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Proton NMR studies of peptide conformations

P BALARAM

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Abstract. This article reviews recent ^{1}H NMR studies on peptides carried out in the author's laboratory. Methods for the delineation of hydrogen bonded NH groups are discussed and exemplified by studies of conformationally constrained peptides. The application of concentration dependences of NMR parameters for NH groups in peptides to the study of peptide aggregation in apolar solvents is considered. Investigations on chemotactic peptide analogs and suzukacillin fragments are briefly summarized. Nuclear-Overhauser effect studies on β-sheet and β-turn conformational models are described. NMR studies on conformational transitions accompanying dissolution of single crystals are illustrated by a study of a peptide containing a -Pro-Pro- segment.

Keywords. Nuclear magnetic resonance; peptide conformation; hydrogen bonding; peptide aggregation; nuclear-Overhauser effects.

1. Introduction

The potential importance of NMR methods in determining the solution conformations of peptides was first emphasized in early studies of the cyclic decapeptide gramicidin S (Stern *et al* 1968; Wyssbrod and Gibbons 1973). In the last decade there has been an explosive increase in the use of NMR methods in peptide conformational analysis, fuelled in part, by the dramatic revolution in NMR spectroscopy, brought about by the ready accessibility of high field spectrometers and more recently, by the introduction of powerful multiple pulse methods, including two-dimensional NMR spectroscopy. The recognition of the important role of peptides in mediating a striking variety of biological processes has provided a major stimulus for these studies. While x-ray diffraction remains the method of choice for determining peptide structures in the solid state (Karle 1981; Benedetti 1982), high resolution NMR is preeminent in analysis of solution conformations (Wüthrich 1976; Deslauriers and Smith 1980). In solution, the dynamic, flexible nature of oligopeptides can result in the population of a number of conformational states, with rapid interconversions being possible, resulting in ambiguities in the interpretation of NMR parameters, in terms of specific conformations (Jardetsky 1980). The use of model peptides with limited conformations can help to define the limits of applicability of various NMR techniques and also serve to calibrate ancillary spectroscopic techniques like IR, CD and Raman methods, using the NMR results as the basis for conformational interpretations. This article briefly reviews some results obtained at Bangalore over the past few years, which illustrate the application of NMR methods to the study of the conformations and aggregation of model peptides in organic solvents.

2. Assignment of resonances

^{1}H NMR studies of peptide conformations are contingent upon the assignment of resonances to protons (NH, $C^{\alpha}H$, $C^{\beta}H$ etc) on specific amino acid residues. $C^{\beta}H$

hydrogens on many residues can be identified unambiguously on the basis of chemical shift values (Wüthrich 1976). Sequential double resonance experiments then permit the connectivity to the $C^{\alpha}H$ and NH protons to be established. The availability of very high field NMR spectrometers (500 or 600 MHz for ^1H) provides excellent resolution of complex spectra and recent two-dimensional (2D) NMR techniques permit ready assignment of specific spin systems (Benn and Gunther 1983; Bax 1982). Figures 1 and 2 exemplify the use of 2D correlated spectroscopy (COSY) (Aue *et al* 1976; Kumar *et al* 1980), in assignment of oligopeptide ^1H NMR spectra. The peptide Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe was synthesized as a model for investigating

the conformations of 20-membered disulfide loops (R Kishore and S Raghothama, unpublished). Boc-Asp(OBzl)-Leu-Thr-Gly-Gly-Gly-Val-OBzl is a synthetic fragment

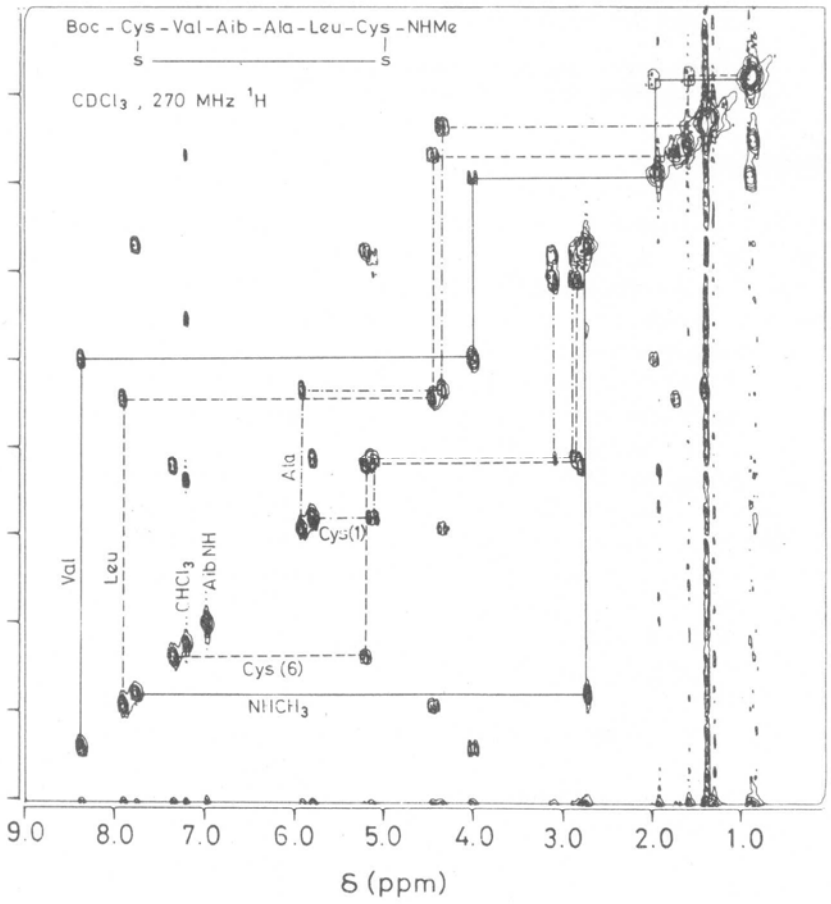

Figure 1. 270 MHz ^1H COSY spectrum (contour plot) of the peptide Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe in $CDCl_3$. Connectivities of NH-C^{α}-H-$C^{\beta}H_2$ protons

for individual residues are shown.

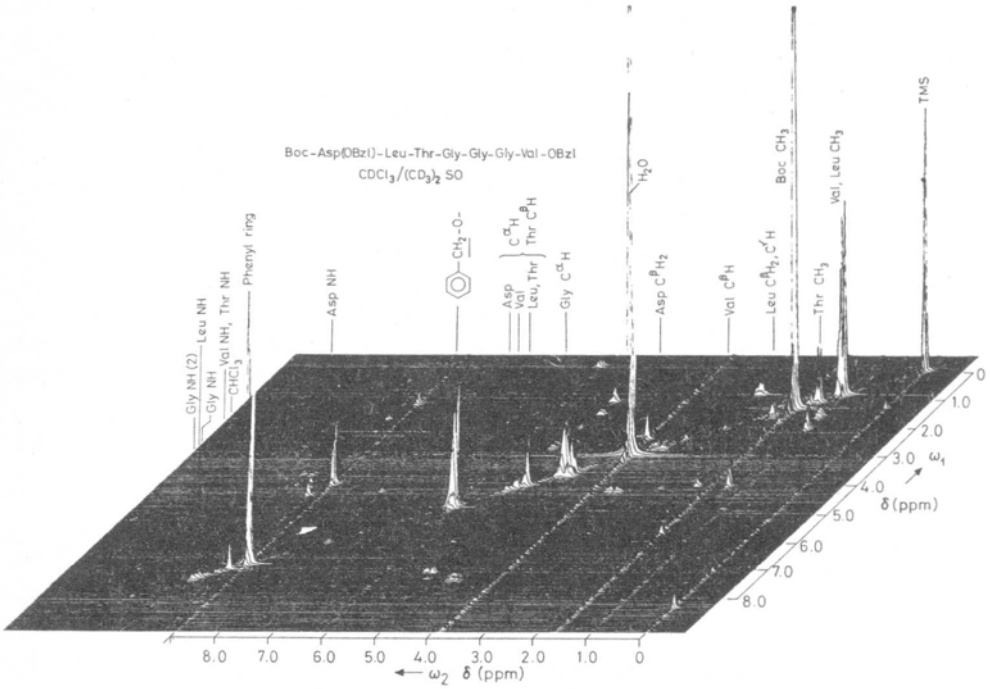

Figure 2. 270 MHz ^{1}H COSY spectrum (stacked plot) of Boc-Asp(Bzl)-Leu-Thr-Gly-Gly-Gly-Val-OBzl in a $CDCl_3$-$(CD_3)_2SO$ mixture.

of a naturally occurring sperm activating peptide (Balaram 1984). It is clearly seen from figures 1 and 2 that the spin systems of individual amino acid residues can be discerned. In cases where the same amino acid occurs more than once in an oligopeptide, assignments of resonances to specific residues are more difficult. In favourable situations, where interresidue nuclear Overhauser effects (NOE's) are observable (*vide infra*) between adjacent amino acids in a sequence, assignments may be made to specific residues (Wagner *et al* 1981; Billeter *et al* 1982). The use of long range spin-spin couplings may also aid in tracing the sequence of amino acids in peptide chains (Wynants and Van Binst 1984). Unambiguous assignments in homooligopeptides can also be made by selective introduction of C^{α}-deuterated amino acids by synthesis.

3. Hydrogen bonding

Folded peptide structures are often stabilized by intramolecular C=O---H–N hydrogen bonds (Toniolo 1980). Typical folded conformations are illustrated in figure 3. A major objective of NMR studies of peptides is to delineate the CO and NH groups involved in intramolecular hydrogen bonding. The extent of solvent exposure of NH groups may be probed by a variety of methods. The more commonly employed techniques include the following:

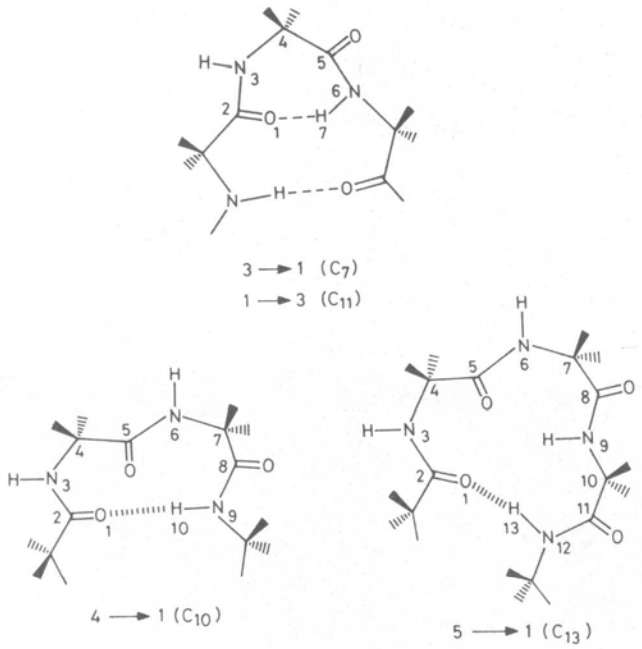

Figure 3. Representative intramolecularly hydrogen bonded conformations in peptides.

3.1 *Temperature coefficients of chemical shifts*

Temperature dependence of NH chemical shifts are usually measured in polar hydrogen bonding solvents like dimethylsulphoxide (DMSO), methanol (MeOH) or water. NH resonances generally move *upfield* with increasing temperature, reflecting breaking of solute–solvent hydrogen bonds. Temperature dependences are invariably linear and the temperature coefficient ($d\delta/dT$) values have been used to characterize solvent exposed and shielded NH groups. $d\delta/dT$ values $< 0{\cdot}003$ ppm/K in solvents like DMSO are characteristic of solvent shielded (presumably intramolecularly hydrogen bonded NH groups), while values $> 0{\cdot}005$ ppm/K are typical of solvent exposed (free) NH groups (Kopple *et al* 1969; Hruby 1974; Venkatachalapathi and Balaram 1981a). Intermediate $d\delta/dT$ values are more difficult to interpret definitively and have sometimes been taken as indicative of weakly intramolecularly hydrogen bonded NH groups (Ravi *et al* 1983). Temperature dependences in aromatic solvents have also proved useful in delineating different kinds of NH groups, probably due to specific solvation of the peptide moiety by these solvents (Venkatachalapathi and Balaram 1981a). $d\delta/dT$ values in apolar solvents like chloroform, cannot distinguish between intramolecularly hydrogen bonded and free NH groups, but can be diagnostic of intermolecular association (Stevens *et al* 1980; Iqbal and Balaram 1982a).

3.2 *Solvent perturbation effects*

In this method, changes in chemical shifts of NH resonances are monitored on addition of a strongly hydrogen bonding solvent, like MeOH or DMSO, to a peptide solution in a poorly hydrogen bonding solvent like $CHCl_3$ or CF_3CH_2OH. Exposed NH groups

show large downfield shifts in such a solvent titration experiment, whereas shielded NH groups are significantly less affected (Pitner and Urry 1972). Monotonic changes in δ values on altering solvent composition are indicative of the absence of solvent-induced structural changes. Conversely, sharp discontinuities are suggestive of conformational transitions.

3.3 *Paramagnetic radical-induced line broadening*

The addition of stable organic free radicals like nitroxides, can induce line broadening of solvent exposed NH groups by enhancement of relaxation rates (Kopple and Schamper 1972; Kopple and Zhu 1983). This experiment is best carried out in apolar solvents like $CHCl_3$, where the solvent does not compete extensively with the radical for interaction with NH groups.

3.4 *Rates of hydrogen deuterium (H-D) exchange*

Solvent-exposed NH groups show much greater rates of H-D exchange as compared to shielded NH groups (Stern *et al* 1968; Narutis and Kopple 1983). This experiment is particularly informative in pure solvent systems like CD_3OD, D_2O etc. In mixed solvent systems quantitative interpretations are risky.

3.5 *Transfer of saturation from solvent resonances*

In this technique intensity changes in peptide NH resonances are monitored while the exchangeable proton of the solvent is saturated. Intensities of solute resonances originating from exposed and labile hydrogens are diminished by transfer of saturation, due to rapid exchange with the solvent, whereas resonances of 'buried' hydrogens are less affected (Glickson *et al* 1974).

In all the methods considered above a clear distinction *cannot* be made between intramolecularly hydrogen-bonded and sterically shielded NH groups. In peptides lacking unusual structural features, solvent shielded NH groups are frequently considered to be hydrogen-bonded. In conformationally flexible peptides interpretations of such hydrogen bonding studies are often inconclusive. The power of these methods is best illustrated in studies of conformationally constrained peptides.

Figure 4 illustrates the temperature and solvent dependence of NH chemical shifts in the decapeptide Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe. Nine well-resolved NH resonances can be seen at 270 MHz (Iqbal and Balaram 1981a). The data in figure 4 clearly establish that with the exception of one Aib NH group (assigned by virtue of its high field position in $CDCl_3$ to the N-terminal urethane) all other 8 NH groups are solvent-shielded or hydrogen-bonded. Of these one (Aib NH(S_2)) is probably involved in a weaker interaction. These observations, together with the known stereochemical propensity of Aib residues to favour 3_{10} helical structures (Nagaraj and Balaram 1981; Prasad and Balaram 1984) led to the suggestion of the hydrogen bonding scheme shown in figure 5 (Iqbal and Balaram 1981a). The interpretation of the NMR results was well supported by the results of a crystal structure determination (figure 5) (Francis *et al* 1983). Analysis of hydrogen-bonded structures by these methods has been extended to several related decapeptides like Boc-(Aib-X)$_5$-OMe (X = L-Ala, L-Val) (Vijayakumar and Balaram 1983), Boc-Aib-X-Aib-Aib-(X)$_3$-Aib-X-Aib-OMe (X = L-Val, L-Leu) (H Balaram and

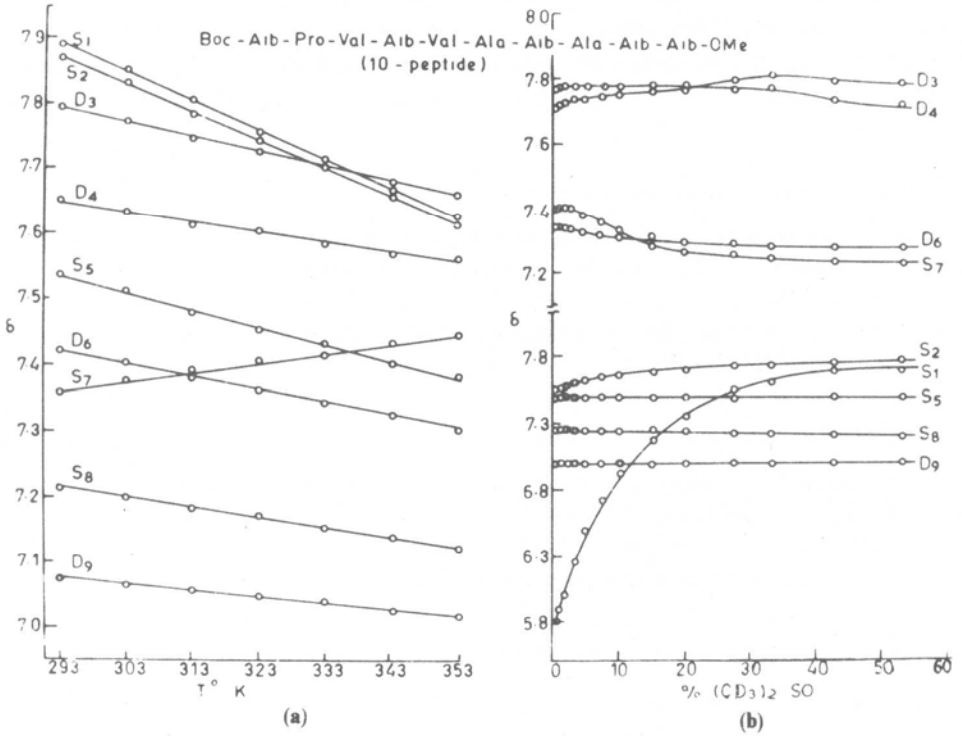

Figure 4. (**a**) Temperature dependence of NH chemical shifts in the decapeptide Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe in $(CD_3)_2SO$. S_n, D_n refer to singlet and doublet NH resonances. The subscript n refers to the order of appearance from low field in $(CD_3)_2SO$. (**b**) Dependence of NH chemical shifts on solvent composition in $CDCl_3$-$(CD_3)_2SO$ mixtures (from Iqbal and Balaram 1981a).

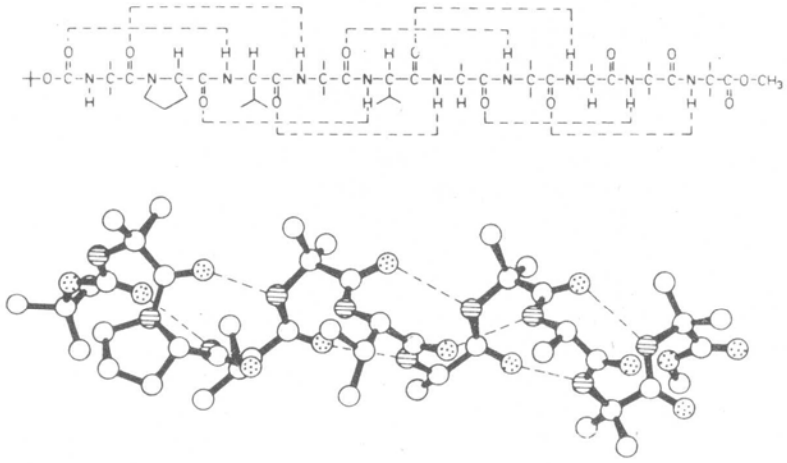

Figure 5. (bottom) Solid state conformation of Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe determined by x-ray diffraction (Francis *et al* 1983). Note 3_{10}-helical ($4 \rightarrow 1$) hydrogen bonding pattern. (top) Intramolecular hydrogen bonding scheme proposed on the basis of ^{1}H NMR studies (Iqbal and Balaram 1981a).

M Sukumar, unpublished), long fragments of suzukacillin ranging in length from 11 to 16 residues (Iqbal and Balaram 1981b, 1982b) and a wide variety of peptide conformational models (Venkatachalapathi and Balaram 1981b; Ravi and Balaram 1984).

4. Peptide aggregation

The concentration dependence of ^{1}H NMR parameters of NH groups can provide valuable information about the intermolecular association of peptides, in solution. Peptide aggregation in apolar solvents like chloroform is often mediated by intermolecular hydrogen bond formation. The possible role of peptide backbone conformation on ease of aggregation has been illustrated in a study of two chemotactic peptide analogs, Formyl-Met-X-Phe-OMe (X = L-Leu, Aib). In the X = Leu peptide no evidence has been obtained for a folded conformation in $CDCl_3$ or $(CD_3)_2SO$. On the contrary in the X = Aib peptide the Phe NH is involved in an intramolecular hydrogen bond, suggesting either a C_7 (γ-turn) conformation centred at Aib or a Met-Aib β-turn (Raj and Balaram 1985). Similar conformations were earlier considered for the analog For-Met-Aib-Phe-OH (Iqbal *et al* 1984). Figure 6 shows the concentration dependence

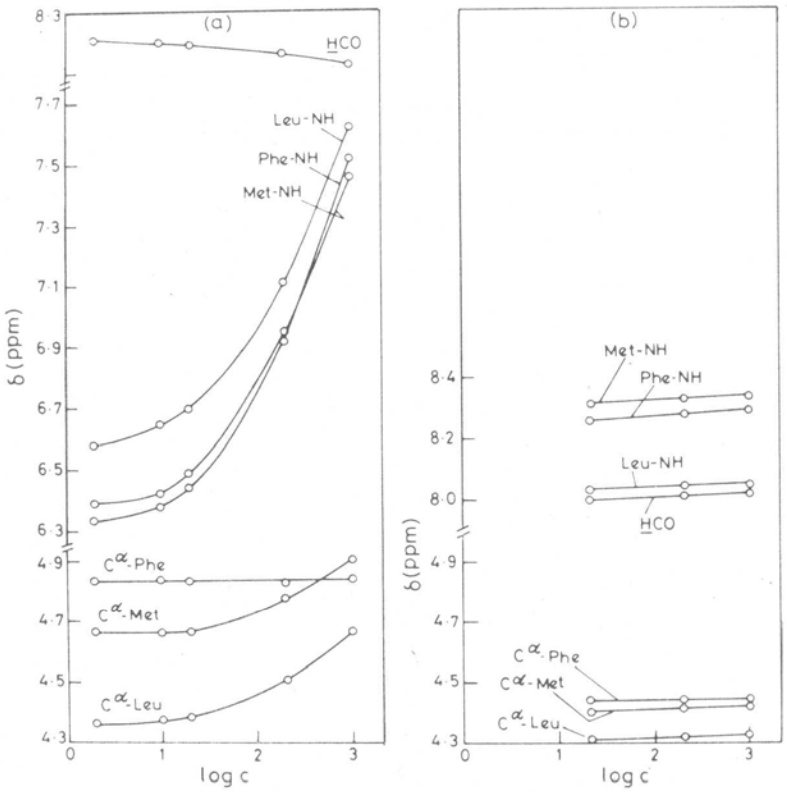

Figure 6. Concentration dependence of chemical shifts (δ) for various resonances in Formyl-Met-Leu-Phe-OMe in $CDCl_3$ (**a**) and $(CD_3)_2SO$ (**b**). Abscissa expresses concentration as log c, where c = molar concentration of peptide $\times 10^4$ (from Raj and Balaram 1985).

of the chemical shifts of NH resonances in Formyl-Met-Leu-Phe-OMe in $CDCl_3$ and $(CD_3)_2SO$. The concentration dependence of temperature coefficients ($d\delta/dT$ values) for both peptides in $CDCl_3$ and $(CD_3)_2SO$ are summarized in figure 7. The data strongly suggest that both peptides are unassociated in $(CD_3)_2SO$ but are aggregated in $CDCl_3$. All three NH groups show large concentration effects in the X = Leu peptide, compatible with the aggregation mode illustrated in figure 8. In the X = Aib case the folded peptide aggregates involve only the exposed Met and Aib NH groups in intermolecular hydrogen bonding.

The possible application of such studies is further illustrated by a comparison of the peptides Boc-X-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (X = Gln 11G, X = Ala 11A). Peptide 11G forms the 7–17 segment of the channel forming ionophore, suzukacillin. In order to evaluate the role of the lone Gln residue in promoting peptide association in apolar solvents, which model membrane environments, the NMR parameters of peptides 11A and 11G were compared.

In peptide 11G nonlinear temperature dependences of chemical shifts are observed for a few NH groups including the Gln sidechain carboxamide protons (figure 9). The nonlinearity is particularly pronounced for the lone Gly NH (T_2) resonance in $(CD_3)_2SO$ at low concentration. A striking feature of the data for the Ala peptide 11A is the absence of such nonlinear behaviour (figure 10). A detailed analysis of the concentration and solvent dependence of NH chemical shifts has permitted the development of a model which ascribes an important role to the Gln sidechain in both intermolecular hydrogen bonding and intramolecular sidechain-backbone interactions (Iqbal and Balaram 1981c). The use of concentration dependences of $d\delta/dT$ values in evaluating the mode of peptide self-association has been described in related studies of a helical decapeptide (Iqbal and Balaram 1982a).

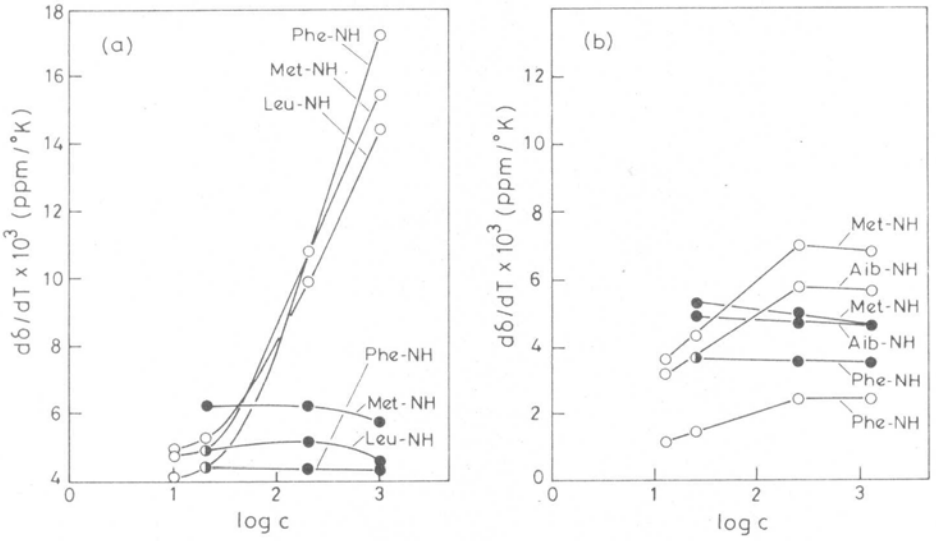

Figure 7. Concentration dependence of temperature coefficients ($d\delta/dT$) of NH resonances in chemotactic peptide analogs. (**a**) Formyl-Met-Leu-Phe-OMe • $(CD_3)_2SO$; o $CDCl_3$ (**b**) Formyl-Met-Aib-Phe-OMe • $(CD_3)_2SO$; o $CDCl_3$-Abscissa defined as in figure 6 (from Raj and Balaram 1985).

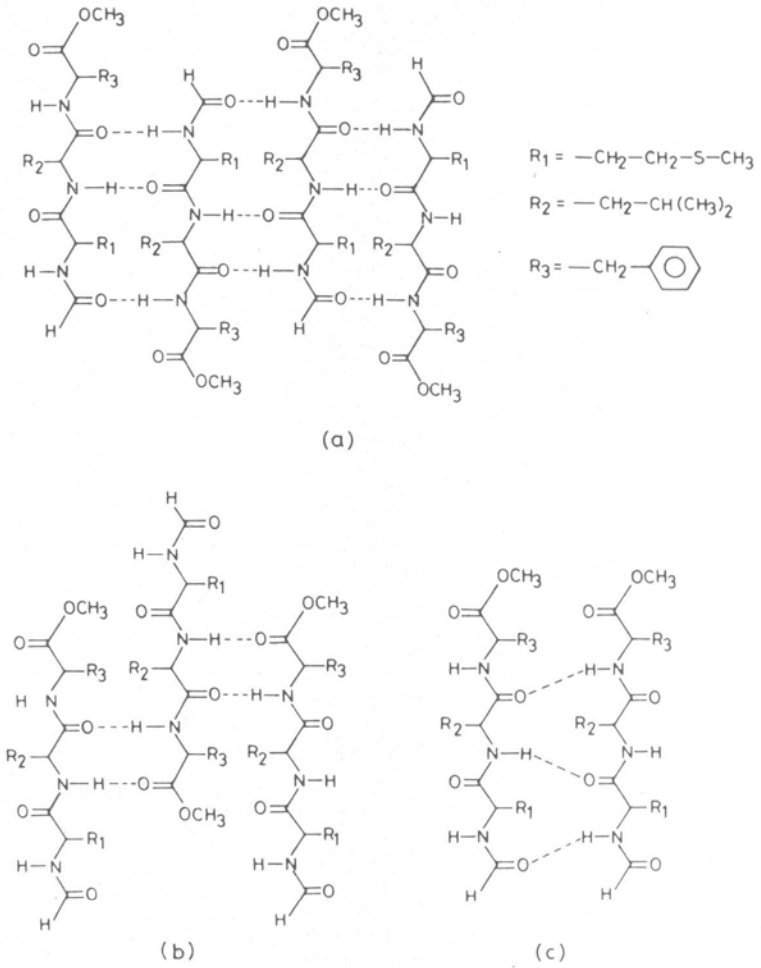

Figure 8. Schematic representation of aggregation modes for Formyl-Met-Leu-Phe-OMe in chloroform, consistent with NMR data. (**a**) antiparallel β-sheets 'in register'. (**b**) antiparallel β-sheets 'out register'. (**c**) parallel β-sheet aggregates.

5. Nuclear-Overhauser effects

Proton-proton NOE's can establish spatial proximity of hydrogen atoms in organic molecules (Noggle and Schirmer 1971; Bothner-By 1979). NOE's are generally observable when the interproton distance is $\leqslant 3{\cdot}0$ Å. Useful interproton distances in studies of peptide conformation are indicated in figure 11. NOE magnitudes are dependent on the correlation time (τ_c) modulating the proton-proton dipolar interaction (Solomon 1955) and are given by the equation

$$f_I(S) = \frac{5 + \omega^2\tau_c^2 - 4\omega^4\tau_c^4}{10 + 23\omega^2\tau_c^2 + 4\omega^4\tau_c^4}, \tag{1}$$

where $f_I(S)$ is the fractional enhancement in resonance intensity of spin S when spin I is

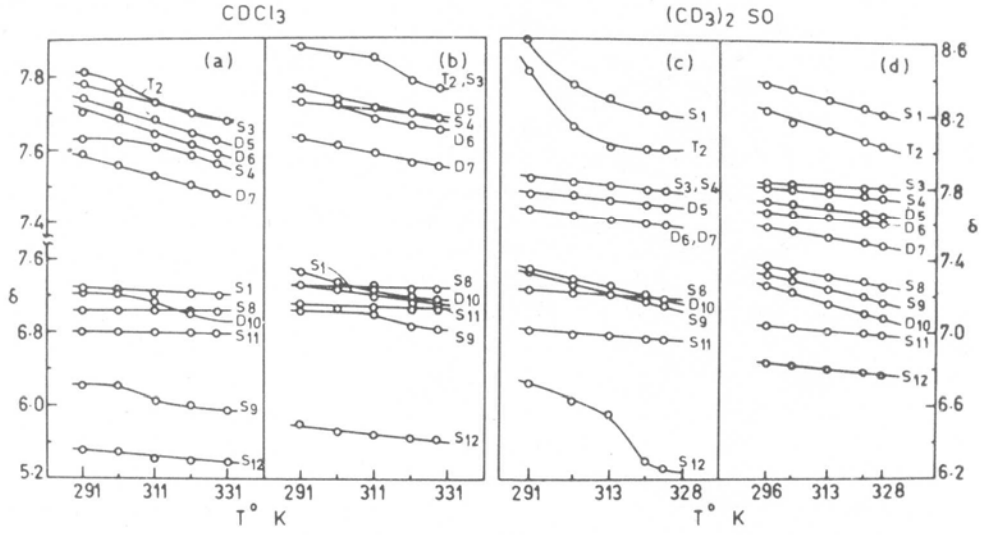

Figure 9. Temperature dependence of NH chemical shifts in the suzukacillin 7-17 fragment Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11G). (**a**) $CDCl_3$, 0·00086 M peptide. (**b**) $CDCl_3$, 0·043 M. (**c**) $(CD_3)_2SO$ 0·00086 M. (**d**) $(CD_3)_2SO$ 0·043 M.

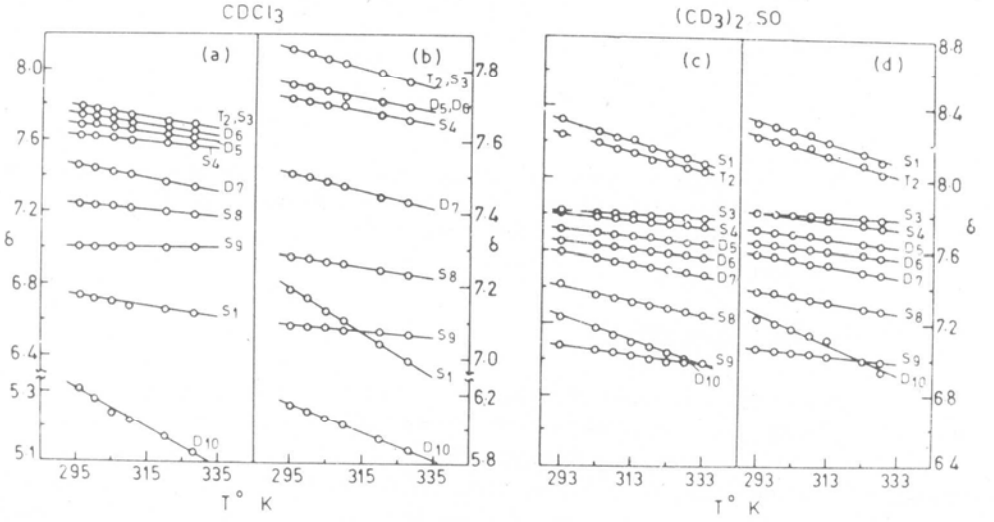

Figure 10. Temperature dependence of NH chemical shifts in the peptide Boc-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11A). (**a**) $CDCl_3$, 0·0018 M peptide. (**b**) $CDCl_3$ 0·045 M. (**c**) $(CD_3)_2SO$, 0·0013 M. (**d**) $(CD_3)_2SO$, 0·045 M.

saturated, ω is the Larmor precession frequency and τ_c is the rotational correlation time, which randomizes the orientation of the internuclear vector $(I - S)$ (Balaram *et al* 1972, 1973). As a consequence of equation (1) NOE's are sharply dependent on τ_c in the region $\omega\tau_c \sim 1$ (figure 12). While positive NOE's are observed in small molecules at all

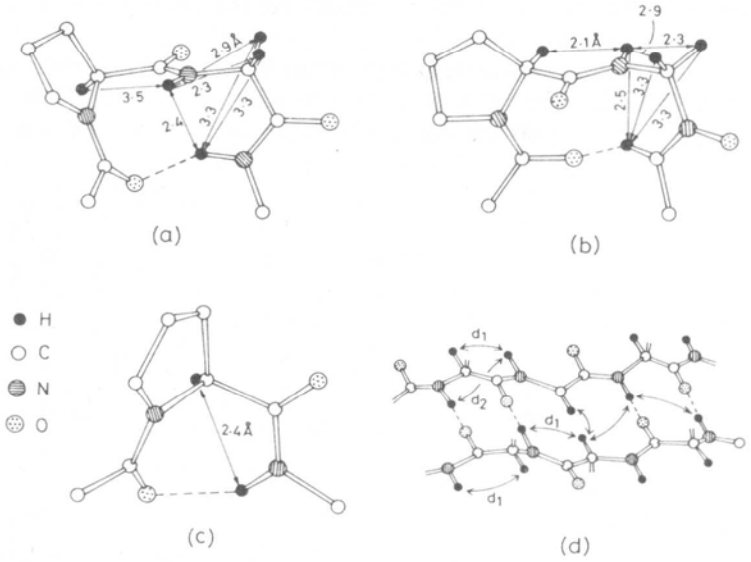

Figure 11. Interproton distances in idealized peptide conformations. (**a**) Pro-Gly type I β-turn. (**b**) Pro-Gly type II β-turn. (**c**) γ-turn (C_7) conformation. (**d**) Antiparallel β-sheet conformation. d_1 (C_iH–N_{i+1}–H) is generally 2·2–2·4 Å and gives rise to large NOEs. d_2 and d_3 are generally $\geqslant$ 3·0 Å in this structure. Transannular NOEs are weak but observable (Kuo and Gibbons 1980; Billeter *et al* 1982; Wagner *et al* 1981).

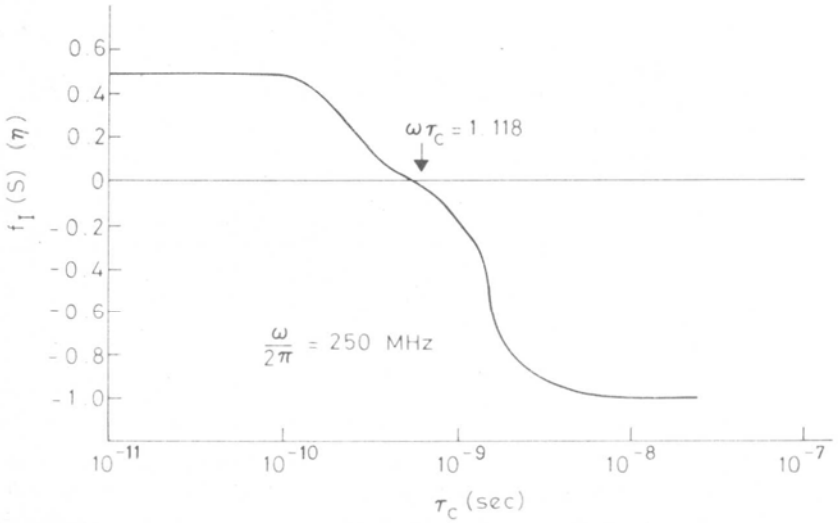

Figure 12. Dependence of ^1H-^1H NOE magnitude (η) on the correlation time τ_c (from Balaram *et al* 1973).

available resonance frequencies, NOE's in macromolecules are generally negative. In oligopeptides both positive and negative NOE's are observed depending on the precise values of τ_c and the spectrometer frequency employed (Glickson *et al* 1976). In unfavourable cases where $\omega\tau_c \sim 1$, NOE magnitudes can be very small making experimental detection difficult. Nevertheless different NOE studies have provided some of the clearest evidence for folded peptide conformations (Rao *et al* 1983; Kuo and Gibbons 1980; Bothner-By 1979).

Illustrative difference NOE experiments on model β-turn peptides, Piv-Pro-X-NHMe are shown in figure 13. Type II β-turns (*e.g.* X = D-Ala, D-Leu, D-Val) are readily recognized by a large NOE between the X-NH and Pro $C^{\alpha}H$ protons. These studies have

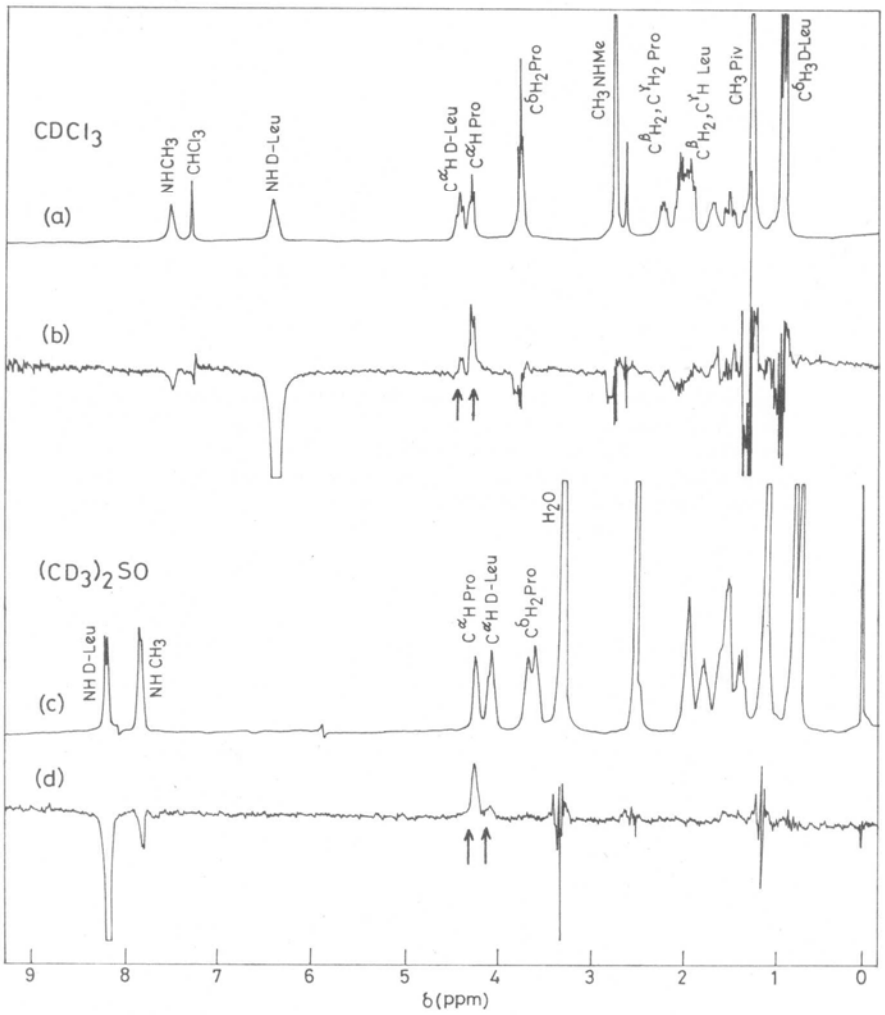

Figure 13. 270 MHz ^{1}H NMR spectra of Pivaloyl-Pro-D-Leu-NHMe in (**a**) $CDCl_3$ and (**c**) $(CD_3)_2SO$. (**b**) Difference NOE spectrum (× 16) in $CDCl_3$ on saturation of D-Leu NH. Note partial saturation of $\underline{NH}CH_3$ (**d**) Difference NOE spectrum (× 4), in $(CD_3)_2SO$ on saturation of D-Leu NH. Note partial saturation of $\underline{NH}CH_3$. Arrows indicate observed NOE's (from Balaram 1984).

permitted a detailed analysis of various β-turn conformations and have aided in the development of correlations with circular dichroism (CD) approaches (Rao *et al* 1983). The potential utility of NOE methods in identifying β-sheet conformations is exemplified by studies on a cyclic hexapeptide disulphide (figures 14, 15). Most relevant interresidue NOE's have been observed, confirming the antiparallel β-sheet conformation, suggested by conventional NMR studies (R Kishore and S Raghothama, unpublished). Extensive NOE studies on several oligopeptides, carried out in this laboratory, suggest that even in hexapeptides NOE magnitudes are sensitive to solvent viscosity and negative NOE's are sometimes observed. In the case of the hexapeptide disulphide mentioned above, negligible NOE's could be detected in DMSO at 20°C. However, heating to 40°C resulted in the observation of small positive NOE's, clearly establishing a correlation time (τ_c) dependence. The routine use of NOE analysis in longer oligopeptides has been greatly facilitated by the development of 2D-NOE spectroscopy (NOESY) (Kumar *et al* 1980; Wider *et al* 1984).

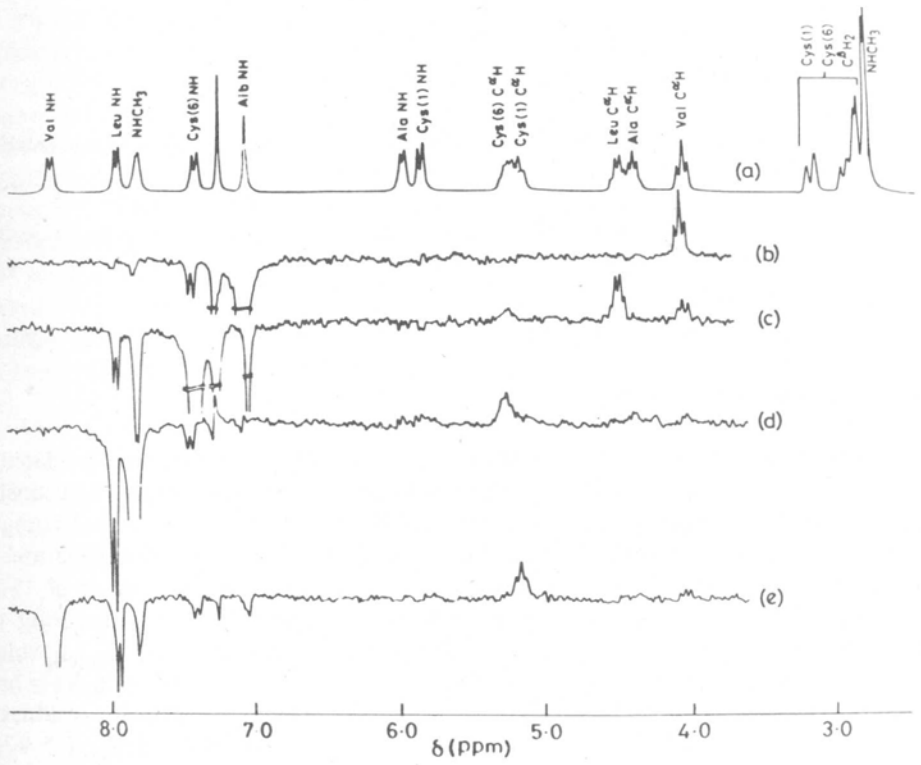

Figure 14. (**a**) Partial 270 MHz ^{1}H NMR spectrum of Boc-Cys-Val-Aib-Ala-Leu-Cys-

NHMe in $CDCl_3$ (**b**)–(**e**) Difference NOE spectra (× 16) obtained by saturation of specific NH resonances. The saturated peak appears as an intense negative signal in the difference spectrum.

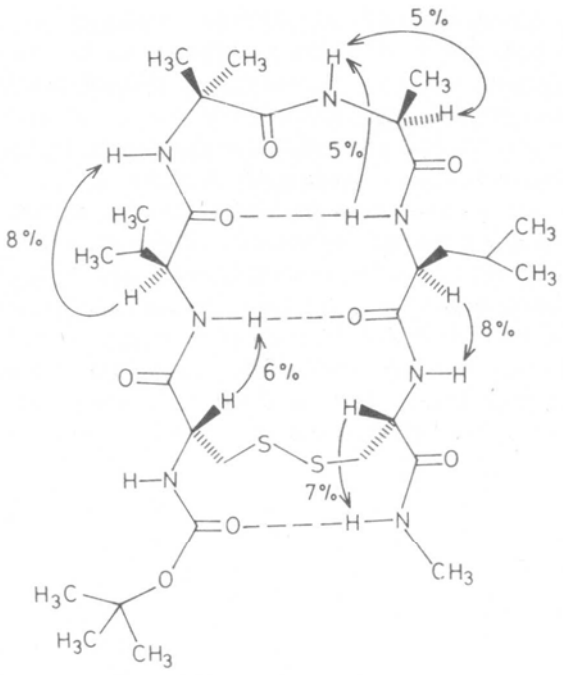

Figure 15. Antiparallel β-sheet conformation of Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe (S—S) consistent with NMR data. Observed NOE's are indicated by arrows linking the two protons. Arrow head indicates irradiated proton.

6. Spin-spin coupling constants

The value of the vicinal coupling constant ($J_{HNC^{\alpha}H}$) in aminoacid residues is related to the dihedral angle about the N–C$^{\alpha}$ bond (ϕ) by the well-known Karplus relationship (Bystrov 1976). A single value of J is compatible with as many as 4 ϕ values leading to ambiguities in interpretation. The combined use of theoretical energy calculations in conjunction with NMR J values can reduce this degeneracy (Gibbons *et al* 1970). Conformational averaging can further influence observed J values, obscuring the stereochemical information (Jardetsky 1980). Despite these difficulties $J_{HNC^{\alpha}H}$ values have been widely used in parallel with other NMR methods. $^{3}J_{HH}$ values of 6–8 Hz have been obtained for residues, where dynamic averaging is likely and also for residues in rigid helical conformations (Iqbal and Balaram 1981a). Large values of $^{3}J_{HH}$ ($\geqslant 9$ Hz) have been observed for peptides in rigid extended β-sheet conformations (Kishore and Balaram 1984). Attempts to use both geminal ($^{2}J_{HH}$) and $^{3}J_{HH}$ values to obtain both ϕ and ψ values for Gly residues in peptides have been reported (Barfield *et al* 1976). The use of heteronuclear vicinal coupling constants (*e.g.* -HC -CO-^{15}N- and ^{13}CO-N-C-H) further expands the possible utility of J values as probes of peptide conformation (Fischman *et al* 1980).

7. Conformational changes on dissolution of single crystals

^{1}H NMR studies of peptide structures in solution are often complicated by the presence of multiple conformational states and the attendant problems of dynamic averaging. A direct comparison with the results of crystal structure determinations is often difficult, since x-ray studies invariably yield only a single solid state conformation. It is thus important to establish the nature of the conformational transitions, which accompany dissolution of single crystals. This problem has been addressed in a study of the model peptide, Boc-Aib-Pro-Pro-NHMe. The presence of a Pro-Pro segment can lead, in principle, to various low energy conformational states, of which four are illustrated in figure 16. These correspond to distinct conformations about the Pro-Pro peptide bond (ω) and the Pro(2) C^α–CO (ψ) bond. Single crystals of the peptide were obtained and an x-ray diffraction study yielded the following conformational angles for the Pro-Pro segment: Pro(2) $\omega = 178{\cdot}7°$ (173·2°), $\phi = -71{\cdot}3°$ (−67·0°), $\psi = 158{\cdot}1°$ (157·1°); Pro(3) $\omega = 179{\cdot}5°$ (172·1°), $\phi = -71{\cdot}8°$ (−62·3°), $\psi = 148{\cdot}6°$ (154·4°), where values in parentheses correspond to the second molecule in the crystallographic asymmetric unit (Balaram *et al* 1983). These values correspond to a *trans* Pro-Pro peptide bond, with a *trans'* conformation at the C^α–CO (ψ) bond of Pro(2).

Figure 17 shows a 270 MHz ^{1}H NMR spectrum of the peptide obtained by dissolving *single crystals* in precooled $CDCl_3$ at 233 K. Resonances due to a single conformation are observed, which must correspond to the *trans-trans'* Pro(2) conformation observed by x-ray diffraction in the solid state. On warming, additional resonances appear, which correspond to population of the *cis* conformer about the Pro-Pro bond and the *cis'* conformer about the Pro(2) C^α–CO bond. Figure 17 illustrates the appearance of as many as three conformers, as observed from the methyl resonance of the N-methylamide group. It is clearly seen that at temperature > 293 K conformers **II** and **III** go into rapid exchange suggestive of a fast equilibration about the Pro(2) C^α–CO bond. This is consistent with a lower barrier for rotation about the C^α–CO (ψ) bond as compared to the Pro-Pro peptide (ω) bond. A 270 MHz ^{1}H NMR spectrum of a *non-crystalline* sample shows the presence of all three conformers at 233 K (figure 17). This

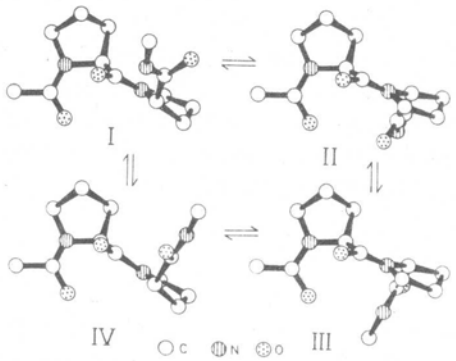

Figure 16. Perspective view of the low energy conformations of a Pro-Pro sequence. Calculations were performed on the model Ac-Pro-Pro-NHMe. **I** (*trans-trans'*); **II** (*cis-trans'*); **III** (*cis-cis'*); **IV** (*trans-cis'*). *Trans* and *cis* refer to the $\omega = 180°$ and $\omega = 0°$ conformations about the Pro-Pro bond, respectively. *Trans'* and *cis'* refer to conformations in the region $\psi \sim 130°$ and $\psi \sim -50°$, respectively for the first Pro residue (from Balaram *et al* 1983).

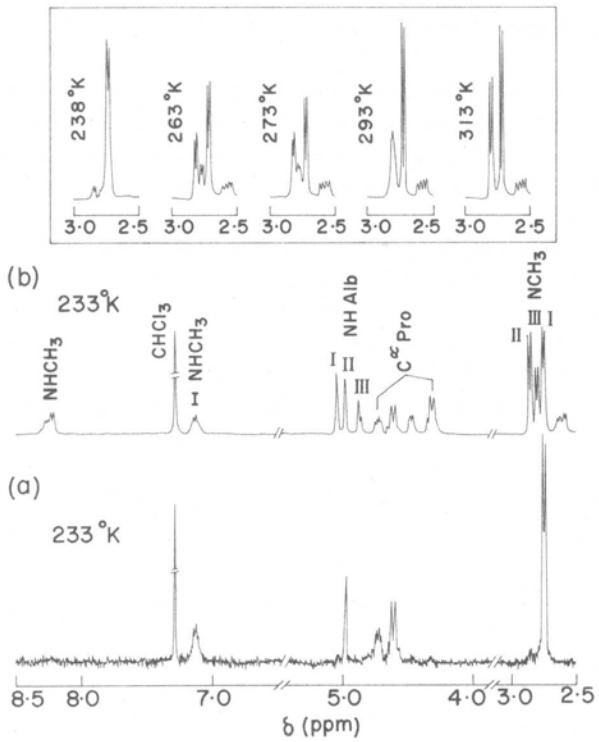

Figure 17. (**a**) Low temperature 270 MHz ^{1}H NMR spectrum of single crystals of Boc-Aib-Pro-Pro-NHMe recorded immediately after dissolution in $CDCl_3$, pre-cooled in liquid N_2 and transferred to the probe at 233 K. (**b**) Low temperature 270 MHz ^{1}H NMR spectrum of a *noncrystalline* sample of Boc-Aib-Pro-Pro-NHMe at 233 K in $CDCl_3$. (Inset) Methylamide CH_3 resonances (doublets) in Boc-Aib-Pro-Pro-NHMe. The sample was prepared as in (**a**) and the spectra recorded at various temperatures. **I, II, III** indicate an assignment to the Pro-Pro conformers described in figure 16 (from Balaram *et al* 1983).

is the first example of the observation of such transitions on dissolution of single crystals of an oligopeptide (Balaram *et al* 1982, 1983).

The studies described above provide a personalized account of ^{1}H NMR studies of peptide conformations, exemplified by problems investigated in the author's laboratory. No attempt has been made to review the burgeoning literature in this area. It is however clear that ^{1}H NMR studies provide the most powerful means of probing the structures and dynamics of peptides in solution.

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