Acta Cryst. (1997). D53, 151–159

Channels in the Gramicidin S-with-Urea Structure and their Possible Relation to Transmembrane Ion Transport

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(*Received 16 January 1996; accepted 10 October 1996*)

Abstract

The structure of membrane-active antibiotic cyclodecapeptide gramicidin S in the crystals of its complex with urea, $C_{60}H_{92}N_{12}O_{10}.0.5[(NH_2)_2CO].7.94H_2O$, has been investigated with three-dimensional X-ray data by the automatic sequential approximation method. The crystals are trigonal, space group $P3_121$, $a = 25.80(3)$, $c = 21.49(2)$ Å, $M_r = 7968$, calculated density $= 1.088\,\mathrm{mg\,m^{-3}}$, $Z = 1$. Conventional R factor: $R1 = 0.0943$, $wR2 = 0.2478$ [$I > 2\sigma(I)$]. The molecule possesses an antiparallel twisted β-structure, with turns involving the Phe–Pro peptides. The Orn side chains extend on one side of the sheet, while the non-polar Val and Leu side chains are located on the other face. One of the Orn residues (namely Orn2) is linked by an intermolecular hydrogen bond to the O atom of Phe4 residue, the other is free. The side chains of the Phe residues have *trans* orientation ($\chi^1 \simeq 180^\circ$) and those of the Val, Orn, Leu residues, except those of Orn2, have the preferential *gauche* orientation with the χ^1 angle close to 60°. Two side chains show statistical disorder and conformation of the Pro residues is C_s—C^β-exo. There is half a urea molecule and also 7.94 water molecules distributed on 13 positions for each antibiotic molecule. A partially occupied and poorly ordered alcohol molecule had been identified. The gramicidin S molecules are arranged around the 3_1 axis in the form of a left-handed double spiral forming suggestive channels. The outer hydrophobic surface of the spiral is made of uncharged side radicals while the inside surface consists of the main-chain atoms, mainly O and N, and of ornithine side chains with N atoms at the ends. By changing the Orn side-chain conformation, the inner diameter of the channels may change from 3.4 to 6.3 Å. Thus, ions and particles of rather large size may pass through the channel. The possibility of the creation of the gramicidin S channels in mitochondrial membranes has been noted by some biochemists. The channel complexes are close-packed in a hexagonal arrangement in the crystal. The Cl^- ions, present in abundance in the mother solution, are not found ordered in the crystals, which may indicate the absence of the charges in the terminal N atoms of the Orn residues.

1. Introduction

The cyclic decapeptide antibiotic gramicidin S [cyclo-(Val-Orn-Leu-D-Phe-Pro)$_2$-] was isolated by Gause & Brazhnikova (1944). The antibiotic acts on the cellular membrane which, in its presence, no longer functions as a penetration barrier for ions. Present knowledge suggests that there are no specific protein receptors for gramicidin S and that its interaction is with the membrane lipid components (Ovchinnikov & Ivano, 1982).

Since the time of isolation of the antibiotic various models have been put forward for its molecule conformation based on the general principles of peptide chain folding, as well as on semi-empirical calculations (Warner, 1961; Vanderkori, Leach, Nemethy, Scott & Scheraga, 1966; Liquori, DeSantis, Kovacs & Mazzarella, 1996; Hodgkin & Oughton, 1957; Schmidt, Hodgkin & Oughton, 1957). The most accepted was the β-model (Hodgkin & Oughton, 1957) according to which the molecule consists of two antiparallel β-chains, joined by proline bridges, and stabilized by four cross-cyclic hydrogen bonds. This model was supported by the investigations of the conformational states of gramicidin S and its N,N' diacetyl derivative in different media by physiochemical, mostly spectral, methods (Kokorin, Zamaraev, Grigorjan, Ivanov & Rozantsev, 1972). A series of crystalline heavy-atom derivatives of gramicidin S with two independent molecules in the asymmetric unit were obtained (Tishchenko & Zykalova, 1963; Tishchenko, Zykalova & Silant'eva, 1964; Tishchenko, Zykalova & Grebenko, 1967; Tishchenko & Zykalova, 1969). However, interpretation of the Patterson maps was not successful. It is probably because of the almost uniform spacing of the gramicidin S molecules and the heavy atoms connected with them, in combination with the high symmetry of the unit cell and a great number of the

atoms in it, Patterson maps were considerably complicated. Various attempts were made to use the heavy-atom method to solve the structure (Schmidt, Hodgkin & Oughton, 1957; De Santis & Liquori, 1971) but up to 1978 the X-ray structure investigations of gramicidin S were unsuccessful.

In 1978, the gramicidin S structure was solved (Hull, Karlsson, Main, Dodson & Woolfson, 1978) by direct methods using *MULTAN*78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978). This was assisted by the successful crystallization of gramicidin S (one molecule in the basic part of the unit cell with space group $P3_121$, $a = 25.8$, $c = 21.49$ Å) from an alcohol solution in the presence of urea and HCl. The structure model was refined using 4902 reflections (completeness 90%) to a resolution of about 1 Å and an R factor of 0.188. However, since the crystals were unstable and several samples were used for data collection the quality of the experimental data was not very high. The standard deviations for the C atoms of the cycle were equal to 0.04 Å and the positions of some atoms were not found.

Good quality crystals have been grown at the Institute of Crystallography, Russian Academy of Sciences, which, in combination with programs for X-ray structure analysis developed at the Institute, has led to a structure of much greater precision.

2. Experimental

The crystals of the gramicidin S complex with urea, suitable for X-ray structure analysis were grown from a water–alcohol solution of the antibiotic in the form of the hydrochloride in the presence of HCl and urea. The space group was $P3_121$ with unit-cell parameters $a = 25.80(3)$, $c = 21.49(2)$ Å [close to those given by Hull *et al.* (1978)], $Z = 6$. As the crystals were unstable in the open air, they were packed in quartz capillaries in the presence of mother liquid. X-ray measurements were carried out on a Syntex $P2_1$ diffractometer at room temperature using Cu $K\alpha_1$ radiation, the ω–2θ scan technique, scan interval $\Delta\omega = 1\ \text{min}^{-1}$, 4786 reflections, $d_{\min} \geq 0.98$ Å completeness = 99.5%. [For the structure investigation 4028 reflections with $I \geq 4\sigma(I)$ were used.] The starting model included 81 atoms of the gramicidin S molecule (Hull, Karlsson, Main, Dodson & Woolfson, 1978). By the automatic sequential approximation method (Andrianov, Shibanova & Simonov, 1987) the positions of the next five peaks were revealed, which were interpreted as one urea and two water molecules. The final synthesis contained many extra peaks, the interpretation of which was quite difficult. Sequential isotropic–anisotropic refinement of the located atoms followed by difference-map calculation and the selection of new peaks by crystallochemical criteria, taking into account possible statistical occupation of some positions by water molecules and by the terminal C atoms of some side chains, allowed us to determine the parameters of 18 more atoms, including atoms of the solvents water and alcohol. But Cl^- ions, the presence of which may be expected taking into consideration the crystallization conditions, were not found. Besides the C, N and O atoms, 59 H atoms were localized in a series of difference maps, and their positional parameters were refined. The positions of 26 more H atoms were calculated geometrically. All H atoms were refined isotropically. The final R factor for this data set was 6.2%.

In the final stages of the refinement the R factor was greatly reduced (from 11%) by the least-squares refinement of the extinction parameter, the final value was 0.725×10^{-4}. In addition to the reduction of the R factor, especially for reflections with small $\sin\theta/\lambda$, the interpretation of the electron-density function became much more straightforward. For this structure the blocked full-matrix 'cascade' least-squares procedure, as implemented in the *AREN* system (Andrianov, 1989) was especially useful.

This procedure allows refinement of structures with up to 430 atoms in the asymmetric unit with the anisotropic approximation on a PC-AT/XT in a reasonable calculation time. All of the calculations were carried out using the program *AREN* on an EC-1045 and *ARENPC* on a PC-AT (Andrianov, 1987).

To obtain e.s.d.'s for the geometrical parameters the refinement was repeated using all the X-ray data with *SHELXL*93 (Sheldrick, 1993). The water occupancies were held to the previous values, but all H-atom positions were calculated. R factor is 0.0943 (see Table 1). Some low-resolution reflections disagree badly which probably suggests that not all the solvent has been modelled.

The atomic coordinates and thermal parameters have been deposited.*

3. Discussion

The structure is determined to a great extent by a complicated system of intra- and intermolecular hydrogen bonds, with and without participation of water, alcohol and urea molecules (see Table 2). First of all there are four cross-cyclic hydrogen bonds, which stabilize the elongated conformation of the molecule cycle (Figs. 1 and 2, Table 2). They link Val1 and Leu8, and Val6 and Leu3. These bonds are not equal, namely the central ones are shorter [2.818(8) and 2.930(8) Å] and the peripheral ones longer [3.256(9)

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory. Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: HE0070).

Table 1. *Crystal data and structure refinement*

Identification code	grm_mos6aa
Empirical formula	C367 H111.68 N78 O112.62
Molecular weight	7968
Temperature (K)	293 (2)
Wavelength (Å)	1.54178
Crystal system	Trigonal
Space group	$P3_12_1$
Unit-cell dimensions (Å,°)	$a = 25.80$ (3) $\alpha = 90$ $b = 25.80$ (3) $\beta = 90$ $c = 21.49$ (2) $\gamma = 120$
Volume (Å^3)	12388 (21)
Z	1
Density (calculated) (mg m^{-3})	1.088
Absorption coefficient (mm^{-1})	0.674
$F(000)$	4392
θ range for data collection (°)	1.98–51.88
Index ranges	$0 \le h \le 22$, $0 \le k \le 13$, $-21 \le l \le 21$
Reflections collected	4786
Independent reflections	4786 [R(int) = 0.0000]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4774/919/934
Goodness-of-fit on F^2	1.076
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0943$, $wR2 = 0.2478$
R indices (all data)	$R1 = 0.0990$, $wR2 = 0.2642$
Absolute structure parameter	1.0 (5)
Extinction coefficient	0.0020 (3)
Largest diffraction peak and hole (e Å^{-3})	0.551 and −0.511

and 3.160 (8) Å], the latter being not quite parallel to each other. The conformation of the molecule is influenced to a smaller degree by the intramolecular hydrogen bond between the terminal NE atom of the Orn2 residue and the O atom of the Phe4 residue

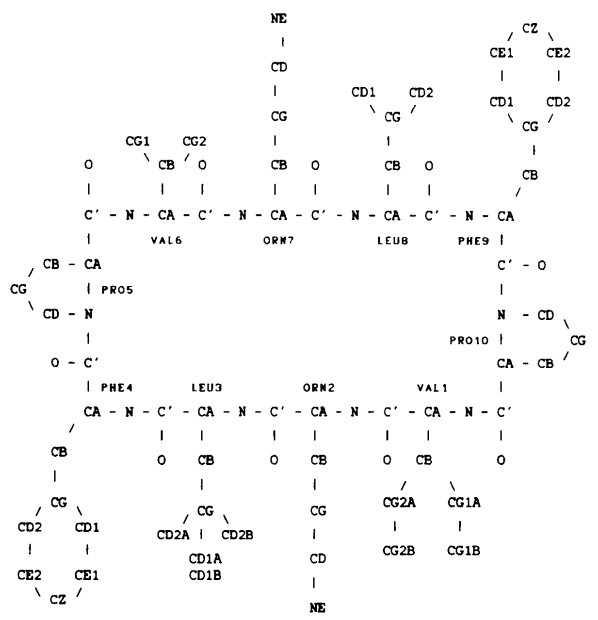


Fig. 1. Chemical structure of the gramicidin S molecule with the numbering of atoms according to the IUPAC-IUB Commission on Biochemical Nomenclature (1971).

Table 2. *Hydrogen bonds*

Within molecule	
Val1 N···Leu8 O	3.154 (0.008)
Val1 O···Leu8 N	2.929 (0.008)
Leu3 O···Pro5 N	3.093 (0.009)
Leu3 N···Val6 O	2.818 (0.008)
Leu3 O···Val6 N	3.257 (0.009)
Orn2 NE···Phe4 O	2.770 (0.022)
Across crystallographic twofold at $z = 1/6$	
Orn2 N···Orn2 O^{i}	3.021 (0.007)
To urea, this lies on crystallographic twofold at $z = -1/3$	
Phe9 N···Ure12 O	2.827 (0.007)
Phe9 O···Ure12 N	2.976 (0.009)
To alcohol, this is partially occupied	
Pro10 O···Alc13 O	2.970 (0.007)
Water network, many disordered 1,3,4 fully occupied 3,4 in special position	
Orn2 NE···Hoh10 O	2.918 (0.041)
Phe4 N···Hoh2 O	2.749 (0.025)
Pro5 O···Hoh1 O^{ii}	2.762 (0.010)
Pro5 O···Hoh3 O	3.047 (0.034)
Orn7 N···Hoh1 O	2.900 (0.009)
Orn7 O···Hoh12 O^{iii}	2.770 (0.022)
Phe9 O···Hoh6 O	2.926 (0.020)
Pro10 O···Hoh6 O	2.722 (0.022)
Ure12 N···Hoh4 O	3.184 (0.016)
Ure12 N···Hoh13 O^{iii}	3.222 (0.061)
Hoh1 O···Hoh5 O^{iii}	2.917 (0.040)
Hoh1 O···Hoh12 O^{iii}	2.847 (0.037)
Hoh3 O···Hoh10 O	2.776 (0.044)
Hoh4 O···Hoh5 O	2.825 (0.057)
Hoh5 O···Hoh7 O	3.159 (0.122)
Hoh5 O···Hoh9 O	2.732 (0.069)
Hoh5 O···Hoh13 O^{iii}	2.038 (0.078)
Hoh6 O···Hoh7 O	2.606 (0.078)
Hoh7 O···Hoh12 O	3.094 (0.119)
Hoh7 O···Hoh13 O^{iii}	3.075 (0.119)
Hoh11 O···Hoh11 O^{iii}	2.713 (0.175)

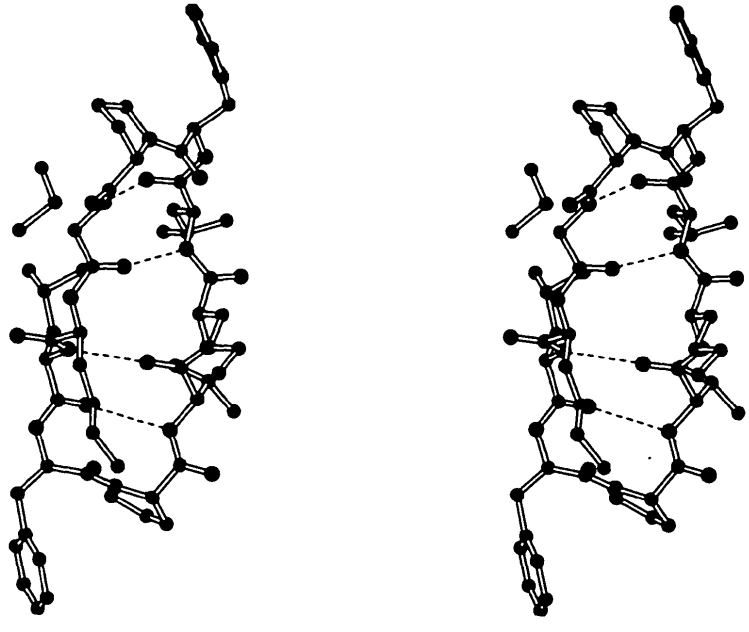
Symmetry operators: (i) $-x, y-x, -z+\frac{1}{3}$; (ii) $x-y, -y, -z-\frac{1}{3}$; (iii) $-x, y-x, -z-\frac{2}{3}$.

[2.793 (22) Å] and also by two intermolecular hydrogen bonds with the urea. The urea molecule joins two gramicidin S molecules across the twofold axis. We have to note that only one intermolecular hydrogen bond occurs directly between the atoms of neighbouring gramicidin S molecules, the water molecules take part in the remainder. The conformation is similar in general outline to the 'pleated sheet' structure suggested by Hodgkin & Oughton (1957), but differs from the latter by the slight twisting of the peptide backbone, similar to that in β-sheets of protein molecules. The molecule has a non-crystallographic twofold axis relating to two β-strands. The r.m.s. deviation of the main-chain atoms is 0.243 Å. The Phe residues are in the *gauche* conformation, allowing the peptide to turn sharply (Fig. 2). The Ramachandran plot (Fig. 3) shows the conformational angles φ and ψ of residues 1 to 3, and 6 to 8 are in the *B* region, and the Phe residues 4 and 9 lie in the *p* region. Conformation φ, ψ and ω angles derived by different techniques are listed in Table 3. The φ

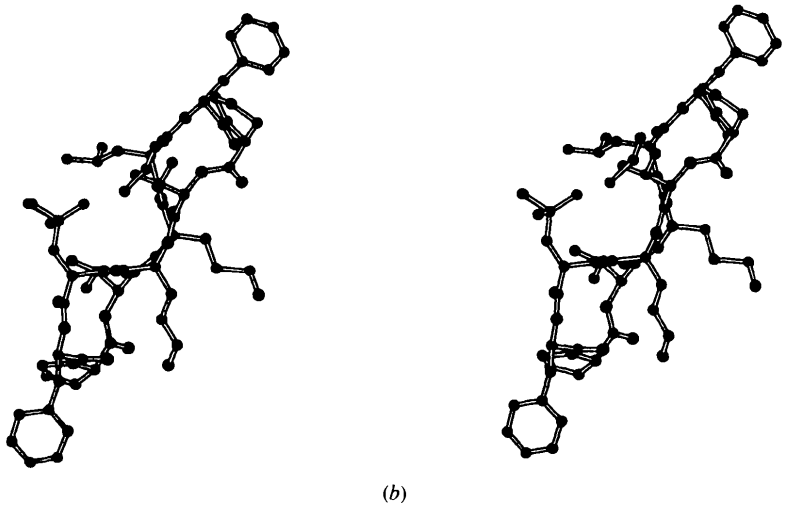
angles derived from the NMR investigation agrees well with the X-ray values, whereas the ψ values differ considerably. At the same time the ψ values from X-ray experiment IV conform slightly better to the data from the calculation in III, then to the spectral data I. The calculated values from study III agree slightly better with the X-ray values than those of study II.

The planes of the Phe9 and Pro10 rings are roughly parallel (the angle between the planes is 29.3°), while those of Phe4 and Pro5 are not (angle 51.2°). There is an intramolecular hydrogen bond [2.79 (22) Å] between the terminal N atom of Orn2 and the carbonyl O atom of Phe4, which cannot be formed between Orn7 and Phe9. The terminal N atom of Orn7 links to the urea molecule.

A distinguishing feature of the molecule is the position of the extended side chains of the ornithine residues on the one side of the molecular cycle. One of these side chains is fastened by an intramolecular hydrogen bond, the other is free (Fig. 2*b*). The distance between the terminal NE atoms of symmetry-related ornithine side chains of Orn7 is about 5.65 Å. The hydrophobic side chains of the Val and Leu residues are oriented in the direction opposite to the ornithine side chains of the peptide. There is statistical disorder in the orientation of the Val1 and Leu3 residue side chains. In the first one CG1 and CG2 atoms occupy two different positions *A* and *B* with occupancies 0.4 and 0.6, in the second one CD1 and CD2 atoms may statistically



(*a*)



(*b*)

Fig. 2. Two stereoviews of the gramicidin S molecule. The dimensions of the peptide are approximately 5 by 14 Å. (*a*) The hydrogen bonds linking the main chains of the peptide. (*b*) The twisting of the backbone.

Table 3. *Experimental and calculated conformational angles (°)*

I, derived from NMR investigation (Ovchinnikov & Ivanov, 1976); II, calculated by DeSantis & Liquori (1971); III, calculated by Dygert, Go & Scheraga (1975); IV, derived from X-ray coordinates. The mean angles are listed with the predicted range in brackets

	φ	ψ	φ	ψ	φ	ψ	φ	ψ
	I (NMR)		II (calc)		III (calc)		IV (X-ray)	
Val	−120	120	−82	137	−89 (2)	125.5 (3.5)	−120	157
							−125	153
Orn	−110	110	−132	152	−143 (5)	128 (2)	−108	131
							−105	136
Leu	−120	110	−143	82	−148 (10)	109 (6.5)	−121	93
							−139	121
Phe	55	−110	58	−116	67.5 (3.5)	−139.5 (0.5)	61	−126
							58	−136
Pro	−60	−40	−68	−31	−75 (0)	−16.5 (1.5)	−81	−2
							−93	11

occupy two positions *A* and *B* with probabilities 0.4 and 0.6, respectively (see deposited material).

The χ angles agree quite well with the expected values. For the aliphatic side chains the torsion angles χ^1 clusters about values 60, 180 and −60°, the last value being most probable (Lakshminarayanan, Sasisekharan & Ramachandran, 1967; Stenkap & Jensen, 1975). In the gramicidin S molecule the angle χ^1 for Val, Orn and Leu is close to the most probable values of + or − 60° (see Table 4) for all the aliphatic side chains except those of Orn2 and position *B* of Val1. For Orn2 this angle deviated from 180° towards negative values [−173.5 (1.1)°] owing to steric influence of the C=O group. In other words for this group *trans* orientation occurs whereas for all others it is *gauche*. The ornithine side chains are in fully extended conformation *i.e.* the χ^2 and χ^3 parameters are close to 180°, the main orientation of the Leu3 and Leu8 radicals is *trans*. The side chains of Phe residues in the gramicidin S molecule are characterized by χ^1 angles close to 180°, χ^{21} and χ^{22} to 90 and −90°, respectively, so the phenyl ring plane is nearly perpendicular to the CA—CD—CG plane. The pirrolidine cycles of the Pro5 and Pro10 residues have identical conformations, namely C_s—C^β-exo (*i.e.* envelope, CB in the bent corner, CB and CG on both sides of the plane of the cycle with symmetry C_s) (Balasubramanian, Lakshminarayanan, Sabesan, Tegoni, Venkatesan & Ramachandran, 1971; Ashida & Kakudo, 1974).

The phenyl rings are planar, with r.m.s. distances 0.011 and 0.021 Å for Phe4 and Phe9, respectively. The Pro5 ring is more 'flat' than Pro10 (see Table 5).

There is one half of urea molecule $OC(NH_2)_2$ for every gramicidin S molecule in the structure, which occupies a special position on the crystallographic twofold axis. There are 7.94 water molecules associated with each antibiotic molecule and distributed on 13 positions, the positions of three of them are fully occupied, two of them being in special positions, the rest have been modelled with occupancies between 0.85 and 0.32. One of the water positions (OW2) is occupied $\frac{2}{3}$ by a water molecule and $\frac{1}{3}$ by an O atom of the alcohol molecule, the C atoms of the alcohol molecule have also been located. The water molecules are hydrogen bonded with antibiotic molecules and with other water molecules (see deposited material). Some of the water positions with occupation less than 1 cannot be filled simultaneously, *i.e.* they belong to different gramicidin S molecules in one channel or even to different channels.

The bond lengths (see deposited material) in the *trans*-peptide groups of all the residues do not differ very much from the standard values of peptides, namely N—CA = 1.454 (7), CA—C′ = 1.520 (6), C′=O = 1.228 (6), N—C′ = 1.334 (3) Å (Tishchenko, 1979). The bond lengths in the side chains in some cases are markedly different from the usual value of 1.54 Å which is connected with large thermal vibration of the terminal atoms and, especially, with statistical disorder of some of them. The full picture of the bond angles of the backbone distribution (see deposited material) is close

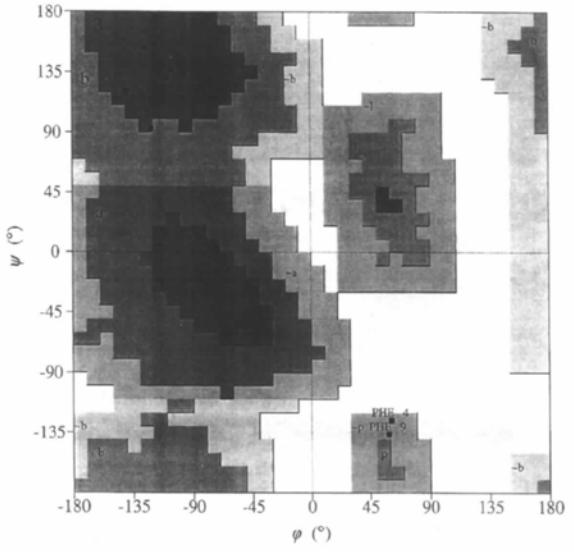


Fig. 3. Ramachandron plot for gramacidin S.

to that expected for peptide chains (Arnott, Dover & Elliott, 1967).

As for the short intra- and intermolecular contacts, they are absent in the structure, especially taking into account ideas about the values of the van der Waals radii of Zefirov & Zorkii (1976).

Of the greatest interest is the packing of the gramicidin S, urea and water molecules. The gramici-

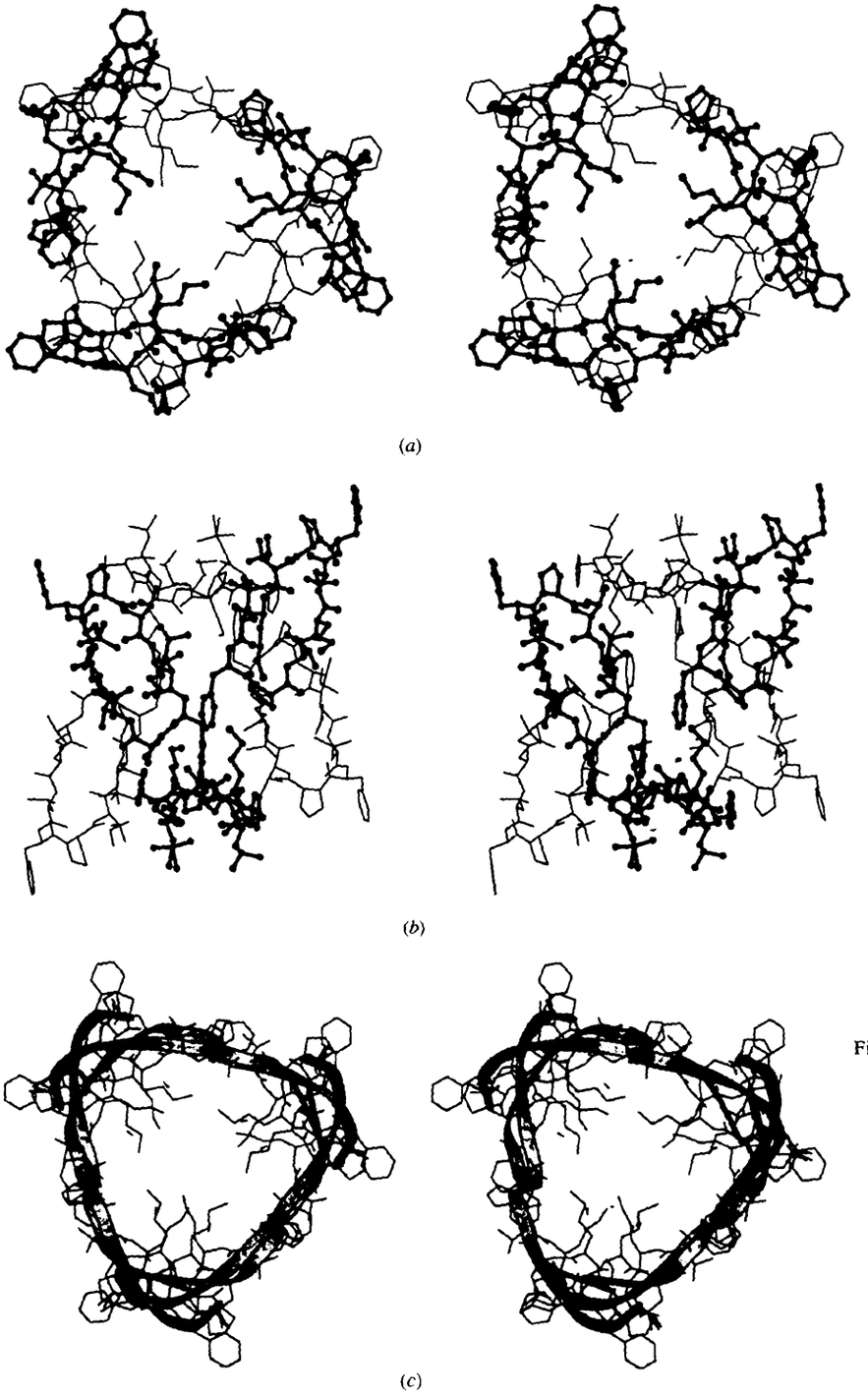


Fig. 4. Stereoviews of the gramicidin S channels formed by six crystallographic equivalent molecules. The urea molecule is present, but water molecules are not shown. (*a*) Viewed along the crystallographic *c* axis showing the orthithine side chains extending into the interior. The backbone is marked by the strand. (*b*) Viewed perpendicular to the *c* axis. Alternate molecules are shown in ball-and-stick, and line mode. The hydrophobic Phe, Pro, Leu and Val side chains all are on the exterior of the complex.

Table 4. *X-ray torsion angles (°)*

Definitions: φ (C—N—CA—C), ψ (N—CA—C—N), ω (CA—C—N—CA), χ^1 (N—CA—CB—CG), χ^2 (CA—CB—CG—CD), χ^3 (CB—CG—CD—NE).

	φ	ψ	ω	χ^{11}	χ^{12}	χ^{21}	χ^{22}	χ^3	
Val1*a*	−119.5	157.0	172.3	−62.5	−177.1	—	—	—	
	(0.8)	(0.7)	(0.7)	(2.1)	(1.4)				
Val1*b*	—	—	—	79.6	−55.9	—	—	—	
				(1.5)	(1.8)				
Orn2	−108.2	131.3	177.2	−174.3	—	179.4	—	−166.0	
	(0.8)	(0.6)	(0.6)	(1.1)		(1.8)		(1.8)	
Leu3*a*	−121.2	92.7	−177.5	−59.5	—	−143.0	−62.0	—	
	(0.7)	(0.7)	(0.7)	(1.1)		(1.9)	(2.5)		
Leu3*b*	—	—	—	—	—	−165.1	90.5	—	
						(2.1)	(1.7)		
D-Phe4	60.6	−126.1	−173.8	173.9	—	−75.1	106.9	−179.6	−178.6
	(0.9)	(0.7)	(0.6)	(0.8)		(1.1)	(1.4)	(0.9)	(1.6)
Pro5	−81.2	−1.5	−174.3	20.9	—	−20.0	—	—	
	(1.0)	(1.3)	(0.8)	(1.4)		(1.8)			
Val6	−125.2	153.0	173.6	58.8	−71.0	—	—	—	
	(0.8)	(0.6)	(0.7)	(1.0)	(0.9)				
Orn7	−105.0	136.4	177.7	−65.9	—	173.1	—	166.9	
	(0.8)	(0.7)	(0.6)	(1.3)		(1.3)		(1.6)	
Leu8	−139.3	120.6	−172.9	−71.6	—	−64.7	—	177.0	
	(0.7)	(0.6)	(0.6)	(1.3)		(2.0)		(1.4)	
D-Phe9	57.7	−135.6	−177.1	175.9	—	−84.8	90.9	175.7	−177.1
	(0.8)	(0.6)	(0.6)	(0.7)		(0.9)	(1.0)	(0.8)	(0.9)
Pro10	−92.6	10.6	−173.0	38.3	—	−37.0	—	21.7	
	(0.7)	(0.9)	(0.6)	(0.7)	—	(0.8)	—	(0.8)	

CG—CD—N—CA angle is 4.6 (1.2)° for Pro5 and 3.6 (0.8)° for Pro10, CD—N—CA—C angle is 105.8 (0.9)° for Pro5 and 93.5 (0.7)° for Pro10.

din S molecules, collected about the 3_1 axis as a left-handed double spiral, form channels (Fig. 4). The outside surface of the spiral is hydrophobic, consisting of uncharged side radicals. The hydrophilic inner surface, forming the channels, is made of main-chain atoms, mainly O and N.

The ornithine tails with the N atoms at the ends are turned inside the channel (Figs. 4*a* and 5). The diameter of the channel can change on account of conformational changes of these tails. The channel is filled by the water molecules, as is clearly seen in Fig. 5. The geometrical characteristics of the spirals are: external diameter about 30–35 Å, internal diameter (without ornithine side chains) about 12 Å. This latter value, the channel diameter, is limited by the terminal N atoms of the ornithine residues, as was already mentioned above and may change at the expense of change of the conformation of the side chain of these residues from about 3.3 to 6.2 Å. Thus, the ions of rather large size may pass through the channel made of gramicidin S molecules inside of membranes, if such channels could exist. Ion transport across a membrane may take place under the action of a transmembrane potential. The very compact packing of the gramicidin

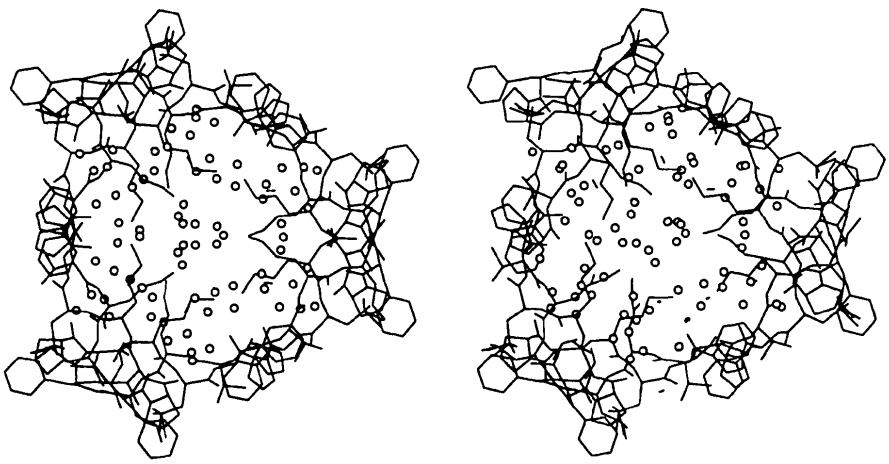


Fig. 5. The arrangement of water molecules in the interior of the gramicidin S channel. Viewed down the 3_1 axis.

Table 5. *Plane equations*

Phe4

6.513 (0.182) x − 2.765 (0.181) y + 20.788 (0.043) z = 2.716 (0.073)

CG	CD1	CE2	CB	CD2	CE1	CZ
0.002	0.006	0.004	−0.012	0.011	0.010	−0.022
(0.009)	(0.009)	(0.018)	(0.008)	(0.017)	(0.011)	(0.017)

R.m.s. deviation of fitted atoms = 0.011

Phe9

0.039 (0.102) x − 2.248 (0.110) y + 21.379 (0.023) z = 7.147 (0.051)

CG	CD1	CE2	CB	CD2	CE1	CZ
0.028	0.026	0.003	−0.033	0.008	−0.015	−0.017
(0.006)	(0.007)	(0.009)	(0.005)	(0.008)	(0.008)	(0.010)

R.m.s. deviation of fitted atoms = 0.021

Proline 'planes'

Pro5

−5.951 (0.171) x − 17.723 (0.115) y + 15.364 (0.094) z = 1.327 (0.062)

N	CA	CB	CG	CD
−0.063	0.100	−0.108	0.074	−0.003
(0.006)	(0.007)	(0.010)	(0.011)	(0.008)

R.m.s. deviation of fitted atoms = 0.079

Pro10

10.351 (0.098) x − 2.585 (0.114) y +18.215 (0.052) z = 5.221 (0.051)

N	CA	CB	CG	CD
−0.088	0.203	−0.244	0.184	−0.056
(0.004)	(0.005)	(0.005)	(0.006)	(0.005)

R.m.s. deviation of fitted atoms = 0.171

S channels in the crystal structure should be noted (Fig. 6).

An important feature of the structure investigated is the absence of the Cl^- ions. There should be two Cl^- ions in the structure associated with each molecule, as gramicidin S usually exists in the form of a salt with positive charges at the terminal N atoms of the Orn residues and the compulsory counter-ion presence, for example, Cl^- ions. We have found no experimental evidence for their presence and, indeed, by no re-interpretation of water locations could we explain their existence as ordered anions.

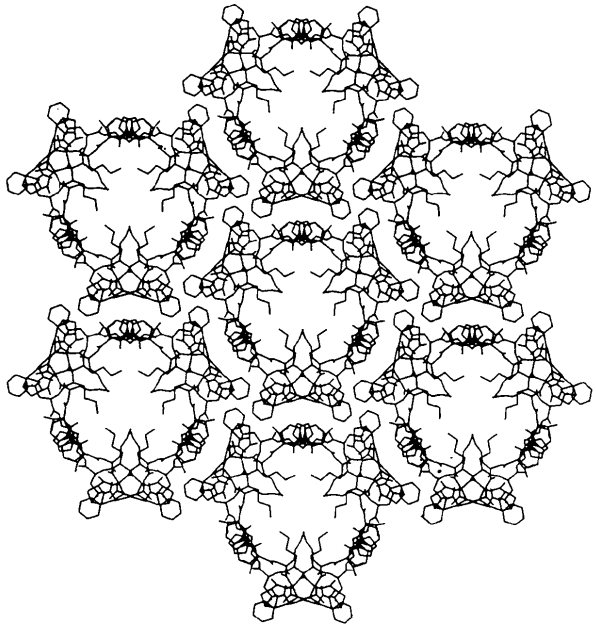


Fig. 6. Packing of the structure viewed along the 3_1 axis.

It should be noted, that the mechanism of the ion transport across the biological membranes effected by gramicidin S has remained unclear until now. In this context, Ovchinnikov & Ivanov (1976) mentioned the proximity of the distance between the NE atoms of the two ornithine residues in the gramicidin S molecule, established by the spectral methods, to the negative charges on the phosphate groups in the phospholipid membranes. The possibility of the creation of the gramicidin S channels was noted by some authors (Sholtz, Solovjeva & Kotelnikova, 1975; Sholtz, 1979; Reznik, 1982), who studied the action of the antibiotic on mitochondrial membranes, though no assumptions were made about the structure of these channels. According to the data of these authors the channels allowed some cations to pass, for example K^+ and Na^+, but the penetrating anions, such as phosphate and acetate, intensified the gramicidin S action on the membranes. There is no noticeable increase in penetration of Cl^- ions in the presence of the antibiotic.

Later it was shown (Heitz, Kaddari, Van Mau, Verducci, Seheno & Lazaro, 1989) that gramicidin S, with some other cyclic peptides, is able to induce the creation of transmembrane ionic channels/pores and the

structure of such pores was proposed based on the aggregation of the molecules.

The authors are thankful to Professor V. T. Ivanov, Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia, for the useful discussion of the results and to Dr Yu. V. Nekrasov, Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia, for help with the data collection.

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