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ION TRANSPORT, ION EXTRACTION, AND ION BINDING BY SYNTHETIC CYCLIC OCTAPEPTIDE

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ABSTRACT. Cyclic octapeptide, cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$ (CGLSP2) was synthesized as an ionophore model. Its ion-transport ability through a chloroform membrane was investigated in connection with ion extractability (K_{ex}) and conformational properties. CGLSP2 transported the picrate salts of Ba^{2+} and Ca^{2+} efficiently. The K_{ex} sequences were $Ba^{2+} > Ca^{2+} >> Mg^{2+}$ and $K^+ > Rb^+ > Na^+$, showing good agreement with the selectivity in ion transport. In addition, cation-binding properties of CGLSP2 to alkali and alkaline earth metal ion were investigated in acetonitrile by CD and NMR spectroscopy. Titration curves obtained from CD data revealed three kinds of CGLSP2/cation complexes. The values of 1:1 complex-formation constants (K_1) decreased in the order $Ba^{2+} > Ca^{2+} > Mg^{2+} > Li^+ >> Na^+ \sim K^+$. ^{1}H- and ^{13}C-NMR data showed that free CGLSP2 exists in at least five different conformational states in acetonitrile. After the addition of equimolar amounts of $Ba(ClO_4)_2$, these conformations converged into a single C_2-symmetric conformation with all-trans peptide bonds.

1. INTRODUCTION

Transmembrane ion transport by cyclic depsipeptide, macrotetrolide actins, and polyether antibiotics is based on "a mobile carrier" [1]. Only few studies, however, have been carried out with regard to carrier-mediated ion-transport by linear or cyclic peptides [2,3]. In comparison, cation-binding properties by synthetic cyclic peptides have been studied extensively [4-6]. We have also synthesized cation-binding cyclic peptides with many N-substituted amino acid residues [7-10]. A new cyclic octapeptide, cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$ (CGLSP2) was synthesized as an ionphore model [11]. It includes two sets of two successive N-substituted peptide bonds, i.e. Lys-Sar and Sar-Pro bonds. This arrangement of peptide bonds is different from those of synthetic cyclic octapeptides studied so far [7,12,13]. Therefore, CGLSP2 is expected to have a ring skeleton that shows a fine balance between flexibility and rigidity. This paper describes an ion transport across chloroform liquid membrane by this cyclic octapeptide. In addition, ion-transport capacity of CGLSP2 is discussed in connection with ion extractability and conformational change at the ion

binding.

2. EXPERIMENTAL

2.1. Synthesis of cyclic octapeptide and related peptides

The preparation and physical characteristic of the cyclic octapeptide, CGLSP2 and related peptides are reported in our preceding paper [11].

2.2. Method of ion transport

Transport of metal picrate across a chloroform liquid membrane was examined in a U-shaped tube at 25°. The source aqueous phase (pH 7.2) contains a metal chloride and picric acid. The alkali metal cations used were Na^+, K^+, and Rb^+, and the alkaline earth metal cations Mg^{2+}, Ca^{2+}, and Ba^{2+}. The chloroform phase contains an ion carrier. The second aqueous phase was adjusted to pH 7.2. Linear octapeptides Boc-[Gly-L-Lys(Z)-Sar-L-Pro]$_2$-OH (LGLSP2-OH) and Boc-[Gly-L-Lys(Z)-Sar-L-Pro]$_2$-OCH_3 (LGLSP2-OM), cyclic tetrapeptide c-[Gly-L-Lys(Z)-Sar-L-Pro] (CGLSP1), and linear tetrapeptide Boc-Gly-L-Lys(Z)-Sar-L-Pro-OH (LGLSP1-OH) were used as related petidic carriers. In addition, valinomycin and dibenzo-18-crown-6 were employed as a K^+ ionophore. The amount of cation transported to the second aqueous phase through membrane was determined spectrophotometrically at 355 nm.

2.3. Procedure of ion extraction

Equal volumes of a chloroform solution containing CGLSP2 and an aqueous solution (pH 7.2) containing a metal picrate were stirred vigorously for 30 min. The mixture was allowed to stand at 25° for 1 h. The absorption of picrate anion in chloroform phase was then determined at 410 nm at 25°. Although a similar extraction was performed with chloroform free from ion carrier, no change of absorption was observed in the chloroform phase.

2.4. Measurement

^{1}H-NMR spectra were obtained at 360 MHz on a NICOLET NT-360 spectrometer with a NIC 1180 Computer Data System. ^{13}C-NMR spectra were recorded on a Varian CFT-20 spectrometer at 20 MHz. CD spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter.

3. RESULTS AND DISCUSSION

3.1. Cation transport across a chloroform liquid membrane

The cumulative amount of transported cation after 10 h by various ion carriers is summarized in Table I. CGLSP2 transported 10.6 μmol Ba^{2+} or 5.5 μmol Ca^{2+} for 10 h. Between cations having similar ionic

Table I Micromoles of cation transported after 10 h

Carrier (diameter,Å)	Na^+ (1.90)	K^+ (2.66)	Rb^+ (2.96)	Mg^{2+} (1.30)	Ca^{2+} (1.98)	Ba^{2+} (2.70)
CGLSP2	0.30	0.54	0.47	0.28	5.5	10.6
LGLSP2-OH	--	0.15	--	0.27	2.6	5.5
LGLSP2-OM	--	0.17	--	--	0.26	1.25
CGLSP1	--	0.14	--	--	--	0.80
LGLSP1-OH	--	0.13	--	--	--	0.10
Valinomycin	--	3.9	--	--	--	0.41
Dibenzo-18-crown-6		9.8	--	--	--	0.18

Phase I : [metal chloride] = 10 mM, [picric acid] = 25 mM, [HEPES] = 10 mM (pH 7.40).
Chloroform Phase : [carrier] = 2.00 X 10^{-4} M.
Phase II : [HEPES] = 10 mM (pH 7.40).

diameter, Ba^{2+} and K^+, the amount of Ba^{2+} transported is about 20 times larger than that of K^+. A similar trend can be seen between Ca^{2+} and Na^+. In addition, CGLSP2 can transport Ba^{2+} at least 2-fold more than LGLSP2-OH, LGLSP2-OM, and CGLSP1. This finding suggests that the number of residues and the cyclic structure is an important factor when designing a peptidic ion carrier. In this way, CGLSP2 was found to be most selective for Ba^{2+}. However, among the physiological cations, it was most selective for Ca^{2+}.

3.2. Cation extraction

Extraction equilibrium constants of CGLSP2 with metal picrates were obtained and shown in Table II. It was confirmed before that CGLSP2 forms a 1:1 complex with cation studied. Consequently, it was found that CGLSP2 can extract Ba^{2+} and Ca^{2+} more efficiently than Mg^{2+}, K^+, Rb^+, and Na^+. The order of the extractability showed good agreement with the selectivity in ion-transport ability.

Table II Extraction equilibrium constants (K_{ex}) of CGLSP2

Cation	K_{ex}	
Na^+	2.28 X 10^2	
K^+	4.73 X 10^2	(M^{-2})
Rb^+	3.65 X 10^2	
Mg^{2+}	2.65 X 10^3	
Ca^{2+}	4.04 X 10^5	(M^{-3})
Ba^{2+}	8.88 X 10^5	

Aqueous Phase : [metal chloride] = 10 mM, [picric acid] = 25 mM, [HEPES] = 10 mM (pH 7.40).
Chloroform Phase : [CGLSP2] = 1.40~7.00 X 10^{-4} M.

This implies that ion transport by CGLSP2 is controlled by ion extraction equilibrium between the source aqueous phase and the chloroform liquid membrane phase.

3.3. Cation binding in acetonitrile

In confirmation of the complexation properties of CGLSP2 in solution, cation-binding studies were accomplished in acetonitrile solution. By analyzing the titration curves obtained from CD spectra [14], 1:1 complex-formation constants (K_1) were evaluated and shown in Table III. In addition, the rate constant of 1:1 complex-formation constants (k_f) was evaluated and also shown in Table III. The K_1 values decrease in the order $Ba^{2+} > Ca^{2+} > Mg^{2+} > Li^+ >> Na^+ \sim K^+$. Doubly charged cations are bound preferentially over monovalent cations. The affinity of Ba^{2+} for CGLSP2 is about 10-fold smaller than that of K^+ for valinomycin. In addition, the Ca^{2+} binding of CGLSP2 is about 10-fold smaller than that of antamanide which is naturally occurring cyclic decapeptide. Between cations having similar ion diameter, the ratio of the binding constants $K_1(Ba^{2+})/K_1(K^+)$ is approximate 10^4. Similar trend can be seen between Ca^{2+} and Na^+. The rate constant (k_f) of 1:1 complex-formation between CGLSP2 and Ba^{2+} is at least 300-fold larger than that reported for complex formation between c-[L-Lys(Z)-L-Pro]$_4$ and Ba^{2+} [6]. However, it is lower than that reported for valinomycin/K^+ complex [15].

Table III 1:1 complex-formation constants (K_1) and rate constants (k_f) of various compounds at 25° in acetonitrile

Compound/Cation	K_1 (M^{-1})	k_f ($M^{-1}min^{-1}$)
CGLSP2/Ba^{2+}	2.7×10^4	$> 4 \times 10^3$
CGLSP2/Ca^{2+}	7.6×10^3	--
CGLSP2/Mg^{2+}	5.6×10^3	--
CGLSP2/Li^+	2.4×10^3	--
CGLSP2/Na^+	very low	--
CGLSP2/K^+	very low	--
c-[Lys(Z)-Sar]$_4$/Ba^{2+}	1.3×10^3(*)	12(*)
c-(Gly-Pro)$_4$/Ba^{2+}	1.6×10^6	--
Antamanide/Ca^{2+}	1×10^5	--
Valinomycin/K^+	3×10^5	2.1×10^9(**)
Nonactin/Na^+	3.8×10^2(**)	$\sim 1.2 \times 10^{10}$(**)

(*) in 95% C_2H_5OH (**) in CH_3OH

Conformational change of the cyclic backbone of CGLSP2 upon complexation was investigated by NMR spectroscopy [14]. ^1H- and ^{13}C-NMR data showed that free CGLSP2 in acetonitrile exists in at least five different conformational states. This feature is ascribed to a cis-trans isomerism around

two sets of two successive N-substituted peptide bonds. A predominant conformer has a C_2-symmetric structure containing two cis Lys-Sar peptide bonds. After the addition of equimolar amounts of $Ba(ClO_4)_2$, those conformations converged into a single C_2-symmetric one with all-trans peptide bonds. This corresponds to a 1:1 complex species. On further addition of $Ba(ClO_4)_2$, CGLSP2 changed the conformation into an asymmetric structure with one cis Lys-Sar peptide bond. This corresponds to a 1:2 complex species. Similar results were obtained for the Ca^{2+}-binding by CGLSP2 in acetonitrile. From the above, the following binding scheme may be proposed, as shown in Fig. 1. Therefore, the slow process of the complex formation between CGLSP2 and Ba^{2+} may be related to a cis-trans isomerization around peptide bonds [16].

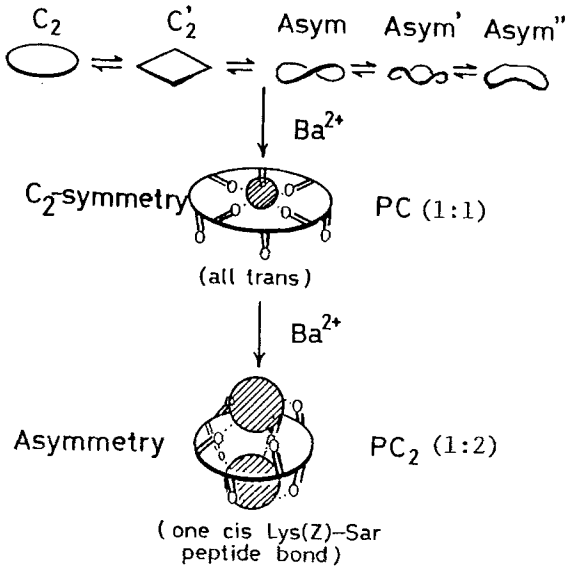

Fig. 1. Scheme for the complexation of CGLSP2 with Ba^{2+}. C_2 and C_2' represent distinct C_2-symmetric conformers. Asym, Asym', and Asym'' represent asymmetric conformers.

Figure 2 shows a proposed conformation of 1:1 complex between CGLSP2 and Ba^{2+}. The Ba^{2+} or Ca^{2+} cation may be bound favorably by the carbonyl groups of the two Sar and the two Gly residues. Here, the

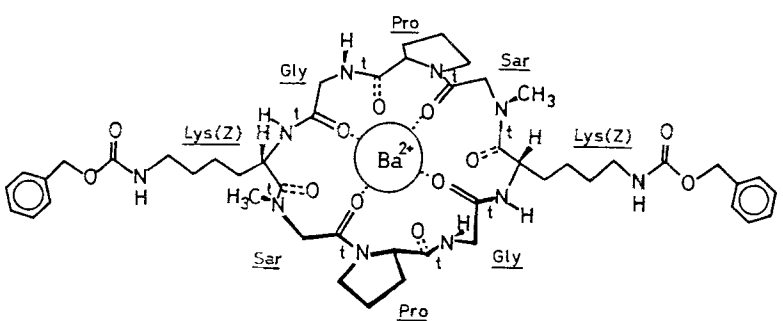

Fig. 2. Schematic representation of a proposed conformation of the 1:1 complex between CGLSP2 and Ba^{2+}.

doubly charged cations enhances the stability of 1:1 complex more effectively than the fitness of the cation size to cavity. However, the Ca^{2+}/Mg^{2+} selectivity of CGLSP2 in the extraction equilibrium disagrees with that of complex formation. It would be attributed to the different solubility of the CGLSP2/cation/picrate-anion into the chloroform phase. Therefore, cation selectivity in transport by CGLSP2 should be closely related to the net solubility [17] of the various cation picrate/CGLSP2 complexes in chloroform, as well as the size and charge number of cation and the cavity size of peptide.

4. REFERENCES

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