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Cation-binding Properties of Ionophorous Cyclic Octapeptide in Acetonitrile Solution

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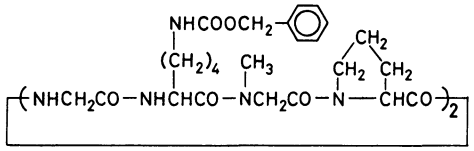
Complex formation of synthetic cyclic octapeptide, cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$, with alkali and alkaline earth metal ion was investigated by CD and NMR spectroscopy. Titration curves obtained from CD data revealed three kinds of CGLSP2(P)/cation(C) complexes. P_2C ("peptide sandwich"), PC ("1 : 1"), and PC_2 ("cation sandwich") complexes occurred in the complexation between CGLSP2 and Ba^{2+}. In the presence of Li^+, Mg^{2+}, or Ca^{2+}, the existence of PC and PC_2 was assumed. K^+ and Na^+ form only a PC species with CGLSP2. By analyzing the titration curves, 1 : 1 complex-formation constants (K_1) were evaluated. The K_1 values decrease in the order $Ba^{2+}>Ca^{2+}>Mg^{2+}>Li^+\gg Na^+$, K^+. ^{1}H- and ^{13}C-NMR data showed that CGLSP2 in acetonitrile exists in at least five different conformational states in a free state. A predominant conformer had a C_2-symmetric structure containing two cis Lys-Sar peptide bonds. After the addition of equimolar amounts of $Ba(ClO_4)_2$, these conformations converged into a single C_2-symmetric one with all-trans peptide bonds. This corresponds to a PC species. On further addition of the salt, CGLSP2 changed the conformation into an asymmetric structure with one cis Lys-Sar peptide bond. This corresponds to a PC_2 species.

Ionophores form stable and lipophilic complexes with Na^+, K^+, and Ca^{2+} *etc.*, and are able to transport them across artificial and biological membranes. Some cyclic oligopeptides have an interesting feature to bind a specific alkali and alkaline earth metal ion.[1-4] In the light of this characteristic, cation extraction,[5] cation transport through a liquid membrane,[6,7] and liquid membrane electrode[8] have been examined by using cyclic peptides. We also reported behavior of cyclic octapeptide, cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$ (CGLSP2) as an ionophorous agent.[9] CGLSP2 could transport or extract Ba^{2+} and Ca^{2+} preferably over Mg^{2+}, K^+, Rb^+, and Na^+. Among physiological cations, Ca^{2+}/Mg^{2+} selectivity in the cation extraction reached 1.5×10^2. Thus, CGLSP2 can compete in this high discrimination ability with naturally occurring antibiotics.[10]

Valinomycin is a macrocyclic dodecadepsipeptide, displaying an exceptionally high selectivity for K^+ over Na^+. The feature can be interpreted in terms of a limited structure of the cyclic backbone in the complexed state.[11] On the other hand, a variety of conformations have been detected for the uncomplexed form in solution.[12] They are found to be highly dependent on solvent polarity. In this way, the cation-binding specificity of valinomycin is based on a conformational rearrangement accompanying complex formation. In particular, CGLSP2 contains four N-substituted peptide bonds (Lys-Sar and Sar-Pro bonds) which are able to take cis as well as trans geometry.[13,14] Therefore, a number of conformations are allowed by random distribution of the cis and trans peptide bonds.

From this point of view, we have studied a cation binding of CGLSP2 with alkali and alkaline earth metal ions in an acetonitrile solution by CD and NMR spectroscopy. Acetonitrile was chosen as a suitable aprotic medium. Its resolution of cation complexes is better than that of protic solvents such as water or methanol. Since CD spectra are very sensitive to peptide conformation upon complexation, they serve to follow a change of complexed species. In addition, it takes the advantage that complex-formation constants can be determined for a certain binding species.[15,16] On the other hand, NMR spectra give information about the structure of that species. Since cis-trans isomerism is slow on the NMR time scale, the conformations can be detected individually by NMR. This paper focused on resolutions and identifications of several complexed species.



cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$ (CGLSP2)

Experimental

The preparation and physical characteristic of the cyclic octapeptide, cyclo[Gly-L-Lys(Z)-Sar-L-Pro]$_2$ (CGLSP2) are reported in our preceding paper.[9]

^{1}H-NMR spectra were obtained at 360 MHz on a NICOLET NT-360 spectrometer with a NIC 1180 Computer Data System, operating in the Fourier transform mode with quadrature detection. ^{13}C-NMR spectra were recorded on a Varian CFT-20 spectrometer at 20 MHz, in the Fourier transform mode with proton-noise decoupling. All the chemical shifts are given in parts per million (ppm) relative to the internal tetramethylsilane.

Circular dichroism spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter equipped with a J-DPY data processor at 20°C. Measurements were carried out using a quartz cell with 0.1-cm path length over 250 nm to 200 nm. Data are represented as molar ellipticities. Unless otherwise noted, spectrophotometric-grade acetonitrile was used as the solvent.

Alkali metal cations used for binding studies were Li^+ (1.20

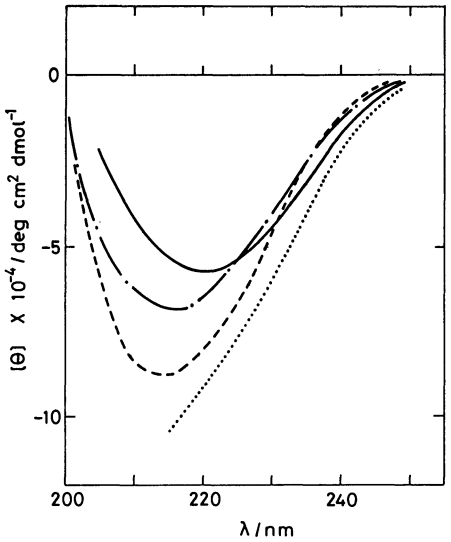


Fig. 1. CD spectra of CGLSP2 in various solvents. Acetonitrile (——), methanol (—·—), 2,2,2-trifluoroethanol (-----), and 1,4-dioxane (·····). Peptide concentration; 6.23×10^{-4} mol dm^{-3}.

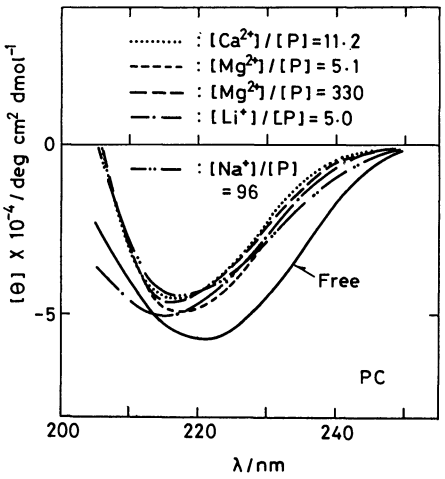


Fig. 2. CD spectra of CGLSP2 in acetonitrile in the presence of lithium, sodium, magnesium, and calcium perchlorate. Molar ratios of a salt to CGLSP2 are shown in the figure. Peptide concentration; 2.47×10^{-4} mol dm^{-3}.

Å), Na^+ (1.90 Å), and K^+ (2.66 Å), and alkaline earth metal cations Mg^{2+} (1.30 Å), Ca^{2+} (1.98Å), and Ba^{2+} (2.70 Å). The value in parentheses indicates each ionic diameter. The cations were dissociated from the corresponding perchlorates. In the case of K^+, acetonitrile/water (95/5, by volume) mixture was used to dissolve the salt. Both CD and NMR measurements were performed after the complexation equilibrium was completely established on addition of each salt.

Results and Discussion

CD Spectra of Cyclic Octapeptide in a Free and a Complexed State. CD spectra of CGLSP2 in various solvents (acetonitrile, methanol, 2,2,2-trifluoroethanol, and 1,4-dioxane) are shown in Fig. 1. Dielectric constant of each solvent is 37.5, 32.7, 26.7, and 2.2, respectively. A large negative Cotton effect appeared at 214—221 nm, which is mainly attributed to an $n\rightarrow\pi^*$ transition of the amide bond. The extremum is shifted to longer wavelength when dielectric constant of the solvent increased. At the same time ellipticity of the negative band decreased. There seems to be no remarkable differences in conformation in these solvents.

When cations smaller than 2.00 Å (Li^+, Na^+, Mg^{2+}, and Ca^{2+}) were added stepwise to a solution of CGLSP2 in acetonitrile, a small spectral change was observed, as shown in Fig. 2. There was a slight decrease of the negative ellipticity. The extremum was shifted 216—218 nm from 221 nm for the free peptide. These spectra are designated type I.

In the presence of cations larger than 2.00 Å [Ba^{2+} (small amounts) and K^+ (large amounts)], the 221-nm negative band decreased in intensity and the extremum was shifted to longer wavelength. In addition,

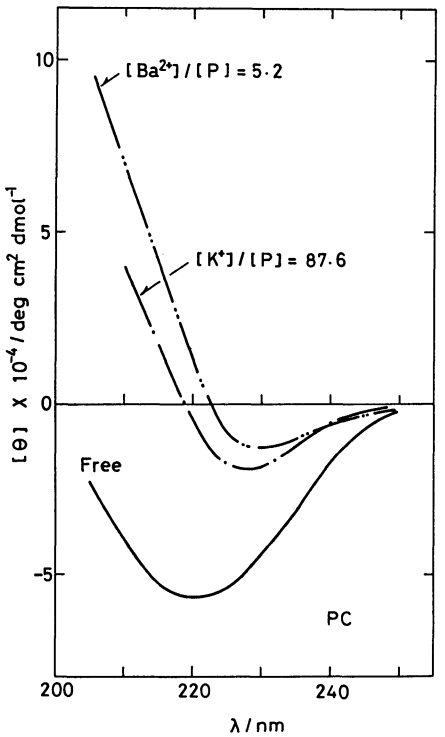


Fig. 3. CD spectra of CGLSP2 in acetonitrile in the presence of potassium and barium perchlorate. Unless otherwise noted, the conditions are the same as those described in Fig. 2.

appearance of a strong positive band around 200—210 nm could be observed (Fig. 3). These are referred to type II. Besides these two types of spectra, a group of spectra designated as type III were found. They were characterized by a small negative ellipticity at 235—250 nm and a relatively sharp negative band at 210—220 nm (Fig. 4). These spectra appeared in the presence of large amounts of Ba^{2+}, Ca^{2+}, and Li^+. The above

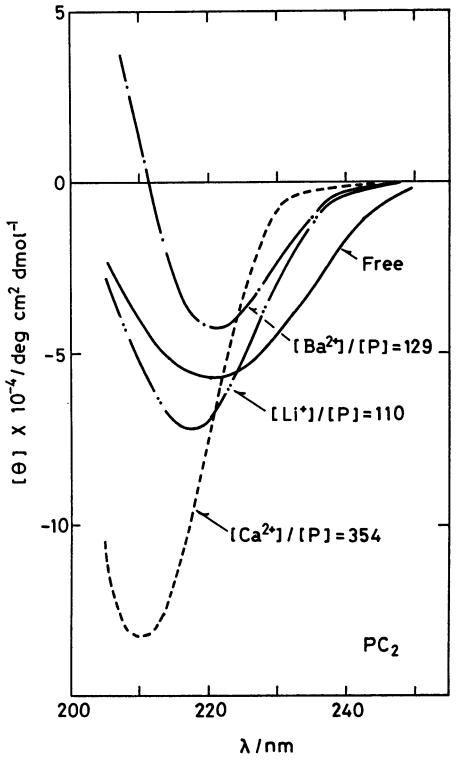


Fig. 4. CD spectra of CGLSP2 in acetonitrile in the presence of lithium, calcium, and barium perchlorate. Unless otherwise noted, the conditions are the same as those described in Fig. 2.

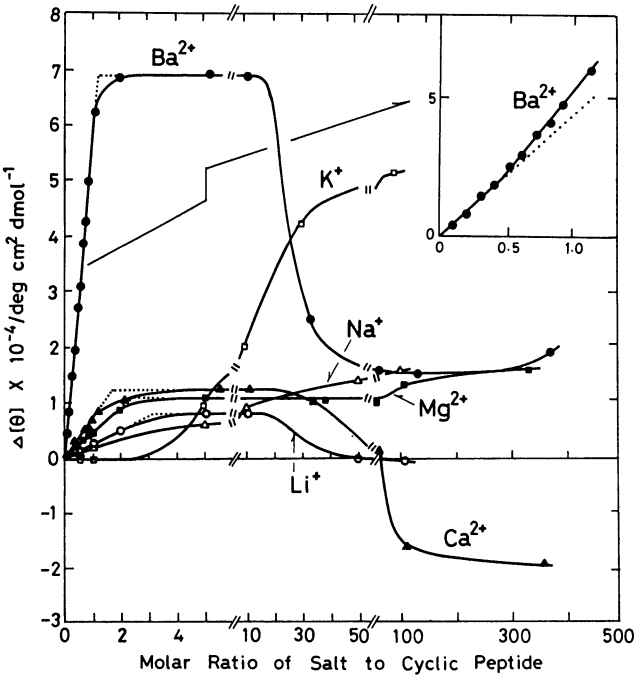


Fig. 5. Titration curves of CGLSP2 for various metal perchlorates in acetonitrile. $\Delta[\theta]$ indicates the difference between the molar ellipticity of a complexed peptide and that of a free peptide.

results suggest that several complexed species exist with different stoichiometries. Moreover, they vary with peptide/cation sets. To clarify these features, we obtained titration curves for each cation from CD spectra.

Titration Curves. Titration curves were depicted, in which the changes in molar ellipticities at a certain wavelength were plotted against the molar ratio of metal salt to cyclic peptide. Titration curves generated from several wavelength (210—230 nm) showed no remarkable differences. Figure 5 indicates the titration curves at 220 nm for Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Ba^{2+}. Three representative curves were depicted according to cations. The first is a titration curve for Ba^{2+}. After adding less than half equivalent of Ba^{2+}, the increase of the ellipticity was linear with the amount of the salt. As the molar ratio further increased (0.5—1.1 equiv), the straight line became another one with a slightly larger slope than that at the early part of titration. After the addition of more than two equivalents of Ba^{2+}, two plateaus that correspond to type II and type III were observed. This finding suggests that CGLSP2 forms at least three types of complexes with Ba^{2+}. At the peptide concentration over the range of 10^{-5}—10^{-3} mol dm^{-3} we have studied, the first species of the complexation may be expressed as P_2C, where P is the cyclic peptide and C is a metal cation. The second and the third step can be interpreted in terms of the formation of a 1:1 PC species and a 1:2 PC_2 species, respectively. The above feature of binding is similar to those found for other cyclic peptides.[16-18]

In the case of Li^+-, Mg^{2+}-, and Ca^{2+}-titration curves, a clear folding could not be observed around the molar ratio of 0.5. Only one plateau is observed within the addition of less than 400 equiv of the cation. It would be associated with the formation of a 1:1 complex. At the late part of titration, the decrease of ellipticity for Li^+ and Ca^{2+}, or the increase of ellipticity for Mg^{2+} indicates an appearance of PC_2 species in place of PC.

K^+- and Na^+-titration curves showed a gradual increase of the ellipticity when the molar ratio increased. The last part of titration with K^+ corresponds to type II which is similar to the case of the first plateau in Ba^{2+} titration (Fig. 3). CD spectrum of CGLSP2 in the presence of 96 equiv of Na^+ resembles closely to that of a 1:1 complex between CGLSP2 and Ca^{2+} (Fig. 2). The above results support that a single complexation step in K^+- and Na^+-titration curve is associated with the formation of a 1:1 complex.

Complex-formation Constants. The data obtained from CD spectra and CD-titration curves revealed the following. (1) P_2C ("peptide sandwich"), PC ("1:1"), and PC_2 ("cation sandwich") complexes occur in the complexation between CGLSP2 and Ba^{2+}. (2) In the Li^+, Mg^{2+}, and Ca^{2+} systems, the existence of PC and PC_2 is assumed. (3) K^+ and Na^+ form only a PC species with CGLSP2. Of course, one can not exclude an existence of alternate complexed species in the presence of larger amount of cations than that we have

Table 1. Complex-formation constants (K_1) of CGLSP2 with various metal perchlorates in acetonitrile at 20°C

Cation	Charge density$\times10^{-10}$/s A m^{-3}	K_1/mol^{-1} dm^3
Li^+	12.1	$(2.4\pm0.2)\times10^3$
Na^+	4.46	Very low
K^+	1.62	Very low
Mg^{2+}	26.8	$(5.6\pm0.9)\times10^3$
Ca^{2+}	7.90	$(7.6\pm1.9)\times10^3$
Ba^{2+}	3.17	$(2.7\pm0.7)\times10^4$

studied. From the region of a 1:1 complex formation in the titration curves, complex-formation constants (K_1) were determined so as to satisfy a normalized α-ϕ curves calculated by Prestegard and Chan.[19] The obtained values are given in Table 1. The affinity of Ba^{2+} for CGLSP2 in acetonitrile ($K_1\approx2.7\times10^4$ mol^{-1} dm^3) is *ca.* 10-fold smaller than that of K^+ for valinomycin ($K_1\approx3\times10^5$ mol^{-1} dm^3 in CH_3CN).[20] In addition, CGLSP2 binds Ca^{2+} favorably ($K_1\approx7.6\times10^3$ mol^{-1} dm^3). This affinity is *ca.* 10-fold smaller than that of a naturally occurring cyclic decapeptide, antamanide which forms a very stable complex with Ca^{2+} ($K_1=1\times10^5$ mol^{-1} dm^3 in CH_3CN).[21] The K_1 sequences were $Ba^{2+}>Ca^{2+}>Mg^{2+}>Li^+\gg Na^+$, K^+. It can be clearly seen that doubly charged cations are bound preferentially over monovalent cations. Between cations having similar ionic diameter (Ba^{2+} and K^+), the ratio of the binding constants, $K_1(Ba^{2+}):K_1(K^+)$, is approximate 10^4. Similar trend could be seen between Ca^{2+} and Na^+ [$K_1(Ca^{2+}):K_1(Na^+)\approx10^3$]. The binding of both Na^+ and K^+ is much lower than that of the other cations, even the smaller cations, Li^+ or Mg^{2+}. As observed with the complex formation between cyclo-(L-Val-Gly-Gly-L-Pro)$_3$ and Li^+, or Mg^{2+},[16] high charge density of the cations may serve to form a stable 1:1 complex.

Rate of Complex Formation. Kinetics of a 1:1 complex formation between CGLSP2 and $Ba(ClO_4)_2$ was investigated in acetonitrile by CD spectroscopy. The complexing reaction was started by combining solutions of the salt (1.14 equiv) and the cyclic peptide. Immediately after mixing, the ellipticity at 220 nm was measured at 1-min intervals. Under these conditions, formation of a 1:1 complex is clearly demonstrated by the titration curves shown in Fig. 5. Consequently, equilibrium was already reached even after 1 min. This result indicates that the rate of complex formation between CGLSP2 and Ba^{2+} is fairly rapid. Formation and dissociation of the complex can be expressed by the following scheme;

$$P + C \underset{k_d}{\overset{k_f}{\rightleftharpoons}} PC$$

where k_f and k_d is rate constants of the complex formation and dissociation, respectively. Assuming the half-life of free cyclic peptide (P) is 1 min, we can

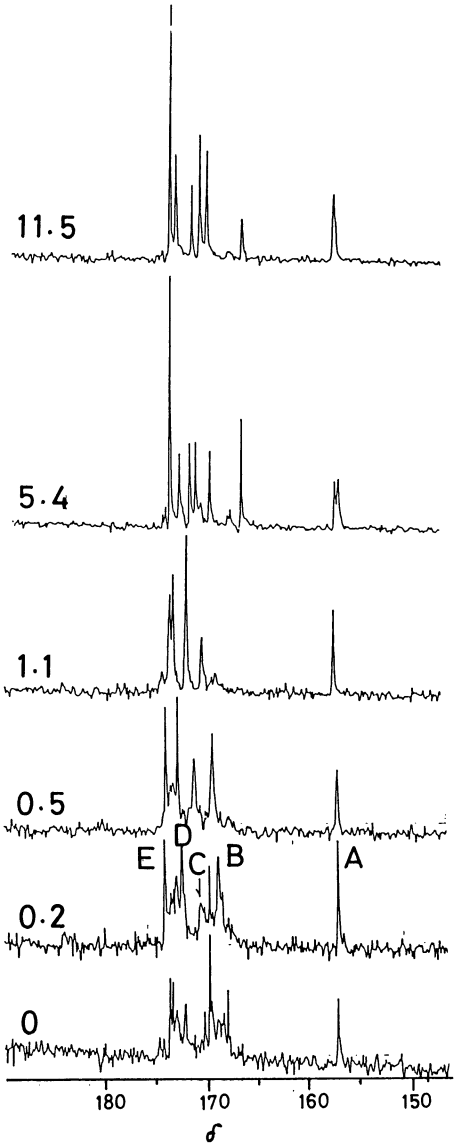


Fig. 6. ^{13}C-NMR spectra of C=O region of CGLSP2 in acetonitrile-d_3 when barium perchlorate was added. Numbers represent the molar ratio of salt to peptide. Peptide concentration; 54.2 g dm^{-3}. Temperature; 25°C.

evaluate the rate constant (k_f) roughly in the same manner as described by Kimura and Imanishi.[4] The k_f value obtained was larger than 66 (mol^{-1} dm^3 s^{-1}) at 20°C. This value appears to be lower than that reported for valinomycin/K^+ complex (3.5×10^7 mol^{-1} dm^3 s^{-1} in methanol).[22] However, it is at least 300-fold larger than that reported for complex formation between cyclo[L-Lys(Z)-L-Pro]$_4$ and Ba^{2+} [0.2 mol^{-1} dm^3 s^{-1} in ethanol/ water (95/5, v/v) mixture].[4]

NMR Investigation. Titration of CGLSP2 with $Ba(ClO_4)_2$ was investigated in acetonitrile-d_3 by NMR spectroscopy (^{1}H and ^{13}C). The number of both C=O carbon resonances and N-CH_3 proton resonances serves to clarify the symmetry of the ring. In addition, the chemical shifts of Pro C^β and C^γ carbon are indica-

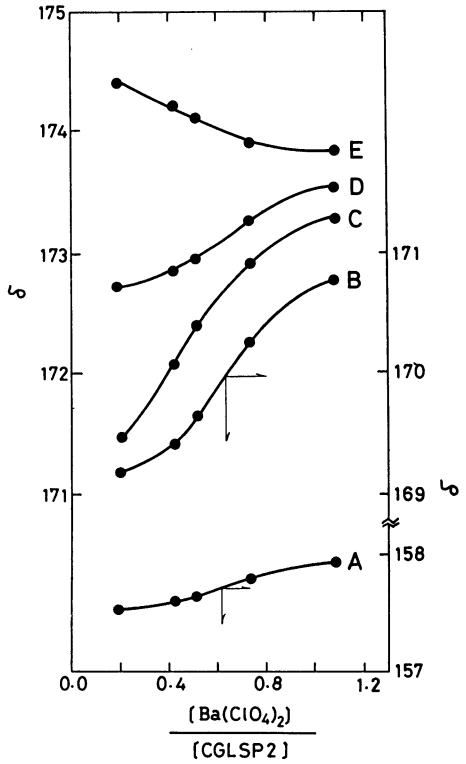


Fig. 7. Shift of carbonyl resonances of CGLSP2 in acetonitrile-d_3 when barium perchlorate was added.

tive of a cis-trans isomerism around Sar-Pro peptide bonds.[13,23] The chemical shifts of Sar N-CH_3 protons reflect a cis-trans isomerism around Lys-Sar peptide bonds.[23,24] In Fig. 6, spectral change of carbonyl resonance region in ^{13}C-NMR is presented. A large number of resonances appeared in the absence of barium salt. When the salt was added stepwise to the solution (<1.1 equiv of Ba^{2+}), five signals became pronounced with increasing in concentration of the salt. This finding indicates that CGLSP2 converged into a single C_2-symmetric conformation from multiple conformations in a free state by the complex formation with Ba^{2+}. That species in a complexed state corresponds to PC that is responsible for type I spectrum in CD. Figure 7 indicates the shift of the five carbonyl carbon resonances caused by the addition of $Ba(ClO_4)_2$. A downfield shift was observed for the signals A, B, C, and D except the signal E. This downfield shift is considered to be caused by ion-dipole interactions between Ba^{2+} and carbonyl groups.[4,17,25] Accordingly, differences in their chemical shifts ($\Delta\delta$) between the cases containing 0.2 and 1.1 equiv of Ba^{2+} were determined. The difference ($\Delta\delta$) of the signals B and C is at least two times larger than that of the other signals A, D, and E. Assignment of the signals were carried out by comparison with the signals of a precursory linear peptides and related compounds,[9] and on the basis of literature information.[26] The Pro C=O, Lys(Z) C=O, Gly or Sar C=O, and Lys(Z) NHCOO carbon resonances must be located in this order from the lowfield. Thus,

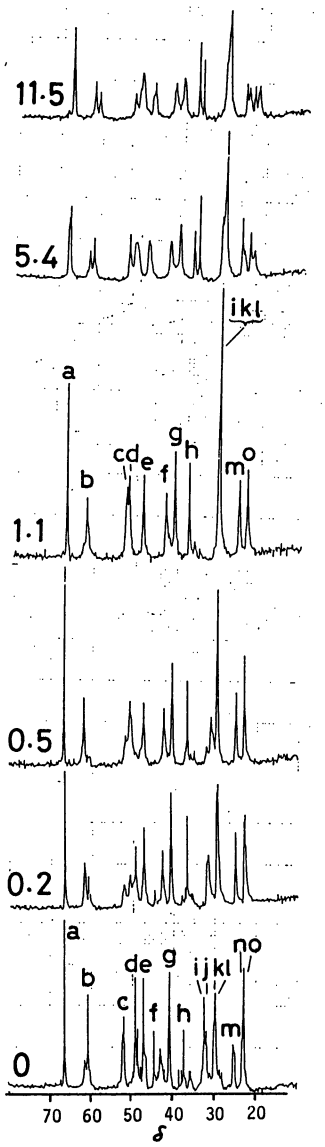


Fig. 8. ^{13}C-NMR spectra of upfield regions of CGLSP2 in acetonitrile-d_3 when barium perchlorate was added. Numbers represent the molar ratio of salt to peptide. Tentative resonance assignments were indicated; (a) $\underline{C}H_2$-C_6H_5, (b) Pro C^{α}, (c) Pro C^{δ}, (d) Lys C^{α}, (e) Sar C^{α}, (f) Gly C^{α}, (g) Lys C^{ε}, (h) Sar N-CH_3, (i) Lys C^{δ}, (j) Pro C^{β}(cis), (k) Pro C^{β}(trans), (l) Lys C^{β}, (m) Pro C^{γ}(trans), (n) Pro C^{γ}(cis), and (o) Lys C^{γ}.

the signals B and C can be attributed to Gly C=O and Sar C=O carbon. Therefore, four carbonyl oxygens of Gly and Sar residues are considered to participate to bind Ba^{2+} cooperatively in the C_2-symmetric conformer. At this time, the side-chain carbonyl groups of Lys residue do not involve in complexation, since the signal A can be assigned to carbonyl carbon of benzyloxycarbonyl group of Lys.

Further addition of more than 1.1 equiv of the salt produced another pattern of signals which was ascribable to a new complexed species. As judged from the number and peak area of resonances in the pres-

ence of 5.4 equiv of the salt, the new species possesses an asymmetric conformation. This conformer corresponds to PC_2 that is responsible for type III spectrum in CD.

Upfield regions of the ^{13}C-NMR spectra of CGLSP2 are given in Fig. 8. Remarkable spectral changes were observed in Pro C^{β} and C^{γ} resonance regions (δ 20—35). As the molar ratio of salt to peptide increases, the Pro C^{β} and Pro C^{γ} carbon signals (j and n) involving in a cis Sar–Pro peptide bond in free peptide diminished and alternatively signals (k and m) arising from the trans peptide bond became predominant. In the presence of 1.1 equiv of the salt, only one signal attributed to the trans Sar–Pro peptide bond appeared at δ 30.31 for Pro C^{β} carbon and δ 25.31 for Pro C^{γ} carbon.[13,24] Even when the salt was further added, the resonances of Pro C^{β} and Pro C^{γ} carbon remained within the region associated with trans Sar–Pro bond.

Similarly, ^1H-NMR spectra were investigated while $Ba(ClO_4)_2$ was added stepwise to CGLSP2 in acetonitrile-d_3. They also suggested a conformational convergence into the single C_2-symmetric conformation. In Fig. 9 , spectral change of N–CH_3 resonance region is shown. In the absence of the salt, a strong signal appears at δ 2.87, in addition to a single resonance at δ 2.92 and three pairs of six signals at δ 2.83, 2.90, 2.96, 2.98, 3.17, and 3.20. Therefore, free CGLSP2 exists in at least five different conformational states (two C_2-symmetric conformers and three asymmetric ones) in acetonitrile. As previously reported,[9] conformational distribution of these conformers is similar to that in chloroform. Moreover, a predominant C_2-symmetric conformer includes two cis Lys–Sar peptide bonds that are the same in chloroform. The temperature dependences of predominant NH proton resonances (not shown) were found to be $\approx$0 ppm deg^{-1} for Lys NH protons and $\approx 7\times10^{-3}$ ppm deg^{-1} for Gly NH protons in acetonitrile-d_3. This result suggests that Lys NH protons of the predominant conformer are shielded from the solvent. ^{13}C-NMR measurement showed that Pro C=O carbon resonance (the signal E) is shifted to upfield upon complexation (Fig. 7). Together with these results, Lys NH protons could be intramolecularly hydrogen-bonded to Pro C=O oxygens in (some) conformers of the free peptide. Consequently, the upfield shift of Pro C=O carbon resonance may be attributed to the destruction of hydrogen bonds in the complex formation.[6]

With increasing in Ba^{2+} concentration (<1.0 equiv of Ba^{2+}), a strong N–CH_3 signal (○) at δ 2.87 disappeared gradually. At the same time, a new N–CH_3 signal (▲) appeared at δ 3.10 upon the addition of 0.25 equiv of Ba^{2+}, and its intensity increased. In the presence of 0.5—1.0 equiv of Ba^{2+}, one major signal was observed at δ 3.09. Further addition of the salt (>1.0 equiv of Ba^{2+}) produced a new pair of signals (●) at δ 3.03 and 2.84. In the presence of more than 5.6 equiv of Ba^{2+}, only this

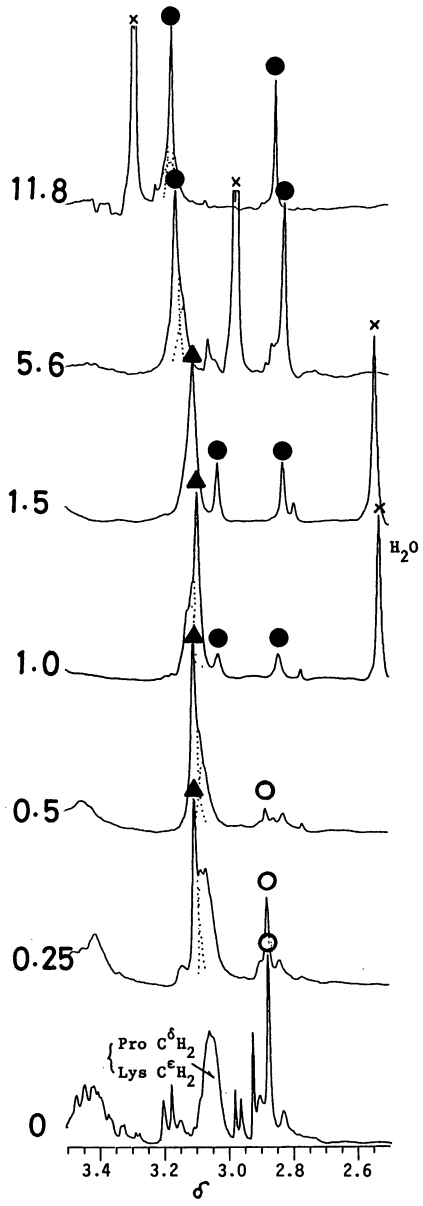


Fig. 9. ^1H-NMR spectra of N–CH_3 region of CGLSP2 in acetonitrile-d_3 when barium perchlorate was added. Numbers represent the molar ratio of salt to peptide. Peptide concentration; 32.0 g dm^{-3}. Temperature; 20°C.

$$C_2(ctttctttt) \rightleftharpoons C_2' \rightleftharpoons A' \rightleftharpoons A'' \rightleftharpoons A'''$$

c: cis, t: trans ↓

$$C_2(ttttttttt)\cdot M^{2+} \qquad [PC]$$

↓

$$M^{2+}\ddagger A(ctttttttt)\cdot M^{2+} \qquad [PC_2]$$

Fig. 10. Scheme for the complexation of CGLSP2 with $Ba(ClO_4)_2$ or $Ca(ClO_4)_2$, which was deduced from ^1H- and ^{13}C-NMR spectroscopy. C_2 and C_2' represent distinct C_2-symmetric conformers. A, A′, A″, and A‴ represent asymmetric conformers. M^{2+} means Ba^{2+} or Ca^{2+}. Peptide-bond sequences in parentheses are given for the linkage of Lys(Z)-Sar-Pro-Gly-Lys(Z)-Sar-Pro-Gly-Lys(Z) on the ring.

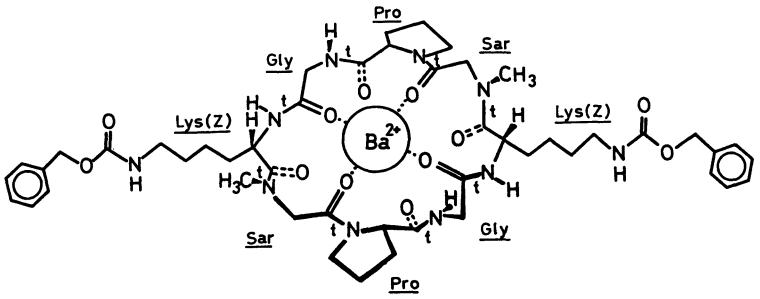


Fig. 11. Schematic representation of a proposed conformation of the 1:1 complex between CGLSP2 and barium cation.

pair of signals exist. These features are consistent with those observed in ^{13}C-NMR measurement, which demonstrates the existence of two complexation steps. However, in ^{1}H- and ^{13}C-NMR spectra, we could not resolve and identify the P_2C species that was detected in a CD spectrum. It has been reported that a resonance for N-CH_3 protons involved in a trans peptide bond occurs in a lower field region than that involved in a cis peptide bond.[24,27] Thus, the N-CH_3 signal marked with (▲) could be associated with trans Lys-Sar peptide bond. In a similar way, the lowfield signal of the two marked with (●) is ascribed to N-CH_3 protons associated with the trans Lys-Sar bond, and the highfield signal is ascribed to those of the cis Lys-Sar bond.

By the combination of the above ^{1}H- and ^{13}C-NMR data, the cis-trans arrangements of peptide bonds in CGLSP2 can be deduced for each complexed species in Ba^{2+} binding. A PC (1:1) complex has an all-trans peptide bonds. In contrast to this, a P_2C (2:1) complex contains one cis Lys-Sar peptide bond. Both conformations are absent in a free state. Moreover, it is of interest that the structure of CGLSP2/Ba^{2+} (1:1) complex in acetonitrile is different from that of CGLSP2/Ca^{2+} (1:1) complex in chloroform/acetonitrile (5/3, v/v) mixture.[9] In order to obtain further details, we measured ^{13}C- and ^{1}H-NMR spectra of the 1:1 Ca^{2+} complex with CGLSP2 in acetonitrile. As a result, the chemical shifts of Sar N-CH_3, Pro C^{β}, and Pro C^{γ} resonances are indicative of the occurrence of all-trans peptide bond arrangement. This fact indicates that the structural difference between Ca^{2+}- and Ba^{2+} -complex is not affected by the nature of cation but by that of solvent. From the above, the following scheme may be proposed for the Ba^{2+} binding and the Ca^{2+} binding by CGLSP2 in acetonitrile. It is shown in Fig. 10.

Inspection of a CPK molecular model having all-trans peptide bonds revealed that four coplanar carbonyl groups form a cavity for binding a cation. The barium or calcium ion may be bound favorably by the carbonyl groups of the two Sar and two Gly residues. This is supported by the chemical shift change of each carbonyl resonances upon complexation (Fig. 7). Figure 11 indicates a proposed conformation of 1:1 complex between CGLSP2 and Ba^{2+}, in which the divalent charge of the cations enhances the stability of 1:1 complex more effectively than the fitness of the cation size to cavity. Actually, CGLSP2 had poor affinity for K^{+} that is suitable for the cavity size.

Cyclo(L-Pro-Sar)$_4$ showed a conformational multiplicity that is based on cis/trans isomerism of all N-substituted peptide bonds.[14,28,29] In this respect, CGLSP2 has a localized distribution of N-substituted peptide bonds,[9] when compared with other cyclic octapeptides.[7,29] Therefore, this approach might provide CGLSP2 with a relatively limited structure of the cyclic backbone similarly to that of valinomycin. However, the ratio of the complex-formation constants, $K_1(Ca^{2+}):K_1(Mg^{2+})$, was very low ($\approx$1.4) in acetonitrile (Table 1). We have reported that the Ca^{2+} transport and extraction by CGLSP2 is much higher than Mg^{2+}.[9] Particularly, the ratio of the extraction equilibrium constants, $K_{ex}(Ca^{2+}):K_{ex}(Mg^{2+})$, was about 150. This high Ca^{2+}/Mg^{2+} selectivity disagrees with that of complex formation. It can be interpreted in terms of three constituent equilibria of the extraction process;[30] (1) distribution of CGLSP2 between the two phases, (2) complex formation of CGLSP2 with the metal cation in the aqueous phase, and (3) ion-pair extraction of CGLSP2/metal cation/picrate-anion species. The Ca^{2+}/Mg^{2+} selectivity of CGLSP2 has no relation with equilibrium (1). Therefore, the Ca^{2+} -selective transport and extraction by CGLSP2 may be closely related to the equilibria (2) and/or (3). With regard to the equilibrium (2), it could be owing to the high Ca^{2+}/Mg^{2+} selectivity of complex formation in the aqueous solution. This could not be confirmed experimentally because CGLSP2 was insoluble in water. With regard to the equilibrium (3), it could be attributed to the fairly poor solubility of the CGLSP2/Mg^{2+}/picrate-anion species into the chloroform phase in comparison with the corresponding Ca^{2+}-complexed ion pair.

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