Int. J. Peptide Protein Res. **32**, 1988, 167–174

Molecular conformation of alamethicin in dimethylsulfoxide solution

A two-dimensional n.m.r. study

K. CHANDRASEKHAR[a], MANOJ K. DAS[c], ANIL KUMAR[a,b] and P. BALARAM[c]

[a]*Department of Physics,* [b]*Sophisticated Instruments Facility and* [c]*Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India*

Received 27 October 1987, accepted for publication 8 March 1988

The solution conformation of alamethicin, a 20-residue antibiotic peptide, has been investigated using two-dimensional n.m.r. spectroscopy. Complete proton resonance assignments of this peptide have been carried out using COSY, SUPERCOSY, RELAY COSY and NOESY two-dimensional spectroscopies. Observation of a large number of nuclear Overhauser effects between sequential backbone amide protons, between backbone amide protons and $C^{\beta}H$ protons of preceding residues and extensive intramolecular hydrogen bonding patterns of NH protons has established that this polypeptide is in a largely helical conformation. This result is in conformity with earlier reported solid state X-ray results and a recent n.m.r. study in methanol solution (Esposito *et al.* (1987) *Biochemistry* **26**, 1043–1050) but is at variance with an earlier study which favored an extended conformation for the *C*-terminal half of alamethicin (Bannerjee *et al.* (1983) *J. Mol. Biol.* **165**, 757–775).

Key words: alamethicin; n.m.r.; peptide conformation

Alamethicin, a 20-residue antibiotic peptide produced by the fungus *Trichoderma viride* (1), has the sequence Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol (2, 3). Alamethicin forms voltage gated transmembrane channels in lipid bilayers (4), a property that has attracted widespread interest (5–9).

The presence of a large number of α-aminoisobutyric acid (Aib, single-letter designation used is U) residues in the sequence has also stimulated considerable interest in the structural chemistry of alamethicin and model Aib-containing peptides (10–21). Accumulated evidence provides overwhelming support for the tendency of Aib-rich sequences to adopt helical conformations (10–20) and the largely helical nature of alamethicin in organic solvents was indeed inferred from early CD studies (22, 23). A crystal structure of alamethicin at 1.5 Å resolution established a largely α-helical conformation over the entire sequence (24).

However, an n.m.r. study of alamethicin in methanol solutions based on an analysis of spin-spin coupling constants ($J_{NHC^{\alpha}H}$) and

Abbreviations recommended by IUPAC-IUB Commission of Biochemical Nomenclature [(1974), *Pure Appl. Chem.* **40**, 314] have been used. Other abbreviations used are: n.m.r., nuclear magnetic resonance; CD, circular dichroism; COSY, correlated spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; HPLC, high performance liquid chromatography; ppm, parts per million.

limited nuclear Overhauser effect (n.O.e) data suggested an α-helical *N*-terminus and an extended structure ("open or β-sheet") for the *C*-terminal half of the molecule (25). Indeed, such a mixed helical $-\beta$-sheet structure has been incorporated into a channel model (9). The stereochemical difficulty involved in incorporating several Aib residues into extended conformations (12) provided the stimulus for us to reexamine the solution conformation of alamethicin using two-dimensional n.m.r. methods. In this report we establish a largely helical conformation for alamethicin in dimethylsulfoxide solutions and compare our results with those of a recently published study in methanol (26).

MATERIALS AND METHODS

Alamethicin was a gift from Upjohn Co. (Kalamazoo, MI) and was used without further purification. A solution of 6.4 mM in $(CD_3)_2SO$ was used in all experiments.

Two-dimensional proton n.m.r. [COSY and SUPERCOSY (27–29)] spectra were recorded on a Bruker WH-270 spectrometer equipped with an Aspect-2000 computer system. 512 t_1 values with 64 transients for each t_1 value were collected. Low-power irradiation was used at all times except during acquisition to suppress the water signal in $(CD_3)_2SO$. Single channel detection was used. The 512 W × 1 K data matrix was zerofilled to 2 K × 2 K and multiplied by unshifted sine-bell filter function in both dimensions before Fourier transformation, yielding a final digital resolution of 2.9 Hz/Pt. The experiments were performed at room temperature (293 K).

Phase sensitive NOESY spectra (500 MHz) were recorded using standard procedures (30) on a Bruker AM-500 spectrometer equipped with an Aspect-3000 computer system. Quadrature detection was employed in both dimensions with the carrier placed at one end of the spectrum and quadrature in t_1 achieved by the time-proportional phase increments method (31). 512 t_1 values with 64 transients per t_1 value were acquired. The 512 W × 2 K data matrix was zerofilled to 2 K × 2 K and multiplied by phase-shifted sine-bell ($\pi/4$ shifted) in both dimensions before Fourier transformation. The final digital resolution was 9.4 Hz/Pt in both domains. A random variation of 5% of the mixing time was used to eliminate coherent contributions from zero quantum coherences in the NOESY spectrum (30).

Experiments to obtain temperature coefficients of various amide resonances were carried out on the Bruker WH-270 spectrometer. The temperature was varied between 290 K and 340 K in steps of 10 K and one-dimensional spectra were acquired with 8 K data points.

RESULTS AND DISCUSSION

Resonance assignments

The ^1H n.m.r. spectrum of natural alamethicin has been reported to resemble that of the HPLC purified peptide (25). Therefore, in the present study, the natural peptide has been used without further purification. The spectra obtained are in agreement with the previously reported data (25, 32). Fig. 1 shows the SUPERCOSY spectrum of alamethicin in $(CD_3)_2SO$. The spectrum displays a large number of crosspeaks leading to detailed assignments, some of which are outlined below. The 10 expected intraresidue N_iH-C^{α}_iH connectivities of Ala(4, 6), Gln(7, 19), Val(9, 15), Gly(11), Leu(12), Glu(18) and Phol (20) are clearly seen. The Gly (11) NH (8.10 ppm) can be recognized by its characteristic triplet nature and coupling to its two $C^{\alpha}H$ resonances (at 3.48 and 3.73 ppm)(Fig. 1c). The NH resonance of Gly(11) shows an n.O.e. to two other NH resonances at 7.69 and 7.99 ppm (Fig. 2). The resonance at 7.99 ppm shows no further COSY connectivity and is therefore assigned to NH of Aib(10). The NH at 7.69 ppm, assigned to Leu(12), shows a COSY connectivity to its $C^{\alpha}H$ at 4.28 ppm (Fig. 1c) and from 4.28 ppm, a crosspeak is seen to $C^{\beta}H_2$ at 1.75 ppm. From $C^{\beta}H_2$ resonance at 1.75 ppm a COSY connectivity is seen to a resonance at 0.82 ppm, which is characteristic of the chemical shift of $C^{\delta}H_3$ resonances of Leu. Since Leu $C^{\beta}H_2$ and $C^{\gamma}H$ resonances are generally overlapped (33, 34, Fig. 2 of ref. 35), the resonance at 1.75 ppm is

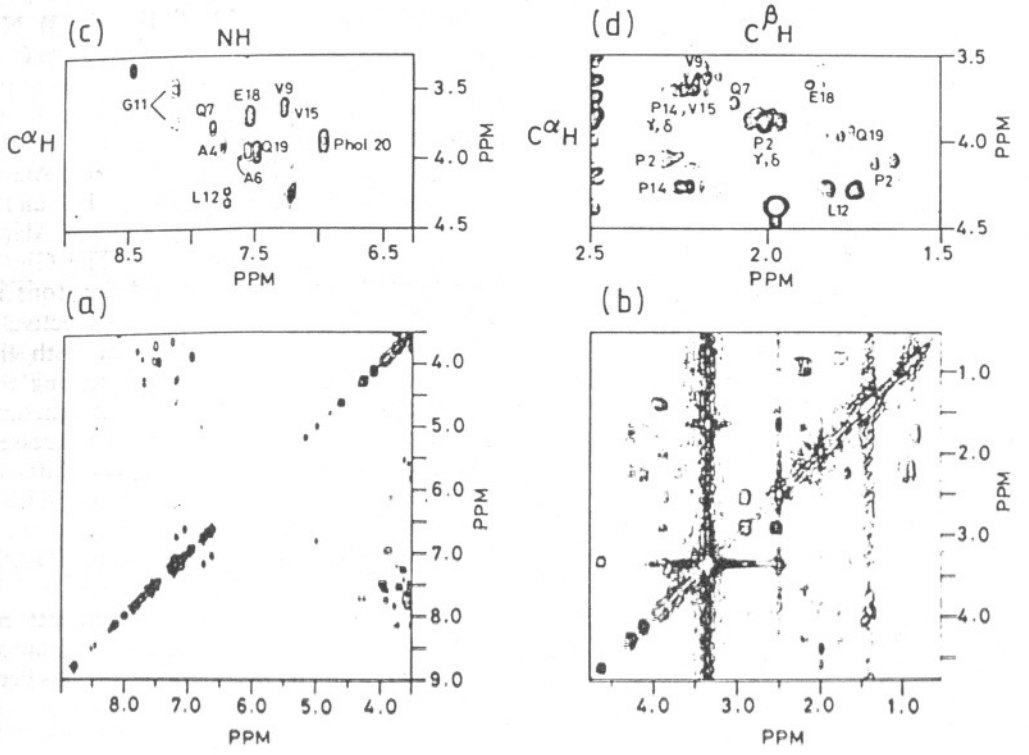

FIGURE 1

Contour plot of the ^1H 270 MHz SUPERCOSY spectrum of 6.4 mM alamethicin in $(CD_3)_2SO$. Diagonal and crosspeak regions of (a) 9.0 to 3.5 ppm and (b) 4.75 to 0.5 ppm and expanded regions of (c) NH-$C^{\alpha}H$ crosspeaks and (d) $C^{\alpha}H$-$C^{\beta}H$ crosspeaks. Specific residue assignments are indicated in single-letter code. In (d), except for P2 (γ, δ) and P14 (γ, δ) connectivities all other crosspeaks represent $C^{\alpha}H$-$C^{\beta}H$ connectivities. SUPERCOSY delay of 10 ms was used in t_1 to enhance crosspeak intensities.

assigned to $C^{\beta}H_2$ and $C^{\gamma}H$ and that at 0.82 to $C^{\delta}H_3$ and $C^{\delta'}H_3$ of Leu(12).

Starting from the highest field NH resonance of Phol(20), the entire spin system is readily recognised from the NH-$C^{\alpha}H$-$C^{\beta}H$; $C^{\alpha}H$-$C^{\beta'}H$-OH COSY connectivities. The spin systems of the two Ala residues were identified with the help of a Relay COSY experiment (not shown). Further assignments and sequence specific assignments need analysis of the NOESY spectra shown in Figs. 2 and 3. A large number of sequential N_iH-$N_{i+1}H$ (Fig. 2) and N_iH-$C^{\beta}_{i-1}H$ and N_iH-$C^{\alpha}_{i-3}H$ (Fig. 3) n.O.e.s have been observed. For example, one of the Ala NH protons shows an NH-NH n.O.e. connectivity to a resonance at 7.82 ppm, which in turn shows a COSY connectivity to a peak at 3.78 ppm. Since Aib has no NH–$C^{\alpha}H$ COSY connectivity and Ala (4) has Aib residues on both sides, this fixes the assignment of Gln (7) and the resonance at 7.55 ppm to Ala (6). Interactive use of the spectra shown in Figs. 1–3 leads to a large number of assignments of alamethicin, which are summarized in Table 1.

Sequential n.O.e.s of the type N_iH-$N_{i+1}H$ indicative of helical structures have been seen over a large number of residues. These are marked in Fig. 2 and are schematically shown in Fig. 4. The largest stretch of these n.O.e.s is Aib(8)-Val(9)-Aib(10)-Gly(11)-Leu(12)-Aib(13). Close proximity of NH resonances of Gln(7) with Aib(8) and Aib(17) with Glu(18) and Gln(19) precludes observation of these crosspeaks. In Pro, this sequential connectivity is observed from $Pro_i C^{\delta}H$-$X_{i-1}NH$. This has been seen from both Pro(2) $C^{\delta}H$-

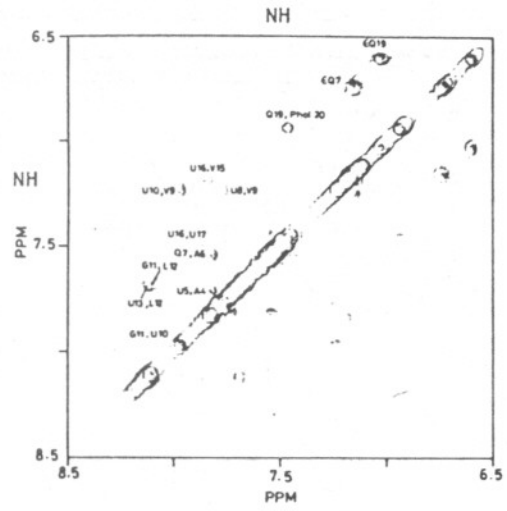

FIGURE 2

Contour plot of the ^{1}H 500 MHz NOESY spectrum of alamethicin in $(CD_3)_2SO$ showing the low field region from 6.5 to 8.5 ppm. The sequential N_iH-$N_{i+1}H$ connectivities are indicated (in single-letter code). The numbers indicate their positions in the peptide sequence. The chemical shift of the N_iH resonance corresponds to the ppm value on the X-axis while that of the $N_{i+1}H$ corresponds to the ppm value on the Y-axis. The mixing time was 200 ms.

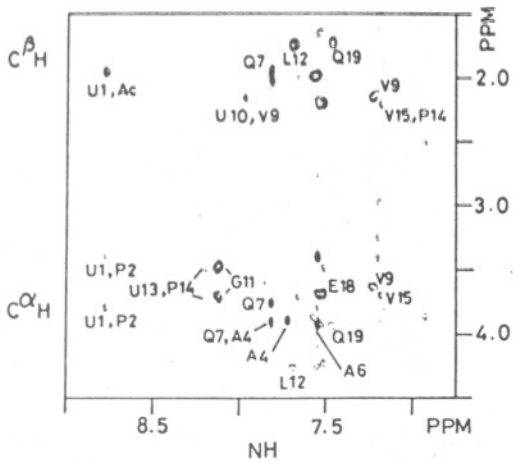

FIGURE 3

Expansion of the ^{1}H 500 MHz NOESY spectrum showing the NH-C^{α}H (3.0–4.5 ppm) and NH-C^{β}H (1.5–2.5 ppm) crosspeak regions. The peaks identified by a single residue code are intraresidue crosspeaks. Four of the indicated crosspeaks are from Aib N_iH to Pro $C^{\delta}_{i+1}H$. Other details are same as in Fig. 2.

Aib(1) NH and Pro(14) C^{δ}H-Aib(13) NH (Figs. 3 and 4). The identification of Pro C^{δ}H resonances leads to further assignments of the Pro(2) and Pro(14) spin systems. Fig. 3 also illustrates other interresidue n.O.e. crosspeaks like Val(9) C^{β}H-Aib(10) NH, Ala(4) C^{α}H-Gln(7) NH and Pro(14) C^{β}H-Val(15) NH and intraresidue n.O.e.s such as Ala(6) NH-C^{α}H and Gln(7) NH-C^{β}H. The Gln(7) and Gln(19) side chain ε-amide protons at 6.63, 7.06, 6.75 and 7.19 ppm respectively, give rise to strong crosspeaks in both the COSY and NOESY spectra, indicating the existence of geminal coupling and magnetization transfer by chemical exchange processes.

Fig. 5 summarizes the chemical shifts of NH resonances of alamethicin in $(CD_3)_2SO$ obtained in this study and compares them with the chemical shifts obtained in CD_3OH (26) and also with the chemical shifts of NH resonances in four synthetic fragments reported earlier (36). The Aib (3) NH resonance, not showing any n.O.e. connectivity, has been identified after assigning all the other NH protons in the spectrum. Most of the NH resonances are shifted to higher field in $(CD_3)_2SO$ relative to CD_3OH with the exception of Aib(3), Ala(4) and Aib(16). The NH chemical shifts in the synthetic fragments also exhibit values similar to those determined for alamethicin in this study except for the *N*- and *C*-terminal residues of the fragments.

Conformation

The involvement of NH groups in intramolecular hydrogen bonding was probed by studying the temperature dependence of NH chemical shifts (37–39). Table 2 lists the temperature coefficients of the NH resonances. Most of the NH resonances exhibit low temperature coefficients with the exception of Aib (1), Aib (13), two ε-amide protons of Gln (19) and one of Gln (7). This indicates that most of the backbone NH groups in alamethicin are intramolecularly hydrogen bonded. Such extensive intramolecular hydrogen bonding patterns are characteristic of helical conformations (20, 36). In studies of polypeptides the interresidue n.O.e. connectivities C^{α}_iH-$N_{i+1}H$ ($d_{\alpha N}$), N_iH-$N_{i+1}H$ (d_{NN}) and C^{β}_iH-$N_{i+1}H$ ($d_{\beta N}$) provide information on the

TABLE 1

Chemical shifts (in ppm) of assigned ^1H resonances in alamethicin[a]

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	Others
Aib(1)	8.78		1.35	
Pro(2)		4.12	2.25	$C^{\gamma}H_2$ 1.83
			1.68	$C^{\delta}H_2$ 3.82
				$C^{\delta}H_2$ 3.42
Aib(3)	7.71		1.37	
Ala(4)	7.72	3.90	1.39	
Aib(5)	7.83		1.38	
Ala(6)	7.55	3.94	1.39	
Gln(7)	7.82	3.78	2.10	$C^{\gamma}H_2$ 2.34
				ε-NH syn 6.63
				ε-NH anti 7.06
Aib(8)	7.73		1.42	
Val(9)	7.25	3.63	2.18	$C^{\gamma}H_3$ 0.92
				$C^{\gamma'}H_3$ 0.92
Aib(10)	7.99		1.40	
Gly(11)	8.11	3.48		
		3.73		
Leu(12)	7.69	4.28	1.75	$C^{\gamma}H$ 1.75
				$C^{\delta}H_3$ 0.82
				$C^{\delta'}H_3$ 0.82
Aib(13)	8.16		1.40	
Pro(14)		4.28	2.18	$C^{\gamma}H_2$ 1.84
			1.63	$C^{\delta}H_2$ 3.73
				$C^{\delta}H_2$ 3.48
Val(15)	7.20	3.72	2.21	$C^{\gamma}H_3$ 0.92
				$C^{\gamma'}H_3$ 0.92
Aib(16)	7.83		1.43	
Aib(17)	7.57		1.36	
Glu(18)	7.52	3.70	1.87	
Gln(19)	7.48	3.97	1.79	$C^{\gamma}H_2$ 2.05
				ε-NH syn 6.75
				ε-NH anti 7.19
Phol(20)	6.94	3.88	2.90	$C^{\beta}H_2$ 3.38
			2.52	OH 4.64

[a] Peptide concentration, 6.4 mM in $(CD_3)_2SO$. Chemical shifts reported (at room temperature) with reference to internal tetramethylsilane.

backbone conformations (39). In particular, successive $d_{\alpha N}$ connectivities are indicative of extended strands, while successive d_{NN} connectivities are characteristic of helical structures. The NOESY results on alamethicin clearly demonstrate that several d_{NN} connectivities are observed while there is no evidence for $d_{\alpha N}$ connectivities. These observations are consistent with an almost completely helical conformation for the entire molecule. The observation of the sequential Pro $C_i^{\delta}H$-$X_{i-1}NH$ n.O.e.s at both Pro (2) and Pro (14) confirms that the helical structure is continued at both the Pro positions as well. Incorporation of Pro residues into 3_{10} helical structures has been suggested by earlier studies of $(Aib\text{-}Pro)_n$ sequence (40, 41) and confirmed by recent crystal structure analysis of a 16-residue peptide which accommodates as many as three proline residues in a helical secondary structure (19). For the entire range of allowed polypeptide conformations the $d_{\alpha N}$ distance ranges from 2.2 to 3.5 Å, the shorter distances being characteristic of extended

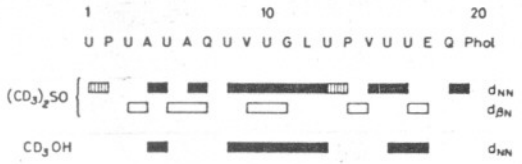

FIGURE 4

Comparison of observed helical NOESY connectivities in CD_3OH (26) with those in $(CD_3)_2SO$ (this study). The connectivities shown are N_iH-$N_{i+1}H$ (d_{NN}, filled bars), $C_i^{\beta}H$-$N_{i+1}H$ ($d_{\beta N}$, unfilled bars) and Aib N_iH-Pro $C_{i+1}^{\delta}H$ (striped bars). From residues Aib(17)–Gln(19) the NH-NH connectivities were not observed due to severe overlap of the NH resonances in this region.

strands and the longer distances being representative of α-helices. The d_{NN} distance ranges from 2.8 Å for an α-helical structure to 4.3 Å in an antiparallel β-sheet. The shortest $d_{\beta N}$ ($C_i^{\beta}H$-$N_{i+1}H$) distance for a helical structure is 2.5 Å, while for an extended strand the shortest $d_{\beta N}$ distance observed is 3.2 Å. In the present study a large number of d_{NN} and $d_{\beta N}$ connectivities have been observed (Fig. 4) while no $d_{\alpha N}$ connectivities could be detected. These results strongly support a largely helical conformation for alamethicin.

Fig. 4 illustrates the n.O.e. connectivities observed for alamethicin in $(CD_3)_2SO$ and compares the results with those obtained in CD_3OH (26). It is seen that the tracing of the helical structure by sequential n.O.e.s is more complete in this study. In CD_3OH an n.O.e. was observed between Gln(19) C$^{\alpha}$H and Phol(20) NH, which suggested an extended conformation for the *C*-terminal dipeptide. In the present study a strong n.O.e. has been observed between Gln(19) NH and Phol(20) NH, suggesting that a helical conformation is maintained even over this segment. These differences may be a consequence of the dif-

TABLE 2

Temperature coefficients ($d\delta/dT$, ppm/K × 10^3)[a] of various amide resonances

Residue	dδ/dT	Residue	dδ/dT
Aib(1)	3.47	Gly(11)	2.14
Aib(3)	1.53	Leu(12)	1.84
Ala(4)	2.65	Aib(13)	3.88
Aib(5)	1.71	Val(15)	0.50
Ala(6)	1.63	Aib(16)	1.71
Gln(7)	1.59	Aib(17)	0.71
Aib(8)	2.65	Glu(18)	2.04
Val(9)	1.35	Gln(19)	1.22
Aib(10)	2.86	Phol(20)	0.92
Gln(7)		Gln(19)	
ε-NH syn	4.87	ε-NH syn	4.82
ε-NH anti	2.69	ε-NH anti	4.49

[a] Measurements were made at 270 MHz over the range 290–340 K in steps of 10 K.

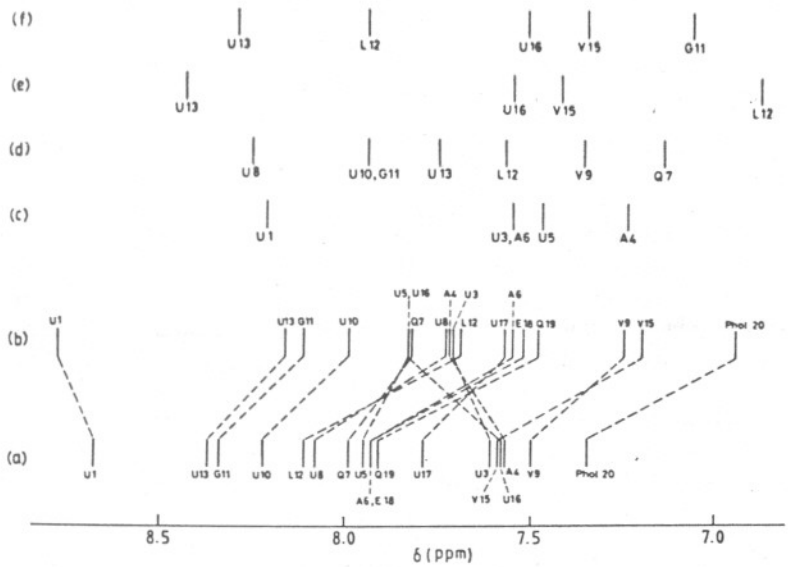

FIGURE 5

Comparison of the NH resonance chemical shifts of alamethicin (a) in CD_3OH (26) with those obtained from this study (b) in $(CD_3)_2SO$ and earlier studies on the following synthetic fragments in $(CD_3)_2SO$ (36). (c) Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe. (d) Boc-Gln-Aib-Val-Aib-Gly-Leu-Aib-OMe. (e) Boc-Leu-Aib-Pro-Val-Aib-OMe. (f) Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe. Residue numbering of the resonances corresponds to the original alamethicin sequence.

ferent states of protonation of the Glu(18) γ-carboxyl group.

Due to extensive overlap in the one-dimensional spectrum $J_{HNC^{\beta}H}$ values are not measurable accurately, except for Leu(12) 7.7 $\pm$ 0.5 Hz, Gln(19) 8.1 $\pm$ 0.5 Hz, Val(9) 5.9 $\pm$ 0.5 Hz and Phol(20) 9.1 $\pm$ 0.5 Hz. The others, measured from cross-sections of the two-dimensional spectrum recorded with lower digital resolution, are Ala(4) 6.0 $\pm$ 2.0 Hz, Gln(7) 6.9 $\pm$ 2.0 Hz, Val(15) 6.4 $\pm$ 2.0 Hz and Glu(18) 7.5 $\pm$ 2.0 Hz. These values are similar to those obtained for alamethicin in methanol, suggestive of a similar distribution of helical conformations in methanol and dimethylsulfoxide. While a succession of low $J_{HNC^{\beta}H}$ values (< 6 Hz) are generally characteristic of helical conformations, the observed values for the *C*-terminal half of alamethicin in both solvents are appreciably larger. However, it should be noted that even small changes in ϕ values of $\sim \pm 20$ can have a large effect on the J values observed for conformations close to $\phi \sim -60$ due to the steep dependence of J on ϕ in this region.

Alamethicin appears to favor helical conformations in organic solvents which are probably very similar to those observed in crystals. This is not surprising since the large number of Aib residues undoubtedly serve to stabilize helical folding and also restrict conformational mobility. The occurrence of appreciable amounts of β-strand structures as suggested earlier (9, 25) is highly unlikely, and the present study together with earlier reports (24, 26) provides strong evidence for largely helical structures.

ACKNOWLEDGMENTS

The authors thank the Upjohn Co. (Kalamazoo, Michigan) for the kind gift of the alamethicin sample. The facilities provided by the 500 MHz FT-NMR National Facility at the Tata Institute of Fundamental Research, Bombay, are gratefully acknowledged. K.C. thanks the University Grants Commission for a research fellowship. This work is supported by a grant from the Department of Science and Technology, India.

REFERENCES

1. Meyer, C.E. & Reusser, F. (1967) *Experientia* **23**, 85–86
2. Martin, D.R. & Williams, R.J.P. (1976) *Biochem. J.* **153**, 181–190
3. Pandey, R.C., Cook, J.C., Jr. & Rinehart, K.L., Jr. (1977) *J. Am. Chem. Soc.* **99**, 8469–8483
4. Mueller, P. & Rudin, D.O. (1968) *Nature* **217**, 713–719
5. Mathew, M.K. & Balaram, P. (1983) *Mol. Cell. Biochem.* **50**, 47–64
6. Menestrina, G., Voges, K-P., Jung, G. & Boheim, G. (1986) *J. Membrane Biol.* **93**, 111–132
7. Boheim, G., Hanke, W. & Jung, G. (1983) *Biophys. Struct. Mech.* **9**, 181–191
8. Mathew, M.K. & Balaram, P. (1983) *FEBS Lett.* **157**, 1–5
9. Hall, J.E., Vodyanoy, I., Balasubramanian, T.M. & Marshall, G.R. (1984) *Biophys. J.* **45**, 233–247
10. Nagaraj, R. & Balaram, P. (1981) *Acc. Chem. Res.* **14**, 356–362
11. Jung, G., Brückner, H. & Schmitt, H. (1981) in *Structure and Activity of Natural Peptides* (Voelter, W. & Weitzel, G., eds.), pp. 75–114, Walter de Gruyter, Berlin
12. Prasad, B.V.V. & Balaram, P. (1984) *CRC Crit. Revs. Biochem.* **16**, 307–348
13. Toniolo, C., Bonora, G.M., Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V. & Pedone, C. (1983) *Biopolymers* **22**, 205–215
14. Bosch, R., Jung, G., Schmitt, H. & Winter, W. (1985) *Biopolymers* **24**, 961–978
15. Bosch, R., Jung, G., Schmitt, H. & Winter, W. (1985) *Biopolymers* **24**, 979–999
16. Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V., Pedone, C., Toniolo, C. & Bonora, G.M. (1986) *Proc. Natl. Acad. Sci. US* **83**, 1988–1992
17. Toniolo, C., Bonora, G.M., Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V. & Pedone, C. (1986) *Macromolecules* **19**, 472–479
18. Karle, I.L., Sukumar, M. & Balaram, P. (1986) *Proc. Natl. Acad. Sci. US* **83**, 9284–9288
19. Karle, I.L., Flippen-Anderson, J., Sukumar, M. & Balaram, P. (1987) *Proc. Natl. Acad. Sci. US* **84**, 5087–5091
20. Das, M.K., Raghothama, S. & Balaram, P. (1986) *Biochemistry* **25**, 7110–7177
21. Bodo, B., Rebuffat, S., El Hajji, M. & Davoust, D. (1985) *J. Am. Chem. Soc.* **107**, 6011–6017
22. McMullen, A.I. & Stirrup, J.A. (1971) *Biochim. Biophys. Acta* **241**, 807–814
23. Jung, G., Dubischar, N. & Leibfritz, D. (1975) *European J. Biochem.* **54**, 395–409
24. Fox, R.O., Jr. & Richards, F.M. (1982) *Nature* **300**, 325–330

25. Bannerjee, U., Tsui, F.P., Balasubramanian, T.M., Marshall, G.R. & Chan, S.I. (1983) *J. Mol. Biol.* **165**, 757–775
26. Esposito, G., Carver, J.A., Boyd, J. & Campbell, I.D. (1987) *Biochemistry* **26**, 1043–1050
27. Anil Kumar, Hosur, R.V. & Chandrasekhar, K. (1984) *J. Magn. Resonance* **60**, 143–148
28. Anil Kumar, Hosur, R.V., Chandrasekhar, K. & Murali, N. (1985) in *Magnetic Resonance in Biology and Medicine* (Govil, G., Khetrapal, C.L. & Saran, A., eds.), pp. 3–16, Tata McGraw-Hill, New Delhi
29. Chandrasekhar, K. & Anil Kumar (1987) *J. Magn. Resonance* **73**, 417–422
30. Wider, G., Macura, S., Anil Kumar, Ernst, R.R. & Wüthrich, K. (1984) *J. Magn. Resonance* **56**, 207–234
31. Marion, D. & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.* **113**, 967–974
32. Gisin, B.F., Davis, D.G., Borowska, Z.K., Hall, J.E. & Kobayashi, S. (1981) *J. Am. Chem. Soc.* **103**, 6373–6377
33. Nagayama, K. & Wüthrich, K. (1981) *European J. Biochem.* **114**, 356–374
34. Davoust, D., Bodo, B., Rebuffat, S. & Platzer, N. (1983) *Biochem. Biophys. Res. Commun.* **116**, 1–8
35. Kessler, H., Bermel, W., Muller, A. & Pook, K. (1985) in *The Peptides* (Hruby, V.J., ed.), Vol. 7 Chap. 9, pp. 438–473, Academic Press, Orlando
36. Nagaraj, R. & Balaram, P. (1981) *Biochemistry* **20**, 2828–2835
37. Kessler, H. (1982) *Angew. Chem. Int. Ed. Engl.* **21**, 512–523
38. Balaram, P. (1985) *Proc. Ind. Acad. Sci. Chem. Sci.* **95**, 21–38
39. Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley-Interscience, New York
40. Prasad, B.V.V. & Balaram, P. (1982) *Int. J. Biol. Macromol.* **4**, 99–102
41. Venkatachalapathi, Y.V. & Balaram, P. (1981) *Biopolymers*, **20**, 1137–1145
42. Pardi, A., Billeter, M. & Wüthrich, K. (1984) *J. Mol. Biol.* **180**, 741–751

Address:

Dr. *P. Balaram*
Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560012
India