

## Chapter 5.9

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# THE USE OF D-AMINO ACIDS IN PEPTIDE DESIGN

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***Radhakrishnan Mahalakshmi and Padmanabhan Balaram\****

Molecular Biophysics Unit, Indian Institute of Science, India.

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## 1. INTRODUCTION

Proteins and most naturally occurring peptides are composed of amino acids of the L-configuration. D-amino acids are found as constituents of natural peptides produced primarily, by microorganisms, using a non-ribosomal mechanism of synthesis. Research in this field dates back to over 60 years ago when Lipmann *et al* noted the presence of D-amino acids in tyrocidines and gramicidins [1]. Post-translational epimerization is an infrequently used mechanism in higher organisms for the introduction of D-amino acids into polypeptide chains [2]. Some notable examples are the production of opioid peptides, namely dermorphins and deltorphins from the skin of various frog species belonging to the Phyllomedusinae sub-family [3] and the contryphans produced by cone snails via post-translational epimerization [4]. Recently, total chemical synthesis of proteins containing all-D residues has been carried out and the characteristics and enzymatic activity of such enantiomeric proteins has been analyzed [5-7]. The incorporation of D-amino acids into polypeptide chains imposes local conformational constraints [8]. The purpose of this review is to examine the use of D-amino acids in the design of peptides adopting well-defined folded conformations.

## 2. CONFORMATIONAL CHARACTERISTICS OF D-AMINO ACIDS

The effects of residue configuration on polypeptide chain conformation are readily understood by considering the Ramachandran maps for N-acetyl-L-Ala-NHMe and N-acetyl-D-Ala-NHMe shown in Fig. 1A & 1B [9-12]. The map for the D-residue is derived by inverting the classical L-Ala map through the origin. Most of the stereochemically allowed regions for the L-residues correspond to negative values of  $\phi$ , while for D-residues, the allowed regions correspond to positive values of  $\phi$ . The classical  $3_{10}$  and  $\alpha$ -helical

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\* Correspondence concerning this article should be addressed to Padmanabhan Balaram, E-mail: pb@mbu.iisc.ernet.in, Fax: +91-80-23600535.

structures formed by all-L polypeptides adopt a right-handed twist ( $3_{10-R}/\alpha_R$ ), while the corresponding D-polypeptides form helices with a left-handed twist ( $3_{10-L}/\alpha_L$ ). Notably, there are small sterically allowed regions, which correspond to positive  $\phi$  values for L-residues and negative  $\phi$  values for D-residues. L-Proline is the most constrained of the amino acids occurring in proteins. The restraints imposed by the formation of the pyrrolidine ring restrict the allowed range of  $\phi$  values in L-Pro to  $-60 \pm 20^\circ$  (Fig. 1C) [11,12]. By extension, the allowed values for D-Pro are  $+60 \pm 20^\circ$  (Fig. 1D). D-Pro can be used to advantage in restricting the local conformation of peptide chains, facilitating the formation of prime  $\beta$ -turns. The only achiral amino acid occurring in proteins is glycine, a residue for which considerably larger regions of  $\phi$ - $\psi$  space are sterically allowed, as a consequence of the absence of a substituent at the  $C^\alpha$  atom (Fig. 2A). The Gly residue can adopt conformations on either side of the  $\phi$ - $\psi$  map. The observed distribution of Gly residues in proteins (Fig. 2B) establishes an approximately symmetric distribution about the origin [13].  $\alpha$ -Aminoisobutyric acid (Aib) is an achiral residue, which contains a pair of methyl groups at the  $C^\alpha$  carbon atom. This residue may be formally viewed as a combination of L-Ala and D-Ala residues. The stereochemically allowed regions of Ramachandran space for Aib are readily derived by considering only the regions of overlap between the L-Ala and D-Ala  $\phi$ - $\psi$  maps (Fig. 2C). Clearly, the Aib residue is largely limited to the right ( $\alpha_R$ ) and left ( $\alpha_L$ ) handed helical structures. The observed distribution of crystallographically obtained conformations for Aib residues in peptides provides overwhelming support for this expectation (Fig. 2D) [11,12]. Achiral amino acids can, therefore, also be viewed as surrogate D-amino acids in peptide design because they can adopt conformations on both sides of the  $\phi$ - $\psi$  map. While the achiral Gly and Aib residues can support conformations with both positive and negative values of  $\phi$ , D-amino acids may be used in design when positive  $\phi$  values are required to form a desired structure.

### 3. $\beta$ -HAIRPIN DESIGN

$\beta$ -Hairpins are widely present in proteins with antiparallel  $\beta$ -sheet formation being facilitated by a nucleating reverse turn. Two-residue  $\beta$ -turns belonging to the type II' and I' category are most often observed as nucleating features for hairpins in proteins [14]. In the prime turns, the residue at position  $i+1$  necessarily adopts the conformation of  $\phi \sim +60^\circ$ , while  $\psi$  values of  $\sim -120^\circ$  and  $\sim +30^\circ$  are observed for type II' and I' turns, respectively [15]. Fig.3A illustrates a view of an ideal hairpin formed by a centrally positioned nucleating type II'  $\beta$ -turn. When the stereochemistry of the  $\beta$ -turn is type II ( $\phi_{i+1} = -60^\circ$ ,  $\psi_{i+1} = +120^\circ$ ), registry of the antiparallel strands is not readily achieved. Although chain reversal occurs, the antiparallel strands drift away from one another (Fig. 3B).

In the design of  $\beta$ -hairpins,  $^D\text{Pro-Xxx}$  sequences can provide a site for nucleation of prime turns. Indeed, insertion of  $^D\text{Pro}$  residues has proved to be a successful strategy for peptide hairpin design [8,11,12,16-21]. Fig. 4 illustrates the superposition of the crystal structures of six designed peptide hairpins containing a central  $^D\text{Pro-Xxx}$  type II'  $\beta$ -turn.  $^D\text{Pro}$  residues provide a strong conformational determinant, when inserted into L-amino acid sequences. In ongoing studies, centrally positioned  $^D\text{Pro-Gly}$  sequences have been used to generate well-defined  $\beta$ -hairpin scaffolds which permit spectroscopic probing of cross-strand interactions between aromatic side chains (Fig. 5A & 5B) [22]. Multiple  $^D\text{Pro-Xxx}$  segments may be used to nucleate multistranded antiparallel  $\beta$ -sheet structures as exemplified by the design of three, four and five stranded  $\beta$ -sheets (Fig. 5C & 5D) [23-26].

In principle, hairpin nucleation may be achieved by using sequences with a high propensity to form prime  $\beta$ -turns. Specifically, the Aib- $^D\text{Ala}$  segment has been used to nucleate the formation of a  $\beta$ -hairpin in the octapeptide Boc-Leu-Phe-Val-Aib- $^D\text{Ala}$ -Leu-Phe-Val-OMe (Fig. 6A) [27]. In this case, crystallographic studies establish that the Aib- $^D\text{Ala}$   $\beta$ -turn adopts a type I' conformation, undoubtedly as a consequence of the tendency of the Aib residue to form  $\alpha_R$  or  $\alpha_L$  conformations. Thus, D-amino acid containing dipeptide segments may be used to generate both type II' and I' structures. While both turn

types can sustain  $\beta$ -hairpin formation, the orientations of the strands are significantly different, as illustrated in Fig. 6C & 6D.

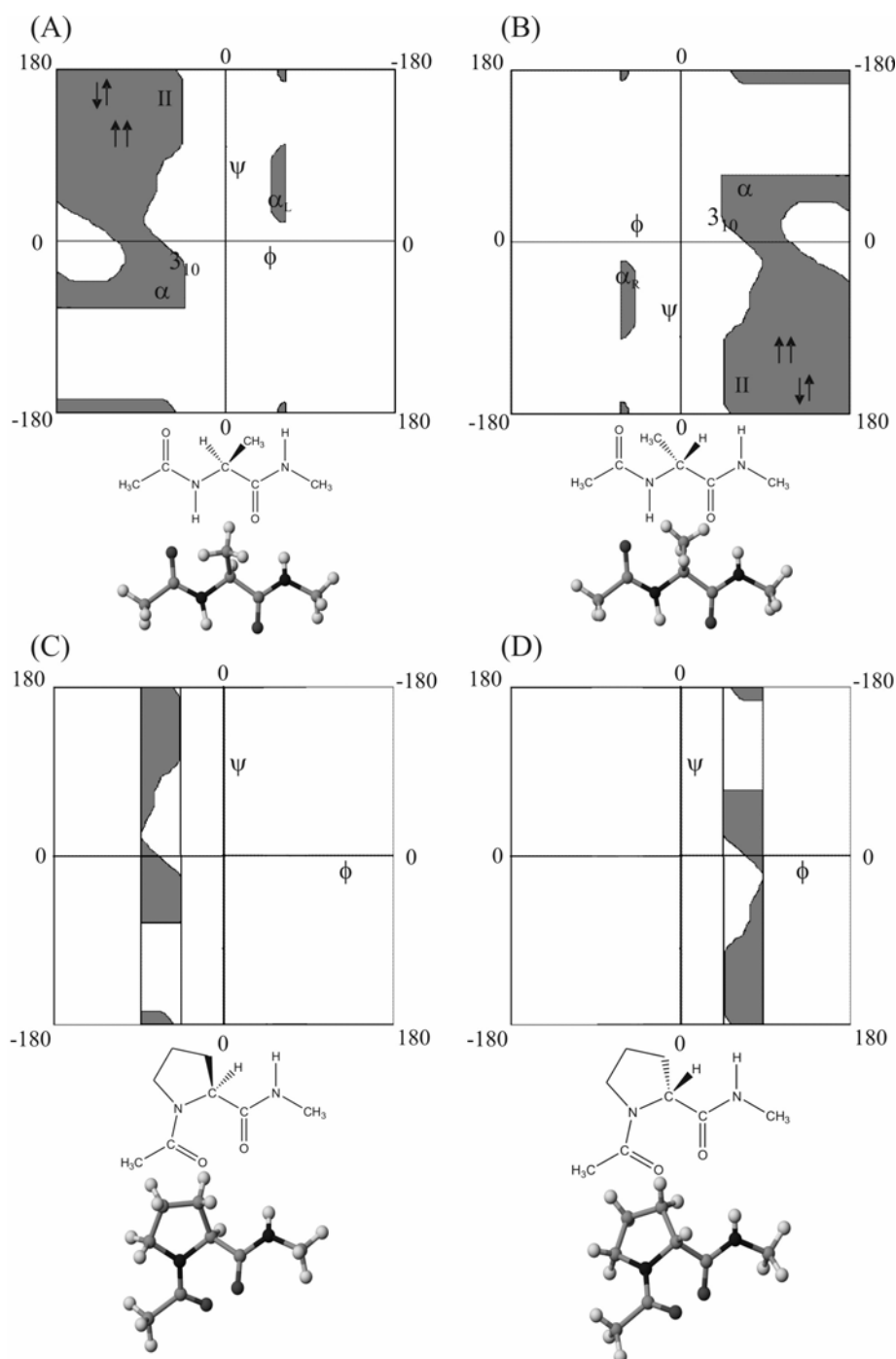


Figure 1. Ramachandran maps showing allowed regions for (A) L-Ala (B) D-Ala (C) L-Pro (D) D-Pro residues, respectively. The chemical formula and a ball-and-stick notation of N-acetyl-amino acyl-NHMe (fully extended conformation) for each amino acid are also shown below their respective maps. Figures have been adapted from [11]. Map in Figure (A) reprinted with permission from [11]; Copyright (2001) American Chemical Society.

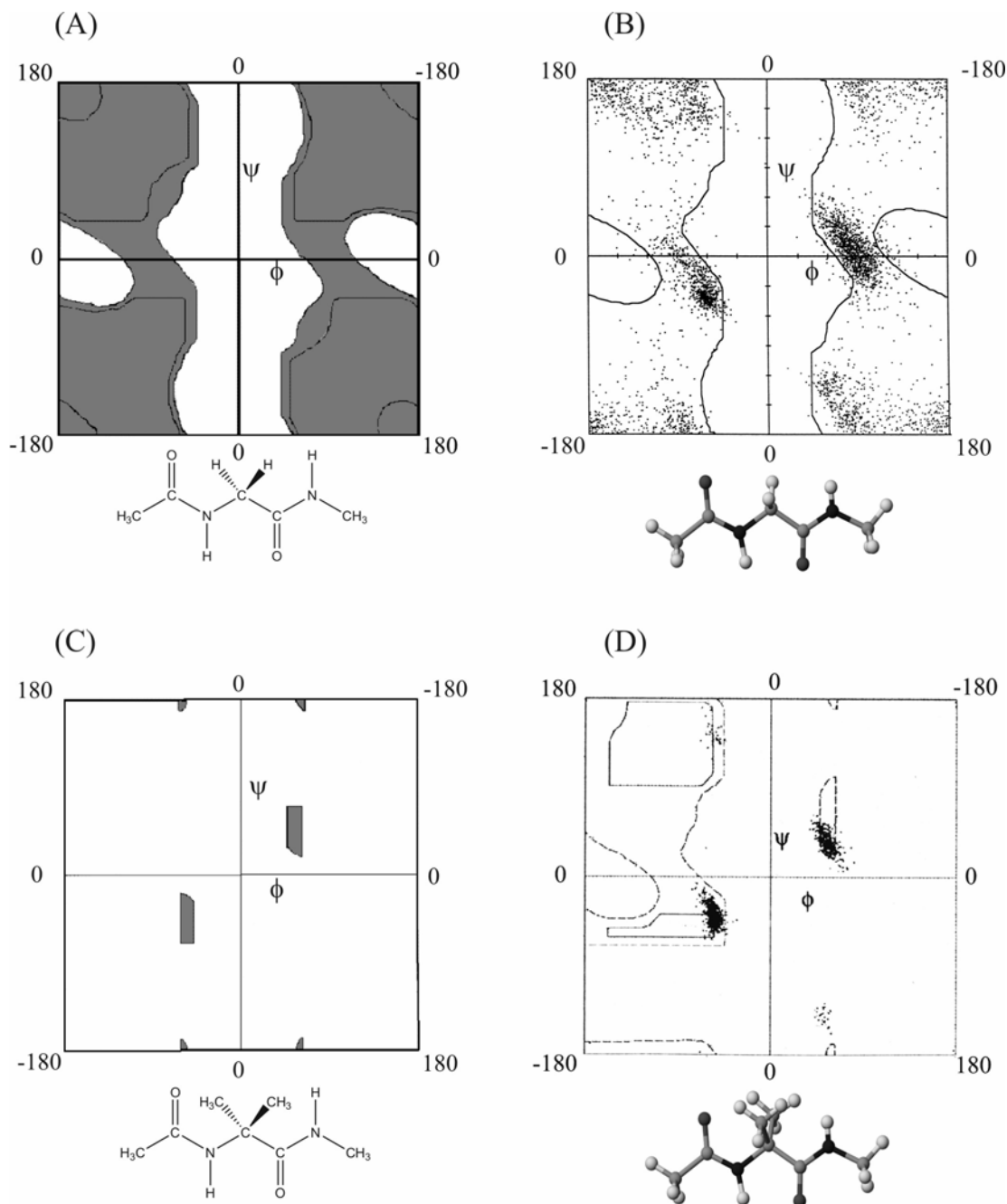


Figure 2. Allowed regions in the Ramachandran plot for Gly (A) and Aib (C) residues compared with  $\phi$ - $\psi$  values obtained from crystal structures of proteins (B) for Gly and peptides (D) for Aib. Also indicated are the chemical structures and ball-and-stick notations for these residues. Figures (A) and (C) reprinted with permission from [11]; Copyright (2001) American Chemical Society. Figures (B) and (D) reprinted with permission from [12]; Copyright (2003) Indian Academy of Sciences.

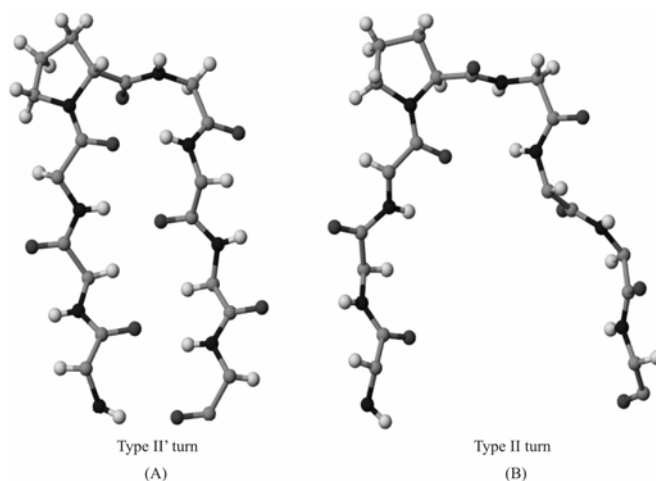


Figure 3. (A) Peptide hairpin with a type II' turn formed using  $^D\text{Pro-Gly}$  as a turn nucleator. Shown is the crystal structure of the peptide Boc-Leu-Val-Val- $^D\text{Pro-Gly}$ -Leu-Val-Val-OMe [17]. Only the peptide backbone and pyrrolidine ring of  $^D\text{Pro}$  residue are shown. (B)  $^D\text{Pro}$  in the previous peptide was replaced by  $^L\text{Pro}$  and the  $\phi$ - $\psi$  values of the turn segment were altered to ideal type II values. Although turn nucleation does occur, it is evident that the two strand segments fray away from each other. This clearly establishes the superiority of  $^D\text{Pro}$  to nucleate tight  $\beta$ -turns compared to its L-analog.

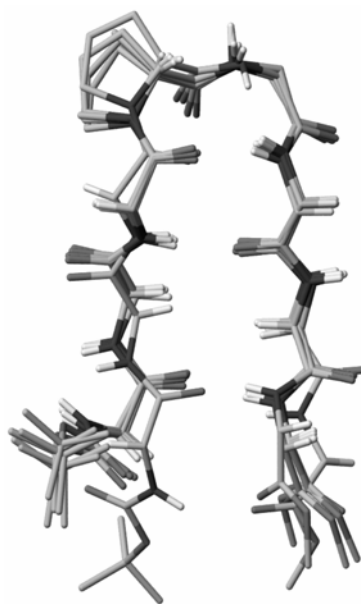


Figure 4. Superposition of crystal structures of six peptide hairpins obtained using a nucleating  $^D\text{Pro-Xxx}$  segment. All the peptides shown, form type II' turns. Sequences: Boc-Leu-Val-Val- $^D\text{Pro-Gly}$ -Leu-Val-Val-OMe [17], Boc-Leu-Val-Val- $^D\text{Pro-Ala}$ -Leu-Val-Val-OMe (2 molecules) [20], Boc-Leu-Phe-Val- $^D\text{Pro-Ala}$ -Leu-Phe-Val-OMe [21], Boc-Leu-Val-Val- $^D\text{Pro-Gly}$ -Leu-Phe-Val-OMe [21], Boc-Leu-Val-Val- $^D\text{Pro-Aib}$ -Leu-Val-Val-OMe [21]. These crystal structures provide experimental evidence for the turn-nucleating ability of  $^D\text{Pro-Xxx}$  segments, which subsequently propagates to well-registered strand segments.

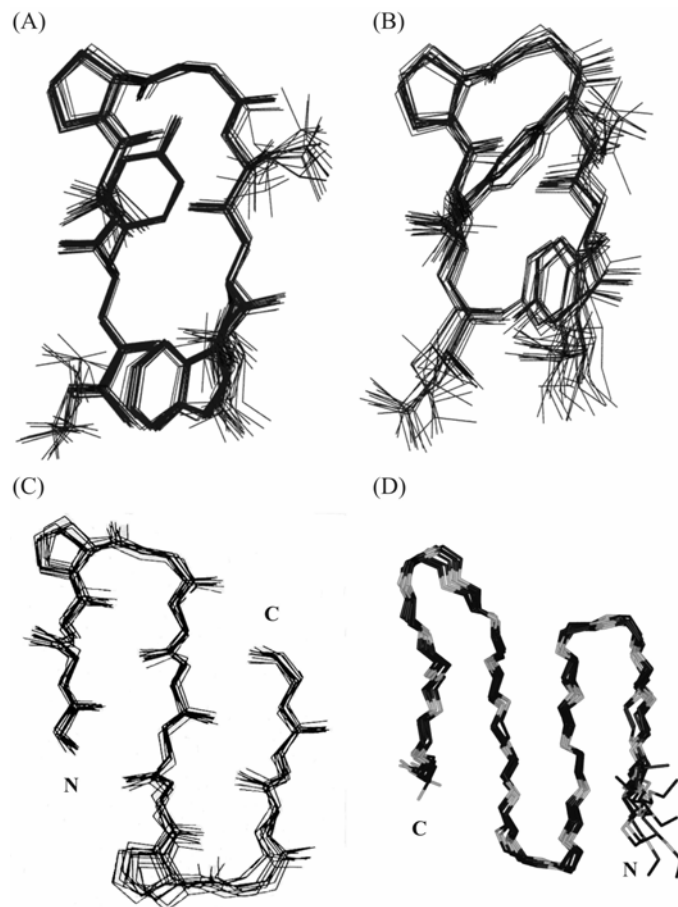


Figure 5. (A and B) Solution NMR structures of octapeptide hairpins formed using  $^D\text{Pro-Gly}$  as a turn nucleator [22]. The tight turn forming ability of  $^D\text{Pro-Gly}$  segments can be exploited for the construction of predefined hairpin scaffolds, which can then be made use of, for the study of tertiary interactions, such as aromatic interactions, in solution, as is demonstrated in these examples. (C and D) The ability of  $^D\text{Pro-Xxx}$  segments to nucleate tight turns can be exploited for the construction of multistranded  $\beta$ -sheet structures. Shown are the solution NMR structures of a 14-residue three-stranded  $\beta$ -sheet [23] and a 26-residue four-stranded  $\beta$ -sheet [24]. Figures C and D reprinted with permission from [11]; Copyright (2001) American Chemical Society.

#### 4. D-AMINO ACID AS HELIX TERMINATION SIGNALS

Polypeptide helices in proteins are characterized by a succession of residues, which adopt  $\phi$ - $\psi$  values that lie in the right handed helical ( $\alpha_R$ ) region of the Ramachandran map. Helix termination at the N- and C-terminal ends occurs when residues drift away to other regions of  $\phi$ - $\psi$  space, most notably, the extended ( $\beta$ -sheet ( $\beta$ )/ polyproline ( $P_{II}$ )) and left handed helical ( $\alpha_L$ ) regions (Fig. 1A). In proteins, a widely observed structural feature at the C-terminal end of helices is the Schellman motif, which is formed when the helix terminating residue adopts an  $\alpha_L$  conformation [28,29]. In proteins structures this mode of termination predominantly involves the occurrence of Gly or Asn residues at the C-terminal position. This is a consequence of the fact that the achiral Gly residue can be readily accommodated in  $\alpha_L$  conformations, while Asn has the greatest  $\alpha_L$  propensity of the remaining chiral residues [30]. The sequence of residue conformations  $\alpha_R \alpha_R \alpha_R \alpha_R \alpha_L$  leads to helix termination with the simultaneous formation of a pair of 4 $\rightarrow$ 1 and 6 $\rightarrow$ 1 hydrogen bonds (Fig. 7A).

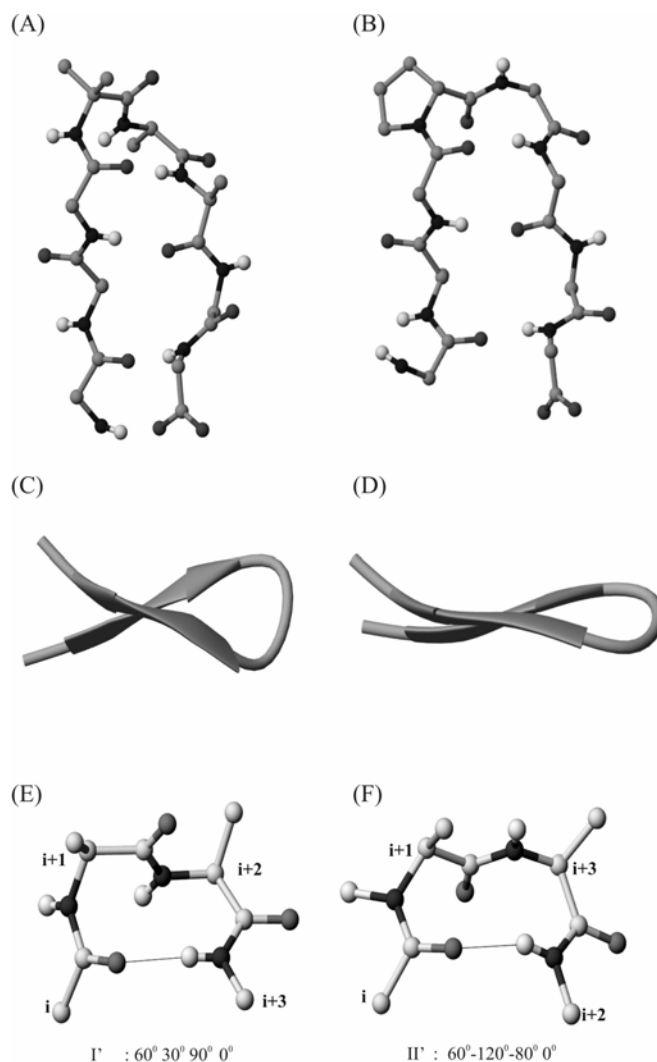


Figure 6. (A) Peptide hairpin nucleated by Aib-<sup>D</sup>Ala segment (Boc-Leu-Phe-Val-Aib-<sup>D</sup>Ala-Leu-Phe-Val-OMe) (type I' turn) [27] compared with a peptide hairpin formed using <sup>D</sup>Pro-Gly (Boc-Leu-Val-Val-<sup>D</sup>Pro-Gly-Leu-Val-Val-OMe) (type II' turn) [17] (B). Side chains of only the turn residues have been included in the figure. (C and D) Strand orientations in both peptides are however different, as illustrated by a side view of the ribbon diagram of the two hairpins. Also shown are ball-and-stick notations of the two turn types: I' (E) and II (F).

In synthetic peptides, helix termination by a Schellman motif can be readily achieved by placement of an achiral residue or a D-residue towards the C-terminal of a potentially helical segment consisting of L-amino acids. Crystallographic characterization of Schellman motifs in peptides have been achieved in sequences containing the achiral Aib residue at the penultimate position from the C-terminus [31]. In the heptapeptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-OMe, the molecule forms a right handed helix over the segment residues 1-5. Aib6 adopts an  $\alpha_L$  conformation resulting in helix termination (Fig. 7B) [32]. Extension of this sequence to the level of a 10-residue peptide by addition of the tripeptide sequence <sup>D</sup>Ala-<sup>D</sup>Leu-Aib-OMe results in an extension of the N-terminal helical segment with termination occurring by Schellman motif formation with <sup>D</sup>Ala8 adopting an  $\alpha_L$  conformation (Fig. 7C) [33]. This example illustrates the energetic advantage of propagating the helix with concomitant formation of intramolecular hydrogen bonds and the propensity of the <sup>D</sup>Ala residue to adopt  $\alpha_L$  conformation. Unexpectedly, the structure of this peptide also revealed an interesting chain reversal stabilized by the formation of a C-H...O hydrogen bond between the Ala4 C $\alpha$ H and <sup>D</sup>Leu9 C=O groups. This structural feature is a consequence of

the extended conformation at the  $^D\text{Leu}9$  residue with a positive value of  $\phi$  and a negative value of  $\psi$ . The generality of this structural feature has been confirmed in the crystal structure of the decapeptide analog, where Ala4 is replaced by Gly. Most importantly, replacement of  $^L\text{Ala}4$  by  $^D\text{Ala}4$  or Aib4 results in a completely different peptide conformation, establishing the importance of the C-H...O interaction in stabilizing this conformational feature [34]. A similar structural motif has also been identified in proteins [35].

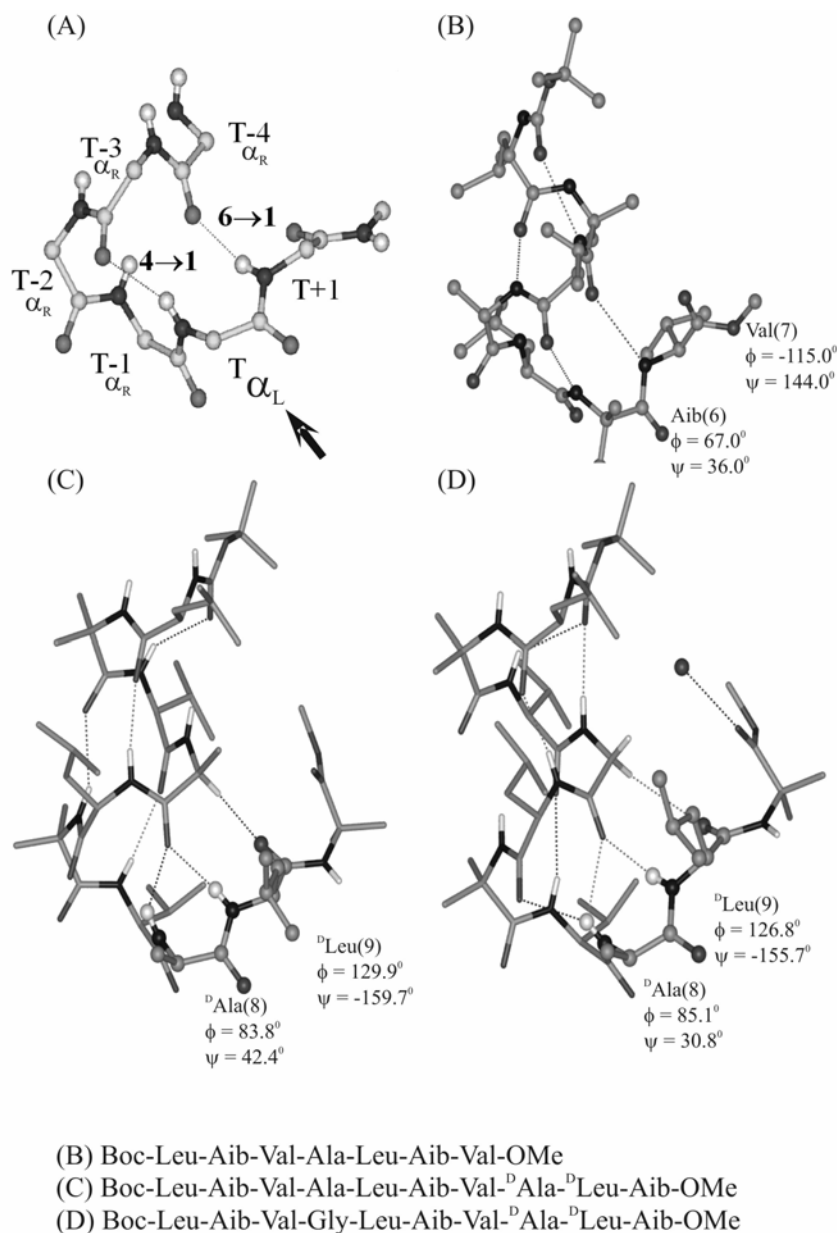


Figure 7. (A) Helix-terminating Schellman motif obtained when the terminal residue (T) adopts an  $\alpha_L$  conformation (indicated by an arrow). A 4 $\rightarrow$ 1 and 6 $\rightarrow$ 1 hydrogen bond formation stabilizes this motif [32]. (B) Crystal structure of a heptapeptide forming the Schellman motif with Aib6 adopting an  $\alpha_L$  conformation [32]. (C and D) Decapeptides containing D-amino acid residues, which function to signal helix termination [33, 34]. Figures (A), (B), (D) reprinted with permission from [12]; Copyright Indian Academy of Sciences. Figure (C) reprinted with permission from [34]; Copyright (2003) American Chemical Society.

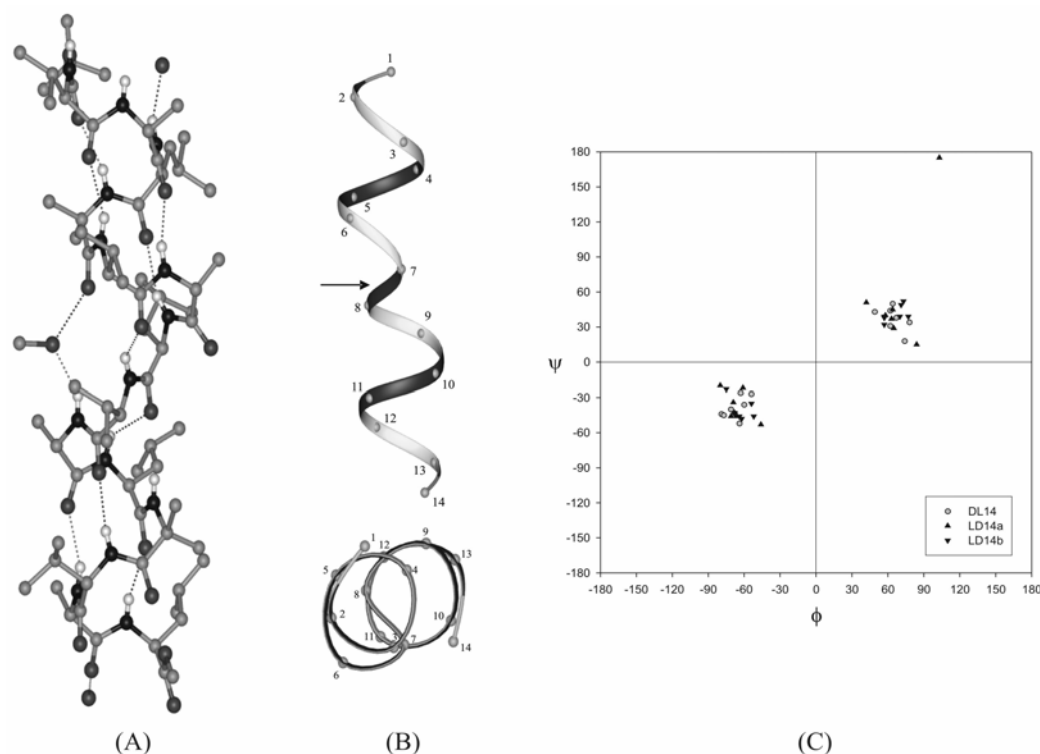


Figure 8. Ambidextrous helices formed by fusing left- and right-handed helical segments. (A) Ball-and-stick notation of a 14-residue peptide Boc-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (DL14) [36]. The DL junction, indicated by an arrow bears the Schellman motif. (B) Ribbon diagram of the peptide viewed along the helix axis and down the helix axis. (C) Plot of the  $\phi$ - $\psi$  values obtained from the crystal structures of two ambidextrous helices. One of the peptides (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-OMe, LD14) crystallized in two polymorphic forms [37]. Figures (A) and (B) reprinted from [12]; Copyright (2003) Indian Academy of Sciences.

The ability of D-amino acid residues to facilitate Schellman motif mediated helix termination may be used to advantage in creating ambidextrous structures, which consist of directly fused right-handed and left-handed helical segments. Fig. 8 illustrates the structure of a 14-residue peptide (Boc-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe) (DL14), which consists of two distinct heptapeptide segments containing amino acid sequences of opposite chirality [36]. The achiral Aib residue at positions 4 and 11 in the sequence nucleates helix formation with the handedness of the helix being determined by the configuration of the flanking amino acid sequences. Careful inspection of the crystal structure in Fig. 8 reveals that the molecule is composed of two distinct helical segments; a left-handed helical segment for residues 1-7 and a right handed helical segment for residues 8-14. Residue 8, which adopts an  $\alpha_R$  conformation, acts as the helix terminating residue with respect to the N-terminal *left handed* helical ( $\alpha_L$ ) segment. The Schellman motif is distorted by the insertion of a methanol molecule into the 6→1 hydrogen bond. The robustness of this fold is further confirmed by the crystal structure of the enantiomeric peptide (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-<sup>D</sup>Val-<sup>D</sup>Ala-<sup>D</sup>Leu-OMe) (LD14), which curiously crystallized in a polymorphic form containing two molecules in the crystallographic asymmetric unit [37]. Comparison of the  $\phi$ - $\psi$  values (Table AII of [37]) confirms the similarity of the folding pattern of these sequences containing separate blocks of L- and D-amino acids.

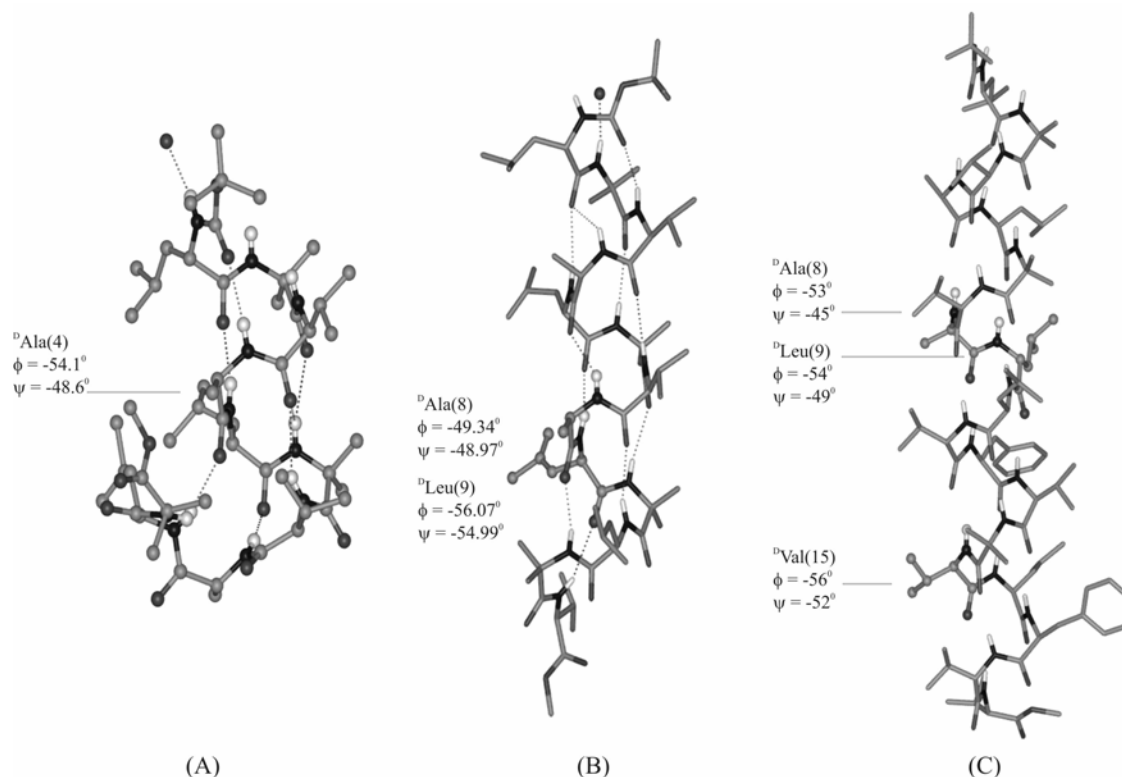


Figure 9. Examples of peptide helices containing D-amino acids incorporated in the right handed helical scaffold. (A) One D-amino acid in the right-handed helix (Boc-Leu-Aib-Val-<sup>D</sup>Ala-Leu-Aib-Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-OMe) [34]. (B) Two D-amino acids in the right-handed helix (Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-Leu-Aib-Val-OMe) [40]. (C) Three D-amino acids in the right-handed helix (Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Leu-Val-Phe-Val-Aib-<sup>D</sup>Val-Leu-Phe-Val-Val-OMe) [41]. Figures (A) and (B) reprinted from [12]; Copyright (2003) Indian Academy of Sciences.

## 5. GUEST D-AMINO ACIDS IN RIGHT HANDED HELICAL SEGMENTS

In the synthesis of analogs of biologically active peptides, it is often desirable to introduce D-amino acids into host sequences composed of L-amino acids. The site of D-amino acid insertion becomes resistant to proteolytic cleavage, thereby resulting in greater *in vivo* stability of such analogs [38]. In designing analogs, it is necessary to retain the conformational features, which are critical for biological activity. Thus, the site of D-amino acid replacement must be carefully chosen in order to minimize the possibility of major conformational changes. The helix is one of the two most widely distributed secondary structures in peptides and proteins. It is therefore instructive to examine the consequences of inserting D-amino acids into the center of potential helical sequences. Inspection of the conformational maps in Fig. 1B clearly establishes that there is a small 'allowed window' for D-amino acids in the  $\alpha_R$  region of conformational space, suggesting that, in principle, D-amino acids can be accommodated into *right-handed* helical segments. The relatively small energy penalty for adopting positive  $\phi$  values can be readily offset by compensating interactions. Fairman *et al* have estimated the helix-forming propensity of D-residues in right handed  $\alpha$ -helices and find a penalty of 0.95kcal/mol for D-Ala when compared to the L-Ala standard [39]. Fig. 9 shows examples of synthetic peptide helices in which D-amino acids have been incorporated without any disruption of the right handed twist of the polypeptide chain. In the decapeptide (Boc-Leu-Aib-Val-<sup>D</sup>Ala-Leu-Aib-Val-<sup>D</sup>Ala-<sup>D</sup>Leu-Aib-OMe), the <sup>D</sup>Ala residue at position 4 adopts an  $\alpha_R$  conformation and is

incorporated into the right-handed helix formed by the segments 1-7. In this case, the observed  $\phi$ - $\psi$  values for the  $^D\text{Ala}$  residue in the two crystallographically independent molecules are  $-46.6$ ,  $-52.9$  and  $-54.1$ ,  $-48.6$  respectively [34]. The 13-residue peptide (Boc-Leu-Aib-Val-Ala-Leu-Aib-Val- $^D\text{Ala}$ - $^D\text{Leu}$ -Aib-Leu-Aib-Val-OMe) provides an interesting example of a double D-segment ( $^D\text{Ala}_8$ ,  $^D\text{Leu}_9$ ) incorporated into a right-handed helix [40]. The crystal structure of the 19-residue peptide (Boc-Leu-Aib-Val-Ala-Leu-Aib-Val- $^D\text{Ala}$ - $^D\text{Leu}$ -Leu-Val-Phe-Val-Aib- $^D\text{Val}$ -Leu-Phe-Val-Val-OMe) is a dramatic example of the accommodation of as many as three D-amino acids into a right-handed helical scaffold [41]. These examples clearly emphasize the fact that right-handed helices can readily accommodate D-residues. Selective D-amino acid substitution in strongly helical sequences may provide a convenient means of enhancing proteolytic stability, without significant disruption of overall secondary structure.

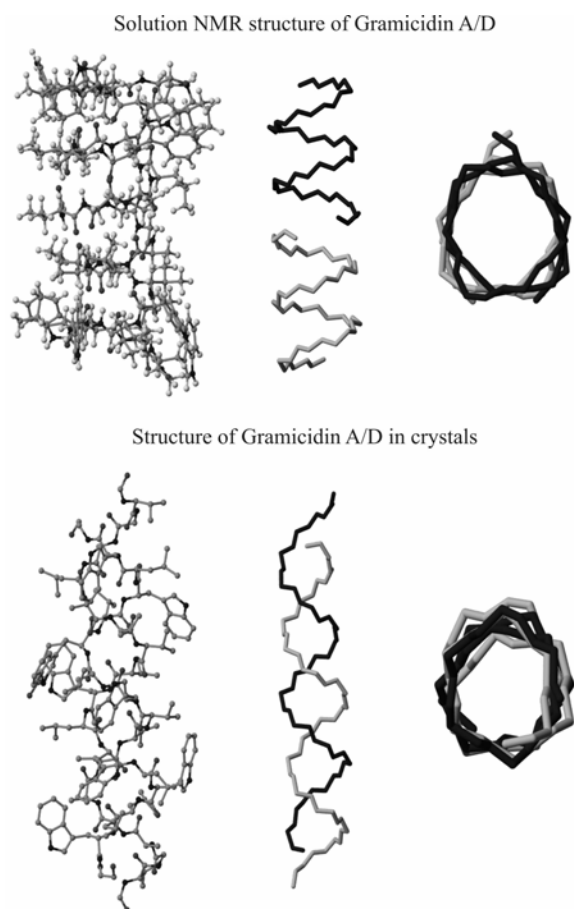


Figure 10. LD peptide helices of gramicidin. Sequence: For- $^L\text{Val}$ -Gly- $^L\text{Ala}$ - $^D\text{Leu}$ - $^L\text{Ala}$ - $^D\text{Val}$ - $^L\text{Val}$ - $^D\text{Val}$ - $^L\text{Trp}$ - $^D\text{Leu}$ - $^L\text{Trp}$ - $^D\text{Leu}$ - $^L\text{Trp}$ -Eta. Shown are the NMR- and X-ray derived structures of gramicidin A/D (PDB coordinates 1JNO [45] and 1ALZ [47], respectively). Note that although the gramicidin structures have been named 'A' and 'D', their sequences are essentially the same. Interestingly, the NMR structure reveals a  $\pi_{LD}$  helical structure for the peptide, whereas the crystal structure is a double helix.

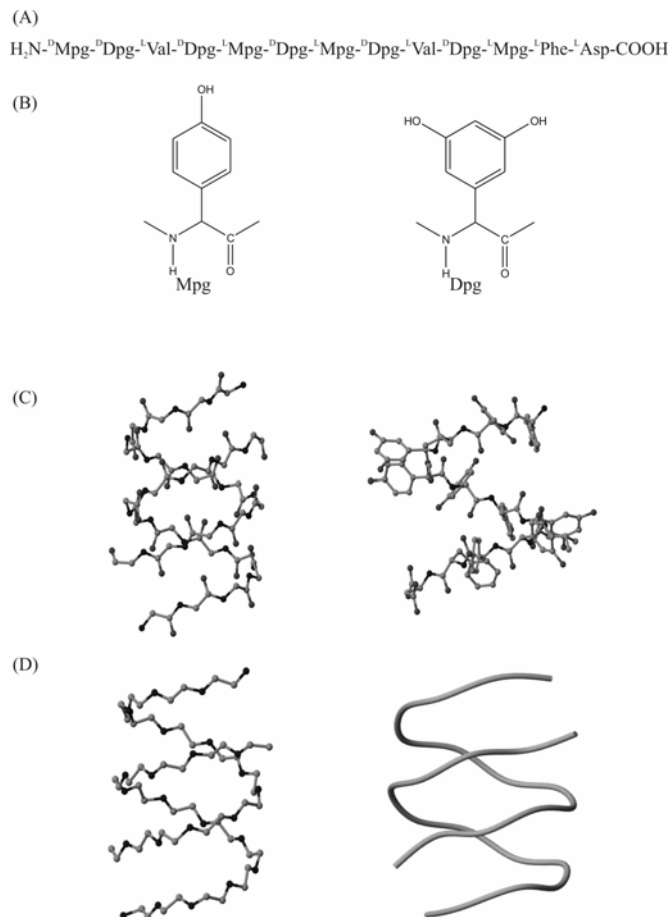


Figure 11. Feglymycin (A) sequence (B) chemical structure of the unnatural amino acids (C) different views of the double helix. Also shown is the ball-and-stick notation of the single molecule. PDB code: 1W7Q [48].

## 6. ALTERNATING LD SEQUENCES

Any discussion of the conformational effects of D-residues in peptide sequences will be incomplete without a consideration of LD polypeptides. Regular alternation of configuration is observed in the linear gramicidins, which are membrane channel forming polypeptides produced by *Bacillus brevis*. Early theoretical studies suggested that alternating LD sequences would adopt wide-bore helical structures in which an approximately extended conformation of the polypeptide was wound around a helical axis. The  $\pi_{\text{LD}}$  ( $\text{LD}_n$  helices, where  $n=3,4,5$ ) helices so generated may be viewed as helices formed by repeating LD dipeptides, in which the two residues adopt  $\beta$ -sheet conformations that lie on opposite sides of the  $\phi$ - $\psi$  map. The helices so formed are stabilized by parallel  $\beta$ -sheet hydrogen bonds between segments of the polypeptide chain on adjacent helical turns [42]. The NMR study of Gramicidin A provides confirmatory evidence for the  $\pi_{\text{LD}}$  helix (Fig. 10A) [43-45]. In crystals, an interesting variant of the  $\beta$ -helical conformation is observed with two antiparallel chains forming a double helical structure (Fig. 10B) [46, 47]. The unique structures formed by alternating LD sequences are also highlighted by a recent structure determination of the 13-residue peptide Feglymycin [48]. In this case also, an antiparallel double helical structure is formed (Fig. 11). Fig. 12 summarizes the distribution of  $\phi$ - $\psi$  values observed for the alternating LD segments. The LD helices are characterized by the formation of a central pore, which can permit passage of cations, a property that is of functional significance in these natural peptides [49].

## 7. CONCLUSIONS

D-amino acids can be effectively used in peptide design since their conformational preferences can facilitate formation of specific folded structures.  $\beta$ -Turn formation is favored in dipeptide sequences of alternating configuration (LD peptides).  $^D\text{Pro}$ , a constrained residue, can be used to force the formation of prime turns (type II'/I'), which in turn serves as nuclei for antiparallel  $\beta$ -hairpin formation in designed peptides. Multiple  $^D\text{Pro-Xxx}$  segments may be used to promote the formation of multistranded  $\beta$ -sheets. D-amino acids can also be used to mimic helix termination signals like the Schellman motif. Peptides containing building blocks of L- and D- amino acids can be induced to form ambidextrous helices in which right- and left-handed segments are fused. Single D-amino acids and double D-segments may be incorporated into synthetic peptides forming right-handed helical structures. The energy penalties for insertion of a D-residue into a right-handed helix are small and readily compensated by minor structural alterations. Alternating LD sequences provide a special example of the formation of  $\beta$ -helices, which may be viewed as structures generated by winding an extended polypeptide chain around a cylinder. Defining the role of D-amino acids on peptide conformations is of importance in the rational design of analogs of biologically active peptides, which may possess a high degree of resistance to proteolysis.

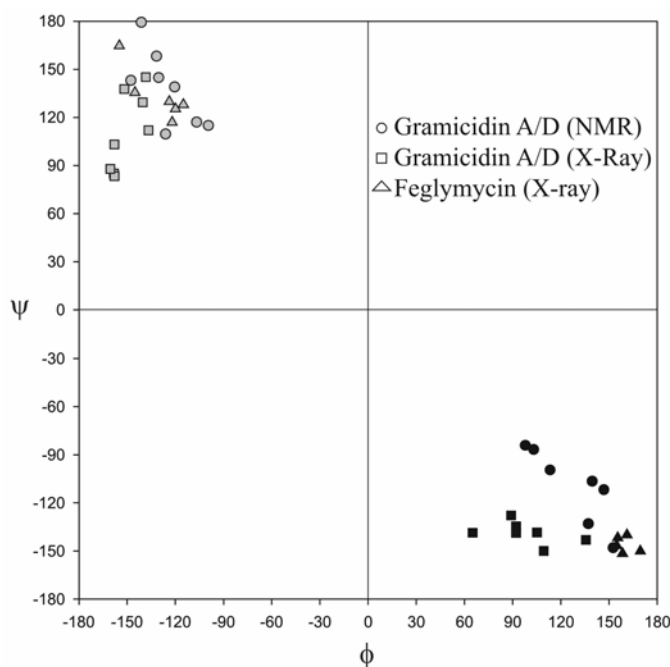


Figure 12. Plot of the observed  $\phi$ - $\psi$  values for the two structures of gramicidin A/D and the crystal structure of feglymycin. It is evident that the dihedral angles of the individual residues populate the extended region of the  $\phi$ - $\psi$  map, although the overall structure formed is helical.

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