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# Identification of Novel Stereogeometrical Features in β - Ala Residue

**Raghuvansh Kishore***
Institute of Microbial Technology, Sector - 39- A, Chandigarh - 160 036 INDIA

**Abstract** : Conformational investigations of β - alanine containing peptides, **1** and **2**, in solution as well as in crystalline state, have revealed the preferences for chirality induced stereogeometrical features which restricted conformational averaging in solution. Evidences for the influences of these conformational features on circular dichroism (CD) spectral behavior of a chiral Ala residue have been provided.

Conformational adaptability of unconstrained non- coding amino - acid, beta - alanine (β - Ala) continues to attract a considerable attention as an analog of α - amino acid[1] for the design and development of peptides incorporating novel conformational features[2]. While X- ray diffraction studies provide an unambiguous means of establishing the conformation in solid state, their conformation in solution have often been inferred and strengthened by their characteristic CD spectral patterns. In addition, the CD technique has been shown to be a powerful spectroscopic probe for evaluation of stereochemical features.

In this communication, we wish to describe the results of $^1H$ NMR, CD and crystal structure investigations on two model peptides, Boc - β - Ala - L - Ala - $NHCH_3$ , **1** and Boc - β - Ala - D - Ala - $NHCH_3$ , **2** which clearly demonstrated, for the first time, the chirality induced accommodation of stereogeometrical features in β - Ala residue which suggested to play a decisive role in maintaining the stable conformation.

The $^1H$ NMR spectral behavior of **1** and **2**, in $CDCl_3$, are clearly very similar and data do not distinguish them (data not shown), indicating similarities in their molecular geometries[3]. The observed vicinal coupling $^3J_{HN\text{-}C^{\alpha}H}$ values ~ 7.4 Hz, in $CDCl_3$, for chiral Ala residues are more consistent with $\phi_{Ala}$ values close to semi - extended conformation ($\phi \approx \pm 100 \pm 20°$) which are in agreement with the $\phi$ values $\approx \pm 116°$, determined in crystalline state[4]. The appearance of $^1H$ NMR $C^{\beta}H_2$ and $C^{\alpha}H_2$ resonances of β - Ala residue in **1** and **2**, is an indicative of $A_2M_2X$ spin system. Interestingly, similar spin system for $C^{\beta}H_2$ and $C^{\alpha}H_2$ resonances was also observed in Boc - β - Ala - NHMe and several other related peptides which appears to be a diagnostic feature of fully extended, trans - conformation : $\mu = \pm 180 \pm 20°$, involving $C^{\beta}$ - $C^{\alpha}$ rotation (manuscript under preparation).

Crystallographic analysis of **1** and **2**, indicated that the backbone of β - Ala adopts an extended conformation with dihedral angles ϕ, μ and ψ close to skew, trans and skew, respectively, while a semi - extended conformation have been characterized for chiral Ala residues[5,6], an observation fully consistent with NMR data. It may be underlined that all the backbone dihedral angles in these peptides are almost comparable in magnitudes except were of opposite signs, clearly indicating that the chirality dictates the signs of all the torsion angles of β - Ala residue. Another important observation of the crystallographic data which need to be stressed is that the β - Ala residue exhibits forbidden signs of ϕ, ψ values that may fall in the L or D - region of the Ramachandran map[7]. Interestingly, while the ϕ, ψ torsion angles of the chiral Ala residue, in **1**, are expected to fall in the L - region of the Ramachandran map, the torsion angles of β - Ala residue, unexpectedly, lie in the D - region. Similarly, the reverse trends in signs of dihedral angles for β -Ala and D - Ala residues are observed for **2**. These observations provide compelling evidences for the presence of a non - superimposable, stereogeometric feature around μ rotation, though devoid of any asymmetric element and give rise to a mirror image relationship between **1** and **2**, and therefore may be regarded as `conformational enantiomorphs' (Fig. 1).

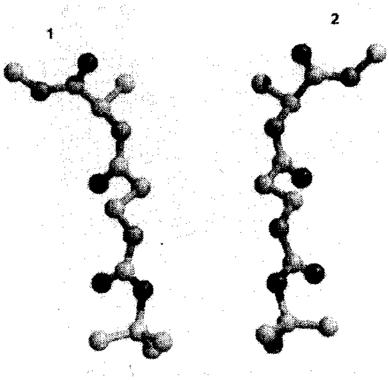

**Figure 1 :** Perspective diagrams of molecular structures of 1 (left)[4a] and 2 (right)[4b], generated from their atomic coordinates, obtained from X - ray diffraction studies, by using program RASMOL.

To investigate whether, the non - superimposable stereogeometrical features of β - Ala residue in **1** and **2** is a stable structural feature, CD measurement were made in a series of solvents of varying polarities (Table). In all the solvents studied, the CD spectra of **1** and **2**, are dominated by a strong negative band (right handed, P form) and a strong positive band (left handed, M form), respectively. Under identical conditions, the CD spectra of these peptides are almost comparable in their shapes, magnitudes and band positions, except were of opposite signs indicating similarities in their principal conformational distributions (Fig. 2).

**Table** : Circular dichroism band positions (nm) and intensities (molar ellipticity $[\theta]_M$ in deg. $cm^2$. $dmol^{-1}$.) in different solvents for **1** and **2**. (MeOH : methanol, TFE : trifluoroethanol, Dioxane : 1,4 - dioxane, TMP : trimethylphosphite). (s) Indicates shoulder

| **Solvents** | **λ(nm)** | **1** | **2** |
|---|---|---|---|
| MeOH | 198 | - 18660 | + 18120 |
| TFE | 200 | - 13630 | + 13500 |
| Dioxane | 217 | - 10840 | + 13500 |
| TMP | 220 | - 12270 | + 12690 |
| | 225 (s) | - 2710 | + 2910 |

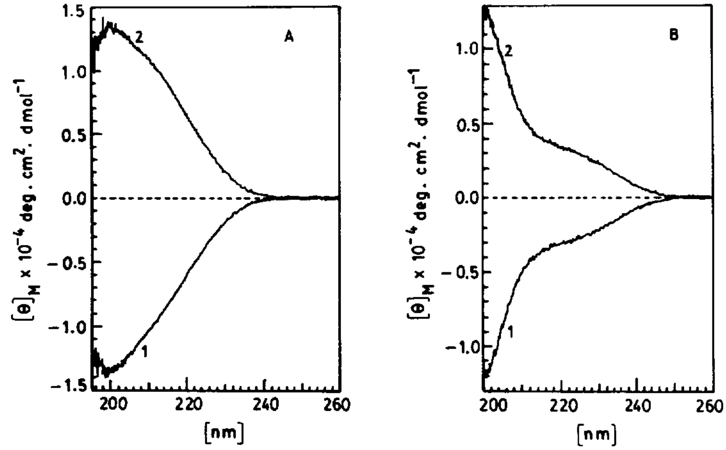

**Figure 2** : Comparisons of CD spectra of **1** and **2** in (A) TFE and (B) TMP

The CD data do not reflect conformational averaging otherwise, significantly low ellipticity value would have been expected. These observations provide a strong experimental support which suggest the retention of the trans - stereogeometrical characteristics of β - Ala residue around μ rotation, similar to those described in crystalline states. On the contrary, the CD spectra of Ac - L- Ala - $NHCH_3$ exhibited a strong solvent dependence. These CD behavior, in conjunction with IR and NMR studies, have been interpreted in terms of specific conformations and attributed to the shift of their relative populations of intramolecularly hydrogen bonded structures, from $C_5$ to $C_7$ as the apolarity of the medium increases[8]. The absence of these conformational characteristics in **1** and **2** suggest that the observed stereogeometrical features of the β - Ala residue around μ rotation are capable of influencing the conformational as well as the CD spectral behavior of chiral Ala residue.

In this investigation, we have been able to establish the presence of a novel non - superimposable stereogeometrical feature originated due to restricted rotation around $C^{\beta}$ - $C^{\alpha}$ torsion angle. Both the signs as well as the magnitudes of all the backbone dihedral angles of this unusual amino - acid residue may be influenced in a restricted manner by the chirality of neighboring residue and probably the constrained imposed. An establishment of dissymmetric conformational features of β - Ala residue would be of great importance to understand and to stimulate further interests in modelling as well as in exploring conformationally complex, unconstrained peptidomimetics containing β - Ala residue and its higher homologues[2c], in general.

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[3] **1.** White Solid, m.p. 140° C, $[\alpha]_D = + 23.9$ (c = 0.23 in MeOH), $R_f = 0.25$. **2.** White solid, m.p. 141°C, $[\alpha]_D = - 26.8$ (c = 0.22 in MeOH), $R_f = 0.25$ ($R_f$ values in 5% MeOH in$CHCl_3$). $^1H$ NMR (300 MHz, $CDCl_3$, 25°C, 10mg / ml, TMS ) **1** : $\delta = 1.37$ (d, 3H), 1.43 (s, 9H), 2.44 (t, 2H), 2.80 (d, 3H), 3.39 (q, 2H), 4.49 (m, 1H), 5.25 (t,1H), 6.68 (q, 1H) & 6.79 (d, J=7.6 Hz, 1H). **2** : $\delta = 1.37$ (d, 3H), 1.43 (s, 9H), 2.44 (t, 2H), 2.80 (d, 3H), 3.40 (q, 3H), 4.48 (m, 1H), 5.23 (t,1H), 6.62 (q, 1H) & 6.75 (d, J=7.3 Hz, 1H).

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[5] **a)** Backbone dihedral angles are defined according to the IUPAC - *IUB Commission on Biochemical Nomenclature, Biochemistry*, **1970**, *9*, 3471 **b)** I.L. Karle, B. K. Handa and C. H. Hassal, *Acta. Cryst.*, **1975**, *B 31*, 555; **c)** The backbone conformational angles for ß-Ala ($-NH-C^{\beta}H_2-C^{\alpha}H_2-CO-$) are $\phi$, $\mu$, $\psi$ correspond to $C'O-N-C^{\beta}-C^{\alpha}$,$N-C^{\beta}-C^{\alpha}-C'O$ and $C^{\beta}-C^{\alpha}-C'O-N$ rotations respectively ; **d) [4] a)** .

[6] **a)** Backbone torsion angles (°) **1.** ß-Ala : $\phi_1 = + 120.6$, $\mu_1 = -167.1$ , $\psi_1 = -118.2$ ; L-Ala : $\phi_2 = - 116.4$, $\psi_2 = + 115.8$ { **[4] a)** } **2.** ß-Ala : $\phi_1 = - 120.9$, $\mu_1 = + 167.2$ , $\psi_1 = + 117.7$ ; D-Ala : $\phi_2 = + 116.6$, $\psi_2 = - 116.2$ { **[4] b)** }.

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