INFRARED SPECTROSCOPY AS A PROBE FOR THE DEVELOPMENT OF SECONDARY STRUCTURE IN THE AMINO-TERMINAL SEGMENT OF ALAMETHICIN

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1. Introduction

Peptides containing α-aminoisobutyryl (Aib) residues have been shown to adopt well-defined structures in solution, by ^{1}H NMR methods [1]. Theoretical studies suggest that the presence of geminal methyl substituents at C^α imposes severe restrictions on the conformations accessible to Aib residues [2,3]. Single crystal X-ray diffraction studies of Z-Aib-Pro-NHMe [4], Z-Aib-Pro-Aib-Ala-OMe [5] and Tosyl-$(Aib)_5$-OMe [6] have clearly established the tendency of Aib residues *to* adopt 3_{10} helical conformations and to initiate the formation of type III β bends, stabilised by 4 → 1 intramolecular hydrogen bonds. Interest in the stereochemistry of Aib containing peptides, stems from the fact that alamethicin [7,8] and the related polypeptide ionophores antianioebin [9], emmerimicin [10], suzukacillin [11] and trichotoxin [12] contain high proportions of Aib residues. Here, we report the results of an infrared spectroscopic study of synthetic alamethicin fragments and demonstrate the development of a 3_{10} helical structure at the amino-terminus of the antibiotic.

2. Experimental procedures

The peptides were synthesised by solution phase methods using DCC mediated couplings. Specific procedures have been described in [1]. All compounds were chromatographically homogeneous, by thin-layer chromatography on silica gel and yielded 100 and 270 MHz ^{1}H NMR spectra fully consistent with their structures. Infrared spectra were recorded on a Perkin Elmer 580 spectrometer. Spectra were recorded in chloroform and carbon tetrachloride using peptide at 5×10^{-3} M and 1×10^{-4} **M**, respectively. The solvents were dried and distilled immediately before use. For studies in $CHCl_3$ a variable pathlength cell with NaCl windows was used and the pathlengths employed were 3.4 mm and 2.2 mm. Spectra in CCl_4 were recorded using a 10 cm pathlength, fused silica cell. Integral band intensities were calculated as in [13].

3. Results and discussion

Figure 1 shows the 3250 – 3500 cm^{-1} region of the infrared spectra of the peptides Z-Aib-Pro-OMe (I), Z-Aib-Pro-NHMe (II), Z-Aib-Pro-Aib-OMe (III), Z-Aib-Pro-Aib-Ala-OMe (IV), Z-Aib-Pro-Aib-Ala-Aib-OMe (V) and Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe (VI) in dilute solution in $CHCl_3$. The band at 3430 -3440 cm^{-1} corresponds to the free NH stretching frequency (ν_{N-H} free) while the bands between 3330 – 3370 cm^{-1} correspond to hydrogen-bonded NH groups (ν_{N-H} bonded). A striking feature in fig.1 is the almost constant intensity of the ν_{NH} free while the ν_{N-H} bonded increases in intensity, with increasing length of the peptide chain. This suggests that while the Aib [1] NH group is free in all the peptides, the remaining amide NH groups are involved in intramolecular hydrogen bonding. The peak positions,

Abbreviations: Aib, a-aminoisobutyric acid; **Z**, benzyloxycarbonyl; Tosyl, p-toluenesulfonyl; DCC, dicyclohexylcarbodiimide

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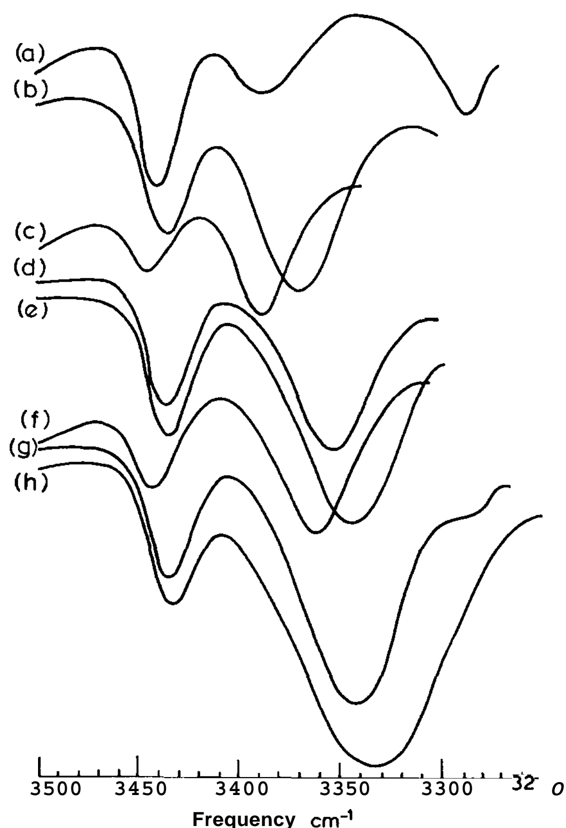

Fig.1. Infrared spectra in the region **3250–3500** cm^{-1} for the peptides in solution. (a) I, CHCl,; (b) II, $CHCl_3$; (c) II, CCl_4; (d) III, $CHCl_3$; (e) IV, $CHCl_3$; (f) IV, CCl_4; (g) V, $CHCl_3$; (h) VI-L, $CHCl_3$.

widths at half heights ($\Delta\nu_{1/2}$) and the integral band intensities for the peptides examined in this study are listed in table 1.

^{1}H NMR studies in $CDCl_3$ have clearly established that Z-Aib-Pro-Aib-OMe (III) and Z-Aib-Pro-Aib-Ala-OMe (IV) contain 1 and 2 hydrogen-bonded NH groups, respectively [1]. Further, the tetrapeptide IV has been shown to have two consecutive β-turns in the solid state, stabilised by two intramolecular hydrogen bonds involving the Aib [3] NH and Ala [4] NH groups. These results suggest that the conformations obtained in the solid state are maintained in solution for the relatively inflexible Aib containing peptides. Consequently it is a reasonable assumption that the conformation of the tetrapeptide IV, observed by IR methods in dilute $CHCl_3$ solution, also corresponds to the consecutive β-turn or incipient 3_{10} helical structure. Using a value of 2 hydrogen bonds for IV, the observed intensities of the 3330–3370 cm^{-1} band for the other peptides yield, 1 intramolecular hydrogen bond in II and III, 3 hydrogen bonds in V and 4 hydrogen bonds in VI. The value obtained for II and III are in excellent agreement with the results of X-ray [4] and 'H NMR studies [1], respectively. The infrared studies have been carried out in $CHCl_3$ at $4.5-5 \times 10^{-3}$ M, when intermolecular associations are likely to be minimal. However, in order to establish this conclusively the peptides II, III, IV and V were studied at significantly lower concentrations in CCl_4 (1×10^{-4} M). The spectra obtained in CCl_4 were almost identical in relative band intensities to the $CHCl_3$ spectra (see fig.1c,f). The results are summarised in table 1.

Further support for our contention that the 3330–3370 cm^{-1} band corresponds to a 10 atom hydrogen structure, comes from our studies on model Aib containing peptides. It was observed that this infrared band occurs only in tripeptide esters and higher peptides which are capable of forming 0-turns but not in dipeptide esters, which cannot form the 10 atom hydrogen bond. Infrared studes of Ac-Aib-NHMe also suggest that structures involving 5 atom (C,) and 7 atom (C_7) hydrogen bonded rings are present in CCl_4 [14]. These conformations have been suggested earlier for acetyl-amino acid-methylamides [15,16]. An interesting feature of fig.1 is the weak band at 3385 cm^{-1} observed for Z-Aib-Pro-OMe (I). In I, the 10 atom hydrogen bond and the C_7 structure are both not possible. The 3385 cm^{-1} peak must therefore arise due to the formation of a C_5 structure (fig.2a) or the 8 atom hydrogen bonded conformation, shown in fig.2b. It may be noted that the latter requires a *cis* Aib-Pro bond. The low intensity may be due to the small fraction of molecules populating the hydrogen bonded state and also due to the reduced strength of the 5 or 8 atom hydrogen bonds relative to the 10 atom hydrogen bond. Broad, weak bands at 3395 cm^{-1} and 3400 cm^{-1} were also observed in $CHCl_3$ for the model dipeptides Z-Aib-Aib-OMe and Z-Aib-Ala-OMe, which can in principle, form C_5 or C_7 structures. These results suggest that the C_5 and C_7 forms occur in non-polar media for molecules which cannot form β-turns, stabilised by 10 atom hydrogen bonds.

Table 1
Infrared spectral parameters for alamethicin fragments

Peptide[a]	Solvent	Concentration	ν_{N-H} bonded			Number[c] of H-bonds	ν_{N-H} free			Number[d] of free NH
			Position (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	Intensity[b]		Position (cm^{-1})	$\Delta\nu_{1/2}$ (cm^{-1})	Intensity	
I[e]	$CHCl_3$	5.75×10^{-3}	3385	21	0.152		3437	18	0.287	
II	$CHCl_3$	5.04×10^{-3}	3368	36	1.445	0.99	3434	24	0.686	1.1
	CCl_4	1.0×10^{-4}	3388	24	0.906	1.08	3443	18	0.301	0.74
III	$CHCl_3$	4.76×10^{-3}	3352	37	1.394	0.96	3435	23	0.665	1.0
	CCl_4	1.06×10^{-4}	3368	28	1.002	1.19	3444	16.5	0.323	0.79
IV	$CHCl_3$	4.90×10^{-3}	3344	44	2.901	2.0	3433	23	0.625	1.0
	CCl_4	0.99×10^{-4}	3360	34	1.676	2.0	3441	18	0.405	1.0
V-L	$CHCl_3$	4.74×10^{-3}	3332	51	4.922	3.39	3433	24	0.692	1.11
	CCl_4	1.02×10^{-4}	3350	36	2.645	3.16	3442	17	0.244	0.60
VI-L	$CHCl_3$	4.3×10^{-3}	3332	56	5.370	3.70	3432	25	0.562	0.90
VII	$CHCl_3$	4.73×10^{-3}	3356	45	2.815	1.94	3424	25	1.104	1.77
V-D	$CHCl_3$	4.57×10^{-3}	3335	53	4.296	2.96	3432	23	0.666	1.07
VI-D	$CHCl_3$	4.88×10^{-3}	3332	52	6.008	4.14	3432	25	0.685	1.1

[a] I Z-Aib-Pro-Ohle; II Z-Aib-Pro-NHMe; III Z-Aib-Pro-Aib-OMe; IV Z-Aib-Pro-Aib-Ala-OMe; V Z-Aib-Pro-Aib-Ala-Aib-OMe; VI Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe; VII Z-Aib-Aib-Ala-NHMe; V-D Z-Aib-Pro-Aib-D-Ala-Aib-OMe; VI-D Z-Aib-Pro-Aib-D-Ala-Aib-Ala-OMe

[b] Unit mol^{-1} liter $cm^{-1} \times 10^{-4}$

[c] Calculated using a value of 2.0 **for IV**

[d] Calculated using a value of 1.0 for IV

[e] Number of hydrogen bonds not indicated as β-turn structnres are not possible

Figure *3* shows a plot of the integral infrared intensities of the *3330–3370* cm^{-1} band as a function of the number of hydrogen bonds. **A** remarkably good correlation exists for II, III and IV whose conformations have been unambiguously established [1,4,5]. The model tripeptide amide Z-Aib-Aib-Ala-$NHMe_3$ (VII) is expected to possess 2 consecutive -Aib-Aib and -Aib-Ala- β-turns on the basis of the infrared data. This is in agreement with the established tendency of Aib residues to initiate β-turns, which has been conclusively demonstrated by the

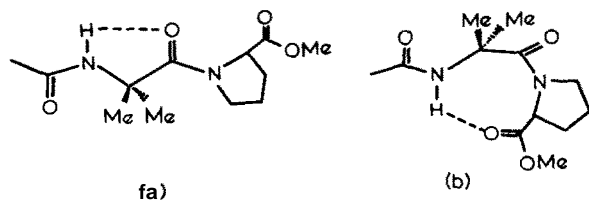

Fig.2. Possible hydrogen bonded structures *for* I. (a) 5 atom; (b) 8 atom.

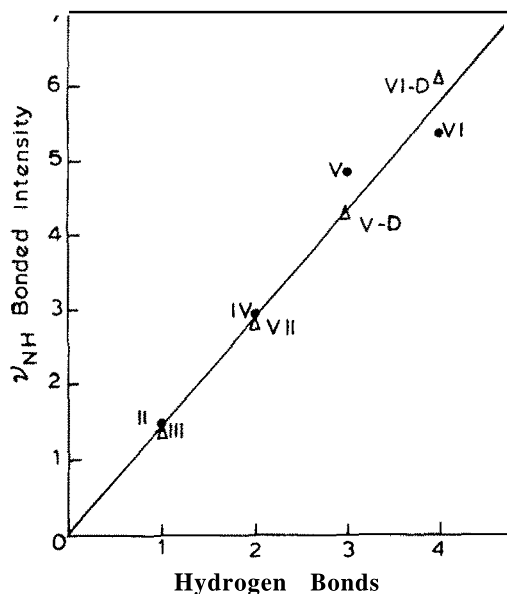

Fig.3. Plot of ν_{N-H} bonded band intensity as a function of the number of hydrogen bonds.

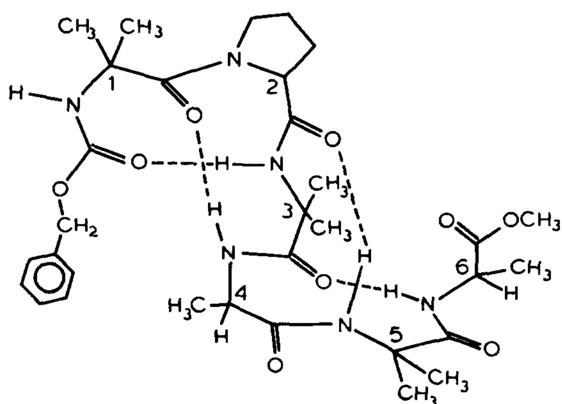

Fig.4. Schematic representation of the hydrogen bonded conformation of the hexapeptide **VI.**

3_{10} helical conformation of Tosyl-$(Aib)_5$-OMe, possessing 3 intramolecular hydrogen bonds [6]. The data in table 1 and fig.3 further suggest that the amino-terminal penta (V) and hexapeptide (VI) of alamethicin contain 3 and 4 intramolecular hydrogen bonds. A schematic representation of a hexapeptide conformation involving 4 consecutive hydrogen-bonded β-turns is shown in fig.4.

Since Aib residues can adopt either right or left handed 3_{10} helical conformations ($\phi = -60^\circ$, $\psi = -30''$ and $\phi = +60^\circ$, $\psi = +30^\circ$) it was of interest to see the effect of changing the configuration of one of the optically active amino acids. Table 1 lists the infrared band intensities for the (D-Ala^4) pentapeptide (V-D) and the (D-Ala^4) hexapeptide **(VI-D).** It is seen that the change in configuration at position 4 is largely without effect on the number of intramolecular hydrogen bonds. This suggests that the right-handed folding of the polypeptide chain observed in the solid state for IV [5] is maintained in V-D and VI-D. Presumably the Pro^2 residue dictates the sense of twist as $\phi = +60^\circ$ is not possible for L-proline. On the other hand, D-Ala can indeed adopt values in the region $\phi = -60^\circ$, $\psi = -30''$.

Infrared studies of the amino-terminal fragments of alamethicin reveal that a 3_{10} helical structure is nucleated in this part of the molecule. Further, -Aib-Pro- , -Pro-X- and -Aib-X- sequences (where X is any amino acid) can be accommodated at the corners of type III (or type I) β-turns [18], as evidenced by the steady increase in the number of hydrogen bonds on going from II to VI. This provides strong support for our earlier contention that alamethicin may indeed adopt a largely 3_{10} helical structure [5]. While considerable evidence has accumulated for the formation of transmembrane channels by alamethicin [19], individual alamethicin molecules are unlikely to adopt helical conformations with large internal diameters. The closely related 3_{10} and α-helical structures [20], which appear to be the conformations most accessible to alamethicin, are incapable of allowing passage of cations through the inside of the helix. Alamethicin aggregates have been implicated in its unique membrane activity [19]. The results of these structural studies suggest that the association of largely 3_{10} helical structures needs to be considered. The results presented here constitute the first clear demonstration of the systematic development of secondary structure in a growing polypeptide chain. The infrared technique outlined above provides a quick and reliable method of quantitating the number of hydrogen bonds in alamethicin fragments. This method may be used to advantage in studies of stereochemically constrained peptides, where the structures determined in the solid state also persist in solution. In order to test the predictions of the infrared method the crystal structures of Z-Aib-Aib-Ala-NHMe and the hexapeptide **Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe** are currently under investigation.

Acknowledgements

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