Synthesis and Ion Channel Formation of Novel Cyclic Peptides Containing a Non-natural Amino Acid

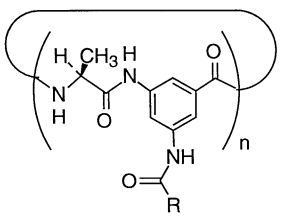
Hitoshi Ishida,*† Kiyoshi Donowaki, †† Yoshihisa Inoue,†,†† Zhi Qi,††† and Masahiro Sokabe†††
†*Inoue Photochirogenesis Project, ERATO, JST, 4-6-3 Kamishinden, Toyonaka 565*
††*Department of Molecular Chemistry, Faculty of Engineering, Osaka University, 2-1 Yamada-oka, Suita 565*
†††*Department of Physiology, Nagoya University School of Medicine, 65 Tsurumai, Showa, Nagoya 466*

(Received June 10, 1997; CL-970444)

A series of novel cyclic peptides possessing an alternate natural / non-natural amino acid sequence were prepared from L-alanine and 5-(*N*-acylamino)-3-aminobenzoic acid with a long acyl chain. Upon examination by the voltage clamp method, membranes composed of the synthetic cyclic peptides with varying ring sizes and side-chain lengths were found to form cation selective channels with a conductance of *ca.* 9 pS in aqueous KCl (500 mM).

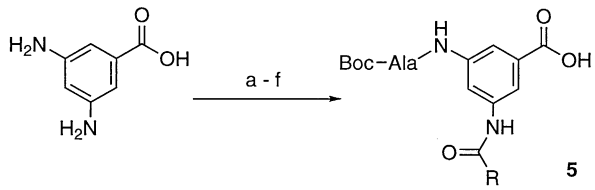
Artificial ion channels have recently received much attention, since they not only serve as simplified models for elucidating the structure and function of native ion channels but are also applicable to nano-scale devices.[1,2] Systematic synthesis of artificial ion channels is considered to be the most promising direct approach to the insights into the structure-function relationship in native ion channels. In this context, it seems somewhat curious that only a limited amount of effort has hitherto been devoted to the systematic synthesis and evaluation of potential ion channels with well-defined structures, except for the recent work by Fyles *et al.*[1]

We have investigated the molecular design of functional peptides, employing a rigid non-natural amino acid, *i.e.* 3-aminobenzoic acid (Aba).[3] The cyclic peptides that consist of alternating natural amino acid (AA) and non-natural Aba, *cyclo*(-AA-Aba-)$_n$ (n = 3, 4), were found to give a structure with stable pores due to the fixed orientation of AA compelled by the rigidity of Aba. Utilizing such a stable pore structure, one can design a variety of novel cyclic peptides that possess desired pores for ion permeation and long alkyl chains for smooth incorporation into bilayer membranes. These peptides are expected to function as artificial ion channels with varying pore size and shape as well as lipophilicity just by changing the amino acid, ring size, and/or alkyl chain length. We report herein the synthesis of a series of novel cyclic peptides (**1** - **4**) and their single ion channel behavior examined by the voltage clamp method.



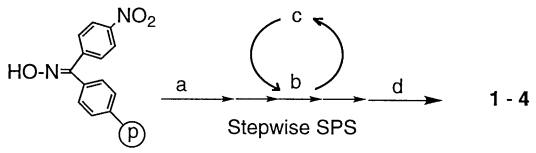
	R	n
1	*n*-C_9H_{19}	3
2	*n*-C_9H_{19}	4
3	*n*-$C_{15}H_{31}$	4
4	*n*-$C_{15}H_{31}$	5

The cyclic peptides **1** - **4** were synthesized by stepwise coupling of the component dipeptides (AA-Aba) prepared in advance, because diaminobenzoic acid is readily oxidized when the amino group is not protected. The syntheses of dipeptides **5** containing an L-alanine (Ala) residue and various *N*-acyl groups at the 5-amino group were performed; see Scheme 1. The target cyclic peptides (**1**-**4**) were synthesized by stepwise coupling of these dipeptides and cyclization on an oxime resin.[4] As shown in Scheme 2, the dipeptide **5** was first introduced to the resin by



a) $SOCl_2$, C_2H_5OH; b) $(Boc)_2O$, Dioxane / H_2O; c) R-COOH, DCC, HOBt, $CHCl_3$; d) TFA; e) Boc-L-Ala-OH, DCC, HOBt, $CHCl_3$; f) 1N-NaOH, EtOH.

Scheme 1.



Oxime resin (P = styrene polymer)

a) **5**, DCC, CH_2Cl_2; b) 25 % TFA, CH_2Cl_2; c) **5**, Bop, HOBt, DMF; d) Et_3N, AcOH, DMF.

Scheme 2.

using dicyclohexylcarbodiimide (DCC) in CH_2Cl_2, which was followed by successive coupling of the second portion of **5** using [(benzotriazol-1-yl)oxy]tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and 1-hydroxybenzotriazole (HOBt) in DMF. After the stepwise coupling being completed, *N*-Boc group in the peptide was deprotected by treating with 25%-trifuluoroacetic acid (TFA) in CH_2Cl_2 for 30 min. Then, the oxime resin was washed successively with CH_2Cl_2, 2-propanol, and CH_2Cl_2. The resin was shaken in an acetic acid/triethylamine/DMF solution for 1 day to yield the target cyclic peptides (**1**-**4**). These compounds were identified by means of the mass and NMR spectroscopy.[5] Especially, their cyclic structures were confirmed by the NMR spectra showing simple symmetrical structures and also by the ninhydrin test indicating the absence of free amino groups in the peptides.

The single channel currents were measured under the voltage clamp conditions with composite membranes incorporating the cyclic peptides into planar lipid bilayers prepared from soybean lecithin.[2c] Typical current patterns recorded for the cyclic peptide **3** are shown in Figure 1. All of the cyclic peptides exhibited similar current patterns showing stepwise changes with a constant amplitude. Since such patterns are characteristic to single ion channels and no appreciable currents were observed in the

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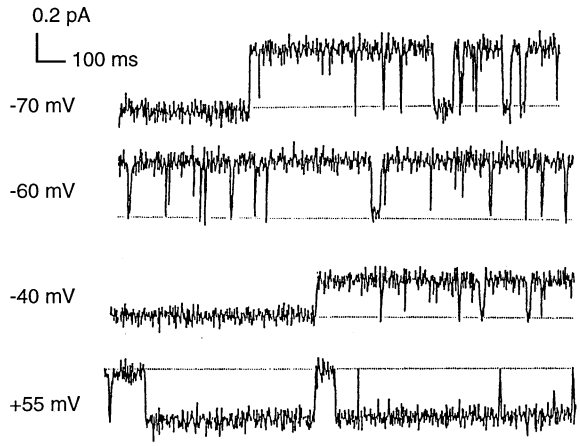

Figure 1. Single-channel currents of **3** at various voltages in symmetric 500 mM KCl solutions. Dotted lines indicate the zero current level.

absence of the cyclic peptides, it is clear that the present results are neither artifacts nor nonspecific leakage currents arising from possible structural disturbance of the bilayer by the peptides. These current patterns also exclude the migration mechanism involving the peptides as ion carriers. This is because the ion transport rate across membranes by single ion carrier is much slower, and accordingly, carrier-mediated currents are too small to be measured with the present method. Table 1 summarizes the averaged single channel conductances, which were calculated from at least 6 membranes for each peptide. The conductance values seem to be very similar among peptides. The cation *versus* anion selectivity of the channel **3** as a typical case was also measured by using asymmetric KCl solutions at both sides of the membrane (500 mM / 100 mM). Fitting the I-V curve under this condition to the Goldman-Hodgikin-Katz equation[6] gave a permeability ratio (P_K / P_{Cl}) of 7.14, indicating that the peptide **3** forms a cation permeable channel. Other three peptides (**1**, **2**, **4**) gave nearly the same values as that of peptide **3**. Taken together the conductance and ion selectivity of the channels seem to be independent of the pore size and the length of the acyl chains in the components for the peptides employed in the present study. This result may indicate that the acyl chains of the peptide line along the inner wall of the channel[2b] and therefore the rate of ion permeation is limited at this part of the channel. Syntheses of artificial ion channels containing other natural amino acids (*e.g.*, L-aspartic acid, L-lysine, and L-serine) and further investigations of the detailed properties of their artificial ion channels are currently in progress.

Table 1. Average single-channel conductance of the membranes containing the cyclic peptides **1**-**4**

Peptides	Conductance / pS[a]
1	9.09 ± 2.20
2	8.38 ± 0.90
3	9.14 ± 1.98
4	8.80 ± 1.83

[a] Determined by more than six independent measurements with membranes from separate preparations.

This work was funded in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture (HI, MS), by a grant from the Japan Society for the Promotion of Sciences (MS), and by the Naito Memorial Science Foundation (HI).

References

1 T. M. Fyles, T. D. James, and K. C. Kaye, *J. Am. Chem. Soc.*, **115**, 12315 (1993) and references cited therein.

2 a) Y. Kobuke, K. Ueda, and M. Sokabe, *Chem. Lett.*, **1995**, 435; b) Y. Tanaka, Y. Kobuke, and M. Sokabe, *Angew. Chem., Int. Ed. Engl.*, **34**, 693 (1995); c) Y. Kobuke, K. Ueda, and M. Sokabe, *J. Am. Chem. Soc.*, **114**, 7618 (1992).

3 H. Ishida, K. Donowaki, M. Suga, K. Shimose, and K. Ohkubo, *Tetrahedron Lett.*, **36**, 8987 (1995); H. Ishida, M. Suga, K. Donowaki, and K. Ohkubo, *J. Org. Chem.*, **60**, 5374 (1995).

4 N. Nishino, M. Xu, H. Mihara, and T. Fujimoto, *Bull. Chem. Soc. Jpn.*, **65**, 991 (1992).

5 **1**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (3H, s), 10.05 (3H, s), 8.31 (3H, d, J = 7.32 Hz), 8.26 (3H, s), 7.98(3H, s), 7.61 (3H, s), 4.70 (3H, m), 2.33 (6H, t), 1.59 (6H, br), 1.44 (9H, d, J = 7.33 Hz), 1.28 (36H, br), 0.87 (9H, t). MS (FAB) *m/z* calcd for (M + Na) $C_{60}H_{84}O_9N_9$ 1101.4, found 1100.6. **2**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10(4H, s), 10.01(4H, s), 8.47(4H, d, J = 6.83 Hz), 7.99(4H, s), 7.91(4H, s), 7.64(4H, s), 4.53(4H, q), 2.29(8H, t, J = 6.83 Hz), 1.59(8H, br), 1.40(12H, d, J = 7.33 Hz), 1.25(48H, br), 0.83(12H, t, J = 6.35 Hz). MS (FAB) *m/z* calcd for (M + Na) $C_{80}H_{112}O_{12}N_{12}$ 1456.8, found 1457.2. **3**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33-10.01(6H, m), 8.48 (4H, m), 8.19-7.58(12H, m), 4.70 (4H, br), 2.33-2.29(8H, m), 1.59(8H, br), 1.43(9H, br), 1.25(36H, br), 0.83(9H, t, J = 6.35 Hz). MS (FAB) *m/z* calcd for (M + Na) $C_{104}H_{164}O_{12}N_{12}$ 1797.5, found 1797.1. **4**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (5H, s), 10.03 (5H, s), 8.53 (5H, br), 8.10-7.66(15H, br), 4.55 (5H, m), 2.29 (10H, br), 1.57 (10H, br), 1.40 (15H, d, J = 5.86 Hz), 1.22 (120H, br), 0.85 (15H, t). MS (FAB) *m/z* calcd for (M + Na) $C_{130}H_{205}O_{15}N_{15}$ 2241.1, found 2240.7.

6 B. Hille, "Ionic Channels of Excitable Membranes", 2nd ed., Sinauer, Sunderland, MA, (1992).