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Cyclo(L-prolylglycyl)$_3$ and Its Sodium, Potassium, and Calcium Ion Complexes: A Raman Spectroscopic Study†

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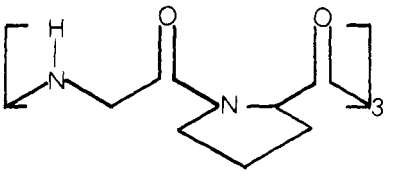
ABSTRACT: Raman spectra of the cyclic hexapeptide cyclo(L-prolylglycyl)$_3$ and its Na^+, K^+, and Ca^{2+} complexes are reported for the solid state and for samples in solution. Model compounds and N-deuteration were used to aid mode identification. Spectra of the uncomplexed ionophore in solution are consistent with previously proposed solution conformations and permit the identification of spectral lines characteristic of proline-containing peptide bonds in the trans and the cis conformations. Upon cation complexation the prolyl carbonyl stretch bands sharpen and upshift 20–30 cm^{-1} (to 1690–1700 cm^{-1}). The glycyl carbonyl stretch band is unaffected by Na^+ complexation, upshifted ~15 cm^{-1} by K^+ complexation, and downshifted ~20 cm^{-1} (to 1619 cm^{-1}) by Ca^{2+} complexation. Arguments supporting the involvement of prolyl carbonyl groups in cation complexation are noted. Spectra of the Na^+ complex of the tetramer cyclo(L-prolylglycyl)$_4$ suggest an asymmetric structure.

Due to their biological activity and structural simplicity, considerable interest has been aroused in the conformations and ion-binding characteristics of synthetic cyclic polypeptides. In particular, the cyclic hexapeptide cyclo(L-prolylglycyl)$_3$ (see Chart I), hereinafter abbreviated c(PG)$_3$, is known (Madison et al., 1974; Deber et al., 1976) to bind Li^+ and Na^+ selectively over K^+ and Rb^+. It also forms several complexes of different stoichiometry with Mg^{2+}, which it binds selectively over Ba^{2+} and Ca^{2+}. These complexes have been studied by proton and ^{13}C nuclear magnetic resonance (NMR) (Bartman et al., 1977), circular dichroism, and conformational minimum en-

Chart I



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ergy calculations (Madison, 1973).

Studies of the conformational and spectroscopic properties of cyclic polymers are simplified by the restricted range of accessible structures and the absence of end effects; the latter also enhances their usefulness as model compounds for proteins. In proline-containing peptides, where rotation about the N–C^{α} bond of the prolyl residue is limited by the pyrrolidine ring, the range of available conformations is further reduced. Because of steric restrictions, c(PG)$_3$ is unlikely to form β turns.

In H_2O and dimethyl sulfoxide, uncomplexed c(PG)$_3$ apparently adopts an asymmetric (A) conformation with one cis Gly–Pro linkage (Madison et al., 1974). In contrast, in dioxane and $CHCl_3$ solution, it apparently adopts a C_3 symmetric (S) conformation with all trans peptide bonds. No comparable data have been available previously on solid-state conformations. Cation complexes of c(PG)$_3$ are believed to have a C_3 symmetric (S*) conformation which does not contain intramolecular hydrogen bonds. A molecule with C_3 symmetry will exhibit vibrational modes of species A (symmetric, nondegenerate) and E (asymmetric, twofold degenerate) in equal numbers. The A and E modes should be both infrared and Raman active.

Experimental Section presents methods and materials. The first three sections of Results report Raman spectra of c(PG)$_3$ in the solid state and in various solvents both before and after complexation with NaSCN, KSCN, and $Ca(ClO_4)_2$. The last section of Results briefly discusses the NaSCN complex of the tetramer cyclo(L-prolylglycyl)$_4$, hereinafter denoted c(PG)$_4$. Discussion relates our measurements to various proposed conformations of c(PG)$_3$ and its cation complexes. Our spectra were interpreted by comparison with known group frequencies and with a variety of model compounds, some novel to Raman spectroscopic study. These compounds, listed in Table I with their amide I frequencies, represent both secondary and tertiary prolyl, glycyl, and alanyl amide carbonyl groups.

Experimental Section

Raman spectra were obtained by using a SPEX Ramalog 4 double-grating monochromator with a cryogenically cooled RCA GaAs photomultiplier tube and a Spectra-Physics Model 164-03 argon ion laser. Laser plasma lines were eliminated with a Claasen filter. The sample-containing capillaries were mounted perpendicular to the scattering plane and parallel to the polarization vector of the incident laser light. A polarization scrambler was permanently mounted in front of the first monochromator slit. Most measurements were performed by using the 5145- and 4880-Å laser lines; in some cases, interference from sample luminescence was reduced by using the 4579-Å laser line. Typical operating conditions were incident power, 60–160 mW, resolution, 2–5 cm^{-1}, and scanning speed, 60 cm^{-1}/min.

Spectra were obtained from uncomplexed c(PG)$_3$ in the solid state and in D_2O, H_2O, $CHCl_3$, and a 2:1 (v/v) CH_3OH–$CHCl_3$ solution and from the NaSCN complex, both in a 2:1 (v/v) $CHCl_3$–CH_3OH solution and in the solid state after recrystallization from that solution. Spectra were also obtained from solid-state samples of the c(PG)$_3$–$Ca(ClO_4)_2$, c(PG)$_3$–KSCN, and c(PG)$_4$–NaSCN complexes. Some samples were later N-deuterated by exchange against D_2O. Spectra were also obtained from the model compounds glycyl-L-proline (Gly-Pro), L-prolylglycine (Pro-Gly), and cyclo(L-prolyl)$_3$ [hereinafter c(Pro)$_3$].

The c(PG)$_3$, c(PG)$_4$, and c(Pro)$_3$ were synthesized by C. M. Deber and E. R. Blout at the Department of Biological Chemistry, Harvard Medical School, using the procedure of Deber & Blout (1974). Spectra of the alanyl diamides were obtained from samples obtained through the laboratory of V. T. Ivanov, Shemyakin Institute of Bio-Organic Chemistry, Moscow, USSR, as part of an ongoing study of their conformations. The Pro-Gly and Gly-Pro were obtained from Sigma Chemical Co. (St. Louis, MO). All were used without further purification. All solvents were spectroscopic grade. Solutions were prepared directly in Kimax glass capillaries (1.7-mm i.d.) and then lightly centrifuged. NaSCN complexes were made by adding the salt to the c(PG)$_3$ solution; solid-state samples were made by slow evaporation to dryness. A few confirmatory spectra were taken in collaboration with Dr. Ira Levin, using a computerized Ramalog system in the National Institutes of Health, National Arthritis, Metabolism and Digestive Diseases Laboratory of Chemical Physics.

Results

Uncomplexed c(PG)$_3$: Solid-State Results. Figure 1 and Table II present Raman spectra and line assignments for solid-state samples of uncomplexed c(PG)$_3$ and several model compounds. The prominent 3429-cm^{-1} band of c(PG)$_3$ represents free NH stretch of the secondary amide (Pro-Gly) group. It is present, although weak, in Pro-Gly but not in Gly-Pro, c(Pro)$_3$, or deuterated c(PG)$_3$. The broad, weak band near 3270 cm^{-1} may indicate the presence of some hydrogen-bonded NH groups (compare polyglycine I) (Small et al., 1970). In the CH stretch region (2800–3050 cm^{-1}), the asymmetric stretch modes of the pyrrolidine methylene groups appear somewhat higher (2985–3004 cm^{-1}) than those of the glycyl methylene groups.

Among our model compounds (Table I), tertiary amides typically have 1644–1651-cm^{-1} carbonyl stretch frequencies, whereas those of secondary amides with little or no hydrogen bonding are typically 1669–1677 cm^{-1}. Simple secondary alkyl acetamides also have carbonyl stretch frequencies 10–15 cm^{-1} higher than those of their tertiary counterparts (Dollish et al., 1974). Similarly, recently published (Stimson et al., 1977) infrared spectra of deuterated *N*-acetyl-*N'*-methylprolylglycinamide (two secondary amide groups, one tertiary amide group) show a doublet near 1670–1690 cm^{-1} and a singlet near 1625 cm^{-1}. These model compounds strongly suggest that the 1671-cm^{-1} singlet of c(PG)$_3$ represents three identical secondary carbonyl groups, while the 1634, 1645-cm^{-1} doublet represents the tertiary carbonyl modes, split slightly by coupling or differences in the local environment (steric and contact forces). Identification of the methylene bend (1424 and 1449 cm^{-1}), methylene wag (1349 and 1329 cm^{-1}), and $C^{\alpha}H$ bend (1319 cm^{-1}) frequencies is straightforward.

The amide III mode is a complex secondary amide vibration which includes NC stretch and in-plane NH bend. It appears in the 1220–1320-cm^{-1} region and is highly conformation sensitive (Koenig, 1972). The corresponding tertiary amide mode is less well studied. Amide III modes may be reliably differentiated from CH bands of similar frequency by N-deuteration, which reduces the former by several hundred cm^{-1}. A comparison of solid-state, H_2O, and D_2O spectra confirms that c(PG)$_3$ has amide III activity near 1264 cm^{-1}. In contrast, the residual activity near 1274 cm^{-1} is apparently methylene twist–wag as that in c(Pro)$_3$. Similar comparisons suggest that the weak band near 1288 cm^{-1} in solid-state samples (1299 cm^{-1} in H_2O solution) also represents secondary amide activity. Pro-Gly and Gly-Pro exhibit fortuitous peaks near 1264 cm^{-1}. Deuteration of Pro-Gly has little effect on that peak, demonstrating it to be a methylene twist band in this compound. Similarly, although the 1295-cm^{-1} infrared-active peak of polyglycine is believed to be amide III (supported by normal

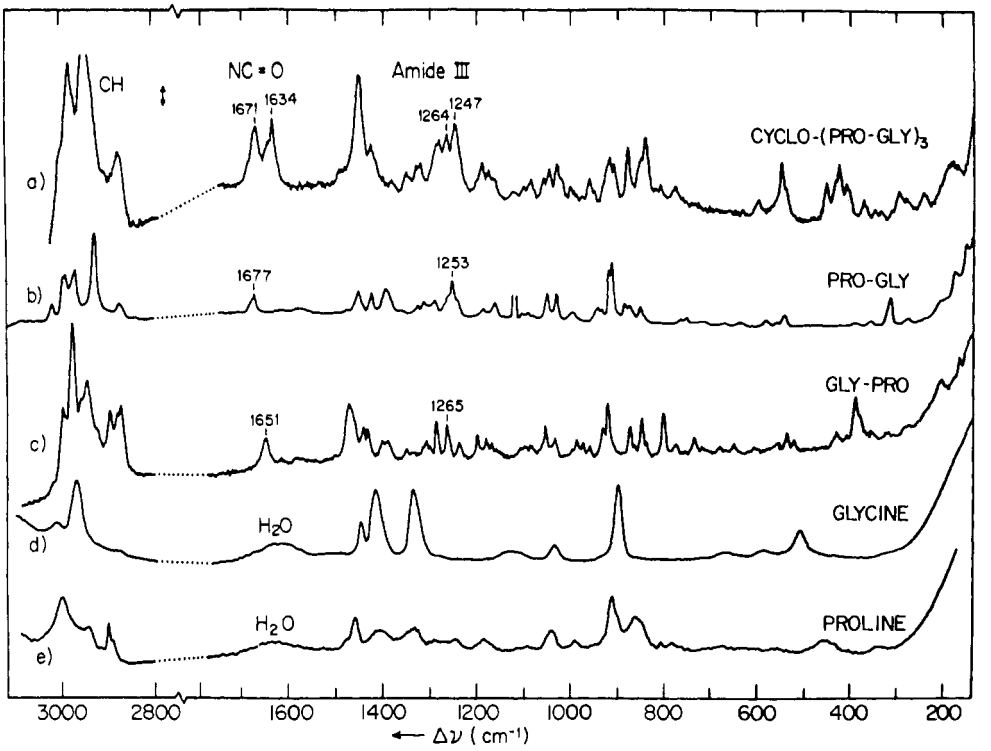

FIGURE 1: Raman spectra of (a) c(Pro-Gly)$_3$, (b) Pro-Gly, and (c) Gly-Pro in the solid state and (d) glycine and (e) proline in concentrated solution in H_2O by using a laser power of 60–160 mW at 4880 or 5145 Å. Resolution is (a–c) 2.5 and (d–e) 5.0 cm^{-1}, with a scanning speed of (a–d) 1.0 and (e) 2.0 cm^{-1}/s. Arrow (upper left-hand corner) shows the count rate, which is (a) 300, (b and c) 3000, and (d and e) 1000 photons/s.

Table I: Model Compounds for Carbonyl Stretch in Cyclo(L-Pro-Gly)$_3$

compound	carbonyl	amide	main ν (C=O) (cm^{-1})	reference
cyclo(Pro)$_3$	prolyl	tertiary	1644	this paper
poly(Pro) (I)	prolyl	tertiary	1647	Rippon et al. (1970)
N-acetyl-*N*-methyl-*N'*-dimethyl-L-alanylamide	alanyl	tertiary	1647	I. M. Asher et al. (unpublished experiments)
Gly-Pro	glycyl	tertiary	1651	this paper
Pro-Gly	prolyl	secondary	1677	this paper
N-acetyl-*N'*-methyl-L-alanylamide	alanyl	secondary	1669[a]	I. M. Asher et al. (unpublished experiments)
poly(Gly) (I)	glycyl	secondary	1674	Small et al. (1970)

[a] The solid state (but not $CHCl_3$ solution) also displays a band near 1654 cm^{-1} corresponding to H-bonded carbonyl groups.

mode calculations), the 1290- and 1287-cm^{-1} peaks of the tertiary amides c(Pro)$_3$ and Gly-Pro, respectively, clearly are not. Our results emphasize once again the need for N-deuteration to identify amide III modes.

Many of the peaks of the 800–1200-cm^{-1} region are localized, conformation-insensitive methylene twist, rock, and wag and pyrrolidine ring stretch vibrations which appear in most of the model compounds (Simons et al., 1972; Rippon et al., 1970; Small et al., 1970). A few conformation-sensitive skeletal stretch modes are also found. Rippon et al. (1970) ascribe the 957- and 1000-cm^{-1} frequencies of poly-L-prolines I and II to cis and trans conformers, respectively. The 962- and 1001-cm^{-1} peaks appear in c(PG)$_3$ and survive dissolution, deuteration, and complexation; however, their relative intensities change appreciably. The 961-cm^{-1} mode appears in Gly-Pro but not in Pro-Gly. Although the model compounds display several important modes between 600 and 775 cm^{-1} (identified as CN torsion and amides IV and V in polyglycine I), there is no c(PG)$_3$ activity in this region.

The 594-cm^{-1} mode represents skeletal deformation. It has been identified as NC=O deformation in tertiary dialkyl acetamides (Dollish et al., 1974) and as C–CN deformation in polyglycine I (Small et al., 1970; Moore & Krimm, 1976). The amide IV and VI modes, in which carbonyl in-plane and out-of-plane bends, respectively, predominate, presumably appear near 536–560 cm^{-1}. The lowest frequency region (150–450 cm^{-1}), which contains delocalized structural deformation and torsion vibrations, is a sensitive probe of polypeptide conformation and composition. Thus, the model peptide spectra (Table II) vary considerably in this region, but no peaks appear in Raman spectra of the free amino acids.

Uncomplexed c(PG)$_3$ in Solution. Substantial spectral changes occur upon dissolving c(PG)$_3$ in $CHCl_3$ (Figure 2b). The tertiary amide peak upshifts from 1634 cm^{-1}, increasing the activity near 1650–1655 cm^{-1} and leaving behind a 1641-cm^{-1} shoulder, whose intensity is consistent with the 1645-cm^{-1} shoulder seen in the solid state. The secondary amide carbonyl stretch band at 1674 cm^{-1} is not appreciably

Table II: Raman Line Frequencies (in cm^{-1}) of Uncomplexed Cyclo(L-Pro-Gly)$_3$ and Model Compounds[a]

solid state	deuterated solid state[b]	$CHCl_3$ soln	H_2O soln	Pro-Gly (secondary)	Gly-Pro (tertiary)	cyclo(Pro)$_3$ (tertiary)	assignments
3429		(3425) br	*c*	(3420) br			NH stretch (free)
(3270) br		3280	*c*	(3252) br			NH stretch (H bonded)
				(3082) br			
				3023		3027	
(3004) sh	2994		2995	2995	2998	2999	CH_2 asym. stretch (Pro)
2985		*c*		2974	2978	2973	
2952			2955			2960	
2947	2950 br	2944 br			2946	(2945)	CH_2 asym. stretch (Gly)
				2933	(sh)	(2936)	
2907	(sl)				2896		
2880	2889	2883	2890	2879	2881	2887	CH_2 sym. stretch
					2873		
		(2846)					2 × CH_2 bend
		(2833)					
1671		1674		1677			prolyl (secondary amide)
	1658 br		1663 br			(1653) sh	carbonyl stretch
						1644	
1645 sh		1641 sh			1651	1622	glycyl (tertiary amide)
1634	(1629) sh		(1632) br				carbonyl stretch
				(1617) br	(1615) br		CO_2^- stretch
		(1585) d		(1587) br	(1581) br		amide II
sl	1472 sh		(1470) sh	1480	1472	1475	CH_2 bend
1449	1455	1453	1455	1457	1443	(1457) sh	CH_2 bend
		(1435) sh		1427	1433	1441	
1424	1417		1421	1395	1402	1411	CH_2 wag plus skeletal
				(1389)	1390		
1349	1350		1349		1352		
1329		1338		1328	(1335)	1339	CH_2 wag
1319	1323	(1317) sh	1322	1316	1311	1313	C^{α}–H bend
(1288) sh		1283	1299	1291	1287	1290	amide III (not tert)
1281	1274		(1273) br	1261	1265	1275	CH_2 twist
1264			sl				amide III
1247	1249		(1250) br	1253			CH_2 twist
	1211	*c*		1244	1239	1232	
1190	1193		1194	1191	1201	(1197) br	ring deform. (Pro)
1175			1180		1181		
1164	(1164) sh	1162	1166	1165	1169	(1159) br	skeletal stretch
1123	(1130) br	1122					
1100 sh			1101	1107	1102		
1086	(1096)[d]	1090	(1087) sh	1093	1087	1085	CH_2 rock (Pro)
1057				1052	1054	1061	
1046	1043	1049	1050				CH_2 wag (Pro) plus
1030	1032	1032	1030	1033	1034	1034	skeletal stretch
1001	1003	1001	1007	999	(999) sh	995	Gly-Pro (trans)
		982	969		988		or Pro-Pro
					975		
962	962	(961)	(960)		961		Gly-Pro (cis)
				946		951	
(sl)	933	(930) sh	936	935			CH_2 rock plus skeletal (Gly)
919	923	918	926	919	924	924	ring breathing (Pro),
910	906		913	913	911	898	CC stretch
880		884		884		(sh)	skeletal stretch
	875	(864) sh	876	875	875	862	
(sh)				853	852	(sh)	
840	843[d]	(844)	846		840		CH_2 rock (Pro)
809	814	*c*	817		802		
779	779			766	779	(770) br	CH_2 twist (Pro)
				753	(757)		
				(719) br	(719) br	(708) br	CN torsion, NH
				671	684		out-of-plane bend
				639	653		
594	593	609		582	608		skeletal deform.
(560) sh	561 sh	(575) sh		(560) sh	560		amide VI
547	542	542 sh		543	539		amide IV and VI
536 sh						530	skeletal deform.
		522			522		
		473				501	
446		(459)					skeletal modes
(429) sh					433		
421	422 br						
403				(391) br	389	392	
367	(352) br				382 sh		
(343)				357	364	346	
(331)		*c*			324	321	
				317		314	

Table II (*Continued*)

solid state	deuterated solid state[b]	$CHCl_3$ soln	H_2O soln	Pro-Gly (secondary)	Gly-Pro (tertiary)	cyclo(Pro)$_3$ (tertiary)	assignments
292	284						
273				278	277	260	
236[d]	*c*						
(223)				211 br	205	205	
(188) sh				179		181	
165	168			157	165		

[a] br = broad; sh = shoulder; sl = slant; w = weak; parentheses = frequency uncertain; sym = symmetric; asym = asymmetric. [b] Recrystallized from D_2O. [c] Solvent lines obscure this region of the spectrum. [d] Unresolved doublet.

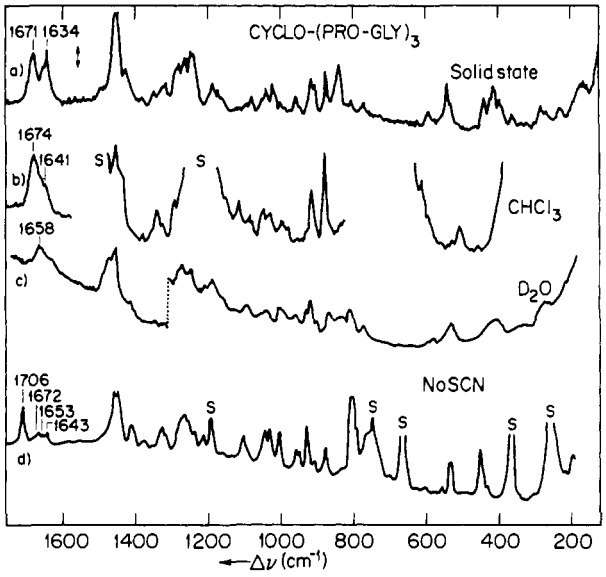

FIGURE 2: Raman spectra of c(Pro-Gly)$_3$ (a) in the solid state, (b) in solution in $CHCl_3$, (c) recrystallized from D_2O, and (d) complexed with NaSCN [solid-state sample from a 2:1 (v/v) CH_3OH–$CHCl_3$ solution]. Spectra were obtained with a laser power of 60–150 mW at 4880 or 5145 Å. (a) is identical with Figure 1a; for the other spectra, resolution is 5 cm^{-1}/s, scanning speeds are (b) 0.5 and (c and d) 1.0 cm^{-1}/s, and count rates (scale indicated by vertical arrow) are (b and d) 300 and (c) 1000 photons/s. (b) is at a concentration of approximately 10 g/L.

affected by dissolution in $CHCl_3$. This interaction of the tertiary but not secondary amide groups with solvent has possible implications for c(PG)$_3$ complexation (see Na^+, K^+, and Ca^{2+} Complexes). The frequencies and relative intensities of the methylene bend (1415–1460 cm^{-1}) and wag (1325–1350 cm^{-1}) bands are also affected, presumably due to the absence of crystalline intermolecular contact forces. The amide III region is obscured by solvent lines. Adding CH_3OH to obtain a 2:1 (v/v) CH_3OH–$CHCl_3$ solution reduces the tertiary amide carbonyl stretch frequency ~5 cm^{-1} and produces a shoulder near 1653 cm^{-1}. The only other change on addition of CH_3OH is a 6-cm^{-1} downshift in the 982-cm^{-1} band.

Considerably different spectra are obtained from c(PG)$_3$ in H_2O or D_2O (Table II). In the conformation-sensitive 200–500-, 1240–1350-, and 1600–1700-cm^{-1} regions, peaks which are multiplets in the solid state collapse into broad bands. In H_2O, solvent interactions (hydrogen bonding) lower both the secondary and tertiary amide carbonyl stretch bands 10 cm^{-1} below their values in $CHCl_3$. The tertiary amide band appears only as a broad, unresolved asymmetry on the secondary amide band. These features persist in moist solid-state samples recrystallized from aqueous solution (Figure 2c). In H_2O, the amide III region shows sharp edges near 1250 and 1273 cm^{-1} which correspond to methylene twist vibrations; they persist in D_2O. The intense, sharp skeletal stretch lines observed near 884 and 918 cm^{-1} in $CHCl_3$ are replaced by a weaker, but still sharp, series near 876, 913, 926 and 936 cm^{-1}. In D_2O solution, Raman activity is lost near 1265 cm^{-1}, implying secondary amide activity in these regions of the H_2O spectrum. N-Deuteration lowers the secondary amide carbonyl stretch frequency 5 cm^{-1} from its value in H_2O solution.

Rippon et al. (1970) assign the 957- and 1000-cm^{-1} lines of poly-L-prolines I and II to cis and trans peptide bonds, respectively (R. C. Lord, MIT, personal communication). Despite changes in relative intensity, we observe some activity at both frequencies in all systems studied. In the solid state, the 962-cm^{-1} line is half again as strong as the 1001-cm^{-1} band. In contrast the 1007-cm^{-1} peak is 40% stronger in H_2O or D_2O solution. In $CHCl_3$ solution, the 961-cm^{-1} peak is only ~14% of the 1001-cm^{-1} line, although substantial activity is seen near 982 cm^{-1}. This 982-cm^{-1} line is downshifted by the addition of hydrogen-bonding solvents: to 976 cm^{-1} in 2:1 (v/v) CH_3OH–$CHCl_3$ and to 969 cm^{-1} in H_2O. In contrast, the 1001-cm^{-1} band is seen at essentially the same frequency in all solvents.

Na^+, K^+, and Ca^{2+} Complexes. c(PG)$_3$ is known to form C_3-symmetric 1:1 complexes with K^+, Li^+, Rb^+, and Cs^+. It also forms complexes with Mg^{2+}, Ca^{2+}, Ba^{2+}, and Mn^{2+}, respectively (Deber et al., 1972; Madison et al., 1974; Deber et al., 1976; Bartman et al., 1977). We find that c(PG)$_3$ in 2:1 (v/v) CH_3OH–$CHCl_3$ readily complexes with NaSCN. Complexation dramatically sharpens the broad secondary carbonyl stretch band, which upshifts from 1674 to 1692 cm^{-1}. In heavily solvated crystals of the complex, this band becomes a sharp, intense peak at the remarkably high frequency of 1706 cm^{-1} (Figure 2d). There is also a weak satellite band near 1672 cm^{-1}; however, since no 1634-cm^{-1} activity is seen, the satellite band is presumably not due to a residue of uncomplexed c(PG)$_3$. The tertiary carbonyl stretch band is less affected by complexation. It appears near 1639 cm^{-1} in solution and splits into a 1643, 1653-cm^{-1} doublet in the solid state.

In 2:1 (v/v) CH_3OH–$CHCl_3$, the amide III region of the Na^+ complex resembles that of uncomplexed c(PG)$_3$ in H_2O or D_2O rather than that of uncomplexed ionophore in $CHCl_3$. A comparison of the solid-state spectra (Figure 2a,d) shows a major rearrangement of this region upon complexation. The intense 1247- and 1281-cm^{-1} peaks of crystalline uncomplexed c(PG)$_3$ disappear or shift in frequency, while the band near 1264 cm^{-1} (amide III) broadens. A broad NH stretch band appears near 3300 cm^{-1}, typical of hydrogen-bonded secondary amide groups.

Replacing Na^+ by K^+ further increases the carbonyl stretch frequencies. In a 2:1 (v/v) CH_3OH–$CHCl_3$ solution, these appear near 1700 and 1655 cm^{-1}, respectively, 8 and 16 cm^{-1} above the corresponding Na^+ values. The 850- and 486-cm^{-1} peaks of the Na^+ complex are not observed in solutions of the K^+ complex.

Spectral differences between crystalline uncomplexed c(PG)$_3$ and crystalline samples of its Na^+ or K^+ complexes include

Table III: Raman Line Frequencies (in cm^{-1}) of Cyclo(L-Pro-Gly)$_3$ Complexes[a]

uncomplexed, solid state	NaSCN complex, solid state	NaSCN complex, 2:1 (v/v) CH_3OH–$CHCl_3$	KSCN, 2:1 (v/v) CH_3OH–$CHCl_3$	$Ca(ClO_4)_2$ complex, solid state[e]	c(Pro-Gly)$_4$–NaSCN, solid state	assignments
3429						NH stretch (free)
	3300 br					NH stretch (H bonded)
(3270) br						
(3004) sh	3022			2998		CH_2 asym. stretch (Pro)
2985	2984				2983	
	2972	*c*		(2970) sh		
2952						CH_2 asym. stretch
2947	2929			2939	2943	
2907	2887					CH stretch (Pro)
2880	2874			2886	2880	CH_2 sym. stretch
	2835					$2 \times CH_2$ bend
	1706	(sl)				
1671	1672	1692	1700	1690	1683	prolyl (secondary amide)
			(w)		1667	carbonyl stretch
1645	1653	1639	1655	1656	1652	glycyl (tertiary amide)
1634	1643			1619	1631	carbonyl stretch
				(1590) br		
				1486		
	1458	*c*	*c*	1454	1452	CH_2 bend
1449	1449					
1424				1424		
	1412			1418	1408	CH_2 wag plus skeletal
	1381 br					
1349				(1354) sh	1344	
1329	1325			1340	(1322)	CH_2 wag
1319	1317 sh			1317	(1310)	C^{α}–H bend
(1288) sh	(1286) sh					amide III
1281		1275		1272	(1280)	CH_2 twist
1264	1267				1267	amide III
1247	(1257) sh	(1250) br		1254	1248	CH_2 twist
	1240					
	(1217)	(1218)		1214 sh		
1190	1192	1193		1198	1190	ring deform. (Pro)
1175				1180	(w)	
				1169	(w)	skeletal stretch
				(1150)		
1123				1120 br	1123 br	
1100 sh	1102	*c*	*c*	1098	1093	CH_2 rock (Pro)
1086						
1057				1047	1049	
1046	1048				1043	CH_2 wag (Pro)
1030	1031				1027	Gly-Pro (trans)
1001	1008			1011	(1005) br	
962	960	961	961	sl	(970) br	Gly-Pro (cis)
	950	949	(~)			
(sl)	930	935	934			
919					922	CH_2 rock plus skeletal (Gly)
910	913	(913) sh	914	(sl)		ring breathing (Pro)
						CC stretch
880	877	879	879	876	877	skeletal stretch
(sh)		850 w		(849)?	862	
840	(842) sh			837	841 br	
			814		815	CH_2 rock–twist (Pro)
809	805	804	*c*	807		
	795				*b*	
779				782		CH_2 twist
				770	772	
				758	(755) br	
	(748)			724		
				625		
594	601	601	(w)	598	574	skeletal deform.
(560) sh	559	561	558	567	557	
547				544		amide IV and VI
536 sh	534	537	537		(535) br	skeletal deform.
		(486) w		518	506	
446	449	451	452		448 br	
(429)	432	(sh)				skeletal modes
421						
403					409	
367	*c*	*c*	*c*	374 br	367	
(343)				(341) br	339	
(331)						
292	(310)					
273				280	275	

Table III (*Continued*)

uncomplexed, solid state	NaSCN complex, solid state	NaSCN complex, 2:1 (v/v) CH_3OH-$CHCl_3$	KSCN, 2:1 (v/v) CH_3OH-$CHCl_3$	$Ca(ClO_4)_2$ complex, solid state[e]	c(Pro-Gly)$_4$-NaSCN, solid state	assignments
236	*c*	*c*	*c*	249	265	
(223)	219			(sh)		
(188) sh	198			166		
165	167					

[a] br = broad; sh = shoulder; parentheses = frequency uncertain; sym = symmetric; asym = asymmetric. [b] Precipitate from solution. [c] Solvent lines interfere with observations in this region. [d] Unresolved doublet. [e] The ClO_4^- ion has strong bands near 935, 630, and 460 cm^{-1} and weaker activity near 1170–1050 cm^{-1}.

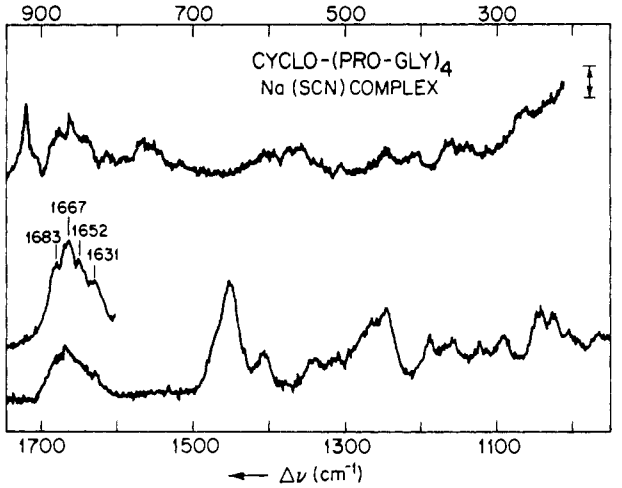

FIGURE 3: Raman spectra of the crystalline c(Pro-Gly)$_3$ divalent complex with $Ca(ClO_4)_2$. Samples (a–c) were recrystallized from D_2O and differ in amounts of uncomplexed c(Pro-Gly)$_3$ activity observed near 1656 cm^{-1}. Conditions for spectra a and b were as follows: laser power, 50 mW at 4579 Å; resolution, 5 cm^{-1}; scanning speed, 0.5 cm^{-1}/s; count rate (scale indicated by vertical arrow), (a) 3000 and (b) 2000 (different spectrometer) photons/s. Spectrum c was taken on a computerized system (nine-point smooth of 11 runs at 5 cm^{-1}/s).

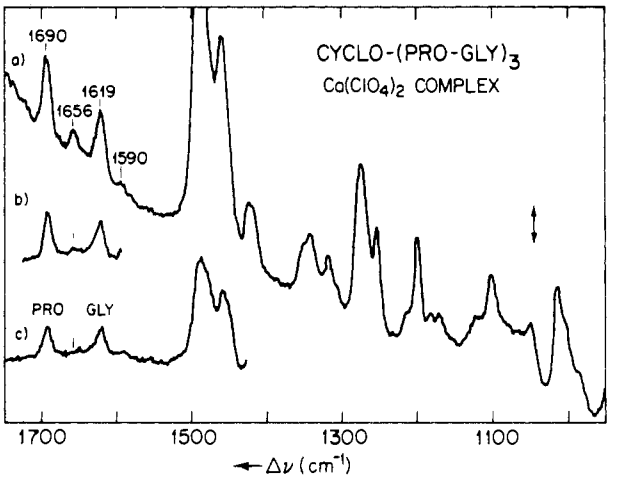

FIGURE 4: Raman spectra of the crystalline c(Pro-Gly)$_4$–NaSCN complex. The tetramer displays great complexity in the carbonyl stretch region (partially resolved in the insert). Conditions were as follows: laser power, 50 mW at 5145 Å; resolution, 4 cm^{-1}; scanning speed, 0.2 cm^{-1}/s; count rate (scale indicated by vertical arrow), 100 photons/s for the main spectra. Resolution of the insert is 3 cm^{-1} (scale, 30 photons/s).

substantial changes in the 2870–3020- (methylene stretch), 1340–1460- (bend/deformation), and 840- and 915–930-cm^{-1} (skeletal stretch) regions. The 547-cm^{-1} band is absent in the complexes. In contrast, the 960- and 1008-cm^{-1} modes are little changed by complexation.

There are few spectral differences between the c(PG)$_3$–Na^+ complex in solution and that in the solid state, consistent with the general observation that cation complexation substantially stabilizes ionophore conformation. The prolyl carbonyl stretch frequency increases to 1706 cm^{-1}. In the solid state, weak new activity appears near 486 and 850 cm^{-1}; there is a general sharpening of the spectral peaks.

Spectra were also obtained from a crystalline sample of c(PG)$_3$ complexed with $Ca(ClO_4)_2$ (Figure 3). It had been previously used in proton NMR studies and had been recrystallized from D_2O solution. Although the peaks were sharp and no D_2O bands were observed, the amide groups were fully N-deuterated; i.e., no NH stretch (3300–3450 cm^{-1}) or amide III (1230–1290 cm^{-1}) vibrations were seen. Complexation with Ca^{2+} has substantial effects on the solid-state spectrum (Figure 3 and Table III). The *tertiary* amide carbonyl stretch frequency is reduced to 1619 cm^{-1}, some 20–36 cm^{-1} lower than in the monovalent complexes. Since N-deuteration alone has little effect on the tertiary carbonyl stretch frequency of uncomplexed c(PG)$_3$ samples (see Uncomplexed c(PG)$_3$ in Solution), this dramatic shift is presumably due to replacing Na^+/K^+ with Ca^{2+}. In contrast, the *secondary* amide carbonyl stretch frequency (1690 cm^{-1}) of the Ca^{2+} complex is similar to that of the monovalent complexes. (The small observed downshift, compared to the Na^+ complex, is most likely a deuteration effect.) A notch seen in the amide III region of the spectrum of the deuterated Ca^{2+} complex near 1260 cm^{-1} is consistent with amide III activity [compare deuterated and undeuterated uncomplexed c(PG)$_3$ at this frequency].

The methylene stretch (three intense bands near 2886, 2939, and 2998 cm^{-1} with shoulders), methylene bend (additional peak near 1486 cm^{-1}), and methylene wag (upshifted to 1340 cm^{-1}) regions also change upon Ca^{2+} complexation. Other changes include the disappearance of the 960-cm^{-1} stretch band, with an intense band being seen near 1011 cm^{-1}. The 930- and 1031-cm^{-1} skeletal stretch bands of the Na^+ complex are absent in the Ca^{2+} complex, but two new stretch bands appear near 1169 and 1180 cm^{-1}. The Ca^{2+} complex resembles uncomplexed c(PG)$_3$ by displaying peaks near 544, 782, and 837 cm^{-1} and by lacking a 795-cm^{-1} peak. The Ca^{2+} complex exhibits little activity between 290 and 440 cm^{-1} or near 1658 cm^{-1}, features which are also absent in N-deuterated uncomplexed c(PG)$_3$. New features characteristic of the Ca^{2+} complex include bands at 518, 625, 724, 758, and 770 cm^{-1}.

Cyclo(L-*Pro-Gly*)$_4$*–NaSCN Complex.* The tetramer cyclo(L-Pro-Gly)$_4$ also forms a complex with NaSCN (Figure 4). In the carbonyl stretch region, this compound shows a broad, complex band near 1630-1685 cm^{-1}, with identifiable peaks near 1631, 1652, 1667, and 1683 cm^{-1}. The breadth and multiplicity of lines in this band may be contrasted with the isolated sharp peaks seen in the c(PG)$_3$–Na^+ complex; the solid-state c(PG)$_4$ complex is apparently characterized by a variety of environments for each type of carbonyl group. The sharp, high frequency carbonyl stretch peak seen near 1700–1706 cm^{-1} in the c(PG)$_3$–Na^+ and c(PG)$_3$–K^+ complexes

is absent in the spectrum of the tetramer complex, suggesting that the high frequency of the 1706-cm^{-1} band may not be due directly to cation–carbonyl interactions [which presumably are very similar in $c(PG)_3$ and $c(PG)_4$]; the high frequency of the 1706-cm^{-1} band might, e.g., be due to steric effects in $c(PG)_3$.

Discussion

In this section we interpret our Raman spectroscopic data in terms of the conformations assumed by $c(PG)_3$ in different environments. Our data also identify the carbonyl groups responsible for cation binding in solution and in the solid state. In general, our solution data are consistent with conformations previously proposed for $c(PG)_3$ on the basis of proton and ^{13}C NMR, circular dichroism, and minimum energy calculation studies (Madison et al., 1974; Deber et al., 1972; Bartman et al., 1977; Madison, 1973). There are no previous studies of $c(PG)_3$ or its cation complexes in the solid state with which to compare our data.

From the work of Madison et al. (1974), uncomplexed $c(PG)_3$ in $CHCl_3$ is in conformation S, a C_3 symmetric conformation with three intramolecular 3 → 1 hydrogen bonds between adjacent glycyl (secondary) amide and glycyl carbonyl groups. The addition of CH_3OH to $CHCl_3$ introduces a 1653-cm^{-1} shoulder on the 1646-cm^{-1} glycyl (tertiary amide) carbonyl stretch band. This is consistent with breaking some of the glycyl–glycyl (3 → 1) hydrogen bonds and converting some of the Gly–Pro peptide linkages from a trans to a cis conformation as would occur if conformation A were being formed in CH_3OH–$CHCl_3$ mixtures. Madison et al. (1974) found that in solvents which are strong H-bond acceptors (H_2O, dimethyl sulfoxide) $c(PG)_3$ assumes the asymmetric conformation A, so our result is not totally unexpected. The trend continues as more CH_3OH is added. In a 3:1 (v/v) CH_3OH–$CHCl_3$ solution, the bands near 1641 and 1658 cm^{-1} have approximately the same intensity. In contrast, the 1674-cm^{-1} prolyl carbonyl vibration is virtually unaffected by adding CH_3OH to $CHCl_3$.

Water reduces both the prolyl and glycyl carbonyl stretching frequencies of uncomplexed $c(PG)_3$ from their values in CH_3OH–$CHCl_3$. The 1200–1350-cm^{-1} (amide III) and 850–950-cm^{-1} (N–C^{α}–C stretch) regions also change substantially from $CHCl_3$ to H_2O solution. The S → A transition, which ruptures three intramolecular hydrogen bonds, would be expected to affect these regions.

The presence of the A conformation in H_2O, as demonstrated by Madison et al. (1974), is supported by changes elsewhere in the spectrum. In $CHCl_3$, the 961- and 1001-cm^{-1} lines (thought to be characteristic of cis and trans peptide bonds involving proline) are in the ratio 1.0:7.5, suggesting that most bonds are in the trans conformation. In H_2O, this ratio becomes 1.4:1, which would indicate a large increase in the number of cis peptide bonds if the line were primarily sensitive to conformation around the Gly–Pro rather than the Pro–Gly link. In the A conformation, the actual ratio of cis/trans Pro–Gly bonds is only 1:2. The observed discrepancy between the last two ratios may reflect differences in the Raman cross sections of the two conformations.

The weak 961-cm^{-1} line seen in the $CHCl_3$ solution could be accounted for if a few percent of the $c(PG)_3$ molecules were in the A conformation (due to moisture or an intrinsic equilibrium between conformations). Similarly, in $CHCl_3$, the 3280-cm^{-1} NH stretch band (H-bonded NH groups) predominates, consistent with conformer S. However, a weak band is also seen at 3425 cm^{-1} (free NH groups), consistent with a minority conformer more open than S, such as A.

Raman studies of the macrocyclic ionophore valinomycin have shown that uncomplexed valinomycin does not retain its solid-state conformation in solution (Asher et al., 1977b; Rothschild et al., 1977). Similarly, the many differences between Raman spectra of uncomplexed $c(PG)_3$ in solution and those in the solid state indicate that the solid-state conformation of $c(PG)_3$ is not found in all solvents.

Uncomplexed $c(PG)_3$ shows both a narrow ~3429-cm^{-1} line and a broad 3270-cm^{-1} band, whether in the solid state or in solution. Although the 3270-cm^{-1} band is at a frequency consistent with being an overtone of the 1634-cm^{-1} peak, the data in the amide I region suggest that it is a true amide stretch band. However, the ~3429-cm^{-1} band is relatively more prominent in the solid state than in $CHCl_3$ solution, as would be expected if the pattern of hydrogen bonding had changed.

The carbonyl stretch bands are appreciably broader in $CHCl_3$ than in the solid state. In H_2O the carbonyl stretch frequencies are appreciably reduced, as would be expected due to hydrogen bonding to the solvent. The glycyl carbonyl stretch band of uncomplexed $c(PG)_3$ is lower in the solid state than in $CHCl_3$, even though in solution the glycyl carbonyl groups all receive intramolecular hydrogen bonds, which would lower their stretch frequency. The solid-state value is comparable to the frequencies found in aqueous solution, indicating strong, perhaps intermolecular, hydrogen bonding of the glycyl carbonyl groups in the solid state. The 1645-cm^{-1} shoulder may be attributed either to a variation in the strength of the hydrogen bonds or to symmetry splitting of the A and E modes.

From the 962- and 1001-cm^{-1} lines, it would appear that $c(PG)_3$ in the solid state may contain a limited number of cis Gly–Pro bonds. By comparison with H_2O solution, the 1:1.5 intensity ratio would indicate that roughly a fifth of the bonds is in this conformation, although direct extrapolation from solution to solid state contains substantial uncertainties.

In 2:1 (v/v) CH_3OH–$CHCl_3$, $c(PG)_3$ readily forms a 1:1 complex with Na^+. Na^+ complexation substantially sharpens the carbonyl stretch bands. The frequency of the prolyl carbonyl line increases by 18 cm^{-1}, while the glycyl carbonyl band shifts by 5 cm^{-1}. In the complex, the prolyl and glycyl carbonyl bands are both singlets, consistent with C_3 symmetry for the complex. Interpretation of these effects is aided by comparison with cation complexes of the macrocyclic ionophores valinomycin (Asher et al., 1974), nonactin, monactin, and dinactin (Phillies et al., 1975; Asher et al., 1977a). In these compounds, cation complexation in solution systematically sharpens Raman lines, as would be expected if the cation served to stabilize a single conformation of the ionophore. Furthermore, in solution cation complexation changes the stretch frequency of the carbonyl groups responsible for cation binding, frequency changes being −5, +2, and +9 cm^{-1} for the valinomycin–K^+ complex in C_2H_5OH, $CHCl_3$, and CCl_4, respectively, and +3 to −10 cm^{-1} for the monovalent cation complexes of the nactins. By contrast, the solid-state nactin–Ba^{2+} complexes show a split carbonyl stretch band, with shifts of −10 and −30 cm^{-1}.

The spectra suggest that in $c(PG)_3$ the prolyl carbonyl groups are responsible for Na^+ and K^+ complexation. On formation of the Na^+ and K^+ complexes, the prolyl carbonyl stretch band changes from a broad ~1674-cm^{-1} band to an extremely sharp single line centered at 1692 and 1700 cm^{-1}, respectively. In contrast, on complexation the glycyl carbonyl stretch band shows much smaller changes in frequency and does not sharpen markedly, consistent with the prolyl groups being more strongly involved with a bound monovalent cation than the glycyl carbonyl groups.

This argument is not entirely unambiguous. For example, the Na^+ complex of the tetramer c(PG)$_4$ does not show a sharp prolyl carbonyl stretch peak in the very high (1690–1710 cm^{-1}) frequency range seen in the c(PG)$_3$ complexes; the 1690–1709-cm^{-1} band of the c(PG)$_3$ complexes could therefore be reinterpreted as arising from steric effects in the smaller c(PG)$_3$ ring rather than from a direct cation–prolyl carbonyl interaction. Furthermore, in nonactin–cation complexes (which involve ester carbonyl groups), the carbonyl stretch frequency is little changed in monovalent cation complexes but is reduced 10–30 cm^{-1} in divalent cation complexes (Asher et al., 1977a). Similarly, the glycyl carbonyl stretch frequency of the c-(PG)$_3$–Ca^{2+} complex is ~20 cm^{-1} lower than that of the c(PG)$_3$–Na^+ complex. Such a comparison of divalent complexes suggests that c(PG)$_3$ binds divalent ions with glycyl carbonyl groups, a result consistent with the finding (Bartmann et al., 1974) that the glycyl carbonyl groups are responsible for binding Mn^{2+} in the c(PG)$_3$–Mn^{2+} complex.

The 962- and ~1000-cm^{-1} lines of the Na^+ complex have the same intensity ratio as those of uncomplexed c(PG)$_3$ in $CHCl_3$, implying that the bulk of the Pro-Gly (secondary amide) groups are trans. However, the amide III and 870–940-cm^{-1} regions of the complex are like those of c(PG)$_3$ in H_2O. The amide III region is known to be sensitive to hydrogen bonding, so the hydrogen-bonding pattern of the complex probably resembles that seen in H_2O; i.e., there are no internal hydrogen bonds in the complex.

In the solid-state Na^+ complex, no activity is seen near 3420 cm^{-1}, indicating that all amide protons are hydrogen bonded. In the S_1* conformation, these bonds cannot all be intramolecular, but intermolecular bonds or bonds to solvent molecules cannot readily be ruled out. The prolyl carbonyl stretch frequency (1706 cm^{-1}) of the solid-state c(PG)$_3$–Na^+ complex is extraordinarily high, while the glycyl carbonyl stretch frequency also increases appreciably between solution and solid state. The resultant carbonyl stretching bands resemble those seen for the K^+ complex in solution; complexation with K^+ increases the frequency of the prolyl and the glycyl stretch frequencies by 26 and 14 cm^{-1}, respectively.

In summary, we find that the Raman spectra of uncomplexed c(PG)$_3$ change substantially between the solid state and $CHCl_3$, $CHCl_3$–CH_3OH, and H_2O solutions. These changes are consistent with the disappearance of the intermolecular hydrogen bonds of the solid-state samples and with the conformational forms previously proposed for uncomplexed c(PG)$_3$ in solution. Solid-state data on c(PG)$_3$ and its Na^+, K^+, and Ca^{2+} complexes are presented for the first time. Comparison with model dipeptides and derivatives indicates that the glycyl carbonyl groups are involved in binding Ca^{2+} in solution but that prolyl carbonyl groups are also involved in binding monovalent ions, especially in the solid state.

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