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Study of the Conformational Profile of Selected Unnatural Amino Acid Residues Derived from L-Phenylalanine

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Abstract: The present work reports the results of a conformational study performed on seven unnatural amino acid residues and on its natural precursor, investigated by means of computational methods at the molecular mechanics level. Amino acid residues selected for the present study are derivatives of L-phenylalanine substituted at the α and/or β carbons. This series is composed of different linear analogs, including α-methyl, β-methyl and β-phenyl substituted with different stereochemistry. Analysis of the Ramachandran maps of the corresponding dipeptides *in vacuo* reveals their conformational preferences, to be used as guidance for the synthesis of constrained peptide analogs with desired conformational propensities. The available conformational space for every dipeptide is also analysed. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: phenylalanine analogs; unnatural amino acids; conformational study; AMBER

INTRODUCTION

Peptides are highly flexible molecules that exhibit availability for an ample conformational space. However, these molecules interact with their receptors in very precise conformations. The bioactive conformation is not usually known, unless X-ray diffraction or NMR spectroscopy studies conducted on the ligand–receptor complex are available. Unfortunately, the number of structures of ligand–receptor complexes available is very limited or even nonexistent, as in the case of peptides interacting with G-protein coupled receptors. An indirect strategy used for years to determine the features of the bioactive form consist of a trial and error procedure in which different peptide analogs, including conformationally constrained ones are synthesized and tested. Restraints imposed on constrained analogs can be global by cyclization of peptide backbone or local, using conformational constrained residues. This last approach has been extensively employed through the use of unnatural amino acids with structural modifications that impose some degree of conformational constraint to the peptide [1–3].

Unnatural amino acid residues greatly amplify the field of peptide design and protein engineering. Expansion of the repertoire of available amino acids, permits the synthesis of bioactive peptide analogs with improved pharmacokinetic properties and furthermore, with the possibility to add new side-chain functionality or exhibit a constrained stereochemistry [3,4]. These analogs are very helpful in the elucidation of the bioactive conformation of natural bioactive peptides and consequently in the design of nonpeptide molecules with similar rigid structures that can be used as lead compounds in the development of new drugs.

In the last years a great deal of information has been gathered about the conformational preferences of some of these unnatural residues [5]. Several modifications of natural amino acids have been pro-

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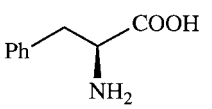
posed in the literature. Methylation of the Cα is one of the simplest, the aminoisobutyric acid (Aib) being the amino acid residue of this class most extensively investigated. This residue exhibits a strong tendency to stabilize 3_{10} and α-helical conformations, as has been established by the analysis of a large number of synthetic peptides by crystallographic and theoretical studies [6–10]. Another possibility to generate unnatural amino acid residues is to include the Cα in a cyclic structure. A peculiar residue of this category inducing conformational rigidity, extensively studied is the 1-aminocyclopropanecarboxylic acid (Ac_3c). X-ray structures of some peptide analogs containing cyclopropane amino acids reveal that they adopt preferentially a conformation with ϕ near $\pm 80°$ and ψ near 0° or, in some cases, 180° [11,12]. This peculiarity confers the amino acid the property to induce β-turn secondary structures in peptides [13,14].

Other ways to generate conformationally constrained unnatural amino acids include Nα-Cα cyclization in a similar fashion as found in proline, or Nα-Cα and Cα-Cδ cyclization in the case of aromatic residues. This latter category of amino acid residues has been successfully used in recent years to design selective analogs. For example, 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) has recently been used to construct high affinity analogs of the opioid or bradykinin receptors [15,16]. These modifications as well as Nα-methylation [17] enhance *cis*/*trans* isomerization of the preceding peptide bond. Another modification described in the literature includes α,β-unsaturated amino acid residues, used to confer helicity to peptides [18]. Modifications like β,β-dimethylsubstitution, β-methylation, β-substituted prolines, 2′,6′-*R* groups on Phe and Tyr and β-isopropyl correspond to other unnatural amino acids also described [5].

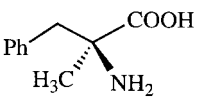
As a part of a wider research project devoted to understand the conformational features induced by different phenylalanine derivatives, in the present work we report the results of the conformational analysis performed on the natural amino acid residue and seven unnatural derivatives, investigated by means of computational methods. Our ultimate goal is to study their potential utility as building blocks in the design of bioactive peptides, by the induction of local conformational constraints. All the amino acids studied in this work are shown in Figure 1. They are derivatives of L-phenylalanine substituted in the α and/or β carbons and most of them have been previously synthesized [6,19–23]. This series concerns linear structures, including α-methyl, β-methyl and β-phenyl substituted derivatives with different stereochemistry.

METHODS

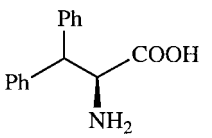
Bidimensional maps of the energy as a function of the ϕ and ψ torsion dihedrals (Ramachandran



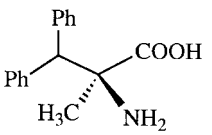
(1) L-Phe



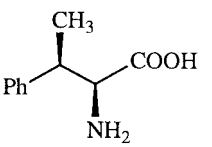
(2) L-(α-Me)Phe



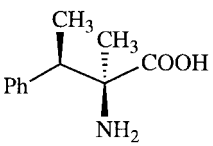
(3) L-Dip



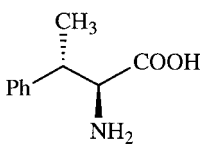
(4) L-(α-Me)Dip



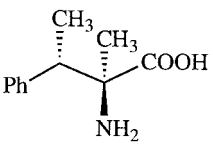
(5) (2S,3R)-(β-Me)Phe



(6) (2S,3R)-(α,β-diMe)Phe



(7) (2S,3S)-(β-Me)Phe



(8) (2S,3S)-(α,β-diMe)Phe

Figure 1 Unnatural L-phenylalanine derivatives selected for the present study. Phenylalanine has also been included for the sake of completeness.

maps) were computed for the respective *N*-acetylmethylamides (dipeptides) of the natural and the seven unnatural amino acids selected for this work as well as that of L-phenylalanine, for the sake of completeness. The AMBER 4.0 program [24] using the parm91 parametrization of the force field [25] was used to carry out these computations. For each of the dipeptides the following steps were followed: (i) construction of the dipeptide in the all-*trans* conformation; (ii) atomic charges consistent with the parm91 set of parameters of the AMBER force field were computed by fitting the molecular electrostatic potential computed at the HartreeFock level with a STO-3G basis set on the all-*trans* geometry; (iii) computation of the Ramachandran plots using a 10° grid was carried out using the SANDER module of AMBER. At each point, the energy was computed by freezing the values of ϕ and ψ and fully optimizing the rest of the geometry; (iv) when local minima in the Ramachandran maps were apparent, complete optimizations of these geometries were carried out. Minima were also searched for the *gauche* +, *gauche* − and *trans* as starting geometries for the χ_1 dihedral angle.

RESULTS

The AMBER computed Ramachandran maps of the eight dipeptides studied are shown in Figure 2a–h. Inspection of the maps suggests that local minima are located on six different areas or catchment regions [26], labeled as indicated in Figure 3.

Low energy regions A to F have also been characterized as low energy regions in natural amino acid dipeptides [27–29] and they are related to peptide secondary structures. Thus, minima in regions A and B correspond to C7 cyclic conformations not usually observed in proteins, but close to the torsions that characterize direct and inverse β-turns, respectively; regions C and D are close to the torsions of right- and left-handed helices, respectively, but are also compatible with other peptide secondary structures as the 3_{10} helix and the $i+1$ position of a β-turn of types I, I′, III and III′; region E corresponds to C5 conformations close to the extended, β-sheet structure and region F is close to the dihedral angles characteristic of the $i+1$ position of a type II β-turn.

Although the energy of all the points of the grid used to construct the Ramachandran maps (Figure 2a–h) were calculated by relaxing the geometries while keeping fixed ϕ and ψ, this methodology does not guarantee that the lowest energy minimum is necessarily found, since side chains may be trapped in local minima. In other words, the minimizer tends to maintain the χ_1 dihedral angle of the side chain in the same staggered conformation all over the map. In order to determine the effect of the χ_1 torsion on the maps, minima already characterized were reminimized with initial values of the χ_1 dihedral increased or decreased by 120°. These calculations showed that for every dipeptide, with the exception of (α-Me)Phe, low energy conformations exhibit always the same value of the χ_1 dihedral. Therefore, the computed Ramachandran maps are acceptable bidimensional projections of the multidimensional energy space of these dipeptides. In the case of (α-Me)Phe the lowest energy minimum is located on region B and exhibits the χ_1 dihedral around 180°, however, the lowest energy minimum of region A exhibits $\chi_1 = 58°$; in region C, $\chi_1 = 66°$ and in region D, $\chi_1 = -53°$.

Energies and values of the ϕ, ψ, χ_1 dihedrals for all the local minima found for the eight amino acids are shown in Table 1. In addition, as a measure of the degree of conformational restriction exhibited by each amino acid, the percentage of the (ϕ, ψ) surface under 5 kcal/mol above the lowest energy minimum for each residue is also shown. Figure 4 shows a bar diagram representing the relative energies of the different minima for each analog, taking as reference its respective global minimum.

DISCUSSION

It is known that conformational energy calculations of dipeptides *in vacuo* systematically yield C7 type structures (regions A and B) as low energy conformations [30]. These conformations are stabilized by intramolecular hydrogen bonds conforming seven member rings. However, this kind of secondary structure is rarely observed in proteins or peptides in solution. Indeed, the stability of the C7 geometries is a consequence of the lack of a polar solvent, favoring the formation of intramolecular hydrogen bonds. This effect is enhanced by the short length of the dipeptide chain precluding the interactions between noncontiguous residues. These facts may introduce some artifacts in interpreting the Ramachandran maps although, in any case, interesting inferences can be drawn out from the comparative analysis of the different residues.

The effect of α-methylation can be deduced from the comparative analysis of the corresponding α-

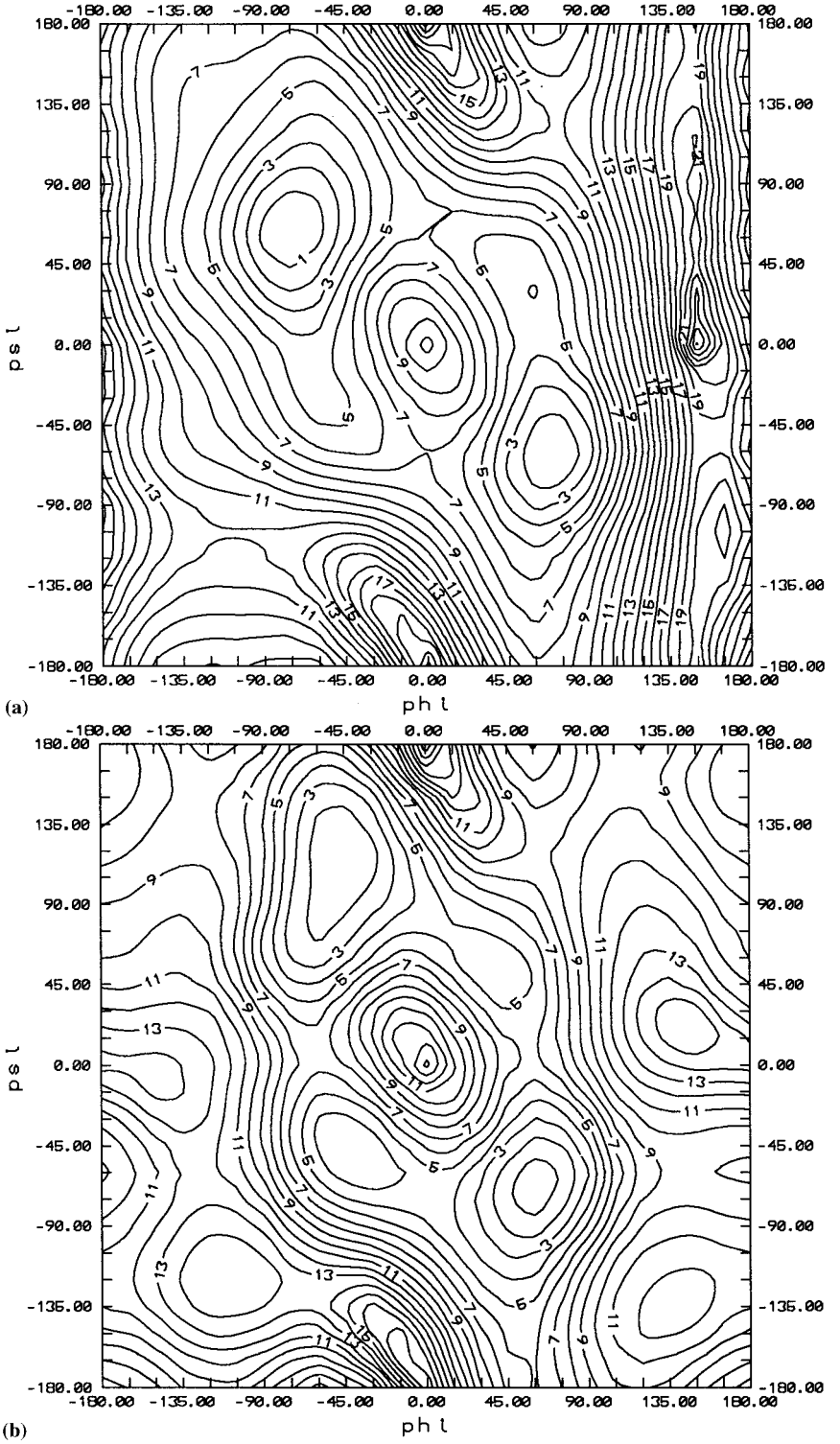


Figure 2 Ramachandran maps of the amino acid residues studied in the present work. Energies are in kcal/mol in respect to the lowest energy minimum of each amino acid. Contours are plotted every kcal/mol. (a) L-Phe (with $\chi_1 = -50°$) (b) L-(α-Me)Phe (with $\chi_1 = 180°$); (c) L-Dip; (d) L-(α-Me)Dip; (e) (2*S*,3*R*)-(β-Me)Phe; (f) (2*S*,3*R*)-(α,β-diMe)Phe; (g) (2*S*,3*S*)-(β-Me)Phe; (h) (2*S*,3*S*)-(α,β-diMe)Phe. Maps of Figure 2a and b were computed by fixing $\chi_1 = -50°$ and $\chi_1 = 180°$, respectively. The rest of the maps were computed with χ_1 at the values of the respective minima (see text).

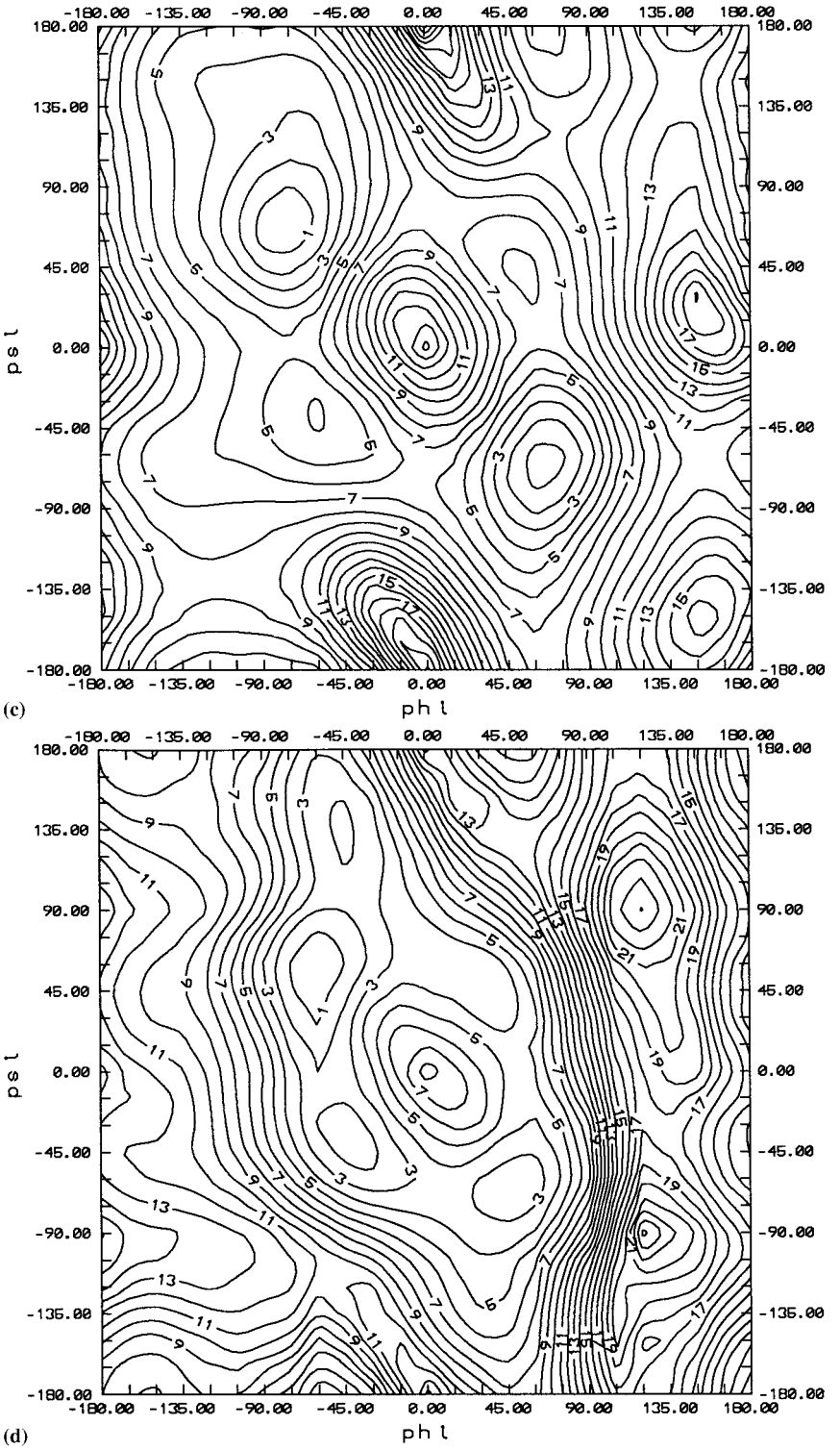
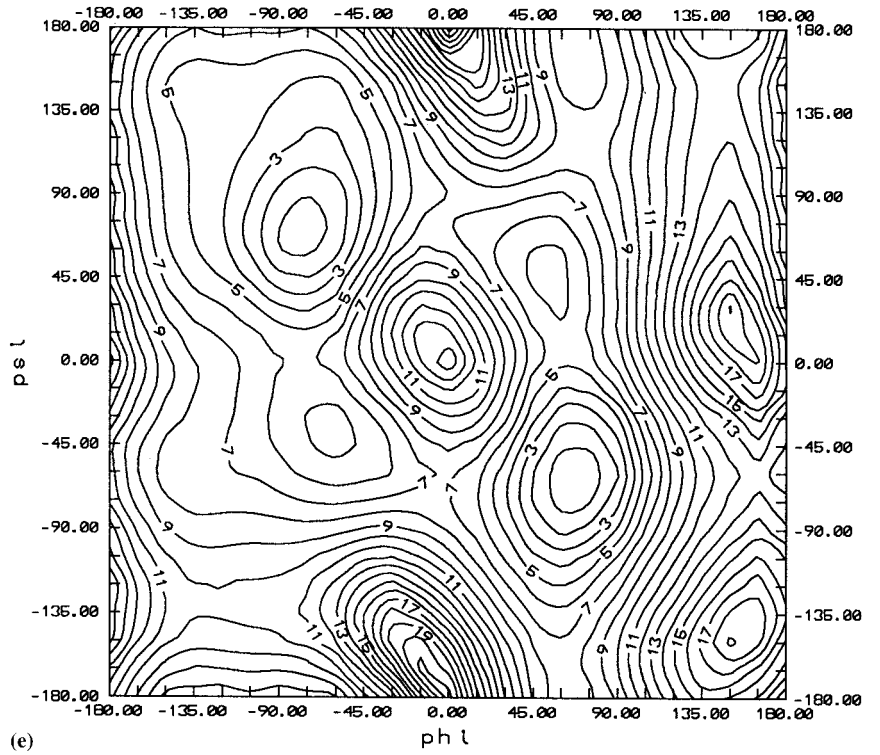


Figure 2 (*Continued*)

methyl derivatives with their parent compounds, i.e. pairs (1, 2), (3, 4), (5, 6) and (7, 8). As can be seen from an inspection of Figure 4 the lowest energy minimum for all the derivatives considered, lie either in regions A or B. Taking for each residue the corresponding lowest energy minimum as reference,



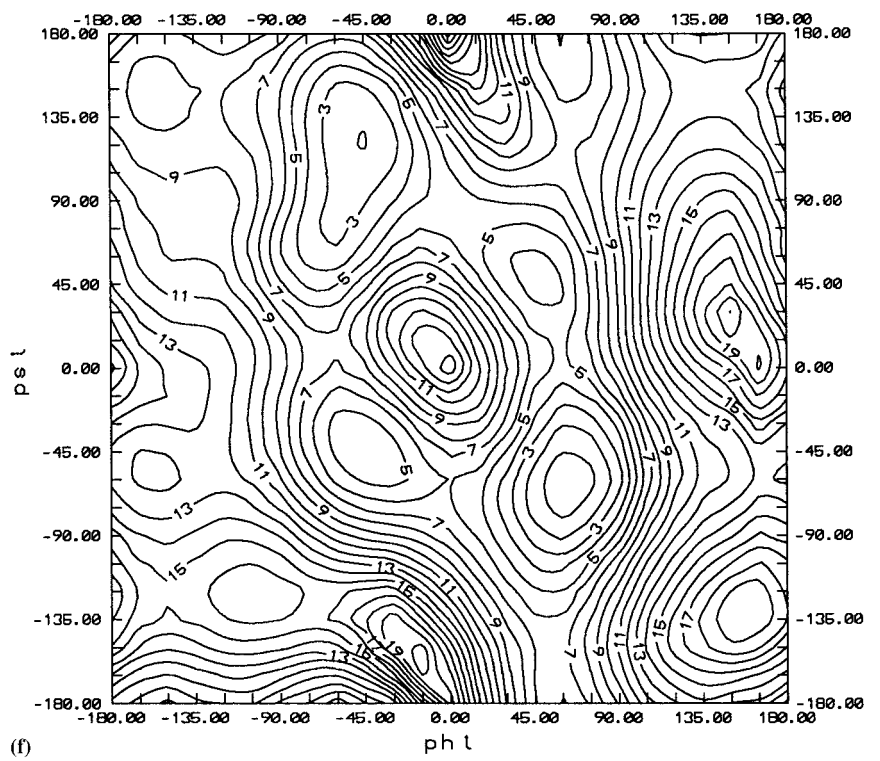


Figure 2 (*Continued*)

α-methyl derivatives exhibit helical conformations favored in regard to the parent compounds (regions C and D). This result correlates well with the experimental observation that α-methyl substituted amino acids tend to form helical structures [6,7]. Another consequence of α-methylation is the ap-

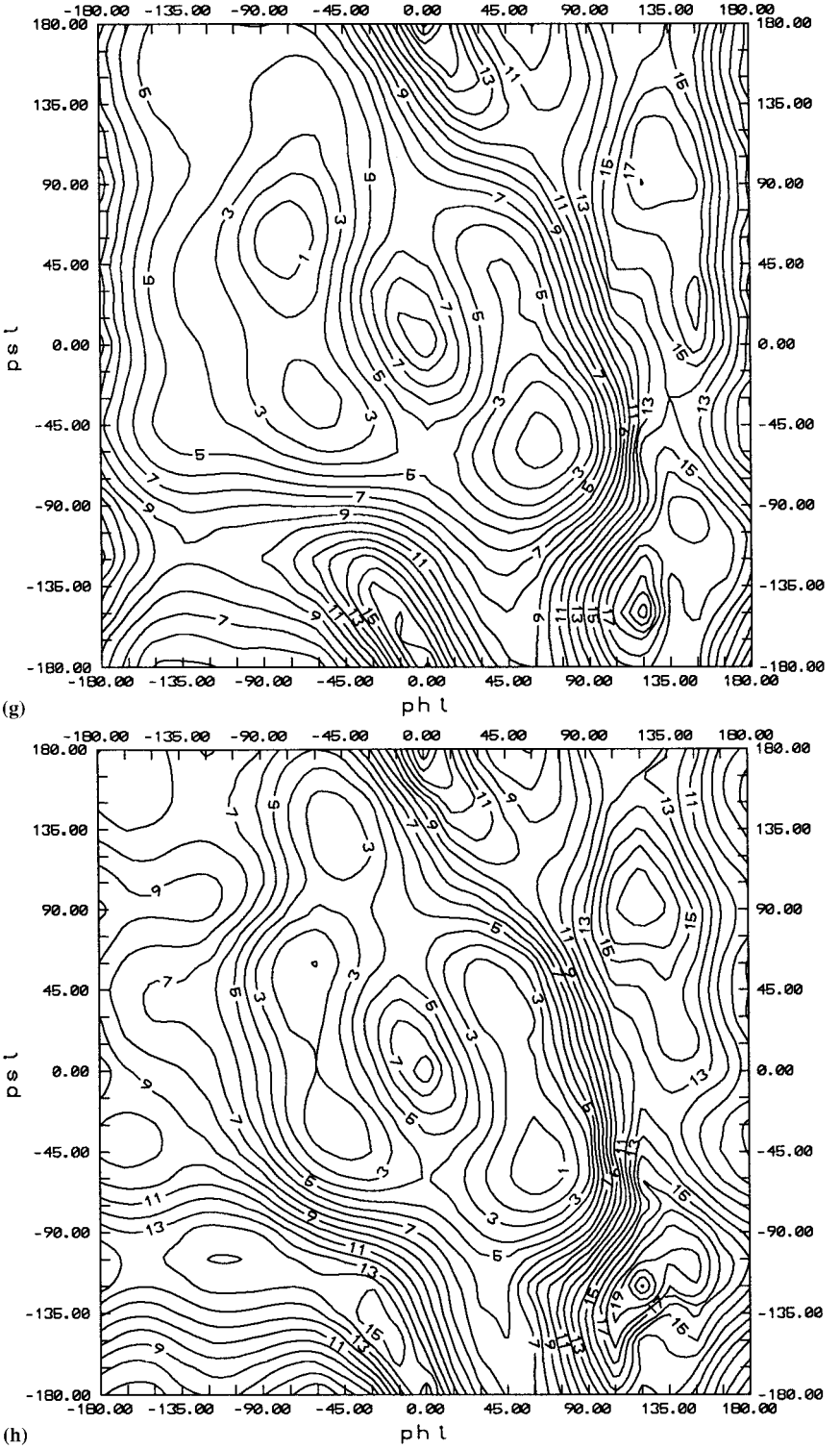


Figure 2 (*Continued*)

pearance of a minimum in region F. In the case of (2S,3S)-(α,β-diMe)Phe (**8**) minima in regions F and A are almost isoenergetic, whereas for the (2S,3*R*) isomer (**6**) the minimum of region A disappears to form part of the minimum F catchment region. Values of the backbone dihedral angles of region F are

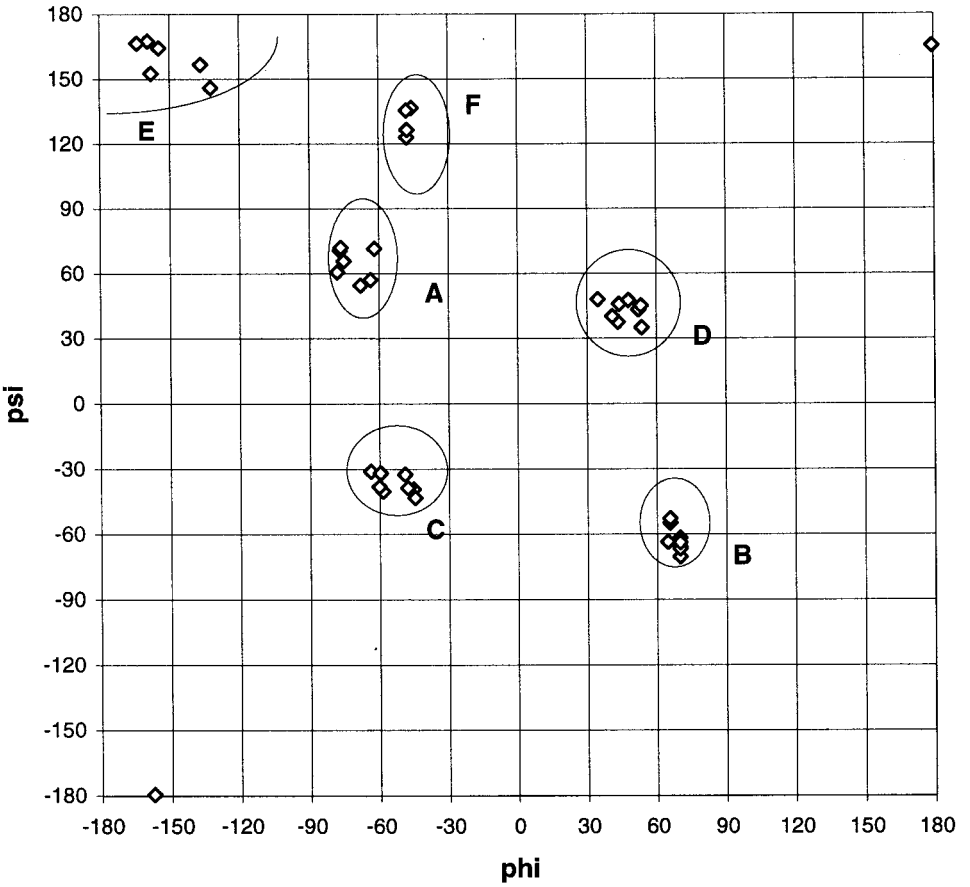


Figure 3 Representation of the different chatment regions of the amino acid residues studied in the present work. Capital letters represent regular catchment regions of most of the amino acid residues.

close to the optimal values for the $i+1$ residue in a type II β-turn. Consequently, present results suggest that α-methyl substituted amino acids, and specifically (2*S*,3*R*)-(α,β-diMe)Phe favor this kind of secondary structure in peptides. Finally, it is also observed a strong destabilization of the extended conformation (region E) in all the α-methyl derivatives in regard to the parent compounds. All these features observed in α-methyl substituted dipeptides can be rationalized on the basis of the steric destabilization introduced by the additional α-substituent on the conformations of region A with ψ around 70°.

Available X-ray structures of peptides containing (α-Me)Phe are in acceptable concordance with the previous analysis of α-methyl substituted residues [6]. The sets of (ϕ, ψ) torsion angles for this amino acid in a series of peptides containing other chiral or achiral residues, are largely found in the helical region. Interestingly, the fully extended C5 conformation has never been observed.

The effect of β-methylation markedly depends on the stereochemistry of the analogs. When the β-methyl group is introduced in an anti position with regard to the amino group (pairs (1, 7) and (2, 8)) helical regions C and D with χ_1 dihedral angles in the range of 60–80° are stabilized. On the other hand, when the β-methyl and the amino groups are in *syn* (pairs (1, 5) and (2, 6)) only C7 type conformations (regions A and B) with $\chi_1 = 180°$ are feasible. The β-methyl substitution introduces a major steric constraint as can be deduced from the fraction of the Ramachandran surface available under 5 kcal/mol above the corresponding lowest energy minimum (Table 1). For the *anti* isomers this is around 25%, whereas for the *syn* isomers this percentage is reduced to a 15%. Therefore, the *anti* isomers are prone to favor secondary structures in region C (right-handed α-helix, 3_{10} helix and type I and III β-turns). However, the respective *syn* stereoisomers tend to favor conformations in region A (left-handed α-helix and type I′ and III′ β-turns).

Table 1 Characteristics of the Minima Located in the AMBER Ramachandran Map for the Amino Acid Dipeptides

Residue		Region A	Region B	Region C	Region D	Region E	Region F	%<5 kcal/mol[a]
L-Phe	Energy	−43.0	−42.0	−39.6	−39.1	−39.5		14.9
	ϕ, ψ	−75.2, 65.7	70.0, −61.7	−63.8, −31.2	53.8, 34.9	−159.3, 167.5		
	χ_1	−50.5	−51.1	51.1	−50.3	−176.1		
L-(α-Me)Phe	Energy	−33.8	−34.8	−34.1	−33.4	−28.6	−33.7	15.9
	ϕ, ψ	−62.0, 71.5	60.5, −70.4	−45.4, −39.3	44.0, 45.7	179.4, 165.1	47.7, 123.0	
	χ_1	−57.5	178.7	65.6	−52.6	170.5	174.5	
L-Dip	Energy	−39.0	−38.7	−35.3	−33.5	−32.9		17.2
	ϕ, ψ	−76.9, 70.5	67.5, −66.0	−58.5, −40.3	52.4, 43.1	−154.5, 164.3		
	χ_{11}, χ_{12}	−177.4, −51.0	177.6, −55.1	178.9, −54.3	−173.0, −45.6	177.3, 43.5		
L-(α-Me)Dip	Energy	−32.9	−30.8	−31.6	−30.24	−26.2	−31.4	22.7
	ϕ, ψ	−63.6, 57.0	48.7, −66.8	−47.8, −38.7	34.8, 47.8	−157.6, −179.6	−45.7, 134.2	
	χ_{11}, χ_{12}	73.4, −58.9	68.1, −67.4	62.3, −72.2	64.6, −69.6	80.6, −51.2	76.0, −57.6	
L-(2*S*,3*R*)-(β-Me)Phe	Energy	−44.4	−44.6	−40.1	−40.2	−40.1		15.9
	ϕ, ψ	−76.4, 71.9	68.2, −63.9	−60.2, −38.1	53.5, 44.9	−132.2, 145.9		
	χ_1	−179.1	180.0	175.4	−171.5	176.6		
L-(2*S*,3*R*)-(α,β-diMe)Phe	Energy		−33.7	−29.9	−30.4	−26.3	−32.0	13.5
	ϕ, ψ		64.8, −63.6	−44.7, 43.3	48.0, 47.5	−157.9, 152.5	−47.6, 126.4	
	χ_1		173.1	162.3	−176.5	164.7	165.0	
L-(2*S*,3*S*)-(β-Me)Phe	Energy	−43.9	43.6	−42.6	−40.3	−40.0		29.7
	ϕ, ψ	−77.9, 60.5	65.7, −54.9	−59.6, −31.9	43.4, 37.4	−136.6, 156.6		
	χ_1	64.2	91.9	74.5	69.5	74.9		
L-(2*S*,3*S*)-(α,β-diMe)Phe	Energy	−31.4	−32.2	−31.3	−31.4	−26.5	−30.2	23.0
	ϕ, ψ	−68.0, 54.3	65.7, −52.9	−49.0, −32.5	41.1, 0.1	−163.9, 166.5	−47.9, 135.3	
	χ_1	61.5	99.3	67.3	64.8	69.4	66.6	

Energy in kcal/mol. The different regions of the map are labeled as indicated in Figure 3.
[a] Percentage of the (ϕ, ψ) surface accessible under 5 kcal/mol above the global minimum.

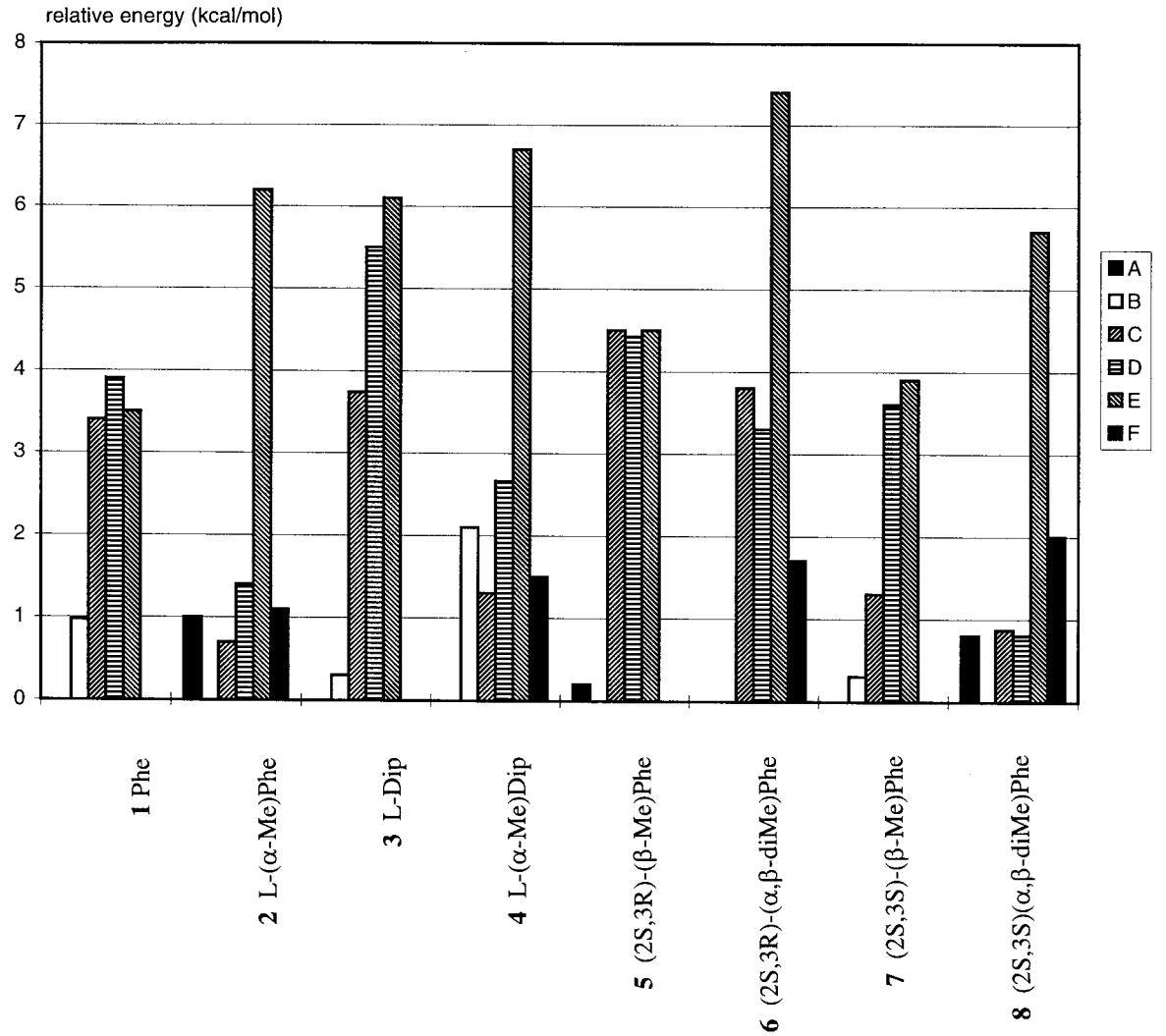


Figure 4 Comparison of the energies of the conformations of the different amino acids residues studied in the present work, relative to the corresponding lowest energy conformation. Asterisks indicate that there is no minimum in that region for the specified amino acid residue.

In order to understand the effects produced by a group larger than methyl, the effect of a β-phenyl substitution was also investigated. Comparison of the pair (1, 3), suggests that the effects introduced by the β-phenyl group are similar to those observed for (2S,3*R*)-(β-Me)Phe, although in the present case helical structures (region C) are not destabilized with regard to the parent compound.

These results can be extended to all possible stereoisomers of the analogs of phenylalanine studied here. The maps of two enantiomers are symmetry related in such a way that a point (ϕ, ψ) on the Ramachandran map of an analog corresponds to the point $(-\phi, -\psi)$ of its enantiomer. Thus, maps of different L and D amino acids can be obtained by this simple transformation. Similarly, the maps of the (2*R*,3*R*) and (2*R*,3*S*) stereoisomers are related in the same way to those of the (2S,3S) and (2S,3*R*) derivatives, respectively.

CONCLUSIONS

The present work reports the results of the conformational analysis performed on seven nonproteogenic amino acids, investigated by means of computational methods. This study was designed to provide a tool that can be used as guidance for the

design of peptide analogs with desired conformational propensities. The amino acids studied are derivatives of L-phenylalanine, substituted in the α and/or β carbons. The residues reported in the present work include different types of linear structures including α-methyl, β-methyl and β-phenyl substituted with different stereochemistry.

Amino acid residues with a methyl group substituted on Cα preferably induce helical and type I and III β-turn structures in the case where the residue is in position $i+1$. Furthermore, these derivatives exhibit as favorable the F catchment region characteristic of the $i+1$ position in type II β-turns. On the contrary, extended structures are clearly destabilized in these derivatives.

Introduction of a methyl group on Cβ reduces considerably the conformational space available. Its effect on the preferred conformations clearly depends on the stereochemistry attained. In the case of the *anti* stereoisomers helical conformations are favored, whereas in the case of the *syn* stereoisomers C7 type conformations are favored.

The β-phenyl substitution favor C7 type conformations, although helical structures are as stable as observed in the parent compounds.

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