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Aspects of Peptide Folding and Aggregation

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Introduction

Good characterizations of the geometrical parameters in molecular systems are indispensable for the design of new molecules for therapeutic purposes and material science, for molecular modeling, for supramolecular assemblies, and for computational chemistry.[1] The helix is a ubiquitous form that occurs as a major shape of the backbone in proteins, in many peptides, and in many fibrous polymers. The geometric features of helices have been characterized by various physical methods, particularly by X-ray diffraction of single crystals. Over the years, parameters of interest have been compiled using results from those protein crystals that yielded diffraction data of the best quality, that is, a resolution of ∼2.0 Å.[2,3] Due to a lack of atomic resolution, protein structures are generally refined with "restrained" or "annealed" least squares that include model building for unresolved portions and poorly defined solvent molecules.[4,5] Many peptide molecules having up to 20 residues have been found to produce very good crystals that scatter X-rays to resolutions of 0.9 Å or better, even those with several independent molecules in the asymmetrtic unit of the unit cell (that is, molecules not related by crystallographic symmetry). The peptide crystals generally have limited hydration. The molecules are quite rigid with low thermal motion and minimal disorder and can be refined by least-squares procedures, without any restraints, to crystallographic *R* values of 5−10%. The special class of helical peptides is particularly suited for the major focus of this Account. Fortuitously, the α-aminoisobutyric acid residue (Aib) that has two methyl groups on the C^{α} atom (also called dimethylglycine and methylalanine), found in peptides occurring in microbial sources such as fungi and spores, is a very strong helix inducer.[6−10] Aib-containing peptides, such as the antibiotics alamethicin and zervamicin obtained from soil samples, and up to several hundred synthetic peptides prepared in the laboratory, for example, the laboratory of Professor P. Balaram at the Indian Institute of Science, have provided crystals for rather precise structure analyses. The topics that have been addressed are multiple conformations, the folding of the helix, helix transitions from one type to another, distortions in the helix by insertion of known helix breakers, unfolding by solvent insertion, hydrogen bonding and its absence where expected, mimetic hydrogen bonding, aggregation to form ion channels, and an ion transport mechanism.

Isabella L. Karle was born in Detroit, MI, in 1921. She received her B.S. degree in chemistry in 1941 and Ph.D. degree in 1944 from the University of Michigan with Professor L. O. Brockway as her thesis advisor in the field of electron diffraction of gases. She synthesized plutonium chloride at the Manhattan Project, University of Chicago, 1944. During 1945−1946, she was an Instructor in Chemistry at the University of Michigan. She and her husband, Jerome Karle, joined the Naval Research Laboratory in 1946, where both continue to be active in research concerning the structure of matter. Since the mid 1950s she has specialized in X-ray crystallography, first in procedures for solving crystal structures, later in applications to natural products and peptides.

Multiple Conformations

In 1963 the crystal structure of cyclic hexagylcyl,[11] synthesized by Ballard et al.,[12] was determined, in which the cyclic hexapeptide assumed four distinctly different conformations in each unit cell, with an occurrence of 4:2:1:1 for *a*, *b*, *c*, and *d*, Figure 1. Two hydrogen bonds, spanning chain reversals, were present in the most prevalent conformer *a*, providing the first experimentally determined values for the torsional angles and hydrogen bond lengths in β-turns. Soon afterward, a different type of hydrogen bond at a chain reversal was found in the structure of ferrichrome A.[13] In 1968, Venkatachalam published stereochemical criteria for conformations for systems of three linked peptide units in which he found the two possibilities for chain reversal that have 4 → 1 hydrogen bonding.[14]

Multiple conformations have since been encountered in many peptide crystals, attesting to the flexibility of peptides and to the similarity of the potential energy values for the conformers. The conformers may occur side-by-side in the same crystal or in separate crystals with entirely different symmetry elements. An example of multiple conformations for cyclic peptides is valinomycin,[15,16] for linear peptides there are the many forms of enkephalin (see Figure 2a),[17,18] and for helical peptides an example is Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe that alternates between folding into an α-helix in one molecule and having extended backbone/3_{10}-helical/α-helical segments in succession in a neighboring molecule.[19] In all cases, the multiple conformers fall within the allowable regions computed for ϕ and ψ torsional angles ($\phi \equiv C'_{i-1}-N_i-C_i^{\alpha}-C'_i$ and $\psi \equiv N_i-C_i^{\alpha}-C'_i-N_{i+1}$) in Ramachandran conformational maps.[20]

The Robust Helix

As already indicated in the Introduction, the Aib residue is an efficient helix former. Among the very many peptides studied, up to 20 residues, which contain either many Aib residues in the sequence or only one Aib residue, all form 3_{10}-helices, mixed 3_{10}/α-helices, or α-helices, with only two exceptions.[21,22] Helices containing Aib residues are ideal to a considerable extent, with intrahelical hydrogen bonds usually having N···O distances of 2.8−3.1 Å.

An example of a nearly ideal α-helix formed by a 15-residue apolar peptide with the sequence Boc-Val-Ala-

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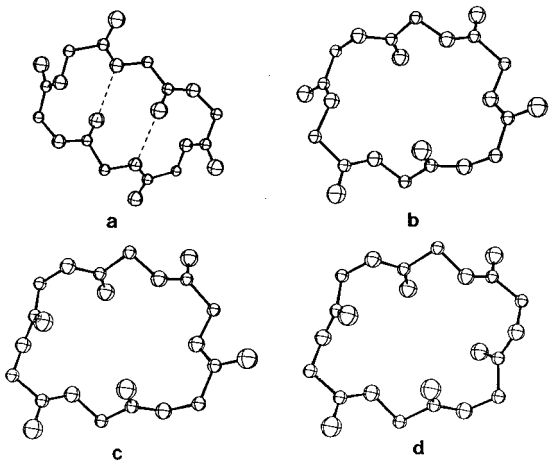

FIGURE 1. Four conformers of cyclic hexaglycyl crystallized side-by-side in the same unit cell.[11]

Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe[23] is shown in Figure 3a, and a nearly ideal 3_{10}-helix is formed by the 9-residue peptide Boc-D-Phe-(Aib)$_4$-Gly-L-Leu-(Aib)$_2$-OMe[24] despite the presence of a D-residue at the N-terminus, Figure 3b. The structures of many 3_{10}-helices have been published for the homopeptides X-(Aib)$_n$-Y.[25,26] Generally, the longer sequences with few Aib residues favor α-helix formation, whereas 3_{10}-helix formation is favored by shorter sequences and a larger number of Aib residues,[9] although there appear to be many minor factors that may also affect the type of helix formed.[10] The transition between 3_{10}- and α-helix types is often readily achieved, facilitated by changing the length of the peptide by a single residue, changing the end group, or changing the crystallizing medium. Other $C^{\alpha,\alpha}$-disubstituted glycine residues, such as Dpg (dipropylglycine) or Dbg (dibutylglycine), can readily replace Aib in heteropeptides and retain the helical backbone[27] as shown in Figure 3c for Ac-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe.

The robust helices described above were formed by sequences of α-amino acids with L-chirality, and containing at least one Aib or other $C^{\alpha,\alpha}$-disubstituted glycines. Effects on helix-forming propensity, helix distortions, helix reversals, helix bending, and helix fraying have been investigated structurally by insertions of possible helix breakers into the backbone, alterations of chirality, removal of hydrogen-bonding potential, and solvation of the backbone. Some of those results are presented here.

Insertions. The incorporation of amino acids with contradictory conformational characteristics, such as the introduction of a central helix-destabilizing Gly-Gly segment between two helix-promoting Aib residues in the Boc-Leu-Aib-Val-Gly-Gly-Leu-Aib-Val-OMe sequence,[28] has not disturbed the formation of a 3_{10}-type helix, Figure 4a. Helix retention has also been shown for the naturally occurring tricogen A IV and a synthetic antibiotic analogue,[29,30] each containing a central Gly-Gly segment between Aib segments.

Helix reversal at the penultimate Aib residue is a feature present in the peptide shown in Figure 4a. Often, 6 → 1 type hydrogen bonds are associated with helix reversals near the C-terminals of peptides and proteins, caused by achiral residues in the penultimate position.[31−34] In this particular example, the potential 6 → 1 hydrogen bond has an insertion of the OH group of a methanol molecule. Although the helix reversal and the accompanying solvation are not directly associated with the Gly-Gly insert, they are also present in the glycine-rich example shown in Figure 4b, where the sequence is Boc-Gly-Dpg-Gly-Gly-Dpg-Gly-NHMe.[35] All the residues are achiral. The hexapeptide adopts a novel, hydrated multiple turn structure, consisting of an initial β-turn, followed by one turn of a 3_{10}-helix, then a helix reversal at Dpg(5), and a hydrated 6 → 1 hydrogen bond. Clearly, the conformation is no longer a simple helix.

The peptide Boc-(Val)$_2$-Aib-Pro-(Val)$_3$-OMe was synthesized to investigate the effect of introducing a strong β-turn-promoting guest segment into an oligopeptide sequence with a tendency to form extended structures. Nevertheless, in a crystal grown from a methanol solution, the peptide assumed the form of a somewhat distorted right-handed 3_{10}-helix, Figure 5.[36] The introduction of a single Aib residue was sufficient to override the otherwise unfavorable sequence for helix formation. In the absence of any Aib residue in a similar sequence, Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe, an almost ideal β-hairpin molecular structure assembling into an extended β-sheet instead of a helix has been obtained.[37]

The introduction of $(-CH_2-)_n$ groups into a backbone in a central position was accomplished by the use of the β-Ala-γ-Abu segment (β-Ala ≡ β-alanine and γ-Abu ≡ γ-aminobutyric acid) that occupies the same length as a Gly-Gly-Gly segment. This kind of insertion replaces one scissile peptide bond by a proteolytically stable C−C bond. Further, it has been shown in crystals that the helical motif of the backbone has been retained, albeit with some minor bulges. The conformation of Boc-Leu-Aib-Val-β-Ala-γ-Abu-Leu-Aib-Val-Ala-Leu-Aib-OMe[38] is shown in Figure 6 with several modified types of 4 → 1 and 5 → 1 hydrogen bonds. The β-Ala-γ-Abu segment was readily accommodated into the helical structure by adopting gauche conformations about specific C−C bonds.

In an effort to form U-shaped two-helix bundles, an ϵ-aminocaproic acid (Acp) linker was inserted between two seven-residue helical segments (helix−linker−helix). Two assemblies were synthesized in which the chiralities in the two helical segments were L,L in the first case and L,D in the second case. The two helices in both the L,L and L,D analogues are displaced laterally by the linker, but in neither case has the linker folded the molecule into the desired U-conformation.[39] The L,D analogue, shown in Figure 7, has a right-handed upper helix with $-\phi$, $-\psi$ torsional angles and a left-handed lower helix with $+\phi$, $+\psi$ torsional angles. The linker also assumes a helical form.

Mixed L- and D-Residues. The achiral Aib residue, with one or several present in helical heteropeptides where the remaining residues have the L-hand, assumes the torsional angles of the L-hand. Presumably, the Aib residues would assume the D-hand in helical peptides if the remaining residues have the D-hand. A single D-residue substituted

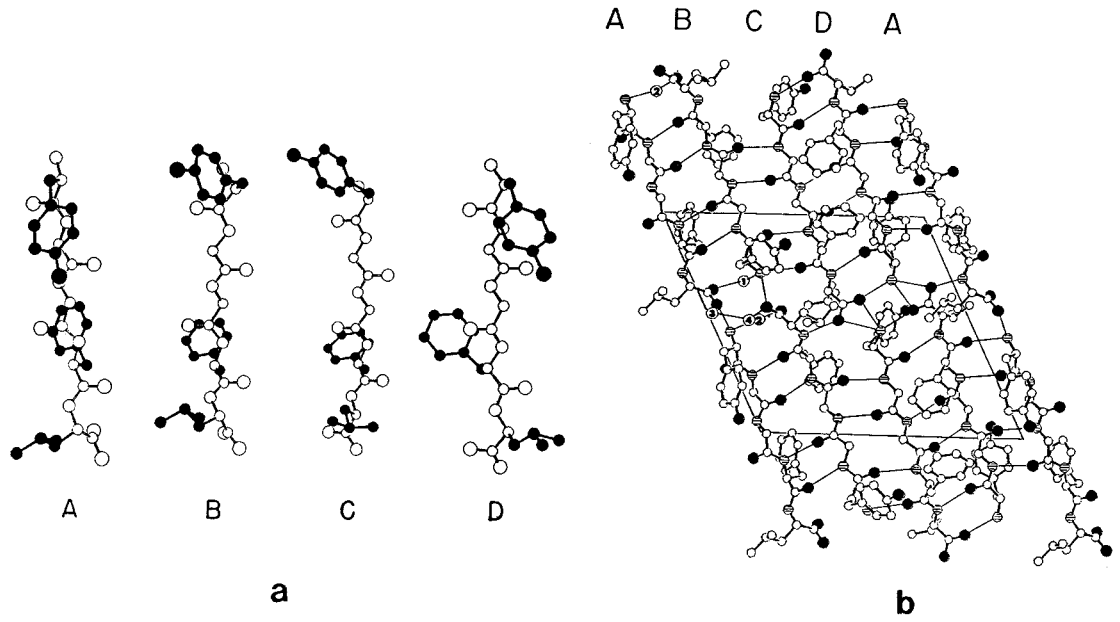

FIGURE 2. (a) Four conformers of Leu-enkephalin in the extended form coexisting in the same cell. (b) Assembly of the four conformers into a β-sheet.[17]

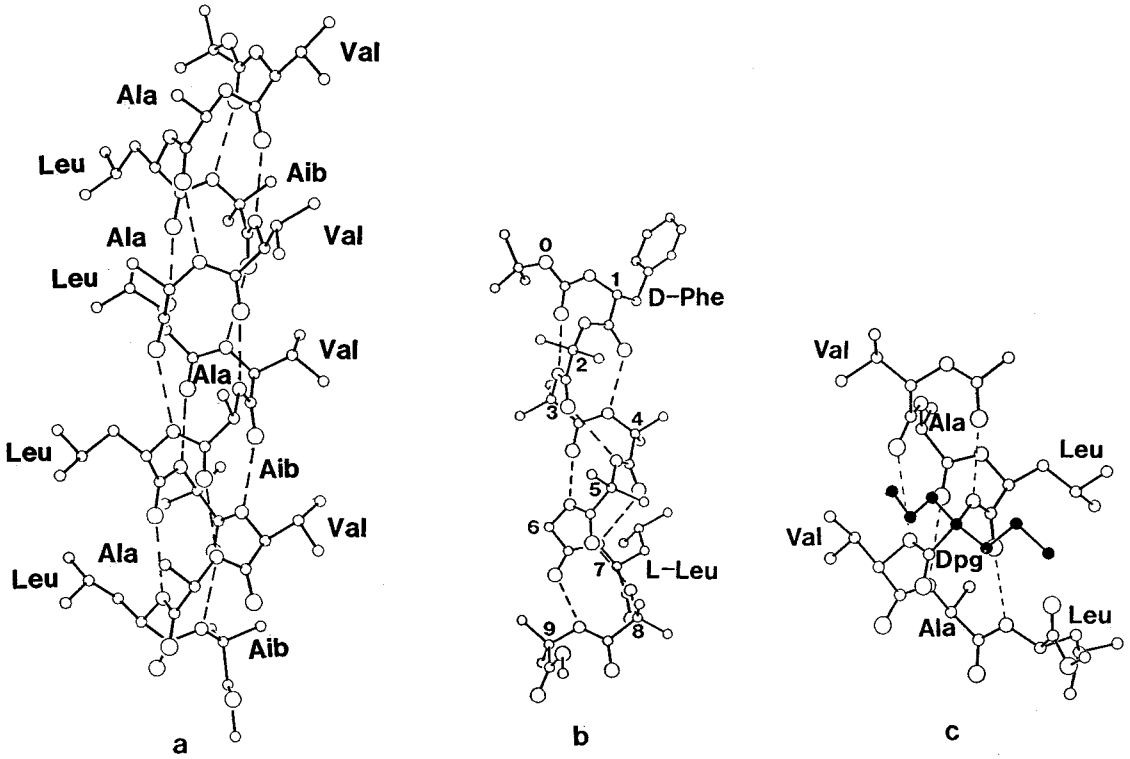

FIGURE 3. (a) A nearly ideal α-helix in a 15-residue peptide containing three Aib residues.[23] (b) A nearly ideal 3_{10}-helix in the nonapeptide Boc-D-Phe-$(Aib)_4$-Gly-L-Leu-$(Aib)_2$-OMe.[24] (c) α-Helix formed by Ac-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe, where Dpg is the helix promoter.[40] (Dpg is darkened.)

for an L-residue in helices of heteropeptides has been observed to assume values for the torsional angles as if it were an L-residue, that is, $-\phi$ and $-\psi$. One example has already been shown in Figure 3b, where the chiral substitution is at the beginning of the sequence.[24] Another example is Boc-Leu-Dpg-Val-D-Ala-Gly-Leu-Dpg-Val-OMe,[40] where the D-Ala-Gly segment is centrally located. The 3_{10}-type helix is right-handed, characteristic for an ordinary helix with all L-residues, with the D-Ala[4] having torsional angle values, $\phi = -57°$ and $\psi = -25°$, comparable to those of residues with an L-hand.

A class of peptides with helix-forming propensities, composed of a block of L-residues followed by a block of D-residues, or vice versa, has been investigated for conformational characteristics. The L-helix−linker−D-helix has been described above and shown in Figure 7. For the same sequence without the linker, Boc-L-(Val-Ala-Leu-Aib-Val-Ala-Leu)-D-(Val-Ala-Leu-Aib-Val-Ala-Leu)-OMe, as well as

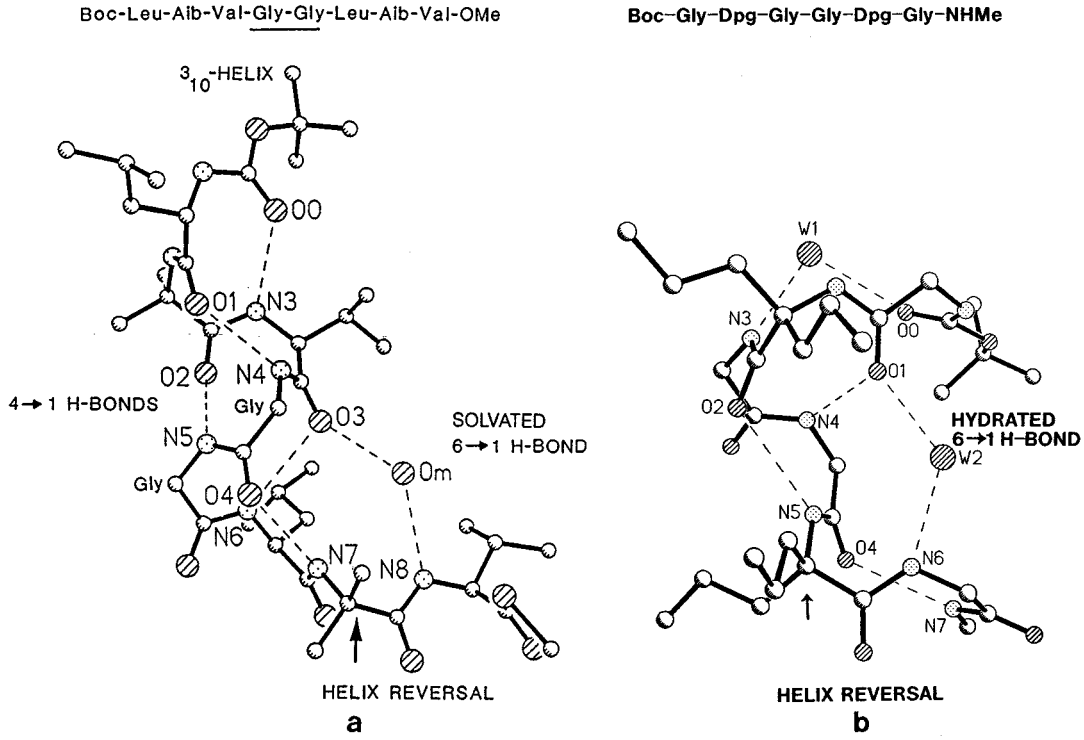

FIGURE 4. (a) Helix reversal at a penultimate Aib residue with a solvated 6 → 1 hydrogen bond.[28] (b) Hydrated, multiple turn structure of the achiral Boc-Gly-Dpg-Gly-Gly-Dpg-Gly-NHMe.[35]

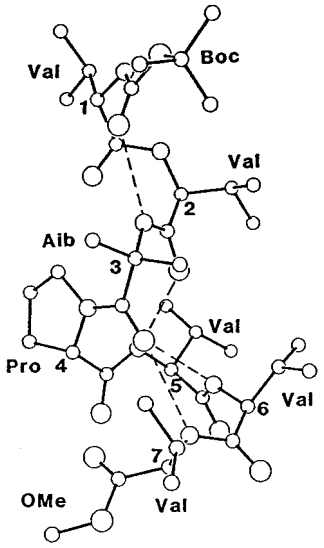

FIGURE 5. Distorted helix with an Aib[3]-Pro[4] segment.[36]

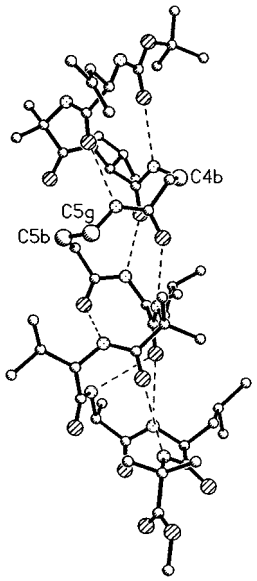

FIGURE 6. Retention of a helical form despite additional $-CH_2-$ moieties (C4b, C5b, and C5g) in the backbone provided by the β-Ala[4]-γ-Abu[5] insertion.[38]

the enantiomeric D,L sequence, the opposing chiral blocks were fused directly to each other.[41] The ambidextrous cylinders formed by the LD and DL enantiomers are mirror images of each other, as expected for enantiomers. The DL enantiomer is shown in Figure 8. At the level of the helix reversal between D-Leu[7] and L-Val[8], which corresponds to the change of chirality, the crystal structure shows that the D-Val[5] CO and L-Leu[8] NH are positioned to permit formation of a solvated 6 → 1 hydrogen bond where the OH of a cocrystallized MeOH bridges the hydrogen bonds. This feature has already been seen in Figure 4, where the helix reversal occurred at the penultimate position of an achiral Aib residue.

Lack of Hydrogen-Bonding Potential. The substitution of a lactic acid residue (Lac) for an amino acid residue in a peptide sequence results in replacing an NH group with an −O− linkage. Without a H atom in this location, the new depsipeptide is deprived of one hydrogen bond. The crystal structures of several helical peptides showed that the peptides maintained their helical form despite the replacement of Ala with Lac and the consequent loss of a hydrogen bond. The conformation of one such depsipeptide, Boc-Val-Ala-Leu-Aib-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe,[40] is shown in Figure 9. The helix has been only

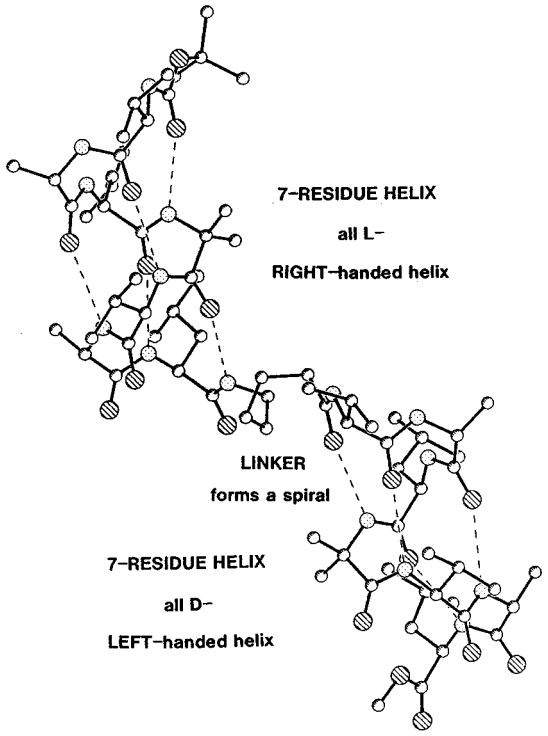

FIGURE 7. L-Helix—linker—D-helix.[39]

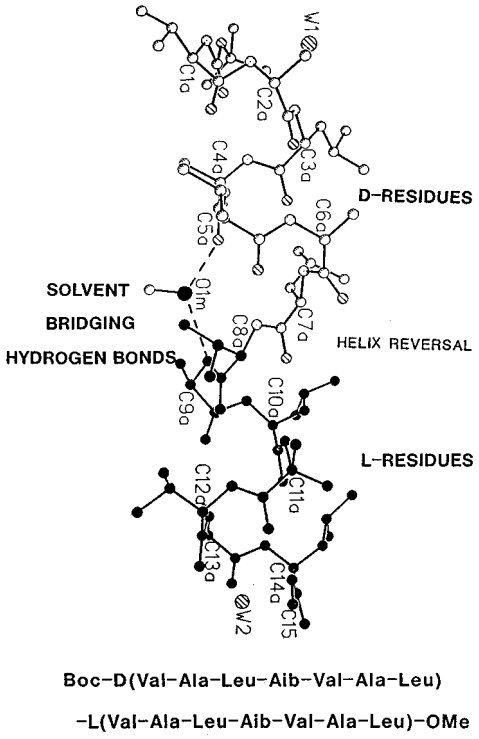

FIGURE 8. D-Helix joined covalently to an L-helix containing a solvated 6 → 1 hydrogen bond at the level of the joint.[41]

mildly distorted from ideal geometry despite the loss of a hydrogen bond at O6L in the middle of the helix.

Ion Channels

The properties and function of a substance often are dependent upon the assembly of several molecules in a specific manner. For example, several long helical molecules may be arranged in the crystal so that a channel is formed with polar groups lining the inner wall and hydrophobic groups covering the exterior wall, a condition that may closely approximate the requirements for K^+ or Na^+ ion transport through membranes. Generally, channel studies are mostly descriptive, given the enormous diversity and complexity in channel type.[42] With rare exception,[43] three-dimensional structures are generally lacking. Channels formed by antibiotic peptides are simpler than those in which proteins are involved and more amenable to providing structural information on an atomic scale, especially with data resolution of 1.0−0.9 Å.

A family of antibiotic peptides, derived from the fungus *Emericellopsis salmosynnemata*, form voltage-gated channels in phospholipid bilayer membranes.[44,46] The sequence for one of the peptides, zervamicin, is Ac-Leu-Ile-Gln-Iva-Ile[5]-Thr-Aib-Leu-Aib-Hyp[10]-*Gln*-Aib-Hyp-Aib-Pro[15]-Phol, where residues with polar side chains are underlined and where four Aib residues plus one Iva residue ensure a helical backbone in the molecule. The helical backbone has an axis that is severely curved, a curvature that is caused by the presence of prolyl rings in the backbone. As expected,[47] all the polar side chains are aligned on one side of the curved helix, the convex side. The glutamine

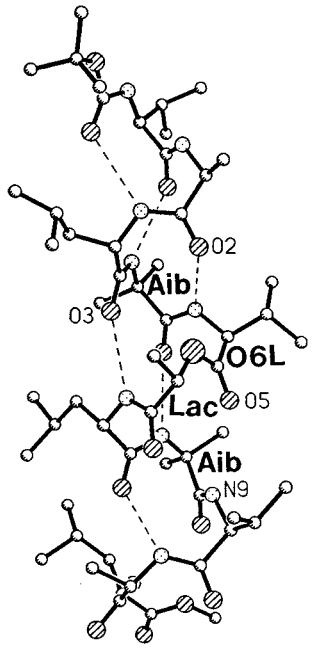

FIGURE 9. Substitution of Lac[6] for Ala[6] and loss of hydrogen bond potential at the depsipeptide position O6L does not disturb the robust helix.[40]

11 (italicized) should have been an exception, since it does not follow the pattern of polar residues occurring at every third or fourth position in the sequence; however, its side chain is long enough to wrap around the helix, away from the hydrophobic side, and project its polar end to complete the polar face.[48,49] Three zervamicin molecules assemble to form an hourglass-shaped channel, Figure 10, with a polar interior and nonpolar exterior. The channel is filled with water molecules. The constriction in the

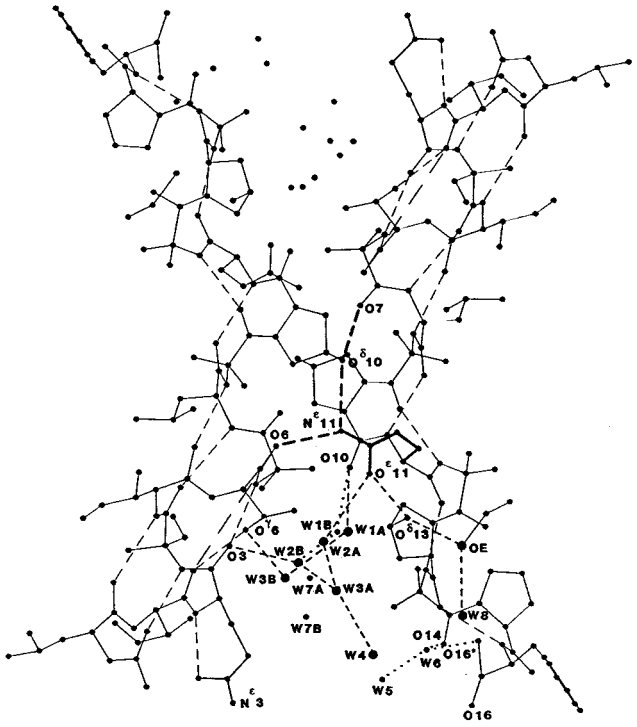

FIGURE 10. Water-filled polar channel formed by Leu-zervamicin in the crystal.[48,49] The constriction in the hourglass-shaped channel is closed by hydrogen bonds involving the NH_2 at the end of the side chain of Gln[11] (darkened). A third Leu-zervamicin molecule that completes the walls of the channel is omitted for clarity.

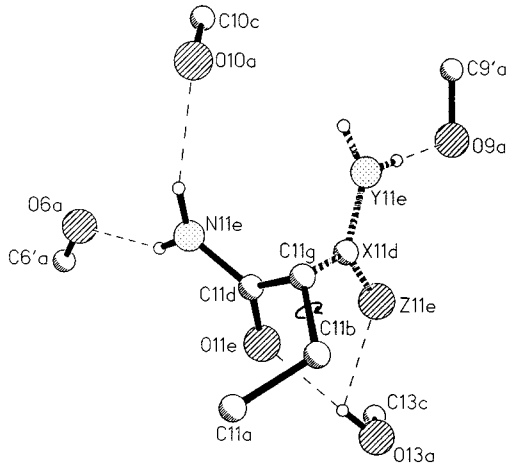

FIGURE 11. Details of the closed channel (solid lines) and open channel (striated lines) that involve a single rotation about the $C^{\beta}-C^{\gamma}$ bond of Gln[11] in zervamicin.[49]

middle of the channel is closed by a hydrogen bond between $N^{\epsilon}11$, from the tip of the side chain in glutamine 11 (darkened), and O6 in such a way that ions or water molecules are blocked from passage through the channel. However, in the same crystal, about 20% of the molecules have been found with an open channel, created by rotation of the side chain of glutamine 11 about the $C^{\beta}-C^{\gamma}$ bond by 96°. Details in the region of the constricted channel and the gating function of the glutamine side chain that closes and opens the channel are shown in Figure 11. Two hydrogen bonds to $N^{\epsilon}11$ that exist in the

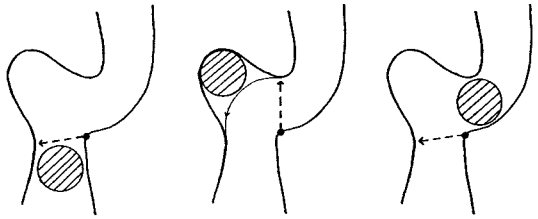

FIGURE 12. Schematic of double-gating in the channel that allows passage of one ion per each opening and closing.

closed position are broken and one is re-formed in the open position with a neighboring molecule.

Since the dimensions and shape of the channel were established in the crystal structure determination, it was possible to calculate a suitable path for the passage of a potassium ion represented by a sphere with 5.6 Å diameter. Starting at position A, in Figure 12, the sphere has sufficient space to travel upward to position B, at which point it has to change direction horizontally. At position B there is insufficient space for the sphere to move further with the gate in the open position; however, at this point, it is possible for the gate to swing from open to closed, or vice versa. After the gate is closed, the sphere is free to travel upward until it reaches the wide part of the channel. For every ion, the gate must open with an applied potential, allowing the ion to pass into a holding cavity while the gate closes, and finally the ion continues its passage through the channel. This very probable double-gating mechanism is consistent with the concept of double-gating already proposed by investigators in entirely different experiments in which ion conductance through cell membranes is measured.

Ion channels are active in a membrane environment. Fortuitously, antiamoebin, Ac-Phe-Aib-Aib-Aib-Iva[5]-Gly-Leu-Aib-Aib-Hyp[10]-Gln-Iva-Hyp-Aib-Pro[15]-Phol, an ionophore similar to zervamicin, cocrystallized with octanol. Despite differences in sequence and local polarity, both molecules fold in an almost identical fashion[50] and the antiamoebin molecules associate to form hourglass-shaped channels in the same manner and shape as the zervamicin, Figure 13. Additionally, the cocrystallized octanol molecules surround the hydrophobic faces on either side of the channel assembly and appear to mimic membrane material. The space-filling diagram in Figure 14 shows a layer of 1-octanol molecules covering the hydrophobic surface of antiamoebin. The arrangement in a crystal having both peptide and octanol molecules may be close to the physical condition in which an ionophore is embedded in a membrane, thus providing information on contacts between membrane components and ionophores.

Concluding Remarks

Many of the conformations adopted by helical peptides containing Aib residues have been described as well as ion channels formed by assembly of helical peptides. The stability of the helix (α, 3_{10}, or mixed) has been emphasized, particularly if one or more Aib or other dialkyl $C^{\alpha,\alpha}$-substituted residues are present in the sequence, despite

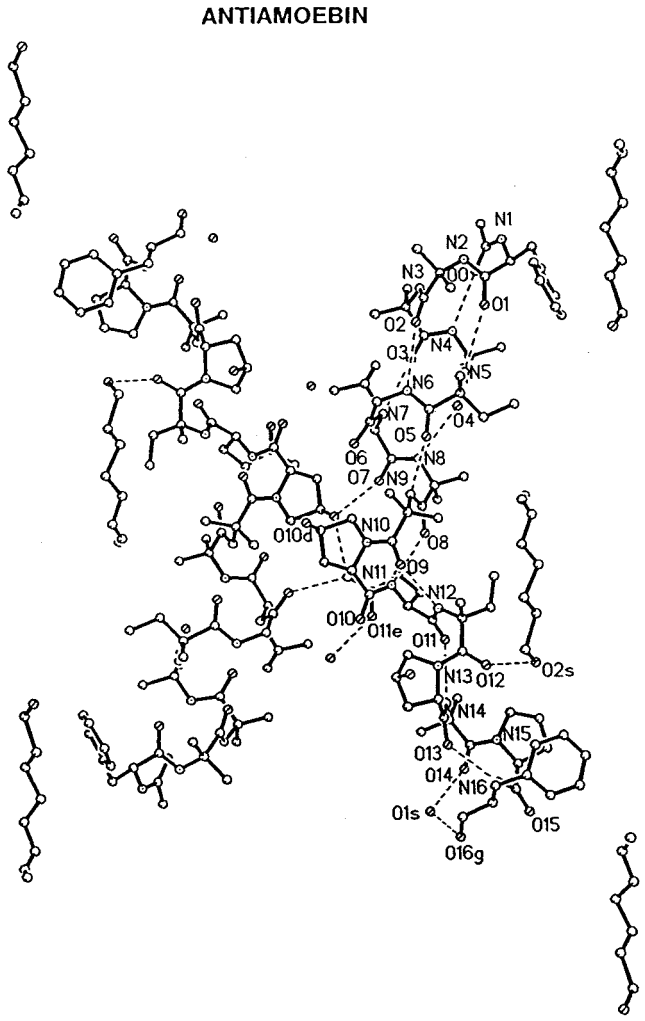

FIGURE 13. Hourglass-shaped channel formed by antiamoebin surrounded by cocrystallized 1-octanol[50] that may mimic membrane material.

a variety of insertions, substitutions, and chirality changes in the backbone that cause local distortions. On the other hand, the flexibility of peptides has been made evident by the multiple isoenergetic conformers that may coexist in the same crystal or in polymorphic crystals. In this Account only structures in the solid state have been discussed. Studies of conformations of the same peptides in solution by spectroscopic procedures have confirmed or strongly suggested the retention of helical backbones or multiple turns in $CHCl_3$ solution, but not necessarily in DMSO solution.[28,35,36,38,41]

Other fascinating and remarkable helical folding patterns have been established for designed β-, γ-, and δ-peptides,[51−54] and for hybrid molecules made with peptides attached to scaffolds.[55] Structures of double-stranded, antiparallel peptide β-helices have been published for peptides having D,L,D,L alternating chirality in the sequence.[56−58] Tubules formed from small cyclic peptides with alternating L,D-residues[59,60] or alternating α,β-residues that self-assemble by complementary hydrogen bonds are receiving much deserved attention at the present time, although some had been conceived and observed at an earlier time.[61,62] New concepts for forming

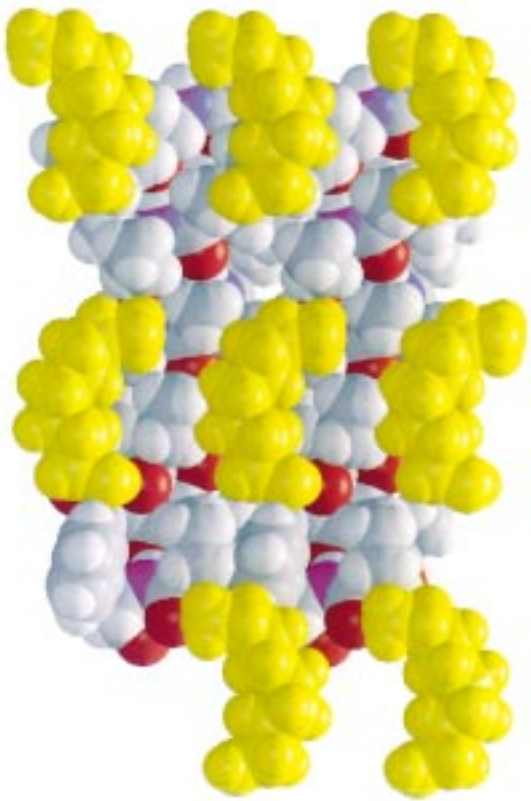

FIGURE 14. A layer of 1-octanol molecules (yellow) in contact with the hydrophobic (concave) side of two antiamoebin molecules (underneath). The view is perpendicular (from the side) to that shown in Figure 13.[65]

tubules by aromatic interactions[63] and hybrid peptides[64] are being developed. Continuing challenges to discover the myriad of new conformations and new properties of still-to-be-designed peptides and hybrid peptides auger an exciting future.

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References

(1) Gavezzotti, A.; Filippini, G. Self-Organization in Molecular Crystals, Liquids and Solutions: Computer Studies. In *Current Challenges on Large Supramolecular Assemblies;* Tsoucaris, G., Ed., Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999; pp 249−263.

(2) McDonald, I. K.; Thornton, J. M. Satisfying Hydrogen Bonding Potential in Proteins. *J. Mol. Biol.* **1994**, *238*, 777−793.

(3) Janin, J.; Wodak, S.; Levitt, M.; Maigret, B. Conformation of Amino Acid Side-Chains in Proteins. *J. Mol. Biol.* **1978**, *125*, 357−386.

(4) Hendrickson, W. A.; Konnert, J. H. Incorporation of Stereochemical Information into Crystallographic Refinement. In *Computing in Crystallography*; Diamond, R., Ramaseshan, S., Venketasan, K., Eds.; Indian Institute of Science: Bangalore, 1980; pp 13.01−13.23.
(5) Brünger, A. T. Crystallographic phasing and refinement of macromolecules. *Curr. Opin. Struct. Biol.* **1991**, *1*, 1016−1022.
(6) Marshall, G.; Bosshard, H. E. Studies on the Biologically Active Conformation. *Circ. Res. 30/31 (Suppl. II)* **1972**, 143−150.
(7) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. Preferred Conformation of Peptides Containing α,α-Disubstituted α-Amino Acids. *Biopolymers* **1983**, *22*, 205−215.
(8) Balaram, P. Peptides as Biorganic Models. *Proc. Indian Acad. Sci.* **1984**, *93*, 703−717.
(9) Karle, I., L.; Balaram, P. Structural Characteristics of α-Helical Peptide Molecules Containing Aib Residues. *Biochemistry* **1990**, *29*, 6748−6756.
(10) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. Factors governing helical preference of peptides containing multiple α,α-dialkyl amino acids. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487−491.
(11) Karle, I. L.; Karle, J. An Application of a New Phase Determination Procedure to the Structure of Cyclo-(hexaglycyl) Hemihydrate. *Acta Crystallogr.* **1963**, *16*, 969−975.
(12) Ballard, D. G. H.; Bamford, C. H.; Weymouth, F. J. Studies in polymerization. VIII. Reactions of N-carboxy-α-amino acid anhydrides initiated by metal cations. *Proc. R. Soc. London, A* **1955**, *227*, 155−183.
(13) Zalkin, A.; Forrester, J. D.; Templeton, D. H. Ferrichrome A Tetrahydrate. Determination of Crystal and Molecular Structure. *J. Am. Chem. Soc.* **1966**, *88*, 1810−1814.
(14) Venkatachalam, C. M. Stereochemical Criteria of Polypeptides and Proteins. V. Conformation of a System of Three Linked Peptide Units. *Biopolymers* **1968**, *6*, 1425−1436.
(15) Smith, G. D.; Duax, W. L.; Langs, D. A.; DeTitta, G. T.; Edmonds, J. W.; Rohrer, D. C.; Weeks, C. M. The Crystal and Molecular Structure of the Triclinic and Monoclinic Forms of Valinomycin, $C_{54}H_{90}N_6O_{18}$. *J. Am. Chem. Soc.* **1975**, *97*, 7242−7247.
(16) Karle, I. L.; Flippen-Anderson, J. L. A New Conformation Exhibiting Near-Threefold Symmetry for Uncomplexed Valinomycin in Crystals from Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1988**, *110*, 3243−3257.
(17) Karle, I. L.; Karle, J.; Mastropaolo, D.; Camerman, A.; Camerman, N. [Leu^5]enkephalin: Four Cocrystallizing Conformers with Extended Backbones that Form an Antiparallel β-sheet. *Acta Crystallogr.* **1983**, *B39*, 625−637.
(18) Smith, G. D.; Griffin, J. F. Conformation of [Leu^5]-Enkephalin from X-ray Diffraction: Features Important for Recognition at Opiate Receptor. *Science* **1978**, 199, 1214−1216.
(19) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, H.; Balaram, P. α-Helix and mixed 3_{10}/α-helix in cocrystallized conformers of Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 765−769.
(20) Ramachandran, G. N.; Sasisekharan, V. Conformation of Polypeptides and Proteins. In *Advances in Protein Chemistry*; Anfinsen, C. B., Jr., Anson, M. L., Edsall, J. T., Richards, F. M., Eds.; Academic Press: New York, 1968; pp 283−438.
(21) Flippen, J. L.; Karle, I. L. Conformation of the Cyclic Tetrapeptide Dihydrochlamydocin Iabu-L-Phe-D-Pro-LX, and Experimental Values for 3→1 Intramolecular Hydrogen Bonds by X-ray Diffraction. *Biopolymers* **1976**, *15*, 1081−1092.
(22) Karle, I. L.; Kishore R.; Raghothama, S.; Balaram, P. Cyclic Cystine Peptides. Antiparallel β-sheet Conformation for the 20-Membered Ring in Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe. *J. Am. Chem. Soc.* **1988**, *110*, 1958−1963.
(23) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Sukumar, M.; Balaram, P. Modular Design of Synthetic Protein Mimics. Crystal Structures, Assembly and Hydration of Two 15- and 16-Residue Apolar, Leucyl-Rich Helical Peptides. *J. Am. Chem. Soc.* **1990**, *112*, 9350−9356.
(24) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. Accommodation of a D-Phe Residue into a Right-Handed 3_{10}-Helix: Structure of Boc-D-Phe-$(Aib)_4$-Gly-L-Leu-$(Aib)_2$-OMe, an Analogue of the Amino Terminal Segment of Antiamoebins and Emerimicins. *Biopolymers* **1993**, *33*, 401−407.
(25) Toniolo, C.; Crisma, M.; Bonora, G. M.; Benedetti, E.; di Blasio, B.; Pavone, V.; Pedone, C.; Santini, A. Preferred Conformation of the Terminally Blocked $(Aib)_{10}$ Homo-oligopeptide: A Long, Regular 3_{10}−Helix. *Biopolymers* **1991** *31*, 129−138.
(26) Benedetti, E. X.-Ray Crystallography of Peptides: The Contributions of the Italian Laboratories. *Biopolym. (Pept. Sci.)* **1996**, *40*, 3−44.
(27) Karle, I. L.; Rao, R. B.; Prasad, S.; Kaul, R.; Balaram, P. Nonstandard Amino Acids in Conformational Design of Peptides. Helical Structures of Crystals of 5−10 Residue Peptides Containing Dipropylglycine and Dibutylglycine. *J. Am. Chem. Soc.* **1994**, *116*, 10355−10361.
(28) Karle, I. L.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. Solid State and Solution Conformations of a Helical Peptide with a Central Gly-Gly Segment. *Biopolymers* **1996**, *38*, 515−526.
(29) Toniolo, C.; Peggion, C.; Crisma, M.; Formaggio, F.; Shui, X.; Eggleston, D. S. Structure determination of racemic trichogin A IV using centrosymmetric crystals. *Nat. Struct. Biol.* **1994**, *1*, 908−914.
(30) Crisma, M.; Monaco, V.; Formaggio, F.; Toniolo, C.; George, C.; Flippen-Anderson, J. L. Crystallographic structure of a helical lipopeptaibol antibiotic analogue. *Lett. Pept. Sci.* **1997**, *4*, 213-218.
(31) Schellman, C. The .alpha.L conformation at the ends of helices. In *Protein Folding*; Janenicke, R., Ed.; Elsevier: Amsterdam, 1980; pp 53−61.
(32) Milner-White, E. J. Recurring Loop Motif in Proteins that Occurs in Right-handed and Left-handed Forms. Its Relationship with Alpha-helices and Beta-bulge Loops. *J. Mol. Biol.* **1988**, *199*, 503−511.
(33) Nagarajaram, H. A.; Sowdhamini, R.; Ramakrishnan, C.; Balaram, P. Rules for α-helix termination by glycine. *FEBS Lett.* **1993**, *321*, 79−83.
(34) Aurora, R.; Srinivasan, R.; Rose, G. D. Termination of right-handed helices in proteins by residues in left-handed helical conformations. *Science* **1994**, *264*, 1126−1130.
(35) Karle, I. L.; Kaul, R.; Rao, R. Balaji; Raghothama, S.; Balaram, P. J. Stereochemical Analysis of Higher α,α-Dialkylglycine Containing Peptides. Character-

istization of Local Helical Conformations at Dipropylglycine Residues and Observation of a Novel Hydrated Multiple β-Turn Structure in Crystals of a Glycine-rich Peptide. *J. Am. Chem. Soc.* **1997**, *119*, 12048−12054.
(36) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, H.; Balaram, P. Peptide Design: Influence of a Guest Aib-Pro Segment on the Stereochemistry of an Oligo-Val Sequence−Solution Conformations and Crystal Structure of Boc-$(Val)_2$-Aib-Pro-$(Val)_3$-OMe. *Biopolymers* **1990**, *29*, 1433−1442.
(37) Karle, I. L.; Awasthi, S. K.; Balaram, P. A designed β-hairpin peptide in crystals. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8189−8193.
(38) Karle, I. L.; Pramanik, A.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. ω-amino Acids in Peptide Design. Crystal Structures and Solution Conformations of Peptide Helices Containing a β-Alanyl-γ-Aminobutyryl-Segment. *J. Am. Chem. Soc.* **1997**, *119*, 9087−9095.
(39) Karle, I. L.; Banerjee, A.; Balaram, P. Design of two-helix motifs in peptides: crystal structure of a system of linked helices of opposite chirality and a model helix-linker peptide. *Folding Des.* **1997**, *2*, 203-210.
(40) Balaram, P.; Karle, I. L. To be published.
(41) Banerjee, A.; Raghothama, S. R.; Karle, I. L.; Balaram, P. Ambidextrous Molecules: Cylindrical Peptide Structures Formed by Fusing Left- and Right-Handed Helices. *Biopolymers* **1996**, *39*, 279−285.
(42) Eisenberg, B. Ionic Channels in Biological Membranes: Natural Nanotubes. *Acc. Chem. Res.* **1998**, *31*, 117−123.
(43) Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Haitt, B. T.; MacKinnon, R. The Structure of the Potassium Channel: Molecular Basis of K^+ Conduction and Selectivity. *Science* **1998**, *280*, 69−77.
(44) Argoudelis, A. D.; Johnson, L. E. Emerimicins II, III and IV, antibiotics produced by Emericellopsis microspora in media supplemented with *trans*-4-n-propyl-L-proline. *J. Antibiot.* **1974**, *27*, 274−282.
(45) Rinehart, K. L., Jr.; Gaudioso, L. A.; Moore, M. L.; Pandey, R. C.; Cook, J. C., Jr.; Barber, M.; Sedgwick, R. D.; Bordoli, R. S.; Tyler, A. N.; Green, B. N. Structures of Eleven Zervamicin and Two Emerimicin Peptide Antibiotics Studied by Fast Bombardment Mass Spectroscopy. *J. Am. Chem. Soc.* **1981**, *103*, 6517−6520.
(46) Balaram, P.; Krishna, K.; Sukumar, M.; Mellor, I. R.; Sansom, M. S. P. The properties of ion channels formed by zervamicins. *Eur. Biophys. J.* **1992**, *21*, 117−128.
(47) Melittin, a membrane surface active peptide, forms a helix that bends at a central Pro residue and has chiefly nonpolar residues on the inside of the curve and polar residues on the outside. Terwilliger, T. C.; Weissman, L.; Eisenberg, D. The crystal Structure of Melittin in the Form I Crystals and Its Implication for Melittin's Lytic and Surface Activities. *Biophys. J.* **1982**, *37*, 353−361.
(48) Karle, I. L.; Flippen-Anderson, J. L.; Agarwalla, S.; Balaram, P. Crystal structure of [Leu^1zervamicin, a membrane ion-channel peptide. Implications for gating mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5307−5311.
(49) Karle, I. L.; Flippen-Anderson, J. L.; Agarwalla, S.; Balaram, P. Conformation of the Flexible Bent Helix of Leu^1-Zervamicin in Crystal C and a Possible Gating Action for Ion Passage. *Biopolymers* **1994**, *34*, 721−735.
(50) Karle, I. L.; Perozzo, M. A.; Mishra, V. K.; Balaram, P. Crystal structure of the channel forming polypeptide antiamoebin in a membrane mimetic environment. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5501−5504.
(51) Borman, S. Peptides Do The Twist. *Chem. Eng. News* **1998** (Aug 31), 24−25.
(52) Gellman, S. H. Foldamers: A Manifesto. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(53) Seebach, D.; Matthews, J. F. β-Peptides: a surprise at every turn. *Chem. Commun.* **1997**, 2015−2022.
(54) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. γ-Peptides Forming More Stable Secondary Structures than α-Peptides: Synthesis and Helical NMR−Solution Structure of the γ-Hexapeptide Analogue of H−$(Val\text{-}Ala\text{-}Leu)^2$OH. *Helv. Chim. Acta* **1998**, *81*, 983−1002.
(55) Karle, I. L.; Ranganathan, D.; Haridas, V. Crystal Structure of Cyclo$(Adm\text{-}Cyst)_3$: An example of a Topologically Defined Double-Helical Cystine Cyclic Peptide. *J. Am. Chem. Soc.* **1996**, *118*, 10916−10917.
(56) Benedetti, E.; Di Blasio, B.; Pedone, C.; Lorenzi, G. P.; Tomasic, L.; Gramlich, V. A double-stranded β-helix with antiparallel chains in a crystalline oligo-L-D-peptide. *Nature (London)* **1979**, *282*, 630.
(57) Pavone, V.; Benedetti, E.; Di Blasio, B.; Lombardi, A.; Pedone, C.; Tomasich, L.; Lorenzi, G. P. Regularly Alternating L, D.-Peptides. III. *Biopolymers* **1989**, *28*, 215−223 and references therein.
(58) Langs, D. A.; Smith, G. D.; Courseille, C.; Precigoux, G.; Hospital, M. Monoclinic uncomplexed double-stranded, antiparallel, left-handed $\beta^{5.6}$-helix ($\downarrow\uparrow\beta^{5.6}$) structure of gramicidin A: Alternate patterns of helical association and deformation. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5345−5349.
(59) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. Self-assembling organic nanotubes based on a cyclic peptide architecture. *Nature* **1993**, *366*, 324−327.
(60) Hartgerink, J. D.; Granja, J. R.; Milligan, R.; Ghadiri, M. R. Self-Assembling Peptide Nanotubes. *J. Am. Chem. Soc.* **1996**, *118*, 43−50.
(61) Karle, I. L.; Handa, B., K.; Hassall, C. H. The Conformation of the Cyclic Tetrapeptide L-Ser(O−t-Bu)-β-Ala-Gly-L-β-Asp(OMe) Containing a 14-Membered Ring. *Acta Crystallogr.* **1975**, *B31*, 555−560.
(62) Chiang, C. C.; Karle, I. L. Crystal structure of the 1:1 mixture of cyclic (L-Ala-L-Pro-L-Phe-L-Pro) and cyclic (L-Ala-L-Pro-D-Phe-L-Pro). *Int. J. Pept. Protein Res.* **1982**, *20*, 133−138.
(63) Ranganathan, D.; Haridas, V.; Gilardi, R.; Karle, I. L. Self-Assembling Aromatic- Bridged Serine-Based Cyclodepsipeptides (Serinophanes): A Demonstration of Tubular Structures Formed Through Aromatic Π−Π Interactions. *J. Am. Chem. Soc.* **1998,** *120*, 10793−10800.
(64) Ranganathan, D.; Karle, I. L. To be published.
(65) The colored figure was prepared by M. A. Perozzo[50] using the graphics program of Merritt, E. A.; Bacon, D. J. Raster 3D: Photorealistic Molecular Graphics. *Methods Enzymol.* **1997**, *227*, 505−524.

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