Synthesis and a Structural Study of an Ion-Binding Cyclic Peptide Analogue of Valinomycin, *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$[†]

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Abstract: The synthesis and the 270-MHz nuclear magnetic resonance investigation of an ion binding cyclic peptide analogue of valinomycin [*cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ (c-(AGPhP)$_3$)] and its cation complexes are reported. In $CDCl_3$ the peptide exists in a C_3 symmetric "propeller" structure containing six (1 ← 4) intramolecular hydrogen bonds. Cation peptide complexes are formed in this solvent. However, the observed broadening of the spectral lines prevented the study of the conformations of these complexes. In CD_3CN, *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ exists as a mixture of several conformers with no evidence for any preferred intramolecular hydrogen bonds. Titration studies in this solvent show that Ba^{2+}, K^+, Rb^+, Cs^+, and Tl^+ form 1/1 ion–peptide complexes while Na^+ and Li^+ form 1/2 ion–peptide complexes. From these studies, it was found that the lifetimes of the 1/1 ion–peptide complexes are as follows: $Ba^{2+} > K^+ \simeq Rb^+ \simeq Cs^+ > Tl^+$. These complexes have conformations of the bracelet type similar to that found for the valinomycin–K^+ complex. From temperature-variation and isotope-exchange experiments, the various complexes were found to have different degrees of rigidity, which correlate well with the corresponding lifetimes. Isotope-exchange studies also provided evidence for a cation release–capture mechanism which involves structural changes in the L-Ala-Gly segment of the molecule and breaking of three of the six hydrogen bonds which stabilize the complex.

There has been considerable interest during the last few years in the structure and conformation of synthetic cyclic peptides and peptide antibiotics which are capable of binding cations.[1-14] The main goal of these studies relates to the understanding of the mechanism of ion transport through biological membranes as mediated by small macrocyclic molecules such as valinomycin.[2] Valinomycin, which was first described by Brockmann[15] and synthesized by Shemyakin,[16] is a cyclic dodecapeptide which is known to alter the ionic permeabilities of artificial[17] and natural[18-20] lipid membranes. Extensive conformational studies using spectroscopic techniques (NMR, CD, ORD, UV, and IR), theoretical calculations, and X-ray diffraction have been carried out[21] and have shown that the ion selectivity of this compound depends not only on the nature of the ligands but also on the conformational state of the whole molecule. It was felt that the study of adequately chosen synthetic analogues of valinomycin might contribute to a better understanding of the mechanism of cation complex formation and transport of these ionophores.

Three such ionophores, *cyclo*(L-Pro-L-Val-D-Pro-D-Val)$_3$ or PV,[22] *cyclo*(L-Pro-L-Val-D-Ala-D-Val)$_3$ or PVAV,[23] and *cyclo*-(L-Val-Gly-Gly-L-Pro)$_3$ or c-(VGGP)[13] have been synthesized. Nuclear magnetic resonance (NMR) studies[11,12,14,24] have shown that all three compounds bind cations and form complexes, in particular with K^+ which are isostructural with the valinomycin–K^+ complex.

In this paper the synthesis of a new cyclododecapeptide related to valinomycin, *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ abbreviated c-(AGPhP)$_3$, is reported.

This peptide has interesting features as compared to the three known cyclododecapeptide ionophores. First, its sequence is made up of alternating dipeptide sequences (L residue-Gly) and (D residue-L residue) combining thus the sequential space features of c-(VGGP) as well as of PV or PVAV. Similarly to these peptides, *cyclo*(Ala-Gly-D-Phe-Pro)$_3$ should then be able to adopt a valinomycin-like bracelet conformation when binding to cations. Second, the sequence D-Phe-L-Pro, absent in the previously cited peptides, should particularly favor the formation of a rigid hairpin turn as in the naturally occurring cyclic peptide gramicidin S.[30] Finally, the presence of aromatic residues in the peptide sequence

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(24) Abbreviations used: c-(AGPhP)$_3$, *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$; c-(VGGP)$_3$, *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$; PV, *cyclo*(L-Pro-L-Val-D-Pro-D-Val)$_3$; PVAV, *cyclo*(L-Pro-L-Val-D-Ala-D-Val)$_3$; CPK, Corey–Pauling–Koltun; BOC, *tert*-butyloxycarbonyl; TOS, *p*-tosylate; OBz, benzyl ester; ONp, *p*-nitrophenyl ester; *p*-Np, *p*-nitrophenol; DCCI, dicyclohexylcarbodimide; MA, mixed anhydride reaction; EtOAc, ethyl acetate.
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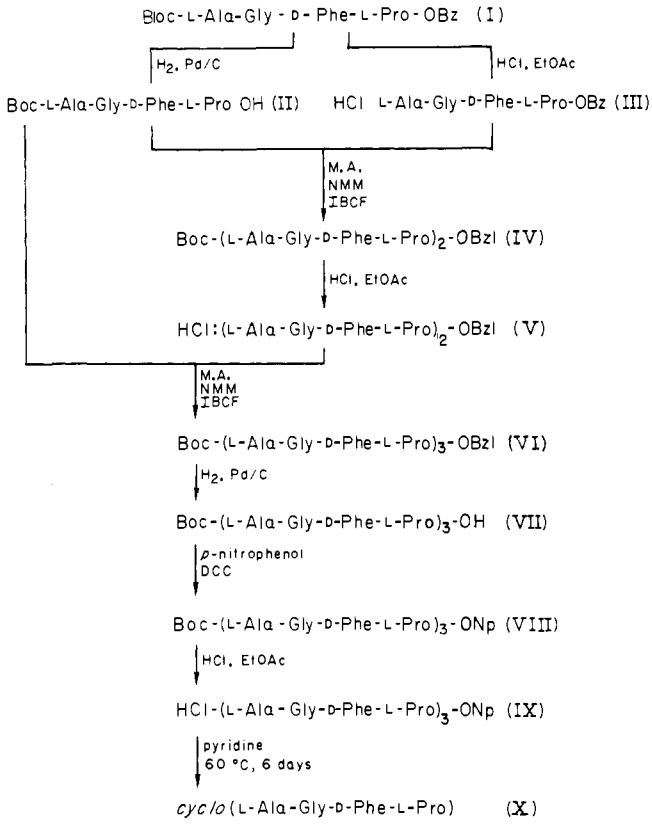
Scheme I



should enhance the lipophilicity of the peptide and therefore its solubility into membranes. Furthermore, the conformational freedom resulting from the Ala-Gly sequence should increase the solubility into aqueous solutions. The amphiphilic character of the potential ionophore *cyclo*(Ala-Gly-D-Phe-Pro)$_3$ makes this peptide a good model for the study of ion complex formation and transport of the complex through membranes.

The structural and dynamic aspects of this compound and its ionic complexes were investigated with NMR spectroscopy. It is shown that in $CDCl_3$ the cyclododecapeptide adopts a "propeller" conformation which is able to bind the various cations Ba^{2+}, K^+, Rb^+, Cs^+, Tl^+, Na^+, and Li^+.

From the salt titrations in CD_3CN, the 1/1 ion–peptide complexes with Ba^{2+}, K^+, Rb^+, Cs^+, and Tl^+ were unambiguously characterized. Their conformations were found to be closely related to the bracelet structure of the valinomycin–K^+ complex and their lifetimes to be in the following order: $Ba^{2+} > K^+ \simeq Rb^+ \simeq Cs^+ > Tl^+$. A correlation was also found between the lifetimes and the structures and susceptibilities to temperature and isotope exchange of these complexes. The isotope-exchange studies also indicated a mechanism of cation release–capture involving the breaking of 3 of the 6 hydrogen bonds which stabilize the bracelet structure of the complex.

Titration studies in CD_3CN showed that Na^+ and Li^+ bind to *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ to form 1/2 ion–peptide complexes.

Preliminary liquid-membrane experiments, undertaken in order to characterize the ion transport properties of the cyclododecapeptide, have shown that the ability for ion transport is as follows: $Tl^+ > K^+ > Ba^{2+}$ (U. Olsher and E. R. Blout, work in progress).

Experimental Section

Synthesis. The cyclic dodecapeptide *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ was obtained by cyclization of the *p*-nitrophenol ester of the linear dodecapeptide HCl-(Ala-Gly-D-Phe-Pro)$_3$-ONp. The overall synthetic pathway is summarized in Scheme I. The identity and purity of the intermediate peptides were checked by TLC, amino acid analysis, infrared spectroscopy, and NMR. TLC was run on Quanta/Gram precoated silica gel plates from Quantum Industries (Fairfield, NJ), and the solvent systems used were chloroform:methanol (93:7 and 80:20) and *n*-butyl alcohol:acetic acid:water (4:1:1). Infrared spectra were recorded on a PE-521 apparatus and NMR spectra on a Varian CFT-20 (^{13}C) and a Varian HA-60 or a Bruker 270 (^1H) spectrometer. Solvents used routinely for synthesis were of analytical grade (Baker). Amino acids and derivatives were purchased from Fox Co. Details of synthetic procedures of intermediate peptides are given below.

Boc-L-Ala-Gly-D-Phe-L-Pro-OBz (I). The synthesis of this protected tetrapeptide has been described.[25] It could not be crystallized from various solvent mixtures and was purified by liquid-column chromatography on silica gel (70–230 mesh) with a mixture of chloroform:methanol (98:2) as elutant. The purity and identity of this peptide were checked by TLC, amino acid analysis, and proton and ^{13}C NMR spectroscopy.

Boc-L-Ala-Gly-D-Phe-L-Pro-OH (II). The tetrapeptide benzyl ester (I) (6.29 g, 10.83 mmol) in solution in 50 mL of *tert*-butyl alcohol was hydrogenated for 16 h under 30 psi of H_2 in the presence of 10% Pd/C as catalyst. Filtration through a millipore LS filter, evaporation of the solvent, and titration in ether yielded a white amorphous solid presenting a single spot on TLC in various solvent systems: yield 5.19 g, 10.48 mmol.

HCl·H-L-Ala-Gly-D-Phe-L-Pro-OBzl (III). Treatment of a solution of the Boc-protected tetrapeptide (I) (5 g, 8.6 mmol) in ethyl acetate (EtOAc) at 0 °C with HCl gas for 45 min yielded, after evaporation of the solvent and triturations with ether, 4.42 g (8.54 mmol) of a white solid (III).

Boc-(L-Ala-Gly-D-Phe-L-Pro)$_2$-OBz (IV). The N- and C-protected octapeptide (IV) was synthesized from the mixed anhydride reaction.[26] A solution of the tetrapeptide acid (II) (3.12 g, 6.36 mmol) in 50 mL of $CHCl_3$ was cooled to -20 °C and treated with 0.71 mL (6.36 mmol) of *N*-methylmorpholine (NMM) and 0.85 mL (6.46 mmol) of isobutyl chloroformate (IBCF). After the mixture was stirred for 10 min, a solution of the tetrapeptide ester (III) (3.29 g, 6.36 mmol) and an additional equivalent of *N*-methylmorphine were added dropwise to the reaction mixture. Magnetic stirring of the reaction mixture at −20 °C (dry ice/CCl4) was allowed to continue for 16 h, the solution warming up slowly to room temperature as the dry ice evaporated. The mixture was successively extracted (3 × 25 mL) with distilled water, 0.1 N HCl, 5% $NaHCO_3$, and saturated NaCl solution. Drying the chloroform layer over anhydrous Na_2SO_4 and evaporation of the solvent yielded a white solid. This product showed one main spot on TLC and was further purified on a silica gel column (2.5 cm × 90 cm) and eluted with a gradient 0–5% methanol in $CHCl_3$. The pure octapeptide was eluted by fractions containing 1.8–2.0% methanol. Yield 4.87 g, 5.11 mmol. The purity was checked by various techniques as described for peptide I.

HCl·H-(L-Ala-Gly-D-Phe-L-Pro)$_2$-OBz (V). The protected octapeptide (IV) (2.6 g, 1.92 mmol) dissolved in ethyl acetate (60 mL) was treated with HCl gas for 30 min at 0 °C. Evaporation of the solvent under reduced pressure and purification by titrations with ether and ether:ethyl acetate (1:1) yielded 2.33 g (1.8 mmol) of octapeptide hydrochloride V.

Boc-(L-Ala-Gly-D-Phe-L-Pro)$_3$-OBzl (VI). This linear dodecapeptide was prepared by the mixed anhydride coupling as already described in the synthesis of IV. A solution of the tetrapeptide acid II (1.55 g, 3.16 mmol) in 30 mL of $CHCl_3$ was cooled to −20 °C in a dry ice/CCl4 bath and *N*-methylmorpholine (0.36 mL, 3.26 mmol) and isobutyl chloroformate (0.43 mL, 3.26 mmol) were added with stirring. After 20 min, the octapeptide hydrochloride V (2.86 g, 3.2 mmol) and an additional equivalent of *N*-methylmorpholine were added. After the mixture was stirred overnight at room temperature, workup as described for IV yielded the dodecapeptide VI as a white amorphous solid which was then dissolved in aqueous methanol 50% solution and treated with Rexyn I-300 ion-exchange resin (Fischer Scientific Co.). Evaporation of the solvent to dryness yielded a white solid (3.37 g, 2.54 mmol) which showed one spot on TLC in various solvent systems.

Boc-(L-Ala-Gly-D-Phe-L-Pro)$_3$-OH (VII). The dodecapeptide benzyl ester IV (3.36 g, 2.53 mmol) was dissolved in 5 mL of methanol and 60 mL of *tert*-butyl alcohol and hydrogenated under 38 psi of H_2 for 48 h, with 10% Pd/C as catalyst. Filtration through Millipore LS and evaporation of the solvent yielded a white solid (2.73 g, 2.2 mmol).

Boc-(L-Ala-Gly-D-Phe-L-Pro)$_3$-ONp (VIII). The dodecapeptide acid VII (2.7 g, 2.18 mmol) was dissolved in 40 mL of chloroform, and the solution was cooled to 0 °C. Dicyclohexylcarbodiimide (DCC) (0.51 g, 2.47 mmol) and *p*-nitrophenol (1.23 g, 8.8 mmol) were added, and the reaction mixture (monitored by TLC) was stirred for 24 h at 0 °C. After removal of the dicyclohexyl urea by filtration and evaporation of the solvent, the resulting solid was purified by titrations with ether to remove

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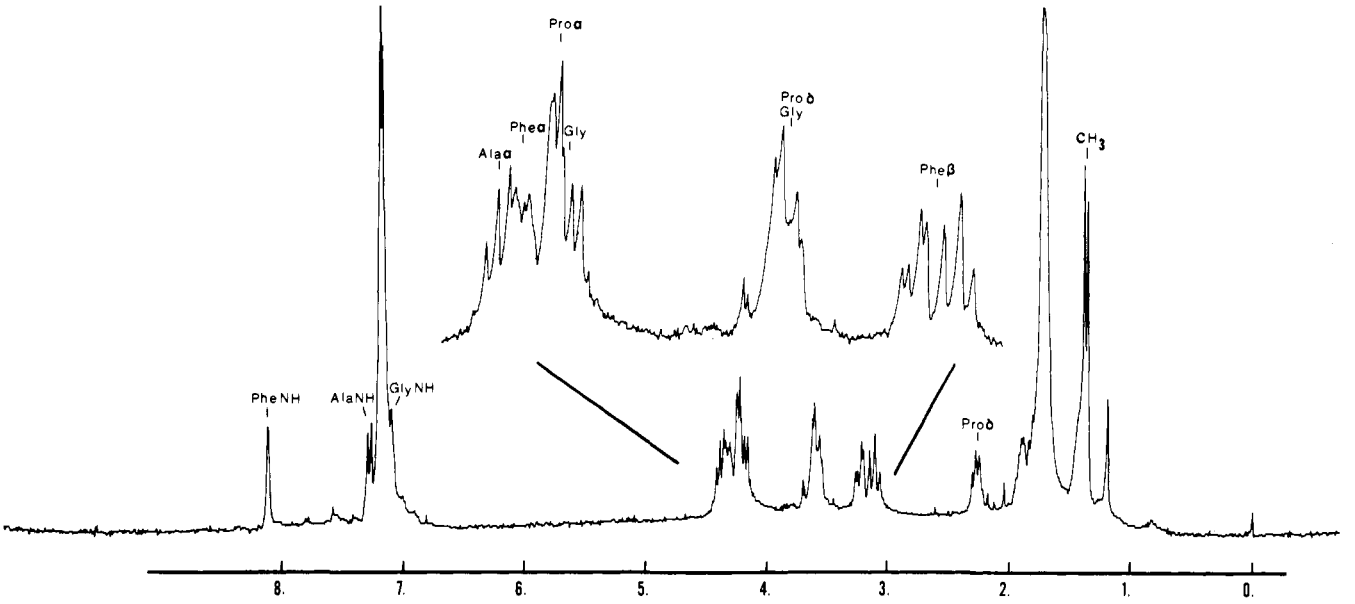


Figure 1. The 270-MHz ^1H NMR spectrum of uncomplexed c-(AGPhP)$_3$ in $CDCl_3$. Concentration 2×10^{-3} M. Temperature 25 °C. Lines at 1.2 and 1.8 ppm are from the solvent.

the excess of *p*-nitrophenol. Yield 2.66 g (1.96 mmol) of the dodecapeptide *p*-nitrophenyl ester VIII.

HCl·H-(L-Ala-Gly-D-Phe-L-Pro)$_3$-ONp (IX). Treatment of 2.6 g (1.92 mmol) of VIII in solution in ethyl acetate (60 mL) with HCl gas for 30 min at 0 °C yielded the dodecapeptide hydrochloride active ester IX which was isolated by evaporation of the solvent and purified by titrations with ether. Yield 2.23 g (1.8 mmol).

***cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ (X).** A solution of IX (2.33 g, 1.8 mmol) in 5 mL of DMF and 20 mL of $CHCl_3$ was added dropwise over a period of 6 h into 1.6 L of freshly distilled pyridine preheated to 75 °C. The reaction mixture was allowed to stir for 6 days at 60 °C. Evaporation of the solvent yielded a brown residue which was triturated with ether, taken up into 60 mL of methanol:water (1/1), and stirred for 2 h with Rexyn I-300 ion-exchange resin. Filtration of the resin and evaporation of the solvent yielded a white solid (1 g) which could not be crystallized. TLC in various solvent systems showed one main (80–90%) spot which was ninhydrin negative and was visualized by the chlorine/tolidine reagent.[27] This crude product was purified by using both high-performance liquid chromatography (HPLC) and gel filtration. A typical example of purification is the following: 80 mg of the crude product (X) was first purified by HPLC on Silica Gel 60[28] with use of a size C (3.8 × 43 cm) Lobar Merck column and a gradient of EtOAc and a mixture BAW (*n*-butyl alcohol:acetic acid:water) (75:10:25) as elutant (10 mL/2 min/tube, back pressure ca. 6 kg/cm^2). Peak detection was performed at 254 nM (ISCO UA-4, 0.2-cm cell). Fractions with 70–80% BAW in EtOAc containing the cyclic dodecapeptide were collected and lyophilized. Yield 53.8 mg. This product (ca. 98% pure) was further purified by gel filtration on Sephadex LH-20 (column 2.5 × 90 cm, UV monitor) with use of methanol as elutant. The main fraction concentrated to dryness yielded 40.8 mg of pure product and showed a single spot on TLC in various solvent systems. Amino acid analysis, after complete hydrolysis in 6 N HCl at 100 °C for 24 h, gave the following ratio: Ala (1.03) Gly (1.00), Phe (1.01), Pro (0.98).

NMR Experiments. Materials. CD_3CN (Bio-Rad) and $CDCl_3$ (Merck & Co.) were dried over molecular sieves before use. CD_3OD was purchased from Merck & Co., and methanol and acetone were from Fisher Scientific Co. Perchlorate and thiocyanate salts were dried in vacuo at 120 °C for at least 3 days.

Titration Studies in CD_3CN. For the highly acetonitrile soluble perchlorate salts of Ba^{2+}, Li^+, Na^+, and the thiocyanate salt of K^+, the titration studies were made by successively adding aliquots of a 50–100 mM salt solution in acetonitrile-d_3 to a CD_3CN solution of the cyclododecapeptide (0.5–4.0 mM) in the NMR tube. The titration was generally stopped after 3 equiv of salt had been added.

For the less soluble (in acetonitrile) salts of K^+, Rb^+, Tl^+, and Cs^+, the following procedure was used. An NMR sample containing the peptide in 400 μL of CD_3CN was prepared (concentration 0.5–2.0 mM). Separate vials, each containing an amount of salt corresponding to 0.2 equiv of the peptide, were prepared by taking an adequate volume of the salt stock acetonitrile solution and evaporating the solvent. After running the spectrum of the free peptide, the solution was transferred to the first vial. After thorough mixing, the solution was transferred back into the NMR tube and the NMR spectrum was run. The procedure was repeated until 3 equiv of salt had been added to the peptide.

Study of the Complexes. In CD_3CN. As the samples corresponding to the salt/peptide ratios 1/1 and 3/1 gave identical spectra (see further in text), the latter ones, which were the final ones obtained in our titration studies, were used for characterizing the complexes. In the particular cases of Ba^{2+}, K^+, and Tl^+ complexes, the complete set of experiments was repeated with samples at salt/peptide ratio 1/1, prepared by mixing equivalent amounts of the perchlorate salt to the peptide (concentration 1.0–2.0 mM).

In $CDCl_3$. Due to the low solubility of the perchlorate salts in this solvent, the complexes were prepared by adding a (4–5)-fold excess of the cation perchlorate salt to the peptide (1.0–2.0 mM) followed by filtration through glass wool into the NMR tube.

The 270-MHz proton NMR spectra were recorded on a Bruker HX-270 spectrometer equipped with a variable-temperature accessory. Spectra were obtained in the Fourier-transform mode and generally were the result of 100–400 accumulations. All the spectra in this paper are given in ppm from tetramethylsilane which was used as an internal reference.

Results

Uncomplexed *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$. A. In $CDCl_3$. Figure 1 shows the 270-MHz spectrum of a 2.0 mM solution of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ in $CDCl_3$ at 25 °C. All resonances correspond to one set of the amino acid residues L-Ala-Gly-D-Phe-L-Pro in the dodecapeptide indicating C_3 symmetry on the NMR time average for this molecule in this solvent. The assignments were made by using the double-resonance technique.

The methylene protons of the glycine are well separated and appear as the AM part of an AMX spin system (where X stands for the Gly NH proton) allowing easy determination of the two $J_{NH-C_\alpha H}$ coupling constants (4.6 and 7.3 Hz). The two other $J_{NH-C_\alpha H}$ coupling constants for Ala and Phe are 8.1 and 2.2 Hz, respectively. The δ-methylene protons of the proline show up as two well-separated patterns, one at 3.65 ppm and the other at 2.32 ppm. The latter one is located at more than 1.0 ppm higher field than the normal resonance of the δ-methylene proline protons in related proline containing dodecapeptides.[11,12,14]

This strong shielding of one of the δ-CH_2 proline protons in *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ must originate from the close proximity of that proton to the aromatic plane of the phenylalanine group.

When the temperature was decreased from 35 to −40 °C, the Ala and Gly NH lines were little affected (0.1×10^{-4} and 0.2×10^{-4} ppm/deg, respectively), while the Phe NH moved downfield by 0.5×10^{-4} ppm/deg. ppm/deg. Upon five fold dilution of the cyclododecapeptide in $CDCl_3$ (from 2.0 to 0.4 mM), no significant change was observed on the Ala and Gly NH chemical shifts, but

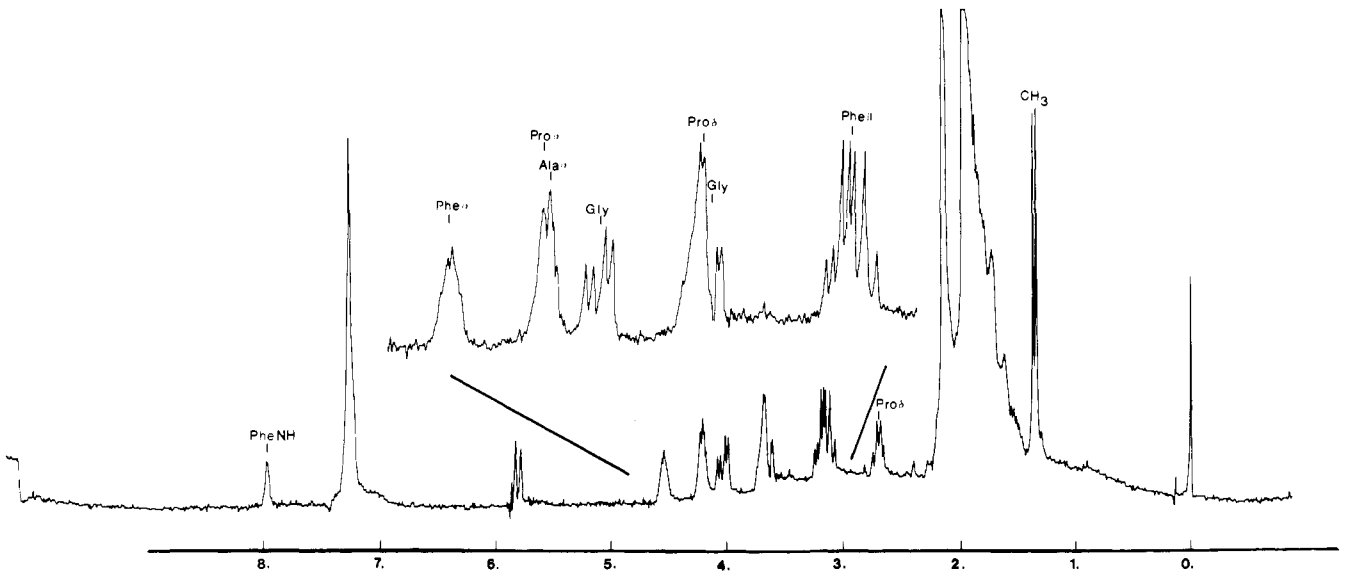


Figure 2. The 270-MHz ^{1}H NMR spectrum of uncomplexed c-(AGPhP)$_3$ in CD_3CN. Concentration 2×10^{-3} M. Temperature 25 °C.

Table I. Vicinal Coupling Constants[a] of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ and Its Ionic Complexes in CD_3CN[b]

	free	Ba^{2+}	K^+	Rb^+	Cs^+	Tl^+
$J_{NH-C_\alpha H}$ Ala	6.7	3.9	4.5	5.0	4.5	4.7
$J_{NH-C_\alpha H}$ Phe	3.9	4.1	4.0	3.7	3.7	3.6
$J_{NH-C_\alpha H_1}$ Gly	6.8	6.2	7.2	7.5	7.2	8.3
$J_{NH-C_\alpha H_2}$ Gly	5.0	6.1	5.2	5.0	5.0	4.8

[a] Given in Hz (±0.2 Hz). [b] Free peptide and complexes were at a concentration of 2×10^{-3} M. Temperature 25 °C.

the Phe NH moved upfield by 0.1 ppm. The dependence of the Phe NH chemical shift upon dilution and temperature variation strongly suggests that this particular NH is involved in intermolecular peptide–peptide interactions and is, therefore, located at the outside of the molecule. The Ala and Gly NH's, on the other hand, are less accessible to these intermolecular interactions, indicating that they are located in the inside of the molecule and may, therefore, be involved in intramolecular hydrogen bonds.

B. In CD_3CN. Figure 2 shows the whole 270-MHz spectrum of a 2.0 mM solution of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ in CD_3CN. The resonances of the free peptide correspond to a C_3 symmetric structure on the NMR time scale. The coupling constants are given in the first column of Table I.

As was observed in $CDCl_3$, one of the δ-methylene protons of proline is located at relatively high field as a result of being close to the aromatic ring of the phenylalanine group. The sharpness of the lines and the positions of the majority of them were not affected when the temperature of the sample was decreased from 45 to −25 °C. However, the high-field δ-methylene proline proton line moved further upfield (≃0.3 ppm) in this temperature range, suggesting a decrease on the average distance between this proton and the aromatic ring of the phenylalanine. The temperature dependence coefficients were 0.26×10^{-4} ppm/deg for Ala NH, 0.33×10^{-4} ppm/deg for Gly NH, and 0.28×10^{-4} ppm/deg for Phe NH (see Table II). It is difficult to draw any conclusions from these values with respect to the involvement of the NH's in any inter- or intramolecular interactions. Indeed, experiments on the ionic complexes of the peptide (which will be dealt with in a later section) as well as experiments on c-(VGGP)$_3$[14] have shown that NH temperature-dependence coefficients in CD_3CN may be as low as 0.1×10^{-4} ppm/deg and as high as 0.4×10^{-4} ppm/deg. The values of the NH chemical-shift changes, upon addition of the hydrogen bond acceptor solvents methanol and acetone to the acetonitrile solution of the peptide, are given in Table II. They seem to indicate some tendency for the Gly NH to be more solvent exposed than the Ala and Phe NH's. No significant change was detected in the lines corresponding to the protons of the backbone suggesting that the conformation (as seen in the NMR spectrum) is not modified upon solvent perturbation.

Table II. Dependence of Amide Proton Chemical Shifts in c-(AGPhP)$_3$ and Its Complexes in CD_3CN[a]

	free				Ba^{2+}			
	A	B	C	D	A	B	C	D
line								
Ala NH	2.6	5.2	2.2	3 min	0.6	4.3	1.3	>>3 h
Gly NH	3.3	20.4	5.4	<2 min	3.5	43.5	14.5	<2 min
Phe NH	2.8	4.6	2.2	3 min	1.3	3.8	1.2	>>3 h
	K^+				Tl^+			
	A	B	C	D	A	B	C	D
line								
Ala NH	0.8	1.2	1.4	>>>3 h	1.0	0.5	2.0	>>3 h
Gly NH	3.3	25.3	13.4	<2 min	3.5	27.8	12.7	<2 min
Phe NH	2.5	1.2	2.0	30 min	3.0	3.2	2.1	8 min

[a] A: Temperature coefficients given as 10^{-3} ppm/deg. In all cases amide protons moved upfield when the temperature increased. B and C: Solvent coefficients given as 10^{-3} ppm/% of methanol (B) or acetone (C) added to the acetonitrile solution (v/v) at 25 °C. In all cases the lines shifted downfield upon addition of either methanol or acetone. D: Halftime values of NH–ND exchange (min for minutes, h for hours). These values were qualitatively obtained by comparing the intensities of the NH lines at different times after addition of methanol-d_4. Inaccuracy is of the order of ±10%. All samples were at a 2×10^{-3} M peptide concentration. The complexes were at a 3/1 ratio of perchlorate salt/peptide.

Rapid NH–ND exchange occurred for the three NH's when 2.5% of methanol-d_4 was added to the peptide in CD_3CN. The exchange of the Gly NH was so fast that the signal had completely disappeared in the first spectrum taken approximately 1 min after addition of the deuterated methanol. On the other hand, the NH–ND exchange halftimes for the Ala and Phe NH's were larger ($\tau_{1/2}$ = 2–3 min). These values, however, are much smaller than the ones found for the exchange of some of the amide protons in the ionic complexes of the peptide (vide infra). The preceding results can be best interpreted by considering that the molecule undergoes conformational changes, during which the Ala and Phe amide protons may become temporarily exposed to the solvent resulting in an actual NH–ND exchange and a subsequent decrease in the intensities of the corresponding lines. The results also suggest that, on the average of the possible conformational states, the Ala and Phe NH's are less exposed to the solvent than the Gly NH. This interpretation is then also consistent with the relatively high temperature coefficients found for the amide protons. Indeed, depending on the conformational state of the molecule, they may be involved in various inter- and intramolecular interactions. Finally, the exchange between the different con-

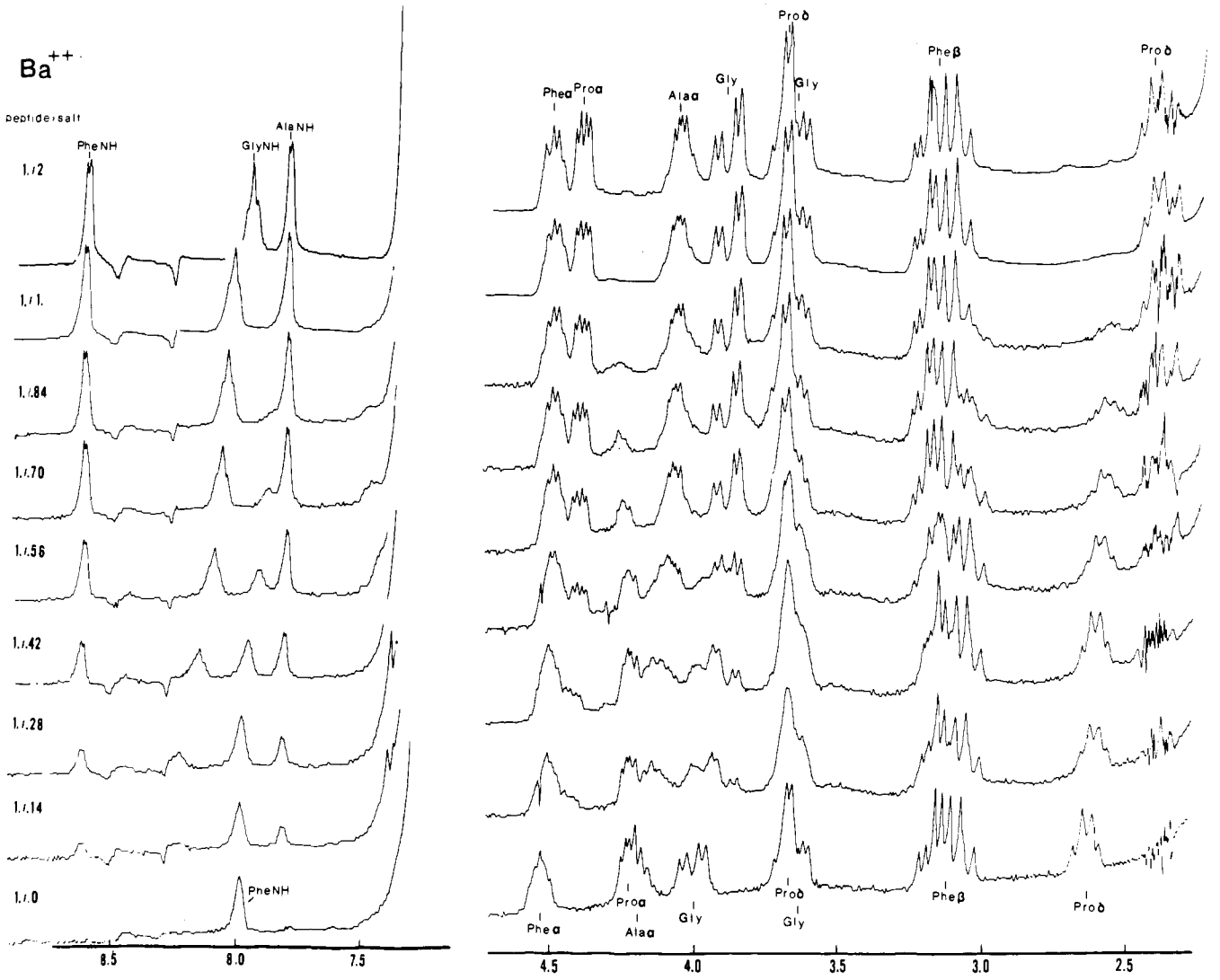


Figure 3. The low-field parts of the 270-MHz ^1H NMR spectra obtained during the titration of 2×10^{-3} M c-(AGPhP)$_3$ with barium percholate in CD_3CN. Temperature 25 °C. The spectral features at 8.5, 8.3, and 2.4 (partially) ppm are spectrometer artifacts.

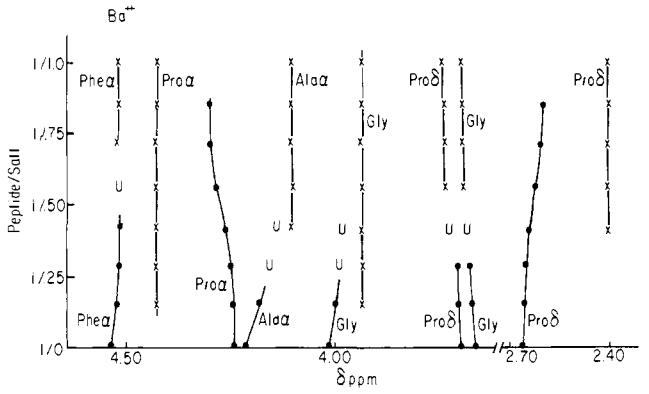


Figure 4. Changes in the line positions during the titration of c-(AGPhP)$_3$ with barium perchlorate in CD_3CN as shown in Figure 3. (●) Free peptide and 1/2 ion–peptide complex lines; (×) 1/1 ion–peptide complex lines; (u) line position could not be given unambiguously.

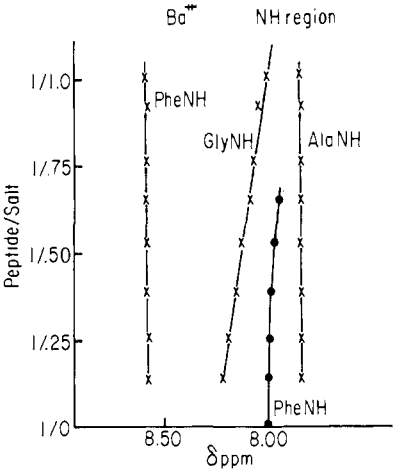


Figure 5. Changes in the amide proton line positions during the titration of c-(AGPhP)$_3$ with barium perchlorate in CD_3CN as shown in Figure 3. Symbols as in Figure 4.

formational states must be rapid on the NMR time scale, as only one set of lines is observed in the NMR spectrum. The energy barrier between the different conformational states must be very low, as there is essentially no line broadening when the temperature is decreased down to −25 °C.

Cation Complexes of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$. A. In CD_3CN. Substantial changes in the positions, widths, and intensities of the lines as well as in the spin–spin coupling constants are observed when the perchlorate salts of Ba^{2+}, K^+, Cs^+, Rb^+, Na^+, Li^+, and Tl^+ are added to the peptide in CD_3CN.

Ba^{2+} Titration. The titration of the cyclododecapeptide with the Ba perchlorate salt is shown in Figures 3, 4, and 5.

The different spectra may be interpreted as resulting from the superposition of two subspectra: one closely related to the free

peptide (as at the 1/0 peptide/salt ratio) and the other related to the ion–peptide complex obtained after addition of 1 equiv of salt to the peptide (as at the ratio 1/1 peptide/salt). On increasing the amount of salt, there is a decrease in the intensities of the lines corresponding to the first subspectrum and an increase in the intensities of the lines corresponding to the second subspectrum. The widths of the lines in both subspectra are very little affected during the titration. It is also immediately apparent that the positions of most of the lines corresponding to the first subspectrum are substantially changed during the process. This is particularly clear for Pro α, Ala α, and the high-field Pro δ, which are respectively located at 4.24, 4.17, and 2.65 ppm in the free peptide spectrum. On the other hand, the positions of all the lines (except Gly NH) corresponding to the second subspectrum are essentially unchanged upon salt titration. When 1 equiv of salt has been added to the peptide, this second subspectrum is the only one present. It is not modified by further addition of salt up to 3 equiv.

The spectral changes which are observed during the titration of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ with the Ba^{2+} perchlorate salt can be interpreted by considering the presence of at least two different peptide-related species in solution. The first subspectrum is related to the free peptide. The modification of the position of some of the lines of this subspectrum upon salt titration results from the ion–peptide complex formation. The second subspectrum is related to a 1/1 type ion/peptide complex. The only line in this subspectrum which has its position changed during salt titration is the Gly NH line, and this may be due to the interaction of the corresponding amide proton with the perchlorate anion. From the line widths at mid-titration, it was estimated that the rate of exchange is less than 5 s^{-1}.

As far as the methyl signal is concerned, its position and width were essentially unaffected during the titration. It always showed up as a well-resolved doublet with 7-Hz splitting. This is not surprising as the positions of the methyl signal in the free peptide and in the 1/1 ion–peptide complex are identical in the range of the experimental error. Therefore, even though the rate of exchange is very low between the two species, no broadening is expected to occur.

K^+, Rb^+, Cs^+ Titration. The titration with the thiocyanate or perchlorate salts of potassium gave essentially the same results, indicating that these two anions are similarly involved in the binding of the potassium ion to the cyclopeptide. In the potassium titration the lines can be classified in two main groups: The first group contains the Phe β and the Pro δ lines located at around 3.15 and 2.65 ppm, respectively. These lines are sharp and well resolved and are only very little affected during the titration. The second group contains all the other lines. They undergo substantial broadening, which is most clearly visible around mid-titration. Furthermore, as in the case of Ba^{2+}, they seem to relate to two subspectra, one closely related to the spectrum obtained for the free peptide (at the 1/0 peptide/salt ratio) and the other related to the spectrum obtained after 1 equiv of salt has been added to the peptide. After addition of 1 equiv of salt to the peptide, the second subspectrum is the only one present, and furthermore, it contains only sharp and well-resolved lines. Further addition of salt up to 3 equiv does not modify the spectrum.

The titration of *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ with the thiocyanate salt of potassium can be best interpreted by considering that there are two major peptide species present in the solution, one corresponding to the free peptide and the other corresponding to a 1/1 type ion–peptide complex. The rate of exchange between the two species is such that it turns out to be rapid for some of the sites of the peptide, resulting in a single set of well-resolved lines for each site (Phe β and Pro δ) and slow intermediate for some of the other sites resulting in two sets of broad lines for each site. From the broadening of the lines we calculated that the rate of exchange is of the order of 20–30 s^{-1}. The spectra obtained upon titration of c-(AGPhP)$_3$ with the perchlorate salts of Rb^+ and Cs^+ are essentially similar to those obtained with potassium thiocyanate.

Titration with Tl^+. The titration was performed with the perchlorate salt of thallium.

For most of the lines a gradual change in their positions was observed when the salt, up to 1 equiv, was added. No further modification was observed in the spectrum when more salt was added up to 3 equiv. The behavior of the lines corresponding to the Gly methylene protons is of particular interest. At the beginning of the titration both sets of lines move toward each other. When approximately 0.35 equiv of salt has been added, these methylene proton signals have the same chemical shifts: some broadening has occurred at this stage. Upon further addition of salt, these lines broaden dramatically and completely disappear between the 1/5 and 1/8 peptide/salt ratios. They finally reappear as well-resolved signals in the spectrum corresponding to the 1/1 peptide/salt ratio. These spectral changes which are observed during the titration of c-(AGPhP)$_3$ with the perchlorate salt of Tl can be best interpreted by considering the presence of at least two different peptide-related species in solution, the free peptide and the 1/1 ion–peptide complex. For most of the proton sites there is a rapid exchange on the NMR time scale between the two species resulting in sharp and well-resolved lines. The slightly different behavior observed for the lines corresponding to the methylene glycine protons can be best explained by considering that, at the beginning of the titration, the free peptide is in rapid equilibrium with another species (i.e., a 1/2 ion–peptide complex) and/or is affected by the presence of the perchlorate anion. As the process goes on further, the 1/1 ion–peptide complex starts forming. In this case, however, the exchange between the 1/1 ion–peptide complex and the free peptide and, eventually, the 1/2 ion–peptide complex is of the intermediate type on the NMR time scale as far as the Gly sites are concerned, resulting in an almost complete disappearance of the corresponding signals. Due to this extensive broadening of the lines, the rate of exchange between the 1/1 ion–peptide complex and the free peptide was estimated to be of the order of 100 Hz on the basis of the chemical-shift differences between the individual Gly CH_2 protons in these two compounds (0.45 and 0.52 ppm). As in all the previous cases, the fact that the spectrum contains sharp and well-resolved lines after 1 equiv of salt has been added and is not changed upon further addition of up to 3 equiv suggests that this spectrum is, indeed, characteristic of the 1/1 ion–peptide complex.

Titration with Na^+ and LI^+. For both ions, the largest changes in the line positions occur during addition of the first 0.5 equiv of salt. After that, small changes are still detectable, even when up to 3 equiv of salt are added. Throughout the titration process, only one set of sharp and well-resolved lines is observed. The preceding observations suggest that the major species formed is the 1/2 ion–peptide complex and that it is in rapid equilibrium on the NMR time scale with the free peptide. However, the fact that further changes occur in the line positions after 0.5 equiv of salt has been added suggests either a relatively low 1/2 ion–peptide formation constant or the presence of another species or both.

Spectral Characteristics of the Ion–Peptide Complexes in CD_3CN. Our titration studies have shown that for the cations Ba^{2+}, K^+, Rb^+, Cs^+, and Tl^+ addition of 1 equiv of salt to the peptide gives a spectrum characteristic of the 1/1 ion–peptide complex, while addition of 0.5 equiv of the Na^+ or Li^+ salts gives a spectrum mainly characteristic of the 1/2 ion–peptide complex.

In all these spectra the sharp and well-resolved lines correspond to one set of amino acid residues (L-Ala-Gly-D-Phe-L-Pro) in the dodecapeptide, indicating C_3 symmetry on the NMR time scale for the complexes in acetonitrile. The assignments were made by using the double-resonance technique. The relevant coupling constants are given in Table I.

It is noteworthy that in all the spectra one of the δ-methylene proline protons is shifted upfield, again indicating, as was the case for the free peptide in CD_3CN and $CDCl_3$, that this particular proton is in very close proximity to the aromatic plane of the phenylalanine group. Significant changes occur in the structure of the peptide upon complex formation. This is best seen from Figure 6, which shows the NH line positions for the free peptide and the different complexes.

In order to obtain more information about the conformations of the complexes, we used variable-temperature, solvent-pertur-

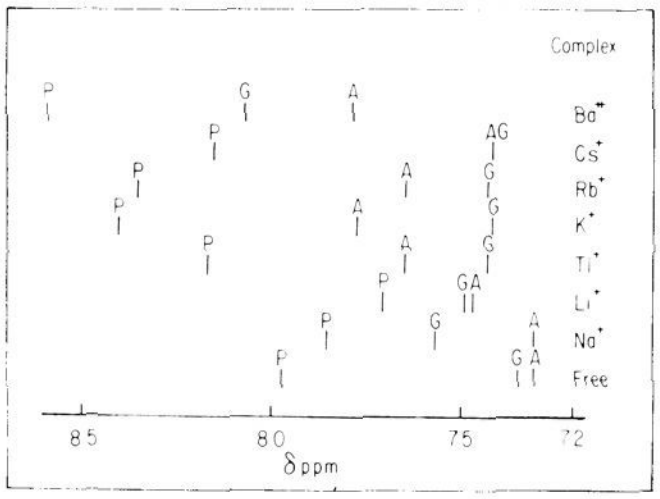


Figure 6. The amide regions of the 270-MHz ^1H NMR spectra of c-(AGPhP)$_3$ and its ion–peptide complexes (obtained after addition of 3 equiv of salt) in CD_3CN. Temperature 25 °C.

bation, and isotope-exchange techniques. However, these experiments were only performed on the well-defined 1/1 ion–peptide complexes with Ba^{2+}, Rb^+, Cs^+, K^+, and Tl^+. The chemical-shift and intensity changes of the amide proton lines of the various 1/1 ion–peptide complexes upon temperature variation, solvent perturbation, and isotope exchange are given in Table II.

In all cases, addition of the hydrogen bond acceptor solvents methanol and acetone, while inducing chemical shift changes on some of the NH lines, did not modify the lines corresponding to the protons of the backbone strongly suggesting that no change in conformation occurred during this process. It is immediately apparent from the temperature-dependence and solvent-perturbation coefficients that in these 1/1 ion–peptide complexes the Gly NH is solvent exposed and probably involved in intermolecular interactions. On the other hand, the low coefficients obtained for Ala NH indicate that this particular NH is shielded from the solvent and located in the inside of the molecules where it may be involved in intramolecular hydrogen bonding. The preceding conclusions were substantiated by the isotope exchange experiments which showed that upon addition of a small amount of methanol-d_4 (2.5%) there was an almost immediate loss of the intensities of the Gly NH lines resulting from NH–ND exchange, while intensities of the Ala NH lines remained virtually unaffected even after several hours.

The fate of the Phe NH is more problematic. In all the complexes, the line corresponding to this amide proton is essentially unaffected when the hydrogen bond acceptor solvents methanol and acetone are added to the acetonitrile solution. This behavior would normally indicate that this NH is solvent shielded and probably located in the inside of the molecule where it may be involved in hydrogen bonding. This seems, indeed, to be confirmed in the case of the Ba^{2+} complex, for which variable-temperature experiments showed a low-temperature coefficient (0.13×10^{-4} ppm/deg) for the Phe amide proton line. However, the temperature coefficient for this line increases gradually and significantly in the series Ba^{2+}, Cs^+, Rb^+, K^+, and Tl^+. In the latter complex, it is almost as high as the Gly NH temperature coefficient. On the other hand, isotope-exchange experiments also showed different rates of NH–ND exchange for the amide proton of the phenylalanine in the various complexes. When 2.5% methanol-d_4 was added to the acetonitrile solution of the Ba^{2+} complex, the intensity of the Phe NH line was very little affected. Only after 4–5 h was some loss of intensity detectable. In the K^+, Rb^+, and Cs^+ complexes, the loss in the intensities of the Phe NH lines was faster with half times of exchange in the range of 20–30 min, while in the Tl^+ complex the exchange is even faster with a half time of exchange of 7 min.

The results of the previous solvent-perturbation and isotope-exchange experiments can be best interpreted by considering that in all the complexes the Phe amide proton, although mainly shielded from the solvent, may become temporarily exposed to it. The particular conformational state of the peptide for which this happens must be of very short lifetime and is probably related to the structural state acquired by the peptide when it releases

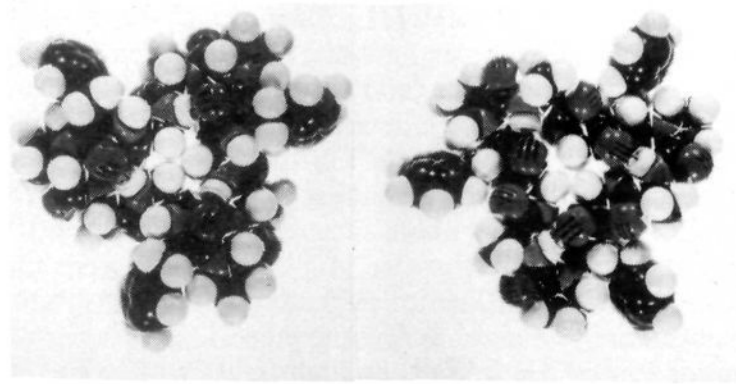


Figure 7. Photographs of a CPK model of the proposed conformation of c-(AGPhP)$_3$ in $CDCl_3$. The two views show the opposite faces of the molecule.

a cation before taking up another one. Assuming that the mechanism of cation release–capture is the same for all the 1/1 ion–peptide complexes, the various NH–ND exchange rates then reflect the actual lifetimes of the different complexes as follows: $Ba^{2+} > K^+ \simeq Cs^+ \simeq Rb^+ > Tl^+$.

B. In $CDCl_3$. Due to the very low solubility of the perchlorate salts in $CDCl_3$, no salt titration studies could be performed in this solvent. However, the spectra of cyclopeptide salt solutions obtained by mixing the peptide with an excess of salt followed by filtration showed that there is a binding of the different cations to the peptide. The extensive broadening of the lines, probably due to aggregation of the complex and/or to the presence of other peptide species in slow intermediate exchange on the NMR time scale, did not allow further study of the complexes.

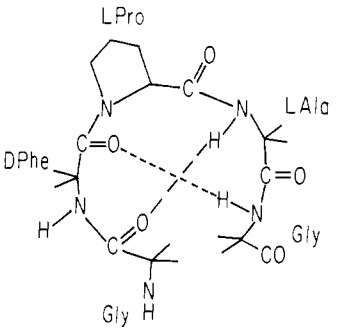
Discussion

Uncomplexed *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$. A. In $CDCl_3$. It is clear from Figure 1 that uncomplexed *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ in $CDCl_3$ has C_3 symmetry on the NMR time scale. The symmetry persisted down to -40 °C. The relatively large temperature coefficient for the Phe NH (0.55×10^{-4} ppm/deg) as compared to the smaller temperature coefficients for the Ala and Gly NH's (0.1 and 0.2×10^{-4} ppm/deg, respectively), as well as the chemical shift change of only the Phe NH line upon solvent dilution, suggests that the Phe NH is involved in peptide–peptide intermolecular interactions and is, therefore, located at the outside of the molecule. On the other hand, the Ala and Gly NH's are located at the inside of the molecule and may, therefore, be involved in intramolecular hydrogen bonding. From the substantial upfield shift of one of the δ-methylene protons of the proline group, it was concluded that the aromatic ring of the phenylalanine group must be in close contact with the proline ring. The only way to bring these two groups close to each other is by having the D-Phe-L-Pro bond trans.

A Corey–Pauling–Koltun (CPK) model was used to construct possible conformations consistent with all the available data for c-(AGPhP)$_3$. Possible angles were deduced from the $J_{NH-C_\alpha H}$ coupling constants.[29] The only conformation found which was satisfactory has the following approximate dihedral angles:

	L-Ala	Gly	D-Phe	L-Pro
ϕ	−145	−90	+45	−60
ψ	+30	+20	+180	20

The ψ values were estimated from both the CPK and Kendrew models of the peptide. Photographs of the model are shown in Figure 7: it is a propeller structure with the glycine methylenes located in the center. There are two intramolecular hydrogen bonds in each repeat unit, one (1 ← 4) between the Gly NH and the preceding Phe CO and the other (1 ← 4) between the Ala NH and the preceding Gly CO as shown in structure A. This conformation is very similar to that of c-(VGGP)$_3$ in CD_2Cl_2 and $CDCl_3$. Such a conformation has not been observed for valinomycin or its synthetic analogues PV and PVAV. In the preceding paper,[14] it was proposed that this unusual conformation results from the presence of additional amide protons as compared to valinomycin. However, such an NH is also present in PVAV, and the resulting basket structure of this peptide in $CDCl_3$ is more



closely related to the bracelet structure of valinomycin than to the propeller structure of c-(AGPhP)$_3$ or c-(VGGP)$_3$. In fact, the latter structure occurs because both the latter peptides contain at least one glycine per peptide unit. Inspection of the c-(AGPhP)$_3$ as well as the c-(VGGP)$_3$ model shows that only methylene groups of glycines can occupy the center of these structures. The constraints imposed by any other bulkier group would probably disrupt this very compact structure.

B. In CD_3CN. The results of our temperature experiments and solvent dependence studies as well as NH–ND exchange experiments strongly suggest that the molecule exists in various conformational states which are in rapid equilibrium on the NMR time scale. The resulting average conformation has C_3 symmetry on the NMR time scale and is probably related to the conformation *C* of valinomycin (which occurs in polar solvents), which is devoid of intramolecular hydrogen bonds. Very similar results were obtained for c-(VGGP)$_3$ in the same solvent.

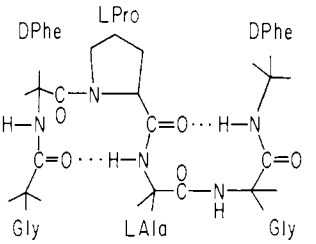
Cation Complexes. A. In CD_3CN. From our titration studies, we can classify the cations studied in two main groups. The first group contains Ba^{2+}, K^+, Cs^+, Rb^+, and Tl^+. For all these ions, it was observed that, after addition of 1 equiv of salt to the peptide, the spectrum was characteristic of a 1/1 ion–peptide complex. The exchange between the latter species and the free peptide covered the complete range between slow and rapid on the NMR time scale. In fact, from the spectra obtained at mid-titration, rates of exchange could be qualitatively appreciated and the following sequence of lifetimes of the complexes was found: $Ba^{2+} > K^+ \simeq Rb^+ \simeq Cs^+ > Tl^+$. The second group contains Na^+ and Li^+. For these ions, the titration curves indicate the formation of a 1/2 ion–peptide complex, which is the major complex present after 0.5 equiv of salt has been added.

The 1/1 ion–peptide complexes were extensively investigated by using solvent-perturbation, temperature-variation, and isotope-exchange techniques. These studies showed that in these complexes the Gly NH is solvent exposed, while the Ala and Phe NH's are solvent shielded. Possible ϕ angles were calculated from the $J_{NH-C_\alpha H}$ with use of Bystrov's equation.[29] CPK models were used to construct possible conformations consistent with all the data for the cation complexes of c-(AGPhP)$_3$ with Ba^{2+}, K^+, Rb^+, Cs^+, and Tl^+.

The conformation of the Ba^{2+} complex has the following approximate dihedral angles:

	L-Ala	Gly	D-Phe	L-Pro
ϕ	−64	77	65	−60
ψ	120	0	−120	−70

This conformation has two (1 ← 4) intramolecular hydrogen bonds per repeat unit L-Ala-Gly-D-Phe-L-Pro, one between the Ala NH and Gly CO and the other between the Phe NH and Pro CO as shown in structure B. The proposed conformation is closely related to the bracelet structure of the valinomycin–K^+ complex and the PV, PVAV, and c-(VGGP)$_3$ cation complexes. Photographs of the model corresponding to this Ba^{2+} complex conformation of c-(AGPhP)$_3$ are shown in Figure 8. The same basic structure is found for the other complexes of c-(AGPhP)$_3$ with K^+ (Rb^+, Cs^+) and Tl^+. Note, however, that, unlike the $J_{NH-C_\alpha H}$ coupling constants of Ala and Phe, which have similar values throughout the complex series, those related to the glycine group show important differences (see Table I). It is interesting that



the variation of the glycine coupling constants correlates well with the variation of the temperature coefficients of the Phe amide proton in the same series.

On the basis of this observation and the results of our titration studies, we propose the following model for the c-(AGPhP)$_3$ complex: The Ba^{2+} complex is expected to have a very rigid structure as a result of the strong interaction between the non-hydrogen-bonded carbonyl oxygens and the double-charged cation. Consequently, the structure will be very little affected upon temperature variation and this seems reflected in the absence of any change in the line position of the CH_α protons of the backbone as well as in the low-temperature coefficients of the Phe and Ala hydrogen-bonded amide protons. On the other hand, the weaker binding of singly charged cations to c-(AGPhP)$_3$ results in a much less stable—and probably more open—structure of the complexes, as reflected in the Gly coupling constants. These complexes will undergo larger structural deformations than the Ba complex. This is indeed confirmed by the relatively high temperature coefficients of the Phe amide protons, as well as the chemical-shift changes of the backbone CH protons upon temperature variation. Noticeable is the fact that the temperature coefficients of the Ala amide protons are low in all the cation complexes.

In our final model picture, we would therefore suggest that the complexes are made up of two segments: the Gly-D-Phe-L-Pro-L-Ala-NH segment, which is very rigid and little affected upon temperature variation and which keeps essentially the same structure throughout the complex series, and the L-Pro-L-Ala-Gly-D-Phe-NH segment, which is more flexible and more susceptible to temperature variation and which is structurally variable in the different complexes. As a result of the greater flexibility of the latter fragment, the bracelet is expected to open most easily from the L-Pro side. This mode of cation release was already proposed in the case of PVAV[12] (which is closely related to c-(AGPhP)$_3$) on the basis of the difference between the complex and the free peptide structures. Our isotope-exchange experiments further substantiate this mechanism. Indeed, we know that in the 1/1 ion–peptide complexes the Phe NH is not exposed to the solvent. The fact that, in going from the Ba^{2+} complex to the K^+ (Rb^+, Cs^+) complex and finally to the Tl^+ complex, the proton of the Phe NH exchanges against deuterium at different rates shows not only that there is some process by which this particular proton becomes momentarily exposed to the solvent but also that the process occurs more often for the Tl^+ complex than for the K^+ (Rb^+, Cs^+) complex and finally for the Ba^{2+} complex. The only plausible process, as depicted in Figure 9, is, in fact, the cation release–capture process during which the cyclopeptide may adopt a temporary conformation which may eventually be closely related to the basket structure proposed for PVAV.[12] Substantiation of the mechanism is given by the fact that the lifetimes of the various complexes—which are directly related to the frequency of the cation release–capture process—correlate very well with the relative rates of NH–ND exchange for the Phe NH in these complexes.

B. In $CDCl_3$. Although it is clear that cation binding occurs in this solvent, no extensive studies could be performed because the lines were generally too broad to allow obtaining accurate coupling constant values and because it was not clear whether or not the final spectrum could be related to a unique complex species.

Conclusions

The proton NMR data presented here indicate that the uncomplexed *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ in $CDCl_3$ has a symmetric, propeller structure with six (1 ← 4) intramolecular hy-

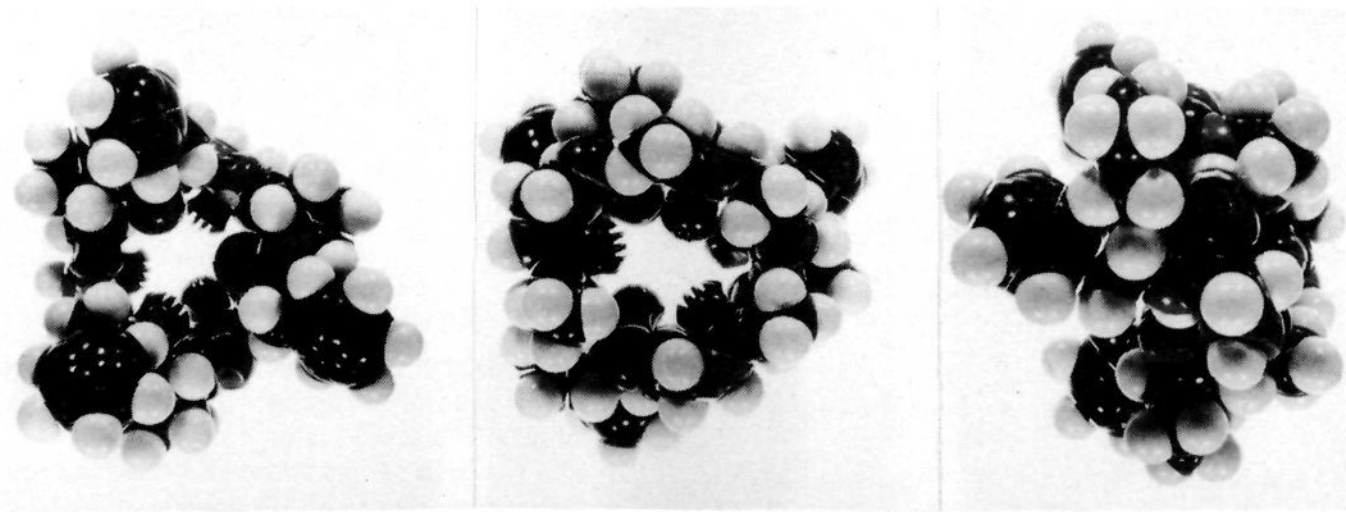
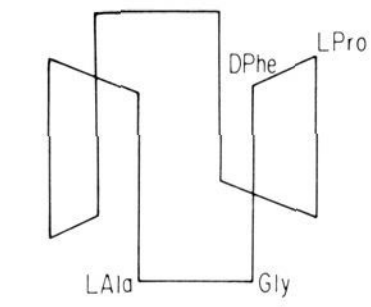


Figure 8. Photographs of a CPK model of the proposed conformation of the c-$(AGPhP)_3$–Ba^{2+} complex in CD_3CN. The left and middle views show opposite faces of the molecule. The right view is a side view showing the two (1 ← 4) hydrogen bonds (see structure B in text).



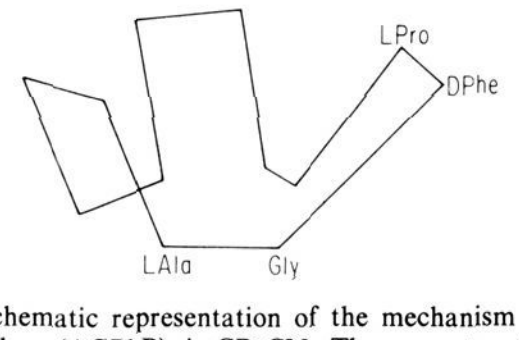


Figure 9. Schematic representation of the mechanism of cation release–capture by c-$(AGPhP)_3$ in CD_3CN. The upper structure represents the ion–peptide complex in the bracelet form. The lower structure represents one possible conformational state of c-$(AGPhP)_3$ in the uncomplexed form. It should be kept in mind that the latter structure, which is actually related to the basket structure of PVAV,[12] is a model which is only supported by the experimental evidence that the Phe NH–CO Pro hydrogen bond is probably broken during the cation release–capture process (see further explanation in text).

drogen bonds, similar to the structure of free c-$(VGGP)_3$ but different from the structures of valinomycin, PV, and PVAV in this solvent. Unlike c-$(VGGP)_3$ the peptide is able to bind various cations in this solvent. However, the spectra were broad and did not allow an extensive study of the complexes.

In CD_3CN uncomplexed *cyclo*(L-Ala-Gly-D-Phe-L-Pro)$_3$ probably exists as a mixture of several conformers in rapid exchange on the NMR time scale. Titration studies of the cyclopeptide with different salts showed that the cations can be classified in two main groups, one containing Ba^{2+}, K^+, Rb^+, Cs^+, Tl^+, for all of which the 1/1 cation–peptide complex formation was highly favored, and the other containing Na^+ and Li^+, for which the 1/2 ion–peptide complex formation was predominant. These titrations also revealed that the lifetimes of the various 1/1 cation–peptide complexes are as follows: $Ba^{2+} > K^+ \simeq Rb^+ \simeq Cs^+ > Tl^+$.

The conformations proposed for the 1/1 ion–peptide complexes are of the bracelet structure type similar to the valinomycin–K^+ complex. It was found that the L-Pro-CO-L-Ala-Gly-D-Phe NH segment of the complex is more susceptible to structural changes than the Gly CO-D-Phe-L-Pro-L-Ala NH segment. Furthermore, the relative susceptibility of the former fragment to structural changes increases in going from the Ba^{2+} complex to the K^+ (Rb^+, Cs^+) complex and finally to the Tl^+ complex. Also owing to the greater susceptibility to structural changes, the bracelet would be expected to open most easily from the Pro side. Isotope experiments gave evidence of a cation release–capture mechanism, the frequency of which was variable in the complex series and related to the lifetimes of the complexes.

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