The crystal structure of a 3_{10} helical decapeptide containing α-aminoisobutyric acid

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Channel forming ionophores Decapeptide α-Aminoisobutyric acid X-ray structure analysis 3_{10} helix

1. INTRODUCTION

Alamethicin and several related α-aminoisobutyric acid (Aib) containing natural and synthetic peptides form voltage-dependent channels across lipid bilayer membranes [1,2]. Their high Aib content constrains these peptides to adopt 3_{10} [2,3] or α-helical conformations [1,4]. Membrane channels are then formed by helical peptide aggregation in the lipid phase [1–3,5], with a major role for the macrodipole moment of the peptide helices in mediating monomer association and channel characteristics [2,6]. The precise helical conformation (3_{10} or a) of Aib peptides is of interest since spatial disposition of side chains are different in the two cases and may lead to significantly different modes of association. This may be relevant in developing detailed molecular models for peptide channels. While 3_{10} helical structures have been observed, except in one case [7], for short Aib containing peptides [2,3,8–10], an ll-residue model peptide [11,12] and the 20-residue natural product alamethicin [13] have been shown to adopt α-helical structures in the solid state. These results have been interpreted as implying that 3_{10} helices are supported only in very short sequences [4,12].

We describe here the solid state conformation of the 10-residue peptide:

Boc-Aib–Pro–Val–Aib–Val–Ala–Aib–Ala–Aib–Aib-OMe

which adopts a 3_{10} helical structure. This result suggests that chain length may not be an overriding criterion in determining the mode of helical folding in these peptides.

2. MATERIALS AND METHODS

The decapeptide, synthesized by solution phase procedures used earlier for alamethicin I [14], was crystallized from a methanol-dimethylsulfoxide mixture. The crystals are orthorhombic, space group $P2_12_12_1$, with 4 molecules in a unit cell of dimensions $a = 8.894(2)$, $b = 15.577(3)$ and $c = 41.810(7)$ Å. The X-ray intensity data were collected on a computer controlled CAD-4 diffractometer, employing $\omega - 2\Theta$ scan using graphite monochromated copper radiation up to a Bragg angle of 60°. The structure was solved by a combination of the vector search methods [15] and the direct methods using the MULTAN program [16]. The atomic parameters were refined by the structure factor least squares method to an R factor of 0.096 for 3374 observed reflections. The details of the X-ray analysis will be presented elsewhere.

3. RESULTS AND DISCUSSION

A perspective view of the decapeptide is shown in fig. 1. The torsion angles which define the main chain conformation [17] are given in table 1. The intramolecular hydrogen-bond parameters are

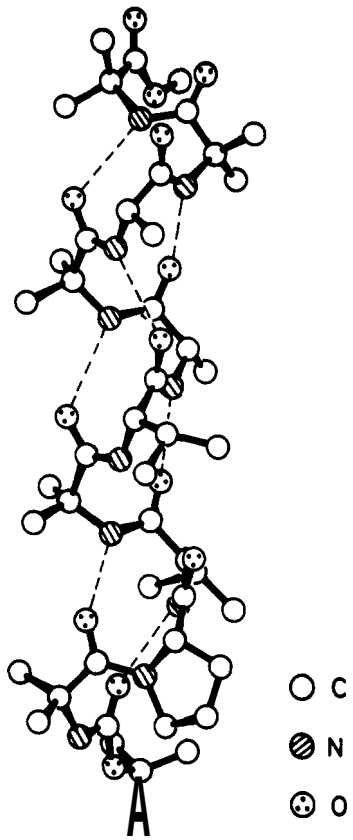

Fig. 1. A perspective view of the decapeptide molecule.

Table 1

Main chain conformational angles (°) in the decapeptide

	ϕ	ψ	ω
Aib	−53	−35	−176
Pro	−59	−27	−179
Val	−70	−16	−178
Aib	−53	−34	−176
Val	**−64**	−12	175
Ala	−61	−16	171
Aib	−50	−33	−177
Ala	−65	−20	176
Aib	−63	−24	−179
Aib	50	**40**[a]	**−176**[a]

[a] ψ and ω for the C-terminal residue are defined by assuming that the methoxyl oxygen is stereochemically equivalent to a peptide nitrogen atom

Table 2

Intramolecular hydrogen bond parameters in the decapeptide

Hydrogen bond	N---O (Å)	H–N---O (°)
Boc CO---HN Val(3)	3.19	9
Aib(1) CO---HN Aib(4)	3.00	15
Val(3) CO---HN Ala(6)	3.02	4
Aib(4) CO---HN Aib(7)	3.13	0
Val(5) CO---HN Ala(8)	2.94	9
Ala(6) CO---HN Aib(9)	3.00	19
Aib(7) CO---HN Aib(10)	3.11	12

listed in table 2. The molecule assumes a nearly regular right-handed 3_{10} helical conformation, with #,+ values close to their ideal values of $\phi = -60°$, $\psi = -30°$. All the intramolecular 4——1 hydrogen bonds appropriate for a 3_{10} helix are formed, with one exception. The NH group of Val(5) is directed towards the CO group of Pro(2) but the observed N---O separation of 3.46 Å is rather large for a good hydrogen bond.

We have determined the crystal structure of the amino (residues **1–5)** and carboxy (residues 6–10) terminal pentapeptide fragments of the decapeptide. The 1–5 pentapeptide [7] assumes a 4-fold helical conformation, whereas the 6–10 fragment is an almost perfect 3_{10} helix [10,18]. However, in the decapeptide an almost uniform 3_{10} helix is observed over the entire length, although some distortion exists in the amino-terminal half, on account of the increased separation of the Pro(2) CO and Val(5) NH groups. A 3_{10} helical conformation with 8 intramolecular N–H---O hydrogen bonds, 7 strong and 1 weak, has been established in $CDCl_3$ and $(CD_3)_2SO$ solutions for this decapeptide, from ^1H NMR studies [19]. The solid state structure is thus in general agreement with the postulated structure in solution.

The decapeptide, whose structure has been described above, corresponds to the amino-terminal sequence originally proposed for the channel forming polypeptide, suzukacillin A [20]. Subsequently this sequence has been revised, by deleting the 2–5 segment of the decapeptide, –Pro–Val–Aib–Val– (G. Jung, personal communication). Nevertheless, the fact that the decapeptide folds into a 3_{10} helix despite the

presence of 2 Val residues, which have a low preference for helical conformations [21], demonstrates the high propensity of Aib residues to dictate helical folding. This study also establishes that 3_{10} helical conformations may be favoured even in relatively large oligopeptides, contrary to earlier suggestions that only a-helical structures are likely to be adopted in peptides longer than *5–6* residues [4,12]. However, as mentioned earlier, an 11-residue Aib-containing peptide [11,12] and the 20-residue peptide alamethicin [13] fold into a-helices. It would therefore appear that the difference between the propensities of Aib residues for promoting 3_{10} and a-helical conformations in peptides, is marginal. The effect of the proportion and precise positioning of Aib in a given peptide, the role of peptide–peptide interactions and the influence of solvent in the choice between 3_{10} and a-helices are, however, yet to be established.

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