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Membrane Channel Forming Polypeptides. 270-MHz Hydrogen-1 Nuclear Magnetic Resonance Studies on the Conformation of the 11–21 Fragment of Suzukacillin†

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ABSTRACT: 270-MHz ^{1}H NMR studies on the synthetic suzukacillin fragments Boc-Leu-Aib-Gly-Leu-Aib-OMe (13–17), Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-OBz (11–17), Boc-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (13–21), and Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11–21) have been carried out in $CDCl_3$ and $(CD_3)_2SO$. The intramolecularly hydrogen-bonded amide hydrogens in these peptides have been identified by using solvent titration experiments and temperature coefficients of NH chemical shifts in $(CD_3)_2SO$. The peptides are shown to favor conformations stabilized by intramolecular 4→1 hydrogen bonds. The 11–21 fragment adopts a highly folded, largely 3_{10} helical conformation stabilized by seven intramolecular hydrogen bonds. An eighth NH group [Gly(5)] appears to be involved in a weaker interaction. Evidence for the possible participation of the Gln side-chain carboxamide group in hydrogen bonding to the peptide backbone is also presented.

The 24-residue, α-aminoisobutyric acid (Aib)[1] containing polypeptide suzukacillin modifies the permeability properties of lipid bilayers by the formation of transmembrane channels (Jung et al., 1976; Boheim et al., 1976). The presence of a large number of Aib residues in the sequence (Figure 1) greatly restricts conformational freedom of the peptide backbone. The tendency of Aib-containing sequences to adopt 3_{10} helical conformations has been clearly established in studies of alamethicin fragments (Nagaraj et al., 1979; Rao et al., 1979, 1980; Nagaraj & Balaram, 1981a) and model peptides (Prasad et al., 1979, 1980; Shamala et al., 1978; Venkatachalapathi et al., 1981; Venkatachalapathi & Balaram, 1981). As part of a continuing program to elucidate the conformational characteristics of Aib-containing membrane active peptides, we have undertaken a detailed study of suzukacillin. An earlier report described the 3_{10} helical folding of the amino-terminal decapeptide (1–10) segment (Iqbal & Balaram, 1981). In the present paper, we summarize the results of 270-MHz ^{1}H NMR studies on the 11–21 suzukacillin fragment and compare the results obtained with studies on smaller fragments. It is clearly shown that the 11–21 fragment is highly folded in solution, and the NMR evidence strongly favors a conformation in which seven NH groups participate in intramolecular hydrogen bonding.

Materials and Methods

The peptides Boc-Leu-Aib-Gly-Leu-Aib-OMe (**1**), Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-OBz (**2**), Boc-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (**3**), and Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (**4**) were synthesized

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[1] Abbreviations used: Aib, α-aminoisobutyric acid; Boc, *tert*-butyloxycarbonyl; OMe, methyl ester; OBz, benzyl ester; TLC, thin-layer chromatography.

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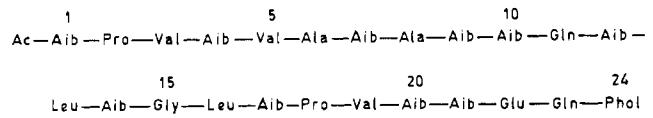

FIGURE 1: Sequence of suzukacillin.

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. The ^2H resonance of $CDCl_3$ and $(CD_3)_2SO$ were used for internal field frequency locking. Spectra were recorded at concentrations of 10 mg/mL, using a sweep width of 3012 Hz with 8K real data points, yielding a digital resolution of 0.367 Hz/point. Solvent titration experiments were carried out by adding a peptide solution in $(CD_3)_2SO$ to a peptide solution in $CDCl_3$. Concentrations of 10 mg/mL were maintained throughout. Variable-temperature measurements were made in $(CD_3)_2SO$ over the range 20–80 °C. The probe temperature was regulated with a B-ST 100/700 temperature controller.

Results

Assignments of NH Resonances. The low-field NH region of the 270-MHz ^1H NMR spectra of the suzukacillin fragments **1** (13–17), **2** (11–17), **3** (13–21), and **4** (11–21) are shown in Figures 2–4. In the pentapeptide **1**, the two Leu NH groups appear as doublets. The high-field doublet in $CDCl_3$ is assigned to the urethane NH (Nagaraj et al., 1979; Nagaraj & Balaram, 1981a). The corresponding assignment in $(CD_3)_2SO$ is based on spectra in $CDCl_3$–$(CD_3)_2SO$ mixtures. The assignment of the Gly NH to the triplet resonance is unequivocal. The Aib(2) and Aib(5) NH singlets are assigned on the basis of conformational arguments, outlined later. In the heptapeptide **2**, the Gln(1) NH is readily assigned to the δ 7.256 doublet in $CDCl_3$ on the basis of its broadening at high temperatures. An unambiguous distinction between the Leu(3) and Leu(6) NH groups is not possible. Similarly the Aib NH resonances cannot be assigned to specific residues. In the nonapeptide **3**, the Leu and Val NH doublets were distinguished by using spin decoupling experiments. An unambiguous assignment of the Val $C^\beta H$ resonance at δ 2.33 is possible by comparing resonances in model peptides containing Leu and Val residues. This permits assignment of the Val $C^\alpha H$ resonance and consequently the NH group. The Leu(1) and Leu(4) NH groups are assigned as in the case of **1**. Once again a unique assignment of the Aib NH resonances to specific residues is not possible. In the undecapeptide **4**, the Leu and Val NH groups were assigned by comparison with the nonapeptide **3**. An unambiguous assignment of the individual Leu and Aib NH resonances is not possible. In the case of peptides **2** and **4**, the side-chain carboxamide protons of Gln could be readily assigned in $(CD_3)_2SO$ by their characteristic broadening at higher temperatures. The chemical shifts of the NH groups in the peptides are summarized in Table I.

Determination of Hydrogen-Bonded NH Groups. The two criteria used for delineation of intramolecularly hydrogen-bonded NH groups were (i) temperature dependence of NH chemical shifts in a hydrogen-bonding solvent like $(CD_3)_2SO$ (Kopple et al., 1969) and (ii) sensitivity of NH chemical shifts to solvent composition in the $CDCl_3$–$(CD_3)_2SO$ system (Pitner & Urry, 1972).

The results of variable temperature and solvent titration experiments for peptides **1**, **2**, **3**, and **4** are summarized in Figures 5–8. In **1**, the Leu(1) NH and one Aib NH [assigned tentatively to Aib(2)] are clearly solvent exposed, as shown by their high-temperature coefficient ($d\delta/dT$) values of $\sim 7 \times 10^{-3}$ ppm/°C. These two groups also show a steep dependence of chemical shifts on solvent composition (Figure 5). The Gly NH and a second Aib NH group also exhibit relatively high $d\delta/dT$ values (4.4×10^{-3} and 3.4×10^{-3} ppm/°C), characteristic of partially solvent-exposed protons. Only the Leu(4) NH group shows a low $d\delta/dT$ value (1.9×10^{-3} ppm/°C) and a marked insensitivity to solvent, suggesting its involvement in an intramolecular hydrogen bond.

In the heptapeptide **2**, both Leu(3) and Leu(5) NH groups show very low $d\delta/dT$ values and solvent shifts. One Aib NH group also exhibits characteristics of an intramolecular hydrogen-bonded group. In addition, the Gly NH and one Aib

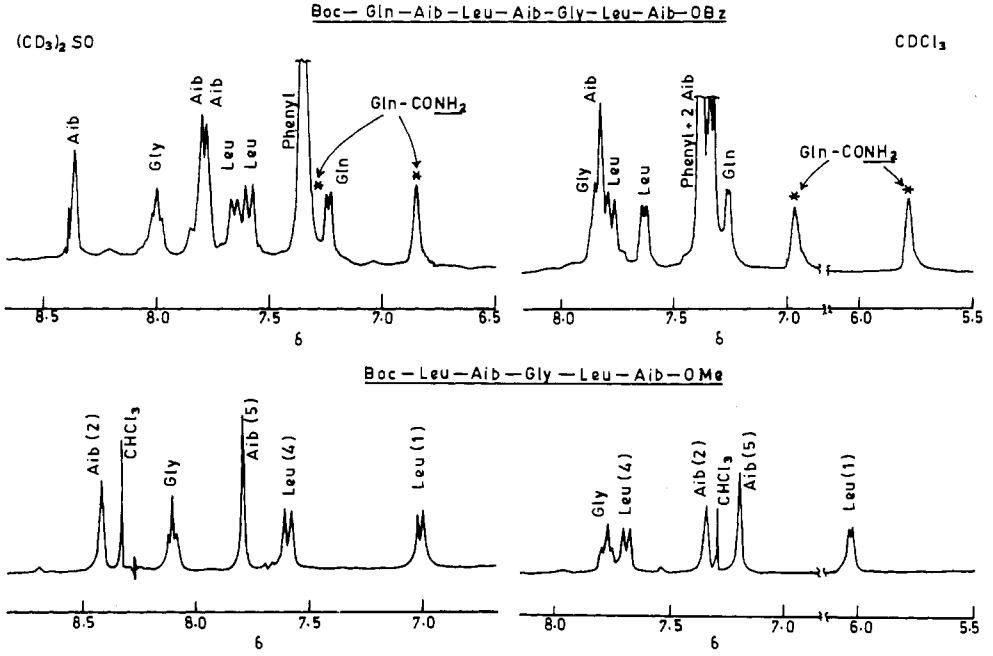

FIGURE 2: 270-MHz ^1H NMR spectra of peptides Boc-Leu-Aib-Gly-Leu-Aib-OMe (**1**) and Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-OBz (**2**) in $CDCl_3$ and $(CD_3)_2SO$. Only the NH resonances are shown.

Table I: ^{1}H NMR Chemical Shifts of NH Groups in Suzukacillin Fragments[a,c]

peptide	Gln	Aib	Leu	Aib	Gly	Leu	Aib	Val	Aib	Aib
Boc-Leu-Aib-Gly-Leu-Aib-OMe (1)			6.038 (4.1)	7.192	7.779	7.695 (7.7)	7.338			
			6.990 (6.9)	8.402	8.084	7.580 (8.8)	7.781			
Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-OBz (2)	7.256 (1.9)	7.804	7.605[b] (5.1)	7.278[b]	7.804	7.763[b] (8.0)	7.368[b]			
	7.247 (5.1)	8.388	7.658[b] (7.8)	7.786[b]	8.029	7.590[b] (8.4)	7.786[b]			
Boc-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (3)			6.186 (4.0)	7.451	7.963	7.742 (7.4)	7.274[b]	7.711 (8.8)	7.661[b]	7.109[b]
			6.991 (7.0)	8.630	8.329	7.605 (7.4)	7.394[b]	7.693 (9.2)	7.856[b]	7.093[b]
Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (4)	7.345 (2.5)	7.269	7.750[b] (7.0)	7.839[b]	7.860	7.617[b] (5.1)	7.116[b]	7.722 (8.1)	7.707[b]	7.100[b]
	7.286 (4.4)	8.411	7.738[b] (8.8)	7.832[b]	8.213	7.593[b] (7.3)	7.378[b]	7.682 (8.0)	7.832[b]	7.069[b]

[a] Values in parentheses are the $J_{HNC^{\alpha}H}$ values in Hz. [b] Assignments are arbitrary as discussed in the text. [c] The first row of δ values are in $CDCl_3$, while the second row are $(CD_3)_2SO$ values for each peptide.

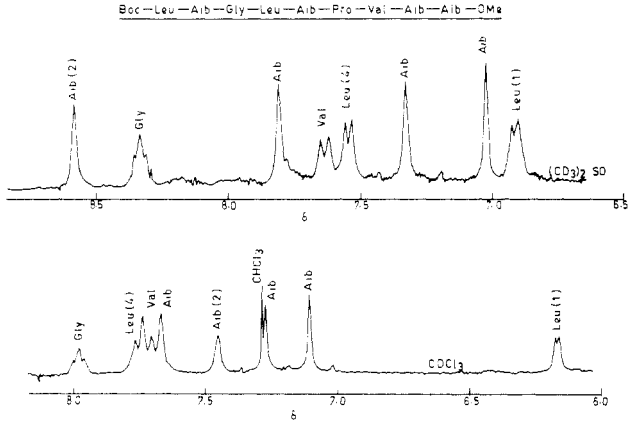

FIGURE 3: NH resonances in Boc-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (3) in $CDCl_3$ and $(CD_3)_2SO$.

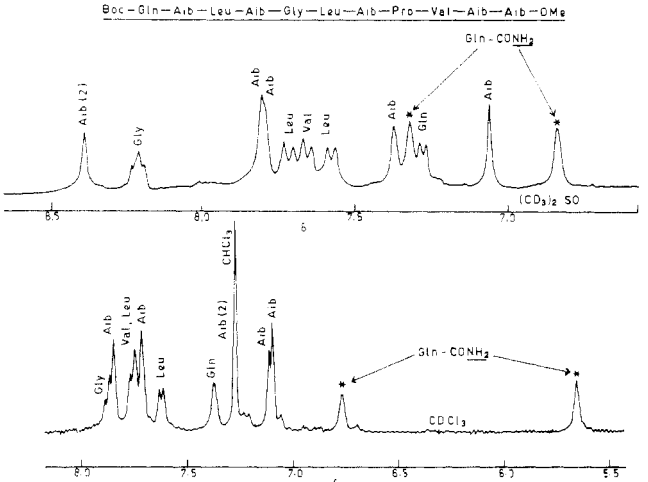

FIGURE 4: NH resonances in Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (4) in $CDCl_3$ and $(CD_3)_2SO$.

NH also yield moderately low $d\delta/dT$ values. The Gln(1) NH has a high $d\delta/dT$ value in $(CD_3)_2SO$ (6.9×10^{-3} ppm/°C) but exhibits very little solvent dependence of chemical shift. In fact, in both peptides **2** and **4**, the Gln(1) HN appears at very low field in $CDCl_3$, suggesting its possible participation in a side-chain–backbone hydrogen bond (see Discussion). Another interesting feature of the solvent titration curve in Figure 6 is that the low-field Aib NH is insensitive to addition of $(CD_3)_2SO$, up to a concentration of 10%, but moves rapidly

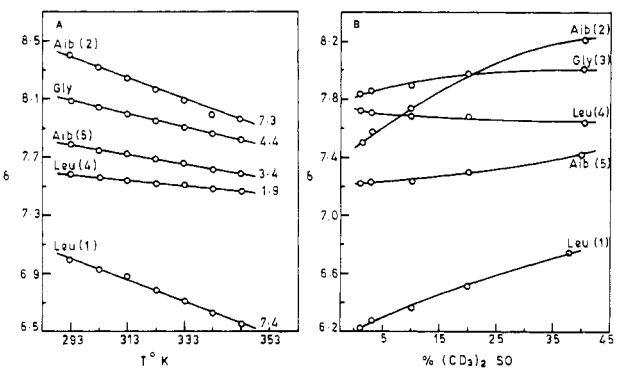

FIGURE 5: (A) Temperature dependence of NH chemical shifts in peptide **1** in $(CD_3)_2SO$ $d\delta/dT$ values ($\times 10^3$ ppm/°C) are indicated. (B) Dependence of NH chemical shifts on solvent composition in **1**.

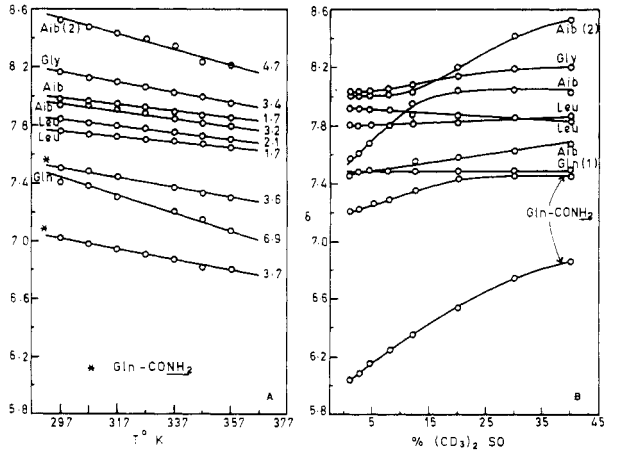

FIGURE 6: (A) Temperature dependence of NH chemical shifts in peptide **2** in $(CD_3)_2SO$ $d\delta/dT$ values ($\times 10^3$ ppm/°C) are indicated. (B) Dependence of NH chemical shifts on solvent composition in **2**.

downfield at higher concentrations. This suggests a possible conformational transition at higher $(CD_3)_2SO$ concentrations.

In the nonapeptide **3**, the Leu(1) NH and one Aib NH are clearly solvent exposed, as shown by their high $d\delta/dT$ values ($>7 \times 10^{-3}$ ppm/°C). Of the other NH groups, only the Gly NH shows a moderately high $d\delta/dT$ value. However, the solvent titration curve for this NH group does not show a simple trend, characteristic of an exposed proton (Figure 7). Once again, a single Aib NH group shows a discontinuity in the solvent titration curve, suggestive of a solvent-dependent conformational change.

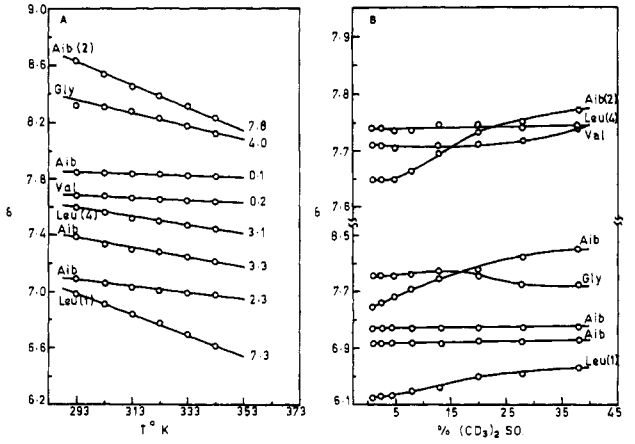

FIGURE 7: (A) Temperature dependence of NH chemical shifts in peptide **3** in $(CD_3)_2SO$. $d\delta/dT$ values ($\times 10^3$ ppm/°C) are indicated. (B) Dependence of NH chemical shifts on solvent composition in **3**.

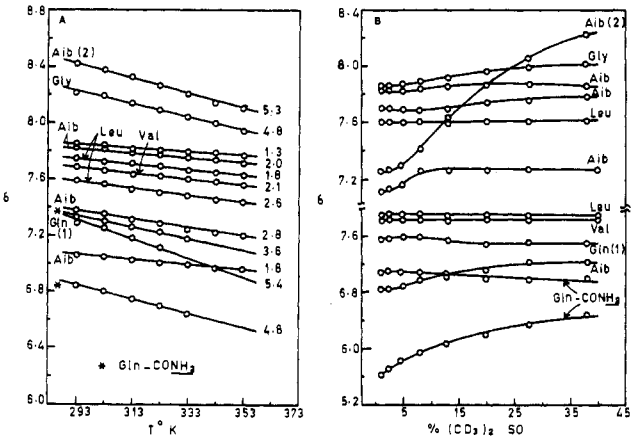

FIGURE 8: (A) Temperature dependence of NH chemical shifts in peptide **4** in $(CD_3)_2SO$. $d\delta/dT$ values ($\times 10^3$ ppm/°C) are indicated. (B) Dependence of NH chemical shifts on solvent composition in **4**.

In the undecapeptide **4**, the Gln(1) and one Aib NH groups are clearly solvent exposed, as shown by their high $d\delta/dT$ values. It should be noted that as in the case of **2**, the Gln(1) NH shows an abnormally low-field resonance position in $CDCl_3$. Seven NH groups yield very low $d\delta/dT$ values (<3 $\times 10^{-3}$ ppm/°C) and also show very little sensitivity to solvent, suggesting their involvement in intramolecular hydrogen bonds. Only the Gly NH exhibits an intermediate $d\delta/dT$ value and shows a small solvent shift. A particularly noteworthy feature of the solvent titration curve (Figure 8) is the behavior of the Aib NH group, assigned to Aib(2). As in the case of the smaller peptides, a distinct transition is observed at $(CD_3)_2SO$ concentrations above 5%.

Discussion

The stereochemical rigidity imposed on the peptide backbone by the presence of Aib residues permits the use of NMR techniques in establishing intramolecularly hydrogen-bonded structures in acyclic peptides. As noted in earlier studies (Nagaraj et al., 1979; Nagaraj & Balaram, 1981a; Venkatachalapathi & Balaram, 1981; Iqbal & Balaram, 1981), excellent correlations between conformations determined by different spectroscopic methods and X-ray studies are obtained in the case of Aib peptides. In particular, the problems of dynamic averaging, which vitiate many NMR studies of linear oligopeptides, are less significant in the case of Aib-containing sequences. The results reported in the preceding section establish the following features for the suzukacillin fragments. (i) In the pentapeptide **1**, the Leu(4) NH group is intramolecularly hydrogen bonded. (ii) The Leu(3), Leu(6), and one Aib NH group are strongly hydrogen bonded in **2**, while the Gly NH and an Aib NH also participate in hydrogen bonding in the heptapeptide. (iii) Five NH groups (Leu(4), Val(7), and three Aib NH groups) are involved in intramolecular hydrogen bonding in the nonapeptide **3**. The NMR data do not strongly favor hydrogen-bond formation involving the Gly(3) NH. (iv) The undecapeptide **4** favors a solution conformation in which seven NH groups are strongly hydrogen bonded. Again, the Gly(5) NH appears to be involved in a weak interaction.

These observations support a structure for **1**, involving an Aib(2)-Gly(3) β turn, with a 4→1 hydrogen bond between Leu(1) CO and Leu(4) NH. This conformation is compatible with the NMR results and also the known tendency of Aib-X sequences to adopt β-turn conformations (Nagaraj et al., 1979; Rao et al., 1980; Nagaraj & Balaram, 1981a). The heptapeptide **2** appears to favor a 3_{10} helical conformation with consecutive type III β turns having Gln(1)-Aib(2), Aib(2)-Leu(3), Leu(3)-Aib(4), Aib(4)-Gly(5), and Gly(5)-Leu(6) as the corner residues. Such a conformation would leave Gln(1) and Aib(2) NH groups free and leads to the earlier assignment of Aib(2) NH. The Gly(5)-Leu(6) β turn may be expected to be more flexible, suggesting that the Aib NH with a relatively high $d\delta/dT$ value (3.2×10^{-3} ppm/°C) may correspond to Aib(7) NH.

In the 11–21 fragment **4**, there are ten backbone NH groups. Of these, seven are involved in strong intramolecular hydrogen bonding, while Gly(5) NH appears to be less shielded from the environment. The exposed NH groups are Gln(1) and Aib(2). The favored conformation for the undecapeptide must therefore be largely 3_{10} helical. The schematic hydrogen-bonding pattern is illustrated in Figure 9. The presence of Pro(8) interrupts a regular sequence of 3_{10} hydrogen bonds and leads to the possibility of an alternative 5→1 hydrogen bond at the -Leu-Aib-Pro-Val-Aib- segment. This is illustrated in Figure 9. The only difference is in the nature of the carbonyl groups involved in hydrogen bonding. The two structural possibilities cannot be distinguished exclusively on the basis of ^1H NMR evidence. An extensive analysis of IR data on alamethicin fragments has, in fact, suggested the possibility of a single 5→1 hydrogen bond in this segment (Rao et al., 1980). A similar possibility is also likely in this suzukacillin fragment, which differs from the corresponding alamethicin fragment only in the replacement of a Val residue by Leu. The ^1H NMR results, however, clearly support a highly folded, largely 3_{10} helical conformation for the 11–21 fragment. Deletion of the Gln-Aib dipeptide in the 13–21 fragment **3** leads to a reduction in the number of intramolecular hydrogen bonds.

In all four peptides, the ^1H NMR parameters of the Gly NH do not unambiguously support its involvement in a hydrogen bond. With the exception of the heptapeptide **2**, all other peptides yield rather high $d\delta/dT$ values for the Gly NH. The solvent shifts are, however, low in every case. This may reflect primary structure effects on NMR parameters. The lack of a C^α substituent may result in anomalous values for the Gly NH parameters in these peptides. Alternatively, a degree of flexibility may be present at the Leu-Gly-Aib segment in these molecules. The Aib NH group showing clearly discontinuous behavior in the solvent titration experiments in the longer peptides **2** and **4** may be assigned to the Aib(2) NH group. It is possible that in both these peptides the presence of Gln(1) may lead to the population of conformational states involving the side-chain carboxamide group with either the

Boc—Gln—Aib—Leu—Aib—Gly—Leu—Aib—Pro—Val—Aib—Aib—OMe

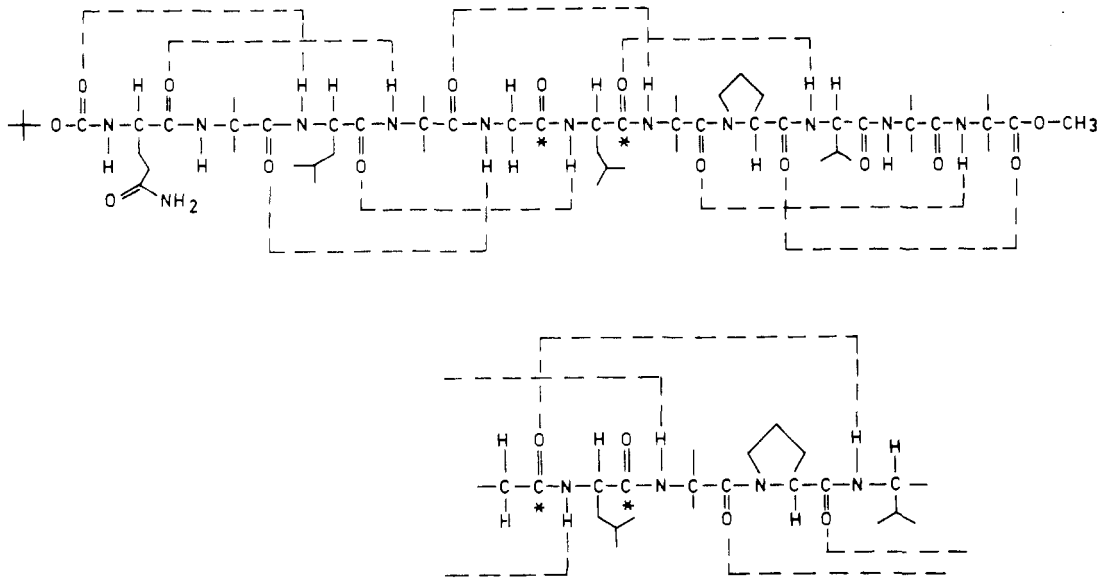

FIGURE 9: (Top) Schematic hydrogen-bonding scheme in **4** showing eight 4→1 hydrogen bonds. (Bottom) Alternative 5→1 hydrogen bonding possibility. The starred CO groups can be differentiated in the two schemes.

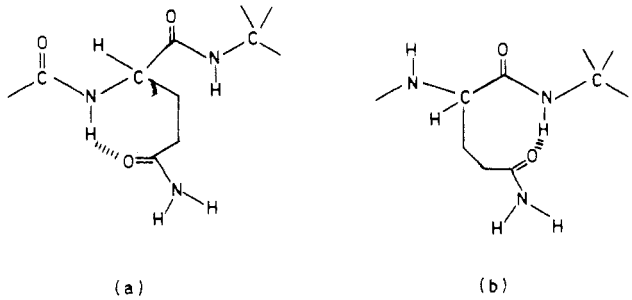

FIGURE 10: Possible hydrogen-bonded structures involving the Gln side-chain and backbone amide groups. (a) Gln NH to side-chain CO. (b) Side-chain CO to Aib(2) NH.

Gln(1) or Aib(2) NH group (Figure 10). The breaking up of such structural features in the polar solvent $(CD_3)_2SO$ may account for the discontinuities in the solvent titration curves of Aib(2) NH. Furthermore, such conformations may account for the low-field position of Gln(1) NH in $CDCl_3$. In the interpretation of the NMR data, we have ignored the possible effects of peptide aggregation on NMR parameters. This is a reasonably good assumption for the Aib peptides, at the concentration used, and has been borne out in earlier comparisons of IR, NMR, and X-ray results (Nagaraj et al., 1979; Rao et al., 1980; Nagaraj & Balaram, 1981a). In specific model studies of Aib peptides, aggregation effects have been shown to be minimal in $(CD_3)_2SO$ solution (Venkatachalapathi & Balaram, 1981). However, the possibility that rigid helical peptides aggregate through amino-terminal groups and Gln side-chain functions needs to be considered for these suzukacillin fragments. Further studies in this direction are currently under way.

The NMR studies outlined above strongly support a highly folded, largely 3_{10} helical conformation for the 11–21 segment of suzukacillin. The 1–10 segment has earlier been shown to favor a 3_{10} helical structure stabilized by eight intramolecular hydrogen bonds (Iqbal & Balaram, 1981). It is therefore likely that the 1–21 hydrophobic segment of suzukacillin will prefer a rodlike helical conformation. Two assay systems involving liposomal cation transport (Nagaraj et al., 1980) and uncoupling of oxidative phosphorylation in rat liver mitochondria (Mathew et al., 1981) have been developed in this laboratory for examining the channel forming ability of Aib-containing polypeptides. Recent experiments suggest that the 1–21 suzukacillin fragment forms cation channels in liposomes and also uncouples oxidative phosphorylation (M. K. Mathew, unpublished results). These results imply that the hydrophobic 1–21 suzukacillin fragment can form transmembrane channel structures, which modify membrane properties. Further studies are under way on the modes of aggregation of peptide helices and the relationship between peptide structure and membrane-modifying activity.

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Differential Compartmentalization of Messenger Ribonucleic Acid in Murine Testis†

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ABSTRACT: Messenger ribonucleic acid (mRNA) from fractionated mouse testis has been used as a template in the wheat germ and reticulocyte lysate cell-free translation systems. Cell-free translation products of deproteinized RNA from testis polysomes and from a nonpolysomal fraction (< 80 S) have been compared by one- and two-dimensional polyacrylamide gel electrophoresis, followed by autoradiography. Wheat germ and reticulocyte ribosomes translate both polysomal and nonpolysomal RNA from testis with high efficiency. Analysis of the polypeptide products of these cell-free translation systems indicates a compartmentalization between polysome-bound and nonpolysomal mRNA in testis. With the assumption of equal template efficiency for those RNAs tested, three classes of radiolabeled polypeptide products have been distinguished: (1) polypeptide bands which represent an equal abundance of mRNA in each cell compartment; (2) polypeptide bands which represent a higher abundance of mRNA in the polysomal than in the nonpolysomal compartment; (3) polypeptide bands which represent a higher abundance of mRNA in the nonpolysomal than in the polysomal compartment. Compared with liver, the testis contained a larger proportion of ribosomes present as monosomes. Further, more poly(A)+ RNA and an equal or greater template activity were found to be associated with the nonpolysomal portion of testis cytoplasm than the polysomal fraction, suggesting that testis does not have as large a proportion of its messenger RNAs actively involved in protein synthesis as does liver. A comparison of cell-free translation products of deproteinized and nondeproteinized RNAs obtained from total testis cytoplasm had revealed similar polypeptide profiles with a few minor differences. These data suggest that some form of selective mRNA masking or sequestration in a subcellular compartment may be regulating the loading of specific nonpolysomal mRNAs onto polysomes.

Evidence for the control of gene expression at the level of selective translation has begun to accumulate in recent years. Investigation of the development of sea urchin oocytes (Davidson, 1976), trout testis (Gedamu & Dixon, 1976), and rat testis (Grimes & Kay, 1979), as well as the early embryogenesis of *Spisula* (Rosenthal et al., 1980) and *Xenopus* (Davidson, 1976), have led to the conclusion that nonpolysomal mRNA[1] plays an important role in the regulation of gene expression during development. Further, regulation at this level may be involved in reticulocyte maturation (Jacobs-Lorena & Baglioni, 1972; Civelli et al., 1980) and in Ascites tumor cells (Geoghegan et al., 1978, 1979; McMullen et al., 1979), Friend cells (S. Cereghini, personal communication), and Hela cells (Penman et al., 1968).

Spermatogenesis provides an excellent developmental system in which to study the role of nonpolysomal mRNPs in gene expression. In this well-ordered process, stem cells, spermatogonia, undergo a series of differentiations to produce the highly differentiated spermatozoa. During this prolonged series of events, chromosome pairing and genetic recombination occur during meiotic prophase. This is followed by two divisions yielding haploid spermatids. In an interval of spermatogenesis called spermiogenesis these spermatids will eventually differentiate into spermatozoa. Spermatids are known to be active in protein synthesis (Monesi, 1964; Turkington & Majumder, 1975), but the extent of transcription in the cells is unclear [for review see Monesi et al. (1978)]. Recently, claims have been made that a considerable fraction of ribosomal and poly(A)+ RNA produced by premeiotic and meiotic cells is preserved until late spermiogenesis. Assuming these RNAs play a physiological role in the testis, one might expect that "long-lived" mRNA could be regulated during spermiogenesis by the process of selective translation.

Potential messenger RNA activity can be assayed by translation in the wheat germ cell-free system (Roberts & Paterson, 1973) or in the cell-free reticulocyte lysate system (Pelham & Jackson, 1976). A new method for assessing the messenger template activity of unextracted cell homogenates has been developed (Geoghegan et al., 1979; Rosenthal et al., 1980) to test the regulatory role of phenol-soluble components associated with mRNA. In this report, we compare the polypeptide products derived from cell-free translations of fractionated mouse testis to determine the polypeptides encoded by polysome-bound and nonpolysomal mRNAs. RNA

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[1] Abbreviations used: Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; mRNA, messenger ribonucleic acid; mRNP, messenger ribonucleic acid and associated protein; poly(A), poly(adenylic acid); poly(A)+, containing poly(adenylic acid); poly(A)−, lacking poly(adenylic acid); poly(U), poly(uridylic acid); RNA, ribonucleic acid; RNP, ribonucleic acid and associated proteins; SA, specific activity; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; PPO 2,5-diphenyloxazole; Me_2SO, dimethyl sulfoxide.

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