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CRYSTAL STRUCTURE OF PEPTIDE CYCLO-(D-VAL-L-PRO-L-VAL-D-PRO)$_3$

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SUMMARY

The crystal and molecular structure of the rubidium/picrate complex of the peptide cyclo-(D-val-L-pro-L-val-D-pro)$_3$, called prolinomycin, has been determined by X-ray crystallography and found to be similar to the well known ion-carrier valinomycin. Prolinomycin crystallizes in the triclinic system with two prolinomycin molecules and two each rubidium cations and picrate anions in the unit cell. There are also ordered toluene and chloroform molecules, which are the solvents of crystallization, in the unit cell. The conformation of the two crystallographically independent prolinomycin molecules in the unit cell are very similar. Potential energy calculations show that the cation is bound more strongly in prolinomycin compared to valinomycin. This was also observed in solution (7).

INTRODUCTION

The cyclic dodecadepsipeptide valinomycin has been the subject of many investigations because of its abilities to complex and solubilize monovalent cations into nonaqueous solvents of low polarity. Monovalent cations so complexed retain their +1 charge and can be transported through nonaqueous barriers such as lipid bilayer membranes provided that some mechanism is present to compensate for the movement of charge. The conformation of complexed valinomycin was determined independently by spectroscopic methods (1) and by X-ray crystallography (2). From these studies it was suggested that the geometry of the ester linkages in valinomycin would be little changed by replacement by peptide linkages, and that the solubility in nonaqueous solvents would be preserved as long as peptide hydrogen atoms were not exposed to the exterior. From such considerations Gisin and Merrifield (3) synthesized the proline analogue of valinomycin, Cyclo-(D-val-L-pro-L-val-D-pro)$_3$, also called peptide PV

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or prolinomycin, in which each ester linkage is replaced by the hydrogenless peptide linkage of the same chirality furnished by proline. A series of studies by Gisin, Tosteson and their co-workers (4-7) showed that prolinomycin would complex monovalent cations even more strongly than would valinomycin but would transport cations through lipid bilayer membranes more slowly.

This present work announces the successful determination by X-ray crystallography of the complex of prolinomycin with rubidium picrate.

METHODS AND MATERIALS

Rubidium picrate was dissolved with an equimolar amount of prolinomycin in methylene chloride and evaporated to dryness. The complex was recrystallized from a 1:1 mixture of chloroform:toluene. A single crystal was sealed in a thin-walled capillary tube, space group and unit cell parameters as determined by the diffractometer were as follows:

$a = 16.139(11)$ Å	$b = 16.312(9)$ Å	$c = 18.270(11)$ Å
$\alpha = 106.70(3)°$	$\beta = 86.95(3)°$	$\gamma = 106.70(3)°$

The space group is P1 or $P\bar{1}$ with two molecules in the unit cell. Reflections with h+k+l odd were weak but present. Complete three-dimensional data to 1.0 Å resolution consisting of 7240 unique reflections were collected on the Hilger-Watts four circle diffractometer using filtered copper radiation in the laboratory of Dr. Herman Watson, Department of Biochemistry, University of Bristol, Bristol, England.

Structure determination and refinement

It was assumed initially that the space group was P1, although other space groups were considered because of the unusual cell lengths and angles and the two molecules per unit cell. Inspection of the Patterson synthesis revealed only one possible Rb-Rb vector, at 1/2,1/2,1/2. The statistical distribution of the intensities of the reflections was of no help in making a decision about the space group, nor had it been for the structure of valinomycin. A heavy atom synthesis calculated on the basis of the two Rb atoms was uninterpretable. Application of the direct methods as P1 or $P\bar{1}$ were unsuccessful. The structure was finally solved by our use of rotation function (8). We have had considerable success applying the rotation-translation functions to cycloheptaamylose complexes which again could not be solved by other methods (9). The orientation of the three-fold axes of the two crystallographically independent molecules were determined using the rotation function. The backbone atoms of the two molecules were located from the heavy atom phased electron density map averaged about the molecular three-fold axes. The side chain atoms and the solvent molecules were located from the difference Fourier maps. The structure is being refined as $P\bar{1}$ by block-diagonal least squares using Dr. Stewart's X-ray 76 crystallographic programs. The R-value with 5949 observed reflections and 116 non-hydrogen atoms is 0.164.

RESULTS

Each prolinomycin-Rb^+ complex, which has a $\bar{3}$ molecular symmetry, sits on a center of symmetry of the crystal so that there are two independent half com-

plexes in the crystallographic asymmetric unit. There are two picrate anions in the unit cell sitting at a general position balancing the +1 charge on each prolinomycin-Rb^+ complex. In addition there are three toluene and two chloroform molecules, which are the solvents of crystallization, in the unit cell; the chloroform and two toluenes sit at general positions and the third toluene in the unit cell sits on a center of symmetry. The unit cell therefore contains three toluene molecules and two each: prolinomycin, Rb, picrate and chloroform. The solvent molecules seem to be well ordered although they have a much higher thermal vibration compared to the prolinomycin molecules.

The conformation of the two crystallographically independent molecules, Molecules I and II, are essentially the same. The conformation found for prolinomycin is in general the same as that found for valinomycin with the β-folding, the 1-4 hydrogen bonding, and the valyl carbonyl residues turned inward to coordinate with the metal cation. The similarity between prolinomycin and valinomycin conformations was in fact predicted by Davis, Gisin and Tosteson (6) from their NMR studies. However, prolinomycin, in contrast to valinomycin, has an exact center of symmetry. This requires an internal racemization of the chirality of the amino acid residues resulting necessarily in no optical activity, and $[\alpha]^{29}D - 2.2 \pm 1.2°$ was observed by Gisin and Merrifield (3). Stereo diagrams viewing down the 3-fold axis of Molecules I and II of prolinomycin and the Rb aurichloride complex of valinomycin (2) are shown in Figure 1.

Although the backbone conformation angles, hydrogen bond lengths and angles, and coordination distances of prolinomycin are similar to what was observed with valinomycin complexes, the $\bar{3}$ symmetry of the molecule is in general maintained better in prolinomycin.

The isopropyl groups of the valyl residues are in staggered conformation with the angle χ around ± 60° and 180°. The 3-fold symmetry of the molecules appears to be closely followed by these side chain atoms too.

The picrate anion is on one side of the prolinomycin molecule and does not have any direct interaction with the cation. It seems to be stabilized by the

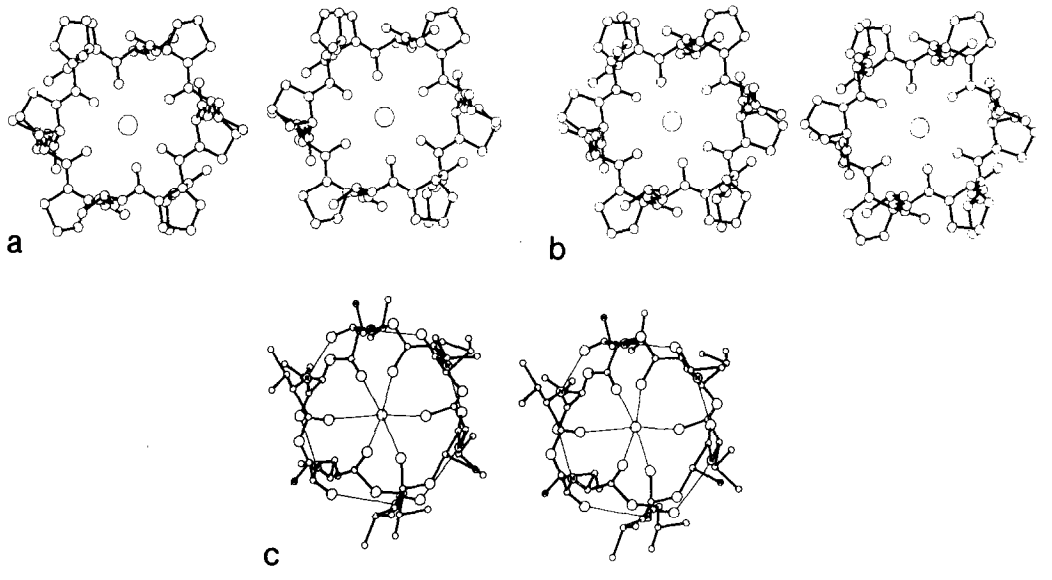

Fig. 1. Stereo view down the molecular three-fold axes of (a) Molecule I of prolinomycin, (b) Molecule II of prolinomycin and (c) valinomycin.

hydrophobic interaction with the proline rings.

It was observed that prolinomycin has a much higher affinity for alkali metal ions and a much poorer efficiency in transporting the cations across lipid-bilayer membranes compared to valinomysin (7). We sought an explanation for this observation from our knowledge of the crystal structure coupled with potential energy calculations (10-12) with the Rb complex of prolinomycin. Similar calculations with other ion-transporting antibiotics have been very effective in understanding the mechanism of complexation (12). We carried out the potential energy calculations (a) between the Rb ion in Molecule I and its encircling prolinomycin molecule, (b) between the Rb ion in Molecule II and its encircling prolinomycin molecule, and (c) between the Rb ion and the encircling valinomycin molecule. The minimum energy position of the cation in prolinomycin turned out to be the same as the one observed in the crystal structure, and the minimum energy values for Molecules I and II are -191 kcal/mole and -193 kcal/mole, respectively. The corresponding value for the Rb complex of valinomycin is -170 kcal/mole. Molecules I and II of prolinomycin are crystallographically

independent determinations and their conformations are similar though not identical; there are several significant differences in their bond lengths and angles. However, the potential energies of the two molecules differ only by 2 kcal/mole and this lends support to the correctness of the constants used in these calculations. If we therefore treat this 2 kcal/mole to be the standard deviation, the difference of 22 kcal/mole observed between prolinomycin and valinomycin should indeed be significant and does not reflect any artifact due to the constants used in the potential energy calculations. Thus the cation appears to be bound stronger in prolinomycin over valinomycin, and this seems to be at least part of the explanation for the higher affinity of prolinomycin to cations observed by Tosteson and co-workers in solution (7).

The differences in the affinities of cations by valinomycin and prolinomycin must also come from the differences in the potential energies of the uncomplexed forms and in the mechanism of conversion from complexed to uncomplexed forms. In prolinomycin the restricted freedom of rotation due to prolyl residues may not allow the insertion or withdrawal of the cation as easily as do the hydroxy acid residues of valinomycin. Another possibility is that prolinomycin is unable to achieve as compact a conformation in the uncomplexed form as does valinomycin, again due to the restricted freedom resulting from the prolyl residues. Indeed it is interesting to compare the complexed and uncomplexed forms of several cyclic host molecules; examples are valinomycin (2, 12,13), beauvericin (14,15), and cyclohexa and cycloheptа amyloses (9,16). In each case the complex form is circular and the uncomplexed form is flattened into an oval. The crystal structure of uncomplexed prolinomycin should throw more light on the structure-function relationship of valinomycin and its peptide analogue.

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