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Context-dependent conformation of diethylglycine residues in peptides

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Key words: diethylglycine residues; extended C_5 conformations; NMR of peptides; peptide conformation; peptide crystal structure

Abstract: Diethylglycine (Deg) residues incorporated into peptides can stabilize fully extended (C_5) or helical conformations. The conformations of three tetrapeptides Boc-Xxx-Deg-Xxx-Deg-OMe (Xxx = Gly, GD4; Leu, LD4 and Pro, PD4) have been investigated by NMR. In the Gly and Leu peptides, NOE data suggest that the local conformations at the Deg residues are fully extended. Low temperature coefficients for the Deg(2) and Deg(4) NH groups are consistent with their inaccessibility to solvent, in a C_5 conformation. NMR evidence supports a folded β-turn conformation involving Deg(2)-Gly(3), stabilized by a 4→1 intramolecular hydrogen bond between Pro(1) CO and Deg(4) NH in the proline containing peptide (PD4). The crystal structure of GD4 reveals a hydrated multiple turn conformation with Gly(1)–Deg(2) adopting a distorted type II/II′ conformation, while the Deg(2)–Pro(3) segment adopts a type III/III′ structure. A lone water molecule is inserted into the potential 4→1 hydrogen bond of the Gly(1)–Deg(2) β-turn.

The incorporation of C^{α}-tetrasubstituted α-amino acids into peptides introduces local backbone stereochemical constraints which are useful in designing predetermined backbone conformations. The α-aminoisobutyryl (Aib) residue has been widely used in the design of β-turn and helical conformations (1–3). In the case of the higher homologs of Aib like α,α-diethylglycine (Deg), α,α-dipropylglycine (Dpg), both fully extended (C_5) and folded helical conformations have been characterized in crystal structures (4–15). Theoretical calculations suggest that for the higher dialkyl glycines the minima in the C_5 and the helical region are

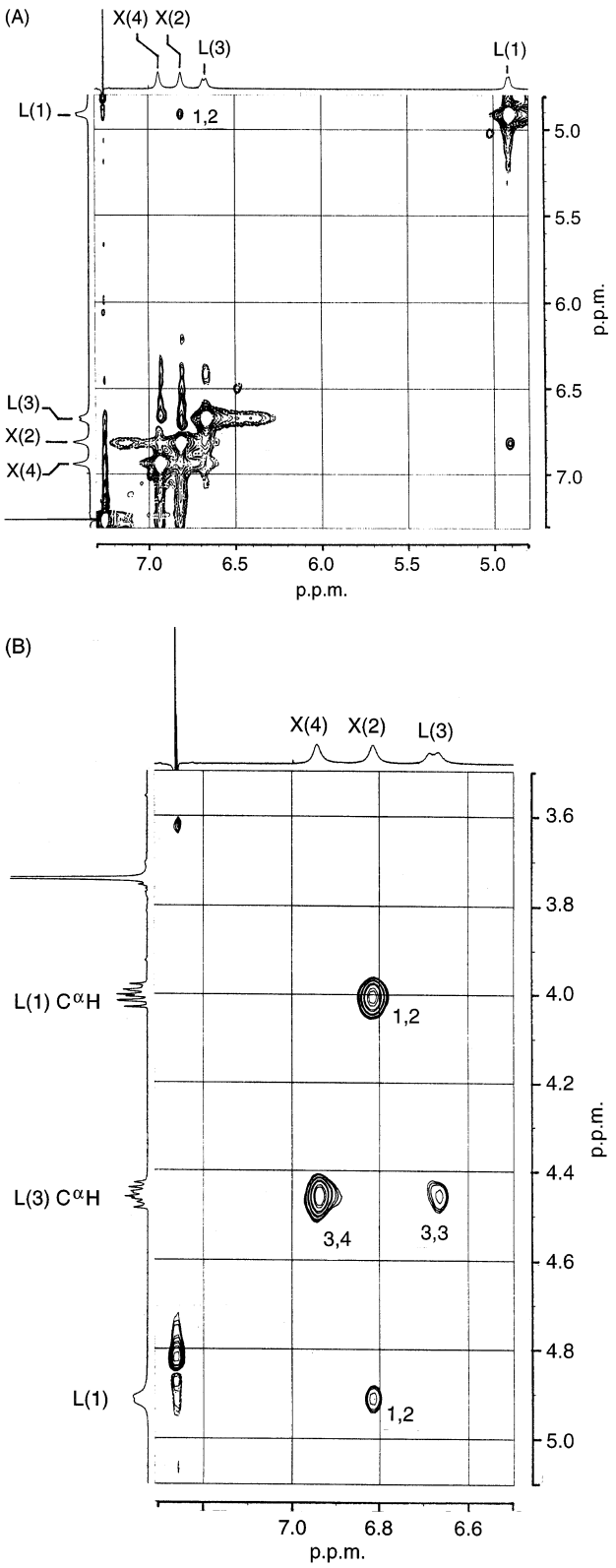

Figure 1. (A) Partial 400 MHz ^{1}H-^{1}H ROESY spectrum of LD4 in $CDCl_3$ showing $N_iH \leftrightarrow N_{i+1}H$ connectivities (X = Deg). (B) Partial 400 MHz ^{1}H-^{1}H ROESY spectrum of LD4 in $CDCl_3$ showing $N_iH \leftrightarrow C^{\alpha}H$ connectivities (X = Deg).

of comparable energy. A pronounced bond angle dependence of the backbone conformation has been suggested by both molecular mechanics (16) and molecular dynamics calculations (17). Local sequence and the environmental factors may play a major role in determining the nature of the conformations adopted at the Dxg residues. As part of a program to probe the context-dependent conformation of Dxg residues, we have investigated short peptides with Deg incorporated at alternate positions. In this report we describe the conformational analysis of three tetrapeptides Boc-Xxx-Deg-Xxx-Deg-OMe (Xxx = Gly, GD4; Leu, LD4 and Pro, PD4). Alternating sequences were chosen to facilitate comparison of the effect of the xxx residue on Deg conformation and also to explore chain length effects. In the present study, attention is focussed primarily on Deg at position 2, since the C-terminus residue is in a conformationally irrelevant position in the tetrapeptide. While the Deg conformations in the Gly and Leu peptides appear to be largely extended in solutions, in the case of the Pro containing sequence, folded conformations appear to be populated. A crystal structure of GD4 reveals a hydrated multiple β-turn conformation, with both Deg residues adopting local helical conformations.

Experimental Procedures

Diethylglycine and derivatives were prepared following procedures described earlier (18). All peptides were synthesized by conventional solution phase procedures, using dicyclohexylcarbodiimide-1-hydroxy benzotriazole mediated couplings, purified by medium pressure liquid chromatography on a reverse phase C_{18} (40–60μ) column and checked for homogeneity by HPLC (C_{18} column, 5μ) (17).

Table 1. **Torsion angles (°) in Boc-Gly-Deg-Gly-Deg-OMe**

Residue	φ	ψ	ω	χ^1	$\chi^{1'}$
Gly (1)	79	177	179		
Deg (2)	–63	–24	179	55	–178
Gly (3)	–83	0	174		
Deg (4)	53	43	176	–59	–174

The torsional angles for rotation about bonds of the peptide backbone (φ, ψ, ω) and about the bonds in the Dpg side chains (χ^1, $\chi^{1'}$) are as defined by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* 1970, **9,** 3471–3479. Estimated standard deviations ≈ 1.0°. Torsion angles are indicated for one enantiomeric conformation of the achiral peptide in the centrosymmetric crystal.

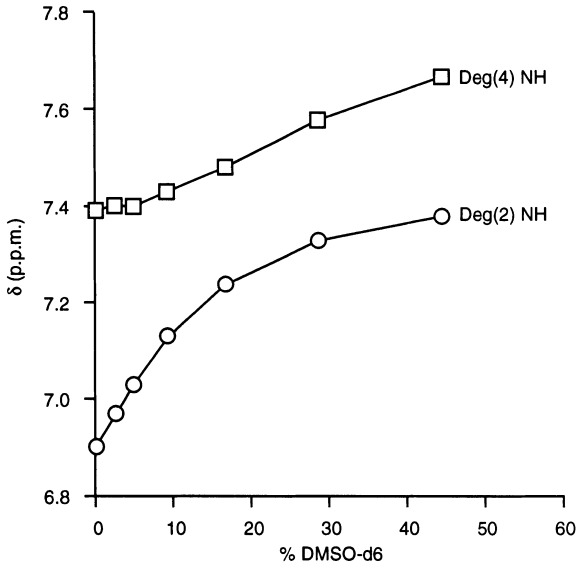

Figure 2. Solvent dependence of NH chemical shifts in peptide PD4 in $CDCl_3$ showing their dependence on $(CD_3)_2SO$ concentration.

The peptides were characterized by complete assignment of 400 MHz ^1H NMR spectra. Attempts were made to grow single crystals of all three peptides by slow evaporation from a variety of solvents. Diffraction quality crystals were obtained only in the case of GD4 from $MeOH/H_2O$.

NMR studies

All NMR studies were carried out on a Bruker AMX-400 spectrometer. Peptide concentrations were in the range of 7–8 mM and the probe temperature was maintained at 298 K. Resonance assignments were done using two dimensional ROESY spectra. 2D data was collected in phase sensitive mode using the time proportional phase incrementation method. A total of 512 data points with 64 transients were collected. Spectral widths were in the range of 4500 Hz with a spin lock time of 300 ms. Zero filling was done to yield data sets of $1\,K \times 1\,K$ using square sine bell window.

Circular dichroism

Circular dichroism studies were carried out on a JASCO 500 spectrometer. A quartz cuvette of 1 mm path length was used for all the experiments. Sample concentrations for the CD experiments were in the range of 1.6–2.4 mM.

X-ray studies

Crystals of Boc-Gly-Deg-Gly-Deg-OMe were grown from $MeOH/H_2O$ by slow evaporation. X-ray diffraction data were collected on a Siemens R3m/V diffractometer (19) with MoKα radiation. Refined unit cell parameters were determined by a least squares fit of the angular setting of 25 accurately determined reflections in the range of $2\theta \leq 25^\circ$. Three dimensional intensity data were collected up to $2\theta = 45^\circ$ using the $\omega - 2\theta$ scan mode. Two standard reflections were monitored during every 98 intensity measurements. The intensity decay was less than 1%. Crystal parameters are: Space group $P2_1/n$, a(Å) = 10.121(1), b(Å) = 22.456(3), c(Å) = 13.281(1), $V(Å^3) = 2889.5(9)$, $D_{calc} = 1.12\ g/cm^3$, $M_W = 488.6$ with $Z = 4$. Out of 3801 unique reflections collected, 1270 reflections had $I > 2\,\sigma(I)$.

The structure was solved by SHELXS-86 (20) and refined isotropically and anisotropically using SHELXL-93 (21). Full matrix least squares refinement was carried out for all the non-hydrogen atoms. All the hydrogen atoms were geometrically fixed and included as riding atoms and the temperature factor for these were assigned as the isotropic equivalent temperature factor of the corresponding non-hydrogen atom to which these were attached. All these hydrogens were included for the structure factor calculation. The function minimized was $\Sigma\, w(|Fo|^2 - |Fc|^2)^2$. For 891 ($I \geq 3\,\sigma\,(I)$) reflections the *R*-value is 7.3%. An *R*-value of 10.3% was obtained using 1270 reflections ($I \geq 2\,\sigma\,(I)$). Coordinates are deposited in the Cambridge Crystallographic Data Center. The torsional angles and hydrogen bonds are listed in Tables 1 and 2, respectively.

Results and Discussion

NMR studies

Assignments of all backbone protons in the peptides GD4, LD4 and PD4 were readily accomplished at 400 MHz in $CDCl_3$ solution using ROESY experiments to establish near neighbor connectivities. The high field chemical shift of the urethane NH (residue 1) in peptides GD4 and LD4 in $CDCl_3$ also provides a convenient starting point for sequential assignments. In the case of PD4 NOEs between the $C^\delta H_2$ protons of Pro residues and Deg NH groups were used to establish sequence specific assignments. The chemical shifts of NH and $C^\alpha H$ protons are summarized in Table 3. In the case of the peptides GD4 and LD4 addition of small amounts of DMSO to $CDCl_3$ solutions resulted in a pronounced downfield shift of the NH protons of residues 1 and 3 (Gly/Leu). In both peptides Deg NH groups of residues 2 and 4 did not show a significant solvent-dependent chemical shift. Hydrogen bonding solvents like DMSO are expected to perturb the chemical shifts

Table 2. **Hydrogen bond parameters in Boc-Gly-Deg-Gly-Deg-OMe**

Type	Donor	Acceptor	N····O(Å)	H····O(Å)	N-H····O(°)
Intramolecular	N4	O_1	3.01	2.17	167
Intermolecular	N1	O_4[a]	3.01	2.35	134
	N2	O1W[b]	2.96	2.11	167
	N3	O1W[c]	2.97	2.22	145
	O1W	O_0	2.77	Hydrogens not not located	

a. Symmetry equivalent at x−1, y, z. b. Symmetry equivalent at −x−1, -y+1, −z+2. c. Symmetry equivalent at x, y, z.

of exposed NH groups, while inaccessible NH groups should remain unaffected (22) The observed pattern of perturbation of chemical shifts of NH is not compatible with classical folded conformations (like β-turns) observed in short peptides, since Deg(2) would be expected to be exposed. The possible involvement of peptide NH groups in intramolecular hydrogen bonds was also probed in DMSO using temperature coefficient chemical shifts (dδ/dt) summarized in Table 3. Once again in GD4 and LD4 the Deg(2) and Deg(4) NH groups exhibit much lower dδ/dt values than Gly/Leu NH groups. Figure 1(A) shows a partial ROESY spectrum of LD4 in $CDCl_3$ which illustrates the relatively low intensity of inter-residue $N_iH\ddot{I}\ N_{i+1}H$ (d_{NN} connectivities). In contrast Fig. 1(B) demonstrates that the inter-residue $C_i\alpha H\ddot{I}\ N_{i+1}H$ NOEs are intense. Furthermore, the inter-residue $d_{\alpha N}$ NOEs are appreciably more intense than the intra-residue $d_{\alpha N}$. Exactly similar results were obtained for peptide GD4. The observed NOE patterns are consistent with an extended peptide structure. Folded helical conformations at Deg residues must yield strong d_{NN} NOEs. Together with the solvent and temperature dependence of NH chemical shifts, the NOE data supports largely extended structures in peptides GD4 and LD4. The apparently solvent shielded nature of the two peptides may be rationalized by noting that in C_5 conformations the NH groups lie very close to the CO group of the same residue, resulting in hindrance to solvation due to steric and electronic effects. The proline containing peptide PD4 behaves in a distinctly different manner. Figure 2 shows the solvent-dependent chemical shifts of the two Deg NH groups upon addition of DMSO and $CDCl_3$ solutions. It is clear that at low concentration of DMSO the Deg(2) NH shows a dramatic downfield shift, while Deg(4) NH shows a discontinuity at ≈10% DMSO suggestive of a solvent induced conformational change. Figure 3 shows the partial ROESY spectrum of peptide PD4 in $CDCl_3$ solution. A strong NOE is observed between Pro(3) $C^{\delta}H_2$ and Deg(4) NH while weaker NOE is observed between Pro(1) $C^{\delta}H$ and Deg(2) NH. These NOEs are characteristic of local helical conformations at the Deg residues. It may be noted that the $C^{\delta}H_2$ group in Pro occupies a position analogous to that of the NH hydrogen in the other amino acids. A strong NOE is also observed between Pro(1) $C^{\alpha}H$ and Deg(2) NH which is consistent with semi-extended Pro(1) ($\psi = 120°$). The NOE data together with the solvent titration results suggest that a significant population of Deg(2)–Pro(3) β-turns may be present in solution, involving Deg(4) NH in an intramole-

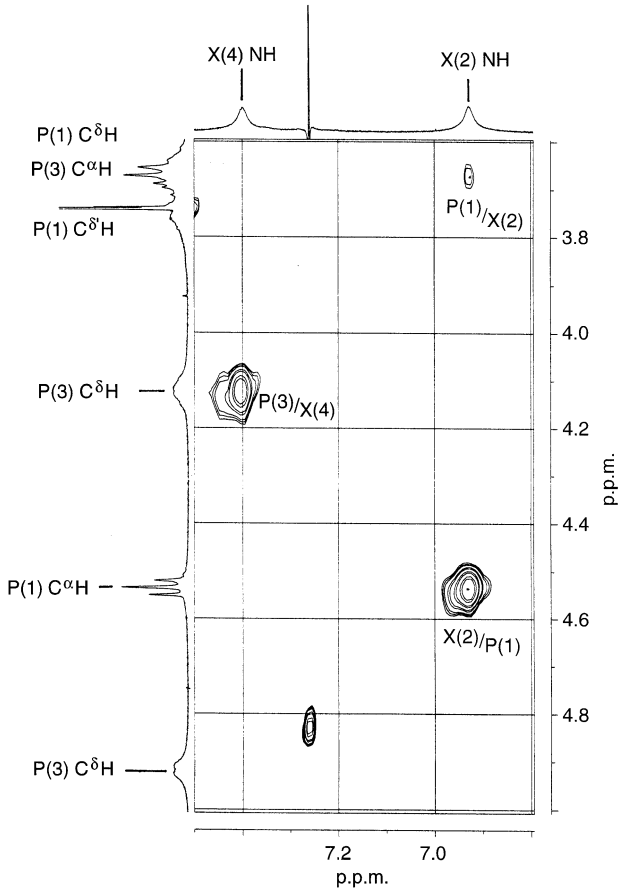

Figure 3. Partial 400 MHz ^1H-^1H ROESY spectrum of PD4 in $CDCl_3$ showing $N_iH \leftrightarrow C^{\alpha}H$, $C^{\beta}H$; $N_iH \leftrightarrow C^{\delta}H$ connectivities.

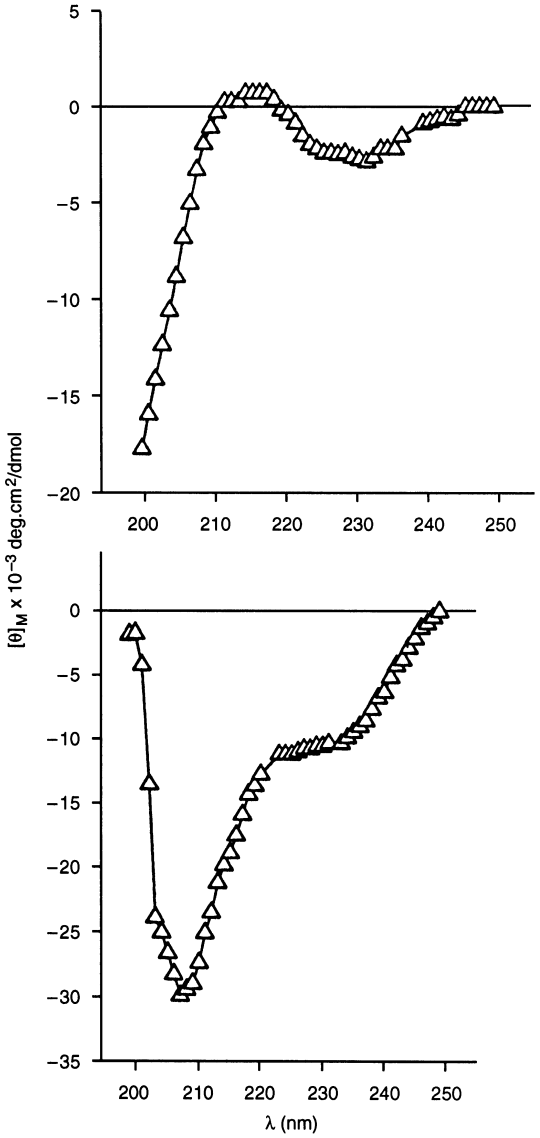

Figure 4. CD spectrum of LD4 in methanol (top) and PD4 in methanol (bottom).

cular 4→1 hydrogen bond with Pro(1) CO. The only hydrogen bonded β-turn type accessible for the Deg-Pro sequence is a type I/III β-turn. Since Deg is unlikely to adopt the $\phi = +60^\circ$, $\psi = -120^\circ$ conformation necessary for a type II′

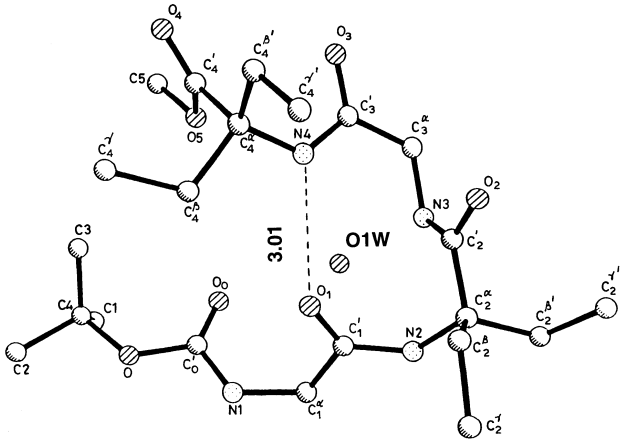

Figure 5. Molecular conformation of Boc-Gly-Deg-Gly-Deg-OMe (GD4) in crystals.

structure which maintain L-Pro in the α_R conformation. Similar type III β-turn conformations have been observed for the Aib-Pro segment in several short peptides (1, 23–25), which require local helical conformation ($\phi \approx -60^\circ$, $\psi \approx -30^\circ$) at both Deg(4) and Pro(5). In DMSO, the observed dδ/dt values for both the Deg NH groups in peptide PD4 are lower, a feature already noticed for the Gly and the Leu analogs. This suggests that in the strongly solvating medium the proline-containing peptide also adopts a fully extended conformation at Deg(2) and Deg(4). Differences in the conformational behavior of peptides LD4 and PD4 are also evident in methanol, where dramatically different CD spectra are obtained (Fig. 4). While the CD spectrum of LD4 is characteristic of extended or unordered peptide structures PD4 exhibits distinct minima at nearly 230 nm and 210 nm suggestive of β-turn conformations (26).

Crystal structure of GD4

Figure 5 shows the molecular conformation in crystals. The backbone torsion angles are summarized in Table 1 and hydrogen bond parameters in Table 2. Figure 6 shows the

Table 3. **NMR parameters for peptides Boc-Xxx-Deg-Xxx-Deg-OMe (Xxx = Gly, Leu, Pro)**

	Chemical shifts δ (p.p.m.) ($CDCl_3$)						−dδ/dt (p.p.b./k)			NOEs (CDCl3)					
	NH			$C^{\alpha}H$			$(CD_3)_2$ SO			$N_iH \leftrightarrow N_{i+1}H$			$Ci^{\alpha}H \leftrightarrow N_{i+1}H$		
Xxx Residues	Gly	Leu	Pro	Gly	Leu	Pro	Gly	Leu	Pro	Gly	Leu	Pro	Gly	Leu	Pro
Xxx	5.42	4.91	–	3.77	4.00	4.55	3.6	5.0	–	w	w	–	s	s	s[a]
Deg	6.95	6.81	6.92	–	–	–	2.6	2.8	2.3	NO	NO	–	–	–	–
Xxx	7.13	6.67	–	3.93	4.45	3.65	3.5	3.8	–	NO	NO	s[b]	s	s	NO
Deg	6.92	6.94	7.40	–	–	–	2.5	2.3	2.6	–	–	–	–	–	–

a. NOE between P(1)$C^{\alpha}H \leftrightarrow$ Deg (2)NH. b. NOE between P(3)$C^{\delta}H \leftrightarrow$ Deg(4)NH.

Table 4. **Observed Deg backbone conformations in peptide crystal structures**

	Torsional angle (°)			
Peptide	**ϕ**	**Ψ**	**τ[a]**	**Reference**
Tfa-Deg-OH	179.0	–179.0	103.3	(6)
Tfa-$(Deg)_2$-OtBu	173.0	–178.0	101.3	(6)
	–55.0	–46.0	110.7	
Tfa-$(Deg)_3$-OtBu	180.0	180.0	102.4	(6)
	180.0	180.0	104.8	
	180.0	180.0	103.2	
Tfa-$(Deg)_4$-OtBu	–178.0	–174.0	101.9	(6)
	–172.0	172.0	102.7	
	179.0	172.0	104.2	
	177.0	–178.0	103.3	
Tfa-$(Deg)_5$-OtBu	179.0	–171.0	102.3	(6)
	176.0	–174.0	101.7	
	–173.0	180.0	102.1	
	179.0	170.0	103.0	
	175.0	–178.0	103.9	
Tfa-$(Deg)_2$-Abu-$(Deg)_2$-OtBu	–54.0	–40.8	109.6	(5)
	–57.8	–19.9	11.7	
	–61.6	–24.0	110.8	
	53.2	44.7	109.5	
Boc-Gly-Deg-Gly-Deg-Ome	–63.0	–24.0	110.0	This study
	53.0	43.0	109.0	

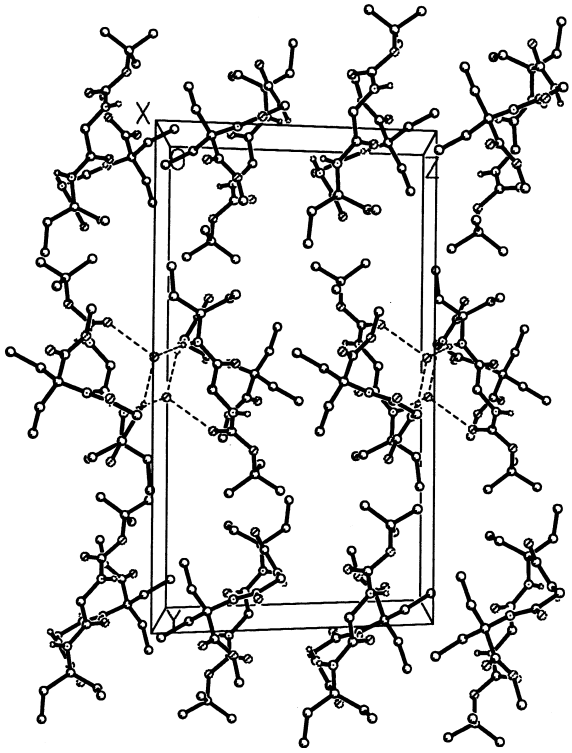

Figure 6. Packing of peptide Boc-Gly-Deg-Gly-Deg-OMe viewed down the '*x*' axis. The broken lines indicate intermolecular hydrogen bonds. Water molecules are depicted as open circles.

view of the molecular packing in crystal down the '*x*' axis. Inspection of the torsion angles in Table 1 reveals that the Deg(2)–Gly(3) segment adopts the type I/I′ β-turn conformation (note that the achiral peptide adopts two enantiomeric folded conformations in the centrosymmetric crystal) stabilized by an intramolecular $4 \rightarrow 1$ hydrogen bond between Gly(1) CO and Deg(4) NH. The conformational angles for Gly(1) and Deg(2) are not far from those expected for a type II/II′ conformation ($\phi_{i+1} = \pm 60^\circ$, $\psi_{i+1} = \pm 120^\circ$; $\phi_{i+2} = \pm 80^\circ$, $\psi_{i+2} = 0^\circ$). The observed distortion of the Gly(1) ψ angle arises from the insertion of a water molecule into the potential $4 \rightarrow 1$ hydrogen bonding site. The observed N...O distance between Gly(3) N and Boc CO is 4.7 Å. The inserted water molecule forms a strong hydrogen bond to both Boc CO and Gly(3) NH groups. Figure 7 shows a view of the water insertion into the potential β-turn. Such water insertions into β-turn and helical structures are not uncommon (10, 27, 28). The structure of GD4 in crystals may be formally described as a hydrated multiple β-turn conformation of the class, type II′–type I or type II–type I′ for the enantiomeric molecules. The bond angles (τ) N-C^α-C′ at the C^α atoms at the two Deg residues are 109.9° and 108.6°,

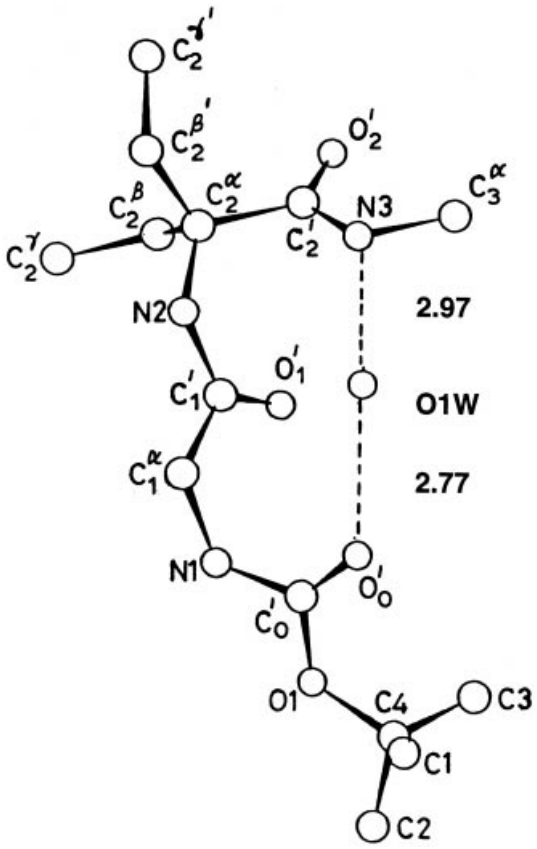

Figure 7. View of the water insertion into the potential β-turn.

respectively. Table 4 compares the observed bond angles at Deg residues reported so far in peptide crystal structures. The relationship between bond angle and local backbone conformation was first noted in the conformational energy calculations for Dxg residues and has also been experimentally observed in crystal structures of Dpg-containing peptides (4). In the case of Deg residues relatively few crystal structures have been reported so far.

Conclusions

The present study reveals that the peptide GD4 adopts distinctly different conformations in solution and in crystals. Extended conformations at Deg appear to predominate in solution, while in crystals both Deg residues adopt local helical conformations. This observation once again emphasizes the fact that the two conformational energy minima are characterized by comparable energies and that environmental effects play a major role in determining the precise structure.

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