Cyclic Biscystine Peptides. Models for Antiparallel β-Sheet Conformations

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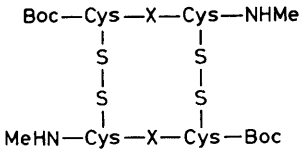
The cyclic biscystine peptides (**1a**) and (**1b**) adopt antiparallel β-sheet conformations in solution, characterized by distinctive ^{1}H n.m.r. spectral parameters.

Disulphide bridges formed between cysteine residues are an important structural determinant in proteins and biologically active polypeptides.[1] The disulphide bond is also an important spectroscopic probe of molecular conformation in these systems and is amenable to direct study by circular dichroism[2] and Raman spectroscopy.[3] There are relatively few studies of conformationally well characterized cystine peptides.[4—6] We describe cyclic biscystine peptides (**1**) as models for the antiparallel β-sheet conformation.[7]

The 22-membered cyclic peptide bis(disulphide)s were formed by Na–liquid NH_3 treatment of the acyclic precursor, Boc-Cys(SCH_2Ph)-X-Cys(SCH_2Ph)-NHMe (X = L-Ala or D-Ala), followed by oxidative cyclodimerization using $K_3Fe(CN)_6$ in aqueous solution.[5] The cyclodimers were obtained on oxidation of solutions having peptide concentrations of 4 and 20 mM, respectively. (**1a**) and (**1b**) were purified by silica gel column chromatography, and shown to be homogeneous by h.p.l.c.; characterization was by 270 MHz ^{1}H and 67.89 MHz ^{13}C n.m.r. spectroscopy (indicative of a C_2 symmetric structure) and mass spectrometry [fast atom bombardment (f.a.b.) MH^+ 813].



(**1**) **a**; X = L-Ala
b; X = D-Ala

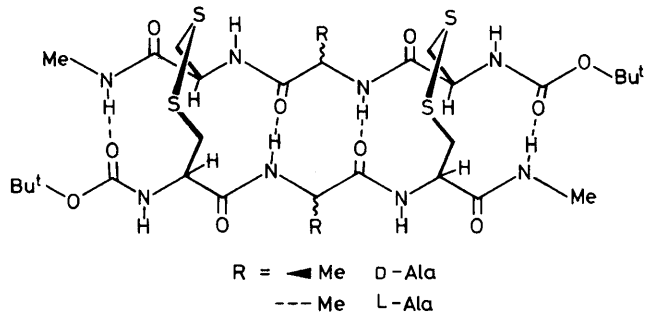


Figure 1. Antiparallel β-sheet conformation proposed for cyclic biscystine peptides (**1a**) and (**1b**).

Table 1. ^{1}H N.m.r. parameters[a] for peptides (**1a**) and (**1b**).

	(**1a**)				(**1b**)			
Residue	Cys(1)	L-Ala(2)	Cys(3)	Methylamide	Cys(1)	D-Ala(2)	Cys(3)	Methylamide
δ(NH)($CDCl_3$)	6.42	9.02	8.04[b]	8.04[b]	6.27	9.09	7.71	8.02
δ(NH)[$(CD_3)_2SO$]	7.19	8.48	8.71	7.83	7.16	8.68	8.99	7.98
dδ/dT[$(CD_3)_2SO$][c]	0.0065	0.0035	0.0067	0.0037	0.0064	0.0024	0.0043	0.0044
δ($C^\alpha H$)($CDCl_3$)	5.38	4.94	5.49	—	5.37	4.90	5.50	—
δ($C^\alpha H$)[$(CD_3)_2SO$]	4.70	4.51	4.84	—	4.80	4.58	4.93	—
J(HN$C^\alpha H$)($CDCl_3$)[d]	9.9	8.1	8.81	—	9.6	6.6	9.2	—
J(HN$C^\alpha H$)[$(CD_3)_2SO$][d]	9.6	7.7	9.2	—	9.9	7.4	9.2	—

[a] δ Values are with respect to internal Me_4Si. [b] Cys(3) N*H* and N*H*Me peaks overlap. [c] dδ/dT Values are expressed as p.p.m./K. [d] J values in Hz. Errors are ±0.4 Hz.

270 MHz ^{1}H N.m.r. data for the two peptides are summarized in Table 1. The extraordinarily low field position of the Ala NH, NHMe, Cys(1) $C^\alpha H$, and Cys(3) $C^\alpha H$ resonances in $CDCl_3$ is noteworthy. The temperature coefficient values (dδ/dT) for the NH resonances in $(CD_3)_2SO$[8] suggest that the Ala NH and NHMe group are hydrogen bonded (solvent shielded) in (**1a**) and (**1b**). A conformation consistent with the n.m.r. results is shown in Figure 1. The high J(HN$C^\alpha H$) values (>9 Hz) observed for Cys(1) and Cys(3) in both $CDCl_3$ and $(CD_3)_2SO$ (Table 1) are strongly indicative of an extended β-sheet conformation (φ values between −130 and −150°).[9] In general, flexible or helical peptides have significantly lower J values (*ca.* 7 Hz). The lower value for the L-Ala and D-Ala NH groups may reflect a distortion from a perfect antiparallel β-sheet conformation owing to close transannular steric interactions between the Ala C=O groups. A parallel dimeric structure cannot simultaneously account for the observed hydrogen bonding pattern and the two-fold symmetry deduced from the n.m.r. data. The low field $C^\alpha H$ resonances of Cys(1) and Cys(3) in $CDCl_3$ may reflect the deshielding effect of the disulphide group, which may adopt an altered orientation in $(CD_3)_2SO$. It is also possible that short $C^\alpha H$ to oxygen distances between non-neighbouring residues in the β-sheet structure are also responsible for the unusual chemical shifts. Such effects have been suggested in proteins.[10,11]

An interesting feature of the biscystine peptides is the similarity of the n.m.r. spectral behaviour of the L- and D-Ala peptides. This suggests that the disulphide bridges force the D-residue into adopting φ,ψ conformational angle values, which are fairly close to that of L-Ala. The conformation shown in Figure 1 suggests that these systems could serve as a means of appropriately positioning functional sidechains on an antiparallel β-sheet backbone. These peptides can also serve as models to characterize further the spectroscopic properties of the –S–S– chromophore and its interaction with the peptide bond.

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