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Transmission of Extramembrane Conformational Change into Current: Construction of Metal-Gated Ion Channel

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Various approaches have been reported for the creation of artificial ion channels based on the design of peptides and nonpeptide molecules.[1] Approaches to utilize the frameworks of natural channels or membrane proteins have also been reported.[2−5] The ultimate goal in the design of these channel molecules is to transmit signals from outside stimuli through the membranes as a channel current. However, most of the channels reported to date have been designed to exert this function by modifying the channel pores. On the other hand, many natural ligand-gated receptor channels, such as the nicotinic acetylcholine receptor channel, have large extramembrane protein segments.[6] These segments are assumed to interact with specific ligands to induce the conformational switch in the channel proteins and eventually transmit the external stimuli by increasing the channel current. However, few approaches in constructing artificial ion channels have employed extramembrane segments as a potential control unit of the channel current.[7,8]

To create channels that have these unique features, we have designed the channel peptide, Alm-[Ida]Fos, as reported herein (Figure 1A−C). This peptide has an alamethicin segment[9] at the N-terminus as a typical channel forming peptide to serve as a transmembrane segment. At the C-terminus, a leucine zipper segment derived from the cFos protein[10] is placed as an extramembrane segment. These segments are connected via a flexible tetraglycine linker so that these segments exert their own function without suffering significant effects from the other ones. This linker also maintains a space between the two segments to allow the ions to flow into the pores. A pair of diiminoacetic acid derivatives of lysine (Ida) residues[11] (Figure 1B) are incorporated into the extramembrane segment (positions 42 and 44 of the peptide) so that they can form complexes with Fe(III) ions.

The peptide design concept is based on our previous report on alamethicin leucine zipper hybrid peptides.[8] The association of the alamethicin segment in the membranes forms ion channel pores, and the conformation of the extramembrane leucine zipper segments significantly influenced the channel current. A peptide bearing an extramembrane segment of a random conformation forms a channel with a larger ion flux as compared with that having a helical conformation. This result suggested that a channel peptide, in which the conformation of the extramembrane segments switches in accordance with extramembrane stimuli, can be an artificial receptor ion channel that transmits the stimuli as changes in the channel current. On the other hand, we have recently shown that the chelation of Fe(III) with a pair of Ida in a leucine zipper peptide produces a significant helix destabilization, when these Ida residues are placed by i and $i + 2$ positioning in the peptide.[12] Fe(II) fails to form a complex with these Ida residues, and the reduction of Fe(III) to Fe(II) or removal of Fe(III) from the systems results in the recovery of the helical conformation of the peptide. This approach can thus be utilized for the reversible control of the extramembrane helical peptide structure.

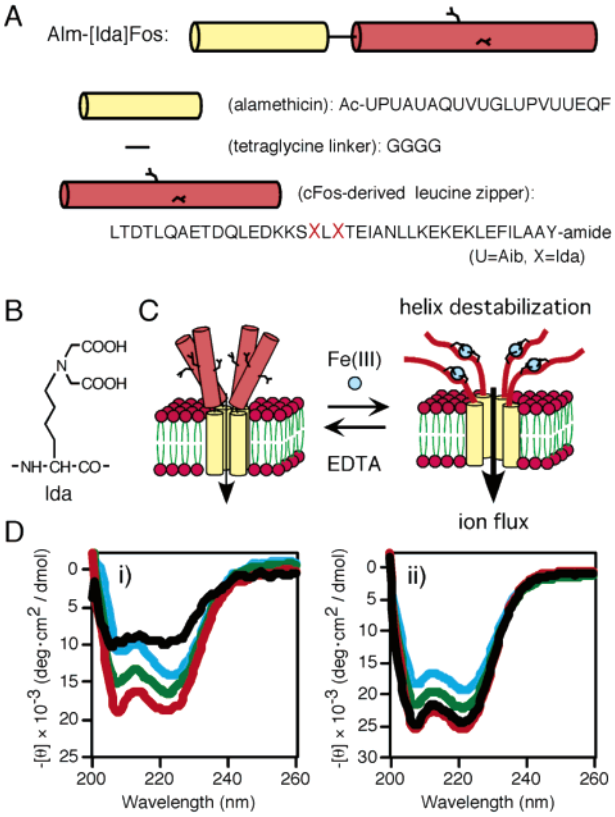

Figure 1. (A) Design and structure of artificial metal-gated ion channel peptide, Alm-[Ida]Fos. (B) Structure of Ida. (C) Schematic representation of the artificial receptor channel that transmits outside stimuli (metal) to inside the membrane as an increase in the ion flux. (D) CD spectra of the Alm-[Ida]Fos (10 μM) (i) and the [Ida]Fos (20 μM) peptides (ii). Black, peptides in 1 M KCl containing 10 mM HEPES and 1 μM EDTA (pH 7.0); red, in the presence of liposomes; green and blue, with 5 and 10 equiv of Fe(III) ($FeCl_3$) in the presence of liposomes, respectively. Liposomes were prepared from egg yolk phosphatidylcholine in the presence of 1 M KCl containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 1 μM EDTA (pH 7.0). Lipid concentration = ∼1 mM.

The peptide chain was constructed by Fmoc-solid-phase peptide synthesis on a Rink amide resin followed by on-resin conversion of lysine at positions 42 and 44 to Ida. Treatment of the peptide resin with trifluoroacetic acid and HPLC purification of the sample yielded a high purity peptide of 64 residues (see Supporting Information).

The conformation of Alm-[Ida]Fos was analyzed by measuring its CD spectra in the absence and presence of liposomes prepared from egg yolk phosphatidylcholine (∼1 mM) (Figure 1D-i). In the absence of liposomes, the CD spectrum of the Alm-[Ida]Fos had a double minima around 205 and 222 nm, suggestive of a helical structure. The helical content of the peptide was judged about 30%, based on the $[\theta]_{222}$ value as a measure of the helical content

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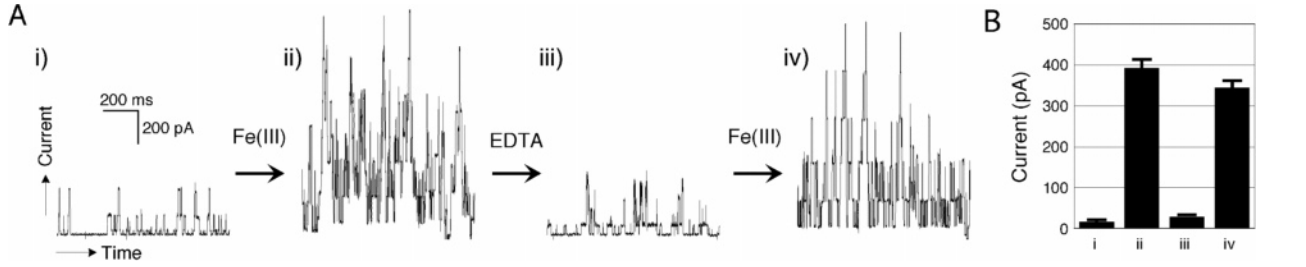

Figure 2. (A) Channel current records of Alm-[Ida]Fos in the absence of Fe(III) (i); after the addition of 2 μM Fe(III) (ii); after the addition of 10 μM EDTA (iii); and after the addition of 12 μM Fe(III) (iv). Peptide concentration = 2.5 nM; voltage = +160 mV; electrolyte = 1 M KCl containing 10 mM HEPES and 1 μM EDTA (pH 7.0). (B) Average channel current going through the membranes and the standard errors of 25 recordings of the channel states corresponding to i−iv in (A). See Supporting Information for details.

(-1.0×10^4 deg cm^2 dmol^{-1}). On the other hand, the $[\theta]_{222}$ value in the presence of the liposomes was twice as high as that in the absence of liposomes (-1.9×10^4 deg cm^2 dmol^{-1}). These facts suggest that Alm-[Ida]Fos would cause a certain structural change by the interaction with membranes, and the interaction of alamethicin segment with membranes would liberate the [Ida]Fos segment onto the membrane surface.[13] The addition of 10 equiv of Fe(III) to the solution of Alm-[Ida]Fos (10 μM) in the presence of liposomes induced destabilization of the helical structure (Figure 1D).[14]

The channel current of Alm-[Ida]Fos was then analyzed using the planar lipid bilayer method.[8] This method has a high sensitivity equivalent to that of the patch-clamp method and enables us to monitor the ion flux (i.e., channel current) going through a channel pore in real time. In the absence of Fe(III), the Alm-[Ida]Fos forms a channel having conductances of 0.13 and 0.72 nS (and less frequently 1.73 and 2.90 nS)[15] (Figure 2A-i and Supporting Information Figure S4). With the addition of Fe(III) (2 μM), a significant and spontaneous increase in the channel current level was observed (Figure 2A-ii). This indicates the fact that the conformational change in the extramembrane segment induced by the interaction with Fe(III) is effectively transmitted as the channel current.[16] Removal of Fe(III) resulted in the reduction of the channel current; by the addition of an excess amount of EDTA (final concentration 10 μM), the channel current decreased to the original level in the absence of Fe(III) (Figure 2A-iii).[17] The further addition of Fe(III) again yielded the higher current level (Figure 2A-iv). Therefore, the channel current was repeatedly controlled by Fe(III). The average channel current and the standard error for states i−iv were calculated as 15.8 ± 5.1, 392 ± 22, 27.6 ± 6.0, and 332 ± 17 pA, respectively (Figure 2B). Therefore, the addition of Fe(III) yields more than a 10−20 times difference in the channel current as compared with that in its absence.

In this study, we have designed an artificial channel peptide Alm-[Ida]Fos and clearly demonstrated that the addition of Fe(III) led to the conformational switch in the extramembrane segment and the eventual increase in the channel current. There are several reports on the creation of artificial ion channels that have a sensing function of the external ligands.[1] However, most of them have been designed so that the interaction with ligands leads to a decreased channel current by plugging the channel pores, and very few of them have a function that can detect the ligand with the increased membrane current as are usually seen in natural ligand-gated channels. The system established here is rather simple and may need further sophistication. However, we believe that this concept can extensively be applicable for the creation of various ligand-gated ion channels with novel receptor functions.

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Supporting Information Available: Synthesis, characterization, and experimental conditions for CD and channel measurement of Alm-[Ida]Fos. Single channel records of Alm-[Ida]Fos and Alm-Fos. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The CD spectra of [Ida]Fos (the peptide corresponding to the extramembrane segment of Alm-[Ida]Fos) in the presence and absence of liposomes are almost identical with each other, suggesting that the [Ida]Fos segment has little interaction with the lipid membranes (Figure 1D-ii).

(14) A greater effect (24% decrease) on the addition of Fe(III) was observed for the [Ida]Fos peptide (Figure 1D-ii). Considering that the alamethicin segment takes a helical structure in the membrane ($[\theta]_{222}$, -1.4×10^4 deg cm^2 dmol^{-1}),[9] the $[\theta]_{222}$ value of Alm-[Ida]Fos in the presence of liposomes is almost the sum of those for alamethicin in the membranes and for the [Ida]Fos peptide in the absence and presence of Fe(III). This suggests that the change in CD spectra of Alm-[Ida]Fos would mainly reflect the structural alternation of the extramembrane segment.

(15) These current levels are comparable with those of Alm-Fos,[8] a peptide having the same amino acid sequence as Alm-[Ida]Fos except that Ida at positions 42 and 44 are Ala and Gln, respectively (Supporting Information Figure S5).

(16) Alm-Fos does not contain Ida residues, and the addition of Fe(III) caused no significant increase in the channel current levels (Supporting Information Figure S5).

(17) Channel conductances of 0.08, 0.41, 1.44, and 1.88 nS were observed. These channel current levels are sometimes observed even in the absence of Fe(III) presumably due to the subtle difference in the assembly states or conformation of the peptide, and this difference may not be due to the interaction of Fe(III) with the channel pore.

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