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NMR Analysis of a Conformational Transition in an Acyclic Peptide. Model System for Studying Helix Unfolding

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The stabilization of helical structures in short apolar peptides is readily achieved by introduction of α,α-dialkylamino acids. The use of stereochemically constrained residues in conjunction with conformationally flexible segments permits the design of peptides that are poised to undergo structural transitions. The octapeptide Boc-Leu-Ac_8c-Val-Gly-Gly-Leu-Ac_8c-Val-OMe (Ac_8c = 1-aminocyclooctane-1-carboxylic acid) incorporates residues with contradictory conformational tendencies. NMR analysis in $CDCl_3$, using nuclear Overhauser effects and delineation of hydrogen-bonded NH groups establishes a 3_{10}-helical conformation. In a polar strongly solvating medium, like DMSO, the helix unfolds. Studies in $CDCl_3$/DMSO mixtures provide clear evidence for a solvent dependent conformational transition. Amide NH chemical shifts and temperature coefficients at varying solvent composition allow a detailed structural analysis of the unfolding process. The intrinsic fragility of the octapeptide helix provides an opportunity to examine invasion of the helix backbone by water molecules. Studies in DMSO solution containing low concentrations of water establish that preferential water peptide interactions may indeed be present.

Introduction

The potential energy surfaces for short acyclic peptides possess multiple minima separated by relatively low activation barriers[1]. As a consequence, short linear peptide sequences exist in solution as interconverting mixtures of multiple conformational species. Conformational analysis by NMR methods is then complicated by averaging of spectroscopic parameters.[2,3] Experimental studies that permit characterization of distinct conformational states are of value in probing stereochemical space and in characterizing molecular conformations associated with various energy minima. Molecular dynamics simulations, using increasingly efficient algorithms, provide a means of theoretically exploring conformational space.[4] In contrast, experimental approaches are limited to characterization of multiple conformations in crystals[5−7] or in interpretations of conformational transitions in short peptides.[8,9] In this report we describe solvent dependent structural transitions in a designed octapeptide Boc-Leu-Ac_8c-Val-Gly-Gly-Leu-Ac_8c-Val-OMe, **1** (Ac_8c = 1-aminocyclooctane-1-carboxylic acid).

The incorporation of nonprotein amino acid residues may be used to impart conformational rigidity to polypeptide backbones.[10−12] In particular α,α dialkylated residues have been widely studied as stabilizers of helical conformations in oligopeptides.[13−16] In peptide **1**, the incorporation of 1-aminocyclooctane-1-carboxylic acid at positions 2 and 7 constrains the local residue conformation to the right- or left-handed 3_{10}/α-helical region of $\phi-\psi$ space. The central Gly-Gly segment was chosen to provide an element of structural flexibility.[17,18] The juxtaposition of residues with contrasting stereochemical properties permits the setting up of a model situation where a conformational transition is realized by modulating solvent conditions. The studies described in this report establish a conversion from a 3_{10}-helical conformation in a poorly hydrogen-bonding solvent $CDCl_3$ to a multiple turn conformation in a strongly hydrogen-bonding solvent, DMSO. The use of organic solvents permits an evaluation of the effect of solvent−solute hydrogen bonding on the helix unfolding process.

Experimental Section

1-Aminocyclooctane-1-carboxylic acid and its derivatives were synthesized as previously described.[19] Peptide **1** was synthesized by conventional solution phase procedures, as described earlier for the Aib analogue,[18] and purified by medium-pressure liquid chromatography on a reverse phase column (C_{18}, 40−60 μm) using methanol−water gradients. The peptide was obtained as a white crystalline solid, homogeneous by analytical HPLC on a C_{18}, 5 μm column. The peptide was characterized by complete assignment of the 400 MHz ^{1}H NMR spectrum.

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 DQF COSY[20,21] and ROESY[22,23] spectra. All 2D data were collected in phase sensitive mode using the time proportional phase incrementation (TPPI) method.[24] Sets of 1024 and 512 data points were used in the t2 and t1 dimensions, respectively. For DQFC and ROESY 24 and 64 transients were collected, respectively. Spectral widths were about 4500 Hz. A spin lock time of 300 ms was used in ROESY. Zero filling was done to finally yield a data set of 1K × 1K. A shifted square sine bell window was used before processing.

The peptide model was built on a Silicon Graphics workstation using the INSIGHTII module. The Ac_8c residue was built separately and energy minimized. This was appended at positions 2 and 7 along with the rest of the residues. Protecting groups, *tert*-butyloxycarbonyl (Boc) and methoxyl (OMe) groups were attached at the N and C termini, respectively. Ideal 3_{10}-helical ϕ, ψ (−60°, −30°) values were used to generate the model prior to minimization.

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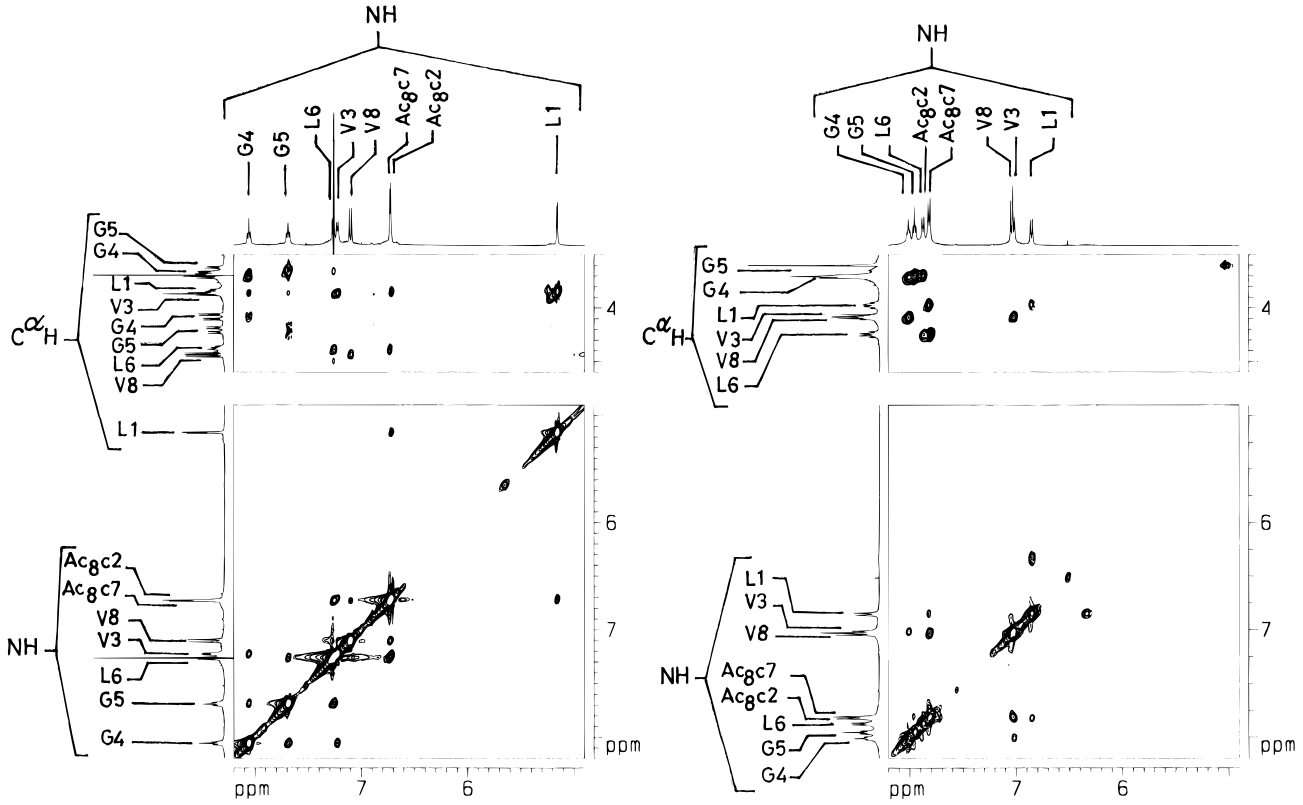

Figure 1. Partial 400 MHz ROESY spectra of peptide **1** in (left) $CDCl_3$ and (right) DMSO. $C^{\alpha}H \leftrightarrow NH$ and $NH \leftrightarrow NH$ connectivities are shown. Resonance assignments are marked on the 1D spectra.

TABLE 1: NMR Parameters for Protons in Peptide 1

	chemical shifts (δ ppm)[a]						
residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	others[c]	$d\delta/dT$[b] (ppb/K)
Leu(1)	5.16 (6.85)	3.84 (3.79)	~1.5 (~1.4)	~1.5 (~1.6)	0.98, 0.96 (~0.9)		6.0
$Ac_8c(2)$	7.72 (7.83)					~2.3−1.3 (2.1−1.4)	6.1
Val(3)	7.15 (7.02)	3.87 (4.08)	2.25 (~2.0)	1.04, 1.01 (~0.8)			0.9
Gly(4)	8.06 (8.01)	4.08, 3.69 (3.70)					3.6
Gly(5)	7.69 (7.95)	4.02, 3.64 (3.69)					4.3
Leu(6)	7.26 (7.87)	4.39 (4.25)	~1.8 (1.45)	~1.8 (1.57)	0.91, 0.86 (0.85)		4.5
$Ac_8c(7)$	6.73 (7.81)					~2.3−1.3 (2.1−1.4)	6.8
Val(8)	7.10 (7.04)	4.44 (4.08)	2.15 (~2.0)	0.93, 0.91 (0.8)			1.0

[a] Chemical shift values of proton resonances in $CDCl_3$ and DMSO. Values in parentheses correspond to DMSO. [b] $d\delta/dT$ is the temperature coefficient of NH chemical shifts in DMSO. [c] Methylene resonances of Ac_8c residues.

Results and Discussion

NMR Studies. *Assignments*. ^1H NMR assignments in $CDCl_3$ and DMSO solutions were carried out using a combination of DQF COSY and ROESY spectra.[25] Partial ROESY spectra for the peptide in the two solvents are illustrated in Figure 1. Resonance assignments are summarized in Table 1. NH and $C^{\alpha}H$ proton assignments are also marked on the one-dimensional spectra shown in Figure 1.

Conformational Analysis in $CDCl_3$. Inspection of the ROESY spectrum in Figure 1 reveals that all sequential $N_iH \leftrightarrow N_{i+1}H$ NOEs (d_{NN}) are observed. The d_{NN} NOEs are appreciably more intense than the corresponding $d_{\alpha N}$ NOEs ($C^{\alpha}_iH \leftrightarrow N_{i+1}H$ connectivities). The observation of strong sequential NH ↔ NH NOEs in peptides is clearly diagnostic of a continuous folded helical conformation.[25,26]

Figure 2 shows the effect of addition of the paramagnetic nitroxide, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), which enhances the relaxation rate of solvent-exposed NH groups in peptides.[27,28] Two NH groups in peptide **1**, Leu(1) NH and $Ac_8c(2)$ NH are significantly broadened, while the remaining six NH groups are much less perturbed. This observation is consistent with a 3_{10}-helical conformation for peptide **1** in $CDCl_3$ stabilized by six successive $4 \rightarrow 1$ hydrogen bonds involving the NH groups of residues 3−8. The only non-hydrogen-bonded, solvent-exposed NH groups are those of Leu(1) and $Ac_8c(2)$.

The observed $^3J_{NHC^{\alpha}H}$ values of Leu(1), 3.0, Val(3), 5.4, and Leu(6), 7.7 Hz are indeed compatible with the values of the torsion angle ϕ (N−C^{α}) of $-60° \pm 30°$. Figure 3 shows an energy-minimized conformation obtained by starting with an "ideal right-handed 3_{10}-helical conformation" ($\phi_i = -60°$, $\psi_i = -30°$). The minimization converged rapidly to a conformation with the following dihedral angles (ϕ, ψ), in degrees: L(1), −67, −56; $Ac_8c(2)$, −49, −40; V(3), −60, −52; G(4), −55, −45; G(5), −58, −34; L(6), −73, −51; $Ac_8c(7)$, −50, −46; V(8), −77, −53. This conformation corresponds to neither an ideal 3_{10}-helix nor an α-helix. Indeed, crystal structures frequently reveal mixed helical conformations. This model is consistent with the known tendency of 1-aminocycloalkane-1-carboxylic acid residues (Ac_nc) to stabilize helical conformation in short peptides. Indeed, there have been several reports of helical conformations of Ac_nc residues where n has ranged from

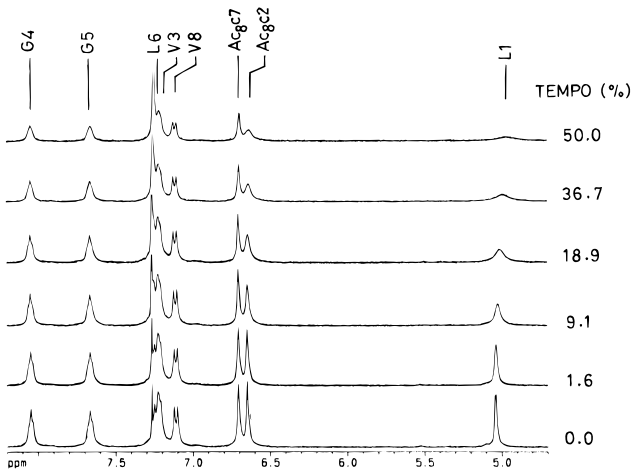

Figure 2. Partial 400 MHz ^{1}H NMR spectra in $CDCl_3$ showing the effect of TEMPO on the line width of NH resonance in peptide **1**. Resonance assignments are indicated. TEMPO concentrations (w/(v %)) are marked on the spectra. Peptide concentration was ~8 mM.

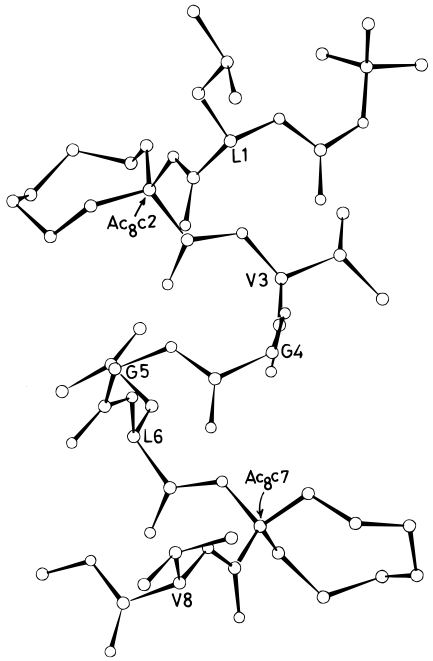

Figure 3. Energy-minimized structure of peptide **1**. Peptide model was built using a Silicon Graphics workstation using INSIGHT II module. Ideal 3_{10}-helical ϕ, ψ ($-60°$, $-30°$)values were used to generate the model prior to minimization.

5 to 7.[19,29−33] The present study provides the first example of helix formation in an Ac_8c peptide. The incorporation of the Gly-Gly segment in the center of a short helix has also been observed earlier in the crystal structure of the related octapeptide Boc-Leu-Aib-Val-Gly-Gly-Leu-Aib-Val-OMe.[18]

Conformational Analysis in DMSO. Inspection of the ROESY spectrum in Figure 1 reveals that in DMSO a continuous stretch of $N_iH-N_{i+1}H$ (d_{NN}) connectivities is absent. Indeed, the only d_{NN} connectivities observed are those between Leu(1) and $Ac_8c(2)$, Val(3) and Gly(4), and $Ac_8c(7)$ and Val(8). The observed interresidue $d_{\alpha N}$ NOEs are stronger than the limited d_{NN} connectivities. The comparison of relative intensities of d_{NN} and $d_{\alpha N}$ NOEs observed in $CDCl_3$ and DMSO strongly suggests a major difference between the conformations observed in the two solvents. The solvent exposure of NH groups in DMSO was probed by determining the temperature coefficient of NH groups ($d\delta/dT$). All NH groups moved linearly upfield with temperature (data not shown). The values

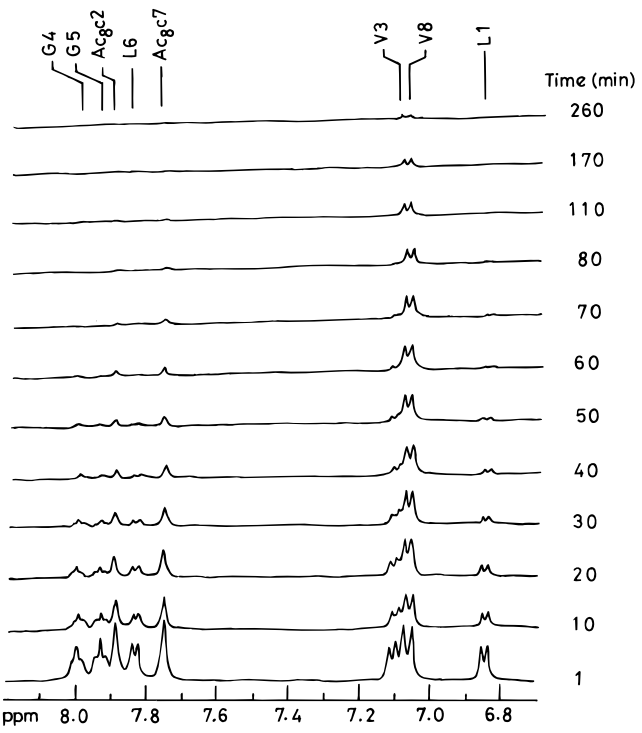

Figure 4. Partial 400 MHz ^{1}H NMR spectra showing time course of H−D exchange of NH resonances following addition of ~9% D_2O (v/v) to a peptide solution in DMSO (~8 mM).

of $d\delta/dT$ are summarized in Table 1. Only two NH groups Val(3) NH and Val(8) NH show very low $d\delta/dT$ values (<3 ppb/K) characteristic of strong solvent shielding. Gly(4) NH has a moderate temperature coefficient (3.6 ppb/K), while the remaining five NH groups have $d\delta/dT$ values >4.5 ppb/K, indicating their exposure to solvent.

Hydrogen−deuterium exchange studies were performed to further characterize the degree of solvent accessibility of peptide NH groups. The results in Figure 4 demonstrate that Val(8) NH does indeed have the slowest exchange rate. Somewhat surprisingly, Val(3) NH exchanges relatively quickly, although the temperature coefficient in DMSO is low. An interesting feature in Figure 4 is that the addition of small amounts of D_2O (~9% v/v) results in chemical shift changes of the resonances. This is discussed subsequently.

The low $d\delta/dT$ values of the Val(3) and Val(8) NH groups suggest that both N and C termini of Leu-Ac_8c-Val segments form β-turns stabilized by 4 → 1 hydrogen bonds (Boc CO···HN Val (3) and Gly(5) CO···HN Val(8)). The ROESY spectrum in Figure 1 shows the presence of Leu(1) NH ↔ Ac_8c(2) NH and Val(3)NH ↔ Gly (4) NH NOEs, which are supportive of a population of type III β-turns involving the Leu(1)-Ac_8c(2)-Val(3) segment. A consecutive type III β-turn segment involving the Leu(1)-Ac_8c(2)-Val(3) segment would leave the Val(3) and Gly(4) NH groups solvent shielded. Such a conformation would in fact constitute one turn of a 3_{10}-helix at the N-terminus. It must be noted that strong $d_{\alpha N}$ connectivities are also observed between Leu(1) $C^{\alpha}H$ ↔ Ac_8c(2) NH and Val(3) $C^{\alpha}H$ ↔ Gly(4) NH. The simultaneous observation of both d_{NN} and $d_{\alpha N}$ NOEs in L-residues in the peptide is generally indicative of conformational averaging involving partially folded helical conformations and extended structures. A single d_{NN} connectivity is observed at the C-terminus, Ac_8c(7) NH ↔ Val(8) NH. The presence of a strong $d_{\alpha N}$ connectivity between Leu(6) $C^{\alpha}H$ and Ac_8c(7) NH and the absence of the d_{NN} connectivity between these residues, together with the strong solvent-shielded Val(8) NH, are strongly supportive of

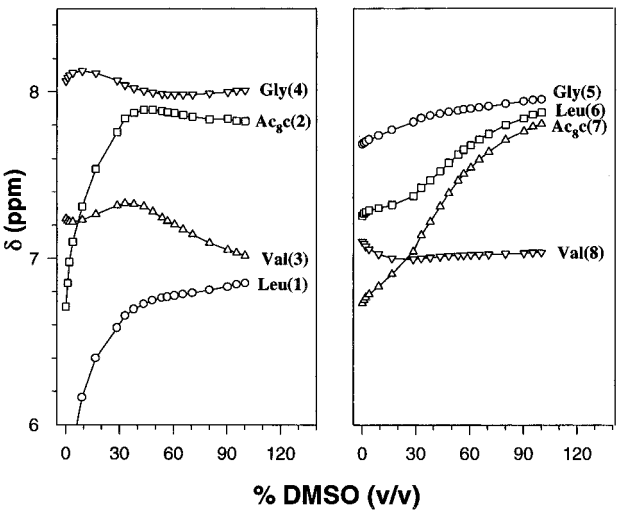

Figure 5. Solvent dependence of NH chemical shifts in $CDCl_3$/DMSO mixtures. Peptide concentration was ~8 mM.

a type II β-turn for the Leu(6)-Ac_8c(7) segment. The absence of d_{NN} connectivites in the Gly(4)-Gly(5)-Leu(6) segment clearly demonstrates that the continuous helix observed in chloroform has been broken in DMSO. Folded conformations are now observed for only the N and C terminal segments, with the former appearing to be somewhat more fragile as indicated by NOE and temperature coefficient data. The observed type II β-turn conformation with Leu(6) as the $i + 1$ residue also involves a significant conformational change at the C terminus as compared to the $CDCl_3$ structure.

Solvent Dependent Structural Transition. The NMR studies in the pure solvents $CDCl_3$ and DMSO establish a 3_{10}-helical conformation in the former, which is unfolded in the latter. Although the N-terminus shows a residual helical turn in DMSO, a single isolated type II β-turn is observed for the C-terminus Leu(6)-Ac_8c(7)-Val(8) segment. To characterize this conformational transition, amide NH chemical shifts were monitored as a function of solvent composition in $CDCl_3$/DMSO mixtures (Figure 5). In peptides, addition of strong hydrogen-bond-accepting solvents such as DMSO usually results in a downfield shift of solvent-exposed NH groups. This is clearly borne out by the observed data for the Leu(1) and Ac_8c(2) NH groups (Figure 5). Val (3) NH behaves anomalously. At low DMSO concentrations, there is a small downfield shift, whereas above 40% (v/v) DMSO the resonance begin to move upfield once again. Anomalous chemical shift behavior is also observed for Gly(4) NH, although the upfield shift is less dramatic compared to Val(3) NH. Inspection of the data in Figure 5 shows that at low DMSO concentration {<20% (v/v)}, only Leu(1) and Ac_8c-(2) NH groups show appreciable chemical shift changes consistent with a 3_{10}-helical fold. Above a DMSO concentration of 30% (v/v), Leu(6) and Ac_8c(7) NH groups begin to show appreciable downfield shifts suggesting their solvent exposure. Both Leu(6) and Ac_8c(7) show a discontinuity at about 28% (v/v), which is indicative of a structural transition. Interestingly, the upfield shift of Val(3) NH is observed at significantly higher DMSO concentration {>40% (v/v)}, suggesting that structural changes at the N- and C-termini take place at different solvent compositions.

To probe the solvent exposure of NH groups in $CDCl_3$/DMSO mixtures of varying composition, temperature coefficients of NH chemical shifts $d\delta/dT$ were determined at DMSO concentrations of 9.09%, 28.5%, 53.5%, and 100% (v/v). In all cases the observed temperature dependences were linear (data not shown). The $d\delta/dT$ values are summarized in Figure 6. Leu-(1) exhibits a high $d\delta/dT$ (>6 ppb/K) indicative of its solvent exposure at all solvent compositions. Val(8) NH has a very low value of $d\delta/dT$ (<1 ppb/K), indicating its solvent-shielded nature over the entire range of solvent composition. Gly(4) and Gly(5) NH groups exhibit intermediate $d\delta/dT$ values with slightly higher temperature coefficients being observed at higher DMSO concentration. Leu(6) NH has a relatively low $d\delta/dT$ at a DMSO concentration less than 30% (v/v) but has a significantly higher value at concentrations greater than 50% (v/v). The two NH groups that show the most pronounced differences are Val(3) and Ac_8c(7). Although Val(3) has an intermediate $d\delta/dT$ value at low DMSO concentrations, in pure DMSO it appears to be solvent shielded. This behavior is surprising, since solvation may be expected to result in greater exposure of the NH group. In contrast, the Ac_8c(7) NH group has a low $d\delta/dT$ value at low DMSO concentration but shows a dramatic increase at 28.5% and 53.5% (v/v) DMSO. This clearly favors a structural transition in which the Ac_8c(7) NH is transformed from a hydrogen-bonded conformation to an exposed situation. The results in Figure 6 provides further support for a definite structural transition occurring at both N- and C- termini of the peptide. The relatively small differences observed for the central Gly(4) and Gly(5) segments suggest that the changes are limited to solvent invasion of the helical backbone characterized in $CDCl_3$, with no major changes in the orientation of peptide planes.

A molecular interpretation for the observed spectroscopic changes in $CDCl_3$/DMSO mixtures may be provided as follows. At low concentrations of DMSO the 3_{10}-helix observed in $CDCl_3$ is transformed into an α-helical structure resulting in a large $d\delta/dT$ value of Val(3) NH. Such 3_{10}- to α-helix transitions have indeed been observed on going from $CDCl_3$ to DMSO in small model helical peptides.[34,35] It should be noted that the transition between helix subtypes are relatively facile, with chain length and sequence effects having being studied in solution[36] and in crystals of oligopeptides.[14,37−39] Several theoretical analyses of 3_{10}- to α-helical transitions have also been reported.[40−46] Calculations of potentials of mean force for an Aib decamer suggest that the α-helix becomes more stable than the 3_{10}-helix as the solvent dielectric increases,[42,43] consistent with the results observed in the present study for the model octapeptide. At high DMSO concentrations there is likely to be a population of Leu(1)-Ac_8c(2) type II β-turns formed by a flip of the peptide bond between residues 1 and 2. In this structure, Val(3) NH is strongly hydrogen bonded ($4 \rightarrow 1$ hydrogen bond with Boc CO), resulting in a much lower $d\delta/dT$ value. The NMR data in DMSO are clearly compatible with a heterogeneous population of conformations for the N-terminus segment involving both type II and type III β-turns. At the C-terminus Val(8) NH maintains its hydrogen-bonded nature over the entire range of solvent composition, suggesting that the conformational transition is not accompanied by a breakage of the hydrogen bond.

In the 3_{10}-helical structure in $CDCl_3$, the Leu(6)-Ac_8c(7) residues occupy the $i + 1$ and $i + 2$ position of a type III β-turn involving Val(8) NH in $4 \rightarrow 1$ hydrogen bond with Gly(5) CO. The results in DMSO establish a type II β-turn at the Leu(6)-Ac_8c(7) segment. This transition is effected by an approximately 180° flip of the peptide bond connecting residues 6 and 7, which may be formally viewed as a concerted rotation about ϕ_6 and ψ_7 without breaking the $4 \rightarrow 1$ hydrogen bond with Val (8) NH. Such a type III−type II interconversion has been shown to be effectively barrierless in theoretical calculations within an AM1 framework.[47]

Peptide Solvation. During the course of the hydrogen−deuterium exchange experiment, addition of small amounts of

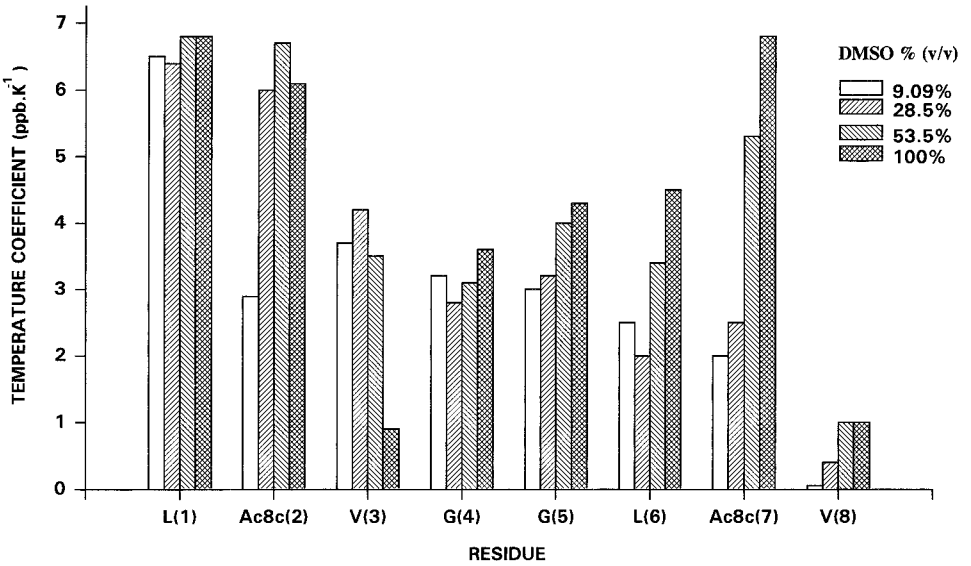

Figure 6. Bar diagram showing the temperature coefficient values of NH resonances in peptide **1** at different concentrations of DMSO in $CDCl_3$.

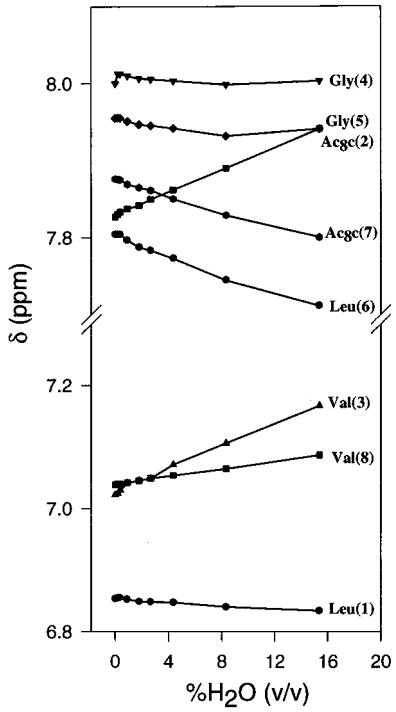

Figure 7. Plot of NH chemical shifts as a function of H_2O concentration in DMSO solution. Peptide concentration was ~8 mM.

D_2O to DMSO solutions of peptide **1** resulted in changes in the chemical shifts of some NH resonances. This observation is interesting, since a low concentration of water was not expected to compete effectively with DMSO for interaction with peptide NH groups. Figure 7 summarizes the changes in NH chemical shifts upon controlled addition of water to DMSO solutions of the octapeptide. Leu(1), Val(8), Gly(4), and Gly(5) do not show major chemical shift changes. $Ac_8c(2)$ and Val(3) NH groups show appreciable downfield chemical shifts, while Leu(6) and $Ac_8c(7)$ move significantly upfield over a concentration range of 0−15% water. A possible explanation is that NH groups, which are strongly intramolecularly hydrogen bonded [Val(8)], or those that are exposed and strongly hydrogen bonded to DMSO [Leu(1), Gly(4), Gly(5)] are unaffected by addition of water. The NH groups that show changes in chemical shifts are probably those that are not strongly interacting with DMSO for local stereoelectronic reasons. For example, proximity to a peptide carbonyl group may electrostatically shield the NH group from the DMSO solvent molecule. Water molecules may in fact approach such NH groups preferentially because of the ability of water to act as both hydrogen bond donor and acceptor, in contrast to DMSO, which is only a strong hydrogen bond acceptor. The $Ac_8c(2)$ and Val(3) NH groups probably do not interact strongly with DMSO but bind preferentially to water with concomitant downfield chemical shifts upon increasing the water concentration. In the case of Leu(6) and $Ac_8c(7)$, water molecules may in fact displace DMSO molecules, rationalizing the observed upfield shifts. Crystal structures frequently reveal insertion of water molecules into peptide backbones even when crystallization is carried out in DMSO or methanol.[6,48,49]

Conclusions

The present study provides firm evidence for a well-defined conformational transition in an apolar octapeptide mediated by a change of solvent. In a poorly solvating medium like $CDCl_3$ intramolecular hydrogen bonding plays a decisive role in stabilizing a folded 3_{10}-helical conformation. In DMSO, the strong hydrogen-bond-accepting property of the solvent displaces the conformational equilibrium toward structures that are unfolded at the conformationally flexible central Gly-Gly segment. Residual secondary structures stabilized by intramolecular hydrogen bonds are indeed maintained at both the N- and C- terminal ends of the molecule, which contain the conformationally constrained Ac_8c residues. In addition to unfolding, peptide bond flips resulting in type III to type II β-turn transitions are established in DMSO. Studies in aqueous DMSO mixtures provide some evidence for peptide hydration even at low concentrations of water. The present example suggests that the use of conformationally constrained and flexible residues in conjunction with each other can lead to the design of peptide systems that are finely poised to undergo conformational transitions. This should permit detailed characterization of multiple conformational states under different solvent conditions.

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