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Study of the influence of neighboring amino acids on proline conformation

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Abstract: DFT calculations made at the B3LYP/6-31+G(d) level were used to investigate how the incorporation of a second amino acid into the backbone affects the conformational preferences of proline. Specifically, the this research studied the second amino acids L-proline and L-alanine and the trans isomerism of the peptide bonds. The lowest energy minimum has been found to have a different conformation for the two systems investigated; while the third presents a different conformation. The results obtained offer evidence of the influence of these systems on the conformational preference of proline.

Keywords: N-acetyl-N´-methylamide; Ac-L-Pro-NHMe; geometry optimizations; proline; alanine.

Introduction

Among the amino acids whose structural rigidity can be exploited in the design of peptides with well-defined backbone conformations are the α-amino acids [1].

Proline and alanine are α-amino acids, of which alanine is one of the smallest, and often found in helices [2], while proline is one of the most restricted.

The cyclic structure of proline makes it unique, presenting a null rotation around the N-C^{α}, with the φ torsion angle restricted to values of approximately -60°. Consequently, proline is mainly found in the α-helical $[(\varphi,\psi) \approx (-60^{o},-30^{o})]$ and semi-extended regions $[(\varphi,\psi) \approx (-60^{o},-140^{o})]$, and also encourages γ-turn conformations $[(\varphi,\psi) \approx (-70^{o},60^{o})]$[16d] of the conformational map[16]. The conformation of proline also has some biomedical applications [3-4].

This study used Density Functional Theory (DFT) methods to research the intrinsic conformational preferences of the proline attached to other amino acids, such as L-proline and L-alanine. Calculations were performed on N-acetyl-N´-methylamide (Ac-L-Pro-NHMe), hereafter referred to as Ac-L-Pro-L-Amino acid –NHMe, incorporating L-proline, L-alanine, and Ac-L-Amino acid-L-Pro-NHMe (Scheme 1). The conformational preferences of the structure of proline, which can be ascertained by attaching a second amino acid to the backbone, may have significant structural consequences for the following reasons: (i) Proline presents restrained conformational properties; and, (ii) Alanina is a flexible amino acid.

The influence of an amino acid can be determined by means of a comparison using N-acetyl-N´-methylamide, denoted here as Ac-L-Pro-NHMe, using the same quantum mechanical method. Specifically, this study examines how the incorporated amino acid affects both the preferred backbone conformation and the cis/trans disposition of the amide bonds.

Experimental Section

Computational Details. All calculations were carried out using the Gaussian 09 computer program[5]. DFT calculations were performed using the 6-31+G(d) basis set. Geometry optimization was performed utilizing Becke's hybrid three-parameter functional (B3) [6], and the Lee, Yang and Parr (LYP)[7] expression for nonlocal correlation (B3LYP).

These computational procedures provided a very satisfactory description of the conformational properties of cyclically constrained amino acids, including pro, and their analogues and applications [8-12].

Thus, the B3LYP method combined with the 6-31+G(d)[13] basis set were used for all the calculations presented in this paper.

The backbone (ω0, φ, ψ, ω, φ′,ψ′, ω′) (see Scheme 1) and dihedral angles of the Ac-L-Pro-L-Amino acid-NHMe are defined in Figure 1. Each amide bond (ω0, ω ,ω′) can be organized in a trans conformation. This study considered the trans state

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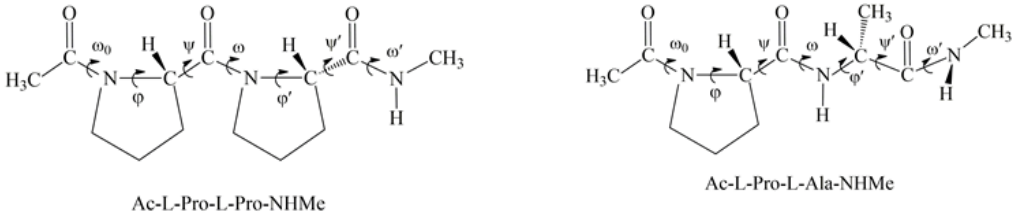
of the amide bond formed by the proline carbonyl (the methylcarboxamide group, –CONHMe, given by ωo, ω), with the aim of exploring how the second amino acid affects the amide linkage isomerism.



Scheme 1. Compounds studied in this research: a) Ac-L-Pro-L-Pro-NHMe; and, b) Ac-L-Pro-L-Ala-NHMe.

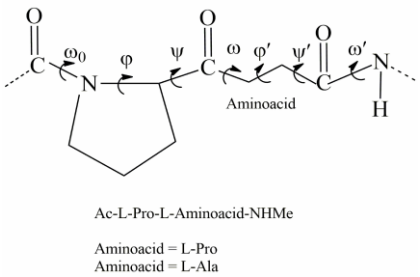


Figure 1. Dihedral angles used to identify the conformations of Ac-L-Pro-L-Amino acid-NHMe studied here. The dihedral angles ω_0, φ, ψ, ω, φ', ψ' and ω' are defined using backbone atoms. In particular, the sequences of atoms used to define φ and φ', are C(=O)–N–C^{α}–C(=O) and C(=O)–C–C–C(=O) respectively.

Nomenclature and Parameters.

The minimum energy conformations of the two dipeptides studied in this research have been denoted using a two label code that specifies the arrangement of the trans isomerization (ω0 ω, ω′), and the conformation of the backbone (φ,ψ, φ′,ψ′). The first letter refers to the trans (t) arrangement of the peptide bond between the first amino acid (ω0 ω) and the second amino acid (ω, ω′). The second label identifies the backbone conformation using the nomenclature introduced by Perczel et al.[14] more than fifteen years ago. Accordingly, in the potential energy surface, E = E(φ,ψ,), nine different backbone conformations can be found: γD, δD, αL, εD, βL, εL, αD, δL, and γL.

Results and Discussion

Ac-L-Pro-L-Pro-NHMe. Table 1 shows the most important structural parameters, together with the relative energy (ΔEgp) in the gas phase for the three minimum energy conformations characterized for Ac-L-Pro-L-Pro-NHMe (Figure 2).

Table 1. Backbone dihedral angles (in degrees), and the relative energies (ΔE^{gp}; in kcal/mol) of the minimum energy conformations of Ac-L-Pro-L-Pro-NHMe with the two peptide bonds in trans calculated at the B3LYP/6-31+G(d) level in the gas phase.

conformation	ω_0	φ	ψ	ω	φ'	ψ'	ω'	ΔE^{gp}
t-εL-t	178.2	-63.4	128.3	179.5	-80.4	79.9	-176.1	0.0[a]
t-εL-t	177.4	-62.6	129.2	-178.3	-76.2	-18.1	177.3	3.3[b]
t-αL-t	-174.1	-54.7	-31.1	177.5	-71.1	-13.6	178.6	3.5[c]

[a] E= -898.0435558, [b] E= -898.0382411, [c] E=-898.0378535.

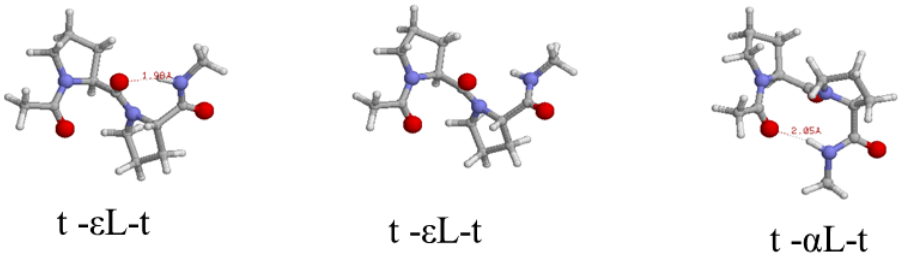


Figure 2. Minimum energy conformations of Ac-L-Pro-L-Pro-NHMe at the B3LYP/6-31+G(d) level: t-εL-t, t-εL-t, t-αL-t.

Two of these, the global minimum and the most stable local minimum, correspond to the t-εL-t, with specifically, the local minimum t-εL-t being 3.3 kcal/mol less stable than the global minimum. The global minimum is stabilized by an intramolecular hydrogen bond occurring in the seven-membered hydrogen-bonded ring: [d(H···O) = 2.712 Å, ∠N–H···O = 120.7º] and [d(H···O) = 2.712 Å, ∠N–H···O = 120.7º]. The conformation of the local minimum does not involve any intramolecular hydrogen bond and is unfavored compared to the global minimum by 3.5 kcal/mol. These minima are distributed as 3 trans-trans according to the cis/trans state of the peptide bonds. According to (φ,ψ), values in the εL region correspond to polyproline II conformation (εL)[15d], which is known to be among those preferred by proline[15]. Calculations of Ac-L-Pro-NHMe at similar theoretical levels to those used in this research locate the conformation as t-γL-t [16].

Ac-L-Pro-L-Ala-NHMe. Figure 3 shows eight characterizations of the energy conformations for Ac-L-Pro-L-Ala-NHMe in the gas phase, while Table 2 shows their structural and energy data. These minima are distributed as 8 trans-trans according to the cis/trans state of the peptide bonds.

Table 2. Backbone dihedral angles (in degrees) and relative energies (ΔE^{gp}; in kcal/mol) of the minimum energy conformations of Ac-L-Pro-L-Ala-NHMe with the two peptide bonds in trans calculated at the B3LYP/6-31+G(d) level in the gas phase.

conformation	ω_0	φ	ψ	ω	φ'	ψ'	ω'	ΔE^{gp}
t-γL-t	-172.9	-82.2	73.4	-174.1	-84.3	70.5	-176.4	0.0[a]
t-αL-t	-170.4	-68.0	-16.7	176.3	-93.8	3.8	176.8	1.4[b]
t-γL-t	-173.2	-82.3	76.5	-170.0	-117.6	6.1	176.8	1.5[c]
t-γL-t	-173.4	-81.6	79.2	-171.0	-102.0	1.2	176.8	1.7[d]
t-γL-t	-172.8	-82.3	71.9	176.9	70.6	-54.0	-177.5	1.8[e]
t-γL-t	-172.6	-82.2	71.2	176.6	70.5	-53.3	-177.4	1.9[f]
t-αL-t	-171.7	-74.9	-21.5	-177.2	-83.4	73.1	-172.7	2.8[g]
t-αL-t	-173.8	-73.1	-20.8	175.4	-150.5	157.1	179.5	3.8[h]

[a]E= -820.6322572, [b]E= -820.6299252, [c]E= -820.6297634,[d]E= -820.6295274, [e]E= -820.6292501, [f]E= -820.6292282, [g]E= -820.6277116, [h]E -820.6261436

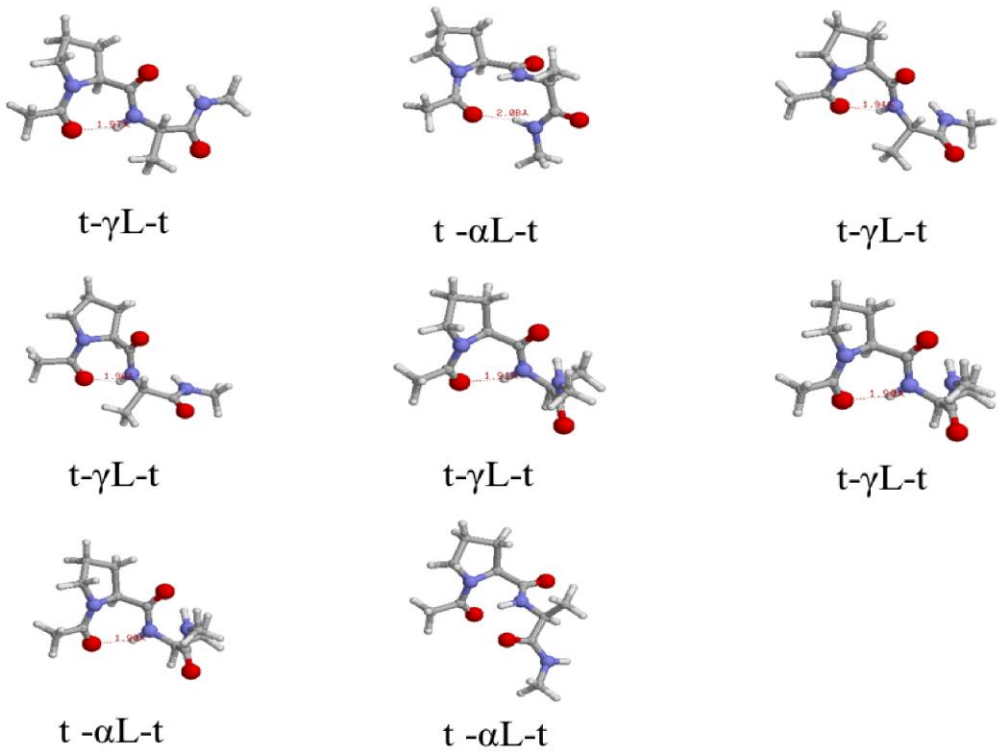


Figure 3. Minimum energy conformations of Ac-L-Pro-L-Ala-NHMe at the B3LYP/6-31+G(d) level: t-γL-t, t-αL-t, t-γL-t, t-γL-t, t-γL-t, t-γL-t, t-αL-t, t-αL-t.

The lowest energy conformation characterized for Ac-L-Pro-L-Ala-NHMe in the gas phase corresponds to a t-γL-t conformer, also identified as the global minimum for the Ac-L-Pro-NHMe. This conformation is stabilized by an intramolecular hydrogen bond, which takes place in the seven-

membered hydrogen bonded ring [d(H···O) = 1.973 Å, ∠N–H···O = 121.2º], geometric parameters which indicate that this intramolecular interaction is very similar to that obtained for Ac-L-Pro-NHMe. According to (φ,ψ), values corresponding to the global minimum correspond to the γ-turn region conformation (γL)[15d]. Interestingly, the conformation for Ac-L-Pro-NHMe at similar theoretical levels to those used here located the global minimum conformation in the γL[16] region.

The flexibility conformation is reflected in Figure 4, which compares the distribution of the φ,ψ, backbone dihedral angles of Ac-L-Pro-L-Pro-NHMe and Ac-L-Pro-L-Ala-NHMe, for the minimum with relative energies lower than 4 kcal/mol.

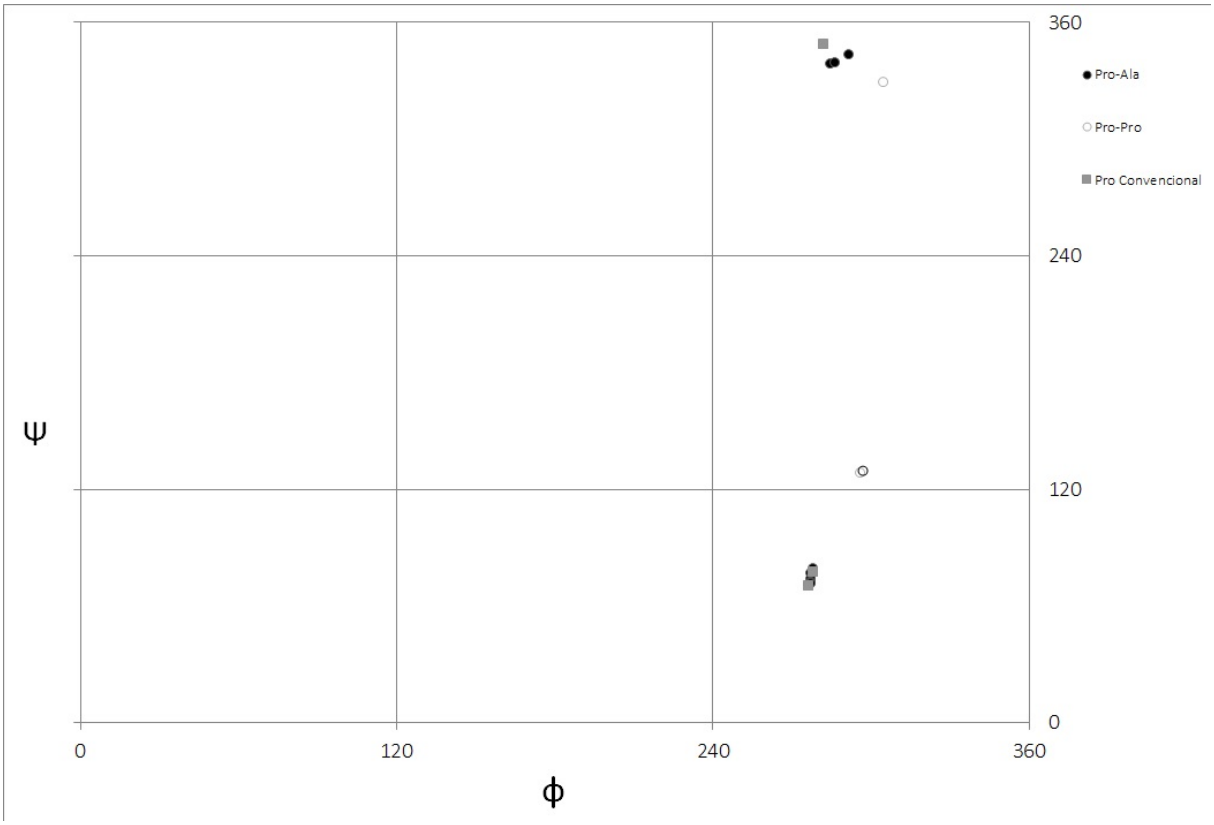


Figure 4. Ramachandran plot distribution. Compares the distribution of the *φ, ψ* backbone dihedral of Pro-Pro (open circles), considering the more representative minimum energy structures, *i.e.* those within a relative internal energy of 4 kcal/mol.

Conclusion

DFT calculations at the B3LYP/6-31+G(d) level have been used to explore the conformational preferences of Ac-L-Pro-L–Amino acid-NHMe and Ac- L–Amino acid-L-Pro-NHMe. The comparison of the results with those obtained for Ac-L-Pro-NHMe at the same theoretical level allows the following conclusions:

(i) The εL conformation with two trans amide bonds was found to be accessible for the Ac-L-Pro-L-Pro-NHMe, but was not found to be an energy minimum for Ac-L-Pro-NHMe. This trend seems to be related to fact than proline does not act as a constrained amino acid attached to itself.

(ii) The γL conformation is the lowest energy minimum for Ac-L-Pro-L-Ala-NHMe. This trend seems to be related to fact than proline acts as a constrained amino acid attached a flexible amino acid such as alanine.

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