The Conformational Characteristics of Dipeptides

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ABSTRACT: The conformational energies of the diastereoisomeric dipeptides L,L- and L,D-alanylalanine are calculated for the zwitterionic state, taking into account torsional and van der Waals potentials, monopole–monopole electrostatic interactions, and coulombic forces between the charged ends. The vicinal coupling $J_{N\alpha}$ of the peptide NH and C-terminal α-proton is computed for each isomer by taking an average over the calculated conformations and assuming a reasonable Karplus-like dependence of $J_{N\alpha}$ on the dihedral angle φ'. The calculated couplings approximate the values observed in aqueous solution. A recently proposed conformational model of diastereoisomeric dipeptides is discussed in the light of the present results.

L,L- and L,D-dipeptides exhibit differences in their physical properties, *i.e.*, dipole moments,[1] melting points,[2] crystalline forms,[2] gas chromatographic[2] and gel filtration[3] mobilities, and nmr spectra.[4-6] The molar polarization and infrared spectroscopic measurements carried out by Lande[2] on L,L- and L,D-α-chloropropionylalanine methyl ester indicate that the amide bond is *trans* ($\omega = 0°$ [7]) in both diastereoisomers, confirming other experimental findings for N-monosubstituted amides.[8] Therefore, the observed differences in the physical properties of such isomers are a consequence of conformational differences at the Cα—C (ψ_1,ψ_2[7]) and N—C$^\alpha$ (φ_2[7]) bonds (see Figure 1). This conclusion is supported by the hard-sphere model studies of Leach, Nemethy, and Scheraga[9] and by the excellent agreement between the measured dipole moments[1] of the 14 diastereoisomers of the di-, tri-, and tetrapeptides of alanine and the values calculated by Flory and Schimmel assuming all amide bonds to be *trans*.[10]

Lande[2] has proposed two conformations of minimum energy for each diastereoisomeric dipeptide which are consistent with all of their physical properties measured to date. In each of these conformations the α-hydrogen atoms are situated in the plane of the intervening amide group and are *trans* to each other. This proposal was based on a study of molecular models.

The purpose of the present investigation was to test these proposals by calculating the conformational energies of L,L-alanylalanine which are related by symmetry to the conformational energies of the L,D isomer. In addition, the vicinal couplings of the peptide NH and C-terminal α-H, designated $J_{N\alpha}$, were measured for each isomer and compared with the values calculated from Lande's model and from the complete contour map of conformational energy.

Method of Calculation

A schematic representation of L,L- and L,D-alanylalanine in planar zigzag form ($\varphi_2 = \psi_1 = \psi_2 = \omega = 0$; these angles assume positive values for right-handed rotations[7]) is shown in Figure 1. The rigid, *trans*-planar peptide group[11-13] restricts the conformational freedom of such dipeptides to rotations about the bonds joining the α-carbon atoms. Specification of the rotation angles ψ_1, φ_2, and ψ_2 determines the conformations. Their conformational energies are therefore a function of these three angles.

If the coulombic attraction between the charged end groups NH_3^+ and CO_2^- is ignored, then the conformational energy of L,L- and L,D-alanylalanine, $E(\psi_1,\varphi_2,\psi_2)$, can be separated into two independent contributions, one for each residue, $E(\psi_1)$ and $E(\varphi_2,\psi_2)$, because the atoms and groups in each residue are sufficiently separated by the intervening peptide group. Flory and Schimmel[10] have calculated $E(\psi_1)$ and $E(\varphi_2,\psi_2)$ for these diastereoisomers. They considered the nonbonded van der Waals repulsions and London attractions (6–12 potential function) and the electrostatic interactions (monopole approximation) associated with the rotations ψ_1, φ_2, and ψ_2. In addition, the intrinsic torsional potentials associated with the ψ_1 and φ_2 rotations were included. However, they did not present a tabulation of the conformational energies or an energy map, but instead used them to evaluate the mean-square dipole moments. In the present study, their calculations were repeated with the minor modification of including the torsional potential associated with ψ_2, and the conformational energies were mapped.

In a second calculation the contribution to the conformational energies of L,L- and L,D-alanylalanine made by the coulombic attraction between NH_3^+ and CO_2^- was evaluated. The charges were placed at the nitro-

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gen atom and midway between the carboxyl oxygens, and the dielectric constant ϵ was assigned values of 80, 40, and 20. The coulombic attraction was calculated as a function of the charge separation, which depends only on ψ_1 and φ_2 (Figure 1).

The relationships of Karplus[14] and of Bystrov, *et al.*,[6] were used to evaluate the average value of $J_{N\alpha}$, as defined in the previous section, for each diastereoisomer

$$J_{N\alpha}(\text{Hz}) = \begin{cases} 8.5\cos^2\varphi' & (0° \leq \varphi' \leq 90°) \\ 9.5\cos^2\varphi' & (90° \leq \varphi' \leq 180°) \end{cases} \quad (1)^{14}$$

$$J_{N\alpha}(\text{Hz}) = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi' \quad (2)^6$$

where φ' is the dihedral angle between N–H and C^α–H^α, as customarily defined. Since φ' is obviously directly related to φ_2, $\cos^2\varphi'$ was averaged[10,15–17] separately over all conformations, *i.e.*, over all (φ_2,ψ_2) corresponding to $0° \leq \varphi' \leq 180°$ for use in eq 1. For use in eq 2, $\cos^2\varphi'$, $\cos\varphi'$, and $\sin^2\varphi'$ were averaged over all (φ_2,ψ_2). In addition, the conformational averages of ψ_1 and φ_2 were determined for both dipeptides. For reasons arising from experimental restrictions (see Experimental Section), the averaging was carried out assuming a temperature of 268°K.

Experimental Section

L,L-Alanylalanine and L,D-alanylalanine were obtained from Cyclo Chemical Co., Los Angeles, Calif., and were observed in 10% (w/v) solutions in H_2O at 100 MHz using a Varian HA-100 spectrometer. The H_2O peak was employed for field-frequency locking. The solutions were adjusted to pH 6.0. At ambient temperature (*ca.* 30°) the NH resonances of both isomers appeared broad and ill-defined and did not yield values of $J_{N\alpha}$. Lowering the temperature to −5° (268°K) produced adequately well-resolved doublets owing to increased ^{14}N quadrupolar relaxation.[18–20] The differences in the chemical shifts observed between the spectra of L,L- and L,D-alanylalanine and the absence in either spectrum of any peaks in trace amounts at the same positions as the major peaks in the spectrum of the other isomer confirmed the stereochemical purity of the samples investigated.

A third diastereoisomer, D,L-alanylalanine, was also observed, although for reasons to be indicated in the Results, it was not specifically included in the conformational energy calculations. (The remaining isomer, D,D, is obviously enantiomorphous with the L,L isomer and does not require separate study; note, however, that the D,L and L,D isomers do not form a mirror-image pair.)

Results

The contour map of the conformational energy of L,L-alanylalanine is shown in Figure 2. The energy contours $E_{L,L}(\varphi_2,\psi_2)$ are plotted in intervals of 1.0 kcal/mol relative to the minimum potential energies marked as × (at *ca.* $\varphi_2 = 120°$, $\psi_2 = 300°$, 120°), and

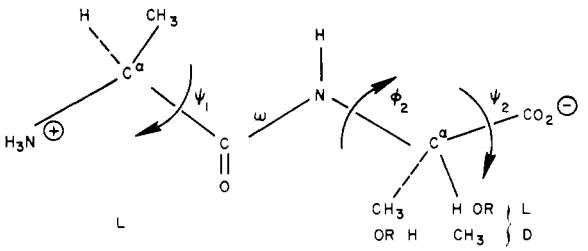

Figure 1. A schematic representation of L,L- and L,D-alanylalanine in the planar zigzag zwitterionic state. If NH_3^+ and CO_2^- are replaced by Cl and CO_2CH_3, respectively, then Figure 1 becomes a schematic representation of the dipeptides (L,L- and L,D-α-chloropriopionylalanine methyl ester) studied by Lande.[2]

were obtained by varying φ_2 and ψ_2 in 30° increments without taking into account the coulombic interactions between the end groups. When these are included ($\epsilon = 80$) the following minor changes over the values in Figure 2 result: for $\varphi_2 = 0$, 30, 60, 90, 270, 300, and 330°, energies of 0.53, 0.50, 0.42, 0.26, 0.26, 0.42, 0.50 kcal, respectively, must be added to $E_{L,L}(\varphi_2,\psi_2)$; for $\varphi_2 = 150$, 210, and 180°, values of 0.29, 0.29, and 0.39 kcal, respectively, must be subtracted from $E_{L,L}(\varphi_2,\psi_2)$. Steric overlaps involving NH_3^+ preclude all values of ψ_1 except 90–100 and 260–270° if the van der Waals radius of nitrogen (1.55 Å) is adopted[10] for the NH_3^+ end group. Consequently, the values of the coulombic interactions between the end groups presented above were obtained by averaging the coulombic interaction for each φ_2 over $\psi_1 = 90$, 100, 260, and 270°.

The coupling constants $J_{N\alpha}$, calculated at 268°K and averaged over 30° intervals in both φ_2 and ψ_2, are found to be identical, as indeed they must be, since $E_{L,D}(\varphi_2,\psi_2) = E_{L,L}(-\varphi_2,-\psi_2)$ and φ_2 and $-\varphi_2$ correspond to the same φ'. In fact, $E_{D,D}(-\varphi_2,-\psi_2) = E_{D,L}(\varphi_2,\psi_2) = E_{L,L}(\varphi_2,\psi_2)$ and all four diastereoisomers must

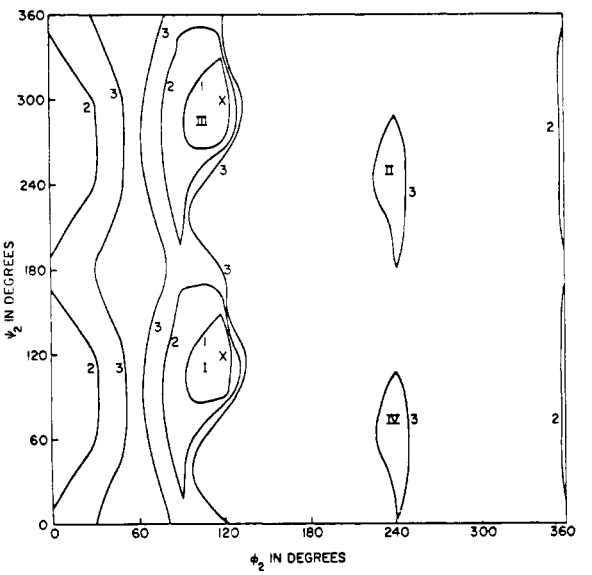

Figure 2. Contour map of the conformational energy of L,L-alanylalanine, $E_{L,L}(\varphi_2,\psi_2)$, calculated without inclusion of the coulombic attraction between NH_3^+ and CO_2^- by varying φ_2 and ψ_2 in 30° increments. The absolute minima at the ×'s correspond to 0.061 kcal/mol; contours are drawn at intervals of 1.0 kcal/mol relative to these minima.

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TABLE I
THE CONFORMATIONAL CHARACTERISTICS OF L,L- AND L,D-ALANYLALANINE

Method	$\langle\psi_1\rangle_{L,L;L,D}$, deg	$\langle\varphi_2\rangle_{L,L}$, deg	$\langle\varphi_2\rangle_{L,D}$, deg	$\langle J_{N\alpha}\rangle_{L,L;L,D}$ Eq 1	$\langle J_{N\alpha}\rangle_{L,L;L,D}$ Eq 2
Calculated from conformational energies	100	131 (149)[a]	182 (191)[a]	4.9 (5.0)[a]	5.4 (5.5)[a]
Obtained from Lande's models[d]	210	150	210	9.0	8.9
Experimental				6.0,[b] 6.5[c]	

[a] Obtained from the calculated conformational energies with inclusion of the coulombic attraction between NH_3^+ and CO_2^-. [b] L,L-Alanylalanine. [c] L,D- and D,L-alanylalanine. [d] See ref 2.

have the same coupling constants if $E(\varphi_2,\psi_2)$ is assumed to be independent of ψ_1. It follows also that $\langle J_{N\alpha}\rangle$ = 4.9 Hz (5.0 Hz with coulombic interaction included) from eq 1 and 5.4 Hz (5.5 Hz with coulombic interaction included) from eq 2.[21] Measured values are 6.0 Hz for the L,L and 6.5 Hz for the L,D and D,L diastereoisomers.

The conformational averages of ψ_1 and φ_2 at 268°K were calculated[22] to be $\langle\psi_1\rangle_{L,L;L,D}$ = 100°, $\langle\varphi_2\rangle_{L,L}$ = 131° (149° with the coulombic term included), and $\langle\varphi_2\rangle_{L,D}$ = 182° (191° with the coulombic term included).

Discussion

The conformationally averaged quantities $\langle\psi_1\rangle_{L,L;L,D}$, $\langle\varphi_2\rangle_{L,L}$, $\langle\varphi_2\rangle_{L,D}$, and $\langle J_{N\alpha}\rangle_{L,L;L,D}$ obtained in the present work are compared in Table I with the corresponding average values derived from Lande's conformational models. The following values of (ψ_1,φ_2) characterize these latter: $(\psi_1,\varphi_2)_{L,L}$ = (120°,240°) or (300°,60°); $(\psi_1,\varphi_2)_{L,D}$ = (120°,120°) or (300°,300°). If each of the dipeptide residues resides equally in each of the two conformations thus assigned, the calculated dipole moments agree with experiment.[2] However, the same two conformer models lead to values of $\langle J_{N\alpha}\rangle_{L,L;L,D}$ of 9.0 Hz (eq 1) or 8.9 Hz (eq 2), rather than to the experimentally observed values of *ca.* 6 Hz. On the other hand, the conformational energies presented here and calculated previously by Flory and Schimmel[10] correctly predict both the experimentally observed dipole moments and values of $J_{N\alpha}$ for both diastereoisomers (and $\langle J_{N\alpha}\rangle$ for the D,L isomer). Thus, Lande's proposed model is clearly in error. As Figure 2 shows, neither dipeptide is confined to just two conformations.

Although the conformational energies presented here lead to calculated couplings (5.0–5.5 Hz) which agree approximately with the values (6.0–6.5 Hz) observed experimentally and refute the conformational models proposed by Lande, the agreement remains outside the probable experimental error in $J_{N\alpha}$. The approximate nature of the potential functions and vicinal coupling constant relations employed together with the assumed independence of residue energies would seem to be reasons sufficient to explain this disparity. However, these same approximations lead to excellent quantitative agreement with experiment when applied to random-coil polypeptides in solution (see preceding paper[17]). The source of this quantitative disparity in the case of the dipeptides is the subject of work currently in progress..

(21) It is found that for lower (and probably more realistic) values of ϵ (ϵ <80), which take into account the fact that a portion of the electric lines of force pass through the molecule itself rather than the solvent, $\langle J_{N\alpha}\rangle$ remains virtually independent of ϵ at 0.1 Hz above the value calculated without inclusion of the coulombic term.

(22) That part of the total conformational energy [$E(\psi_1,\varphi_2,\psi_2) = E(\psi_1) + E(\varphi_2,\psi_2)$] which depends solely on ψ_1 is not presented here because the averaged vicinal coupling constant is virtually independent of $E(\psi_1)$.