Note Added in Proof. After this work was completed and in press, a particularly attractive group theoretical treatment of methyl relaxation has appeared (G. B. Matsen, *J. Chem. Phys.*, in press). This treatment leads to spectral density functions identified with symmetry labels rather than with auto- and cross-correlation. The advantage is to provide a physical interpretation for the pre- and postexponential terms in methyl relaxation behavior characterized by multiple exponentials. Subsequent work from this laboratory will use this development.

Acknowledgments. We wish to thank Dr. L. G. Werbelow (Department of Chemistry, University of Utah, Salt Lake City, Utah) for his thorough and helpful comments and suggestions. Thanks are also due to Dr. J. S. Morley (ICI, Ltd., Pharmaceuticals Division, England) for his generous gift of the tetragastrin used in this study, to Ms. Christine Reid and Mr. Marc Huppert for their assistance in sample preparation and thin layer chromatography, and to Dr. Hermann E. Bleich for helpful discussions. We wish to especially thank Mr. Mark Latina for his aid in the computational aspects of this work.

This work was performed at the New England Area NMR Research Resource Facility and supported by NIH Grant RR 00639 to J.A.G.

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Proton Magnetic Resonance and Conformational Energy Calculations of Repeat Peptides of Tropoelastin: the Tetrapeptide

Md. Abu Khaled, V. Renugopalakrishnan, and Dan W. Urry*

Contribution from the Laboratory of Molecular Biophysics and the Cardiovascular Research and Training Center, University of Alabama Medical Center, Birmingham, Alabama 35294. *Received April 8, 1976*

Abstract: The detailed conformation of a tetrapeptide of tropoelastin, *t*-Boc-L-Val_1-L-Pro_2-Gly_3-Gly_4-OMe in $CDCl_3$, has been obtained from a combined analysis of ^1H NMR spectra and conformational energy calculations. The observations of Gly_3 and Gly_4 methylene protons as ABX spin systems indicate a fixed conformation similar to a cyclic peptide stabilized by hydrogen bond formation. Temperature dependence and solvent perturbation of NH protons and conformational energy calculations each showed the presence of a β-turn, a ten atom hydrogen-bonded ring involving the Gly_4 NH and Val_1 C=O, and a segment of an antiparallel β-pleated sheet stabilized by a hydrogen bond between the Val_1 NH and the Gly_4 C=O. Conformational angles obtained from the observed $^3J_{\alpha CH\text{-}NH}$ coupling constants and from conformational energy calculations were in good agreement. The secondary structure of this tetramer is shown to be the same as previously proposed for the high polymer of the tetramer in water at elevated temperature.

An understanding of the essential functional role of elastin in vascular wall and its molecular pathology in atherosclerosis requires an understanding of its conformation and dynamics. Structural features at the molecular level are largely responsible for the dynamic properties and interactions of elastin. Gray, Sandberg, and co-workers[1,2] have shown that soluble

tropoelastin, the precursor protein of fibrous elastin,[3-6] contains repeating sequences, a tetrapeptide (L-Val_1-L-Pro_2-Gly_3-Gly_4, VPGG), a pentapeptide (L-Val_1-L-Pro_2-Gly_3-L-Val_4-Gly_5, VPGVG), and a hexapeptide (L-Ala_1-L-Pro_2-Gly_3-L-Val_4-Gly_5-L-Val_6, APGVGV). The repeat peptides, their oligomers, and high polymers have been synthesized in this laboratory.[7-9] From extensive studies by NMR using temperature coefficient[10-13] and solvent perturbation[14-16] methods, it has been shown that there is a β-turn in each monomeric unit of these repeat peptides as well as additional secondary structural features and new β-spiral type conformations for the high polymers of the penta- and hexapeptides of elastin have been proposed.[17-19] Recently conformational energy calculations on the pentamer[20] and nuclear Overhauser enhancement measurements on all three repeat peptides[21] have confirmed that this β-turn, containing Pro_2 and Gly_3 at the corners of the ten-membered ring, is type II. Of special interest to the present work, Dale and Titlestad[22] have reported the ^1H NMR spectral behavior of methylene protons (CH_2) of glycine or sarcosine contained in cyclic oligopeptides where the CH_2 protons appear as ABX or AB spin patterns. The ring constraints make the methylene protons nonequivalent[22] and result in the AB type spin pattern. Therefore, if the glycine protons in the repeat peptides of elastin are relatively fixed by a β-turn and additional secondary structural features, they should also be nonequivalent and exhibit ABX spin patterns.

Useful information relative to conformation can be derived by analyzing the ABX spectrum and by using the Karplus relationship[23,24]

$$^3J = A\cos^2\theta + B\cos\theta + C \ldots \quad (1)$$

where A, B, and C are constants. Several workers[25-29] have obtained the values A, B, and C for peptide systems by semiempirical and theoretical methods. The widespread application of this relationship has been to evaluate the angles ϕ and χ of peptide backbone and side chains[30,31] by observing $^3J_{\alpha CH\text{-}NH}$ and $^3J_{\alpha CH\text{-}\beta CH}$, respectively. The limitations of the method are due to the approximate $\cos^2\theta$ dependence of the Karplus relation and the multiple numerical solutions of θ for a single value of ^3J. Therefore, studies of peptide conformations have effectively utilized a combination of the observed ^3J values and conformational energy calculations[32-36] to limit the range of possible conformations. This approach proved to be particularly successful for the valinomycin-K^+ complex,[36] where the x-ray structure obtained some years later showed no atom of this dodecamer to be out of place by more than 0.5 Å.[37] In this study we investigate the detailed conformation of the tetramer of elastin, VPGG, by the combined results of ^1H NMR spectral analysis and conformational energy calculations. The conformational energy calculations utilize the "free space approximation" and the partitioned potential energy method. Such calculations have been applied in the past to many biologically important molecules.[38-44] Recently it has been argued[45] that conformational energy calculations in vacuo approximate conformations observed in solvents of the low polarity, i.e., of low dielectric constants. In this paper we report the congruity of calculated low-energy conformations and ^1H NMR conformational studies in chloroform for a tetrapeptide of elastin. While previous studies on the tetrapeptide of tropoelastin have already led to a proposal for the occurrence of a type II β-turn involving a Val_1-C-O···HN-Gly_4 hydrogen bond and placing Pro_2 and Gly_3 at the corners[7,16,21] and under special circumstances the occurrence of an additional Val_1-NH···O-C-Gly_4 hydrogen bond,[19] the present effort confirms these proposals mainly by means of detailed analysis of the glycyl CH_2 coupling constants in chloroform and by conformational energy calculations which together provide specification of preferred dihedral angles and a plotted perspective of the conformation.

Experimental Section

The tetrapeptide of elastin, t-Boc-Val_1-Pro_2-Gly_3-Gly_4-OMe, was synthesized in this laboratory.[7] ^1H NMR spectra were obtained with a 0.1 M solution in $CDCl_3$. To facilitate spectral analysis, 20% by volume C_6D_6 was added (see Figure 1A and B). ^1H NMR measurements were made on a Varian HR-220 spectrometer operating at a probe temperature of 21 °C and equipped with an SS-100 computer system. Simulated spectra were obtained by using a Varian data machine spin simulation program. All the double resonance experiments were performed on a JEOL PS-100 spectrometer operating at a probe temperature of 22 °C and in the internal lock mode. Variable temperature experiments were made with the PS-100 spectrometer equipped with a JEOL JNM VT-38 temperature controller.

Conformational Energy Calculations. The partitioned potential energy method has been used for the conformational energy calculations, the program for which originated in the Ramachandran laboratory[46] and has since been extensively modified.[47] Total conformational energy, E_{Total}, is expressed as a sum of four separate terms:

$$E_{Total} = E_{van\ der\ Waals} + E_{electrostatic} + E_{torsion} + E_{H\text{-}bonding} \quad (2)$$

$E_{van\ der\ Waals}$ was calculated using Buckingham's "6-exp" potential function with parameters suggested by Ramachandran and Sasisekharan.[38] $E_{electrostatic}$ was calculated assuming a dielectric constant of unity, up to the monopole term,[48] using ab initio minimal basis set (STO-3G) net charges for glycyl, L-valyl, and L-prolyl residues and OCH_3 group, whereas CNDO/2[44] net charges were used for the *tert*-butyloxycarbonyl group. Net charges for glycyl, L-valyl, and L-prolyl residues were derived from an ab initio study of N-acetylglycine-N-methylamide,[45] N-acetyl-L-valineamide, and N-formyl-L-prolineamide,[49] Net charges for the OCH_3 group were taken from an ab initio study of methanol[50] and for t-Boc groups were derived from a CNDO/2 calculation on t-Boc-L-Val NH_2. The net charges for VPGG were assembled from those of the fragments, taking care to preserve the overall electroneutrality of the peptide. $E_{torsion}$ was calculated using a threefold torsional potential with torsional barriers of 0.6 and 0.2 kcal/mol, respectively, for C^α-N and C^α-C' bonds.[51] $E_{H\text{-}bonding}$ was calculated using the following empirical potential function derived by Ramachandran et al.[52]

$$E_{H\text{-}bonding} = E_{min} + p_1\Delta^2 + q_1\theta^2 \quad (3)$$

with $\Delta = R - R_{min}$ where the best values of the parameters E_{min}, p_1, and q_1 were obtained by comparing the theoretically expected distribution in hydrogen bond length R and hydrogen bond angle, θ, i.e., angle between N···O and N-H directions, with existing experimental data. The best values obtained[52] were, $p_1 = 25$ and $q_1 = 0.001$, with $R_{min} = 2.95$ Å and $E_{min} = -4.5$ kcal/mol. The above hydrogen bond potential function has been applied to seven-membered (C_7) and ten-membered (C_{10}) hydrogen-bonded ring systems, i.e., γ-turns and β-turns in peptides.[53] A fully extended conformation of VPGG was constructed using standard dimensions for the peptide group,[38] so that all the backbone angles ϕ and ψ were equal to 180°.[54] Peptide groups were assumed to be planar and trans with all values of ω equal to 180°. For the valyl side chain C^α-C^β, C^β-$C^{\gamma 1}$, and C^β-$C^{\gamma 2}$ bond lengths were assumed to be 1.54 Å and all C-H bonds were taken to be 1.10 Å. Bond angles N-C^α-C^β, C^α-C^β-$C^{\gamma 1}$, and C^α-C^β-$C^{\gamma 2}$ in the valyl side chain were taken to be 110, 112, and 112°, respectively,[55] whereas tetrahedral values of 109.47° were used for all C-C-H and H-C-H bond angles. Initially a trans conformation with $\chi_1{}^1 = 180°$ was assumed for the valyl side chain.[56] We have assumed a C^γ exo conformation for L-proline with $\chi_2{}^1$, i.e., the N-C^α-C^β-C^γ torsion angle, equal to about −17° as found in p-Br-Cbz-Gly-L-Pro-L-Leu-Gly(OH).[57] Hydrogen atoms were fixed at standard tetrahedral bond angles of 109.47° and bond lengths of 1.09 Å to the L-prolyl side chain. The t-Boc group was assumed to be staggered about the C-O-C plane and was constructed with the dimensions reported by Kashino et al.[58] for N-*tert*-butyloxycarbonyl-S-benzylcysteinylglycine methyl ester. The methoxy group was similarly assumed to be staggered about the C-O-C plane and was constructed with a bond angle for C-O-C equal to 110° and a tetrahedral geometry for the methyl group.[59] A bond length of 1.42 Å was assumed for the C-O bond. The torsion angles necessary for a description of the backbone peptide chain of VPGG are shown in Figure 5 (below) where ϕ_1 and ψ_1 torsion angles are designated for Val_1, ϕ_2 and ψ_2 for Pro_2, ϕ_3 and ψ_3 for Gly_3, and ϕ_4 and ψ_4 for Gly_4 residues. The ϕ and ψ rotations around the single

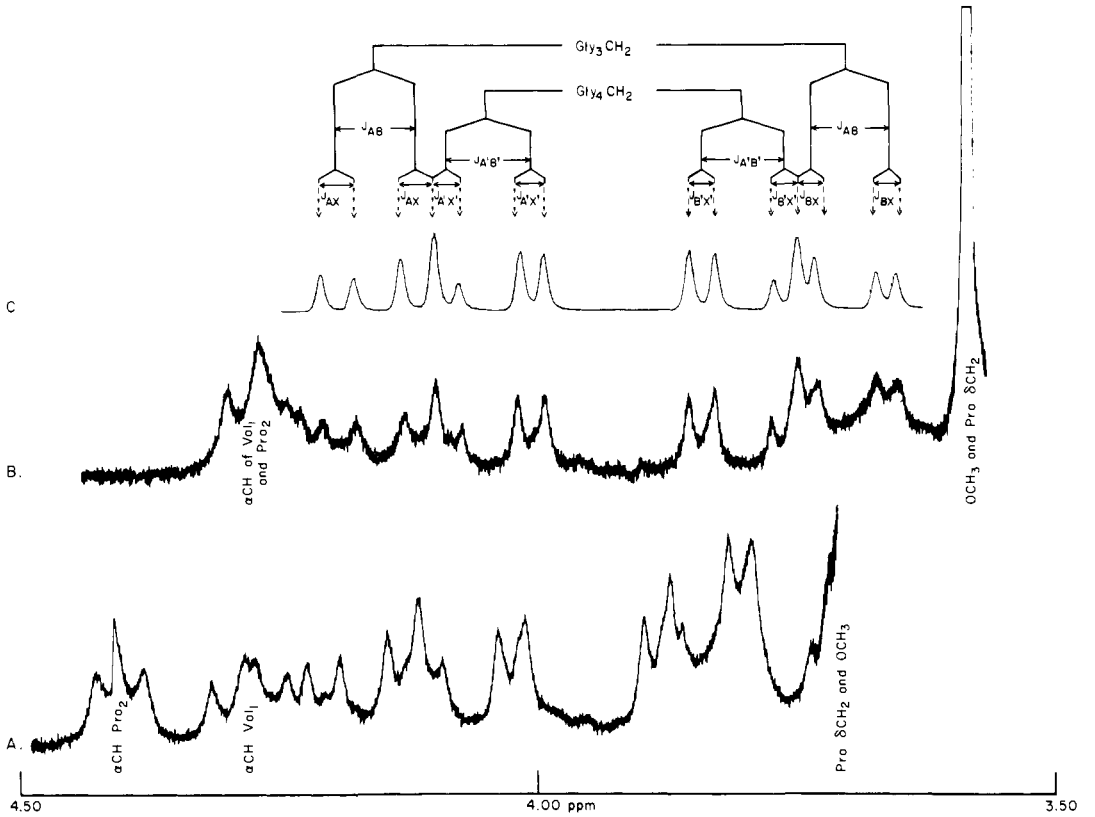

Figure 1. 220-MHz ^{1}H NMR spectra of *t*-Boc-Val$_1$-Pro$_2$-Gly$_3$-Gly$_4$-OMe (αCH region only): (A) in $CDCl_3$; (B) 80% $CDCl_3$ and 20% C_6D_6 (by volume); and (C) computer simulated spectrum of Gly$_3$ and Gly$_4$ CH_2 protons (analyzed as an ABX spin system).

bonds of the backbone, i.e., N-C$^\alpha$ and C$^\alpha$-C′ in VPGG, were systematically performed starting with the coordinates of the fully extended conformation generated as described above. Due to the rigid conformation adopted by proline, ϕ_2 is fixed near $-60°$,[56] the conformational energy of VPGG is a function of seven torsion angles ϕ_1, ψ_1, ψ_2, ϕ_3, ψ_3, ϕ_4, and ψ_4, not considering torsion angles necessary to describe the puckering mode of L-prolyl residue. In a later stage of the calculations the side chain torsion angle $\chi_1{}^1$ was varied in order to obtain the preferred conformation of L-Val$_1$ side chain.

Results

Proton Magnetic Resonance. The 220-MHz ^{1}H NMR spectra of the α-proton region (expanded to 5 Hz/cm) of *t*-Boc-Val$_1$-Pro$_2$-Gly$_3$-Gly$_4$-OMe (VPGG) are shown in Figure 1. Figure 1A shows the signals for α protons obtained in $CDCl_3$, the αCH of Pro$_2$ at 4.43 ppm and αCH of Val$_1$ at 4.32 ppm were easily assigned from their fine structure and by double resonance experiments. Irradiation of the Boc-Val$_1$NH, a 9.5-Hz doublet at 5.51 ppm (not shown in the spectrum), collapsed the doublet of a doublet (9.5 and 7.5 Hz) at 4.32 ppm into a doublet showing a 7.5-Hz coupling between the αCH and the βCH protons of Val$_1$. The Gly$_3$ and Gly$_4$ methylene protons (CH_2) appeared more complicated and overlapped. Addition of 20% C_6D_6 (by volume) resulted in all the glycyl methylene proton signals being well-spread and analyzable (see Figures 1B and 1C). The broad triplet of the Pro$_2$ αCH moved upfield and overlapped with the signal of the Val$_1$ αCH. All the chemical shifts and coupling constants are obtained in this solvent mixture (80% $CDCl_3$ + 20% C_6D_6) and recorded in Table I.

The assignments of the two NH signals for Gly$_3$ and Gly$_4$ in $CDCl_3$ were made by means of a $CDCl_3 \rightarrow Me_2SO$-$d_6$ solvent titration and by comparison with previously determined temperature dependences and solvent perturbations.[7] The temperature coefficients ($d\delta/dT$) observed in $CDCl_3$ are as follows: Gly NH (a pseudotriplet) at 7.69 ppm shows $d\delta/dT$ = 0.0041 ppm/°C; Gly NH (a broad triplet) at 7.52 ppm shows $d\delta/dT$ = 0.0091 ppm/°C, and Val$_1$ NH (a doublet, 9.5 Hz) at 5.44 ppm shows $d\delta/dT$ = 0.0052 ppm/°C. Solvent perturbation on going from chloroform (with 20% C_6D_6) to dimethyl sulfoxide at 21 °C showed that the broad triplet of the Gly NH at 7.52 ppm is the most perturbed one. In previous studies it was found that the Gly$_4$ NH is shielded from the solvent[7,15,60] and that this shielding is due to H bding to the Val$_1$ C=O, giving a ten-membered ring (β-turn).[7,15] Since the peptide proton at 7.69 ppm has a low temperature coeffi-

Table I. ^{1}H NMR Parameters for *t*-Boc-Val$_1$-Pro$_2$-Gly$_3$-Gly$_4$-OMe in $CDCl_3$ with 20% C_6D_6 (v/v)

AMINO ACID RESIDUES	PROTON(S)[a]	CHEMICAL SHIFT (δ) IN PPM (± .005)	COUPLING CONSTANT (J) IN HZ		
			$^3J_{\alpha CH-NH}$	$^3J_{\beta CH-\alpha CH}$	$^3J_{\beta CH-\gamma CH}$
t-Boc	CH_3	1.386 (s)			
L-Val$_1$	$^1\gamma CH_3$	0.864 (d)			7.0 ± 0.1
	$^2\gamma CH_3$	0.955 (d)			7.0 ± 0.1
	βCH	2.363 (m)		7.5 ± 0.1	7.0 ± 0.1
	αCH	4.285 (dd)	9.5 ± 0.1	7.5 ± 0.1	
	NH	5.443 (d)	9.5 ± 0.1		
L-Pro$_2$	γ&βCH_2	1.6 - 2.0 (m)		a	a
	$^\delta CH_2$	c			
	αCH	4.268 (bt)		a	a
				GEMINAL COUPLING	
Gly$_3$	αCH (A)	4.159 (dd)	7.0	-17.0	
	αCH (B)	3.712 (dd)	4.0	-17.0	
	NH (X)	7.523 (bt)	e		
Gly$_4$	αCH (A')	4.047 (dd)	5.0	-18.0	
	αCH (B')	3.814 (dd)	5.5	-18.0	
	NH (X')	7.693 (pt)	e		
OMe	CH_3	3.600 (s)			

[a] Not analyzed. [c] Overlapped with OMe signal: s = singlet, d = doublet, m = multiplet, dd = doublet of a doublet, bt = broad triplet, pt = pseudotriplet. [e] Values obtained by ABX spin analysis of CH_2 proton signals.

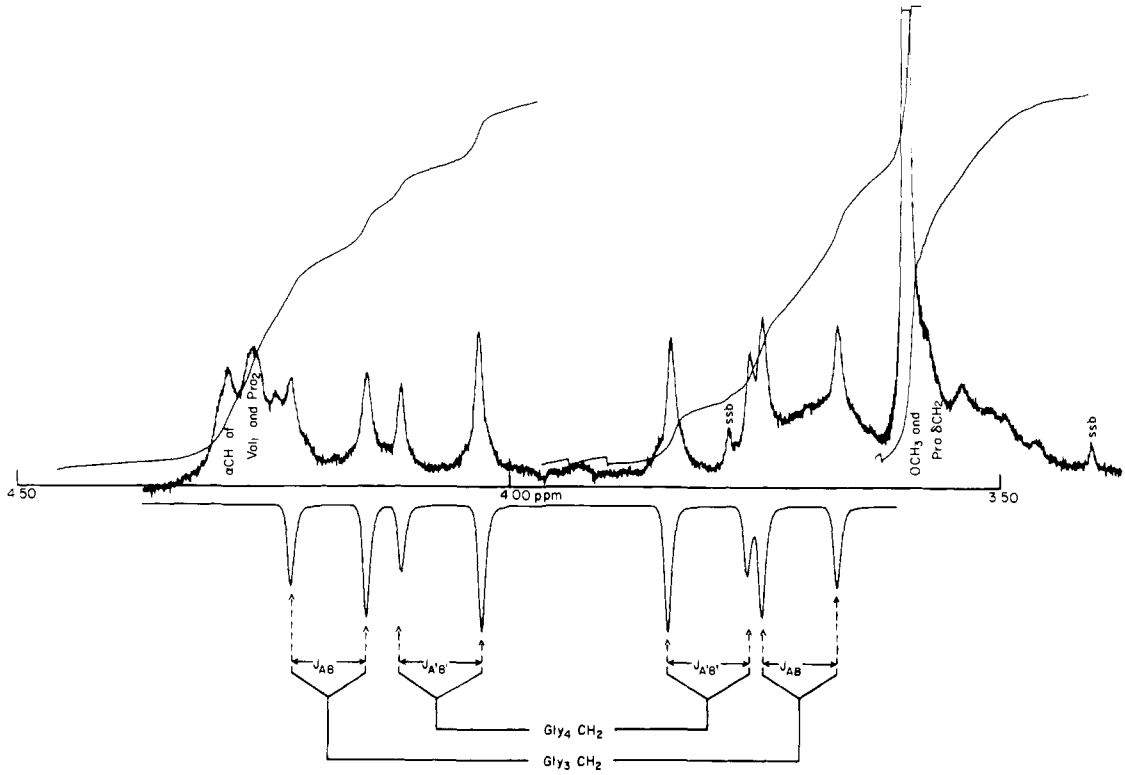

Figure 2. 220-MHz ^{1}H NMR spectrum of *t*-Boc-Val$_1$-Pro$_2$-Gly$_3$-Gly$_4$-OMe (deuterated to remove αCH–NH couplings) in 80% $CDCl_3$ and 20% C_6D_6. The αCH region is shown with the computer simulated spectrum for Gly_3 and Gly_4 CH_2 protons (analyzed as an AB spin system).

cient[10-13] and is less solvent perturbed,[14,15] indicative of H-bond formation, we therefore assigned this pseudotriplet signal at 7.69 ppm to Gly_4 NH. These assignments were confirmed by a $CDCl_3 \rightarrow Me_2SO$-$d_6$ solvent titration on comparison with previous assignments[7] made in Me_2SO-d_6. It can be seen in Figure 1B that the methylene protons of both Gly_3 and Gly_4 appeared as two ABX spin systems comprising 16 lines in the spectrum. The signals of Gly_3 CH_2 are designated as ABX, while those of Gly_4 CH_2 as A′B′X′ (Figure 1B and 1C). The assignments of ABX and A′B′X′ were made by the double resonance experiments, such that irradiation of Gly_3 NH at 7.52 ppm collapses the outer eight lines into a four-line AB spectrum. Similarly, by irradiating Gly_4 NH at 7.69 ppm the inner eight lines collapsed into a four-line A′B′ spectrum. This phenomenon can be best noted in Figure 2, where the two ABX spin systems have been transformed into two AB spin systems (AB for Gly_3 and A′B′ for Gly_4) comprised of eight lines upon deuteration of the NH protons. During this experiment it was also observed that the Gly_3 NH proton exchanged with deuterium faster than the other two NH protons in the spectrum. This is in agreement with a structure in which the Gly_3 NH proton is solvent exposed. The approximate values of chemical shifts and coupling constants were calculated by analyzing the spectrum as an ABX spin system[61] and then by using the approximate values in a computer simulated spectrum. The correct values of chemical shifts and coupling constants were recorded (Table I) when the computer simulated spectrum exactly matched with the experimentally obtained ^{1}H NMR spectrum (Figure 1B and 1C).

The conformational angle, ϕ, for Val_1 (ϕ_1) was calculated by using the Karplus like relation (see eq 1) with the coefficients of Bystrov et al.,[28] i.e.,

$$^3J_{\alpha CH\text{-}NH} = 9.4 \cos^2 \theta - 1.1 \cos \theta + 0.4 \ldots \quad (4)$$

The torsion angles for Gly_3 (ϕ_3) and Gly_4 (ϕ_4) were also calculated by using the coefficients of Bystrov et al.[28] for the glycyl residue

$$^3J_{\alpha CH\text{-}NH} + {}^3J_{\alpha CH'\text{-}NH} = -9.8 \cos^2 \phi - 1.3 \cos \phi + 15.0 \quad (5)$$

Since the $\cos^2$ dependence gives several values of ϕ for a single value of $^3J_{\alpha CH\text{-}NH}$, only those values of ϕ are recorded in Table II, which are in agreement with angles obtained by conformational energy calculations. Using these ϕ torsion angles, the torsion angles ψ were obtained from the Dreiding model of VPGG. These values are also given in Table II.

Table II. Backbone Torsion Angles of VPGG Obtained from ^{1}H NMR and Conformational Energy Calculations[a]

METHODS	L-Val$_1$		L-Pro$_2$		Gly$_3$		Gly$_4$	
	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_4
PMR	-143	120[b]	-60[b]	120[b]	55	30[b]	-128	140[b]
THEORETICAL CALCULATIONS	-130	130	-60	100-110	80-90	40	-130	120

[a] Torsion angles are given in accordance with IUAPC–IUB[54] convention. [b] Angles obtained from the Dreiding model of VPGG.

Low-Energy Conformations. Energy surfaces corresponding to pairs of torsion angles ϕ_1–ψ_1, ϕ_3–ψ_3, and ϕ_4–ψ_4 for Val_1, Gly_3, and Gly_4, respectively, are shown in Figure 3. The energy surface ϕ_1–ψ_1 for Val_1 was initially obtained assuming $\chi_1{}^1$ equal to 180°. As stated above, in the later stages of the calculations the side chain torsion angle $\chi_1{}^1 = 150°$ was found to be preferred. A torsional barrier of 2.8 kcal/mol had been assumed for the side chain rotation.[55] A plot of energy as a function of $\chi_1{}^1$ and ψ_2 for the valyl side chain and Pro_2, respectively, are shown in Figure 4. The ϕ_1–ψ_1 energy surface was recomputed using the new value of $\chi_1{}^1 = 150°$ and was found not to vary from that shown in Figure 3A. Since a tetrahedral bond angle of 109.47° was assumed for the bond angles τ, i.e., C′–C$^\alpha$–N, an attempt was made to relax this criterion using a total minimization scheme originally de-

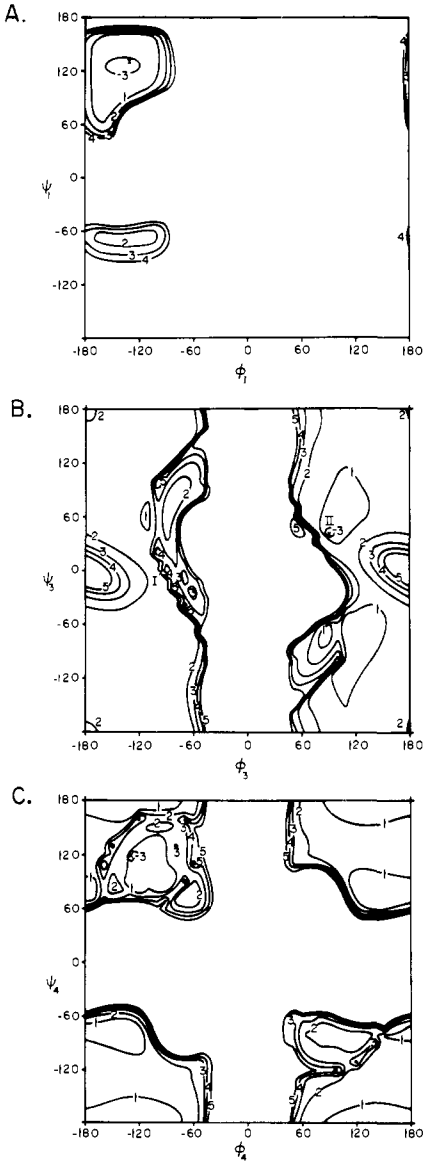

Figure 3. (A) ϕ_1–ψ_1 energy surface in kilocalories/mole relative to the global minimum marked X. Hydrogen-bonded region is indicated by negative contour level. (B) ϕ_3–ψ_3 energy surface in kilocalories/mole relative to the global minimum marked X. Hydrogen-bonded region is indicated by negative contour level. Types I and II β-turns are also marked. (C) ϕ_4–ψ_4 energy surface in kilocalories/mole relative to the global minimum marked X. Hydrogen-bonded region is indicated by negative contour level.

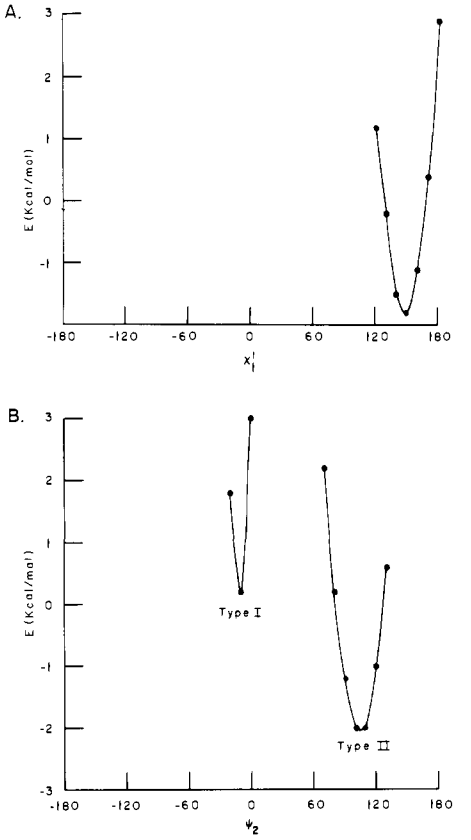

Figure 4. (A) Energy in kilocalories/mole as a function of χ_1' for Val_1 side chain rotation. (B) Energy in kilocalories/mole as a function of ψ_2 for Pro_2 assuming $\phi_2 = -60°$. Types I and II β-turn are also marked.

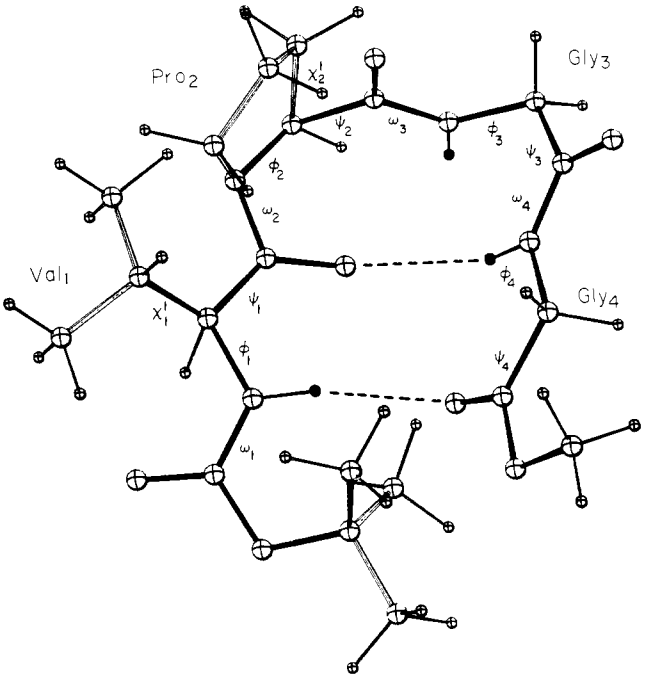

Figure 5. Stereoscopic perspective for the minimum energy conformation of VPGG. 1 → 4 and 4 → 1 hydrogen bonds are also shown.

scribed by Boyd[62] and subsequently adapted for peptides.[43] The stereoscopic drawing of the minimum energy conformation with the stabilizing hydrogen bonds so obtained is shown in Figure 5. In Table II the torsion angles of the minimum energy conformation are compared with torsion angles derived from proton magnetic resonance studies on VPGG. The torsion angles are given in accordance with the IUPAC-IUB convention.[54]

Discussion

Two secondary structural features of VPGG can be derived from the ^1H NMR spectral analysis as well as from conformational energy calculations of this molecule. The nonequivalence of both the Gly_3 CH_2 and Gly_4 CH_2 protons indicates that the molecule takes up a fixed conformation, similar to a cyclic peptide[63] with the formation of two H bonds. A ten-membered (C_{10}) H bond,[64,65] commonly referred to as a β-turn, involving Val_1 C=O and Gly_4 NH are apparent from the temperature dependence and solvent perturbation of both Val_1 NH and Gly_4 NH protons (see Results section). This conformation also emerges from an analysis of the results of the theoretical calculations presented in Figures 3 and 4. A β-turn in a linear peptide can occur in two forms,[64,65] type I and type II, which are related by an approximate 180° rotation of the peptide moiety between residues two and three of the β-turn.

From the values of torsion angles (Table II) ϕ_2 and ψ_2 of Pro_2 (Figure 4B) and ϕ_3 and ψ_3 of Gly_3 (Figure 3B), a β-turn of type II can be assigned to VPGG involving Pro_2 and Gly_3 at the corners as shown in Figure 5. This observation is in agreement with the proposal of Venkatachalam[64] and Geddes et al.[65] that a type II β-turn is formed when the sequence L-X-Gly occurs at the corners.

Experimentally, nuclear Overhauser enhancement (NOE) measurements on repeat tetra-, penta-, and hexapeptides of elastin[21] and conformational energy calculations on a repeat pentapeptide of elastin[20] have demonstrated the occurrence of this Pro_2-Gly_3 type II β-turn. The theoretical calculations on VPGG have shown that the type I β-turn is less stable by ~2.1 kcal/mol than the type II β-turn (see Figure 4B). In Figure 3B type I and type II β-turns are also indicated and the same approximate energy difference is seen between the two forms. However this result is dependent on the dielectric constant chosen for the calculation of the electrostatic energy component. In view of the ^{1}H NMR studies carried out in $CDCl_3$, which has a low dielectric constant, the comparison of the theoretical calculations with experimental studies is expected to be valid as argued in a previous study.[45] The observation of geminal coupling constants (see Table I) of −17.0 Hz for the Gly_3 CH_2 and −18.0 Hz for the Gly_4 CH_2 protons is also indicative[66,67] of a fixed structure with intramolecular H bonds. It is observed from the theoretical calculations that the 4 → 1 and 1 → 4 H bonds are both nonlinear with hydrogen bond lengths of 2.90–3.05 Å and hydrogen bond angle θ near 30°. Hydrogen bond energies ranging from −2.50 to −3.40 kcal/mol were obtained for the 1 → 4 and 4 → 1 hydrogen bonds, depending on the hydrogen bond length R and hydrogen bond angle θ, thereby contributing significantly to the stability of H-bonded conformations. A similar study of the energetics of β-turn conformations in a tripeptide with alanyl side chains by Chandrasekharan et al.[53] suggests that the minimum energy conformation usually has a somewhat nonlinear H bond and the observations on VPGG are in line with their conclusions.

The observations of $^3J_{\alpha CH\text{-}NH}$ for Val_1, Gly_3, and Gly_4 give an approximate torsion angle, ϕ, for these amino acid residues. Since multiple angles can be derived for a single value of $^3J_{\alpha CH\text{-}NH}$, the most probable values of the torsion angle, ϕ, have been obtained by comparison with the theoretically calculated torsion angles (Table II). Using these ϕ angles and taking into account the formation of the two H bonds (mentioned above), a Drieding model of VPGG was constructed from which the corresponding approximate values of the torsion angle, ψ, were obtained and also listed in Table II. The theoretically derived results presented in Figure 3 show a cluster of low energy conformations with slight differences in the backbone torsion angles. Torsion angles corresponding to the minimum energy conformation are included in Table II for comparison with the angles obtained from ^{1}H NMR results. From this comparison it can be observed that the torsion angles ϕ_1, ψ_1, ϕ_4, and ψ_4 indicate the formation of an antiparallel β-pleated sheet (Figure 5). The $^2J_{A'B'}$ of −18.0 Hz for Gly_4 CH_2 protons also indicates that the two methylene protons of Gly_4 are staggered with respect to its adjacent C=O group,[67] giving an extended type conformation[63] for the Gly_4 residue. This is also supported by measurement of ϕ_4 from its $^3J_{\alpha CH\text{-}NH}$ values (Tables I and II). The observation of $^3J_{\alpha CH\text{-}NH}$ of 9.5 Hz for Val_1 strongly suggests a β-pleated conformation which in combination with Gly_4 (through the formation of two H bonds, discussed earlier) gives rise to an antiparallel β-pleated sheet[32] for *t*-Boc-VPGG-OMe in solution. The reason for Val_1 and Gly_4 residues adopting an antiparallel β-pleated sheet conformation can be understood from a detailed study of the ϕ_1–ψ_1, ϕ_3–ψ_3, and ϕ_4–ψ_4 energy surfaces presented in Figure 3. The ϕ_1–ψ_1 energy surface (Figure 3A) is somewhat similar to the ϕ–ψ plot for an alanyl residue preceding a prolyl residue (see ref 56 and literature cited therein). The ϕ_1–ψ_1 energy surface (Figure 3A) shows an allowed low energy region on the upper left side commonly known as β-pleated sheet region around ϕ_1 = −70 to −180° and ψ_1 = 40–170° where the minimum occurs. This region is preferentially stabilized by the occurrence of hydrogen bonding. In addition a secondary region, 2–4 kcal/mol higher than the global minimum, occurs around ϕ_1 = −180 to −85° and ψ_1 = −50 to −90°. The 1 → 4 H bond (C_{14}) occurs from a combination of the local conformational feature of Val_1 residue and the strong electrostatic and van der Waals attractive interaction between Val_1 NH and Gly_4 C=O. The interaction of the Val_1 side chain with the prolyl side chain and the bulky tertiary butyl group is in large part responsible for this conformational feature.

The observation of a 7.5-Hz (Table I) coupling constant between αCH and βCH protons of Val_1 indicates either a 55% resident time of its side chain (angle χ_1^1) in trans form[68] or the angle χ_1^1 is locked in an off trans conformation. From the conformational energy calculations for the Val_1 side chain, χ_1^1, shown in Figure 4A, it is evident that the appearance of a low energy conformation around χ_1^1 = 150° is in agreement with the observed value of $^3J_{\alpha CH\text{-}\beta CH}$ of 7.5 Hz and a fixed side chain orientation.

Conclusion

The results of ^{1}H NMR spectral analysis and conformational energy calculations together show a unique antiparallel β-pleated sheet conformation for *t*-Boc-VPGG-OMe in $CDCl_3$, with a type II β-turn involving Pro_2 and Gly_3 at the corners. From a detailed study of temperature dependence of peptide NH protons and carbonyl carbons of poly VPGG (PTP) it was proposed[19] that this polypeptide (PTP) in water at an elevated temperature adopts a "cross-β-structure" showing each tetramer unit essentially as an antiparallel β-pleated sheet. The results reported here for the conformation of tetramer VPGG in $CDCl_3$ lends support to the previous conclusions of the β-turn and the proposal of a cross-β-structure (see Figure 7C of ref 19). The spectral features of Gly_3 CH_2 and Gly_4 CH_2 protons are of primary importance in detailing the total conformation of this molecule. The two CH_2 groups are quite different. In the case of Gly_3, one αCH is cyclohexane-like[69] ("quasiequatorial") and the other is "quasiaxial" with respect to the adjacent C=O group. In the case of Gly_4, the αCH_2 protons are more or less staggered with respect to the adjacent C=O group.[67] The chemical shift differences between the two methylene protons of both Gly_3 and Gly_4 (Table I) show the similarity with those of cyclic peptides containing glycine as one of the residues.[63,70,71] This is largely due to the magnetic anisotropy effect of the adjacent C=O group[71,72] which shows that the proton in the plane of the C=O group is shielded more than the one which is out of the plane. This is in good agreement with the previous findings.[63,70-73] This effect will find increasing application in future work on the investigation of conformation of peptides in solution.

It bears reemphasis that the secondary structure derived in detail in this work for the tetrapeptide in $CDCl_3$ by proton magnetic resonance and in vacuo by conformational energy calculations is the same as previously derived for the high polymer of the tetrapeptide in water by means of proton and carbon-13 magnetic resonance studies which were specifically designed for determining secondary structure (see Figure 7C of ref 19). Thus, while solvent does vary the probability of occurrence of the conformation, the β-turn has been shown to be the preferred conformation in solvents as diverse as chloroform, methanol, dimethyl sulfoxide, and water. Specifically for the polytetrapeptide in water, the β-turn (the ten atom

hydrogen-bonded ring) is approximated to occur 70% of the time below 50 °C and 90% above 50 °C and the 14 atom hydrogen-bonded ring is approximated to occur 60% of the time above 50 °C (see Table IV of ref 19).

Acknowledgment. This work was supported by National Institutes of Health Grant HL-11310. Our thanks to Drs. D. A. Buckingham and G. J. Gainsford, Australian National University, Canberra, Australia for providing us a copy of the minimization program.

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