THE CRYSTAL AND MOLECULAR STRUCTURE OF THE AMINO TERMINAL TETRAPEPTIDE OF ALAMETHICIN. A NOVEL 3_{10} HELICAL CONFORMATION+

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SUMMARY. The molecular structure of N-benzyloxycarbonyl-α-aminoisobutyryl-prolyl-α-aminoisobutyryl-alanyl methyl ester (Z-Aib-Pro-Aib-Ala-OMe), the amino terminal tetrapeptide of alamethicin is reported. The molecule contains two consecutive β-turns with Aib-Pro and Pro-Aib at the corners, forming an incipient 3_{10} helix. This constitutes the first example of an X_2-Pro_3 β-turn in the crystal structure of a small peptide.

INTRODUCTION.

The linear polypeptide antibiotic alamethicin, which affects the electrical properties of membranes uniquely (1), contains a high proportion α-aminoisobutyric acid (Aib)* (2). The presence of Aib residues is likely to impart considerable conformational rigidity to the alamethicin molecule. Theoretical investigations suggest that Aib residues will be constrained to conformations close to the right and left handed α-helical regions (3). As part of a program to study the conformations of alamethicin and its synthetic fragments, we have prepared the amino terminal tetrapeptide, Z-Aib-Pro-Aib-Ala-OMe (I).

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* Abbreviations: Aib, α-aminoisobutyric acid; Z, benzyloxy-carbonyl.

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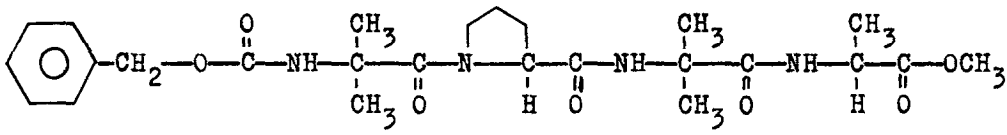


^{1}H NMR studies in chloroform and dimethylsulfoxide, using deuterium exchange and solvent perturbation methods, indicated that the Aib(3) and Ala(4) NH groups were solvent shielded, suggesting that the tetrapeptide adopts a well defined, folded conformation in solution (4). In this communication we describe the crystal structure of the tetrapeptide (I), which shows a novel structural feature involving two consecutive β-bends corresponding to an incipient 3_{10} helix.

MATERIALS AND METHODS.

The tetrapeptide (I) was synthesised by stepwise addition of aminoacid methyl esters to Z-Aib, using dicyclohexylcarbodiimide as the condensing agent. The details are described elsewhere (4). Crystals of I grown from an ether-ethylacetate mixture were clear stout prisms, crystallising in the orthorhombic system with the space group $P2_12_12_1$. The cell parameters were a = 9.694 Å, b = 15.034 Å, c = 18.595 Å, with one molecule in the asymmetric unit. The intensities of 2524 reflections were measured on a CAD-4 diffractometer with MoK_α radiation (λ = 0.710). However, only 2378 reflections with $I > 3\sigma$ were used for the structure determination and refinement. The structure was determined by the multisolution technique (MULTAN) of Germain, Main and Woolfson (5), using about 350 E values. The E-map with the highest combined figure of merit revealed 30 atoms out of 36 non-hydrogen atoms in the structure. The remaining atoms were recovered from successive difference-Fourier syntheses. The structure was refined to an R value of 0.107, with isotropic temperature factors and the unit weighting scheme, using the block-diagonal least squares program of Shiono (6). Further refinement of the structure with anisotropic temperature factors and a suitable weighting scheme, is in progress.

RESULTS AND DISCUSSION.

The projection of the molecule down the a-axis is shown in Fig 1. The most notable feature of the structure is the

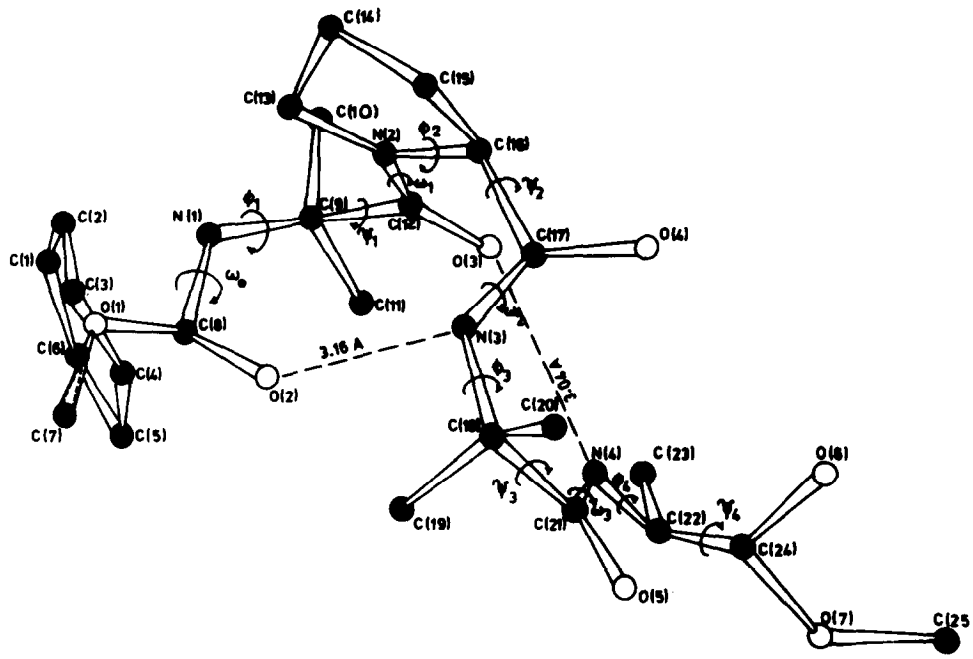


Fig 1. Structure of the tetrapeptide, Z-Aib-Pro-Aib-Ala-OMe

presence of two intramolecular hydrogen bonds. The amide nitrogen N(3) of Aib(3) is hydrogen bonded to O(2) of the urethane protecting group, while N(4) of Ala(4) is hydrogen bonded to O(3) of the carbonyl group of Aib(1). The N---O distances obtained are 3.16 Å for N(3)—O(2) and 3.06 Å for N(4)—O(3). Similar distances have been observed for hydrogen bonds in cyclo(Gly-Gly-Gly-Gly-DAla-DAla) (7), Pro-Leu-Gly-NH_2 (8), Li^+-antamanide (9) and valinomycin (P_1) (10). Only one intermolecular hydrogen bond is observed between N(1) and O(4) of two tetrapeptide molecules with a N(1)—O(4) distance of 3.12 Å. The tetrapeptide structure is composed of two consecutive 10-atom hydrogen bonded β-turns (Fig 2) with Aib(1) and Pro(2) at the corners of the first β-bend and Pro(2) and Aib(3) forming the corners of the second bend. The conformational angles (11) obtained are summarised in Table I.

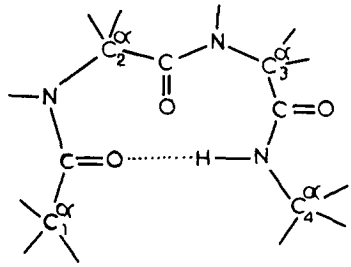
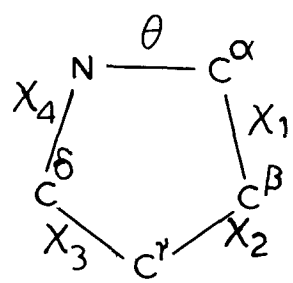


Fig 2. Schematic representation of a β-turn.

It is seen that the ∅,Ψ values for Aib(1) lie close to a right handed α-helix (-58°,-47°) while Aib(3) has dihedral angles moderately removed from the α-helical region. The two β-turns formed in this structure fall into the Type III category (12) and the resulting folding pattern forms an incipient 3_{10} helix (13). The observed conformational angles represent a structure distorted from an ideal 3_{10} helix ($\emptyset \sim -60°, \Psi \sim -30°$). The low Ψ value of -11° obtained for Aib(3) is consistent with the generally low Ψ_3 values ranging from -8° to +13° observed in the crystal structures of small peptides containing β-bends (14). The presence of Pro(2) with the higher Ψ value of -37.9° allows initiation of the second β-bend. The torsional angles for the pyrrolidine ring, defined below are $\chi_1 = -26.8°$, $\chi_2 = -36.7°$, $\chi_3 = -31.0°$, $\chi_4 = 13.6°$ and



$\theta = 7.5°$. The C^{β} and C^{γ} atoms are displaced on either side of the plane $NC^{\alpha}C^{\delta}$ by 0.19 Å and 0.36 Å respectively.

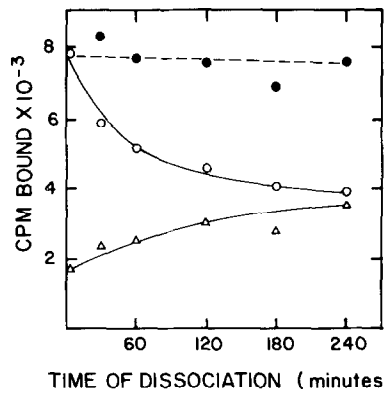


Figure 4: Recycling of NGF receptors.

Cultures of A875 were grown to a density of 2.7 x 10^5 cells per 20 cm^2 petri dish. After washing two times with the binding buffer, 20 ng of [^{125}I]NGF (33,000 cpm) was added in 1.5 ml to half the dishes (o) for a 60-min incubation at room temperature. [^{125}I]NGF was removed and the cells were washed four times with cold binding buffer. 1.5 ml of cold binding buffer was added. The samples were incubated at 4°C and, at various times, duplicates were lysed and the [^{125}I]NGF remaining was determined.

After washing the other dishes twice, 1.5 ml of the binding buffer with unlabeled NGF at 200 ng/ml was added for a 60-min incubation at 22°C (Δ). The cells were washed four times and 1.5 ml of fresh binding buffer was added at 4°C. At various times, the buffer containing dissociated unlabeled NGF was removed from the dishes and 20 ng of radiolabeled NGF in 1.5 ml of binding buffer was incubated with the culture for 60 min at 4°C. The dishes were washed and the bound radioactivity was determined. The counts remaining after dissociation (o), the counts binding to newly available receptors (Δ), and the sum of these (•) are plotted as a function of time of dissociation.

The sum of the counts remaining after dissociation and the counts binding to newly available receptors remained constant as a function of time of dissociation. This implies a one-to-one correspondence in the dissociated receptors' ability to accept new radiolabeled ligands in place of the unlabeled molecules which had dissociated. At 4°C, there is no synthesis of new receptors by the melanoma cells (data not shown).

DISCUSSION: Under the conditions used it is possible to bind NGF, and perhaps other molecules that recognize the NGF receptors, in such a way that both the ligand and the receptor can be dissociated and can function again.

Cells can be fixed with formaldehyde and stored frozen. [^{125}I]NGF which binds to such cells can be dissociated from the fixed receptor. This binding

with the theoretical results. The intramolecular separation between O(2) of the benzyloxycarbonyl group and N(4) of Ala(4) is 4.12 Å ruling out the possibility of a 5⟶1 hydrogen bond corresponding to a single turn of an α-helix.

The 3_{10} helix has often been observed at the ends of α-helical segments in proteins (18) while β-turns often precede helical segments in proteins. It is tempting to speculate that the incipient 3_{10} helical turns formed by consecutive β-bends may serve as nucleation sites for helical folding and that specific tetrapeptide sequences may initiate formation of helices. The precise structural requirements for nucleation of helical structures are yet to be established (19). CD studies of alamethicin have been used to estimate a α-helical content of 40% for the antibiotic in apolar solvents (20). The stereochemical constraints imposed by 8 Aib residues distributed throughout the 19 residue polypeptide would argue for a higher degree of helicity. The results of the structure determination of I show a 3_{10} helical segment forming at the amino terminal. It is conceivable that the polypeptide may continue to fold as a 3_{10} helix. Calculations of CD patterns for β-turns suggest that an unambiguous distinction between the spectra of α-helical structures and specific β-turn conformations may prove difficult (21). Further work is necessary before the CD characteristics of 3_{10} helical structures are established. In order to establish more firmly the conformational preferences of Aib residues in oligopeptides and to further study the development of secondary structure in the alamethicin sequence, we are engaged in the determination of the crystal structures of Tosyl-(-Aib-)$_5$OMe and the amino terminal hexapeptide of alamethicin.

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