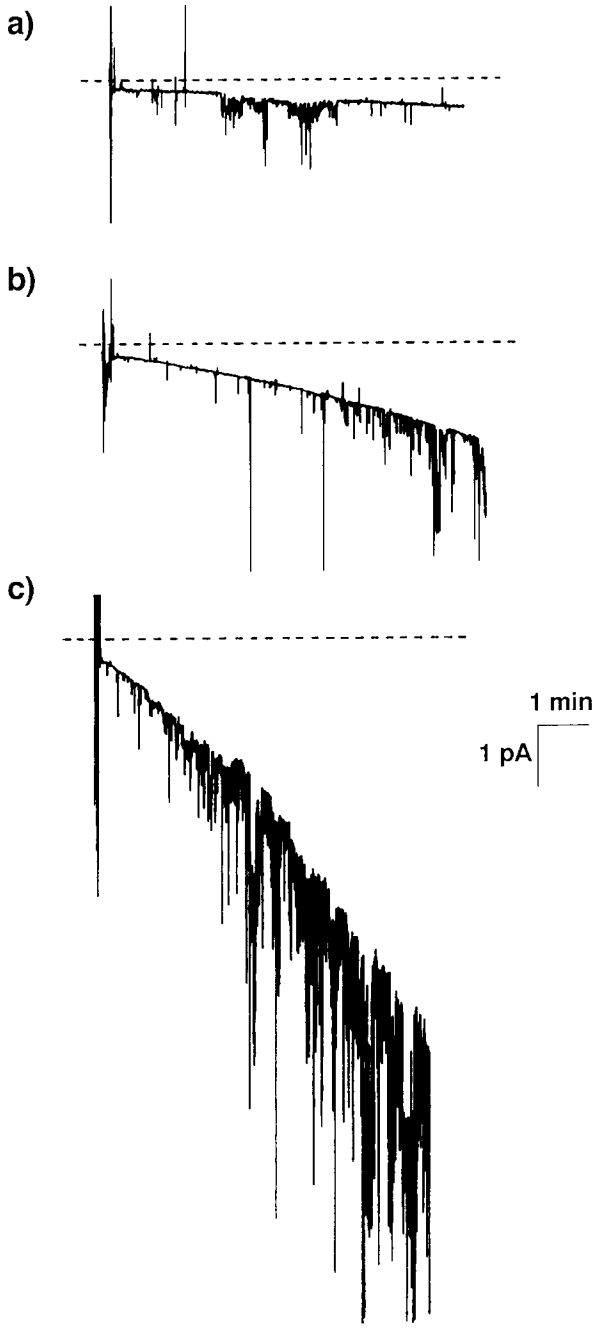


Fig. 5. Effect of the concentration of Glu-4_3 on the change in membrane current. Experiments were done according to the procedure described in Fig. 4 with different concentrations of Glu-4_3, (a) 19.7 μM, (b) 29.0 μM, (c) 38.7 μM. The membrane potential applied was −40 V.

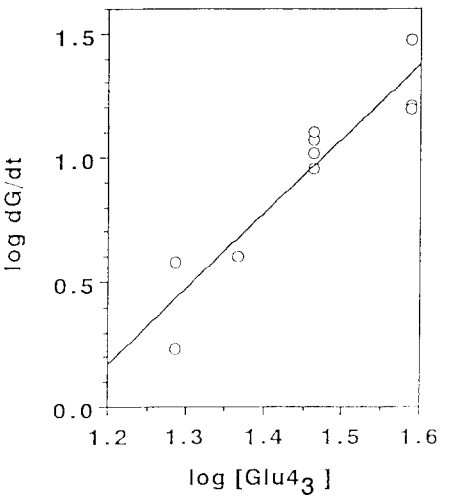


Fig. 6. A double-logarithmic plot of the rate of conductance increase as a function of peptide concentration. The straight line is fitted with a following equation: $\log dG/dt = 3.0 \log[\text{Glu-}4_3] - 3.4$ ($R = 0.91$, correlative coefficient).

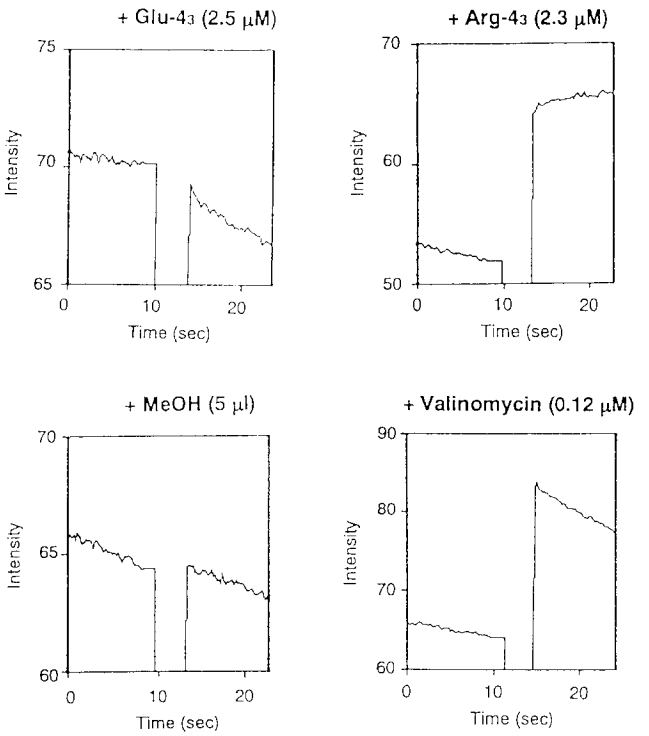


Fig. 7. Effects of Glu-4_3 and Arg-4_3 on the formation of membrane potential in egg PE/egg PC (7:3) small unilamellar liposomes as measure by fluorescence changes of $DiSC_3(5)$. The composition of the solution inside the liposome was 1 mM KCl, 158 mM sucrose, and 10 mM Tris-Hepes (pH 7.4), while that outside the liposome was 90.1 mM KCl, 15.8 mM sucrose, and 10 mM Tris-Hepes (pH 7.4). After 1 μM of $DiSC_3(5)$ was added to the liposomal suspension, a small amount of the sample solution was added and the fluorescence change was monitored at 672 nm with the excitation wavelength at 622 nm. The abscissa of the traces is fluorescence intensity with an arbitrary unit.

is carried by both K^+ and Cl^- ions (non selective), while the mode 2 current is carried dominantly by K^+ ion (cation selective), although the quantitative value of permeability ratio between K^+ and Cl^- was not determined.

To confirm the ion selectivity shown above by using the planar bilayer technique, another type of experi-

ments was done in which effect of Glu-4_3 on the formation of membrane potential on egg PE/egg PC (1:1) liposomes was measured by the fluorescence-chromophore method with $DiSC_3(5)$ [17]. Fig. 7 shows the fluorescence changes by the addition of Glu-4_3 in comparison with the additions of solvent methanol (control), valinomycin (K^+ selective ionophore), and Arg-4_3. Since there was a KCl concentration gradient across the liposomes (1 mM and 90 mM for inside and outside, respectively), the cation-selective permeation would generate inside-positive membrane potential. The sign of the membrane potential (represented by the direction of fluorescence intensity change) generated by Arg-4_3 was the same as that generated by valinomycin, whereas no membrane potential was formed by Glu-4_3. Without the liposomes, no change of the fluorescence intensity was observed by the addition of any sample (data not shown). These results indicate that Arg-4_3 induces the cation-selective ion permeation, whereas Glu-4_3 induces non-selective permeation.

4. Discussion

It has been reported that channels formed by basic peptides display anion-selectivity as observed for an amphipathic antibiotics, such as magainin [4], mellitin [5], cecropin [6], and defensin [7]. Neutral and acidic antimicrobial peptides such as gramicidin A [19], alamethicin [20], δ-toxin [21] and a series of membrane-spanning ion channel-forming segments of cationic channel proteins, display cation-selectivity [22,23]. An acidic amphipathic sequential polypeptide composed of poly(γ-methyl L-glutamate-co-L-glutamic acid) with 30% mol of glutamic acid residue also forms the cation permeable channels into a cationic bilayer membranes [24]. The cation selectivity of the ion pathway in the case of the synthetic polypeptides implies that cation permeates faster than anion. The transmembrane ion movements are determined by the association and dissociation rates of the particular ion within the membrane pathway, depending on the interaction of these ions with the space structure. Amphipathic acidic peptides aggregate each other to form a transmembrane bundle structure having the hydrophobic exterior and hydrophilic inner pore surrounded by anionic surface. Thus, the cation may be transported through the anionic pore. The converse should be also true. However, as described in the 'Introduction', some basic amphiphilic peptides such as Arg-4_3, -4_6 [10,11] and S4 segment [12] of sodium channel were found to form cation-selective ion channels. Therefore, it is interesting to examine whether the acidic peptides having opposite charge groups to the basic peptides can make anion-selective ion channels.

Large differences between Glu- and Arg-4_3 were found in their effects on the increase of membrane current and in the ion selectivity of the ion-conducting structure they formed. The interaction of Glu-4_3 with planar lipid bilayers produced two modes of current change. The rate of the steady increase of the conductance (mode 1) greatly depended on the peptide concentration. The appearance of channel-type openings (mode 2) was also dependent on the peptide concentration. No current change was observed by the addition of solvent methanol only. These results indicate that the two types of current changes are really derived from the interaction of the peptide with the planar bilayer membrane. No ion selectivity was observed in the mode 1 current, whereas the mode 2 current was cation-selective. Interestingly, the application of negative membrane potential induced the mode 1 current, whereas positive membrane potential slowed down and stopped the mode 1 current (Fig. 4b) or even decreased it (data not shown). In addition, perfusion of the *cis* compartment decreased the mode 1 current. These results suggest that the mode 1 current is caused by the slow insertion of Glu-4_3 into the lipid bilayers. Such view is supported by the findings that the mode 1 current was an increase with lowering pH (data not shown), which means that the number of Glu-4_3 molecules in the lipid bilayers becomes larger because of the increase of the hydrophobicity due to the protonation of the peptide-carboxyl groups.

Contrary to Glu-4_3, Arg-4_3 has not shown such two mode current changes [11]. However, some basic peptides, which could not make channels in lipid bilayers, showed a conductance increase somewhat like the mode 1 current, although the current was accompanied with much irregular transient fractuations [11]. Such current change is reminiscent of that generated by hydrophobic and amphiphilic peptides that interact with bilayers but do not form well-defined stable structure in the membranes [25]. As the mode 1 current did not show such irregular transient fractuation, Glu-4_3 is likely to form a certain unknown stable structure which can transport both cations and anions.

Here, assumed that the unknown stable structure might be called semichannel structure (SS) and that unit structure of semichannel is formed by aggregation of n molecules of Glu-4_3 Gl, an equilibrium relation will hold as follows.

$$n\ \text{Glu-}4_3 \underset{K}{\rightleftharpoons} \text{Semichannel structure}$$

$$K = [\text{SS}]/[\text{G}]^n$$

where [ ] indicates molarity of each species and K is the equilibrium constant. The specific conductance G is considered to have a linear relation with the concen-

tration of SS and accordingly with $K[Gl]^n$. Therefore, the rate of increase in [SS] may be expressed as

$$d[SS]/dt = k[SS] = kK[Gl]^n$$

where k is the rate constant.

The specific conductance G of mode 1 increased with time (as is shown in Fig. 5) in parallel with the increase in [SS], therefore the rate can be related to $d[SS]/dt$ as the following equation:

$$dG/dt = k'd[SS]/dt = K'[Gl]^n$$

where k' and K' are constants. Accordingly the next equation is obtained.

$$\log(dG/dt) = \log K' + n \log[Gl]$$

This expression corresponds to the mode 1 curve shown in Fig. 6, from the slope of which the aggregation number n (i.e., stoichiometric coefficient) is estimated as three. Thus, three Glu-4_3 molecules are likely to form a semichannel.

Based on the hypothesis that there exist semichannels allowing ions to pass through the membrane, the current of mode 1 is responsible for semichannels and that of mode 2 which is ion selective corresponds to 'real ion channels'. The energetically and entropically more stable semichannels composed of three Glu-4_3 molecules may have a possibility to incidentally (in lower probability) form the real ion channels. The ion channels formed by chance while the thermal motion of Glu-4_3 molecules, have a free-energetically less stable structure than semichannel so that their life time is very short. The spikes of mode 2 seems to tell us it.

The great difference was also observed in the structure in solution and in the interaction of these peptides with the lipid bilayer under no electric field conditions. CD spectra showed that Glu-4_3 took α-helical structure strongly in buffer solution, whereas Arg-4_3 did only weakly in the same condition as reported previously. The result suggests that the helix-forming property of Glu-4_3 in aqueous medium is larger than that of Arg-4_3. In the presence of egg PC liposomes, the helical content of Glu-4_3 decreased a little in comparison with that in the absence of liposomes, whereas that of Arg-4_3 greatly increased by the presence of liposomes. The extent of ellipticity of Glu-4_3 is, however, almost identical with that of Arg-4_3 [12] in the presence of neutral liposomes, suggesting that neutral phospholipids can interact with the both peptides without discrimination.

The difference between Glu-4_3 and Arg-4_3 was also observed in leakage-inducing abilities of these peptides. Arg-4_3 greatly induced the leakage of carboxyfluorescein from liposomes, whereas Glu-4_3 showed much smaller effects. Comparing the hydrophobicity of these two peptides, Glu-4_3 is more hydrophobic than Arg-4_3, because the hydrophobicity of Glu residue (-0.62 kcal/mol) is larger than that of Arg residue (-1.8 kcal/mol). Since both peptides take α-helical structure with the same content in the presence of neutral liposomes as described above, the magnitude of leakage-inducing ability of these peptides may not be interpreted simply in terms of the intensity of hydrophobicity.

The difference of lipid composition of the planar membrane between egg PC/egg PE (7:3) and DiphytPC did not essentially alter the interaction mode of Glu-4_3 with lipid bilayers, although the rate of the mode 1 current-increase was slightly larger and the conductance of the mode 2 channel current was smaller for the former lipid composition than for the latter. Ion selectivity of the channel (mode 2 current) was similar, indicating that the ion selectivity of the pore is primarily determined by its intrinsic characteristics and appears not to be influenced by properties of the host membranes. Similar observations has been reported by Montal et al. [26].

The present study shows that the channel formed by Glu-4_3 is normal in ion selectivity in contrast to the one formed by Arg-4_3. Since the planar bilayer experiments were performed under the same conditions, such a difference in the property of Glu-4_3 and Arg-4_3 channel should be due to the difference of chain length of Glu and Arg side chain and/or the difference of charge of carboxyl and guanidino groups of Glu and Arg residues, respectively. Concerning chain length, Arg side chain is longer approximately by two methylene length longer than Glu side chain, which may affect the interaction of each peptide with the membrane surface. Moreover, less space may be occupied by the carboxyl group of Glu residue, which is possibly facing the channel lining, than by the guanidino group of Arg residue. Such difference at the pore region of the channel may bring about a difference in the hydration of the pore with water molecules, resulting in alteration in ion selectivity.

Hydrophilic residues with opposite charge also lead to the difference of interaction of these peptides with head groups of lipid bilayers. The anionic groups of Glu-4_3 will interact with the cationic head group (e.g., choline, ethanolamine) of zwitter ionic phospholipids and the cationic groups of Arg-4_3 will interact with the anionic head group (e.g., phosphate) of phospholipids. Such difference of interaction may lead to the difference of insertion of the peptides into lipid bilayers.

Based on the preset results, we speculate the interaction mechanism of Glu-4_3 with lipid bilayers as follows (Fig. 8). A part of Glu-4_3 molecules in solution exist as oligomer, which interact with bilayer surface and then rearranged their conformation to the monomer structure in which the hydrophilic part in peptide is partially embedded in surface lipid bilayers in a parallel manner. Then the monomer penetrates

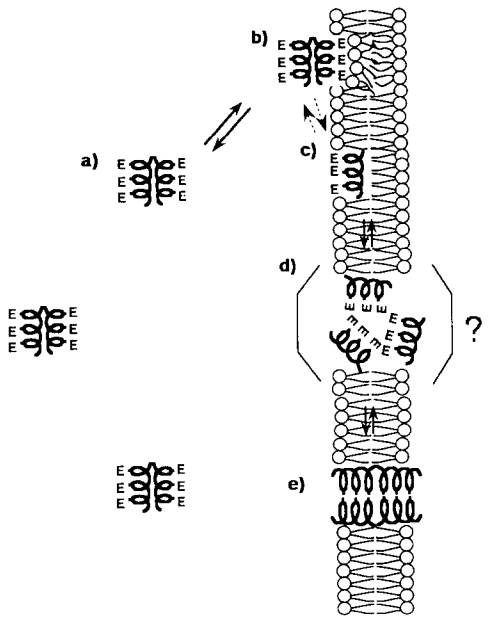


Fig. 8. Proposed mechanism of channel formation by Glu-4_3. (a) aggregated form in bulk solution; (b) charge interaction of peptide with lipid bilayers; (c) partially penetrated form horizontally into lipid bilayer; (d) semichannel; (e) channel.

slowly into the bilayers, perpendicularly, by association of three molecules, for exhibiting a mode 1 current increase. In such way, negative membrane potential enhances the formation of the structure responsible for the mode 1, so-called 'semichannel', by inducing electrophoretic movement of the peptide having negative charges. With the elapse of time, the penetrated oligomeric structure is rearranged to be a short-lived channel which is responsible for the mode 2 current. In such state, the peptide would form an oligomeric bundle of head to tail dimer proposed as a channel model proposed for Arg-4_3, previously [11].

Although initial aim of this study was to investigate the difference in the ion selectivity of the channels formed by the basic peptide (Arg-4_3) and its acidic counterpart (Glu-4_3), unexpectedly, we could not give an answer why some basic amphipathic peptides can form cation selective ion channels. By the study of membrane potential formation in neutral liposomes, we have shown another evidence that Arg-4_3 can exhibit cation selectivity. Since lipid compositions gave no difference in the ion selectivity of the channels formed by Glu-4_3 and Arg-4_3, such difference between Glu-4_3 and Arg-4_3 may be due to the difference of pore size or/and hydration behavior within the pore. We have shown that slight modifications of hydrophobic part in amphipathic peptide can induce large difference in channel conductance [12]. In the present study, we have shown that the alteration of hydrophilic part changes not only the channel properties such as conductance, gating, and ion selectivity, but also interaction of the peptides with lipid bilayer membranes.

5. References

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