Crystal Structure of Boc-Ala-Aib-Ala-Aib-Aib-Methyl Ester, A Pentapeptide Fragment of the Channel-Forming Ionophore Suzukacillin

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Synopsis

t-Butyloxycarbonyl-L-alanyl-α-aminoisobutyryl-L-alanyl-α-aminoisobutyryl-α-aminoisobutyric acid methyl ester (*t*-Boc-L-Ala-Aib-L-Ala-Aib-Aib-OMe), $C_{24}H_{43}N_5O_8$, an end-protected pentapeptide with a sequence corresponding to the 6th through the 10th residues in suzukacillin, crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 11.671$, $b = 14.534$, $c = 17.906$ Å and $z = 4$. The molecule exists as a right-handed 3_{10}-helix with a pitch of 6.026 Å. The helix is stabilized by three 4 → 1 hydrogen bonds with the NH groups of Ala(3), Aib(4), and Aib(5) hydrogen bonding to the carbonyl oxygens of *t*-Boc, Ala(1), and Aib(2), respectively. The helical molecules arrange themselves in a head-to-tail fashion along the *a* direction in such a way that the NH groups of Ala(1) and Aib(2) hydrogen bond to the carbonyl oxygens of Aib(4) and Aib(5), respectively, of a translationally related molecule. The helical columns thus formed close-pack nearly hexagonally to form the crystal.

INTRODUCTION

Membrane channel-forming ionophores like alamethicin[1] and suzukacillin[2] are acyclic polypeptides containing a high proportion of α-aminoisobutyric acid (Aib). ^{1}H-nmr investigations of the solution conformations of oligopeptide fragments of alamethicin[3,4] and suzukacillin,[5,6] in organic solvents, and the crystal structures of several short Aib-containing model peptides[7–12] and small alamethicin fragments[5,13–15] have provided evidence for the tendency of Aib residues to favor 3_{10}-helical conformations accompanied by 4 → 1 hydrogen-bond formation. CD studies[16,17] of a nonadecapeptide analog of alamethicin and smaller fragments bound to polyoxyethylene supports have been interpreted in terms of α-helical conformations, though the chiroptical distinctions between 3_{10}- and α-helices remain to be established. The recent crystal structure determination of a model 11-residue peptide, Boc-L-Ala-(Aib-L-Ala)$_2$-L-Glu(OBz)-(L-Ala-Aib)$_2$-L-Ala-OMe,[18] has, however, established the occurrence of the α-helical conformation, with concomitant formation of 5 → 1 hydrogen bonds. Yet another conformation, similar to the α′-helix,[19] exists in the crystal structure of the N-terminal pentapeptide, Boc-Aib-L-Pro-L-Val-Aib-L-Val-OMe, of suzukacillin.[20] The crystals of this pen-

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tapeptide and the undecapeptide referred to earlier incorporate solvent molecules, in contrast to the known crystals of almost all other Aib-containing peptides. Theoretical investigations[14,19,21,22] have indicated that Aib-containing peptides could assume a variety of helical conformations, including those corresponding to the classical α- and 3_{10}-helices. The role of the precise positioning of Aib residues in a given sequence, the effect of its length and peptide–peptide interactions, and the influence of solvent in determining backbone folding, however, remain to be established.

As part of a continuing investigation of the conformational characteristics of Aib-containing membrane channels,[20,23] we describe in this report the solid-state conformation of the peptide, *t*-Boc-L-Ala-Aib-L-Ala-Aib-Aib-OMe, which corresponds to the 6–10 segment of suzukacillin A. The pentapeptide adopts a classical 3_{10}-helical conformation, stabilized by three intramolecular 4 → 1 hydrogen bonds, in contrast to the N-terminal pentapeptide of suzukacillin,[20] which exhibited structural features somewhat different from those found in the crystal structures of other Aib-containing peptides.

EXPERIMENTAL

t-Butyloxycarbonyl-L-alanyl-α-aminoisobutyryl-L-alanyl-α-aminoisobutyryl-α-aminoisobutyric acid methyl ester (*t*-Boc-L-Ala-Aib-L-Ala-Aib-Aib-OMe), synthesized by solution phase procedures as described for alamethicin,[24] was crystallized from an ethyl acetate–methanol solution. The space group and the unit-cell dimensions determined from x-ray diffraction photographs and the density measured by flotation in an aqueous sodium chloride solution are as follows: space group, $P2_12_12_1$; a = 11.671(4), b = 14.534(1), c = 17.906(4) Å; ρ(measured) = 1.15(1) g cm^{-3}; ρ(calcd.) = 1.16 g cm^{-3}; Z = 4

Intensity data were collected from a specimen of dimensions 0.25 × 0.38 × 0.24 mm on a computer-controlled CAD-4 diffractometer employing ω-2θ scan up to a maximum Bragg angle of 68° using graphite monochromated CuK_α radiation. Of the 3082 reflections collected in this range, 2804 having $I > 2\ \sigma(I)$ were used in refinement. The intensities were corrected for Lorentz and polarization factors but not for absorption (μ = 7.26 cm^{-1} for CuK_α radiation).

The structure was solved using program MULTAN[25] based on direct methods for phase determination, followed by Fourier techniques, and refined by the block-diagonal SFLS method to an R value of 0.065 for 2804 observed reflections. The heavy atoms and the hydrogen atoms were assigned anisotropic and isotropic temperature factors, respectively. The weighting function used had the form $1/(a + bF_0 + cF_0^2)$, where a = 1.48, b = −0.099, and c = 0.011. The scattering factors for the hydrogen and other atoms were taken from Refs. 26 and 27, respectively. The positional parameters of the non-hydrogen atoms are given in Table I. Lists of structure factors, thermal parameters, and hydrogen atom parameters are available on request.

TABLE I
Fractional Coordinates ($\times 10^4$) of Non-Hydrogen Atoms

Atom	x/a	y/b	z/c
C(1)	6756(6)	3151(5)	682(3)
C(2)	4761(6)	2594(5)	474(3)
C(3)	5100(6)	4181(4)	987(3)
C(4)	5525(4)	3190(3)	943(3)
O(5)	5619(2)	2825(2)	1704(2)
C(6)	4709(3)	2704(3)	2145(2)
O(7)	3716(3)	2769(2)	1952(2)
N(8)	5037(3)	2493(2)	2848(2)
C(9)	4187(3)	2234(2)	3394(2)
C(10)	4793(4)	2009(3)	4129(3)
C(11)	3267(3)	2961(2)	3515(2)
O(12)	2272(3)	2740(2)	3635(2)
N(13)	3612(3)	3840(2)	3490(2)
C(14)	2856(4)	4621(3)	3633(3)
C(15)	3510(5)	5491(3)	3415(5)
C(16)	2472(7)	4631(6)	4447(4)
C(17)	1798(3)	4578(2)	3101(2)
O(18)	900(2)	4946(2)	3290(2)
N(19)	1942(3)	4182(2)	2436(2)
C(20)	1026(4)	4180(3)	1886(2)
C(21)	1449(5)	3819(5)	1140(3)
C(22)	−28(4)	3635(3)	2133(2)
O(23)	−926(3)	3752(3)	1778(2)
N(24)	63(3)	3085(2)	2725(2)
C(25)	−907(4)	2632(3)	3084(2)
C(26)	−1461(5)	1918(3)	2561(4)
C(27)	−473(5)	2172(4)	3799(3)
C(28)	−1835(3)	3336(3)	3310(2)
O(29)	−2810(3)	3078(2)	3433(2)
N(30)	−1504(3)	4211(2)	3396(2)
C(31)	−2345(4)	4949(3)	3487(2)
C(32)	−1718(5)	5838(3)	3633(3)
C(33)	−3102(5)	5029(4)	2783(3)
C(34)	−3128(4)	4735(3)	4160(3)
O(35)	−4151(3)	4789(4)	4145(3)
O(36)	−2532(3)	4536(2)	4766(2)
C(37)	−3168(6)	4367(5)	5439(3)

RESULTS AND DISCUSSION

A perspective view of the molecular conformation of the pentapeptide is shown in Fig. 1. The values of the backbone torsion angles (ϕ,ψ)[28] are given in Table II. The parameters of the inter- and intramolecular hydrogen bonds in the crystal structure are listed in Table III. The peptide backbone folds into a right-handed 3_{10}-helix,[29] and the ϕ,ψ values of the first four residues lie very close to the ideal values of $\phi = -60°$, $\psi = -30°$ [30] The torsion angles for the fifth residue lie in the left-handed helical region. This feature has been observed for the C-terminal Aib residue in a number of crystal structures of helical peptides with a terminal ester group.[3,7,9,10,14]

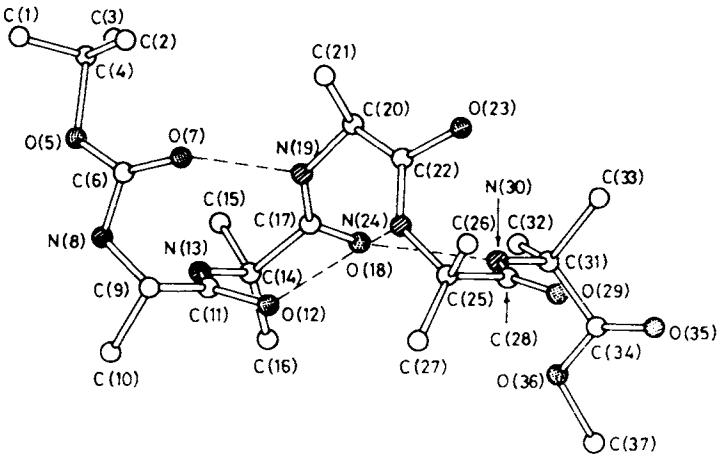

Fig. 1. Perspective view of the molecule along a direction perpendicular to the helix axis. The hydrogen bonds are indicated by broken lines.

The observed pitch of the helix (6.026 Å) is close to that of a classical 3_{10}-helix (6.0 Å), the height per residue varying between 1.949 and 2.066 Å. The unit twist, however, varies between 109.5° and 139.3° with an average value of 119.9°. The helix is stabilized by three intramolecular 4 → 1 hydrogen bonds, with the NH groups of Ala(3), Aib(4), and Aib(5) interacting with the CO moieties of the Boc protecting group, Ala(1), and Aib(2), respectively. The observed hydrogen-bonding pattern is in complete agreement with that postulated for the pentapeptide in solution on the basis of ^{1}H-nmr studies.[5] In the case of stereochemically rigid Aib peptides, excellent agreement between spectroscopic and x-ray diffraction studies has been obtained in several cases,[23] though some disagreement has also been noted in the analysis of the amino terminal pentapeptide of suzukacillin.[20]

The helix axis of the pentapeptide molecule is nearly parallel to the crystallographic a-axis and makes an angle of 6.7° with it. As seen from Fig. 2, the arrangement is such that is permits the NH groups of Ala(1) and Aib(2) to hydrogen-bond to the carbonyl oxygens of Aib(4) and Aib(5), respectively, of a molecule related by the a translation. Thus, the molecules

TABLE II
Main-Chain Torsion Angles

	ω	ϕ	ψ
Ala(1)	−176.7(3)	−57.9(4)	−36.9(4)
Aib(2)	−175.0(3)	−55.5(5)	−29.5(5)
Ala(3)	+170.8(4)	−64.4(5)	−13.0(5)
Aib(4)	+169.8(3)	−54.7(5)	−22.3(5)
Aib(5)	+177.1(4)[a]	+56.4(5)	+51.2(5)[a]

[a] The ester oxygen atom in the protecting group has been treated as geometrically equivalent to an amide nitrogen atom in the calculation of these torsion angles.

TABLE III
Hydrogen-Bond Parameters

Hydrogen Bond	1(N···O)	θ(H-N···O)
N(19)···O(7)	3.043(5)	11(3)
N(24)···O(12)	3.091(4)	13(2)
N(30)···O(18)	3.009(4)	8(3)
N(8)···O(29)[a]	2.852(5)	15(3)
N(13)···O(35)[a]	3.176(5)	36(3)

[a] Atoms belonging to a molecule related by an a translation.

align along the a-axis in a head-to-tail fashion with a hydrogen-bonding pattern almost appropriate to that for an infinitely long peptide helix. These infinitely long helical columns pack together to form the crystal. Similar packing modes have also been observed in the crystal structures of three other 3_{10}-helical pentapeptides, namely, Tosyl-(Aib)$_5$-OMe[7], Z-(Aib)$_5$-OBu^t,[10] and Boc-L-Leu-Aib-L-Pro-L-Val-Aib-OMe (Ch. Pulla Rao and P. Balaram, submitted for publication). The head-to-tail arrangement is dictated by the fact that the NH and CO groups that are not involved in intramolecular hydrogen bonds are positioned at opposite ends of the molecule. The generation of a macrodipole moment in peptide helices may also favor this packing mode.[31,32] It is of interest to note that dipole–dipole

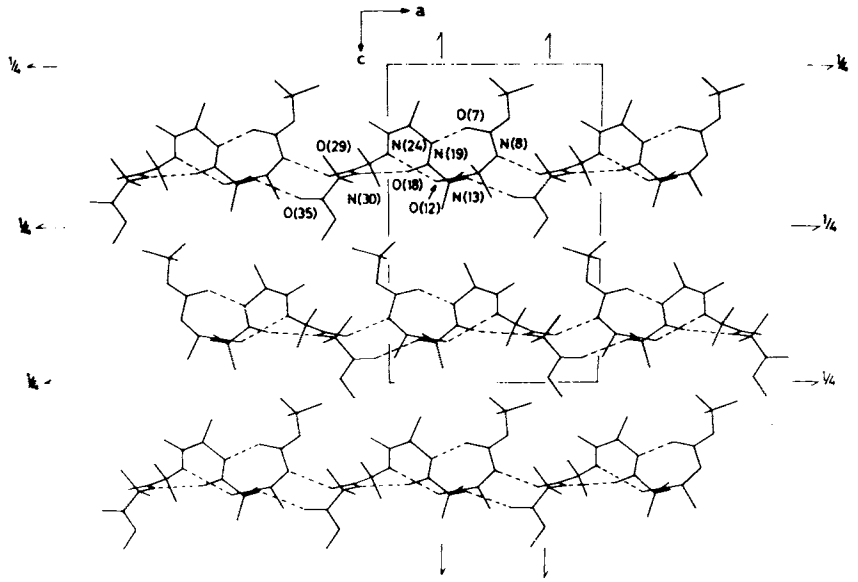

Fig. 2. Head-to-tail arrangement of molecules as viewed along the b direction. Broken lines indicate hydrogen bonds. Only two of the four molecules in the unit cell are shown; the 2_1 screw along b and the two other molecules generated by this symmetry element are not shown for the sake of clarity. The molecules that are not shown form part of helical columns that run in the opposite direction.

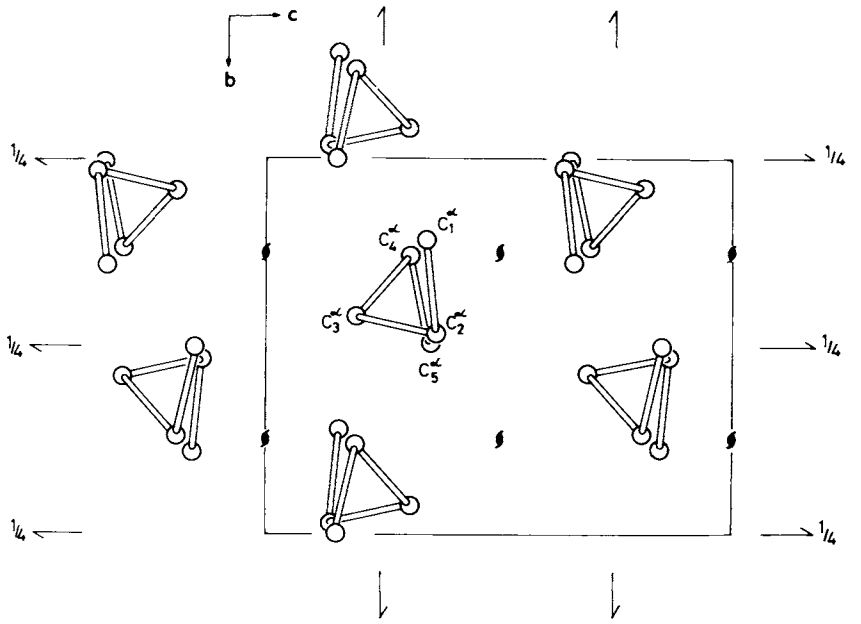

Fig. 3. Schematic representation of the structure as seen along the a-axis. Only α-carbon positions are indicated.

interactions between adjacent peptide helices have been suggested to be an important determinant in the folding of protein chains.

A schematic representation of the crystal structure as viewed along the a-axis is shown in Fig. 3. Assuming two nonbonded atoms to be in contact if the corresponding interatomic distance is less than $(R_1 + R_2) + 0.2$ Å, where R_1 and R_2 are the respective van der Waals radii, each column is in contact with six surrounding columns. This indeed is the most efficient packing mode if a helix can be approximated to a cylinder. Of the six surrounding columns, two have the same direction as that of the one in the middle, whereas the remaining four propagate in the opposite direction. The numbers of contacts with the two parallel columns are 10 and 2, whereas those with the antiparallel columns vary between 3 and 6.

X-ray investigations in the solid state[5,7–15,18,20] and spectroscopic studies in solution,[3–6,16,17] carried out in this laboratory and elsewhere, on the fragments of alamethicin and suzukacillin, and related model compounds, strongly suggest that Aib-containing ionophores have rigid rodlike helical structures. Each peptide helix cannot, by itself, function as an ion-channel because of its very small internal diameter. Aggregation of peptide helices therefore appears to be necessary for the formation of transmembrane channels. Attempts to develop a molecular model for such channels are currently underway.

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