Membrane Channel Forming Polypeptides. 270-MHz Proton Magnetic Resonance Studies of the Aggregation of the 11-21 Fragment of Suzukacillin in Organic Solvents[†]

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ABSTRACT: 270-MHz ¹H NMR studies of the 11-21 suzu-kacillin fragment Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-G) and its analogue Boc-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-A) have been carried out in CDCl₃ and (CD₃)₂SO. The NH chemical shifts and their temperature coefficients have been measured as a function of peptide concentration in both solvents. It is established that replacement of Gln by Ala is without effect on backbone conformation. Both peptides adopt highly folded 3₁0 helical conformations stabilized by seven intramolecular 4→1 hydrogen bonds. Nonlinear temperature dependences are demonstrated for free NH groups in the Gln(1) peptide.

Aggregation is mediated by intermolecular hydrogen bonds formed by solvent-exposed NH groups. A major role for the Gln side chain in peptide association is suggested by differences in the NMR behavior of the Gln(1) and Ala(1) peptides. For the Gln(1) peptide in CDCl₃, the carboxamide side chain carbonyl group forms an intramolecular hydrogen bond to the peptide backbone, while the trans side chain NH shows evidence for intermolecular interactions. In (CD₃)₂SO, the cis carboxamide NH is involved in intermolecular hydrogen bonding. The possible role of the central Gln residue in stabilizing aggregates of peptide channel formers is discussed, and a model for hexameric association is postulated.

 $m{A}$ lamethicin, suzukacillin, and related lpha-aminoisobutyric acid (Aib)1 containing membrane channel forming polypeptides (Figure 1) can alter the permeability properties of artificial lipid membranes (Mueller & Rudin, 1968; Boheim & Kolb, 1978). In particular, alamethicin, suzukacillin, and trichotoxin A-40 (Boheim et al., 1976; Bruckner et al., 1979) form channels that exhibit voltage-dependent conductance characteristics, a feature that is reminiscent of excitable membranes (Hall, 1978). Conformational analysis of synthetic fragments of alamethicin (Nagaraj et al., 1979; Rao et al., 1979, 1980; Prasad et al., 1979; Nagaraj & Balaram, 1981a; Smith et al., 1981) and suzukacillin (Iqbal & Balaram, 1981a,b) has led to the suggestion that these hydrophobic polypeptides adopt largely 3₁₀ helical conformations. Since such helical structures have very small internal diameters, passage of cations through the helix interior is ruled out. Transmembrane channels must then be composed of aggregates of these rodlike helical molecules, to yield a central aqueous core (Mathew et al., 1981; Edmonds, 1979). This proposal is in marked contrast to the suggested models for the gramicidin A channel (Urry, 1971; Veatch et al., 1974; Bradley et al., 1978; Weinstein et al., 1979), where passage of cations occurs through the hydrophilic interior of the helical structures.

An examination of the sequences in Figure 1 shows that these polypeptides are composed of predominantly hydrophobic residues, with polar groups present only at the carboxyl terminal. There is, however, a lone Gln residue approximately in the center of the apolar sequence in alamethicin [Gln(7)], suzukacillin [Gln(11)], hypelcin A [Gln(7)], and trichotoxin A-40 [Gln(6)]. In the shorter peptides, antiamoebins and emerimicins, the Gln residue is closer to the C-terminal. Association of such apolar peptides in the lipid phase may be facilitated by hydrogen bonding involving the carboxamide

group of Gln. A major interest of this laboratory has been to establish the structural characteristics of membrane channels formed by Aib-containing polypeptides. Previous NMR studies have suggested that the 1-10 and 11-21 suzukacillin fragments adopt highly folded 3₁₀ helical conformations, stabilized by intramolecular 4→1 hydrogen bonds in organic solvents (Iqbal & Balaram, 1981a,b). However, the problem of peptide aggregation in both polar and apolar media needs to be addressed. In this report, we examine by 270-MHz ¹H NMR spectroscopy the aggregation behavior of the 11-21 suzukacillin fragment, Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-G), in organic solvents like CDCl₃ and (CD₃)₂SO. This fragment has been chosen as a model system, since excellent resolution of amide NH resonances is obtained. Studies on the peptide Boc-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-A) are also reported to enable an assessment of the role of the Gln side chain in promoting peptide association.

Materials and Methods

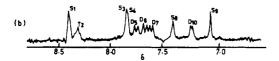
The peptides Boc-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-A) and Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11-G) were synthesized by solution phase procedures as described for alamethicin (Nagaraj & Balaram, 1981b). All peptides were homogeneous by TLC on silica gel and were characterized by 270-MHz ¹H NMR. Detailed synthetic procedures will be described elsewhere.

¹H NMR spectra were recorded on a Bruker WH-270 FT-NMR spectrometer at the Bangalore NMR Facility as described earlier (Iqbal & Balaram, 1981a,b). Peptide concentrations in CDCl₃ and (CD₃)₂SO were varied over the range 1–50 mg/mL. Concentrations were varied by dilution of a concentrated stock solution. Solvent titration experiments were carried out by adding a peptide solution in (CD₃)₂SO in aliquots to a CDCl₃ solution, maintaining a fixed concentration of 10 mg/mL.

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¹ Abbreviations used: Aib, α -aminoisobutyric acid; Boc, *tert*-butyloxycarbonyl; OMe, methyl ester.

FIGURE 1: Sequence of Aib-containing membrane channel forming polypeptides.



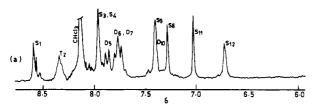
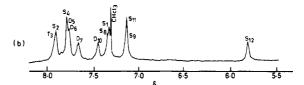


FIGURE 2: Partial 270-MHz 1 H NMR spectra (NH region) of peptides in (CD₃)₂SO: (a) 11-G (0.00086 M, 1 mg/mL). (b) 11-A (0.0013 M, 1.5 mg/mL).

Results

Chemical Shifts of NH Resonances. Figure 2a shows the low-field region of the 270-MHz ¹H NMR spectrum of a solution of peptide 11-G (0.00086 M) in (CD₃)₂SO. Resonances attributable to the 12 NH groups (10 backbone and 2 side chain) are observed. Singlets, doublets, and triplets are identified by the labels S_n , D_n and T_n , respectively, with the subscripts indicating the order of appearance from low-field in (CD₃)₂SO. Assignments of doublets to Leu(3) and Leu(6) (D_5, D_7) and Val(9) (D_6) were made on the basis of decoupling experiments, as described earlier (Iqbal & Balaram, 1981b). An unambiguous distinction between the two Leu residues is not possible. The triplet (T₂) can be unequivocally assigned to Gly(5) while the Gln(1) side-chain carboxamide NH groups $(S_9 \text{ and } S_{12})$ are assigned on the basis of their broadening and disappearance at high temperatures. This feature has been noted in earlier studies of model Gln peptides (Zanacchi & Moore, 1980). The urethane NH of Gln(1) was assigned to the only doublet (D10) that broadened at high temperature in (CD₃)₂SO, a property frequently observed for the urethane NH in a variety of peptides (Iqbal et al., 1981; M. Iqbal, unpublished results). The remaining five singlets (S₁, S₃, S₄, S_8 , and S_{11}) were then assigned to the Aib residues. In our earlier study of the conformation of 11-G, S₁ was assigned to the only non-hydrogen-bonded Aib residue in the 3₁₀ helical structure, i.e., Aib(2) (Iqbal & Balaram, 1981b).

The NH resonances of the peptide 11-A in (CD₃)₂SO (0.0013 M) are shown in Figure 2b. Assignments of reso-



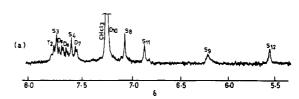
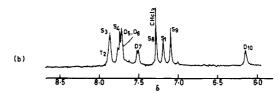


FIGURE 3: Partial 270-MHz ¹H NMR spectra (NH region) of 11-G at different concentrations in CDCl₃: (a) 0.00086 M; (b) 0.043 M.



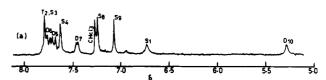


FIGURE 4: Partial 270-MHz ¹H NMR spectra (NH region) of 11-A in CDCl₃: (a) 0.0018 M; (b) 0.045 M.

nances are based on those for 11-G. The Ala(1) NH (urethane, D_{10}) was unambiguously identified by its high-field position in CDCl₃, a characteristic of urethane NH groups (Nagaraj et al., 1979; Nagaraj & Balaram, 1981; Iqbal & Balaram, 1981a). The corresponding resonance in (CD₃)₂SO was identified by solvent titration experiments in CDCl₃-(C-D₃)₂SO mixtures. Figures 3 and 4 show the NH resonances of peptides 11-G and 11-A, respectively, in CDCl₃ at the extreme concentrations used in this study. For 11-G, well-resolved resonances were observed for all 12 NH protons at low concentrations. Some spectral overlap (T₂ and S₃; D₅ and D₆; S₈ and S₁; S₁₁ and S₉) was observed at high concentrations. However, separation of such overlapping peaks could be obtained in temperature variation experiments, permitting as-

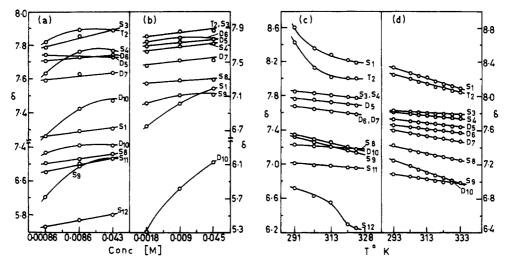


FIGURE 5: (a) Concentration dependence of NH chemical shifts for 11-G in CDCl₃. (b) Concentration dependence of NH chemical shifts for 11-A in CDCl₃. (c) Temperature dependence of NH chemical shifts for 11-G (0.00086 M) in (CD₃)₂SO. (d) Temperature dependence of NH chemical shifts for 11-A (0.0013 M).

Table I: NMR Parameters of NH Groups in Peptide 11-G

NH ^a	$d\delta/dT$ (ppm/°C) × 10 ³ for CDCl ₃ at				$d\delta/dT$ (ppm/°C) × 10 ³ for (CD ₃) ₂ SO at			
	0.000 86	0.0086 M	0.043 M	$\Delta\delta^{m{b}}$	0.00086 M	0.0086 M	0.043 M	$\Delta\delta$ $^{m{b}}$
S, [Aib(2)]	1.7	5.5	5.0	0.042	С	5.3	5.5	-0.213
T_2 [Gly(5)]	c	4.0	c	0.063	c	4.8	5.0	-0.234
S ₃ (Aib)	c	3.0	c	0.090	2.1	1.3	1.1	-0.027
S ₄ (Aib)	1.9	3.5	2.0	0.125	2.1	2.0	1.7	-0.050
D. (Leu)	3.0	1.9	2.2	-0.008	1.9	1.8	2.0	-0.032
D ₆ [Val(9)]	3.5	2.0	2.2	0.032	2.2	2.1	1.9	-0.011
D ₇ (Leu)	2.9	3.0	2.3	0.039	2.1	2.6	3.0	-0.090
S_8 [Aib(7)]	0.3	1.3	1.7	0.241	1.2	2.8	3.3	0.127
S. (trans)	c	9.0	c	0.897	4.2	3.6	4.0	-0.039
D_{10} [Gln(1)]	c	8.5	c	0.159	6.1	5.4	6.1	-0.077
S ₁₁ (Aib)	2.3	1.3	1.5	0.243	1.2	1.8	2.3	0.053
S ₁₂ (cis)	2.7	4.0	4.3	0.235	c	4.8	5.0	0.109

^a Assignments made as discussed earlier (Iqbal & Balaram, 1981b). S_9 and S_{12} are the side-chain NH protons of Gln, trans and cis to the CO group, respectively. ^b $\Delta \delta$ is the difference in chemical shift between the highest and lowest concentration values. Negative values indicate upfield shifts with increasing concentration. ^c $d\delta/dT$ values were not obtained, as the δ vs. T plots are nonlinear.

	$d\delta/dT$ (ppm/°C) × 10^3 for CDCl ₃ at			$d\delta/dT$ (ppm/°C) × 10 ³ for (CD ₃) ₂ SO at			
NH a	0.0018 M	0.045 M	$\Delta \delta^{m{b}}$	0.0013	0.009 M	0.045 M	$_{\Delta\delta}{}^{oldsymbol{b}}$
S, [Aib(2)]	4.7	5.8	0.452	6.1	6.0	6.4	-0.009
$T_{2}[Gly(5)]$	3.1	3.1	0.084	5.2	5.3	5.7	-0.001
\mathbf{S}_3	3.1	3.1	0.084	0.9	1.1	1.0	0.007
S ₄	2.1	2.0	0.099	1.9	2.5	2.3	0.007
D, (Leu)	2.8	1.8	0.070	2.3	2.4	2.5	-0.001
D_6 [Val(9)]	2.4	1.8	0.018	2.3	2.3	2.5	0.004
D ₂ (Leu)	3.8	3.3	0.062	3.1	3.0	3.4	-0.005
S_8 [Aib(7)]	1.6	1.5	0.044	4.1	3.7	4.0	-0.024
S ₉	0.2	0.9	0.019	2.4	2.2	2.5	-0.008
D_{10} [Ala(1)]	5.2	7.8	0.840	7.7	7.3	8.4	-0.015

^a Assignments made as described in text, by comparison with 11·G. b Δδ is defined in footnote to Table I.

signments. In 11-A, exceedingly well-resolved resonances were observed over the entire concentration range. All assignments in CDCl₃ are based on solvent titration experiments. The Gln(1) NH (D_{10}) in 11-G appears at abnormally low field in CDCl₃, over the entire concentration range, supporting our earlier suggestion that this NH group may be involved in a side chain-backbone hydrogen bond with the CO group of the carboxamide side chain of Gln(1) (Iqbal & Balaram, 1981b). The chemical shifts of the various NH resonances were determined as a function of concentration in order to monitor the effect of peptide association for 11-G and 11-A. Repre-

sentative results in CDCl₃ are summarized in Figure 5a,b. The differences in chemical shifts ($\Delta\delta$) at the highest and lowest concentrations studied are presented in Tables I and II for 11-G and 11-A, respectively.

Temperature Dependence of Chemical Shifts. Intramolecularly hydrogen-bonded NH groups are often delineated on the basis of temperature and solvent dependences of NH chemical shifts (Wuthrich, 1976). Low-tempeature coefficients $(d\delta/dT < 4 \times 10^{-3} \text{ ppm/}^{\circ}\text{C})$ in hydrogen bond accepting solvents, like $(CD_3)_2$ SO, are characteristic of solvent shielded and/or intramolecularly hydrogen-bonded NH

groups, while $d\delta/dT$ values $> 6 \times 10^{-3}$ ppm/°C are generally assigned to solvent-exposed, free NH groups (Wuthrich, 1976; Hruby, 1974; Venkatachalapathi & Balaram, 1981a,b). Temperature coefficient data in relatively nonpolar, poorly hydrogen-bonding solvents like CDCl₃ may also provide valuable information on peptide structure and aggregation. Stevens et al. (1980) suggest that low $d\delta/dT$ values (<3 × 10⁻³ ppm/°C) in CDCl₃ may correspond to either strongly solvent-shielded or completely exposed NH groups. High $d\delta/dT$ values, on the other hand, may be interpreted as indicative of NH groups participating in intermolecular hydrogen bonding or in weak intramolecular interactions, both of which are disrupted with increasing temperature. A detailed NMR analysis of hydrogen-bonded peptide structure should be facilitated by a comparison of the concentration and solvent dependences of $d\delta/dT$ values for NH groups. The utility of such studies in approaching the problem of peptide association has been explored in the case of 1-10 suzukacillin fragment (M. Iqbal and P. Balaram, unpublished results). A detailed analysis for the peptides 11-G and 11-A is outlined below. Temperature dependences of NH chemical shifts were measured over the concentration range 0.043-0.00086 M in CDCl₃ and (CD₃)₂SO. In (CD₃)₂SO, 11-G at low concentrations (0.00086 M) shows pronounced curvature of the δ vs. T plots for resonances S_1 [Aib(2)], T_2 [Gly(5)], and S_{12} [the side-chain amide hydrogen of Gln(1), cis to the CO group] (Figure 5c). At 0.043 M, all NH groups show linear temperature dependences. Our earlier study established linear behavior of the NH resonances of 11-G at 0.0086 M in (CD₃)₂SO (Igbal & Balaram, 1981b). At low concentration in CDCl₃, the resonances S₉ (Gln side-chain amide hydrogen trans to the carbonyl group) and D₁₀ [Gln(1)] show a nonlinear temperature dependence of chemical shifts. At 0.043 M, the curve for D_{10} is significantly more linear, while that of S_9 still exhibits a nonlinear temperature dependence. Further, two overlapping NH resonances (T2) and one Aib NH (S3) show slight deviations from linearity. The upfield shifts with increasing temperature are very much smaller for S_3 and T_2 as compared to S₉ and D₁₀. Deviations from linearity may arise due to structural changes with temperature, resulting in the exposure of an originally solvent-shielded NH group. The $d\delta/dT$ values for the various NH resonances in 11-G at different concentrations in CDCl₃ and (CD₃)₂SO are presented in Table I. Seven backbone NH groups (S3, S4, D5, D6, D7, S_8 , and S_{11}) show low $d\delta/dT$ values ($<4 \times 10^{-3} \text{ ppm/}^{\circ}\text{C}$) in (CD₃)₂SO, over the entire range of concentrations. This suggests the involvement of seven NH groups in intramolecular hydrogen bonding and further supports the contention that the 3₁₀ helical conformation proposed earlier (Iqbal & Balaram, 1981b) is largely unaffected by aggregation effects.

All NH resonances in 11-A show linear temperature dependences of chemical shifts in both $CDCl_3$ and $(CD_3)_2SO$, over the entire concentration range studied (0.0018-0.045 M). Figure 5d shows a representative set of data. Further, for 11-A in $(CD_3)_2SO$, seven NH groups $(S_3, S_4, D_5, D_6, D_7, S_8,$ and S_9) have relatively low $d\delta/dT$ values $(<4 \times 10^{-3} \text{ ppm/°C})$ at all concentrations (Table II). Figure 6 shows the dependence of NH chemical shifts in 11-A on solvent composition in $CDCl_3-(CD_3)_2SO$ mixtures. Increasing concentration of the strongly hydrogen bond accepting solvent $(CD_3)_2SO$ perturbs significantly only resonances S_1 and D_{10} while the others are insensitive. These results together with the $d\delta/dT$ values in Table II strongly suggest that 11-A adopts a largely 3_{10} helical conformation, stabilized by seven strong intramolecular $4\rightarrow 1$ hydrogen bonds. This is analogous to the structure suggested

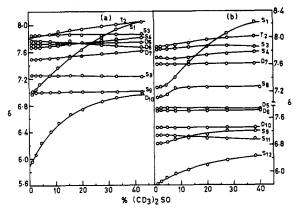


FIGURE 6: Solvent dependence of NH chemical shifts in CDCl₃-(CD₃)₂SO mixtures: (a) 11-A, 0.009 M; (b) 11-G, 0.0086 M.

for 11-G (Iqbal & Balaram, 1981b) and leads to the conclusion that replacement of Gln(1) by Ala is without any major effect on the backbone conformation of the peptide. The solventexposed NH groups in 11-A are Aib(2) (S_1) and Ala(1) (D_{10}). The Gly(5) NH (T₂) in 11-A has a relatively high $d\delta/dT$ value in $(CD_3)_2SO$ [(5.2-5.7) × 10⁻³ ppm/°C] but shows only a small solvent shift in the CDCl₃-(CD₃)₂SO system. As proposed earlier for 11-G (Iqbal & Balaram, 1981b), this NH group may participate in a weaker interaction. In both peptides, the Gly-Leu segment is probably more flexible than the rest of the chain. One Aib NH (S₈) shows a comparatively high $d\delta/dT$ value in $(CD_3)_2SO$ but a rather low value in CDCl₃. S₈ may be assigned to Aib(7) NH, since the Gly-Leu type III β turn could be destabilized in a polar solvent like $(CD_3)_2SO$, resulting in disruption of the $4\rightarrow 1$ hydrogen bond between Aib(4) CO and the NH of Aib(7). In nonpolar media (CDCl₃), the integrity of the 3₁₀ helical structure is probably fully maintained. The corresponding Aib(7) NH in 11-G (S₈) may be assigned similarly, on the basis of its high $d\delta/dT$ value in (CD₃)₂SO at high concentrations. Interestingly, this resonance exhibits a very large concentration dependence of $d\delta/dT$ in (CD₃)₂SO, with an increase from 1.2 × 10⁻³ ppm/°C at 0.00086 M to 3.3×10^{-3} ppm/°C at 0.043 M.

A further point of comparison between 11-G and 11-A may be made from the solvent titration curves in Figure 6. In 11-A, the fully solvent-exposed hydrogens, S₁ and D₁₀, show large, monotonic downfield shifts with increasing (CD₃)₂SO concentration, while all the other NH groups are relatively insensitive. In 11-G, S₁ [Aib(2) NH] shows a large solvent shift at high (CD₃)₂SO concentrations but is insensitive up to about 5% (CD₃)₂SO. Similar discontinuous behavior is also observed for S₈. These results are suggestive of solvent-dependent structural changes and have earlier been attributed, in the case of S₁, to breaking of side chain—backbone hydrogen bonds to the CO of the Gln side chain. The behavior of S₈ probably arises from destabilization of the Gly—Leu type III β turn.

Discussion

Highly folded 3_{10} helical conformations have been suggested for the 1–10 and 11–21 suzukacillin fragments (Iqbal & Balaram, 1981a,b) on the basis of ¹H NMR studies, using solvent titration and temperature coefficient data for delineating hydrogen-bonded NH groups. These structural proposals were also based on the known propensity of Aib residues to favor type III β -turn or 3_{10} helical structures (Nagaraj et al., 1979; Rao et al., 1980; Venkatachalapathi & Balaram, 1981b; Venkatachalapathi et al., 1981; Nagaraj & Balaram, 1981a). The present study was designed to probe the modes of peptide association in both polar and nonpolar solvents and

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FIGURE 7: Schematic representation of inter- and intramolecular hydrogen bonding involving the Gln side chain. (a) Association favored in CDCl₃ with trans carboxamide NH participating in an intermolecular hydrogen bond. (b) Association postulated in (CD₃)₂SO. Cis carboxamide NH participates in intermolecular hydrogen bonding.

to evaluate the role of the Gln residue in stabilizing peptide aggregates.

The results described above establish that replacement of Gln(1) by Ala(1) does not appreciably alter backbone conformation. Both peptides 11-A and 11-G adopted folded, 3_{10} helical structures stabilized by at least seven intramolecular $4\rightarrow 1$ hydrogen bonds. In 11-A, the $d\delta/dT$ values, for the intramolecularly hydrogen-bonded NH groups, are relatively insensitive to changes in concentration in CDCl₃ and (C-D₃)₂SO. 11-G behaves similarly except for resonance S_8 [Aib(7) NH]. This suggests that even if peptide association occurs, the backbone conformations remain largely unaltered. Further support for this conclusion is obtained from studies which establish that these shielded NH groups show very little concentration dependence of chemical shifts in both peptides.

The solvent-exposed (free) NH groups in 11-A and 11-G, however, do exhibit concentration dependence of chemical shifts and temperature coefficients in both solvents. For 11-A in CDCl₃, both free NH groups S₁ [Aib(2)] and D₁₀ [Ala(1)] show increases in $d\delta/dT$ values with increasing temperature. These resonances also show large downfield shifts with increasing peptide concentration in CDCl₃, in marked contrast to the intramolecularly hydrogen-bonded NH groups. These observations suggest that the helical 11-A molecules associate by intermolecular hydrogen bonding involving exposed NH groups. The increase in $d\delta/dT$ values in CDCl₃ with concentration is consistent with an increase in the population of associated species (Stevens et al., 1980; M. Iqbal and P. Balaram, unpublished results). In (CD₃)₂SO, the solvent-exposed NH groups also do not show any concentration dependence of chemical shifts or $d\delta/dT$ values, suggesting that in polar media peptide association is not appreciable.

For 11-G in CDCl₃, the solvent-exposed backbone NH group S_1 [Aib(2)] and D_{10} [Gln(1)] show relatively small downfield shifts with increasing concentration. As noted earlier, the urethane NH (D_{10}) appears at abnormally lowfield in CDCl₃ over the entire concentration range. D_{10} also does not show any concentration dependence of $d\delta/dT$ values in CDCl₃. We propose that Gln(1) NH (D_{10}) is involved in a hydrogen bond with the CO group of the Gln side chain over the entire concentration range studied. At low concentration (0.00086 M), species in which Aib(2) NH (S_1) is hydrogen bonded to the Gln side-chain CO are also populated. With increasing concentration, peptide helices aggregate, with Aib(2) NH becoming involved in intermolecular hydrogen

bonding. The side chain-backbone interaction in which Gln(1) NH participates is retained even in the associated species. This interpretation accounts for the low $\Delta\delta$ value for S_1 (Table I) and also its concentration-dependent $d\delta/dT$ value. The side-chain carboxamide proton S_9 (trans) shows a large downfield shift with increasing concentration in CDCl₃, whereas S_{12} (cis) is much less concentration dependent. These results suggest that peptide association occurs via hydrogen bonds involving S_9 , while S_{12} is relatively free. The nonlinearity in the δ vs. T curves for S_9 would then arise from dissociation of aggregated species at higher temperatures.

For 11-G in (CD₃)₂SO, of the exposed backbone NH groups, S₁ shows a large concentration dependence of the shape of the δ vs. T curves. At low concentration (0.00086 M), there are large deviations from linearity which are abolished at higher peptide concentration (≥ 0.0086 M). D₁₀ exhibits a linear δ vs. T curve, and the $d\delta/dT$ values are unaffected by concentration. The results are consistent with the involvement of S₁ [Aib(2) NH] in intermolecular association. The low concentration dependence of chemcal shifts in this solvent, as compared to CDCl₃, is reasonable since in the free molecules the exposed NH groups would bond to (CD₃)₂SO while in associated species they would interact with a CO group. The chemical shift of the side-chain NH S₂ (trans) shows a linear temperature dependence, and the $d\delta/dT$ values are concentration independent. On the contrary, S₁₂ (cis) shows nonlinear temperature dependence of chemical shift at low concentrations (0.00086 M) in $(CD_3)_2SO$. At higher concentrations, the δ vs. T curves are linear, and the $d\delta/dT$ values are high, characteristic of an exposed NH group. It appears that aggregates in (CD₃)₂SO may be stabilized by hydrogen bonding involving the cis carboxamide NH (S₁₂), in contrast to CDCl₃ where the trans NH (S_0) is involved. This may arise from the fact that in CDCl₃ the aggregates formed retain the hydrogen bond between Gln(1) NH and the CO group of the Gln side chain. In such a situation, the trans NH would be sterically more accessible. The modes of intermolecular association suggest in the two solvents are schematically illustrated in

The present study establishes that the suzukacillin fragment 11-G aggregates over the concentration range 0.00086 (1 mg/mL)-0.0086 M (10 mg/mL) in (CD₃)₂SO. In CDCl₃, aggregated species appear to be present even at the lowest concentration studied (0.00086 M). The mode of aggregation in the two solvents differs but is mediated by intermolecular

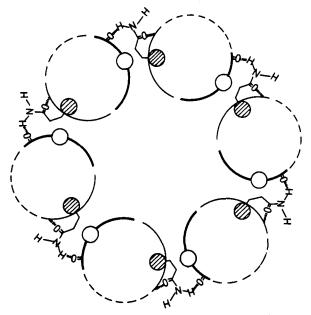


FIGURE 8: Proposed model for a hexameric aggregate of channelforming polypeptides like suzukacillin or alamethcin.

hydrogen bonds involving the solvent-exposed Aib(1) NH and Gln side-chain carboxamide protons. Our results suggest that peptide association is strongly favored in nonpolar media. In the case of 11-A and 11-G, aggregation does not affect backbone conformation, and the molecules probably associate as rigid helical rods. This may, in general, be true for the stereochemically rigid Aib-containing peptides. However, in other cases, aggregation could, in principle, involve large changes in backbone conformation. ¹H NMR studies of peptides in organic solvents should therefore also address the possible role of aggregation and the consequent effects on NMR parameters. The differences in the behavior of 11-A and 11-G clearly establish an important role for the Gln(1) side chain in peptide association. It is likely that the aggregation via intermolecular hydrogen bonding between the Gln side chain of one molecule and a backbone amide group of another may be an important factor in stabilizing channelforming peptide aggregates in the apolar phase of biological membranes.

Two interesting features of the data presented merit comment. The Gly(5) NH (T_2) shows nonlinear δ vs. T plots for 11-G in CDCl₃ at all concentrations studied (figure not shown) and also at 0.00086 M in (CD₃)₂SO (Figure 5c). S₈ [Aib(7)] NH shows a significant increase in $d\delta/dT$, with the peptide concentration in (CD₃)₂SO. We speculate that these anomalies associated with Gly(5) and Aib(7) may arise due to involvement of the central segment, -Aib(4)-Gly(5)-Leu(6)-Aib-(7)-Pro(8)-, in peptide aggregation. A particularly attractive possibility might involve the Gly(5) CO group in intermolecular hydrogen bonding. In a fully folded 3₁₀ helical structure, the regular sequence of 4-1 hydrogen bonds is interrupted by the presence of Pro(8) in 11-G, resulting in a free CO group at Gly(5). We have earlier shown that distorted 3_{10} helical conformations can accomodate a Pro residue in the center of the helix (Venkatachalapathi & Balaram, 1981b; Prasad & Balaram, 1981). The possibility of conformational flexibility in this sequence has also been discussed for the corresponding alamethicin fragments (Rao et al., 1980; Nagaraj & Balaram, 1981a).

An examination of the sequences in Figure 1 shows that for the longer channel formers, alamethicin, suzukacillin, hypelcin, and trichotoxin A-40, the central Gln residue and the Pro residue at the C-terminal end are separated by seven amino acids in every case. This lends some credence to the hypothesis that spatial relationships between these residues is important. In all four polypeptides, the fourth residue after Gln might provide a free CO group for hydrogen bonding. It is tempting to hypothesize that the Gln side-chain amide group of another molecule would serve as the hydrogen donor. Assuming completely 3₁₀ helical conformations for these peptide chains, one can define three distinct helix faces, charcterized by specific side chains. This results from the 3-fold symmetry of the 3₁₀ helix, together with axial translation, which brings every fourth residue to the same side of the helix. Helix association may occur with adjacent chains running parallel or antiparallel. The central position of the Gln residue in the sequences of the longer channel formers (Figure 1) suggests that both possibilities would lead to reasonably close helix packing. The Gln and Gly (or Gln + nour residues) residues now reside in different helix faces, preventing symmetrical dimeric association. The smallest unit where such association is stereochemically satisfactory, with an appreciable central channel, from preliminary model building, appears to be the hexamer where the Gln side chain of one helix is linked to one neighbor and the Gly CO is linked to the other. This arrangement is schematically represented in Figure 8. Folding of the Gln side chain to form a side chain-backbone hydrogen bond, as in 11-G, would allow closer approach of the polypeptide helices, permitting additional stabilization by weak van der Waals forces. Such an arrangement is particularly relevant since hexameric aggregates have been implicated in channel functions (Boheim & Kolb, 1978; Edmonds, 1979). The proposed organization of these polypeptide aggregates leads to a channel interior lined with nonpolar side chains. The presence of ordered water in such channels may result in efficient proton translocation across membranes. Some evidence for this has been obtained in studies which demonstrate that alamethicin and synthetic hydrophobic fragments serve as efficient uncouplers of oxidative phosphorylation in mitochondria (Mathew et al., 1981). The peptides examined in this study are too short to form transmembrane channels (Mathew et al., 1981; Nagaraj et al., 1980), precluding a correlation between ease of aggregation and membrane activity. Further studies on larger suzukacillin fragments aimed at evaluating the role of the Gln residue in modulating membrane activity are currently being pursued in this laboratory.

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Characterization of Rat Liver Oligonucleosomes Enriched in Transcriptionally Active Genes: Evidence for Altered Base Composition and a Shortened Nucleosome Repeat[†]

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ABSTRACT: A transcriptionally active chromatin fraction of oligonucleosome size has been separated and isolated by a modified micrococcal nuclease fractionation procedure. After mild enzymatic digestion, rat liver nuclei were lysed, and the chromatin was separated by centrifugation on linear sucrose gradients. Fractions from four regions of the gradient were pooled and labeled, from the top to the bottom, A, B, C, and D, respectively. Fraction A, which contained 20% or less of the total DNA, was determined to have a mean size of a hexanucleosome. By hybridization with [3H]cDNA transcribed from total cytoplasmic poly(A) mRNA, DNA from

fraction A was shown to be 10–15-fold enriched in transcribing genes when compared with total DNA. This fraction also has a somewhat higher concentration of AT base sequences. Significant differences were observed in nucleosome phasing. Fraction A has the shortest repeat length, fractions B and C are intermediate, and fraction D, which is depleted in transcribing DNA sequences, has the longest. Thus, we have isolated a chromatin fraction of oligonucleosome size enriched in transcribing genes and organized with reduced nucleosome spacing.

In differentiated cells, only a small percentage of the information in chromosomal DNA is expressed (McCarthy et al., 1973). While this transcribed chromatin appears to possess a periodic nucleosome structure (Lacy & Axel, 1975; Mathis

& Gorovsky, 1976; Bellard et al., 1978), there is increasing evidence that transcribing genes exist in an altered conformation. First, transcribing nuclear DNA is preferentially digested by nucleases such as DNase I (Berkowitz & Doty, 1975; Weintraub & Groudine, 1976; Garel & Axel, 1976) and DNase II (Gottesfeld et al., 1974). In addition, micrococcal nuclease cleaves the ovalbumin gene more rapidly than the globin gene in hen oviduct (Bellard et al., 1978), and both DNase I and micrococcal nuclease preferentially cleave ac-

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