Stabilization of Y-Turn Conformations in Peptides by Disulfide Bridging

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The ideal y-turn conformation in peptides is stabilized by the formation of two intramolecular hydrogen bonds.[1,2] These are between the NH of residue i and the C=O of residue i + 2 ($1 \rightarrow 3$, C_{11}) and the C=O of residue i and the NH of residue $i + 2$ ($3 \rightarrow 1$, C_7).[3,4] While this reverse-turn structural feature has been observed in proteins,[1,5] unambiguous characterization of this conformation has yet to be realized in small peptides. Several examples of a single $3 \rightarrow 1 (C_7)$ 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 y-turn conformation in a protected tripeptide, stabilized by formation of a disulfide crosslink.

The peptide

Boc—L—Cys—L—Ala—L—Cys NHMe
S——————S

1

1 was synthesized

from its acyclic precursor, Boc-L-Cys(SBzl)-L-Ala-L-Cys(SBzl) NHMe by Na-liquid NH, reduction, followed by oxidative cyclization using $K_3Fe(CN)_6$ in dilute aqueous solution (3m*M*).[6] 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}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 **1.** 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

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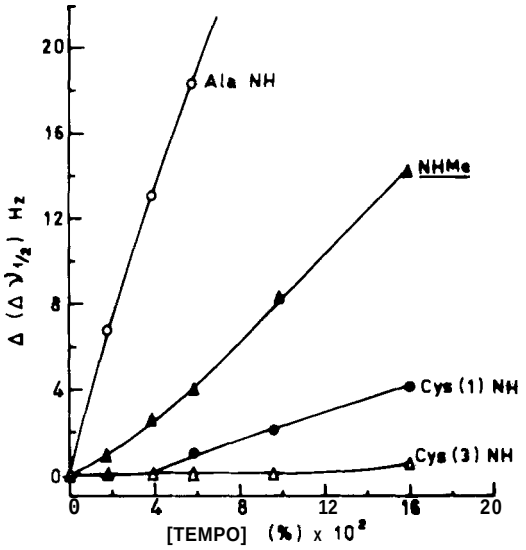

Fig. 1. Effect of the addition of the free radical TEMPO on the linewidth of NH resonances in 1: Ala (○), Me (▲), Cys(1) (●), and Cys(3) (△).

on going from an apolar, poorly hydrogen-bonding solvent like $CDCl_3$ to a polar, strongly hydrogen-bonding solvent like $(CD_3)_2SO$. The other three NH groups move to substantially lower field in $(CD_3)_2SO$, as compared with $CDCl_3$. In $CDCl_3$, 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 $CDCl_3$ could arise from breakage of ***intermolecular*** hydrogen bonding between peptide molecules.[8,9] A study of concentration dependence of NH chemical shifts in $CDCl_3$ 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 $CDCl_3$. This is fully consistent with their involvement in intramolecular hydrogen bonding, as shown in Fig. 2. In polar solvents like

TABLE I

[1]H-NMR Parameters[a] for Peptide NH Groups in Boc-L-Cys-L-Ala-L-Cys-NHMe (1)

	δ (ppm)		dδ/dT	
	$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2SO$
Cys(1)	5.47	6.98	0.0042	0.0095
Ala(2)	6.66	8.85	0.0147	0.0034
Cys(3)	7.69	7.45	0.0042	0.0014
NHMe	7.09	7.98	0.0086	0.0041

[a] Chemical-shift (δ) values are with respect to internal Me_4Si.

[b] $d\delta/dT$ values are expressed as ppm/K.

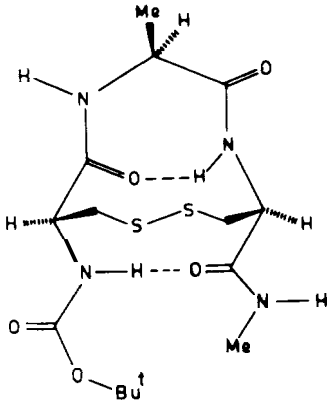

Fig. 2. The y-turn conformation proposed for peptide **1.**

$(CD_3)_2SO$, the **1** → **3** (C_{11}) hydrogen bond is broken and nmr data favor only the **3** → **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 y-turn conformations in proteins possessing 11-membered disulfide loops, like the a-subunit of human chorionic gonadotropin merits further consideration.[10]

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