Designing of peptides with left handed helical structure by incorporating the unusual amino acids

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The conformational behaviour of ΔAla has been investigated by quantum mechanical method PC-IL in the model dipeptide Ac-ΔAla-NHMe and in the model triptides Ac-X-ΔAla-NHMe with X=Gly, Ala, Val, Leu, Abu and Phe and is found to be quite different. The computational results suggest that in the model triptides the most stable conformation corresponds to φ=−30°, ψ=120° and φ=ψ=30° in which the >C=O of the acetyl group is involved in hydrogen bond formation with N-H of the amide group. Similar results were obtained for the conformational behaviour of Δ-Ala in Ac-Δ-Ala-NHMe and Ac-Ala-Δ-Ala-NHMe.

The conformational behaviour of the amino acids ΔAla, Δ-Ala, Val and Aib in model triptides have been utilized in the designing of left handed helical peptides. It is shown that the peptide HCO-(Ala-Δ-Ala)-NHMe can adopt both left and right handed helix whereas in the peptide Ac-(Ala-ΔAla),-NHMe the lowest energy conformer is β-bend ribbon structure. Left handed helical structure with φ=−30°, ψ=60° for Δ-Ala residues and φ=ψ=−30° for Ac-Ala is found to be more stable by 4 kcal mol$^{-1}$ than the corresponding right handed helical structure for the peptide Ac-(Δ-Ala-ΔAla),-NHMe. In both the peptides Ac-(Val-ΔAla),-NHMe and Ac-(Val-Δ-Ala),-NHMe the most stable conformer is the left handed helix. Comparisons of results for Ac-(Δ-Ala-ΔAla),-NHMe and Ac-(Δ-Ala-ΔAla),-NHMe also reveal that the Val residues facilitate the population of 3 left handed helix over the other conformers. It is also shown that the conformational behaviour of Aib residue depends on the chirality of neighboring amino acids, i.e. Ac-(Aib-Δ-Ala),-NHMe adopts right handed helical structure whereas Ac-(Aib-Δ-Ala),-NHMe is found to be in left handed helical structure.

Of great interest in research in peptide chemistry is the rational design and synthesis of natural peptide analogs highly potent, selective and metabolically stable and macromolecular systems with tailor-made structural and functional properties. Conformational preferences are dictated to greater extent by interaction of the atoms of a given side chain with atoms of the backbone as well as the atoms of the neighboring peptide units. In other words, the short range interactions are very important in proteins and the peptides and can adopt a large number of conformations. Therefore, in molecular design, it is necessary to restrict the number of conformations to a minimum. To this end, substitution of non-coded for coded amino acids using α-β dehydropeptides, both natural and resulting from the modification of saturated bioactive peptides with α-β dehydroamino acid residues have been studied. These have attracted increasing interest and frequently occur in natural peptides e.g. in antibiotics, toxins and alkaloids. The commonly occurring dehydroamino acids are dehydroalanine (ΔAla), dehydrovaline (ΔVal), dehydroleucine (ΔLeu), dehydrophenylalanine (ΔPhe), dehydrotryptophan (ΔTrp), dehydroproline (ΔPro) and dehydroaminoiso-butyric acid (Abu) and these play an important role in influencing the backbone conformations to varying magnitude so that stable folded structures are produced. Accumulation of functions i.e. the rigid groups, the amino and carboxyl group and the C=C double bond at α position of an unsaturated residue have considerable chemical, physico-chemical and stereochemical consequences including increased resistance to enzymatic degradation. Consequently, these amino acids can provide conformational constraint on the peptide backbone and restrict the orientation of the side chain β-substituents. In this paper we present work on peptide design using α,β-dehydroamino acid residues and D-amino acids.

The helix forming peptides have recently been shown to form cation selective ion channels across the biological membranes. Therefore, peptides with left handed helical structure have been designed...
to increase the resistance to hydrolytic enzymatic degradation. The basis for designing the membrane active peptides has been the careful analysis of the (\( \phi \), \( \psi \)) energy maps of individual amino acids residues (both usual and unusual) and also in peptides. This study deals with the conformational behaviour of the model tripeptides of the form Ac-X-\( \Delta \)Ala-NHMe with \( X=Gly, Ala, Leu, Val, Abu \) and Phe; Ac-Ala-D-Ala-NHMe and the model heptapeptides- Ac-(L/D-Ala-\( \Delta \)Ala)-NHMe, Ac-(Ala-Ala)-NHMe or HCO-Ala-D-Ala)-NHMe, Ac- (L/D-Val-\( \Delta \)Ala)-NHMe and Ac-(Aib-\( \Delta \)Ala)-NHMe in order to mimic the role of \( \Delta \)Ala and D-amino acids residues. Further, the objective in carrying out this study is based on the fact that gramicidin A is a hydrophobic pentadecapeptide containing L and D amino acids in alternate positions\(^{19,20}\) and the D-amino acids can be substituted by corresponding \( \Delta \)-amino acids residues.

**Computational method**

The geometry of Ac-\( \Delta \)Ala-NHMe was optimized by using potential function of the type suggested by Vinter et al.\(^2^2\) and the optimized bond lengths and bond angles, thus obtained were used in the construction of \( \phi, \psi \) maps in peptides. It must be pointed out that bond angles and bond lengths were in agreement with the crystallographic data on dehydro residues\(^2^3\). Standard bond lengths and bond angles were used for the usual and D-amino acid residues. The energy calculations were performed by the quantum mechanical method, PCIL0\(^2^4\) (Perturbative Configuration Interaction using Localized Orbitals) on VAX-VMS system. The torsional angle corresponding to the cis-planar arrangement in the molecules is taken as zero. The torsional angles of the peptide geometry are taken as trans i.e. 180°. The computations were carried out by systematic variation of two torsional angles (increment between two successive calculations being 30°). The possible \( \chi \) values for usual amino acids were adopted by constructing one dimensional potential energy curves and two dimensional conformational energy maps and then, the rotations around the backbone of the peptides were performed to determine the overall conformation. The isoenergy curves are presented upto 5 kcal/mol above the global energy minimum. It is worth mentioning here that the energy minima in the computational results at the ab initio level for usual amino acids by Lawrence and Thompson\(^2^5\) and for dehydroalanine residue by Aleman\(^2^6,2^7\) are also minima in the PCIL0 calculations\(^2^8,2^9\). Further, the PCIL0 results\(^2^8\) for di and tripeptides containing usual amino acid residues are also in conformity with those of ab initio results by Wiener\(^2^6\), Hoffmann\(^3^1\) and knowledge based crystallographic data\(^3^2-3^4\). Therefore, the conformational states have been generated from the knowledge of global, local and low energy minima in the \( \phi, \psi \) and \( (\chi_\alpha, \chi_\beta) \) energy maps for individual amino acids and from the knowledge of minima in energy maps for amino acid residues in tripeptides. The energies of the generated conformational states have been computed and only the energies for the first few most stable conformations are reported.

**Results and Discussion**

**Conformational behaviour of \( \Delta \)Ala and D-Ala \( \Delta \)Ala**

The geometry of Ac-\( \Delta \)Ala-NHMe was optimized and the various bond lengths and bond angles were found to be in agreement with that of X-ray crystallographic data\(^3^3\). The \( \phi, \psi \) energy map for \( \Delta \)Ala is shown in Fig. 1a. In this conformational energy map the global energy minimum corresponds to the extended structure with \( \phi=\psi=180°\) and is surrounded by isoenergy curves upto 5 kcal/mole. The low energy regions at 0°,±90° lie -3 kcal/mol and the left and right handed helical regions are -4 kcal/mol above the global energy minimum. The NMR studies\(^5,3^5\), crystallographic results\(^3^6-3^8\), \( ab \) initio\(^3^7,3^1\), semi-empirical quantum mechanical methods\(^2^8\) and molecular mechanics results\(^3^9\) are in conformity with the extended structure.

Due to the absence of an asymmetric centre, the \( \phi \), \( \psi \) energy map for \( \Delta \)Ala in Ac-\( \Delta \)Ala-NHMe is symmetric. Therefore, it is logical to explore whether the conformational behaviour of \( \Delta \)Ala when incorporated in the peptides, remains the same or is altered, i.e the effect of chirality of the adjacent amino acid residue. Therefore, the conformational behaviour of model tripeptides Ac-X-\( \Delta \)Ala-NHMe with \( X=Gly, Ala, Val, Leu, Abu \) and Phe, have been investigated. In the \( \phi, \psi \) energy maps for Ala, Val, Leu, Abu and Phe, the global energy minimum lies at -30°, 120° and the local or low energy minima correspond to right handed helical region. The conformational structure with \( \phi=30°\), \( \psi=120°\) for usual amino acids is well established and is preferred over helical structure for peptides containing a maximum of 4 amino acid residue (un-
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Fig. 1—φ,ψ Conformational energy maps for ΔAla. (a): in model peptide Ac-ΔAla-NHMe, the global energy minimum corresponds to the extended structure and (b): in tripeptide, Ac-Ala-ΔAla-NHMe, the global energy minimum lies at φ₂ = ψ₂ = 30° [The conformational behaviour of ΔAla in the model tripeptide is different from the model dipeptide]

published results). This structure is generally found on the surface of proteins stabilized by intermolecular hydrogen bond formation with water molecules 29,34,40.

The conformational behaviour of ΔAla in all the above mentioned peptides (except Ac-Gly-ΔAla-NHMe) was found to be similar with the global energy minimum at φ = ψ = 30° as shown in Fig. 1b. It is apparent from the energy maps in Fig. 1 that the conformational behaviour of ΔAla in the model dipeptide Ac-ΔAla-NHMe and in the model tripeptides Ac-X-ΔAla-NHMe is quite different. However, the conformational behaviour of ΔAla in Ac-Gly-ΔAla-NHMe is almost similar as in the model dipeptide Ac-ΔAla-NHMe. The computational results suggest that these peptides are conformationally labile and the most stable conformation corresponds to φ₁ = -30°, ψ₁ = 120° and φ₂ = ψ₂ = 30° in which the >C=O of the acetyl group is involved in the hydrogen bond formation with N-H (N-O distance being 2.7 Å) of the amide group (Fig. 2).

D-Ala

The φ,ψ maps for D-Ala in Ac-D-Ala-NHMe and Ac-Ala-D-Ala-NHMe are shown in Fig. 3. Despite the similarities, the φ,ψ map for D-Ala reveals interesting features i.e. (i) The propensity or tendency of D-Ala residue to be in the extended structure decreases in peptides (ii) the global energy minimum for D-Ala in Ac-D-Ala NHMe changes from φ = 30°, ψ = -120° to φ = 30°, ψ = 60° in Ac-Ala-D-Ala-NHMe and the low energy minimum at φ = 90°, ψ = -60° is changed to local energy minimum in Ac-Ala-D-Ala-NHMe,
i.e. the global energy minimum lies in the left handed helical region for D-Ala in peptides.

Global minima for Δ-Ala and D-Ala in model tripeptides has been explored in the designing of left handed helix and is also being investigated in the designing of an amphipathic and in particular the left handed amphipathic helix. The amphipathic helix has been envisaged as the fusogenic structure in bringing the fusion of vesicles\textsuperscript{40-41}. It is apparent from Fig. 1 that the \((\phi, \psi)\) energy map for ΔAla in Ac-ΔAla-ΔAla-NMe is more restricted than the \((\phi, \psi)\) energy map for D-Ala in Ac-D-Ala-D-Ala-NMe. This may suggest that the driving capacity for left handed structure may be more for ΔAla than D-Ala residue but it is shown to be dependent on the nature of usual amino acid preceeding D-Ala and ΔAla.

**Conformational studies of model heptapeptides**

Gramicidins are linear pentadecapeptides with uncharged and hydrophobic amino acid of alternating chirality which is produced by the aerobic soil bacterium *Bacillus brevis*\textsuperscript{19-21}. The N-terminal octapeptide of gramicidin A has the sequence HCO-Val-Gly-Ala-D-Leu-Ala-D-Val-D-Val-NHMe. Therefore, conformation of the model peptides, Ac-(ΔAla-ΔAla)$_2$-NHMe, HCO-(ΔAla-D-Ala)$_2$-NHMe or HCO-(Ala-D-Ala)$_2$-Ala-NHMe, Ac-(D-Ala-Aib)$_2$-NHMe, Ac-(Aib-D-Ala)$_2$-NHMe, Ac-(Val-Δ-Ala)$_2$-NHMe and Ac-(D-Val-A-Ala)$_2$-NHMe have been studied by utilizing the information obtained from the \((\phi, \psi)\) and \((\chi_1, \chi_2)\) energy maps for global, local and low energy minima of various amino acids in model dipeptide and tripeptides. The energy calculations for all the peptides have also been carried out by taking \(\phi, \psi\) values for regular secondary structures.

The computational results for some of the stabilized structures with respect to the extended state for the various peptides are summarized in Table I. For the peptide HCO-(ΔAla-D-Ala)$_2$-Ala-NHMe, there is only a difference of \(-1.3\) kcal mol\(^{-1}\) between the left and right handed helical structures. This is due to the imperfect alternating chirality of amino acid, at the C-terminal end. This has been checked by performing computations for the peptide HCO-(Ala-D-Ala)$_2$-NHMe. The helical structures, both the right and left handed, are the most stabilized. Here, it may be pointed out that in Ac-(Ala)$_n$-NHMe, the right handed helical structure is favoured over the left handed helical structure whereas it is the other way in Ac-(D-Ala)$_n$-NHMe but the absolute energy difference in the two cases, i.e. Ac-(Ala)$_n$-NHMe and Ac-(D-Ala)$_n$-NHMe for left and right handed helices is the same.

For the tripeptide, Ac-Ala-ΔAla-NHMe as mentioned above, the lowest energy conformation corresponds to \(\phi_1=30^\circ, \psi_1=120^\circ\) and \(\phi_2=\psi_2=30^\circ\) in which >C=O of the acetyl group and NH of the amide group
are involved in hydrogen bond formation. Therefore, to understand whether the same structure is found/favoured for Ac-(Ala-ΔAla)₃-NHMe or not, various conformational states have been generated and the most stable structure is found to be the one with \( \phi=30^\circ, \psi=120^\circ \) for Ala residues and \( \phi=\psi=30^\circ \) for ΔAla residues. In this structure, alternate peptide bonds are involved in the intramolecular hydrogen

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The energy of stabilization, \( \Delta E \), is with respect to the corresponding extended geometry.
bond formation resulting in the formation of three hydrogen bonds between the >C=O of ith residue and NH of i+3th residue (Fig. 4). But this structure is different from \( \beta \)-helical structure and has been named as \( \beta \)-bend ribbon structure as the \( \phi, \psi \) values of \(-30^\circ, 120^\circ\) is observed in the \( \beta \)-bend type-II structure\(^45\). In other words, there is some regular pattern. The distance between N and O of i+3th and ith residues in \( \beta \)-bend ribbon structure and \( \beta \)-helices\(^46\) are 2.70 Å and 2.76 Å, respectively. Involvement of alternate peptide bond in intramolecular hydrogen bond formation implies that half of the peptide bond may be involved in intermolecular hydrogen bond formation between the \( \beta \)-ribbon strands/structures leading to aggregation. The resulting structure will also have the characteristics of \( \beta \)-helical structures. NMR studies\(^47-57\) do suggest the formation of some type of ordered structure in peptides containing dehydroamino acid residues but does not distinguish between \( \beta \)-helical structure and the ten membered ring structure named as \( \beta \)-bend structure.

In the peptide HCO-(D-Ala-D-Ala)-NHMe, the lowest energy conformers are both left and right handed helices whereas in peptide, Ac-(Ala-DAla)-NHMe, the lowest energy conformer is \( \beta \)-bend ribbon structure and the left handed helix being \(-5\) kcal/mol less stable. Thus, utilizing the conformational behaviour of D-Ala and D-Ala in model tripeptide, the peptide Ac-(D-Ala-DAla)-NHMe has been designed. For this peptide, the lowest energy conformer is found in the left handed helical region with \( \phi=30^\circ, \psi=60^\circ \) for D-Ala residues and \( \phi=\psi=30^\circ \) for \( \Delta \)Ala residues and \( \phi=\psi=30^\circ \) for D-Ala residues and a view of the peptide in this state is shown in Fig. 5a. The right handed helical structure with \( \phi=-30^\circ, \psi=-60^\circ \) for D-Ala residues and \( \phi=\psi=-30^\circ \) for \( \Delta \)Ala residues is less stable by 4 kcal mol\(^{-1}\) and the \( \beta \)-bend ribbon structure is less stable by 5 kcal mol\(^{-1}\) than the left handed helical structure. Crystallographic results for the peptide are also in support of this finding\(^58\).

The other left handed helical designed peptide which is predicted to be the most stable in the \( \beta \)-helical structure are Ac-(Val-\( \Delta \)Ala)-NHMe and Ac-(D-Val-\( \Delta \)Ala)-NHMe. The Val and D-Ala residues adopt \( \phi, \psi \) values of \((30^\circ, 60^\circ)\) and \((30^\circ, 30^\circ)\) in the peptide Ac-(Val-\( \Delta \)Ala)-NHMe whereas in the peptide Ac-(D-Val-\( \Delta \)Ala)-NHMe D-Val and \( \Delta \)Ala were found to adopt \( \phi, \psi \) values of \((49^\circ, 26^\circ)\) and \((30^\circ, 30^\circ)\) respectively. The \( \beta \)-bend ribbon structure and the right handed helix are found to be less stable by \(-12\) kcal mol\(^{-1}\) than the left handed helical structure. The guiding principle in the design of this peptide has
been the careful analysis of \( \phi, \psi \) energy map for Val and D-Ala residues. The global energy minimum for Val residues in Ac-Val-NHMe is at -30°, 120° with low energy minimum at 0°, 90°. It is worth mentioning here that on the energy scale, the left-handed helical structures for the peptides, Ac-(D-Val-Mla)-NHMe and Ac-(Val-Mlak)-NHMe exactly have the same energy but the extended state for Ac-(D-Val-Mlak)-NHMe is more stable than the extended state of Ac-(Val-Mlak)-NHMe. In Table 1, \( \Delta E \) values are depicted with respect to the extended structures. The same energy for the two peptides is due to the equivalent disposition of the side chain of Val residues with respect to the similar and light group at C\( \beta \) position in D-Ala in the left-handed helical structures. The stability of left-handed helical structure with respect to the corresponding extended structure in the peptide Ac-(Val-D-Ala)-NHMe and Ac-(Val-D-Ala)-NHMe clearly reveals that the Val residues facilitate the population of left-handed helix over the other conformers. It may be pointed out here that for the heptapeptides, Ac-(Leu/Abu-D-Ala)-NHMe, the lowest energy conformation is the \( \beta \)-bend ribbon structure not the left-handed helical structure.

The preference of 3\(_{10}\) helix for Aib homooligopeptide is well established by X-ray diffraction studies and by energy calculations. Oligopeptides less rich in Aib have tendency to fold their backbone in the right-handed \( \alpha \)-helix or in a mixed \( \alpha/3_{10} \) helix conformation depending on the main chain length, the Aib content and the specific amino acid sequence. There is hardly any study for peptides containing both Aib and D-amino acids. The \( \phi, \psi \) map for \( \alpha \)-amino isobutyric acid (Aib) residue in Ac-Aib-NHMe shown in Fig. 6 is symmetrical with global minimum at \( \phi = 180° \) and degenerate local minima at \( \phi = \pm 30°, \psi = \pm 60°; \phi = \pm 30°, \psi = \pm 120° \). This implies that the Aib residues have the equal propensity with \( \phi, \psi \) value in helical regions, i.e., left or right handed. Therefore, computations have been carried out for the model heptapeptide Ac-(Aib-D-Ala)-NHMe for various conformations. The results presented in Table 1 clearly reveal that the most stable conformation is the 3\(_{10}\) left-handed helix. Energy of stabilization (\( \Delta E \)) with respect to the corresponding extended states are the same in the two peptides but on the energy scale, peptide, Ac-(Aib-D-Ala)-NHMe is more stable in all the conformational states by ~35 kcal mol\(^{-1}\) than the corresponding conformational states in Ac-(D-Ala-Aib)-NHMe. A graphical view of these peptides shown in Fig. 5b and 5c in the left-handed helical structure also support this observation. This implies that the Aib residue at the first position stabilizes the peptide more effectively and this may account why Aib is found at the first position in some of the peptibol antibiotics-Alamethacin, Hypelein A, Trichotoxins and Suzukacin\(^{64}\). Thus, the conformational behaviour of Aib residue depends on the chirality of neighbouring amino acids.

**Energy minimization**

The conformations within 6 kcal/mole of the most stable conformation have also been subjected to energy minimization by molecular mechanics method, using AMBER software for the peptides Ac-(Ala-D-Ala)-Ala-NHMe and Ac-(Ala-D-Ala)-Ala-NHMe. The molecular mechanics results favours the \( \alpha \)-helix, both right-handed and left-handed helix for the peptide Ac-(Ala-D-Ala)-Ala-NHMe and the left-handed \( \alpha \)-helix for the peptide Ac-(Aib-D-Ala)-Ala-NHMe irrespective of the starting conformational structure whether 3\(_{10}\) or \( \alpha \)-helix. Further, the order of stability of the various conformational structure obtained by quantum mechanical (PICLO) and molecular mechanics results remains unaltered. It is worth mentioning, that the molecular mechanics calculations at all the dielectrics do not favour the 3\(_{10}\) helical structure even for the
well studied: Ac-(Ala-Aib)-NHMe peptide. This is due to the fact, that AMBER potential shows large repulsion for hydrogen bonded structures with O-H distance less than 2 Å. On the other hand quantum mechanics results show stabilization with hydrogen bond distance of 2 Å.

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