Molecular conformation and ion transport of cyclic and linear ionophores

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X-ray crystal structure determinations and energy-minimization techniques provide conformational data on the complexed and uncomplexed forms of ion transport antibiotics of the shuttle and channel types. In the solid state, hexadecaisoleucinomycin (HEXIL), an analogue of valinomycin, is observed as an asymmetric macrocycle stabilized by eight intramolecular (4→1) hydrogen bonds. The structure obtained from energy-minimization procedures exhibits a greater variation in φ and ψ angles of chemically equivalent residues than does the crystallographically observed structure. The structure has eight carbonyl groups directed toward its interior and is capable of providing flexible coordination to a positively charged ion or molecule. These structural findings are consistent with the observed capacity of HEXIL to complex cesium ions, tetramethyl ammonium ions and acetylcholine. Gramicidin A is a pentadecapeptide that functions as a transmembrane channel for transporting monovalent cations. Uncomplexed gramicidin A crystallizes as a left-handed, antiparallel, double-stranded, helical dimer with 5.6 amino acid residues per turn. The helix has an overall length of 31 Å and an average inner channel diameter of 4.8 Å. The channel of this crystalline form does not contain ions or solvent molecules. Transporting ions through this channel could be achieved only by some expansion of the channel opening that would involve breaking and reforming hydrogen bonds that stabilize the double-stranded helix.

Keywords: ion transport, ionophores, membrane channels, gramicidin, isoleucinomycin, valinomycin

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This paper was presented at the Symposium on Molecular Recognition, held in Sopron, Hungary, 24–27 August 1988.

INTRODUCTION

Ionophores are synthetic compounds or naturally occurring antibiotics that induce ion transport across living and model membranes. They include both polypeptides and polyethers and differ widely in their ion specificity and apparent mode of action. Most ionophores either act as shuttle carriers or form hydrophilic channels through the lipid membrane.[1] The shuttle carriers consist of cyclic or linear compounds; contain oxygen atoms in the form of either linkages, hydroxy groups or carbonyl groups; and are capable of providing a coordination sphere for a metal ion. Following the coordination of an ion, minor conformational changes produce a species with a hydrophobic exterior that is capable of traversing the membrane. Release of the ion at the other side of the membrane completes the process.[2] Channel-forming antibiotics, in contrast, are believed to be fixed in the lipid bilayer but may require conformational flexing of the hydrophilic channel interior in order to facilitate ion-specific transport.

Ionophores have been the subject of numerous crystallographic structure determinations.[3] In our laboratories we have compared both complexed and uncomplexed forms in order to identify the conformationally flexible regions of ionophores, to determine the molecular dynamics of ion capture and release and to gain insight into the structural basis for cation selectivity.[4,5] Representative examples of our efforts to model the complexing and transport process of shuttle carriers and channel forming ionophores are provided by recent studies of hexadecaisoleucinomycin (HEXIL) and gramicidin A, respectively.

SHUTTLE CARRIERS

Valinomycin, cyclo[-(L-Val-D-Hyi-D-Val-L-Lac)$_3$-] (Hyi

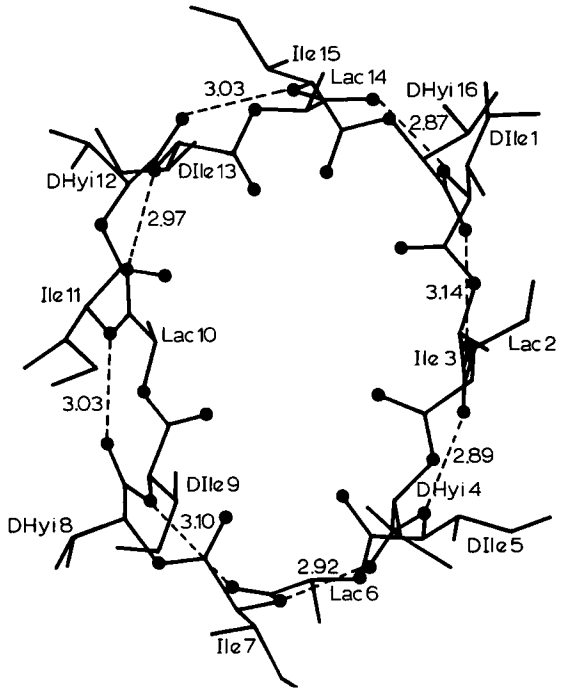

Figure 1. Conformation of hexadecaisoleucinomycin (HEXIL) observed in the solid state

and Lac are the hydroxyacids hydroxyisovalerate and lactate, respectively), is an ionophore that is highly selective for potassium ions.[6] On the basis of a comparison of the crystallographically observed structures of complexed[7] and uncomplexed[8-11] forms of valinomycin, a mechanism for the complexing process has been proposed. Isoleucinomycin, an analogue of valinomycin in which the valine residues have been replaced with isoleucines, was found to have greater capacity for extracting alkali metal ions into organic phases while retaining K^+ selectivity.[12] X-ray analysis revealed the conformation of isoleucinomycin to be intermediate between that observed for the complexed and uncomplexed forms of valinomycin.[13] This conformational difference may be a contributing factor to the enhanced capacity for ion extraction.

In order to investigate the influence of macro-ring size and the effect of the individual peptide residues on ion capture and transport, the octadepsipeptide and hexadecadepsipeptide analogues of valinomycin and isoleucinomycin have been synthesized and studied biochemically and crystallographically. The crystallographic conformation of uncomplexed octaisoleucinomycin, cyclo[-(D-Ile-Lac-Ile-D-Hyi)$_2$-], consists of two chain reversals linked together to produce a rectangular shaped molecule possessing pseudorotational symmetry. The octadepsipeptides exhibit only weak association with Na^+ ions.[12] Hexadecaisoleucinomycin, cyclo [-(D-Ile-Lac-Ile-D-Hyi)$_4$-] (HEXIL), complexes and transports larger cations, such as Cs^+ and tetramethyl ammonium ions, more efficiently than hexadecavalinomycin.[12]

The crystallographically observed conformation of the HEXIL molecule is an asymmetric bracelet that is stabilized by eight intramolecular (4→1) hydrogen bonds (Figure 1). The nitrogen atoms of the D-Ile and Ile residues are hydrogen bond donors to the carbonyl oxygen atoms of Lac and D-Hyi, respectively. The eight carbonyl oxygens of the D-Ile and L-Ile residues are directed toward the inside of the bracelet. These carbonyl oxygens form the boundaries of an elliptical cavity whose dimensions are approximately 7.5 × 5.5 × 4.0 Å.

Using molecular mechanics calculations, Pletnev explored the conformational space of the HEXIL molecule and found several low-energy forms.[14] Initial calculations using the backbone structure and β carbons produced a dozen low-energy forms having eight hydrogen bonds of the 4→1 type and a twofold (or fourfold) symmetry axis. These hydrogen-bonded macro-ring structures were held fixed as the side chains were added and their conformations refined. The molecular conformation corresponding to the lowest energy model is one in which the molecule has a rectangular shape with twofold symmetry and all eight of the D-Ile and L-Ile carbonyl oxygens directed toward the outside of the ring. The next lowest energy conformation (+5.5 kcal/mol) closely approximates the crystallographically observed structure. The φ, ψ and ω angles of this model are compared with those of the crystallographically determined structure in Table 1. All other model structures had calculated energies of more than 13 kcal/mol higher than the minimum.

It is noteworthy that the calculated structures are biased by symmetry constraints that are not present in the crystal structure. The asymmetric shape seen in the crystal is probably stabilized by weak solvent interactions. Although molecules in solution will not be con-

Table 1. Torsion angles in the crystallographically observed structure of HEXIL and in the calculated model that most nearly approximates the observed structure

	D-Ile			L-Lac			L-Ile			D-Hyi		
	φ	ψ	ω	φ	ψ	ω	φ	ψ	ω	φ	ψ	ω
X-ray	67	−131	−174	−112	20	176	−56	138	−179	102	−10	−178
	62	−138	179	−91	6	174	−72	132	177	83	8	177
	64	−132	178	−104	21	177	−53	138	170	89	−1	175
	68	−136	−175	−96	1	179	−64	130	178	91	12	174
Calculated	49	−127	172	−88	51	177	−64	99	179	62	35	177
	59	−108	172	−63	−38	−176	−47	136	177	83	−50	−179

strained to the crystallographically observed conformations, neither will they be constrained to a symmetric shape. It is likely that in solution there will be a flexing of the macro ring in which the crystallographically observed structure constitutes one local minimum-energy conformation.

The X-ray crystal structure determinations of the three isoleucinomycin analogues, cyclo[-(D-Ile-Lac-Ile-D-Hyi)$_n$-] (where n = 2,3,4), provide nine independent observations of the conformation of this tetradepsipeptide sequence. A Ramachandran plot illustrates the conformational flexibility in the φ, ψ values of this sequence (Color Plate 1). The values of φ and ψ that result from the energy-minimized conformation of HEXIL that most nearly approximates the observed structure are also shown in Color Plate 1.

Examination of the φ–ψ plot reveals that the L-Ile and Lac residues have significantly different conformations in the octadepsipeptide than the same residues in the 12- and 16-membered rings. The φ, ψ values for this sequence show greatest internal consistency in the HEXIL structure despite the absence of symmetry constraints in the macrocycle. The φ, ψ values are more uniform in the observed structure of the HEXIL than in the energy-minimized structure.

An examination of the peptide bonds (ω) suggests a relaxation of conformational strain as the ring size is expanded. A substantial distortion exists in the octadepsipeptide where the average absolute value of the eight peptide bonds is 169°. There is some distortion in the dodecadepsipeptide ($<|\omega|>$ = 173°) and little distortion in the hexadecadepsipeptide ($<|\omega|>$ = 176°).

HEXIL contains four Type II (D–L) and four Type II′ (L–D) β-turns. A comparison of the average φ and ψ values for Type II β-turns (−61,134/91,2) with ideal values (−60,120/80,0)[15] shows a consistent increase in the variation of the ψ values of the L-Ile residues and φ values of the D-Hyi residues. Comparison of the average φ, ψ values for the Type II′ turns (65,−134/−100,12) with the ideal values (60,−120/−80,0) shows a similar variation in the ψ values of D-Ile residues and significantly increased variation in the averages for the φ values of the D-Ile residues and in both the φ and ψ values of the L-Lac residues. These differences can be attributed to the presence of oxygen linkages in place of the NH groups normally present in peptides upon which the ideal forms are predicated.

Comparison of the side-chain conformations observed in HEXIL with those previously reported for its octa- and dodecadepsipeptide analogues reveals some interesting patterns. Eight of nine D-Hyi residues, including all of those in HEXIL, have similar conformations in which the $X^{1,1}$ and $X^{1,2}$ torsion angles have approximate values of −60° and +60°, respectively. In proteins the most commonly observed conformation for valine residues is one in which $X^{1,1}$ = −60° and $X^{1,2}$ = 180°.[16] In this conformation the hydrogen atoms on the C_α and C_β carbon are *trans* to one another. Substituting an oxygen in D-Hyi for the NH group in valine alters the torsional relationship across the C_α–C_β bond so that the conformation in which both methyl groups of the D-Hyi are *gauche* (or clinal) to the C–O bond is preferred.

The value of $X^{1,1}$ most commonly observed for L-Ile residues in protein crystal structure determinations is near −60° ($X^{1,2}$ = 180°).[16] The three L-Ile residues in the dodecadepsipeptide and three of the four L-Ile residues in HEXIL have this conformation. The seven D-Ile residues in these larger macrocycle structures have the most stable conformation in which $X^{1,1}$ and $X^{1,2}$ values are approximately +60° and 180°. The signs of the $X^{1,1}$ torsion angles for D-Ile and L-Ile residues in the octadepsipeptide are at variance with the patterns in the protein data, providing another indication that there is greater intramolecular strain in this structure than in the larger macrocycles.

An examination of electron density difference maps indicated the presence of solvent molecules in the HEXIL cavities. The solvent structure forms channels that extend parallel to the axis through the stacks of bracelets. The density was modeled with eleven full or partial occupancy water molecules. The refined positions of these water molecules make fourteen contacts with the eight carbonyl oxygens that line the cavity and range from 2.98 Å to 3.50 Å, but only three of these distances have lengths short enough for typical hydrogen bonds. There is only one close contact between a solvent molecule and a carbon atom of HEXIL. Interatomic contacts among the water molecules include five contacts that have magnitudes between 2.65 Å and 3.1 Å and may constitute ordered hydrogen bonds. Eleven contacts between solvent molecules are less than 2.55 Å and indicate disorder in solvent association and occupancy. Space-filling models of HEXIL with and without the solvent molecules present in the cavity are illustrated in Color Plates 2a and 2b. A stereo diagram of the HEXIL molecule with the encapsulated water is shown in Figure 2.

In summary, the X-ray crystal structure of hexadecaisoleucinomycin reveals an asymmetric macrocycle stabilized by eight intramolecular (4→1) hydrogen bonds. The repeating tetradepsipeptide sequence exhibits less evidence of strain in the cyclic tetramer reported here than in the previously studied cyclic dimer and trimer. The structure obtained from energy minimization exhibits a greater variation in the φ and ψ angles of chemically equivalent residues than does the crystallographically observed structure. Also note that although the observed φ, ψ and ω angles of the tetradepsipeptide sequence are nearly identical, suggesting a four-

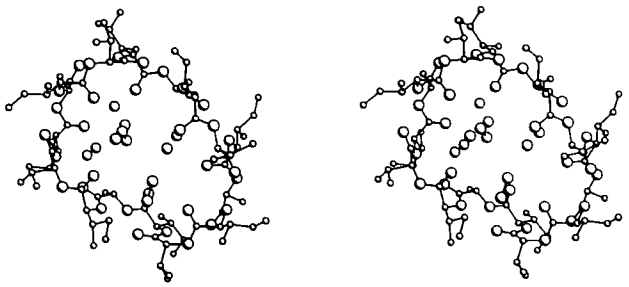

Figure 2. Stereo diagrams of HEXIL with the solvent present in the channel

fold symmetry axis, HEXIL does not appear to possess any obvious molecular symmetry. The structure presents a hydrophobic exterior and has eight carbonyl groups directed toward its interior that are capable of providing flexible coordination to a positively charged ion or molecule. Minor, energetically feasible adjustments could increase the size of the cavity. These structural findings are consistent with the observed capacity of HEXIL to complex cesium ions, tetramethyl ammonium ions and acetylcholine.

ION CHANNELS

The channels that selectively transport ions across biological membranes are constructed from proteins or aggregates of proteins. These channels may represent a pore that is surrounded by adjacent α-helical segments of protein that span the membrane. The selectivity and gating characteristics of the channel may be regulated by conformational changes that vary the diameter of the channel and adjust the positions of the amino acid side chains that line the channel interior. The transmembrane channels formed by antibiotic molecules are known to be much simpler in construction. Gramicidin A is a linear, alternating D–L pentadecapeptide isolated from *Bacillus brevis*[17] that exhibits antibiotic activity primarily against gram-positive bacteria.[18] In membranes, gramicidin A forms channels that are specific for monovalent cations.[19] Numerous models have been proposed for the structure of gramicidin A in various solvents, lipid bilayers and the solid state. These models have been based on NMR, CD, IR and Raman studies. Biochemical studies demonstrate that the active form of the molecule is a dimer.[20] Proposed models include a single-stranded, head-to-head $\beta^{6.3}$ dimer[21] and a double-stranded antiparallel $\beta^{5.6}$ dimer.[20] The peptide strands are hydrogen bonded in a β sheet pattern that is wrapped into a coil. Because of the alternating L- and D-configuration of the polypeptide, all the bulky side chains are on the one face of the β-ribbon. Consequently, the ribbon can be coiled so that the residues are on the outer surface.

The crystal structure of the cesium chloride/gramicidin A complex contains two independent, but structurally similar, molecular dimers. The peptide backbones of each dimer form a left-handed, antiparallel, double-stranded helix with a hydrogen-bonding pattern corresponding to an idealized $\beta^{7.2}$ helix but which has been twisted down to 6.4 residues per turn.[22] Each channel contains two cesium and three chloride ions, in the order Cl-Cs-Cl-Cs-Cl, but the separation between these ions is greater than the sum of their ionic radii. There is some evidence of distortion in the peptide backbone of the channel, with regard to the positioning of carbonyl groups in order to achieve coordination with the cesium ions. Some features of the cesium complex structure, such as solvent association, hydrogen bonding and ion coordination geometry, are not well resolved because the crystals diffracted to only 2.0 Å resolution.

The determination of the structure of the uncomplexed form of gramicidin A at 0.86 Å resolution was based upon intensity data gathered at 120 K using large well-

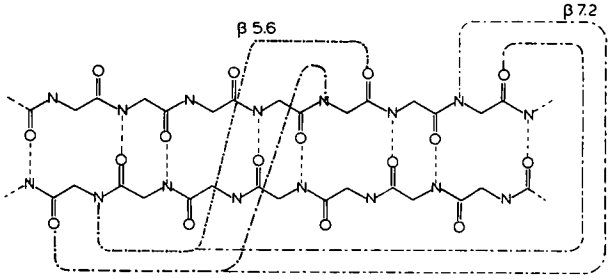

Figure 3. Schematic diagram illustrating the hydrogen-bonding patterns for the uncomplexed $\beta^{5.6}$ (dot-dashed lines) and cesium complexed $\beta^{7.2}$ (dashed lines) molecular models of the gramicidin A channel helices. The uncomplexed form is reported here, and the cesium complex structure is from Ref. 22

formed crystals.[23] The excellent quality of the low-temperature data provides a structure rich in detail. A dimer of the pentadecapeptide forms the same type of double-stranded β-ribbon observed in the complexed form (Color Plate 3). However, in the uncomplexed form the double-strand ribbon is wound more tightly to form a longer channel with a smaller diameter (Color Plate 4). In this case, the left-handed antiparallel double-stranded channel is 31 Å long, has 5.6 amino acids per turn and an approximate diameter of 4.85 Å. The tighter coiling is accomplished by a shift of the hydrogen bonds joining the edges of the double-stranded β-ribbon to an analogous interactive site (Figure 3) two peptide units away.

The averaged φ, ψ torsion angles of the peptide backbone of the uncomplexed channel form are -152 ± 9, 110 ± 22 degrees for the L residues and 101 ± 20, -142 ± 8 degrees for the D residues. These values, compared with -141, 116 and 102, -159 degrees for the idealized $\beta^{5.6}$ helix, indicate the extent to which the pitch of the helix varies from one residue to the next. The idealized helix has a twofold axis normal to the helical axis at the center of the dimer that relates one molecular strand to the other. This diad axis is approximate only in the crystal structure dimer because of the asymmetry in the crystalline environment of these molecules. The average pairwise difference between the φ and ψ torsion angles of residues related by this axis is only 8 degrees. Moreover, the largest discrepancies ($\Delta\psi$ Val^7 = 24 degrees; $\Delta\varphi$ Val^8 = -30 degrees) involve adjacent torsion angles that compensate for one another to help preserve the pseudo twofold symmetry. The root-mean-square displacement between the main chain and β-carbon atoms related by this pseudo twofold axis is 0.55 Å.

The channel is void of solvent molecules but does contain three structurally similar pockets where the diameter exceeds 5.0 Å. These pockets are separated by well-defined constrictions in the helix. A hypothetical potassium ion placed in the center of one of these pockets was found to make four keto oxygen and four peptide nitrogen contacts averaging 2.73 Å, with no oxygen-nitrogen contact less than 2.66 Å. These distances, although reasonable for an eight-coordinate potassium, would be quite unusual if the hydrogen-bonding pattern of the helix were not disrupted. Each of the four oxygen

atoms in the hypothetical coordination sphere is hydrogen bonded pairwise to one of the four peptide nitrogen atoms on the opposite strand of the helix. A potassium ion entering this pocket could form bonds to the four carbonyl oxygen atoms only at the expense of severely weakening or breaking the interstrand hydrogen bonds. While ion transport down an antiparallel $\beta^{5,6}$ helix is not inconceivable and would involve considerable contortion of the channel, it is more likely that this destabilization process might serve as a mechanism to transform the $\beta^{5,6}$ helix into the more efficient ion-conducting single-stranded $\beta^{6,3}$ form.

The potassium complex of valinomycin provides an example of the octahedral coordination frequently observed in which six carbonyl groups are directed toward the potassium ion in the center of the cavity. In the gramicidin A dimer, potential ion binding sites consists of four nitrogen atoms and four oxygen atoms. Although eightfold coordination for potassium is not unusual, it is uncommon for this ion to bind to nitrogen atoms, especially when oxygen donors are available. Additionally, the binding directions of these ligands are not pointed toward the center of these potential binding sites but are directed nearly parallel to the long axis of the helix. Moreover, the coordination geometry of these binding pockets would be expected to be unstable if a potassium ion were present, since it would prefer to bind to the four oxygen atoms at the expense of disrupting the local hydrogen bond pattern connecting the two helical strands. It is conceivable that transitions between the various forms of membrane-bound channel dimers might be facilitated by destabilizing forces such as these.

ACKNOWLEDGEMENT

This investigation was supported in part by Public Health Service grants GM32812 and HL32303.

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