The ideal γ-turn conformation in peptides is stabilized by the formation of two intramolecular hydrogen bonds. These are between the NH of residue \(i\) and the C=O of residue \(i + 2\) (1 → 3, \(C_1\)) and the C=O of residue \(i\) and the NH of residue \(i + 2\) (3 → 1, \(C_3\)). While this reverse-turn structural feature has been observed in proteins, unambiguous characterization of this conformation has yet to be realized in small peptides. Several examples of a single 3 → 1 (\(C_1\)) hydrogen bond have been reported in crystal structures of cyclic peptides and inferred from spectroscopic studies in apolar solvents. We wish to describe the spectroscopic characterization of a γ-turn conformation in a protected tripeptide, stabilized by formation of a disulfide crosslink.

The peptide

\[
\text{Boc-L-Cys-L-Ala-L-Cys NHMe}
\]

was synthesized from its acyclic precursor, Boc-L-Cys(SBzl)-L-Ala-L-Cys(SBzl) NHMe by Na-liquid NH₃ reduction, followed by oxidative cyclization using \(K_3Fe(CN)_6\) in dilute aqueous solution (3 m\(M\)). The cyclic monomer was separated from cyclodimers and higher oligomers by silica gel column chromatography and shown to be homogeneous by reverse-phase HPLC. The peptide was characterized by 270-MHz \(^1\text{H-nmr}\) and mass spectrometry fast-atom bombardment (f.a.b) MH\(^+\) 407.

The involvement of NH groups in intramolecular hydrogen bonding was probed using the temperature and solvent dependence of NH chemical shifts and paramagnetic radical induced broadening of NH resonances. Figure 1 shows the effect of the addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) on the NH resonances of I. Extensive broadening is seen for the Ala NH and NHMe resonances, whereas Cys(1) and Cys(3) NH groups are significantly less affected. Table I summarizes the NH chemical shifts and temperature coefficients \((d\delta/dT)\) in (CD\(_3\))₂SO and CDCl\(_3\). In (CD\(_3\))₂SO, the Cys(3) NH group has a very low \(d\delta/dT\) value, in contrast to the other three NH resonances. The Cys(3) NH resonance also shows a very small change in chemical shift.
on going from an apolar, poorly hydrogen-bonding solvent like CDCl₃ to a polar, strongly hydrogen-bonding solvent like (CD₃)₂SO. The other three NH groups move to substantially lower field in (CD₃)₂SO, as compared with CDCl₃. In CDCl₃, the $d\delta/dT$ value observed for Ala NH and NHMe are markedly larger than for the Cys(1) and Cys(3) NH groups. Large $d\delta/dT$ values in an apolar solvent like CDCl₃ could arise from breakage of intermolecular hydrogen bonding between peptide molecules. A study of concentration dependence of NH chemical shifts in CDCl₃ over the range 2–25 mM suggests that intermolecular effects are significant only for the Ala NH group and, to a lesser extent, for the terminal NHMe group.

The nmr data provide strong support for the solvent-shielded nature of Cys(1) and Cys(3) NH groups in CDCl₃. This is fully consistent with their involvement in intramolecular hydrogen bonding, as shown in Fig. 2. In polar solvents like

\[\begin{array}{c|c|c|c}
\text{TABLE I} \\
\hline
\text{\small 'H-NMR Parameters}^a \text{ for Peptide NH Groups in Boc-L-Cys-L-Ala-L-Cys-NHMe (1)} \\
\hline
\text{NH} & \text{CDCl₃ (ppm)} & \text{(CD₃)₂SO (ppm)} & \text{CDCl₃} & \text{(CD₃)₂SO} \\
\hline
\text{Cys(1)} & 5.47 & 6.98 & 0.0042 & 0.0095 \\
\text{Ala(2)} & 6.66 & 8.85 & 0.0147 & 0.0034 \\
\text{Cys(3)} & 7.69 & 7.45 & 0.0042 & 0.0014 \\
\text{NHMe} & 7.09 & 7.98 & 0.0086 & 0.0041 \\
\hline
\end{array}\]

\(^a\) Chemical-shift ($\delta$) values are with respect to internal Me₅Si.

\(^b\) $d\delta/dT$ values are expressed as ppm/K.
(CD$_3$)$_2$SO, the $1 \rightarrow 3$ (C$_{17}$) hydrogen bond is broken and nmr data favor only the $3 \rightarrow 1$ (C$_7$) hydrogen bond, involving the Cys(3) NH group. These results emphasize the importance of disulfide bridges in stabilizing specific peptide conformations. The occurrence of γ-turn conformations in proteins possessing 11-membered disulfide loops, like the α-subunit of human chorionic gonadotropin merits further consideration.$^{10}$

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References


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