Aggregation of Apolar Peptides in Organic Solvents. Concentration Dependence of ^{1}H-NMR Parameters for Peptide NH Groups in 3_{10} Helical Decapeptide Fragment of Suzukacillin

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Synopsis

Peptide NH chemical shifts and their temperature dependences have been monitored as a function of concentration for the decapeptide, Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe in CDCl$_3$ (0.001–0.06M) and (CD$_3$)$_2$SO (0.001–0.03M). The chemical shifts and temperature coefficients for all nine NH groups show no significant concentration dependence in (CD$_3$)$_2$SO. Seven NH groups yield low values of temperature coefficients over the entire range, while one yields an intermediate value. In CDCl$_3$, the Aib(1) NH group shows a large concentration dependence of both chemical shift and temperature coefficient, in contrast to the other eight NH groups. The data suggest that in (CD$_3$)$_2$SO, the peptide adopts a 3_{10} helical conformation and is monomeric over the entire concentration range. In CDCl$_3$, the 3_{10} helical peptide associates at a concentration of 0.01M, with the Aib(1) NH involved in an intermolecular hydrogen bond. Association does not disrupt the intramolecular hydrogen-bonding pattern in the decapeptide.

^{1}H-nmr spectroscopy has been extensively applied in the conformational analysis of peptides.[1,2] The delineation of intramolecular hydrogen bonds has been a subject of particular interest. The various methods used to determine the solvent exposure of peptide NH groups include (1) rates of hydrogen–deuterium exchange,[3] (2) solvent dependence of chemical shifts,[4] (3) temperature dependence of chemical shifts,[5] (4) paramagnetic radical-induced line broadening,[6] and (5) transfer of saturation from exchangeable solvent protons.[7] Of these, the temperature dependence of NH chemical shifts has probably been applied most extensively. While many studies have been carried out in polar, hydrogen-bonding solvents like (CD$_3$)$_2$SO or CD$_3$CN, a few reports of studies in less polar media like CDCl$_3$ have been reported.[8] It has recently been pointed out that modifications in interpretation may be necessary to analyze temperature coefficient ($d\delta/dT$) data for NH groups in apolar, non-hydrogen-bonding solvents. Using studies on small model acyclic peptides, it has been suggested that low $d\delta/dT$ values (<0.0025 ppm/°C) in CDCl$_3$ may be attributed to free or intramolecularly hydrogen-bonded groups, while high $d\delta/dT$ values are characteristic of intermolecularly hydrogen-bonded groups.[8]

Using 270-MHz ^{1}H-nmr, we have recently established a highly folded conformation for the decapeptide Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-

Ala-Aib-Aib-OMe (1) (Aib = α-aminoisobutyric acid, $H_2N-C(CH_3)_2-$COOH), the amino terminal fragment of the membrane channel former, suzukacillin.[9] The peptide adopts a 3_{10} helical conformation in $CDCl_3$ and $(CD_3)_2SO$ stabilized by eight intramolecular $4 \rightarrow 1$ hydrogen bonds. The identity of the solvent-shielded NH resonances was established using $d\delta/dT$ values in $(CD_3)_2SO$ and the solvent dependence of chemical shifts in $CDCl_3-(CD_3)_2SO$ mixtures. In this report, we examine the concentration dependence of NH chemical shifts and temperature coefficients in $CDCl_3$ and $(CD_3)_2SO$ in an attempt to answer the following questions:

Does the decapeptide aggregate at the concentrations used in these solvents?

Does aggregation affect the backbone conformation?

Is there any correlation between $d\delta/dT$ values obtained in $CDCl_3$ and $(CD_3)_2SO$ for stereochemically rigid peptides?

We show that decapeptide 1 does aggregate in $CDCl_3$ at concentrations $\geqslant 10$ mg/mL, but association occurs only through NH groups not involved in intramolecular hydrogen-bonding. The association of such rigid helical structures in nonpolar media may be relevant in developing models for the aggregation of channel-forming peptides[10] like suzukacillin[11] and alamethicin[12] in the lipid phase of membranes.

EXPERIMENTAL

Peptide 1 was synthesized by solution phase procedures, found to be homogeneous by TLC on silica gel, and yielded 270-MHz ^1H-nmr spectra fully consistent with the sequence. ^1H-nmr studies were carried out on a Bruker WH-270 FT-nmr spectrometer at the Bangalore NMR Facility. The ^2H resonances of $CDCl_3$ and $(CD_3)_2SO$ served as the internal field-frequency lock. Spectral widths of 3012 Hz were used, and after Fourier transformation, the data were stored in 8K memory locations, yielding a digital resolution of 0.367 Hz/point. All chemical shifts are expressed as δ (ppm) downfield from internal tetramethylsilane. Peptide concentrations were varied between 1 and 60 mg/mL (0.001–0.06M). In $(CD_3)_2SO$ the highest concentration that could be attained was 30 mg/mL (0.03M) because of limited solubility of the peptide.

RESULTS

Figure 1 shows the low-field region of the ^1H-nmr spectrum of 1 in $CDCl_3$, as a function of concentration. Nine distinct NH resonances are observable. Of these, five are singlets assignable to the Aib residues, and four are doublets assignable to the Ala and Val residues. As described earlier, the Aib(1) NH can be unequivocally assigned in $CDCl_3$, while the Ala and Val groups are distinguished by spin decoupling. The corresponding resonances in $(CD_3)_2SO$ were identified by solvent titration experiments.[9]
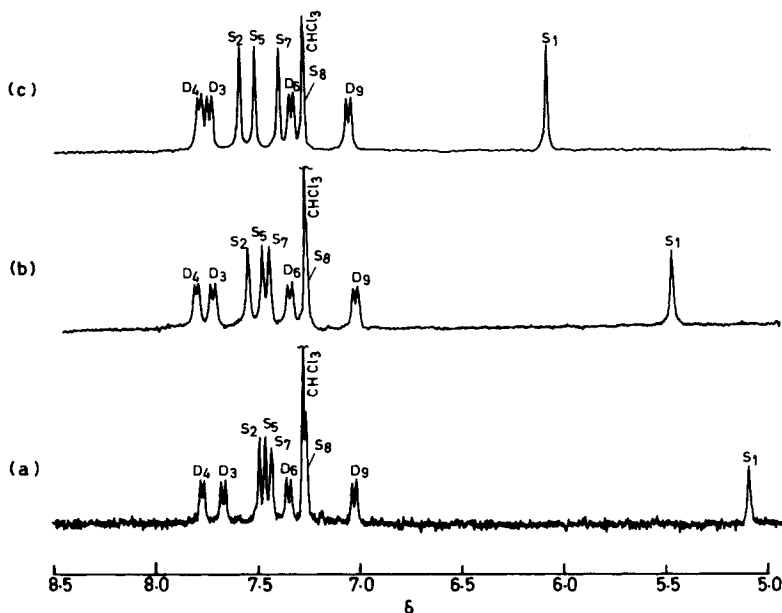
Fig. 1. Partial 270-MHz ^{1}H-nmr spectra (low-field region) of 1 in CDCl$_3$ as a function of concentration: (a) 0.001M (b) 0.01M, and (c) 0.06M. Temperature, 293 K.

Temperature coefficients ($d\delta/dT$) for the various NH resonances were determined as a function of concentration in both solvents. All NH resonances exhibited linear temperature dependences. The results are summarized in Table I. The concentration dependence of NH chemical shifts in both solvents is shown in Fig. 2. The change in chemical shift ($\Delta\delta$) on going from 0.001 to 0.06M in CDCl$_3$ and 0.03M in (CD$_3$)$_2$SO for the various NH groups in 1 are listed in Table I.

DISCUSSION

Our earlier report was based on $d\delta/dT$ values in (CD$_3$)$_2$SO determined at a concentration of 10 mg/mL (0.01M).[9] The $d\delta/dT$ values together with solvent dependence of chemical shifts led us to conclude that 1 adopts a 3_{10} helical conformation, in which eight NH groups are intramolecularly hydrogen bonded. These 4 → 1 hydrogen bonds stabilize consecutive type III β-turns. The Aib(1) NH group is the only one completely exposed to the solvent. One Aib NH (S$_2$), tentatively assigned to Aib(7), has a rather high value in (CD$_3$)$_2$SO, suggesting that the Val(5)-Ala(6) β-turn may be less stable. From the data in Table I, it is clear that $d\delta/dT$ values for 1 in (CD$_3$)$_2$SO are relatively insensitive to concentration. Further, there are only very small changes in chemical shifts ($\Delta\delta$) on going from 0.001 to 0.03M. This suggests that peptide aggregation is not a significant factor in interpreting the nmr parameters in (CD$_3$)$_2$SO. In CDCl$_3$, on the contrary, there is a large concentration dependence of $d\delta/dT$ for the Aib(1)

TABLE I

Concentration Dependence of ^{1}H-NMR Parameters of NH Groups in Peptide 1

	CDCl$_3$				(CD$_3$)$_2$SO			
	$d\delta/dT^a$ (ppm/°C $\times$ 10^3)				$d\delta/dT^a$ (ppm/°C $\times$ 10^3)			
NH	0.001M	0.01M	0.06M	$\Delta\delta^b$	0.001M	0.01M	0.03M	$\Delta\delta^b$
S$_1$	3.3	6.3	8.0	1.016	5.2	4.4	4.9	−0.025
S$_2$	5.5	3.3	3.5	0.083	4.0	4.0	4.3	0.024
D$_3$	3.1	2.6	2.5	0.069	2.6	2.2	2.5	0.000
D$_4$	1.0	1.2	1.7	0.039	1.5	1.6	1.6	−0.010
S$_5$ ^{c}	2.7	2.9	3.0	0.075	2.9	2.6	2.8	−0.003
D$_6$	2.0	1.8	1.6	0.003	1.7	2.0	0.1	0.017
S$_7$ ^{c}	−0.8	1.1	−1.6	−0.015	1.6	−1.0	0.7	0.035
S$_8$	1.6	1.7	2.0	0.039	1.4	1.5	1.5	0.007
D$_9$	1.0	1.0	1.6	0.046	0.8	1.0	1.0	−0.005

^{a} Negative $d\delta/dT$ values correspond to downfield shifts of the NH resonance, with increasing temperature.

^{b} $\Delta\delta$ is the change in NH chemical shift on going from a concentration of 0.06 to 0.001M in CDCl$_3$ and 0.03 to 0.001M in (CD$_3$)$_2$SO at 298 K. Negative values correspond to a downfield shift at lower concentration.

^{c} The assignment of S$_5$ and S$_7$ resonances is arbitrary. Both resonances show small chemical shift variations as a function of concentration, resulting in overlap of the closely spaced peaks under different conditions.

NH group (S$_1$). The temperature coefficient increases with concentration. Aib(1) NH also shows a large concentration dependence of chemical shift (Fig. 2, Table I) as compared to the remaining NH groups. The downfield shift of Aib(1) NH with increasing peptide concentration may be ascribed to aggregation, with formation of intermolecular hydrogen bonds involving the solvent-exposed NH group. The other eight NH groups do not show
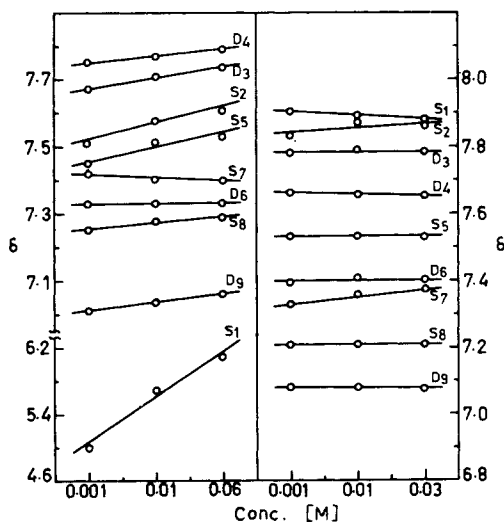

Fig. 2. Concentration dependence of NH chemical shifts in CDCl$_3$ (left) and (CD$_3$)$_2$SO (right).

large concentration-dependent chemical shift changes, suggesting that peptide association does not significantly alter the environment of these groups. A comparison of the low-concentration $(0.001M)$ data in $CDCl_3$ and $(CD_3)_2SO$ shows that with the exception of the resonances S_1 [Aib(1) NH] and S_2 [tentative Aib(7) NH], the $d\delta/dT$ values obtained in the two solvents agree very closely and are all $\lesssim 0.003$ ppm/°C. This is consistent with the earlier suggestion that seven NH groups are involved in strong intramolecular hydrogen-bonding, while one Aib NH (S_2) participates in a weaker interaction.

The interpretation of $d\delta/dT$ values in $(CD_3)_2SO$ is based on the fact that solvent-exposed NH groups are strongly hydrogen-bonded to the sulfoxide moiety of the solvent, in contrast to the groups involved in intramolecular hydrogen-bonding. With increasing temperature, the breaking of solvent–solute hydrogen bonds results in an upfield shift of solvent-exposed NH protons. Typically, $d\delta/dT$ values >0.004 ppm/°C have been ascribed to exposed NH groups.[5,13] In $CDCl_3$, there are no strong hydrogen-bonding interactions between the solvent and solute. Consequently, low values of the temperature coefficients are expected for both solvent-exposed and intramolecularly hydrogen-bonded protons. Stevens et al.[8] have further shown that large temperature dependences are observed when an NH group is initially shielded from solvent but exposed at higher temperature. This corresponds to a situation where intermolecular association occurs or when an ordered conformation is unfolded, with concomitant breakage of intramolecular hydrogen bonds.

The data for peptide 1 (Table I) lead to the following conclusions:

i. In $CDCl_3$ the peptide adopts a 3_{10} helical conformation stabilized by eight intramolecular $4 \rightarrow 1$ hydrogen bonds.

ii. At concentrations of 10 mg/mL $(0.01M)$ in $CDCl_3$, intermolecular association occurs via hydrogen-bonding involving the free NH group of Aib(1). Since the CO groups of residues 1–7 and the urethane CO are involved in intramolecular hydrogen bonds, association may involve the CO groups of residues 8, 9, or 10. It is, therefore, likely that the decapeptide molecules associate in "head-to-tail" fashion.

iii. The decrease in the $d\delta/dT$ value for S_2 with increasing concentration in $CDCl_3$, suggests that aggregation may, in fact, stabilize the helical folding of the molecule by enhancing the likelihood of formation of the Val(5)–Ala(6) type III β-turn. As noted earlier, S_2 has been tentatively assigned to Aib(7) NH.

iv. In $(CD_3)_2SO$ there is no evidence for peptide association up to a concentration of $0.03M$. This is not surprising since solvent–solute interactions are probably more facile than solute–solute ones in this strongly hydrogen-bonding solvent.

v. The $d\delta/dT$ values in $(CD_3)_2SO$ provide clear evidence for seven strong intramolecular hydrogen bonds, with one resonance, S_2[Aib(7) NH], showing evidence for a weaker interaction.

The results presented above suggest that the concentration dependence

of both temperature coefficients and chemical shifts of peptide NH groups in solvents of different hydrogen-bonding characteristics may be used to evaluate both secondary structure and intermolecular association. Such an analysis is particularly facile for stereochemically rigid peptides,[14,15] where predominantly a single conformational state is populated and large strucDpral transitions do not occur with changes in solvent or temperature. The intermolecular association demonstrated for peptide 1 in $CDCl_3$ is of particular interest, since the larger hydrophobic peptides like suzukacillin and alamethicin do, in fact, associate in the nonpolar lipid phase of membranes to form channel structures.

While hydrophobic association of similar apolar peptides has been demonstrated in aqueous solution,[16] the present study suggests that intermolecular hydrogen bonding may be important in determining the structure of the aggregate in nonaqueous media. Association does not appear to result in any marked structural change for 1. This lends support to the view that transmembrane channels of hydrophobic polypeptides may actually be composed of rodlike 3_{10} helical structures aggregated to yield a central core of ordered water.[17-19] Further studies on the aggregation behavior of alamethicin and suzukacillin fragments in both aqueous and nonaqueous media are currently underway in this laboratory. The results will be presented elsewhere.[20,21]

References

1. Craig, L. C., Cowburn, D. & Bleich, H. (1975) *Annu. Rev. Biochem.* **44**, 477–490.

2. Wuthrich, K. (1976) *NMR in Biological Research: Peptides and Proteins*, North-Holland, Amsterdam.

3. Stern, A., Gibbons, W. A. & Craig, L. C. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 734–741.

4. Pitner, T. P. & Urry, D. W. (1972) *J. Am. Chem. Soc.* **94**, 1399–1400.

5. Hruby, V. J. (1974) *Chemical and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. 3, Weinstein, B., Ed., Marcel Dekker, New York, pp. 1–188.

6. Kopple, K. D. & Schamper, T. J. (1972) *J. Am. Chem. Soc.* **94**, 3644–3646.

7. Pitner, T. P., Glickson, J. D., Dadok, J. & Marshall, G. R. (1974) *Nature* **250**, 582–584.

8. Stevens, E. S., Sugawara, N., Bonora, G. M. & Toniolo, C. (1980) *J. Am. Chem. Soc.* **102**, 7048–7050.

9. Iqbal, M. & Balaram, P. (1981) *J. Am. Chem. Soc.* **103**, 5548–5552.

10. Nagaraj, R. & Balaram, P. (1981) *Biochemistry* **20**, 2828–2835.

11. Jung, G., Konig, W. A., Liebfritz, D., Ooka, T., Janko, K. & Boheim, G. (1976) *Biochem. Biophys. Acta* **433**, 164–181.

12. Mueller, P. & Rudin, D. O. (1968) *Nature* **217**, 713–719.

13. Venkatachalapathi, Y. V. & Balaram, P. (1981) *Biopolymers* **20**, 625–628.

14. Nagaraj, R., Shamala, N. & Balaram, P. (1979) *J. Am. Chem. Soc.* **101**, 16–20.

15. Rao, Ch.P., Nagaraj, R., Rao, C. N. R. & Balaram, P. (1980) *Biochemistry* **19**, 425–431.

16. Nagaraj, R. & Balaram, P. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1041–1049.

17. Mathew, M. K., Nagaraj, R. & Balaram, P. (1981) *Biochem. Biophys. Res. Commun.* **98,** 548–555.

18. Iqbal, M. & Balaram, P. (1981) *Biochemistry* **20,** 4866–4871.

19. Nagaraj, R. & Balaram, P. (1981) *Acc. Chem. Res.* **14,** 356–362.

20. Iqbal, M. & Balaram, P. (1981) *Biochemistry* **20,** 7278–7284.

21. Mathew, M. K., Nagaraj, R. & Balaram, P. (1982) *J. Biol. Chem.* **257,** 2170–2176.