Conformations of an Ion-Binding Cyclic Peptide Analogue of Valinomycin, *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$†

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ABSTRACT: A 270-MHz ^{1}H nuclear magnetic resonance investigation of an ion-binding cyclic peptide analogue of valinomycin, *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$, and its cation complexes is reported. In CD_2Cl_2 and $CDCl_3$, the peptide is proposed to occur in a C_3-symmetric conformer with the N–H's of all six glycine residues intramolecularly hydrogen bonded. This conformation is different from the familiar valinomycin bracelet structure and lacks any "cavity". Cations do not bind, or bind only weakly, to the peptide in these solvents. Uncomplexed *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$ in acetonitrile appears to be averaging among several conformations with no evidence found for any preferred intramolecular hydrogen bonds. The strong 1:1 complexes of *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$ with K^+ and Ba^{2+} in acetonitrile are structurally analogous to the bracelet conformation of valinomycin and involve the N–H's of the Val residues and of the Gly's preceding Pro in intramolecular hydrogen bonding. Tl^+ was also found to form strong 1:1 complexes with the dodecapeptide.

In recent years there has been considerable interest in the structure and conformation of various naturally occurring and synthetic cyclic peptides and peptide antibiotics which are capable of binding cations (Ovchinnikov et al., 1974; Ovchinnikov & Ivanov, 1975; Ivanov, 1973; Weiland, 1973; Ivanov et al., 1973; Madison et al., 1974; Prestegard & Chan, 1969; Eisenman et al., 1976; Deber et al., 1977; Sugiwara et al., 1976). Extensive studies have been carried out to understand the molecular basis for the ion transport in biological membranes stimulated by small macrocyclic antibiotics such as valinomycin (Ovchinnikov & Ivanov, 1975). Valinomycin, a cyclic depsipeptide with the sequence (L-Val-D-HyIv-D-Val-L-Lac)$_3$, is known to increase cation permeabilities in biological membranes (Pressman, 1968; Tosteson et al., 1967). Nuclear magnetic resonance (NMR) studies of valinomycin and its cation complexes (Haynes et al., 1969; Ivanov et al., 1969; Urry & Ohnishi, 1970; Ohnishi & Urry, 1969; Ohnishi et al., 1972; Grell et al., 1973; Patel & Tonelli, 1973; Davis & Tosteson, 1975) have shown that ion selectivity of this compound depends not only on the nature of ligands but also on the conformational state of the whole molecule. The conformation of free valinomycin is highly solvent dependent, whereas a cation complex, namely, valinomycin·K^+, is only very weakly solvent dependent and has a "bracelet" structure (Patel & Tonelli, 1973; Ovchinnikov et al., 1974).

In order to understand in more detail the relationship between conformation and the selective cation complexation and transport of these ionophores, it is useful to study a number of synthetic analogues of valinomycin. The proton magnetic resonance spectrum of one such analogue of valinomycin, *cyclo*(L-Val-D-Pro-D-Val-L-Pro)$_3$ (Gisin & Merrifield, 1972), has been reported recently (Davis et al., 1976); this compound binds cations, and its K^+ complex is isostructural with the valinomycin·K^+ complex. It is also reported that *cyclo*(L-Val-D-Pro-D-Val-L-Pro)$_3$ forms stable complexes with Li^+ and Na^+ ions, unlike valinomycin.

Another synthetic analogue of valinomycin, *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$ [*cyclo*(VGGP)$_3$],[1] synthesized in our laboratory (Pease, 1975; Baron et al., 1977), has been investigated using ^{1}H and ^{13}C NMR spectroscopy. In this compound Gly residues replace the two D residues of valinomycin, which may result in greater conformational freedom for the molecule. It should be noted that this cyclic peptide has only amino acids which occur in proteins and thus may serve as a model for certain portions of ion-binding membrane proteins. The decreased number of bulky side chains in this cyclic peptide relative to valinomycin and the earlier analogue may be expected to lead to greater access of complexed ions to solvent. An investigation of *cyclo*(VGGP)$_3$, using circular dichroism (Baron et al., 1977), showed that this peptide forms stable complexes with alkali metal and alkaline earth metals and that the stabilities of the complexes are affected by both size and charges of the cations. It was shown that large cations (K^+, Ca^{2+}, and Ba^{2+}) form the most stable 1:1 peptide–cation complexes and that of these the divalent ions bind more strongly by a large factor. Binding constant data in acetonitrile (CH_3CN) showed that the stabilities of the 1:1 peptide–cation complex of *cyclo*(VGGP)$_3$ followed the order $Ba^{2+} > Ca^{2+} > K^+ > Mg^{2+} > Li^+ > Na^+$.

In the present paper we report the results of a ^{1}H NMR study of the solution conformation of free *cyclo*(VGGP)$_3$ and its cation complexes. A new type of conformation containing six 1←4 hydrogen bonds has been observed for the free peptide in $CDCl_3$ and CD_2Cl_2. The *cyclo*(VGGP)$_3$ complexes with K^+, Ba^{2+}, and Tl^+,[2] however, are closely analogous in con-

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[1] Abbreviations used: *cyclo*(VGGP)$_3$, *cyclo*(L-Val-Gly-Gly-L-Pro)$_3$; CPK, Corey–Pauling–Koltun.

[2] The NMR results obtained for 1:1 complexes of Ca^{2+} with *cyclo*(VGGP)$_3$ suggested that it formed less stable complexes than K^+, Tl^+, or Ba^{2+}, and this is in contradiction to the binding constant data reported by Baron et al. (1977). Since calcium perchlorate is highly hygroscopic, it is possible that the ions were hydrated to differing extents in the CD and NMR studies. As a resuslt, potential problems arose in analyzing the NMR data, and conformational interpretations of the Ca^{2+} data are not presented herein.

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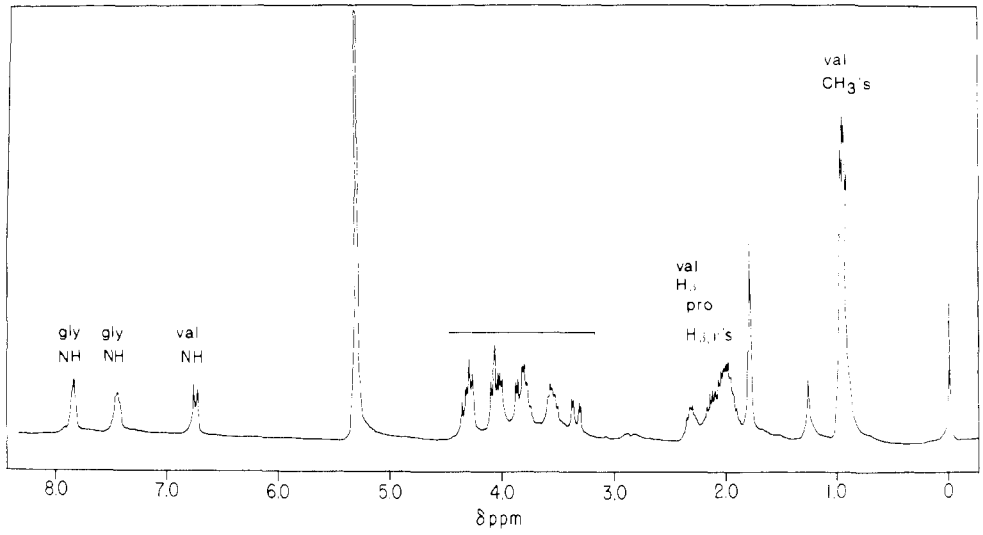


FIGURE 1: The 270-MHz ^1H NMR spectrum of uncomplexed *cyclo*(VGGP)$_3$ in CD_2Cl_2. Peptide concentration was 5×10^{-3} M. Sharp lines at 1.8 and 5.4 ppm are from solvent. The bracketed region is shown expanded in Figure 2. Temperature, 28 °C.

formation to the valinomycin·K^+. ^{13}C NMR results will be presented separately.

Experimental Procedures

The synthesis of *cyclo*(VGGP)$_3$ has been reported (Pease, 1975; Baron et al., 1977). Solutions of complexes were prepared by adding equimolar amounts of the perchlorate salts of the various cations (ions investigated in the present study are Ba^{2+}, K^+, Tl^+, Mg^{2+}, Li^{2+}, and Na^+) to solutions of *cyclo*(VGGP)$_3$ in deuterioacetonitrile (CD_3CN). *cyclo*-(VGGP)$_3$ has low solubility in CD_3CN compared to other nonpolar solvents. The concentration of the peptide was in the range of (1–1.5) $\times 10^{-3}$ M for the different complexes. Prior to use, the perchlorate salts of the various cations were dried in vacuo at 120 °C. The use of perchlorate salts reduces potential NH–anion and cation–anion interactions. CD_3CN (Thompson Packard Co.) was used as the solvent for studies of the cation complexes, as, besides its lipophilic character, a strong cation–peptide interaction relative to weak solvent–cation interaction is expected in this solvent (Deber et al., 1977). In addition, the data on binding constants (Baron et al., 1977) of *cyclo*(VGGP)$_3$·cation complexes were obtained using this solvent.

The 270-MHz proton NMR spectra were recorded using a Bruker HX-270 spectrometer equipped with a variable temperature accessory. Spectra were obtained in the Fourier transform mode and generally were a result of 500–1000 accumulations.

Results

Uncomplexed cyclo(VGGP)$_3$. Figures 1 and 2 show the 270-MHz ^1H NMR spectrum of *cyclo*(VGGP)$_3$ in CD_2Cl_2; essentially identical spectra were obtained in $CDCl_3$. All resonances correspond to one set of amino acid residues, Val-Gly-Gly-Pro in the dodecapeptide, indicating C_3 symmetry in the NMR time average for this molecule in these solvents. The Val NH line is a clear doublet at 6.78 ppm with separation of 8.5 Hz, and the two Gly NH's appear as a broad doublet at 7.47 ppm and as a pseudotriplet at 7.87 ppm. The methylene protons of both glycines appear as AB portions of ABX spin systems with a total of 16 lines (two doublets of doublets, with further splitting by the NH's). All assignments were made by double-resonance experiments. The chemical shifts and spin–spin coupling constants which could be measured accurately and unambiguously are listed in Table I. Figure 3 shows the temperature dependence of the NH chemical shifts in this solvent. The temperature coefficient of the chemical shift for the Val NH line was 5.8×10^{-3} ppm/deg, and for each of the two Gly NH's, it was 2.5×10^{-3} ppm/deg. In nonpolar, weakly interacting solvents, such as CD_2Cl_2 and CD_3CN, temperature dependences of NH chemical shifts are unlikely to be a result of perturbations of the weak solvent–peptide hydrogen bonding. Rather, at concentrations sufficiently high to favor peptide–peptide interactions, temperature variations perturb the peptide–peptide

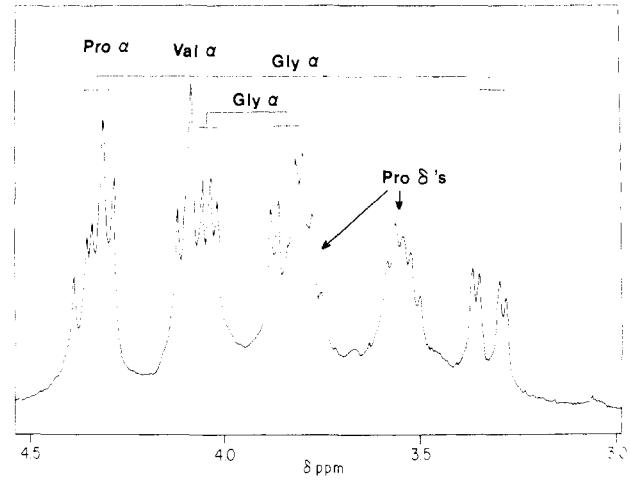


FIGURE 2: The α-CH region of the 270-MHz ^1H NMR spectrum of uncomplexed *cyclo*(VGGP)$_3$ in CD_2Cl_2. Conditions as indicated in Figure 1.

Table I: Proton Chemical Shifts and Vicinal Coupling Constants for *cyclo*(Val-Gly-Gly-Pro)$_3$ in CD_2Cl_2[a]

resi-due	chemical shift (ppm)[b]						J (Hz)		
	NH	H_α	H_β	H_γ	H_δ	CH_3	$J_{NH-C_\alpha H}$	$J_{HH'}$	$J_{\alpha\beta}$
Val	6.78	4.1	2.1			0.95, 0.98	8.4		8.0
Gly	7.47	3.36, 4.34					4.2, 4.2	−18.6	
Gly	7.87	3.86, 4.08					4.6, 4.6	−17.2	
Pro		4.3	2.3	2.0	3.6				

[a] Concentration = 5×10^{-3} M in CD_2Cl_2. [b] In parts per million from Me_4Si.

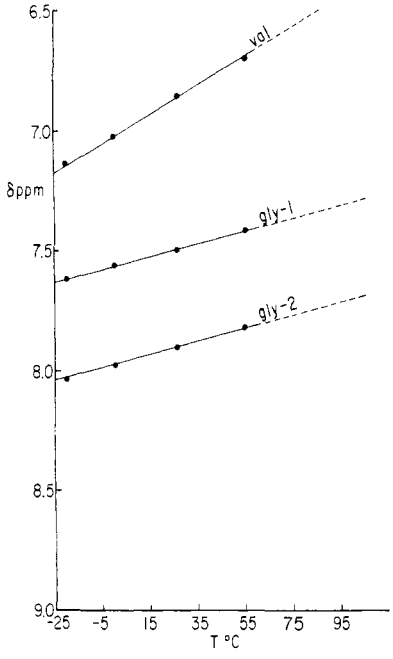


FIGURE 3: The temperature dependence of chemical shift for the amide resonances of free *cyclo*(VGGP)$_3$ in CD_2Cl_2. $\Delta\delta/\Delta T$ for Val NH, 5.8×10^{-3} ppm/deg; for both Gly NH's, 2.5×10^{-3} ppm/deg.

intermolecular hydrogen bonding and, hence, the NH chemical shifts. It was confirmed herein by the concentration dependence of NH resonances that peptide–peptide hydrogen bonding was occurring in these solvents in the range of concentrations studied. Therefore, the temperature dependences' data can be interpreted to indicate that in this solvent the Val NH is more accessible to intermolecular interactions than are the two Gly NH's which may be intramolecularly hydrogen bonded. The addition of a small amount of methanol (CD_3OH), a potential hydrogen-bond acceptor, to the solution of chloroform (up to $CDCl_3$–CD_3OH 90:10 v/v) perturbs the Val NH resonance to a greater extent than the two Gly NH resonances. The Val NH is shifted downfield by 0.72 ppm, and the two Gly's are shifted downfield equally by 0.17 ppm. This result also supports the observation from the temperature dependences of the chemical shifts that Val NH is more exposed and that the two Gly's are sequestered from intermolecular interactions—most likely through intramolecular hydrogen bonding.

In the polar solvent, Me_2SO-d_6, in addition to the major resonances corresponding to one Val-Gly-Gly-Pro unit, additional resonances of smaller intensity were observed. This observation indicates the existence of an additional conformer separated by a high-energy barrier from the major species. No detailed investigations were carried out to understand the nature of these major and minor conformations of *cyclo*(VGGP)$_3$ in Me_2SO-d_6.

In CD_3CN the resonances of the "free" uncomplexed peptide also corresponded to a C_3-symmetric structure (on the NMR time scale). The lines in the α region (see Figure 5) were greatly overlapping, broad, and difficult to assign. This could be due, possibly, to interconversion among different conformational states of *cyclo*(VGGP)$_3$, indicating greater conformational freedom of the peptide in this solvent. In temperature studies, all three N–H resonances displayed the same intermediate temperature dependence ($\Delta\delta/\Delta T = 3.0 \times 10^{-3}$ ppm/deg), and no marked changes were observed in the upfield region.

Cation Complexes of cyclo(VGGP)$_3$. Appreciable changes in the positions of the lines and spin–spin coupling constants

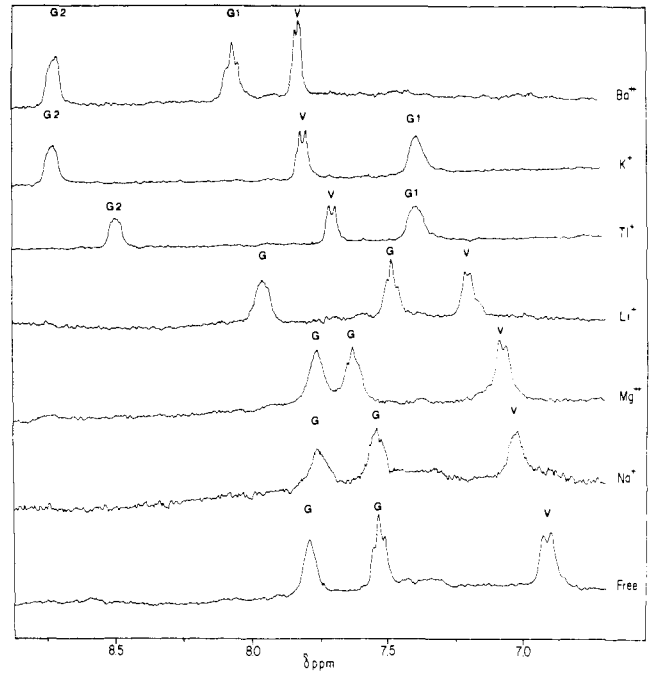


FIGURE 4: The amide region of the 270-MHz ^{1}H NMR spectra of *cyclo*(VGGP)$_3$ and its 1:1 complexes. Ba^{2+}, K^+, Tl^+, Li^+, Mg^{2+}, and Na^+ ions in CD_3CN. Temperature, 28 °C.

for the different cation complexes compared to free *cyclo*(VGGP)$_3$ were observed in CD_3CN. Figure 4 shows the amide region of the 270-MHz ^{1}H NMR spectra of free *cyclo*(VGGP)$_3$ and equimolar solutions of peptide with Ba^{2+}, K^+, Tl^+, Li^+, Mg^{2+}, and Na^+ ions. An examination of Figure 4 shows that for all samples there are only three NH lines, corresponding to one Val-Gly-Gly-Pro unit in solutions of the various cation complexes, indicating the presence of a C_3-symmetric conformer, at least on the NMR time scale. In the case of the K^+ complex, there is a large downfield shift of $\sim$1 ppm for the NH's of Gly-2[3] and Val residues and a small upfield shift of 0.15 ppm for the Gly-1 NH upon binding.

With the Ba^{2+} complex, in addition to the large downfield shifts of $\sim$1 ppm for the Val and Gly-2 NH lines, the Gly-1 NH was also shifted downfield by 0.5 ppm, as compared to free *cyclo*(VGGP)$_3$. In the Tl^+ solution the shifts were similar to those of the K^+ complex. For the Li^+ complex the NH's of Gly-2 and Val were shifted downfield by a small extent (0.12 and 0.24 ppm), and a small upfield shift of 0.06 ppm was observed for Gly-1 NH. The NH chemical shifts for Mg^{2+} and Na^+ solutions were practically the same as for free *cyclo*(VGGP)$_3$.

The α region of the spectra for the free *cyclo*(VGGP)$_3$ and its complexes with Ba^{2+}, K^+, and Tl^+ is shown in Figure 5. The α region of the spectra of Na^+ and Mg^{2+} complexes were not well resolved and greatly overlapped, similar to that in free *cyclo*(VGGP)$_3$. The spectrum of the Li^+ complex was well defined but not so well resolved as those of the Ba^{2+}, K^+, and Tl^+ complexes. All the resonances were again assigned by spin-decoupling studies. The Gly CH_2 protons form the AB

[3] Glycine resonances were initially tentatively assigned to Gly-1 or Gly-2 in the *cyclo*(VGGP)$_3$ sequence. This assignment was then considered in terms of fit of the data (temperature and solvent dependences of NH resonances, coupling constants) to possible conformational models. The assignment used throughout is a result of this process of interpretation. It is clear that no unambiguous determination of which resonances are attributable to a particular glycine in the sequence is possible without isotopic substitution. Therefore, none of the conformational conclusions arrived at herein depends on the specific glycine assignment.

Table II: Proton Chemical Shifts and Coupling Constants for Free and Cation Complexes[a] of *cyclo*(Val-Gly-Gly-Pro)$_3$ in Acetonitrile[b]

ion	dia-meter (Å)	chemical shift (ppm)												J (Hz)				
		Val				Gly-1		Gly-2		Pro				Val		Gly-1	Gly-2	
		NH	H_α	H_β	CH_3	NH	H_α	NH	H_α	H_α	H_β	H_γ	H_δ	$J_{NH-C_\alpha H}$	$J_{\alpha\beta}$	$J_{NH-C_\alpha H}$	$J_{NH-C_\alpha H}$	
none		6.9				7.55		7.8		4.2								
K^+ (1.5×10^{-3} M)	2.66	7.88	3.48	~2.2	1.03, 0.96	7.4	~4.1, 3.41	8.77	~4.05 3.26	4.26	*c*	*c*	3.6	6.0	10.7	7.7, 4.8	3.0, 7.0	
Tl^+ (1.07×10^{-3} M)	2.94	7.71	3.5	~2.2	1.03, 0.95	7.4	4.25, 3.43	8.5	4.03, 3.29	4.32	*c*	*c*	3.5	6.2	10.3	8.8, 4.5	~3.7, 7.2	
Ba^{2+} (1.08×10^{-3} M)	2.7	7.84	3.65	~2.2	1.04, 0.94	8.07	3.9, ~3.65	8.74	4.10, 3.44	4.3	*c*	*c*	3.6	4.4	10.7	6.0, ~2.5	3.0, 7.0	

[a] 1:1 complexes of peptide-cation perchlorate salts. [b] All chemical shifts measured with respect to Me_4Si as internal standard. [c] The chemical shifts of Pro β and Pro γ protons are between 1.6 and 2.0 ppm.

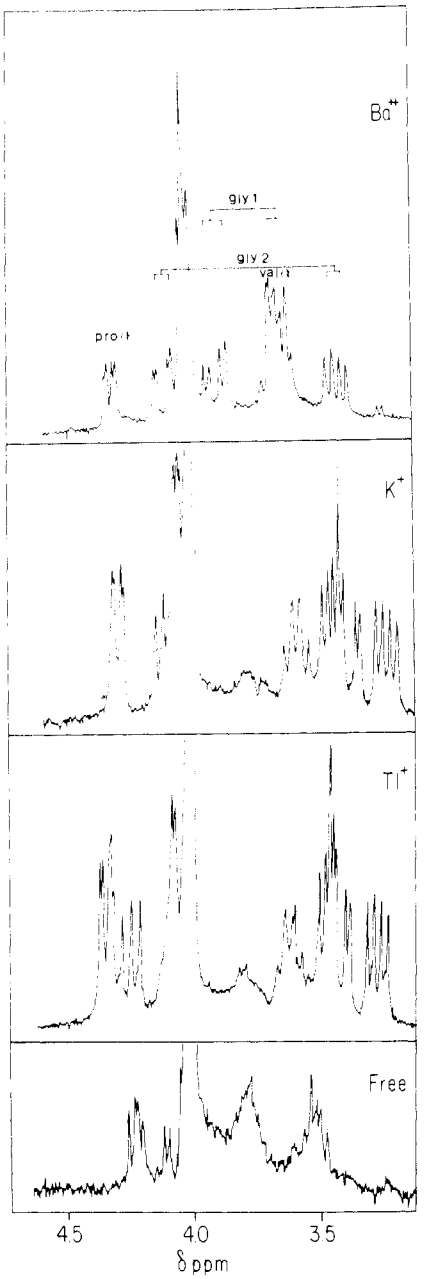


FIGURE 5: The α-CH region of the 270-MHz ^1H NMR spectra of *cyclo*(VGGP)$_3$ and its 1:1 complexes with Ba^{2+}, K^+, and Tl^+ ions in CD_3CN. Temperature, 28 °C. The peak centered at 4.05 is due to residual water.

part of an ABX system with one set of two well-resolved quartets for Gly-1 and another set of two quartets for Gly-2.

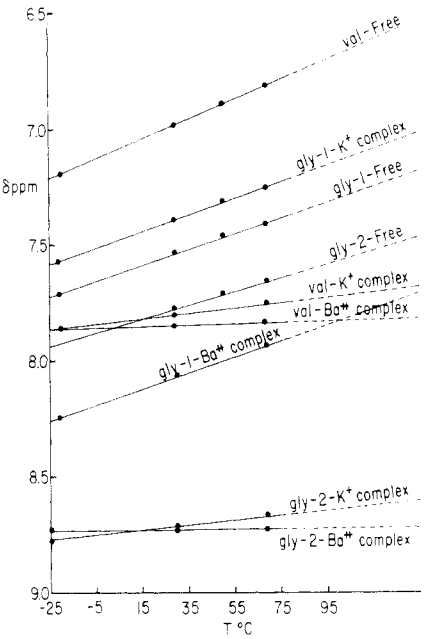


FIGURE 6: The temperature dependence of chemical shift for the amide resonances of free *cyclo*(VGGP)$_3$ and its 1:1 complexes with Ba^{2+} and K^+ ions in CD_3CN. For free *cyclo*(VGGP)$_3$, $\Delta\delta/\Delta T = 3.0 \times 10^{-3}$ ppm/deg for all three NH's; for the K^+ complex, $\Delta\delta/\Delta T$ for the Val NH and the Gly-2 NH is 1×10^{-3} ppm/deg; for the Gly-1 NH, $\Delta\delta/\Delta T = 3.0 \times 10^{-3}$ ppm/deg; for the Ba^{2+} complex, $\Delta\delta/\Delta T$ for the Val NH and Gly-2 NH is near zero; for the Gly-1 NH, $\Delta\delta/\Delta T = 3.5 \times 10^{-3}$ ppm/deg.

The chemical shifts and spin–spin coupling constants obtained for free *cyclo*(VGGP)$_3$ and the Ba^{2+}, K^+, and Tl^+ cation complexes in CD_3CN are listed in Table II.

Figure 6 shows the temperature dependences of the chemical shifts of the three NH lines for free *cyclo*(VGGP)$_3$ and the complexes of K^+ and Ba^{2+} ions in CD_3CN. The $\Delta\delta/\Delta T$ values for all three NH's had nearly the same value of 3.0×10^{-3} ppm/deg in free *cyclo*(VGGP)$_3$, whereas for the Ba^{2+} complex $\Delta\delta/\Delta T$ for Gly-1 was 3.5×10^{-3} ppm/deg and for Gly-2 and Val NH's approximately zero. For K^+ and Tl^+ complexes the $\Delta\delta/\Delta T$ values obtained were 3.0×10^{-3} ppm/deg for Gly-1 NH, as compared to $\sim 1.0 \times 10^{-3}$ ppm/deg for Gly-2 and Val NH's. The Gly-1 NH gave a $\Delta\delta/\Delta T$ of $\sim 3.0 \times 10^{-3}$ ppm/deg, as compared to $\sim 1.5 \times 10^{-3}$ ppm/deg for Gly-2 and Val NH's in the case of Li^+, Mg^{2+}, and Na^+ complexes. Similar results were obtained by solvent perturbation of the NH lines; i.e., addition of 1% acetone to *cyclo*(VGGP)$_3$ in CD_3CN shifted equally all the three NH's of free cyclic peptide downfield by 0.05 ppm. For the complexes, Gly-1 was the most perturbed resonance with a greater downfield shift than Gly-2 or Val NH's. (For example, in the case of the Ba^{2+}

complex, addition of 1% acetone to the *cyclo*(VGGP)$_3$·Ba^{2+} complex in CD_3CN shifted the Gly-1 resonance at 8.07 ppm downfield by 0.1 ppm, whereas the Gly-2 at 8.74 ppm and Val NH at 7.84 ppm had a small shift of ~0.01 ppm.) However, the differences between the shifts of the Gly-1 NH and those of the Gly-2 and Val NH's decrease as one goes from the Ba^{2+} complex to the Na^+ complex. For example, in the case of the Na^+ complex, the Gly-1 NH was shifted by 0.08 ppm, as compared to 0.04-ppm shifts of the Gly-2 and Val NH's.

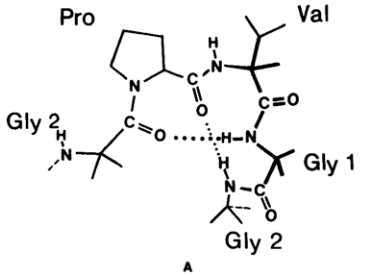
Discussion

Uncomplexed cyclo(VGGP)$_3$. It is clear from Figures 1 and 2 and Table I that uncomplexed *cyclo*(*VGGP*)$_3$ in nonpolar solvents like CD_2Cl_2 has C_3 symmetry on the NMR time scale. The symmetry persisted down to −20 °C. The relatively large temperature coefficients of chemical shifts ($\Delta\delta/\Delta T = 5.8 \times 10^{-3}$ ppm/deg) for the Val NH doublet, as compared to the smaller temperature coefficients ($\Delta\delta/\Delta T = 2.5 \times 10^{-3}$ ppm/deg) for both Gly NH's, suggest that the two Gly NH's are intramolecularly hydrogen bonded. The Gly-Pro peptide bonds are all trans, as shown by ^{13}C NMR data (Easwaran, Pease, and Blout, unpublished data) for *cyclo*(VGGP)$_3$ in $CDCl_3$, in which the chemical shifts of proline C_β and C_γ carbons are 162.9 and 167.32 ppm upfield from external CS_2, respectively.

A Corey–Pauling–Koltun (CPK) model was used to construct possible conformations consistent with all the available data for *cyclo*(VGGP)$_3$ in CD_2Cl_2, in particular, the $J_{N\alpha}$ coupling constants which lead to allowed ϕ angles (Bystrov et al., 1973) of ca. 180° for both glycines and −90, −150, or 40–80° for valine and the low-temperature and solvent dependences of both Gly NH's. The only conformation found which was satisfactory has the following approximate dihedral angles.[4]

	Val	Gly-1	Gly-2	Pro
ϕ	−90	180	180	−60
ψ	30	50	60	−60

The ψ values were estimated from both CPK and Kendrew models of the peptide. The salient features of the proposed conformation are shown in the photograph of the CPK model in Figure 7. It has two intramolecular hydrogen bonds in each repeat unit (a total of six), one (1←4) between the NH of Gly-2 and C=O of the Pro preceding the Val-Gly-1-Gly-2-Pro unit and the other hydrogen bond (1←4) between the NH of Gly-1 and the C=O of the Gly-2 of the preceding unit. This arrangement is shown schematically in structure A.



A

The proposed conformation of *cyclo*(VGGP)$_3$ in CD_2Cl_2 and $CDCl_3$ is different from that of free valinomycin in nonpolar solvents (Ovchinnikov et al., 1974) where a "bracelet"-type structure with six intramolecular hydrogen bonds formed by L-Val and D-Val NH's with type II (L-Val-D-HyIv) and type II′ (D-Val-L-Lac) β turns has been observed (Ivanov et al., 1969; Ohnishi & Urry, 1969; Venkatachalam, 1968; Ramachandran & Chandrasekharan, 1970). With free *cyclo*(VGGP)$_3$ in each Gly-2-Pro-Val-Gly-1-Gly-2 unit, there are essentially two loops of right-handed 3_{10} helix (i.e., two distorted β turns), since hydrogen bonding occurs between residues 1 and 4 and residues 2 and 5. The ϕ,ψ angles indicate that the helix is imperfect (a true right-handed 3_{10} helix has $\phi,\psi = -60,-30°$). The distortion of the helix is likely to be due to the presence of the proline side chain and to the constraints of cyclization. The presence of additional amide protons as compared to valinomycin and *cyclo*(L-Val-D-Pro-D-Val-L-Pro)$_3$ (Gisin & Merrifield, 1972) and the consequent occurrence of the intramolecular hydrogen bonds for the NH's of two consecutive Gly residues possibly lead to this unusual conformation for *cyclo*(VGGP)$_3$ in $CDCl_3$ and CD_2Cl_2.

FIGURE 7: Photograph of a CPK model of the proposed conformation of free *cyclo*(VGGP)$_3$ in CD_2Cl_2. The upper and middle views show opposite faces of the molecule. The lower is a side view showing the two 1←4 hydrogen bonds (see structure A in text).

The proposed conformation of free *cyclo*(VGGP)$_3$ is such that it does not form a good cavity lined with carbonyl oxygens,

[4] IUPAC–IUB Commission on Biochemical Nomenclature (1970), *Biochemistry 9*, 3471.

as does valinomycin in nonpolar solvents. Indeed, exploratory studies with complexes of *cyclo*(VGGP)$_3$ with K^+ and Ba^{2+} in $CDCl_3$ did not show any appreciable changes in the ^1H NMR spectra. However, in a $CDCl_3$–CD_3OH 90:10 (v/v) solvent mixture, the chemical shifts of the NH resonances, the α region, and the J values of a *cyclo*(VGGP)$_3$ plus Ba^{2+} differed substantially from free *cyclo*(VGGP)$_3$. This indicates that in a $CDCl_3$–CD_3OH 90:10 (v/v) solvent mixture the Ba^{2+} ion binds to *cyclo*(VGGP)$_3$ in contrast to K^+ ion, which does not bind or binds weakly to *cyclo*(VGGP)$_3$ in $CDCl_3$ or $CDCl_3$–CD_3OH 90:10 (v/v) solvent mixture.

In CD_3CN the overlapping and poorly defined spectra of the α region for uncomplexed *cyclo*(VGGP)$_3$, the similar values of $\Delta\delta/\Delta T$ (3.0×10^{-3} ppm/deg), and the equal extent of solvent perturbation of the NH lines indicate that *cyclo*-(VGGP)$_3$ has an average conformation with no fixed structure, probably interconverting among different conformational states. The average conformation exhibits C_3 symmetry on the NMR time scale. The structure is probably somewhat similar to conformation C of valinomycin (which occurs in polar solvents, dimethyl sulfoxide and trifluoroethanol–water, 1:2) (Ovchinnikov et al., 1974), i.e., devoid of intramolecular hydrogen bonds. This conformation of *cyclo*(VGGP)$_3$ in CD_3CN—without the constraints of a specific hydrogen-bonding scheme—is potentially more likely to interact favorably to form cation complexes than the conformation which occurs in CD_2Cl_2 (vide infra).

Cation Complexes of cyclo(VGGP)$_3$. In the previous study of *cyclo*(VGGP)$_3$ (Baron et al., 1977), which used CD to analyze binding, three types of cation complexes were observed. These complexes, termed P_2C, PC, and PC_2, had binding constants which depended on the cation. The present NMR study employed equimolar solutions of peptide and cation in an attempt to favor the 1:1 (PC) species. In all cases examined, single sets of resonances were observed in NMR spectra, although calculations using the published binding constants reveal that mixtures of the different uncomplexed species were present to some degree for each cation studied. Hence, the complexed species were in rapid equilibrium (on the NMR time scale) among one another and with free peptide, and the signals observed represented weighted averages of the various species present. In order to interpret the observed NMR data in terms of the conformation of the 1:1 PC complex, it is necessary to establish that this species is predominant under the conditions of the experiment. With Ba^{2+} and K^+ calculations show that such a condition was met. (Tl^+ appears also to be dominated by PC from the similarities with Ba^{2+} and K^+ spectra.) However, the equimolar solutions of *cyclo*(VGGP)$_3$ with Na^+, Li^+, and Mg^{2+} did not contain a predominance of PC. (Indeed, P_2C is probably the major species in the case of Na^+, Li^+, and Mg^{2+}.) The NMR data on these salts, therefore, were not used to deduce the conformations of the 1:1 complexes.

The zero temperature coefficients of chemical shift and lack of solvent perturbation for the Gly-2 and Val NH's compared to the $\Delta\delta/\Delta T$ of 3.5×10^{-3} ppm/deg and large solvent perturbation for the Gly-1 NH's support the conclusion that the Ba^{2+} complex has six intramolecular hydrogen bonds. Similar results for the K^+ and Tl^+ ion complexes also indicated that Gly-2 and Val NH's are likely to be internally hydrogen bonded.

A CPK model was used to construct possible conformations consistent with all data for the cation complexes of *cyclo*-(VGGP)$_3$ with Ba^{2+} and K^+ as given in Table II, in particular, the $J_{N\alpha}$'s and the small temperature coefficient of chemical

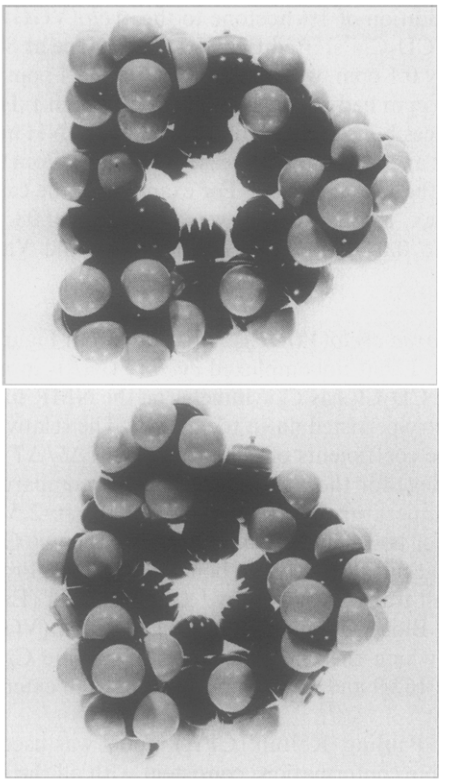


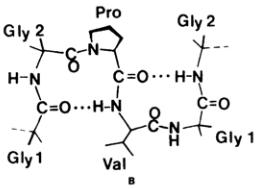
FIGURE 8: Photographs of the CPK model of the proposed conformation of (top) the *cyclo*(VGGP)$_3$·K^+ complex; (bottom) the *cyclo*(VGGP)$_3$·Ba^{2+} complex. Note the change in the position of the Gly-1 (following Val) NH's, which shifts them toward the cavity in the Ba^{2+} complex.

shifts and solvent perturbation of Gly-2 and Val NH's. The conformations for the Ba^{2+} and K^+ ion complexes of *cyclo*-(VGGP)$_3$ found to be satisfactory have the following approximate dihedral angles.

		Val	Gly-1	Gly-2	Pro
Ba^{2+} complex	ϕ	−65	20	50	−60
	ψ	140	90	−170	−70
K^+ complex	ϕ	−70	60	50	−70
	ψ	110	45	−110	−60

ψ angles were estimated from a Kendrew model. (The conformational angles for the Tl^+ complex are very similar to those of the K^+ complex.)

The conformation described by the above dihedral angles has two 1←4 intramolecular hydrogen bonds per Val-Gly-Gly-Pro unit, one between the NH of Val and the C=O of Gly-1 and the other between the NH of Gly-2 and the C=O of Pro, as shown in structure B. The proposed conformations



B

for *cyclo*(VGGP)$_3$·K^+, ·Ba^{2+}, and ·Tl^+ complexes are similar to that of the valinomycin·K^+ complex. They are "bracelet" structures with dihedral angles consistent with a type II β turn for the fragment Pro-Val-Gly-1-Gly-2 and a type II′ β turn for the fragment Gly-1-Gly-2-Pro-Val (Venkatachalam, 1968). However, it is seen that, in going from the Ba^{2+} complex to the K^+ (or Tl^+) complex, the backbone dihedral angles are slightly altered as a consequence of the inward movement of the six carbonyl oxygens in binding the doubly charged cation.

Of particular interest is the movement of the NH of Gly-1 closer to the center of the cavity containing Ba^{2+} ion with Gly-1 ϕ values decreasing from 60 to 20°. Photographs of the CPK model of the proposed conformations consistent with the data are shown in Figure 8.

Although the proposed conformation of the *cyclo*-(VGGP)$_3$·K^+ complex is very similar to the "bracelet" structure proposed for the valinomycin·K^+ complex, the formation of very stable complexes of Ba^{2+} and Tl^+ ions with *cyclo*(VGGP)$_3$ is very different from the behavior of valinomycin. No NMR studies of the Ba^{2+} complexes of valinomycin are reported in the literature. However, the data on stability constants (Ovchinnikov et al., 1974) of valinomycin in methanol with different cations indicate that the binding of Ba^{2+} is weaker (stability constant = 2000) than that of K^+ (stability constant = 80 000). Also, the binding observed herein of Tl^+ ions to *cyclo*(VGGP)$_3$ appears to be more comparable to that of K^+ than in the case of valinomycin, where stability constants reported (Ovchinnikov et al., 1974) are an order of magnitude different (5400 compared to 80 000 for K^+ in methanol). This relatively high stability and binding constant for *cyclo*(VGGP)$_3$·Ba^{2+} and *cyclo*(VGGP)$_3$·Tl^+ complexes, in contrast to the valinomycin·Tl^+ and ·Ba^{2+} complexes, should make this model compound an interesting ionophore.

The results above also indicate that, in general, large cations are preferred for binding by *cyclo*(VGGP)$_3$. In the case of K^+ and Ba^{2+} complexes, the diameters of the cations are nearly the same, and the increased stability of the *cyclo*(VGGP)$_3$·Ba^{2+} complex is due primarily to the increased cationic charge. As is shown in Figure 8, in the Ba^{2+} complex, the six inwardly pointing carbonyls are brought closer together to neutralize the increased cationic charge. In doing so, the amide proton of Gly-1 is moved toward the polar cavity (see Figure 8), and its NMR resonance occurs at lower fields compared to the *cyclo*(VGGP)$_3$·K_+ complex. The observed downfield shift presumably arises from the shift in electron density due to the proximity of the Ba^{2+} cation.

Conclusions

The ^{1}H NMR data presented here indicate that uncomplexed *cyclo*(VGGP)$_3$ has a conformation (in $CDCl_3$ and CD_2Cl_2) very different from that of free valinomycin in the same solvents. *cyclo*(VGGP)$_3$ assumes a conformation consistent with six intramolecular (1←4) hydrogen bonds involving all the Gly NH's. No potential ion-binding cavity is present in this conformation, and stable cation complexes of *cyclo*(VGGP)$_3$ apparently are not formed in these solvents (from the observed lack of perturbation NMR spectra upon addition of salts).

In CD_3CN uncomplexed *cyclo*(VGGP)$_3$ is likely to be averaging among several conformations with no preferred intramolecular hydrogen bonds. The absence of a discrete hydrogen-bonded conformation in this solvent seems to favor cation binding. Binding to Ba^{2+}, K^+, and Tl^+ ions was examined by NMR, and models were proposed for the 1:1 complexes. The *cyclo*(VGGP)$_3$·K^+ complex is essentially isostructural with the "bracelet" structure of the valinomycin·K^+ complex; the Ba^{2+} and Tl^+ complexes with *cyclo*-(VGGP)$_3$ are also very similar in conformation to the K^+ species, with some minor adjustments occurring in the complex with the doubly charged ion (Ba^{2+}).

References

Baron, D., Pease, L. G., & Blout, E. R. (1977) *J. Am. Chem. Soc. 99*, 8299–8306.

Bystrov, V. F., Ivanov, V. T., Portnova, S. L., Balashova, T. A., & Ovchinnikov, Yu. A. (1973) *Tetrahedron 29*, 873–877.

Davis, D. G., & Tosteson, D. C. (1975) *Biochemistry 14*, 3962–3969.

Davis, D. G., Gisin, B. F., & Tosteson, D. C. (1976) *Biochemistry 15*, 768–774.

Deber, C. M., Madison, V., & Blout, E. R. (1977) *J. Am. Chem. Soc. 99*, 4788–4798.

Eisenman, G., Deber, C. M., & Blout, E. R. (1976) *Biophys. Soc. Abstr. 16*, 819.

Gisin, D. F., & Merrifield, R. B. (1972) *J. Am. Chem. Soc. 94*, 6165–6170.

Grell, E., Funck, T., & Sauter, H. (1973) *Eur. J. Biochem. 34*, 415–424.

Haynes, D. H., Kowalsky, A., & Pressman, B. C. (1969) *J. Biol. Chem. 244*, 502–505.

Ivanov, V. T. (1973) *Ann. N.Y. Acad. Sci. 264*, 221–243.

Ivanov, V. T., Laine, I. A., Abdulaev, N. D., Senyavina, L., Popov, E. M., Ovchinnikov, Yu. A., & Shemyakin, M. (1969) *Biochem. Biophys. Res. Commun. 34*, 803–811.

Ivanov, V. T., Evstratov, A. V., Sumskaya, L. V., Melnik, E. I., Chumburidze, T. S., Portnova, S. L., Balashova, T. A., & Ovchinnikov, Yu. A. (1973) *FEBS Lett. 36*, 65–71.

Madison, V., Atreyi, M., Deber, C. M., & Blout, E. R. (1974) *J. Am. Chem. Soc. 96*, 6725–6734.

Niu, C.-H., Madison, V., Pease, L. G., & Blout, E. R. (1978) *Biopolymers 17*, 2747–2751.

Ohnishi, M., & Urry, D. W. (1969) *Biochem. Biophys. Res. Commun. 36*, 194–202.

Ohnishi, M., Fedarko, M. C., Baldeschwieler, J. D., & Johnson, L. F. (1972) *Biochem. Biophys. Res. Commun. 46*, 312.

Ovchinnikov, Yu. A., & Ivanov, V. T. (1975) *Tetrahedron 31*, 2177–2209, and references cited therein.

Ovchinnikov, Yu. A., Ivanov, V. T., & Shkrob, A. M. (1974) "*Membrane Active Complexones*", Elsevier Scientific, Amsterdam.

Patel, D. J., & Tonnelli, A. E. (1973) *Biochemistry 12*, 486–496.

Pease, L. G. (1975) Ph.D. Thesis, Harvard University.

Pressman, B. C. (1968) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 27*, 1283–1288.

Prestegard, J. N., & Chan, S. I. (1969) *Biochemistry 8*, 3921–3927.

Ramachandran, G. N., & Chandrasekharan, R. (1970) *Pept.: Chem. Biochem., Proc. Am. Pept. Symp., 2nd.*

Sugiwara, T., Imanishi, Y., & Higashimur, T. (1976) *Biopolymers 15*, 1529–1542.

Tosteson, D. C., Cook, P., Andreoli, T. E., & Tieffenberg, M. (1967) *J. Gen. Physiol. 50*, 2513–2525.

Urry, D. W., & Ohnishi, M. (1970) in *Spectroscopic Approaches to Biomolecular Conformation* (Urry, D. W., Ed.) pp 263–300, American Medical Association Press, Chicago, IL.

Venkatachalam, C. M. (1968) *Biopolymers 6*, 1425–1436.

Weiland, T. (1973) in *Chemistry and Biology of Peptides* (Meienhofer, J., Ed.) pp 377–396, Ann Arbor Science Publishers, Ann Arbor, MI.