Crystal structure of the channel-forming polypeptide antiamoebin in a membrane-mimetic environment

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ABSTRACT **Crystals of an ion-channel-forming peptaibol peptide in a partial membrane environment have been obtained by cocrystallizing antiamoebin with *n*-octanol. The antiamoebin molecule has a bent helical conformation very similar to that established for Leu-zervamicin, despite a significantly different sequence for residues 1–8. The bent helices assemble to form a polar channel in the shape of an hour glass that is quite comparable to that of Leu-zervamicin. The molecules of cocrystallized octanol are found in two different areas with respect to the assembly of peptide molecules. One octanol molecule mimics a membrane segment along the hydrophobic exterior of the channel assembly. The other octanol molecules fill the channel in such a way that their OH termini satisfy the C═O moieties directed into the interior of the channel. Structure parameters for $C_{82}H_{27}N_{17}O_{20}\cdot 3C_8H_{18}O$ are space group $P2_12_12_1$, $a = 9.143(2)$ Å, $b = 28.590(8)$ Å, $c = 44.289(8)$ Å, $Z = 4$, agreement factor $R_1 = 11.95\%$ for 4,113 observed reflections [$>4\sigma(F)$], resolution ~1.0 Å.**

Peptaibol antibiotics are attractive model systems for investigating the structure and function of membrane-channel-forming polypeptides (1–3). Crystal structure determination of alamethicin (4) and Leu[1]-zervamicin (5–8) have provided details of the stereochemistry of these transmembrane helices and their models of association, leading to suggestions for plausible mechanisms of voltage gating (3–5). Thus far, crystal structures for these membrane-active peptides have been obtained without any cocrystallized lipid component. In this report, we describe the structure of an antiamoebin–octanol solvate at 1.0-Å resolution that reveals a membrane-mimetic environment for the peptaibol. Antiamoebins are a family of compounds closely related to 16-residue fungal polypeptides (9, 10) with anti-protozoal and anthelmintic activities (11).

The sequence of antiamoebin **1** is compared with Leu-zervamicin **2** and alamethicin **3**, as follows (9): Ac-Phe-Aib-Aib-Aib-Iva[5]-Gly-Leu-Aib-Aib-Hyp[10]-Gln-Iva-Hyp-Aib-Pro[15]-Phol (**1**), Ac-Leu-Ile-Gln-Iva-Ile[5]-Thr-Aib-Leu-Aib-Hyp[10]-Gln-Aib-Hyp-Aib-Pro[15]-Phol (**2**), and Ac-Aib-Pro-Aib-Ala-Aib[5]-Ala-Gln-Aib-Val-Aib[10]-Gly-Leu-Aib-Pro-Val[15]-Aib-Aib-Glu-Gln-Phol[20] (**3**), where Aib is α-aminoisobutyric acid, Iva is isovaline (α-ethylalanine), Hyp is 4-hydroxyproline, and Phol is phenylalaninol. For compounds **1** and **2**, the sequences are identical for residues 9–16 except for the change at residue 12 from Aib to Iva. For residues 1–8, the sequences are significantly different in that the polar residues with long side chains Gln[3] and Thr[6] in compound **2** have been replaced with Aib[3] and Gly[6] in compound **1**. Further, the first 8 residues of compound **1** contain four Aib residues and one Iva, compared with one Aib and one Iva in compound **2**. Crystallization and subsequent structure analysis by x-ray diffraction of alamethicin **3** (4) did not show the formation by aggregation of the peptide molecules of any channels lined with polar residues, as might be expected for a membrane ion channel. In the three different crystal forms of Leu-zervamicin (5–7), a channel that was similar in each formed that had a polar interior, an appropriate size for transporting K^+ ions, and a gating mechanism at Gln[11] (7) that strongly suggested a model for ion channels formed in membranes. Cocrystallization of a peptaibol with a lipid component has not yet been achieved; however, crystals have now been obtained containing both antiamoebin and octanol. The octanol appears to serve as a partial mimic for a lipid.

EXPERIMENTAL PROCEDURES

Antiamoebin I, the major polypeptide of microheterogeneous antiamoebin was isolated by HPLC as described (9). Crystals were obtained from a 1:1 methanol/*n*-octanol mixture over a period of several weeks. The x-ray diffraction data were measured at −50°C on a four-circle diffractometer with CuK_α radiation ($\lambda = 1.5428$ Å) from a crystal covered with microscope immersion oil. The $\theta/2\theta$ scan mode was used with a $2.0° + 2\theta(\alpha_1 - \alpha_2)$ scan width, 14°/min scan speed, and $2\theta_{max} = 100°$ (1.0-Å resolution). The crystal data are $C_{82}H_{127}N_{17}O_{20}\cdot 3C_8H_{17}OH$, molecular weight = 1623.0 + 390.7, space group = $P2_12_12_1$, $a = 9.143(2)$ Å, $b = 28.590(8)$ Å, $c = 44.289(8)$ Å, $V = 11578$ Å^3, $Z = 4$, and calculated $d = 1.158$ g/cm$_3$.

The initial placement of the antiamoebin molecules in the unit cell was accomplished by using Brunger's annealing least-squares program X-PLOR (12–14) on a model based on the known structure of Leu-zervamicin in a similar orthorhombic cell (7), although the *c* cell parameter was 4.5 Å longer for the antiamoebin cell. Subsequently, alternate cycles of ordinary least-squares refinement and difference maps corrected the gross misplacement of the phenyl ring in Phe[1] and located the three octanol molecules. The octanol molecule on the nonpolar face of the peptide is relatively rigid and the atomic positions were determined well. In the two octanol molecules located in the hour-glass-shaped channel, the hydroxyl groups participate in three hydrogen bonds with C═O or OH groups on the polar face of the peptide; consequently the hydroxyl oxygens of the octanols had low thermal factors, and their positions in the structure were well determined (Fig. 1). The positions of the successive carbon atoms in the octanol chains became increasingly disordered to the point that the terminal

Abbreviations: Aib, α-aminoisobutyric acid; Hyp, 4-hydroxyproline; Iva, isovaline (α-ethylalanine); Phol, phenylalaninol.

Data deposition: The atomic coordinates have been deposited in the Cambridge Crystallographic Data Base, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K. (accession no. CCDC 101253).

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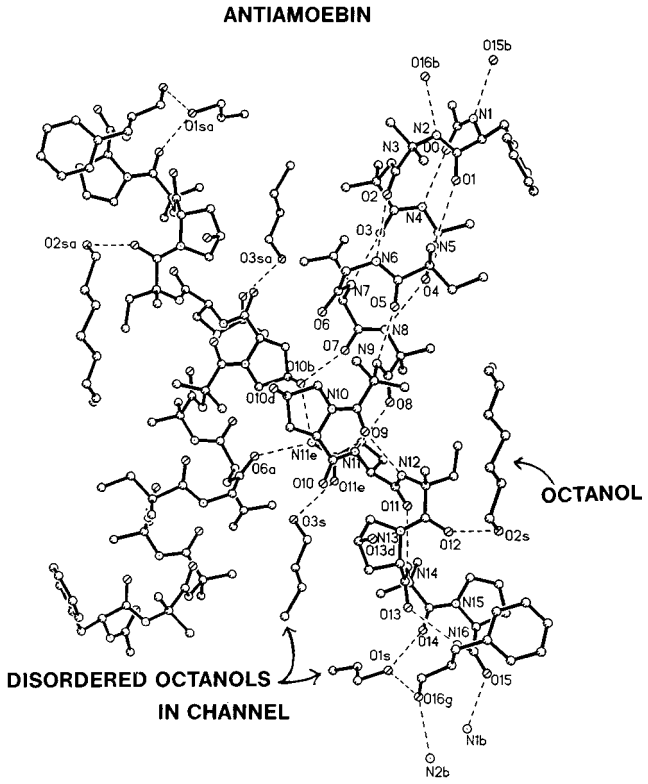

FIG. 1. Conformation of antiamoebin **1** and the channel formed by three symmetry-related peptide molecules. (The third molecule is directly behind one of the molecules shown.) The channel is in the closed position that involves the N(11e)H···O(6a) hydrogen bond. An open channel position was also found in the Leu-zervamicin crystal **2**. The dashed lines indicate hydrogen bonds. A well-ordered octanol molecule is shown on the concave hydrophobic side of the peptide. A symmetry related octanol is shown on the left. Only a portion of two more octanol molecules that reside in the channel is shown because the remainder of the hydrophobic chains of the octanol molecules are disordered to various degrees.

ones could not be located in the difference maps. The final R factor was 11.95% for 4133 F_o values measured $>4.0\sigma$ and 2,325 parameters. (The least-squares program used was Siemens SHELXTL, Version 5.03).

RESULTS AND DISCUSSION

The preponderance of C^{α}-disubstituted amino acid residues in compound **1** suggested a 3_{10}-helix formation (15–17) for the first half of the peptide rather than an α-helix as found in compound **2** (5–7). Actually, despite the high Aib content in compound **1**, the backbone conformations of compounds **1** and **2** are almost identical. A least-squares fit of the positions of 15 C^{α} atoms shows a deviation of only 0.50 Å. Identical pairs of side chains have the same conformation, except for Phol[16], and nonidentical pairs of side chains have similar conformations, except for Phe[1]/Leu[1] (Fig. 2). Torsional angles about the bonds in the backbone and the side chains in compound **1** are listed in Table 1. Values for hydrogen bonds are listed in Table 2, along with the three different types of helical segments that make up the total bent helix. The channels formed by association of the peptide molecules in the crystals of compounds **1** and **2** also are almost identical. Fig. 1 shows the conformation of compound **1**, the hour-glass-shaped channel and octanol molecules flanking the hydrophobic faces of the peptides on both sides of the channel, mimicking a membrane environment.

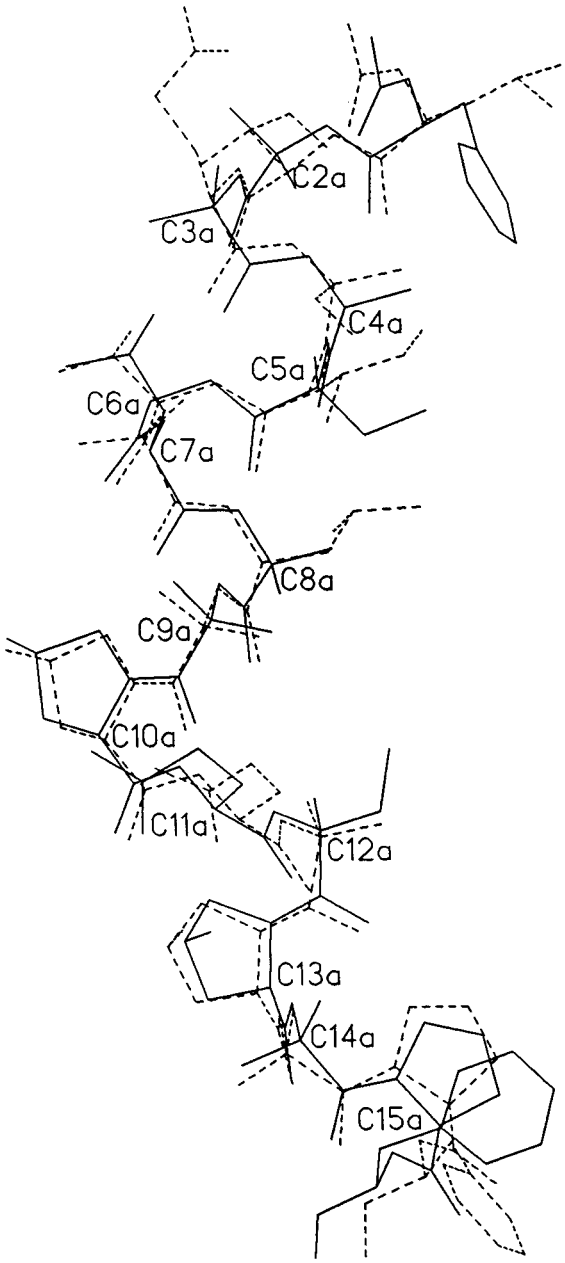

FIG. 2. Superposition of the structure of Leu[1]-zervamicin (7) (dashed lines) and the structure of antiamoebin (solid lines).

The environment of the octanol on the outside of the channel is shown in Figs. 3 and 4. The OH of the octanol forms a hydrogen bond (O···O = 2.83 Å) with carbonyl O12 of Iva[12], the only polar area on the concave side of the bent helix. Similarly in compound **2**, the carbonyl O12 of Aib[12] participates in a hydrogen bond with ethanol, the crystallizing solvent. The nearest C_{oct}···C_{pep} distances range between 3.93 and 4.14 Å, values that are usually observed between hydrocarbon moieties in neighboring molecules. The hydrocarbon chain of the octanol is not completely extended. It is sufficiently flexible for the C8s end to bend easily and follow the contour of the hydrophobic face of the peptide molecule. The C8s atom is 4.01 Å and 4.12 Å away from the ethyl chain of Iva[5] and 4.07 Å away from one CH_3 group of Aib[9] (Figs. 1 and 4). It also approaches the phenyl group of a neighboring peptide molecule by 4.14 Å (Fig. 3). At the hydroxyl terminus, aside from the hydrogen bond to O12, the nearest O···C distances are 3.52 Å and 3.61 Å to phenyl carbon atoms in Phe[1] of a

Table 1. Torsional angles in antiamoebin

Residue	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	§
Phe(1)	−64	−48	−175	+174	−115, +61			
Aib(2)	−56*	−43	180					
Aib(3)	−61	−47	−179					
Aib(4)	−51	−51	−178					
Iva(5)	−57	−51	−172	+64				
Gly(6)	−68	−36*	−174					
Leu(7)	−81*	−34	176	−63	+171, −65			
Aib(8)	−72*	−31	−159*					
Aib(9)	−55	−46	−178					
Hyp(10)	−61	−20	−176	−18	+33	−35	+25	−5
Gln(11)	−87	−11	−174	−47	−53	−40†, +142‡		
Iva(12)	−54	−36	−178	+54				
Hyp(13)	−77	−3*	−174	−27	+44	−44	+29	−2
Aib(14)	−44	−53	−172					
Pro(15)	−76	−15	−177	+6	−10	+10	−6	0
Phol(16)	−103*	+68		−65	−74, +113			

Torsional angles ϕ, ψ, and ω (in degrees) for the backbone and χ^n for the side chains follow the convention presented in ref. 18. The estimated standard deviations are near 2.0°. The symbol § represents the torsion angle $C^{\delta}NC^{\alpha}C^{\beta}$.
*Values for ϕ, ψ, and ω that are more than 10° different than in Leu-zervamicin (7).
†For O^{ϵ}.
‡For N^{ε}.

neighboring peptide and Phol[16] of the same molecule containing O12.

The channel in compound **1** differs from the channel in compound **2** in the polarity of the channel surface. The middle of the hour-glass-shaped channel (Fig. 1) has identical hydrogen bonding in compounds **1** and **2**. The polarity of the lower right side is also identical in compounds **1** and **2**, with carbonyl oxygens O10, O11e, O13, and O14 and hydroxyl oxygens O13d and O16g all extending from the surface into the channel. On the lower left, however, the channel in compound **1** is quite hydrophobic. The side chains of Leu^7 and Aib^3 are very prominent. Thr^6 with its OH group in compound **2** has been replaced with Gly in compound **1**. The N3e moiety of Gln^3 present in the mouth region of **2** has been replaced with Aib^3 in compound **1**. The N(3)H moiety in compound **1** is near the channel surface, does not participate in any hydrogen bonding, and represents the only polarity on the lower left. It is not surprising that the channel in compound **1** is filled with octanol

Table 2. Hydrogen bonds

Type	Donor	Acceptor	O···O and N···O, Å	H*···O and H*···N, Å	CO···N angle, degrees
Head to tail	N1	O15†	2.848	1.96	129
Head to tail	N2	O16G†	2.858	1.99	
	N3††				
5 → 1	N4	O0	2.992	2.13	158
	N5	O1	3.172	2.28	161
	N6	O2	3.095	2.24	157
	N7	O3	2.845	1.98	164
	N8	O4	3.110	2.24	155
	N9	O5	2.944	2.32	153
	N10	(Hyp)			
	O10D	O7‡	2.764		
4 → 1	N11	O8	2.862	2.03	140
	N11E	O6§	2.914	2.07	
	N11E	O10D‡	2.975	2.09	
β-ribbon	N12	O9	3.094	2.21	121
	N13	(Hyp)			
	O13	O11E¶	2.775		
	N14	O11	2.951	2.090	133
	N15	(Pro)			
	N16	O13	2.854	2.09	
	O16G	O1S‖	2.716		
	O3S	O11E	2.818		
	O2S	O12	2.834		
	O1S	O14	2.671		

*Hydrogen atoms were placed in idealized positions with N—H = 0.90 Å.
†At symmetry equivalent $x, 1 + y, z$.
‡At symmetry equivalent $1/2 + x, 1/2 - y, -z$.
§At symmetry equivalent $-1/2 + x, 1/2 - y, -z$.
¶At symmetry equivalent $-1 + x, y, z$.
‖Atoms O1S, O2S and O3S are the O atoms in the OH groups of the three octanol molecules.
††N(3)H and O(10) do not participate in hydrogen bonds.

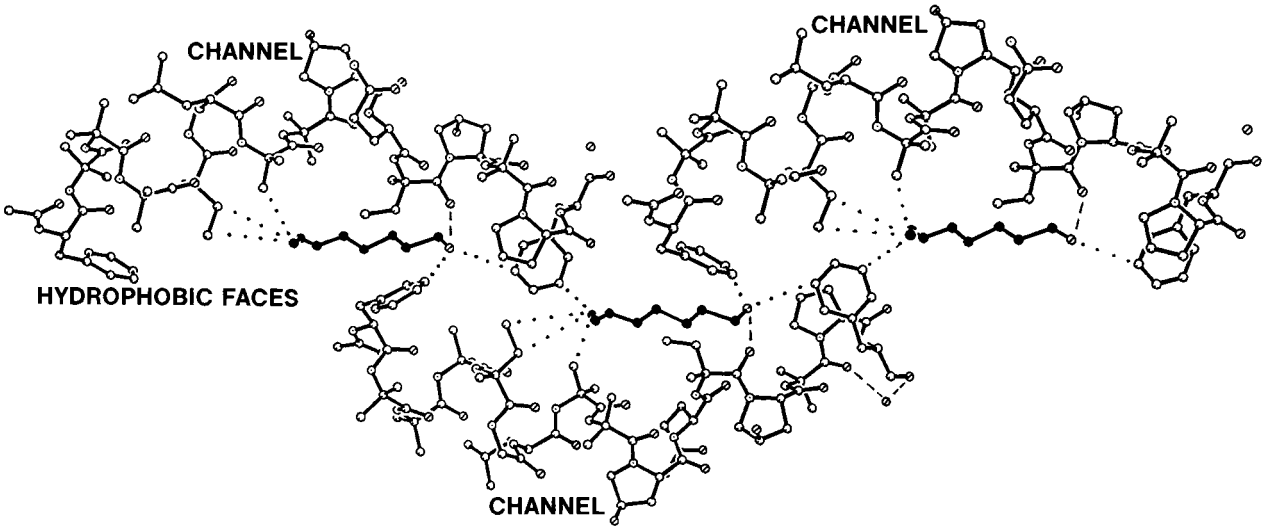

FIG. 3. Environment of the octanol molecules residing in hydrophobic cavities between peptide molecules. Dashed lines represent hydrogen bonds, and dotted lines are nearest van der Waals' approaches.

molecules whose OH groups are satisfied by the hydrogen bonding potential of O11e, O14, and O16g and whose hydrocarbon chains are compatible with the hydrophobic surface. It is not clear how the partially hydrophobic surface of the channel in antiamoebin compares with Leu-zervamicin in attracting K^+ ions.

In conclusion, our experiments demonstrate that the hourglass-shaped ion channel in antiamoebin is almost identical to the polar channel present in three different crystal forms of Leu[1]-zervamicin, despite the hydrophobicity of residues 1–8 in antiamoebin. The narrow part of the hour glass channel where gating may take place in Leu[1]-zervamicin (7), has the same residues and hydrogen bonding scheme in both peptides. The presence of octanol molecules in the crystal provides a membrane-mimetic environment for the ion channels.

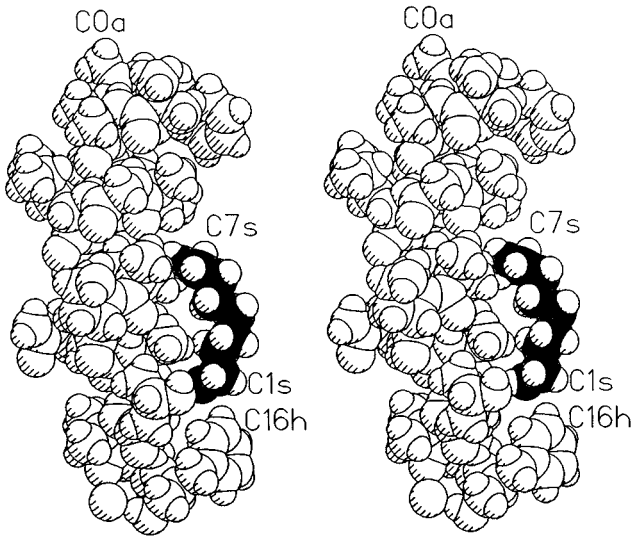

FIG. 4. Stereodiagram of space-filling drawings of peptide **1** and associated octanol molecule (darkened). The view is rotated ~45° from the orientation in Fig. 1.

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1. Mathew, M. K. & Balaram, P. (1983) *Mol. Cell. Biochem.* **50,** 17–61.
2. Marsh, D. (1996) *Biochem. J.* **315,** 345–361.
3. Sansom, M. S. P. (1993) *Q. Rev. Biophys.* **26,** 365–421.
4. Fox, R. O. & Richards, F. M. (1982) *Nature (London)* **300,** 325–330.
5. Karle, I. L., Flippen-Anderson, J. L., Agarwalla, S. & Balaram, P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5307–5311.
6. Karle, I. L., Flippen-Anderson, J. L., Agarwalla, S. & Balaram, P. (1992) in *Proteins: Proceedings of the Seventh Conversation in Biomolecular Stereodynamics,* eds. Sarma, R. H. & Sarma, M. H. (Adenine, Guilderland, NY), Vol. 2, pp. 97–111.
7. Karle, I. L., Flippen-Anderson, J. L., Agarwalla, S. & Balaram, P. (1994) *Biopolymers* **34,** 721–735.
8. Balaram, P. Krishma, K., Sukumar, M., Mellor, J. R. & Sansom, M. S. P. (1992) *Eur. Biophys. J.* **21,** 117–128.
9. Pandey, R. C., Meng, H., Carter Cook, J., Jr., & Rinehart, K. L., Jr. (1977) *J. Am. Chem. Soc.* **99,** 5203–5205.
10. Das, M. K., Raghothama, S. & Balaram, P. (1986) *Biochemistry* **25,** 7110–7117.
11. Thirumalachar, M. J. (1968) *Hindustan. Antibiotics, Bulletin* **10,** 287–289.
12. Brunger, A. T., Kuriyan, J. & Karplus, M. (1987) *Science* **235,** 458–460.
13. Brunger, A. T., Krukowski, A. & Erickson, J. (1990) *Acta Crystallogr. A* **46,** 585–593.
14. Brunger, A. T. (1992) *Nature (London)* **355,** 472–474.
15. Toniolo, C., Bonora, G. M., Bavoso, A., Benedetti, E., di Blasio, B., Pavone, V. & Pedone, C. (1983) *Biopolymers* **22,** 205–215.
16. Marshall, G. R., Hodgkin, E. E., Langs, D. A., Smith, G. D., Zabrocki, J. & Leplawy, M. T. (1990) *Proc. Natl. Acad. Sci. USA* **97,** 487–491.
17. Karle, I. L. & Balaram, P. (1990) *Biochemistry* **29,** 6747–6756.
18. IUPAC-IUB Commission on Biochemical Nomenclature (1970) *Biochemistry* **9,** 3471–3479.