Cystine Peptides. The Antiparallel β-Sheet Conformation of Two Synthetic Cyclic Bis(cystine peptides)

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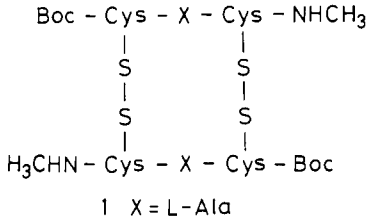
Abstract: Conformational studies on two synthetic cyclic bis(cystine peptides) have been carried out. The NMR data support a C_2-symmetric structure possessing four intramolecular hydrogen bonds in $CDCl_3$ and $(CD_3)_2SO$ solutions. The involvement of the X-*NH* and *NH*Me groups in formation of transannular hydrogen bonds is inferred from the temperature and solvent dependences of NH chemical shifts, hydrogen–deuterium-exchange rates, and radical-induced line-broadening experiments. IR studies over a wide concentration range also favor hydrogen-bonded structures. Unusually low $C^{\alpha}H$ chemical shifts for Cys[1] and Cys[3] residues, high $J_{HNC^{\alpha}H}$ values (≥9 Hz), and the observation of nuclear Overhauser effects between $C_i^{\alpha}H$ and $N_{i+1}H$ protons in **1** provide compelling evidence for an antiparallel β-sheet conformation for the 22-membered cyclic bis(cystine peptides).

Disulfide bridges constitute an important structural element of peptide and protein structures.[1,2] Cystine residues are unique in providing a covalent cross-link between positions on a polypeptide chain, which are far apart in the primary sequence. Disulfides in proteins also play an important functional role in redox processes.[3] Despite their obvious importance, there have been relatively few investigations of the structural properties of peptide disulfides. A substantial body of the published literature in this area has been devoted to studies of the neurohypophyseal hormones oxytocin and vasopressin which contain a 20-membered disulfide loop.[4] As part of a systematic study of the conformations of cystine peptides, we have earlier reported studies of small disulfide loops, containing 14-membered rings.[5-8] Disulfide bridging can be useful in stabilizing specific reverse-turn conformations.[5-9] In this report, we describe studies on 22-membered cyclic bis(cystine peptides) (**1** and **2**), which provide a good model



1 X = L-Ala
2 X = D-Ala

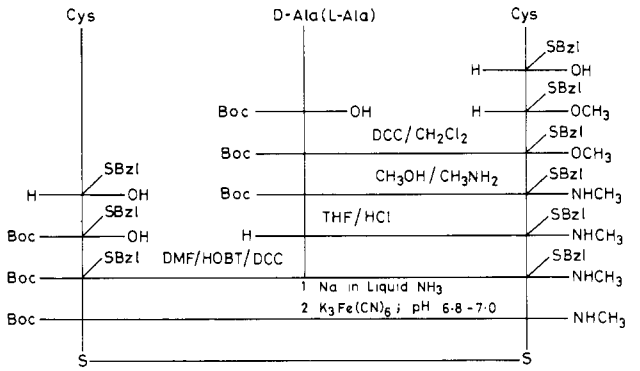
for antiparallel β-sheet conformations, with adjacent polypeptide chains held together by disulfide bridges.[10]

Experimental Section

Synthesis and Characterization of Peptides. Peptides **1** and **2** were synthesized by solution-phase procedures by using the approach summarized in Scheme I. Representative procedures have been detailed for the synthesis of related cyclic peptide disulfides.[5,8] The procedures for the cleavage of the *S*-benzyl groups followed by oxidative cyclization is briefly outlined below.

Boc-Cys(*S*-Bzl)-D-Ala-Cys(*S*-Bzl)-NHMe (1.7 g, 2.9 mmol) was dissolved in 800 mL of freshly distilled liquid ammonia. Freshly cut pieces of metallic sodium were added gradually, with vigorous stirring, until a blue color persisted. The excess Na was destroyed carefully with a few drops of acetic acid. After evaporation of the ammonia under a stream of nitrogen, 1.1 L of distilled water was added and the pH adjusted to 6.8–7.0. The concentration of the dithiol was assumed to be ~4 mM, based on quantitative reduction. Oxidation was effected by gradual addition of a 0.02 M $K_3Fe(CN)_6$ solution (150–200 mL), while maintaining the pH at 6.8–7.0. The aqueous solution was concentrated to 100 mL and extracted successively, with $CHCl_3$ (3 × 50 mL) and EtOAC (3 × 50 mL). The combined organic extracts were dried and evaporated to give a solid. The crude product was chromatographed on a silica gel column ($CHCl_3$ and $CHCl_3$–4% CH_3OH as eluants) to yield **2** as a white solid (200 mg, yield 17%).

Scheme I



Peptide **1** was isolated as 14% yield from the acyclic precursor Boc-Cys(*S*-Bzl)-L-Ala-Cys(*S*-Bzl)-NHMe, by a similar procedure. Both **1** and **2** were shown to be homogeneous by reverse-phase HPLC on a Lichrosorb RP-18 column (linear gradient elution 70–95% CH_3OH/H_2O, 20 min; flow rate 0.8 mL min^{-1}; retention times **1** 12.4 min, **2** 15.8 min). FAB mass spectra of **1** and **2** yielded the MH^+ peak at 813, confirming the dimeric structure (M_r 812). Both **1** and **2** were completely characterized by their 270-MHz ^1H and 67.89-MHz ^{13}C NMR spectra. Representative spectra for **2** are shown in Figure 1.

Spectroscopic Studies. ^1H NMR studies were performed on a Bruker WH-270 spectrometer at the Sophisticated Instruments Facility, Bangalore, or on a Varian FT-80A spectrometer as described previously.[5-9] NOE experiments were carried out at a peptide concentration of 10 mg/mL at a probe temperature of 293 K. Perturbed and normal spectra recorded sequentially in different parts of the memory (8 K each) were obtained by low-

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Table I. ^{1}H NMR Parameters[a] for Peptides **1** and **2**

parameters	1, residue				2, residue			
	Cys^1	L-Ala^2	Cys^3	NHMe	Cys^1	D-Ala^2	Cys^3	NHMe
δ_{NH} ($CDCl_3$)	6.42	9.02	8.04	8.04	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
$d\delta/dT$ ($CDCl_3$)[b]	0.0090	0.0034	0.0138	0.0048	0.0090	0.0047	0.0133	0.0045
$d\delta/dT$ ($CD_3)_2SO$[b]	0.0065	0.0035	0.0067	0.0037	0.0064	0.0024	0.0043	0.0044
$\delta_{C^\alpha H}$ ($CDCl_3$)	5.38	4.94	5.49		5.37	4.90	5.50	
$\delta_{C^\alpha H}$ ($CD_3)_2SO$	4.70	4.51	4.84		4.80	4.58	4.93	
$J_{HNC^\alpha H}$ ($CDCl_3$)[c]	9.9	8.1	8.8		9.6	6.6	9.2	
$J_{HNC^\alpha H}$ ($CD_3)_2SO$[c]	9.6	7.7	9.2		9.9	7.4	9.2	

[a] δ values are expressed as ppm downfield from internal TMS and are reported for a peptide concentration of 12 and 6 mM for peptides **1** and **2**, respectively, in $CDCl_3$ and 12 mM for both **1** and **2** in $(CD_3)_2SO$. [b] $d\delta/dT$ values are expressed as ppm/K, measured at a concentration of 12 and 6 mM for peptides **1** and **2**, respectively, in $CDCl_3$ and 12 mM for both **1** and **2** in $(CD_3)_2SO$. [c] Errors in J values are ±0.4 Hz.

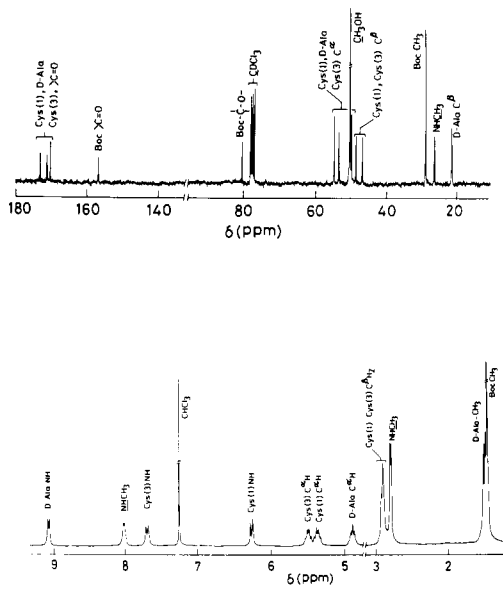

Figure 1. ^{1}H (270 MHz) (bottom) and ^{13}C (67.89 MHz) (top) NMR spectra of peptide **2** in $CDCl_3$.

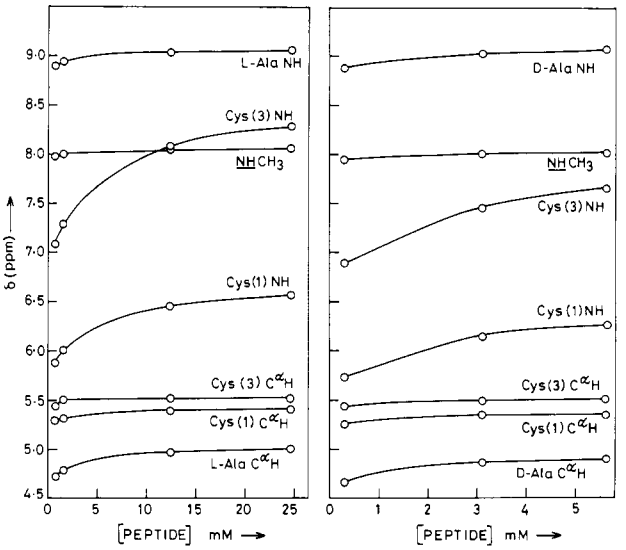

Figure 2. Concentration dependence of NH and C$^\alpha$H chemical shifts in $CDCl_3$ for peptides **1** (left) and **2** (right).

power on-resonance saturation of a peak and by off-resonance shifting of the irradiation frequency, respectively. Typically, 128 accumulations (acquisition time, 1.4 s) were utilized in each, with a delay time of 4.0 s between transients to facilitate buildup of initial equilibrium magnetization. Presaturation times and decoupler power were varied to obtain selectivity of irradiation of closely spaced NH resonances (Cys^{3}NH and NHMe). The difference-free induction decay was multiplied by a decaying exponential before Fourier transformation. Identical filtering of the normal spectrum permitted estimates of NOE magnitudes. Undegassed samples were used in NOE experiments. IR spectra were recorded on a Perkin-Elmer Model 297 spectrometer using cells of path length 4 mm. CD spectra were recorded on a JASCO J-20 spectropolarimeter.

Results and Discussion

The 270-MHz ^{1}H and 67.89-MHz ^{13}C NMR spectra of **2** in $CDCl_3$ are shown in Figure 1. The spectra clearly support a C_2-symmetric structure for the bis(cystine peptide). Similar spectra were obtained for peptide **1**. Assignments of ^{1}H resonances were made by spin-decoupling experiments. The Cys^1 NH (urethane) could be assigned by virtue of its high field position in $CDCl_3$.[5-8] The NMR parameters for the NH and C$^\alpha$H resonances in **1** and **2** are summarized in Table I. In principle, both parallel and antiparallel dimers may be formed on oxidative cyclodimerization of the corresponding dithiols. Both dimers can give rise to NMR spectra characteristic of C_2-symmetric structures. However, the NMR data discussed subsequently favor an intramolecularly hydrogen-bonded conformation for both peptides. For a parallel dimer, stereochemically feasible, hydrogen-bonded structures cannot have C_2 symmetry and would be expected to give rise to NMR spectra corresponding to a peptide composed of six distinct residues. The constraints of C_2 symmetry *together with* the presence of intramolecular hydrogen bonds are consistent only with an antiparallel dimeric structure. Consequently, all subsequent analysis will consider only the antiparallel structures **1** and **2**.

Delineation of Hydrogen-Bonded NH Groups. The delineation of hydrogen-bonded NH groups was carried out by using temperature and solvent dependence of NH chemical shifts, paramagnetic radical-induced line broadening of NH resonances, and hydrogen–deuterium (H–D) exchange studies.[11-13] The temperature dependences of NH chemical shifts in $CDCl_3$ and $(CD_3)_2SO$ were measured over the ranges 293–323 and 293–343 K, respectively, and were found to be completely linear. The temperature coefficient ($d\delta/dT$) values are summarized in Table I. In both solvents, the Ala and methylamide NH groups exhibit significantly lower $d\delta/dT$ values as compared to the Cys^1 and Cys^3 NH groups. In $(CD_3)_2SO$, the observed $d\delta/dT$ values for the Ala and methylamide NH groups are characteristic of moderately solvent-shielded NH protons.[6] Low $d\delta/dT$ values in $CDCl_3$ may be indicative of either intramolecularly hydrogen-bonded NH groups or of solvent-exposed protons. High $d\delta/dT$ values in $CDCl_3$, on the other hand, may be diagnostic of intermolecular hydrogen bonding. These interactions are likely to be broken at elevated temperatures.[14,15] The high $d\delta/dT$ values in $CDCl_3$ of the Cys^1 and Cys^3 NH groups could be indicative of their in-

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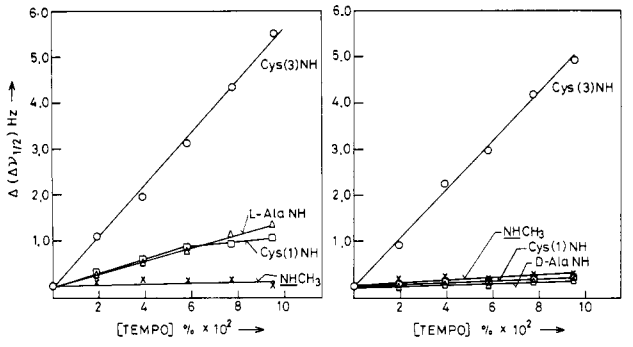

Figure 3. Line broadening of peptide NH resonances as a function of TEMPO concentration in $CDCl_3$ for **1** (left) and **2** (right).

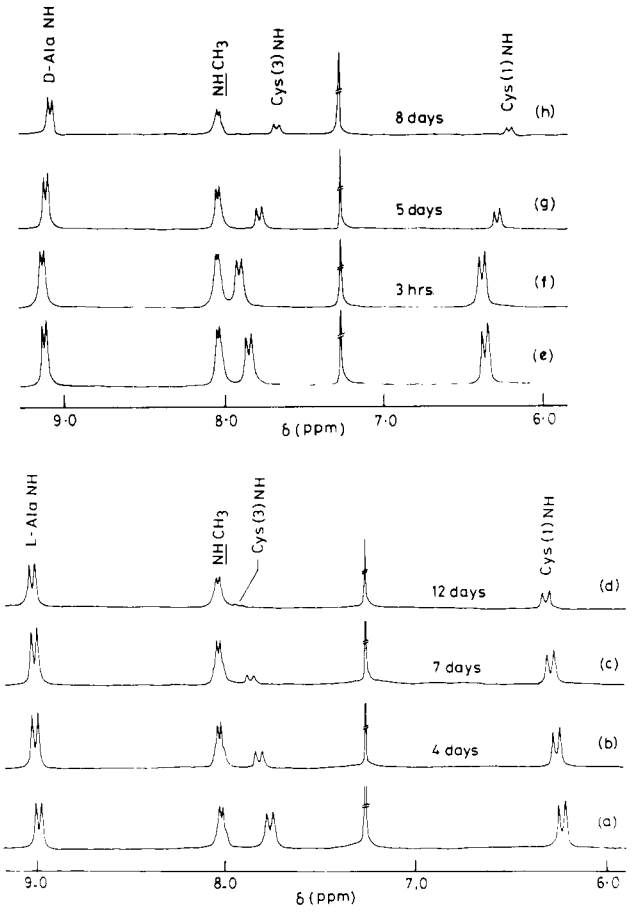

Figure 4. Hydrogen–deuterium exchange of NH resonances in a $CDCl_3$–D_2O mixture: (a–d) **1**, (e–h) **2**. Time intervals after D_2O addition are indicated on the traces.

volvement in stabilizing peptide association, by intermolecular hydrogen-bond formation. Supporting evidence for this is seen from the concentration dependence of NH chemical shifts in $CDCl_3$ (Figure 2). In both peptides **1** and **2**, only the Cys^1 and Cys^3 NH groups show a downfield shift at higher peptide concentrations, while the other two NH groups are considerably less influenced.

Figure 3 illustrates the effect of addition of the nitroxide radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) on the NH resonance line widths in **1** and **2**. The data clearly suggest that the Cys^3 NH is solvent-exposed, as evidenced by the large line broadening, while the remaining three NH groups interact less effectively with the radical in both peptides. Figure 4 shows representative changes in intensity of NH resonances as a function of time after addition of D_2O to $CDCl_3$ solutions of the peptides. In both **1** and **2**, the Cys^3 NH exchanges relatively rapidly, while the Ala and methylamide NH protons exchange very slowly. The Cys^1 NH exchanges almost at the same rate as Cys^3 NH in the

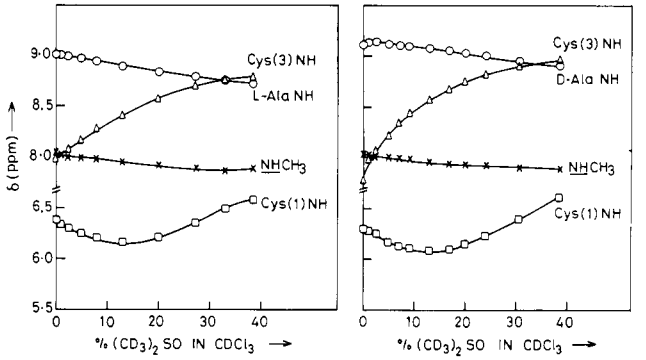

Figure 5. Solvent dependence of NH chemical shifts in $CDCl_3$–$(CD_3)_2SO$ mixtures as a function of solvent composition for (left) **1** and (right) **2**.

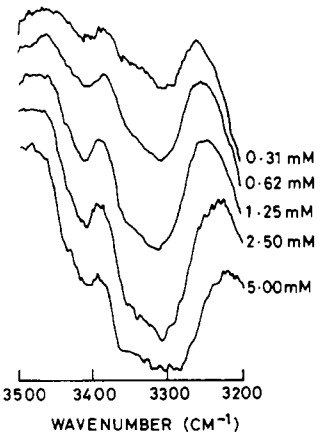

Figure 6. Partial IR spectra (NH stretching region) of **2** as a function of concentration in $CHCl_3$ solution.

D-Ala peptide **2**, whereas its exchange rate is significantly slower in the L-Ala peptide **1**. While quantitative interpretations of H–D-exchange rates in heterogeneous solvent mixtures like $CDCl_3$–D_2O are hazardous, the results provide qualitative support for the solvent-shielded nature of the Ala and methylamide NH groups. The concentration dependence data in Figure 2, however, suggest that intermolecular association via hydrogen-bond formation involving Cys^3 and Cys^1 NH groups is likely in $CDCl_3$. The large downfield shift of Cys^3 NH at higher peptide concentrations suggests that this group is substantially more exposed than Cys^1 NH in both peptides. This is also consistent with the behavior of Cys^1 NH in the radical perturbation and H–D-exchange experiments. It is possible that a specific orientation of the bulky Boc group may result in steric shielding of the Cys^1 NH proton.

Figure 5 shows the effect of adding increasing amounts of the strongly hydrogen-bonding solvent $(CD_3)_2SO$ to peptide solutions in the relatively apolar solvent $CDCl_3$. The Ala and methylamide NH groups show little change in chemical shifts with changes in solvent composition, characteristic of solvent-shielded NH groups. The Cys^3 NH moves rapidly to lower field with increasing $(CD_3)_2SO$ concentration in both **1** and **2**, confirming its exposure to the solvent in both peptides. The Cys^1 NH exhibits anomalous solvent titration curves in both peptides. It shows an initial *upfield* shift on addition of $(CD_3)_2SO$, followed by a moderate downfield shift at higher $(CD_3)_2SO$ concentration. While a definitive explanation for this behavior is not possible at present, it may be noted that the Cys^1 NH group also exhibits distinctive behavior in the radical perturbation and H–D-exchange studies. The involvement of the neighboring Boc group in steric shielding of Cys^1 NH together with solvent-dependent changes in orientation of this moiety may explain the behavior observed in Figure 5.

Infrared Studies. The NMR results presented thus far strongly favor the involvement of at least two NH groups in intramolecular hydrogen bonding in both $CDCl_3$ and $(CD_3)_2SO$. These are the central Ala (L or D) NH and terminal methylamide groups.

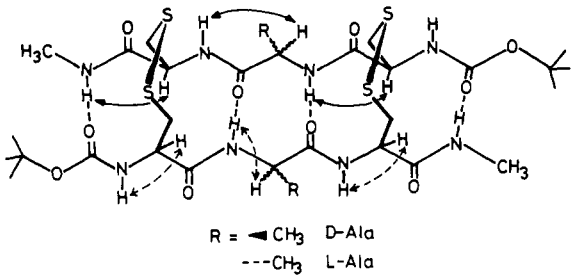

Figure 7. Proposed antiparallel β-sheet conformation of peptides **1** and **2**, showing the hydrogen-bonding pattern. Dark arrows indicate observed interresidue NOE connectivities, while broken arrows indicate observed intraresidue NOE's (see Figure 8).

Further support for the occurrence of intramolecularly hydrogen-bonded conformations comes from IR studies in $CHCl_3$ solutions. Representative spectra of the NH stretching bands in **2** as a function of peptide concentration are illustrated in Figure 6. Bands corresponding to both free NH (ν_{NH}(f)) and hydrogen-bonded NH (ν_{NH}(hb)) stretching vibrations are observed at ~3420 and 3320 cm^{-1}, respectively.[16,17] The ν_{NH}(hb) band is observed over the concentration range 0.31–5 mM, suggesting that intramolecular hydrogen bonds do contribute to this absorption. The ν_{NH}(hb) band has a distinct shoulder at 3360 cm^{-1} and a stronger peak at 3320 cm^{-1}, suggesting that hydrogen bonds of different strengths may stabilize the solution conformation of peptide **2**. In the solid-state (KBr), C=O stretching bands at 1695 (urethane), 1670, and 1635 cm^{-1} (amide I) are observed. The position of the urethane absorption is about 10–20 cm^{-1} lower than that observed in free urethane groups, suggesting its involvement in hydrogen bonding.[16] The position of the amide I bands at 1635 and 1670 cm^{-1} is consistent with an antiparallel β-sheet conformation.[18] Analogous results were obtained with peptide **1**.

Conformations of Peptides 1 and 2. The above analysis of hydrogen bonding in peptides **1** and **2** provides support for the antiparallel β-sheet conformation shown in Figure 7. The unusually low field positions of the $C^{\alpha}H$ protons of Cys^1 and Cys^3 (δ 5.3–5.5) in $CDCl_3$, for both **1** and **2**, are presumably a consequence of this conformation. NMR studies of proteins suggest that low-field $C^{\alpha}H$ and NH resonances are characteristic of β-sheet structures,[19,20] possibly as a consequence of short $C^{\alpha}H$-to-oxygen atom distances, between nonneighboring residues in these structures.[21] The upfield shift of the $C^{\alpha}H$ resonances in $(CD_3)_2SO$ relative to $CDCl_3$ may reflect a distortion of the structure in the more polar solvent. This is likely in view of the greater ability of $(CD_3)_2SO$ to solvate peptide groups, competing with peptide C=O groups for hydrogen-bonding NH sites. While chloroform is a weak hydrogen bond donor, dimethyl sulfoxide is a strong hydrogen bond acceptor. Further, dissociation of aggregated peptide structures in $(CD_3)_2SO$ may facilitate deviations from perfectly extended β-sheet conformations. The high $J_{HNC^{\alpha}H}$ values ($\geq$9 Hz) observed for Cys^1 and Cys^3 NH groups for **1** and **2** in both solvents are also supportive of an extended β-sheet conformation. These values are compatible with $\phi \sim -130°$ to $-150°$, necessary in such conformations.[22] In general, both flexible and helical peptides have significantly lower J values ($\leq$7 Hz).[22,23] The lower $J_{HNC^{\alpha}H}$ value for the L- and D-Ala NH groups may reflect a distortion about the N–C^{α} (ϕ) bond due to close, transannular steric interactions between the Ala C=O groups in an ideal antiparallel β-sheet structure.

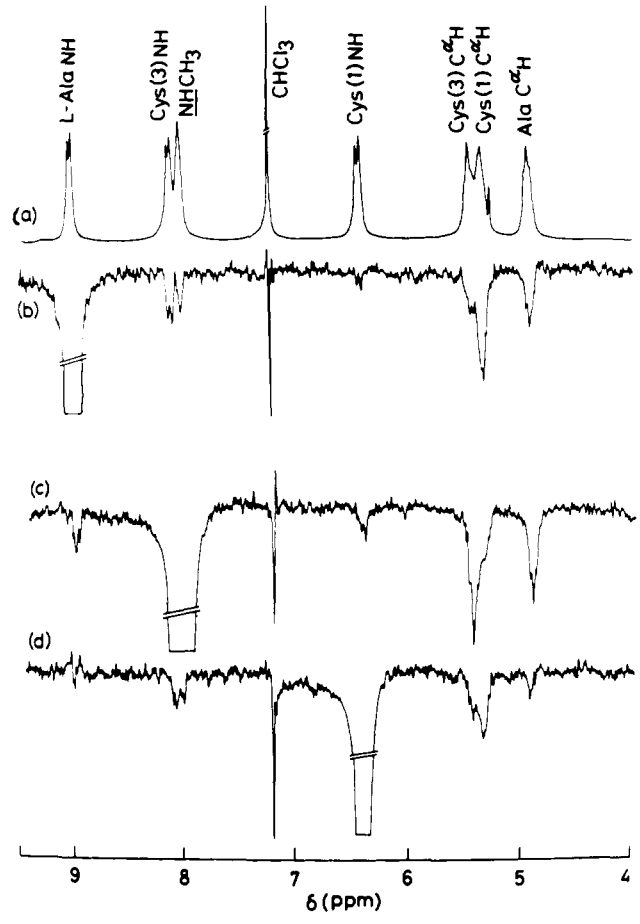

Figure 8. (a) Partial 270-MHz ^1H NMR spectrum of peptide **1** in $CDCl_3$. (b–d) Difference NOE spectra[9] obtained by irradiation of NH resonances (b) Ala NH (c) Cys^3 NH, and $NHCH_3$ (d) Cys^1 NH. The difference spectra are magnified by a factor of 16 (b, d) and 32 (c).

Nuclear Overhauser Effects. Further support for the conformation shown in Figure 7 comes from nuclear Overhauser effect (NOE) studies. Figure 8 shows NOE difference spectra obtained for peptide **1** in $CDCl_3$. Irradiation of Ala^2 NH results in 7% reduction in the intensity of the Cys^1 $C^{\alpha}H$ resonance and a 4% reduction of the Ala^2 $C^{\alpha}H$ peak. The former corresponds to the *inter*residue NOE in the antiparallel β-sheet conformation (see Figure 7), while the latter is the *intra*residue effect. The observation of NOE's between the $C^{\alpha}H$ proton of a particular residue and the NH proton of the succeeding residue is characteristic of antiparallel β-sheets.[24,25] The resonance positions of the Cys^3 and methylamide NH resonances are very close (δ ~8.04). Irradiation of these resonances yields two clear negative NOE's at the Cys^3 $C^{\alpha}H$ (9%) and Ala $C^{\alpha}H$ (6%) protons. This is consistent with the conformation illustrated in Figure 8, where NOE connectivities between the following protons are expected: Cys^3 $C^{\alpha}H$ $\leftrightarrow$ $HNCH_3$ and Ala^2 $C^{\alpha}H$ $\leftrightarrow$ Cys^3 NH. Irradiation of Cys^1 NH yields an appreciable NOE only on Cys^1 $C^{\alpha}H$, which is the intraresidue NOE indicated in Figure 8. In all cases, the observed NOE's are negative, suggesting that rotational correlation times (τ_c) are long enough to be outside the extreme narrowing limit at 270 MHz (i.e., $\omega\tau_c \ll 1$).[26,27] The data described earlier suggest that **1** aggregates in $CDCl_3$ at the concentrations used in the NOE

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studies. Even dimeric species may be expected to yield negative NOE's at 270 MHz. For example, the cyclic 12-residue depsipeptide valinomycin exhibits negative NOE's at 250 MHz.[28] In experiments with the D-Ala peptide **2**, only very weak NOE's (~1%) could be detected. Since all other spectroscopic characteristics of the two peptides are clearly very similar, the nonobservation of appreciable NOE's in **2** could result from correlation times very close to the region of zero NOE. These results suggest that at high frequencies, observation of NOE's in oligopeptides of moderate size, may be rendered difficult in certain cases by unfavorable correlation times.[29]

The spectroscopic results described above provide compelling evidence for the antiparallel β-sheet conformation of the bis(cystine peptides) **1** and **2**. The stability of the structure is undoubtedly influenced by the presence of two disulfide cross-links. The similarity of the spectral properties of **1** and **2** suggests that the conformational angles (ϕ,ψ) of the central L- and D-Ala residues may be similar, reflecting the ability of the two covalent S–S cross-links to impose intrinsically unfavorable conformations on the neighboring residues. While the NMR evidence suggests association of peptides in $CDCl_3$ at the concentrations studied, disaggregation is likely in $(CD_3)_2SO$ with little alteration of backbone conformation. In CD studies of the disulfide $n \rightarrow \sigma^*$ transitions (340–250 nm),[30] in $CHCl_3$ and $(CH_3)_2SO$ the position and sign of the S–S cotton effect ($CHCl_3$, 270 nm, **1** 12 900, **2** 17 620 deg cm^2 dmol^{-1}, $(CH_3)_2SO$, 270 nm, **1** 8120, **2** 12 100 deg cm^2 dmol^{-1}) remain unaltered in the two solvents. This suggests that no major change occurs in disulfide conformations on changing solvent polarity in both peptides. The reduced ellipticity value in $(CH_3)_2SO$ is consistent with enhanced flexibility about the S–S bond in this solvent.

The conformations of **1** and **2** involve transannular hydrogen-bond formation, between the CO groups of the residue preceding Cys and the NH group of the succeeding residue. Such structures are likely to be a general feature of cystine residues, in peptides, unconstrained by other factors. Studies of acyclic cystine peptides containing a single S–S bridge suggest that this is indeed the case[31,32] (Antony Raj, P.; Balaram, P., unpublished results). The results of the present study supporting the occurrence of disulfide bridges across an antiparallel β-sheet structure are at variance with conclusions based on analysis of protein crystal structure data, where the observed S–S conformations have generally precluded interstrand bridging in β-sheets.[2] The cyclic bis(cystine peptide) skeleton provides a relatively rigid structural model for further investigations of interactions between S–S linkages and functional side chains. Studies of peptides with different residues in the central position may yield valuable information.

Acknowledgment. We are grateful to Dr. T. M. Balasubramaniam, Washington State University, for providing the FAB mass spectra. This research was supported by a grant from the Department of Science and Technology, India. The use of the WH-270 NMR spectrometer at the Sophisticated Instruments Facility, Indian Institute of Science, is gratefully acknowledged.

Registry No. 1, 93629-01-7; **2**, 93713-45-2; H-Cys(*S*-Bzl)-OH, 3054-01-1; H-Cys(*S*-Bzl)-OCH_3, 22728-88-7; Boc-L-Ala-OH, 15761-38-3; Boc-D-Ala-OH, 7764-95-6; Boc-L-Ala-Cys(*S*-Bzl)-OCH_3, 98688-00-7; Boc-D-Ala-Cys(*S*-Bzl)-OCH_3, 98688-01-8; Boc-L-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-02-9; Boc-D-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-03-0; H-L-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-04-1; H-D-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-05-2; Boc-Cys(*S*-Bzl)-OH, 5068-28-0; Boc-Cys(*S*-Bzl)-L-Ala-Cys(*S*-Bzl)-NHMe, 98688-06-3; Boc-Cys(*S*-Bzl)-D-Ala-Cys(*S*-Bzl)-NHMe, 98688-07-4.

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Structure Elucidation of Nitrosocimetidine, a Mutagenic Charge-Transfer System

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Abstract: The antiulcer drug cimetidine is nitrosated by using $NaNO_2$ and aqueous HCl to generate a mononitroso product which is isolated in up to 80% yield as either the free base or nitrate salt. The structures of both the free base and nitrate salt were elucidated by using spectral methods. It was found that nitrosation of cimetidine causes a shift in the guanidine π-electron distribution from predominantely conjugated cyanimino-type bonding to a more delocalized arrangement typically observed for unsubstituted guanidines. The free base of nitrosocimetidine in $CHCl_3$ or Me_2SO forms a hydrogen bond between the uncharged imidazole and guanidine moieties. The hydrogen bond can be intramolecular, and its formation is followed by the gradual appearance of charge transfer (CT) between the hydrogen-bonded moieties. Similar CT complexes are formed in $CHCl_3$ from equal molar amounts of 1-methylimidazole and *N*,*N*′-dimethyl-*N*″-cyano-*N*-nitrosoguanidine (DCNG) or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). The charge-transfer transitions for these systems are similar to those of the $n \rightarrow \pi^*$ transitions normally observed for the nitrosoguanidines, suggesting that nearly equivalent orbital energies are occupied by the π electrons of the guanidine and imidazole moieties in the charge-transfer complex. The greater mutagenic activity reported for nitrosocimetidine as the free base compared to its nitrate salt may be related to the presence of an intramolecular complex in the neutral compound.

A. Introduction

Cimetidine (**1**) is a widely prescribed drug for the control of various disorders of the esophagus, stomach, and duodenum.[1,2] The drug blocks the histamine H_2-receptor controlling gastric acid secretion, so it is frequently used to treat peptic ulcer disease. Although it is quite effective in the management of this disease,[3] a possible association of cimetidine therapy with gastric cancer has led to questioning the safety of this drug. Elder and co-

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