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Trans-Bilayer Ion Conduction by Proline Containing Cyclic Hexapeptides and Effects of Amino Acid Substitutions on Ion Conducting Properties

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Several ion channel forming cyclic peptides have been reported over the past two decades and various ion conducting mechanisms have been proposed. In this article, we report on amino acid substitutions in cyclic hexapeptides and their effects on the ion conducting properties of these peptides. Cyclic hexapeptides, cyclo(Pro Xxx Yyy)2, containing two Pro residues, were used as the main framework. The substitution is performed at the Xxx positions with cationic/hydrophilic Lys or hydrophobic Leu. Yyy positions were substituted with D Phe, D Ala, or Gly. The peptides which were absent Lys residues showed ion conducting profiles with clear transitions of electric currents, whereas the peptides containing Lys residues tended to exhibit spiky or burst like profiles. These profiles were altered single state profiles by the protection of ε amino groups with aromatic protecting groups. The protected analogs exhibited significant decrease in ion conductance. These results indicated that peptides containing Lys conduct ions without forming ring stacked tube like structure. Ion channel properties were also affected by conformational changes of the cyclic peptides induced by substitution of the Yyy positions. Enhancement of intramolecular β turn structures of cyclic peptides tended to decrease their ion conductance values.

Conduction of ionic species across phospholipid bilayers is restricted due to the hydrophobic nature of the bilayer core, nonetheless ion conduction via the bilayer is allowed by ion channel peptides.1 Naturally occurring ion channel peptides have generally linear structures consisting of 10 to 20 amino acid residues. Details of relationships between molecular structure and ion conducting mechanisms of linear peptides have been exhaustively investigated.2 A better understanding of fundamental structures of ion channel peptides allows for artificial ion channel design.3-4 Ion channel forming of cyclic peptides has been also reported and utilization of cyclic peptides for the design of artificial ion channels have been attempted.5-7 In comparison with linear peptides, the reduced number of amino acid residues in cyclic peptides makes their design easier. Tailor made ion channel forming cyclic peptides would be not only useful in biophysical studies, but also in other applications such as development of novel anti infective agents, nanosize functional material.8 These channel forming cyclic peptides have been believed to associate and form high ordered pore constructs through noncovalent intermolecular interactions in lipid membranes, meaning that appropriate intermolecular interactions should be required to construct pores with desirable ion conducting properties.9,10 However, there is still no consensus about the principles governing the formation of pores that can assist their design. Additionally, correlation of peptide conformations with ion channel function also needs to be clarified for appropriate engineering of pore constructions. It is thought that steady accumulation of knowledges about the structure activity relationships is the only way to obtain cyclic peptides with desired ion channel properties.

In the present study, a series of Pro containing cyclic hexapeptides were synthesized and ion channel properties were evaluated. It was expected that the consequence of this study would contribute to knowledge to overcome the above issues. Structures of cyclic peptides evaluated in this study are illustrated in Figure 1. Pro containing hexameric cyclic peptide have been utilized as models for conformational studies of ion channel properties.11-14 Comparison of ion channel properties of these cyclic peptides would be a rational way to understand the relationships of peptide conformation and ion channel function. Several reports of nanotube and/or further ion channel formation by cyclic hexapeptides have been published, though effects of peptide side chains for ion channel formation are not well understood.15,16 Cationic and/or hydrophobic characteristics have been considered as important factors for both peptide lipid membrane and peptide peptide interactions.9,10 Therefore, effects of cationic and hydrophobic characteristics of cyclic peptides on ion conducting properties should be
addressed. On the basis of these points, hydrophobic Leu or cationic/hydrophilic Lys were incorporated into Pro containing cyclic hexapeptide constructs and ion conducting properties were compared. The details of peptide design principles are explained in the following sections.

Results and Discussion

Peptide Design. Although cyclo(Pro Xxx Yyy)$_2$, a common sequence of cyclo hexapeptides, has been previously utilized to investigate the properties of β turn structures in cyclic peptides, the ion channel forming ability of these peptide sequences was also expected due to possibilities of intermolecular interactions between peptide molecules and some experimental results.$^{11-15}$ For example, Ramesh et al. reported briefly the ion channel properties of cyclic peptides with the same common sequence.$^{15}$ The presence of the Pro residues not only maintained peptide conformations, but also promoted cyclization steps during peptide synthesis.$^{11}$ The Xxx positions were substituted with hydrophobic Leu residues or cationic/hydrophilic Lys residues. Cationic and hydrophobic characteristics of the peptides are believed to be essential factors for peptide peptide and peptide membrane interactions. In addition, the amphiphilicity of the cyclic peptides and the possibility of intermolecular hydrogen bond formation could result in the clustering and stacking of peptide surfaces.$^{9}$ Consequently, it was expected that the amino acid substitutions would alter the intermolecular interactions and the properties of the resulting ion channel formation in model lipid membranes. In fact, charged residues were found to play a critical role for the association of helical ion channel peptides in lipid bilayers.$^4$ The Lys ε amino group protection of peptides with benzyl oxyacarbonyl (Z) groups was used to verify the roles of the charged amino groups. The Yyy positions were substituted with D Phe, D Ala, or Gly residues to assess conformational effects on ion channel properties. Details of the conformational changes induced by the amino acid substitutions will be discussed in the following section.

Circular Dichroism (CD) Measurements. Structural properties of model peptides were evaluated by CD spectra. Figure 2 exhibits the CD spectra of peptides 1–6 collected in

![cyc(Pro-Xxx-Yyy)$_2$](image1.png)

**Figure 1.** Structures of designed cyclic hexapeptides. Cyclic hexapeptides are composed of a repeat of Pro $^1$ Xxx $^2$ Yyy sequences (left). Side chain compositions of the analogs are illustrated at right. Z groups (COOBz) in the structures of peptides 7, 8, and 9 stands for the benzyl oxyacarbonyl group.

![CD spectra of peptide 1–6](image2.png)

**Figure 2.** CD spectra of peptide 1–6. All spectra were collected in the presence of 2 mM DPPC SUVs. The spectra were collected in the far UV range from 190 to 260 nm. (a) CD Spectra of model peptides containing Leu. (b) Spectra of model peptides containing Lys. Symbols represent peptide 1 (closed triangle), peptide 2 (closed circle), peptide 3 (closed square), peptide 4 (open triangle), peptide 5 (open circle), and peptide 6 (open square), respectively.
the presence of 2 mM dipalmitylophosphatidylcholine (DPPC) small unilamellar vesicles (SUVs). All spectra showed double minima around 200 and 220 nm, respectively, and positive ellipticities were detected around shorter wavelengths (with maxima below 190 nm). Although it was difficult to distinguish the details of conformational differences from the observed spectra, a set of “rules” correlating amino acid sequences with preferences for particular types of \( \beta \) turn structures in the cyclic hexapeptides have been proposed by Giersch et al.\(^\text{13} \) They concluded that the sequence 1 Pro L Yyy Gly (Yyy not Val) leads to type I \( \beta \) turn structures, and sequence 1 Pro L Yyy D Xxx leads to type II\(^\prime \) \( \beta \) turn structures when they repeated in cyclic hexapeptides. According to these rules, the spectra of peptides 3 and 6, possessing Pro Xxx Gly sequence can be characterized as type I \( \beta \) turn conformations. Correspondingly, the spectra of peptides 1, 2, 4, and 5 possessing Pro Xxx D Yyy sequence can be characterized as type II\(^\prime \) \( \beta \) turn structures. CD ellipticities were increased by the substitution of D Ala residues with D Phe residues. Increase in ellipticity could imply an enhancement in the type II\(^\prime \) \( \beta \) turn conformation of the peptides.

**Ion Channel Properties of Cyclic Hexapeptides.** Ion channel activities of model peptides were measured in diphytanoylphosphatidylcholine (DPhPC) bilayers. Typical traces of ion conduction by peptides 1–6 are illustrated in Figure 3 and parameters obtained from the traces are summarized in Table 1. The pore radii were estimated from observed conductance values and specific interactions between the constructed pores and ions were not considered in the calculation (see experimental section). The exhibited traces were reproduced at least three times under comparable conditions. All peptides exhibited current fluctuations with conductance values between 50 200 pS. Generally, carrier or channel conducting mechanisms have been suggested for ion transport by cyclic peptides or peptidomimetics. The carrier ion transporter, such as valinomycin, accepts ions on one side of the membrane, moves them to the opposite side and releases the ion.\(^\text{17} \) The ion conducting velocity and efficiency of carrier molecules are relatively less than that of channel molecules. Observed ion conductance values corresponding to channels

![Figure 3](image-url)  
**Figure 3.** Representative profiles of ion conductions by peptides 1–6. The experiment was carried out by pipette dipping patch clamp at 25 ± 2°C and data were filtered at 1 or 2 kHz frequency. The pipettes were filled with a 0.5 M KCl solution buffered with 5 mM HEPES (pH 7.4) containing 1 μM peptides. Symbols C and O (1, 2, 3, ...) denote the zero (closed) current level and open levels of channels, respectively.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Applied potential (( E )) /mV</th>
<th>Open state</th>
<th>Conductance values (( G ))/pS</th>
<th>Pore radius (( R ))/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+100</td>
<td>1</td>
<td>47</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>+100</td>
<td>1</td>
<td>73</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>1</td>
<td>67</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>95</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>109</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>+100</td>
<td>1</td>
<td>79</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>+100</td>
<td>1</td>
<td>106</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>+100</td>
<td>1</td>
<td>98</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td>+100</td>
<td>1</td>
<td>26</td>
<td>0.06</td>
</tr>
<tr>
<td>8</td>
<td>+100</td>
<td>1</td>
<td>43</td>
<td>0.08</td>
</tr>
<tr>
<td>9</td>
<td>+100</td>
<td>1</td>
<td>40</td>
<td>0.07</td>
</tr>
</tbody>
</table>

a) The conductance values (\( G \)) were calculated as \( G = I/E \).  

b) The pore radii (\( r \)) were estimated according to eq 1. The membrane thickness (\( l \)) is 3.0 nm and the bulk solution resistivity (\( \rho \)) of 500 mM KCl is 0.13 \( \Omega \) m.\(^\text{16} \)

\( 10^7 \) ions/s, but are significantly larger and distinguishable from that of carriers (\( 10^4 \) ions/s).\(^\text{18,19} \)

The single state conductance value of peptides 1 and 2 can be interpreted in accordance to the paradigm of ring stacked tube like ion channels.\(^\text{18} \) It is reasonable to assume that due to structural limitations, the pore diameter of the tubular structures formed by stacking of cyclic peptides remains relatively inflexible and these supramolecular structures could have a single state conductance value. Interestingly, the ion conducting profile of peptide 3, assumed to possess type I \( \beta \) turn conformation, was distinct from those of peptides 1 and 2. The trace with multi state conductance values would imply the existence of conducting substates. Decisive interpretation for the difference could not be done, though the conformational difference of peptide 3 might cause the alteration of ion channel properties.\(^\text{20} \) It was also expected that the presence of two Gly residues, with lower steric hindrance of their side chains, in
peptide 3 can contribute to the flexibility of the pore structures formed by this peptide.

Peptides lacking the Lys residues showed ion conducting profiles with distinct open close transitions, whereas peptides containing Lys residues tended to show conductance profiles with spiky or burst like fluctuations. Generally, positively charged peptides, especially amphiphilic examples, accumulate on both negatively charged, as well as zwitterionic lipid membranes, and some of the peptides show membrane perturbation activities. Non-specific membrane disruption could be considered as one reason for the spiky or burst like patterns. Nonetheless, well defined independent events in millisecond open close time scales can indicate the existence of ion conducting pores within lipid membranes. Additionally, according to amplitude histogram analyses (not shown), multi state like conductance behavior was also detected in the conductance traces of peptides 4 and 5 (Figure 3). Duclozier et al. proposed a few possibilities for the ion conducting mechanism of cyclic peptides and putative traces of those ion conduction mechanisms as follows (Figure 4). These possible mechanisms include: (i) tube like architecture constructed with stacking ring shaped peptides expected to show a single state conductance value and (ii) bundle like structures constructed with aggregation of stacked cyclic peptide columns. These investigators predicted that channels behaving in the latter manner exhibit multi state conductance values. Ghadiri’s group reported that a cyclic peptide series composed of repeated hydrophobic L and D amino acid exhibited single state fluctuations of electrical currents reflecting fixed pore diameters. A self assembled tube like architecture composed of stacked peptide rings with intermolecular hydrogen bonds via peptide main chains, was proposed for the ion conducting mechanisms of these peptides. On the other hand, it was reported that cyclic tetrapeptides tentoxin; cyclo(N Me Ala Leu N Me ΔPhe Gly) and (2S,9S) 2 amino 8 o xo 9,10 epoxydecanoic acid (Aoe) containing HC toxin; cyclo(Ala Δ Ala Aoe Δ Pro) form transmembrane ionic channels without ring stacked tube like structures, since these peptides exhibited a pattern with multi state conductance values. Cylindrical bundle formation by cyclic peptide aggregates has been proposed as a possible ion conducting mechanism for tentoxin and HC toxin. These reports imply that the observed multi state conductance values in this study were consequence of pore formation without ring stacked tube like structures. However, peptide 3 that lacks Lys exhibited multi state conductance values, while peptide 6 that contains Lys exhibited single state values. Clearly understanding this point has not been obtained at the present stage.

**The Effects of Side Chain Amino Groups.** In order to verify whether the characteristic ion conductance profile is mediated by free amino groups of Lys side chains, ion channel activities of peptides 7, 8, and 9, the respective Lys side chain protected versions of peptide 4, 5, and 6, were evaluated. Figure 5 illustrates typical ion conducting profiles of the amino group protected analogs. The profiles were converted to the patterns with single state conductance values and the values were significantly lower than those of the non protected analogs (26 43 pS: see Table 1). If these peptides formed the

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**Figure 4.** Presumed ion channel forming by cyclic peptide. (left) Ion conduction by membrane perturbation. (center) Bundle like structures constructed with aggregation of stacked cyclic peptide column. (right) Nanotube formed by peptide stacking. Filled circles illustrated in this figure represent ionic species.

**Figure 5.** The biophysical properties of peptides 7 9. (a) CD spectra of the peptides. CD spectra were collected in the presence of 2 mM DPPC SUVs. Symbols represent peptide 7 (open triangle), peptide 8 (open circle), and peptide 9 (open square), respectively. No significant changes of the spectra were observed by the side chain protection. (b) Representative patterns of ion conduction by these peptides. In peptides 7 9, the characteristic conductance profiles of the corresponding peptides 4 6 were converted to considerably lower conductance values and different open close transitions by protecting ε amino group.
conventional tubular structures by peptide stacking, the internal structures of the pores should not be affected by the presence or absence of the protecting groups. Therefore, this observation provides additional evidence that relevant cyclic hexapeptides, possessing free amino groups, form ion channels without the tube like structures. Feigin et al. reported that the cyclic lipodepsipeptide, syringomycin E, formed ion channels which show multistate conductance values. Syringomycin E contains three cationic residues and has a total of +2 net charges. These researchers concluded that the pore structure was formed by association of clustered complexes of the peptides and charged residues gathering in the pore lumen. As aforementioned, the details of ion conducting mechanism of Lys containing cyclic hexapeptides are uncertain, though observed changes in the ion conductance corresponding to the amino group protection indicates that amino groups correlated ion channel forming and the cyclic hexapeptides have a potential to convert their ion channel properties with the variation of side chain groups. Nonetheless, the possibility has not been excluded that the conductances were influenced by hydrophobicity or other effects of Z groups. Further investigations of this point remain in future work.

Conclusion

In the present study, structures of cyclic hexapeptide conducting pores were modified on the basis of peptide structures. In spite of the simple amino acid substitutions, these cyclic peptides exhibited drastic changes in their ion channel properties, as suggested by the conversion of ion conduction mechanisms. The ion conducting mechanism of the Lys containing cyclic hexapeptides could not be clearly identified, however it is experimentally suggested that the cyclic peptides are able to form different ion conducting structures. Conformational differences in the cyclic peptide monomers also altered ion conductivity and/or resulted in the formation of substate conducting levels, implying different pores correlated with ion channel properties. Recently, computational chemistry has been utilized to explore the ion channel formation of cyclic peptides. Correspondence between the biophysical and simulation techniques could further clarify the details of the ion conducting mechanisms.

Experimental

Materials. tert Butoxycarbonyl (Boc) protected amino acids, N,N-dicyclohexylcarbodiimide (DCC), 1 hydroxybenzo triazole (HOBr), and 2,2,2 trifluoroacetic acid (TFA) were purchased from Peptide Institute (Osaka, Japan). DPhPC was obtained from Avanti Polar Lipids (Alabaster, AL, U.S.A.) as 50 mg mL⁻¹ chloroform solutions. α cyano 4 hydroximino acid (α CHCA) and DPPC were purchased from Sigma (Tokyo, Japan). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan), and used as received.

Peptide Synthesis. Synthesis of peptide 3 had been reported previously, and the same strategy was applied for the syntheses of peptides 1, 2, 7, 8, and 9. Briefly, coupling reactions of amino acids or peptide fragments were performed with DCC and HOBr in the presence of triethylamine or N methylmorpholine. Removal of Boc groups was achieved with TFA or 4 M HCl in 1,4 dioxane. After the elongations of linear peptides up to the desired sequence, C terminal carboxyl groups of the linear products were converted to succinimide ester with N hydroxysuccinimide and DCC. After deprotection of Boc protected groups on the N termini, cyclization was performed in pyridine under highly diluted conditions. Purification of crude products was carried out by gel filtration chromatography, silica gel chromatography or preparative reverse phase high performance liquid chromatography (RP HPLC) on a WakoSil 5C4 200 column (Wako Pure Chemical Industries). Peptides 4, 5, and 6 were obtained from peptides 7, 8, and 9, respectively, by removal of Z groups on ε amino groups by H₂ reduction catalyzed by Pd/C. Purities of synthetic peptides were confirmed by analytical RP HPLC on a WakoSil 5C4 200 column (Wako Pure Chemical Industries).

HPLC analyses were performed on a system comprised of an 807 IT integrator (JASCO, Tokyo, Japan), two pumps PU 980 (JASCO), a UV 970 detector (JASCO), and a Rheodyne 7125 injector (Rheodyne Inc., CA, U.S.A.). Molecular masses of synthetic peptides were determined by matrix assisted laser desorption ionization time of flight (MALDI TOF) mass spectroscopy on Voyager DERP (PerSeptive Biosystems Inc., Framingham, MA, U.S.A.) using α CHCA as matrix reagent. MALDI TOF MS for cyclo(Pro Leu D Phe)₂ (1): C₂₆H₄₂N₈O₆ [M + H]+ 715.4, found 715.5; cyclo(Pro Leu D Ala)₂ (2): C₂₅H₴₀N₈O₆ [M + H]+ 563.4, found 563.0; cyclo(Pro Leu Gly)₂ (3): C₂₅H₄₀N₈O₆ [M + H]+ 535.3, found 535.9; cyclo(Pro Lys D Phe)₂ (4): C₂₆H₅₀N₈O₆ [M + H]+ 745.4, found 745.9; cyclo(Pro Lys D Ala)₂ (5): C₂₅H₄₀N₈O₆ [M + H]+ 593.4, found 592.9; cyclo(Pro Lys Gly)₂ (6): C₂₆H₅₀N₈O₆ [M + H]+ 565.4, found 566.1; cyclo(Pro Lys(Z) D Phe)₂ (7): C₂₆H₅₀N₉O₁₀Na [M + Na]+ 1035.5, found 1034.6; cyclo(Pro Lys(Z) D Ala)₂ (8): C₂₆H₅₀N₉O₁₀Na [M + Na]+ 883.4, found 884.2; cyclo(Pro Lys(Z) Gly)₂ (9): C₂₆H₅₀N₉O₁₀ [M + H]+ 833.4, found 833.4. Amino acid compositions and peptide concentrations of stock solutions used in CD measurements and ion channel activity measurements were determined by quantitative amino acid analysis as described previously. Amino acid analyses were performed on a Pico Tag Work station (Waters, Milford, MA, U.S.A.) after hydrolysis in constant boiling HCl at 110°C for 24 h.

CD Measurements. CD spectra were recorded on a JASCO J 720 spectropolarimeter (JASCO). A cylindrical cuvette of 2 mm path length was used for the measurements. The measurements were carried out in 2 mM DPPC SUVs. Peptide concentrations were 20 μM. Hydrophobic peptides were dissolved in EtOH as 1 mM stock solutions and then diluted with the SUV containing buffer. Samples were incubated at room temperature for 30 min before measurements. Four scans were averaged for each sample and the averaged blank spectra were subtracted respectively. DPPC SUV was prepared according to a modified Bangham’s method. DPPC lipid was dissolved in chloroform and then dried overnight in vacuo. The lipid film was hydrated with 10 mM phosphate buffer solution (pH 7.4), vortexed at 50°C for 30 min, and then sonicated for 6 min using a Branson Sonifier 250 sonicator (Branson, CT, U.S.A.). Prepared SUV solution was centrifuged for 5 min to remove the titanium particles from sonicator probe tip.
Single-Channel Measurements. Single channel recording was carried out by pipette dipping patch clamp at ambient temperature (25 ± 2 °C). The patch clamp electrodes were prepared by the two pulls method by a pipette puller (Narishige, Tokyo, Japan), without fire polishing, from he matocrit hard glass capillaries (Narishige). Settings in the micropipette puller were adjusted to obtain tip diameter of ca. 1 μm. Pipettes were filled with 0.5 M KCl solution, buffered with 5 mM 2 [4 (2 hydroxyethyl) 1 piperazinyl] ethanesulfonic acid (HEPES) (pH 7.4), containing 1 μM cyclic peptide. The pipette tip was immersed in a disposable Petri dish filled with the same buffer without peptides. After immersion of the pipette, DPhPC monolayer was spread on the surface of the dish solution by carefully adding to the edge of the dish 0.5 1.0 μL of a 10 mg mL⁻¹ solution of the lipid dissolved in n hexane. Before any movement of the electrode, ca. 10 min were allowed for the evaporation of the solvent from the surface of the solution. Single channel currents were amplified using an Axpatch 1D patch clamp amplifier (Axon Instruments, Inc., Union City, CA, U.S.A.) controlled by pClamp 6 (Axon Instruments, Inc.) software. Data was filter at 2 kHz frequency and analyzed using Axograph (Axon Instruments, Inc.). Electric current and conductance values were evaluated from amplitude histogram analyses of the conductance traces.

Calculation of pore diameter was performed using the following equation.¹⁰

\[ G = \pi r^2/\ell (1 + \pi r/2) \]  

Where, \( G \) is the observed conductance value; \( \ell \) is the membrane thickness (or pore length, approximately 0.3 nm); \( r \) is the radius of pore; and \( r' \) is the conductance of the ionic species (0.13 Ωm for 0.5 M KCl solution).¹⁶

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References