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 ¹H 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 CDCl₃ and (CD₃)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 (CD₃)₂SO. The peptides are shown to favor conformations stabilized by intramolecular 4→1 hydrogen bonds. The 11–21 fragment adopts a highly folded, largely 3₁₀ 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 3₁₀ 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 3₁₀ helical folding of the amino-terminal decapeptide (1–10) segment (Iqbal & Balaram, 1981). In the present paper, we summarize the results of 270-MHz ¹H 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.

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 $(\text{CD}_3)_2\text{SO}$. Only the NH resonances are shown.

Table I: ^1H 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) 6.990 (6.9)	7.192 8.402	7.779 8.084	7.695 7.580 (8.8)	7.338 1.781			
Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-OBz (2)	7.256 (1.9) 7.247 (5.1)	7.804 8.388	7.605 ^b (5.1) 7.658 ^b (7.8)	7.278 ^b 7.786 ^b	7.804 8.029	1.163 ^b (8.0) 7.590 ^b (8.4)	7.368 ^b 7.786 ^b			
Boc-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (3)			6.186 (4.0) 6.991 (7.0)	7.451 8.630	7.963 8.329	1.742 (7.4) 7.605 (7.4)	7.274 ^b 7.394 ^b	7.711 (8.8) 1.693 (9.2)	7.661 ^b 7.856 ^b	7.109 ^b 7.093 ^b
Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (4)	7.345 (2.5) 7.286 (4.4)	1.269 8.411	7.750 ^b (7.0) 7.738 ^b (8.8)	7.839 ^b 7.832 ^b	7.860 8.213	7.617 ^b (5.1) 7.593 ^b (7.3)	7.116 ^b 7.378 ^b	7.122 (8.1) 7.682 (8.0)	7.707 ^b 7.832 ^b	7.100 ^b 7.069 ^b

^a Values in parentheses are the $J_{\text{HNC}\alpha\text{H}}$ 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 $(\text{CD}_3)_2\text{SO}$ 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 $(\text{CD}_3)_2\text{SO}$. FIGURE 4: NH resonances in Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (4) in CDCl_3 and $(\text{CD}_3)_2\text{SO}$.

NH also yield moderately low $d\delta/dT$ values. The Gln(1) NH has a high $d\delta/dT$ value in $(\text{CD}_3)_2\text{SO}$ (6.9×10^{-3} ppm/ $^{\circ}\text{C}$) 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 $(\text{CD}_3)_2\text{SO}$, up to a concentration of 10%, but moves rapidly

FIGURE 5: (A) Temperature dependence of NH chemical shifts in peptide 1 in $(\text{CD}_3)_2\text{SO}$ $d\delta/dT$ values ($\times 10^3$ ppm/ $^{\circ}\text{C}$) 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 $(\text{CD}_3)_2\text{SO}$ $d\delta/dT$ values ($\times 10^3$ ppm/ $^{\circ}\text{C}$) 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 $(\text{CD}_3)_2\text{SO}$ 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/ $^{\circ}\text{C}$). 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 (CD₃)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 (CD₃)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 CDCl₃. 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 (CD₃)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 intramo-

lecularly 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 $\rightarrow$ 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 $\rightarrow$ 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 $\rightarrow$ 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

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 ($\text{CD}_3)_2\text{SO}$ 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 $(\text{CD}_3)_2\text{SO}$ 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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