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CONFORMATIONS OF PROLINE-CONTAINING CYCLIC PEPTIDES. II. ASYMMETRIC SOLUTION CONFORMATIONS OF *CYCLO*-(L-PRO$_2$-GLY)$_2$ AND ITS ALKALINE-EARTH METAL COMPLEXES AS STUDIED BY NMR SPECTROSCOPY[1]

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Dedicated to the memories of the late Prof. G. Bruckner on the occasion of his 80th birthday

Abstract: By ^{1}H and ^{13}C NMR methods, the title peptide, either free or complexed with metal ions, is shown to preferentially assume asymmetric solution conformations. Spectral data strongly suggest the occurrence of an unusual β-turn with two Pro residues in consecutive corner positions.

The role of glycine in reverse turns and of proline in restricting the available conformational space of protein structures is conveniently studied in small cyclic model peptides.[2] As part of our systematic investigations into the solution conformations and ion-binding properties of *cyclo*-(Pro$_2$Gly$_n$-Pro$_2$Gly$_m$) (n,m = 1,2,3) series,[1,3] we now report on the major conformational characteristics of the cyclic hexapeptide, *cyclo*-(Pro$_2$-Gly)$_2$ (n,m = 1), as inferred from ^{1}H and ^{13}C NMR observations[4] aided by the use of specifically ^{2}H-, ^{13}C- and ^{15}N-labelled isotopomers[5] and ion-binding experiments. The results outlined below show that, similarly to other members of this series, the free hexapeptide exhibits multiple conformations with well-defined, highly populated conformational states, the populations being subject to the hydrogen-bonding properties of the solvent. Constitutional symmetry of the molecule notwithstanding, the peptide backbone is found to preferentially assume asymmetric conformations that differ in the number and type of *cis* X-Pro bonds. The relevant ^{13}C and ^{1}H NMR data are given in Tables 1 and 2, respectively.

^{13}C NMR spectra reveal that, in $CDCl_3$, the hexapeptide occurs in a single conformational state (conformer A_1) giving rise to three Pro-C^{γ} resonances from 25.0 to 25.6 ppm typical for *trans* X-Pro bonds and a fourth Pro-C^{γ} signal at 22.33 ppm attributable to Pro residue in a *cis* X-Pro sequence.[2,6] The addition of CD_3OD, CD_3CN or DMSO to the chloroform solution of the hexapeptide results in the appearance of a second set of ^{13}C resonances due to another asymmetric species (conformer A_2) characterized by a two-*cis*-two-*trans* X-Pro geometry. In neat methanol, acetonitrile or water, A_1 and A_2 coexist with nearly equal populations whereas in DMSO A_2 becomes the predominant state (approx. 85% at room temp.). The nature of the *cis*-configured X- Pro bonds was readily obtained by recording the carbon-13 spectra of the specifically labelled hexapeptide *cyclo*-(Pro-[γ-$^{2}H_2$]Pro-Gly)$_2$.[5b] As attested by the "missing" Pro-C^{γ} resonances (and accompanying splittings of other ^{13}C signals from the labelled residues, see Table 1), both A_1 and A_2 feature one *cis* Pro-Pro sequence while transition from A_1 to A_2 involves the *trans*-to-*cis* isomerization of a Gly-Pro peptide bond.

Further characterization of conformational states A_1 and A_2 was inferred from ^{1}H NMR data, variable temperature studies and solvent titration experiments. The usual criteria of accessibility of amide NH's to solvent (or intramolecular interactions) indicate that, in A_1, both Gly

Table 1. Relevant ^{13}C NMR data of cyclo-(Pro_2-Gly)$_2$ conformers[a]

	A_1 ($CDCl_3$)		A_2 (DMSO-d_6)		$(pep)_2Mg^{++}$[b] (CD_3CN)	$(pep)Mg^{++}$ (CD_3CN)	$(pep)_2Ca^{++}$ (CD_3CN)
Pro-C^{γ}(3,6)	*22.33* *24.99*		*23.03* *24.92*		*22.45* *23.05*	*22.15*	*22.58*
Pro-C^{γ}(2,5)	25.44 25.59		23.17 25.57		24.94 25.27	25.45	25.38
Pro-C^{β}(2,5)	27.20 28.75		27.63 30.94		28.91 29.79	29.23	29.19
Pro-C^{β}(3,6)	*28.05* *31.73*		*27.98* *33.19*		*31.88* *32.77*	*32.11*	*32.29*
Gly-C^{α}(1,4)	41.85 42.18		41.27 42.09		41.34 41.46	41.55	41.63
Gly-CO(1)	167.41	(0.85)	168.27	(0.73)	167.68	168.00	167.98
Gly-CO(4)	168.04	(1.47)	168.93	(1.03)	168.74		
Pro-CO(2,5)	169.99 170.45	(2.44) (2.12)	170.19 171.30	(0.91) (0.81)	169.86 170.44	170.92	170.32
Pro-CO(3)	170.98[c]	(1.39)	170.72[e]	(0.82)	170.61[g]	178.31	175.00
Pro-CO(6)	171.48[d]	(2.06)	173.65[f]	(1.02)	179.80[h]		

a) At 32°C. Chemical shifts are in δ ppm and were measured relative to internal dioxane the chemical shift of which (67.13 ppm, rel. to internal TMS in $CDCl_3$) was taken to be constant in various solvents. Values in italics refer to carbon atoms in selectively labelled [γ-$^2H_2$]Pro residues. In parentheses: paramagnetic shifts (ppm) after addition of 25% (v/v) TFE. b) For this conformer, the numbering of residues reflects merely the sequence according to Fig. 1. c) $^1J(^{15}N,^{13}C)$ = = 16.32 Hz; d) $^1J(^{15}N,^{13}C)$ = 15.51 Hz; e) $^1J(^{15}N,^{13}C)$ = 15.91 Hz; f) $^1J(^{15}N,^{13}C)$ = 14.63 Hz; g) $^1J(^{15}N,^{13}C)$ = 14.82 Hz; h) $^1J(^{15}N,^{13}C)$ = 15.20 Hz.

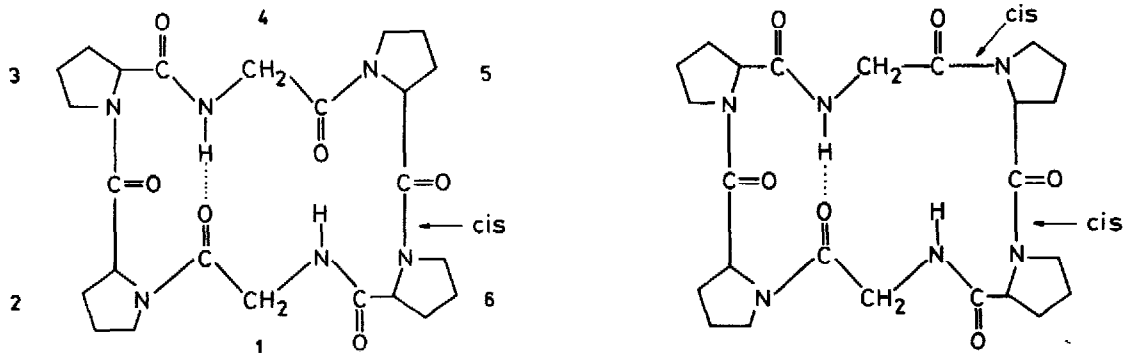
NH's are sequestered. Both NH resonances are essentially independent of temperature in either $CDCl_3$ or DMSO and move *upfield* by a small amount on changing from $CDCl_3$ to the more strongly hydrogen bonding DMSO.[7,8] By contrast, application of the same criteria to the data for A_2 shows that in this conformation one of the NH's becomes clearly exposed while the other remains inaccessible to solvent (see Table 2). The inaccessibility to solvent of Gly NH's may be indicative

Table 2. ^1H NMR parameters[a] *of Gly-NH's in cyclo-(Pro_2-Gly)$_2$ conformers*

Conformer	Solvent	NH(4)	$(\Delta\delta/\Delta T).10^3$	NH(1)	$(\Delta\delta/\Delta T).10^3$
A_1	$CDCl_3$	6.93 (4.9, 2.4 Hz)	1.6	9.15 (4.9, 0.9 Hz)	2.0
	DMSO-d_6	6.83 (4.9, 2.4 Hz)	1.0	8.87 (4.9, 0.9 Hz)	2.0
A_2	DMSO-d_6	7.19 (9.5, 3.2 Hz)	1.8	8.69 (7.0, 5.6 Hz)	4.5
$(pep)_2Mg^{++}$[b]	CD_3CN	7.22 (10.3, 2.5 Hz)	-	8.65 (7.4, 5.3 Hz)	-
$(pep)Mg^{++}$	CD_3CN	8.06 (8.0, 3.5 Hz	(both NH's)		
$(pep)_2Ca^{++}$	CD_3CN	7.71 (6.5, 5.0 Hz)	(both NH's)		

a) At 29°C in 0.01 M solutions. Chemical shifts (in ppm) are relative to internal TMS, $^3J(NH\alpha)$ coupling constants are from high-field measurements. Temperature coefficients (ppm/deg.) were obtained between 29 and 75°C. b) The numbering of residues is arbitrary for this conformer.

Figure 1



of intermolecular H-bonding. The shielding differentials of carbonyl carbons (assigned through the spectra of hexapeptide isotopomers containing ^{15}N- and 1-^{13}C-labelled Gly residues) measured upon addition of trifluoroethanol (TFE) to the $CDCl_3$ solution of A_1 (see Table 1) lend support to this expectation. The remarkably small TFE-induced paramagnetic shift of Gly^1-CO resonance is consistent with this carbonyl group pointing inward, away from the solvent and H-bonding to Gly^4-NH.[8,9] This interpretation is further reinforced by observing a high (16.32 Hz) value of the amide $^1J(^{15}N,^{13}C)$ coupling constant[10] for Pro^3-CO, peptide-linked to Gly^4-^{15}NH, (*vs.* the 15.51 Hz measured for Pro^6-CO) and, also, by the IR spectra (0.03 to 0.003 molar $CHCl_3$ solns) exhibiting two concentration independent bands of equal intensity at 3360 and 3270 cm^{-1} characteristic of free and H-bonded NH vibrations, respectively.[11] A much similar set of observables for A_2 (see Tables 1 and 2) suggests that the transannular H-bond remains unaltered upon solvent-assisted transition from A_1 to A_2 and the latter state, despite its second *cis* X-Pro bond, becomes competitive by virtue of H-bonding of the exposed NH with the solvent. Although our data now available do not allow for an unambiguous determination of the placement of the *cis* Pro-Pro linkage with respect to the intramolecular H-bond, model-buildings suggest it to be located between Pro^5 and Pro^6 as shown schematically in Fig. 1. Clearly, the most remarkable feature of these conformers is the presence of a hitherto unobserved (and theoretically unexpected[12]) 1 ← 4 hydrogen bond encompassing two Pro residues in the i + 1 and i + 2 positions.

While further efforts are being made to locate the position of *cis* Pro-Pro bond within A_1 and A_2, the importance of the intramolecular H-bond in stabilizing the asymmetric conformations A_1 and A_2 may indirectly be assessed by the predominance of two-*cis*(Pro-Pro)-two-*trans*(Gly-Pro) geometries for the alkaline-earth metal complexes of the hexapeptide[13] in which intramolecular hydrophilic interactions are replaced by ion-ligand forces. Of particular relevance is the highly stable, "sandwich" type, (2:1) complex with Mg^{++} ions, $(pep)_2Mg^{++}$, identified through ^1H NMR-monitored titration of the CD_3CN solution of hexapeptide with $Mg(ClO_4)_2$.[14] Unlike the cases of other known peptide sandwiches,[15] the solution form of this complex is portrayed by NMR as a species with inherent asymmetry. Carbon-13 chemical shift data (see Table 1) show that the com-

plexed ligands in $(pep)_2Mg^{++}$ assume two-*cis*(Pro-Pro)-two-*trans*(Gly-Pro) backbone geometry; the number of resonances (six CO's, two Gly-C^{α}'s, *etc.*) attests the lack of overall symmetry. Since any exchange process that involves both ligands and is fast on the NMR time scale would time-average the asymmetry (as it apparently does in most known similar cases), the lack of overall symmetry may be rationalized by assuming that either the complex has the two ligands in the same asymmetric conformations or that the two ligands assume two, non-equivalent C_2 symmetric geometries. In the latter case, the occurrence of only one carbonyl resonance (due to Pro-CO-Gly) with (unusually large) paramagnetic shift attributable to the ion-dipole interaction[16] might suggest that the two ligands are bound to the metal ion *via* different carbonyl groups, and to different extent.[17] It is of interest to note that the $(pep)_2Ca^{++}$ sandwich of the hexapeptide, also having its ligands in a two-*cis*(Pro-Pro)-two-*trans*(Gly-Pro) arrangement, proved to be fully symmetric (on the NMR time scale) (see Table 1) which may reflect the lower stability of this complex.[18] Also the symmetric two-*cis*(Pro-Pro)-two-*trans*(Gly-Pro) conformation is characteristic of the other, 1:1, Mg^{++} complex of the hexapeptide, $(pep)Mg^{++}$. A more detailed account on these and related studies, including the results of X-ray analyses now in progress, will be presented elsewhere.

REFERENCES AND NOTES

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4. Spectra were recorded with Varian XL-100/15 Disk-augmented FT NMR system. Frequencies: 100.1 (^1H), 25.16 (^{13}C), 15.36 (^2H), and 10.14 (^{15}N) MHz. Details of the high-field (400/500 MHz) ^1H NMR studies will be presented elsewhere.
5. (a) For details of the syntheses of *cyclo*-$(Pro_2$-[1-^{13}C]Gly$)_2$ (enriched 2.25 and 90%, respectively), *cyclo*-$(Pro_2$-[^{15}N]Gly$)_2$ (enriched 31%) and *cyclo*-(Pro-[γ-$^2H_2$]Pro-Gly$)_2$ (70% γ-$^2H_2$) see: M. Hollósi, Z. Dobolyi, and G. Bujtás, *Liebigs Ann. Chem.*, 796 (1978). (b) Preparation of the selectively ^2H-labelled [γ-$^2H_2$]- proline will be described in a forthcoming publication.
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14. Overall stability constant, estimated from CD titrations (see Ref. 3): $K(2:1) > 10^7$. This consitutes the most stable metal complex identified for the entire *cyclo*-$(Pro_2$-Gly_m-Pro_2-$Gly_n)$ series.
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18. According to our CD and NMR titrations in CD_3CN, formation of the 1:1 Ca^{++} complex is complete at a 1.5:1 salt-to-peptide molar ratio while the $(pep)_2Mg^{++}$ complex persists even at a fivefold molar excess of $Mg(ClO_4)_2$.

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