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Lipophilic Cyclic Hexapeptide for Calcium Ion-Selective-Electrode

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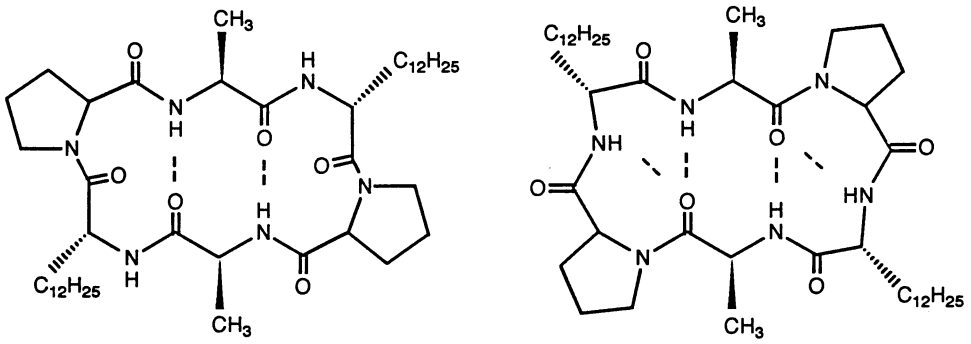
The cyclic hexapeptides containing artificial and lipophilic amino acid, α-aminomyristic acid were designed. The poly(vinyl chloride) (PVC) membrane electrode based on a cyclic hexapeptide exhibited high calcium ion selectivity over sodium and potassium ions.

A number of cyclic peptides are known to bind metal ions with considerable selectivities.[1] Some of them are actually demonstrated to exhibit ion-transport-activity across the chloroform liquid membrane.[2] However, none of the designed cyclic peptides are used for the polymer membrane electrodes, while a natural product, valinomycin is successfully applied for the potassium ion-selective-electrode (ISE).[3] Most of the ionophorous peptides employ naturally occurring hydrophobic amino acids such as Leu, Phe, and in some cases Glu or Lys with side chain protection. The side chains of these amino acids are not hydrophobic enough to cover the polarity of the peptide bond moiety. Accordingly, we chose α-aminomyristic acid[4] (Amy) bearing long chain alkyl group as a lipophilic component. The selective binding capacity for particular cation should be carried by carbonyl oxygen atoms properly organized on the cyclic peptide framework. For this purpose, we selected Pro to make compact β-turn conformation by combination with foregoing or following D-amino acid. The simpler chemical structure may be helpful for the easier determination of the conformation and elucidation of its relationship to the ion selectivity. Thus, cyclic hexapeptides with repeating tripeptide sequences, *cyclo*(-D-Amy-L-Pro-L-Ala-)$_2$ (**1**) and *cyclo*(-L-Ala-L-Pro-D-Amy-)$_2$ (**2**) were designed. According to the literature,[5] these two cyclic peptides **1** and **2** were expected to take different β-turn conformation, type II' and type II, respectively as illustrated in Fig. 1.

The Ac-DL-Amy was treated with *Aspergillus genus* acylase (Tokyo Kasei). After twice treatment, recovered Ac-D-Amy was hydrolyzed with 2 M (1 M=1 mol·dm^{-3}) HCl to give D-Amy; $[\alpha]_D^{25}$ -24.4° (c 2.0,



cyclo(-D-Amy-L-Pro-L-Ala-)$_2$ **1** cyclo(-L-Ala-L-Pro-D-Amy-)$_2$ **2**

Fig. 1. Lipophilic cyclic hexapeptides designed to have different types of β-turn conformations. The broken lines denote hydrogen bonds for β- and γ-turns.

$CHCl_2COOH$). The peptides were assembled by conventional method. The linear hexapeptides, Boc-(-L-Ala-D-Amy-L-Pro-)$_2$-OH and Boc-(-D-Amy-L-Ala-L-Pro-)$_2$-OH were condensed with *N*-hydroxysuccinimide by dicyclohexyl carbodiimide. The peptide active esters were treated with trifluoroacetic acid to remove Boc group. The intermediates were allowed to cyclize in pyridine in high dilution conditions. The desired cyclic hexapeptide **1** was isolated and purified by silica gel chromatography with $CHCl_3$/MeOH (49/1, v/v). Yield of an oil, 36%; FAB-MS, m/z 788 $(M+H)^+$. Another cyclic hexapeptide **2** was obtained as crystalline powder in 59% yield by recrystallization from EtOH; mp 270 °C (decomp.); FAB-MS, m/z 788 $(M+H)^+$. They showed the same R_f value (0.50) on silica-gel TLC with $CHCl_3$/MeOH (9/1, v/v).

The conformations of the cyclic hexapeptides were elucidated by measurements of circular dichroism (CD) and 400 MHz ^{1}H-NMR spectra. Figure 2 shows the CD spectra of cyclic peptides in acetonitrile. The profiles are typical for type II' and type II β-turn conformations for **1** and **2**, respectively.[5] The NMR spectra in $CDCl_3$ gave only two signals for amide protons of each cyclic hexapeptide, assigned as L-Ala (6.84 ppm), D-Amy (7.22 ppm) for **1**, D-Amy (6.48 ppm) and L-Ala (7.97 ppm) for **2**. This fact proves that they have single and perfect C_2-symmetry conformations. Furthermore, the temperature dependences of the chemical shifts of amide protons (Fig. 3) revealed that some of them are engaged in the hydrogen bonds. Comparatively less steep temperature dependence of L-Ala NH in **1** ($\Delta\delta/\Delta T$=-4.55 x 10^{-3} ppm/K) suggests that there could exist weak hydrogen bond probably for the type II' β-turn conformation. On the contrary, both amide protons in **2** are tightly hydrogen bonded, the $\Delta\delta/\Delta T$ values are -1.49 and -1.81 x 10^{-3} ppm/K for L-Ala NH and D-Amy NH, respectively. They form type II β-turn and γ-turn conformations in the cyclic hexapeptide framework, which is rather flat in shape.

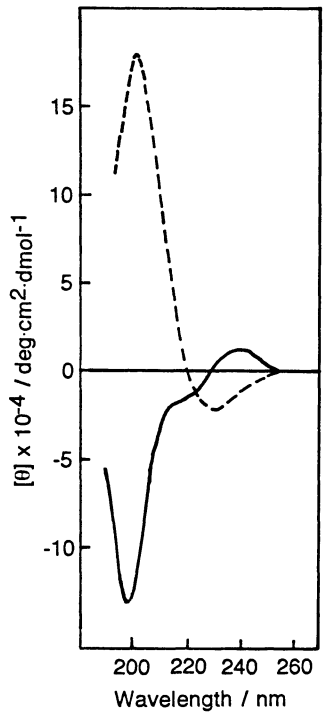


Fig. 2. CD spectra of cyclic hexapeptides, **1** (——) and **2** (– – –) in acetonitrile.

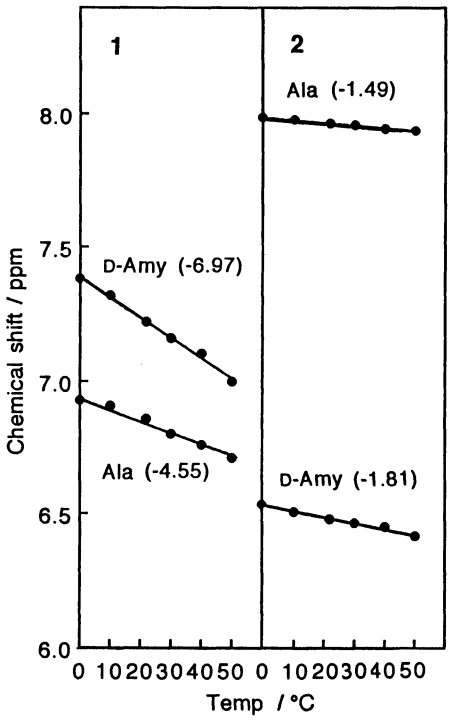


Fig. 3. Temperature dependences of the chemical shifts of amide protons of cyclic hexapeptides.

The electrode membranes were prepared according to the literature[6] and consisted of the cyclic peptide (4.6 wt%), PVC (23.2 wt%), NPPE (2-nitrophenyl phenyl ether, 70.8 wt%), and KTCPB ((potassium tetrakis(4-chlorophenyl)borate, 1.4 wt%). The membranes were conditioned in 1×10^{-3} M of $CaCl_2$ or other chlorides overnight. The EMF measurements were carried out at 25 °C with an electrochemical cell of Ag-AgCl / 1M $CaCl_2$ / PVC membrane / sample solution / 0.1 M NH_4NO_3 / 3 M KCl / AgCl-Ag. The selectivity coefficients were determined by the mixed solution method with background concentrations of 0.2 M for alkali metal ions and 0.1 M for magnesium ion.

Figure 4 shows the typical EMF response of the electrode based on **1** to the calcium activities. Almost ideal Nernstian slope (29.8 mV/decade) for calcium ion was attained in the activity range of 10^{-5}-10^{-2} M. The response was not interfered by the presence of sodium and potassium ions at 0.2 M. The selectivity coefficients for Ca/Na, Ca/K, and Ca/Mg were determined as follows; log $K^{Pot}_{Ca,Na}$ =-3.3, log $K^{Pot}_{Ca,K}$ =-2.6, and log $K^{Pot}_{Ca,Mg}$ =-2.8, respectively. The electrode based on **2** showed no response to any cations. The difference in responsive capacity obviously originates from the conformational differences, which is related to the arrangement of carbonyl oxygens on the cyclic peptide framework.

Although ETH 1001 and ETH 129 are known as excellent responsive agents applicable for the calcium ISE,[7] the cyclic peptide was for the first time successfully utilized in the membrane electrode. The cyclic peptides retain advantages in the strict and controllable conformations, which could adjust the arrangement of the ligands for further finely designed ionophores. The increase in lipophilicity in particular amino acid and replacement of Ala with more complicated amino acid should change the conformation for more selective response.

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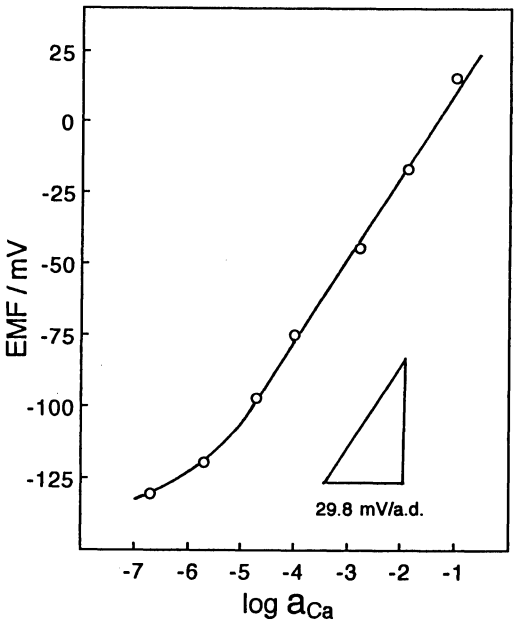

Fig. 4. Response of the membrane electrode based on **1** toward the Ca^{2+} activity.

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