THE γ-TURN AS AN INDEPENDENT CONFORMATIONAL FEATURE IN SOLUTION

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SUMMARY: The γ-turn, an 11-membered hydrogen-bonded ring formed by a 1 → 3 type H-bond, has been observed in chloroform in the tripeptide, t-Boc-Gly_1-L-Val_2-Gly_3-OMe, by proton magnetic resonance spectroscopy. This tripeptide sequence occurs in repeat penta- and hexapeptides of elastin where it also forms a γ-turn. The dihedral angles of γ-turns, which were proposed previously on the bases of theoretical calculations and x-ray diffraction, are compared with the results obtained by proton magnetic resonance. The results reported here demonstrate for the first time that the γ-turn can exist in solution as an independent conformational feature.

INTRODUCTION

A "Turn" which functions as a basis for folding in a protein has been defined in terms of the angles formed by the vectors connecting the α-carbon atoms in sequential peptide residues (1). The most frequently proposed folding structure is the β-turn (2-4) which is stabilized by a 4 → 1 type of H-bond forming a 10-membered ring (C_{10}). This is a hydrogen bond involving the NH of residue 4 and the C-O of residue 1. Printz, et al. (5) have proposed another turn called the "γ-turn" for their models of angiotensin II. This turn has been shown by theoretical calculations (6) to be a preferred conformation in the tripeptide sequence, Gly-Gly-Gly, formed by a 1 → 3 type of H-bond giving rise to an 11-membered ring (C_{11}). X-ray diffraction studies of thermolysin (7) also showed a "γ-turn" formed by residues 25-27. The γ-turn can be described in terms of three sets of φ and ψ dihedral angles labelled in Figure 1 where a 7 atom hydrogen bonded ring is also seen to be possible. On the basis of extensive proton and carbon-13 magnetic resonance studies on peptides of tropoelastin in solution (8-11), 11-membered hydrogen

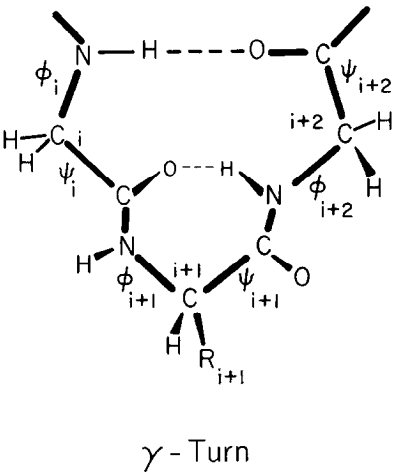

FIGURE 1: General representation of a γ-turn containing glycine as the first and third residues and an L-amino acid as the second residue.

bonded rings were shown to occur in the repeating pentapeptide, Val_1-Pro_2-Gly_3-Val_4-Gly_5 (VPGVG) and hexapeptide, Ala_1-Pro_2-Gly_3-Val_4-Gly_5-Val_6 (APGVGV). The γ-turn occurred in the sequence Gly_3-Val_4-Gly_5. The conformational angles of this sequence within the repeat pentapeptide of elastin (12), VPGVG, differ somewhat from those reported earlier (6,7) (vide infra). In the repeat peptides of tropoelastin there also occurs, as another prominent secondary structural feature, the β-turn involving the NH of residue 4 and the C-O of residue 1. The question, therefore, arises as to whether the γ-turn can occur as an independent conformational feature and, if so, whether the dihedral angles differ from those reported previously (6,7). For these reasons the peptide t-Boc-Gly_1-Val_2-Gly_3-OMe was studied. If the γ-turn exists as a relatively stable structure then it is expected that both the Gly_1 CH_2 and Gly_3 CH_2 proton systems would occur as ABX spin patterns since ring closure would make the methylene protons non-equivalent (13-15). Analysis of the glycine ABX spin patterns provides information on the ϕ_1, ψ_1, ϕ_3 and ψ_3 dihedral angles and, of course, analysis of the valyl αCH quartet provides information on the ϕ_2 and χ_2^1 dihedral angles.

MATERIALS AND METHODS

The tripeptides X-Gly_1-Val_2-Gly_3-OMe (GVG) were synthesized (16) with X as t-butyloxycarbonyl (t-Boc) and acetyl (Ac) in order to assign the Gly_1 protons. Solutions (0.02 M) of each of the t-Boc-GVG-OMe and Ac-GVG-OMe were made using $CDCl_3$ as the solvent. Proton magnetic resonance (PMR) measurements were performed on a Varian HR-220 spectrometer operating at a probe temperature of 20°C and equipped with an SS-100 computer system. Simulated spectra were obtained using a Varian Data Machine spin simulation program. Double resonance and variable temperature experiments were carried out on a JEOL PS-100 spectrometer equipped with a JEOL JNM VT-38 temperature controller.

RESULTS

The 220 MHz spectrum of the expanded α-proton region of t-Boc-Gly_1-L-Val_2-Gly_3-OMe is shown in Figure 2A where it can be seen that both the Gly CH_2 protons appear as ABX spin patterns. The assignments of the signals of these two methylene groups of Gly_1 and Gly_3 were achieved by assigning first their

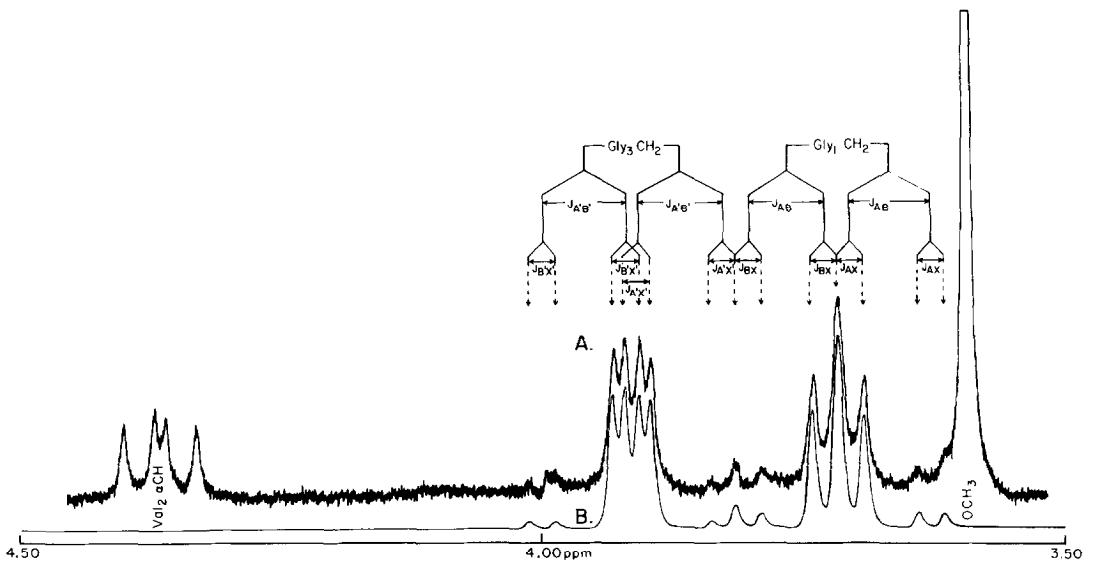

FIGURE 2: A. 200 MHz spectrum of t-Boc-Gly_1-L-Val_2-Gly_3-OMe in $CDCl_3$
B. Computer simulated spectrum. Note the exact correspondence between A and B. Also included is coupling analysis diagram.

corresponding NH proton signals. This was done by comparison of the spectra of t-Boc-GVG-OMe and Ac-GVG-OMe. The signal of Gly_1 NH appears at 5.53 ppm for t-Boc-GVG-OMe while for Ac-GVG-OMe it appeared at 7.26 ppm, whereas the Gly_3 NH occurs at 7.38 ppm in the t-Boc derivative and at 7.37 ppm in the acetyl derivative. The assignments of CH_2 signals were then made by irradiating the respective NH proton signals and observing the multiplet collapses in the αCH region of the spectrum. The values of chemical shifts and coupling constants of both the Gly_1 CH_2 and Gly_3 CH_2 were obtained from a computer simulated spectrum (Figure 2B). The values for Val_2 were readily obtained from the αCH quartet. All the PMR data are given in Table I. The φ dihedral angles for t-Boc-GVG-OMe in Table II were obtained from $^3J_{\alpha CH-NH}$ by using a Karplus-like relationship with the coefficients derived by Bystrov et al. (17).

TABLE I.

PMR PARAMETERS FOR t-BOC-GLY_1-L-VAL_2-GLY_3-OMe IN $CDCl_3$

AMINO ACID RESIDUES	PROTON(S)	CHEMICAL SHIFTS (δ) IN PPM (± .01)	COUPLING CONSTANTS (J) IN Hz		
			$^3J_{\alpha CH-NH}$	$^3J_{\alpha CH-\beta CH}$	$^3J_{\beta CH-\gamma CH}$
t-Boc	CH_3	1.39	-	-	-
L-Val_2	$^1\gamma CH_3$	0.92	-	-	7.0 ± 0.1
	$^2\gamma CH_3$	0.98	-	-	7.0 ± 0.1
	βCH	2.14	-	7.0 ± 0.1	7.0 ± 0.1
	αCH	4.38	7.8 ± 0.1	7.0 ± 0.1	-
	NH	7.13	7.8 ± 0.1	-	-
				GEMINAL COUPLING (^2J)	
Gly_1	CH(A)	3.48	5.5	-16.7	
	CH(B)	3.75	5.5	-16.7	
	NH(X)	5.53	5.5	-	
Gly_3	CH(A')	3.89	5.5	-18.2	
	CH(B')	3.93	5.5	-18.2	
	NH(X')	7.38	5.5	-	
OMe	CH_3	3.59	-	-	

TABLE II.

COMPARISON OF CONFORMATIONAL ANGLES (ϕ AND ψ) FOR A γ-TURN

COMPOUNDS	AMINO ACID RESIDUES INVOLVED IN γ-TURN	CONFORMATIONAL ANGLES*					
	i i+1 i+2	ϕ_i	ψ_i	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
Angiotensin II[5] (Model Building)	$-Val_3-Tyr_4-Val_5-$	175	128	67	-61	-132	-162
Tripeptide[6] (Theoretical calculations)	$-Gly_1-Gly_3-Gly_3-$	172	128	68	-61	-131	162
Thermolysin[7] (x-ray)	$-Ser_{25}-Thr_{26}-Tyr_{27}-$	-148	92	86	-57	-114	148
Repeat Pentapeptide of Elastin[12] (PMR)	$-Gly_3-Val_4-Gly_5-$	56	30[a]	-146	120[a]	61	170[a]
Tripeptide (this work)	$-Gly_1-Val_2-Gly_3$	-140	130[a]	80	-60[a]	-140	180[a]

*See Figure 1.

[a]Obtained from the Drieding Model of Gly-Val-Gly.

The ψ dihedral angles were obtained from a Drieding model. These values can be compared to the work of Barfield et al. (18), which relates $^2J_{AB}$ to both the ϕ and ψ angles. All the values are given in Table II together with values obtained from other sources for other γ-turns.

Non-equivalence of the two Gly CH_2 (Gly_1 and Gly_3 of t-Boc-GVG-OMe) protons (see Figure 2) indicates (13-15) a preferred conformation. Since the temperature dependence of peptide NH chemical shifts ($d\delta/dT$) is one of the criteria (3,8-11) to identify H-bonding, variable temperature experiments were performed which gave the following results: $d\delta/dT$ of Gly_1 NH = 0.0043 ppm/^oC; $d\delta/dT$ of Val_2 NH = 0.0076 ppm/^oC and $d\delta/dT$ of Gly_3 NH = 0.0066 ppm/^oC. This delineation indicates that the Gly_1 NH is essentially completely shielded from the solvent, most reasonably by an intramolecular hydrogen bond which results in a fixing of the glycine dihedral angles.

DISCUSSION

The appearance of the two Gly CH_2 protons of t-Boc-GVG-OMe as ABX spin patterns indicates that both Gly residues are constrained. The temperature dependence of the Gly_1 NH chemical shift of 0.0043 ppm/^oC shows this NH to be shielded from the solvent as occurs in an intramolecular hydrogen bond. Both of these PMR constraints on the structure are satisfied by a hydrogen bond between the NH of Gly_1 and the C-O of Gly_3 as shown in general for a γ-turn in Figure 1. The calculated ϕ dihedral angles using the Bystrov et al. (17) relationships fit for a γ-turn type of structure. These dihedral angles for t-Boc-GVG-OMe are listed in Table II along with other molecular systems where γ-turns have been proposed (5-8,11,12). It can be seen in Table II that there is qualitative agreement between the values of the torsion angles (ϕ and ψ) of thermolysin (7), Gly_1-Gly_2-Gly_3 (6) and Gly_1-Val_2-Gly_3. In Table II it can also be seen that there are differences in the values for the ϕ and ψ dihedral angles between the repeat pentapeptide of elastin ($V_1P_2G_3V_4G_5$) (12) and the tripeptide sequence, Gly_1-Val_2-Gly_3. This difference can be understood if we consider the other structural features that occur in the pentapeptide utilizing residues which are involved in the γ-turn. In the pentapeptide of elastin (Val_1-Pro_2-Gly_3-Val_4-Gly_5) the amino acid sequence Gly_3-Val_4 constitutes part of the γ-turn, part of the β-turn and also part of a 14-membered ring formed by a $1 \rightarrow 4$ type H-bond (8,12).

A short range interaction between the Gly_3 NH and Gly_1 C=O (a $3 \rightarrow 1$ type H-bond) forming a 7-membered ring (C_7) was also shown to be expected on the basis of theoretical calculations for Gly_1-Gly_2-Gly_3 (6) and the ϕ angles of Gly_1-Val_2-Gly_3 are consistent with its presence in this tripeptide. The temperature coefficient of Gly_3 NH of 0.0066 ppm/^oC in Gly_1-Val_2-Gly_3 (vide supra) indicates the presence of significant shielding from the solvent in a manner consistent with a 7-membered ring in this molecule (see Figure 1). This hydrogen bond was not observed in the pentapeptide of elastin (Val_1-Pro_2-Gly_3-Val_4-Gly_5) in $CDCl_3$ (9) but was observed in the same molecule in more

polar solvents (8,11). In non-polar solvents, such as chloroform, there occurs a 1 → 4 type H-bond forming a 14-membered ring between the Val_1 NH and Val_4 C=O of Val_1-Pro_2-Gly_3-Val_4-Gly_5 which disrupts the short range interaction of the 7-membered hydrogen bonded ring. Therefore the γ-turn is not as rigid as the β-turn and can be formed with or without the 7-membered hydrogen bonded ring.

The γ-turn of t-Boc-Gly_1-Val_2-Gly_3-OMe, however, is more stable than the possible β-turn for this sequence which would utilize the t-Boc carbonyl and the Gly_3 NH and would allow the Gly_3 CH_2 to be free to rotate rather than to be constrained as the data indicates. It may also be noted that there is qualitative correspondence between the ϕ and ψ angles of Table II and the values obtained for ϕ_1, ψ_1, ϕ_3 and ψ_3 on the basis of the Barfield et al. (18) ϕ-ψ map with geminal coupling constant ($^2J_{AB}$) contours. Accordingly it is reasonable to conclude that the γ-turn is an independent conformational feature which does not require the presence of associated cooperative interactions for its stability. This has implications specifically as to the nature of chain mobility in the elastin molecule and generally with respect to the initiation of folding in proteins such as thermolysin.

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