Journal of Computer-Aided Molecular Design, 8 (1994) 441–448
ESCOM

J-CAMD 246

A quantum-mechanical study of the chain-length dependent stability of the extended and 3_{10}-helix conformations in dehydroalanine oligopeptides

Jordi Casanovas[a] and Carlos Alemán[b,*]

[a]*Departament de Química-Física, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1, E-08028 Barcelona, Spain*
[b]*Departament d'Enginyeria Química, E.T.S. d'Enginyers Industrials de Barcelona, Universitat Politècnica de Catalunya, Av. Diagonal 647, E-08028 Barcelona, Spain*

Received 16 July 1993
Accepted 14 December 1993

Key words: Dehydroalanine; 3_{10}-Helix; Extended conformation; Conformational analysis; Cooperative effects

SUMMARY

A quantum-chemical study of the chain-length dependent stability of the extended, 2_7-ribbon and 3_{10}-helix conformations in dehydroalanine (ΔAla) oligopeptides has been performed by using both semiempirical AM1 and ab initio 4-31G methodologies. The validity of both methods in the study of the conformational properties of ΔAla oligopeptides was tested first on the dipeptide. The results of this test showed that 4-31G and AM1 calculations are in good agreement with 6-31G* calculations and experimental data. In order to monitor the conformational conversions, ΔAla oligopeptides comprising two to six residues were constructed. Molecular geometries were fully optimized using AM1, and the final conformations were verified to be minima by analysis of the corresponding second-derivative matrices. Conformational studies revealed that the 3_{10}-helix is stabilized with respect to the 2_7-ribbon when the number of residues is three or four, at the AM1 and ab initio 4-31G level respectively, while the extended form is the most stable in all the calculations performed. On the other hand, if a linear behaviour is assumed for longer chains, our calculations show a trend that would predict a conversion from extended form to 3_{10}-helix in oligopeptides with around six (ab initio 4-31G) or eight (AM1) ΔAla residues. In order to explain these conformational changes, the cooperative effects for the different conformers were investigated. Large cooperative energy effects were found for the 3_{10}-helix conformation.

INTRODUCTION

The understanding of the interaction forces that determine the stable conformations of polypeptides is of crucial interest for the rational design of synthetic peptide mimics for structural motifs in proteins [1]. This requires the control of polypeptide chain stereochemistry; however,

*To whom correspondence should be addressed.

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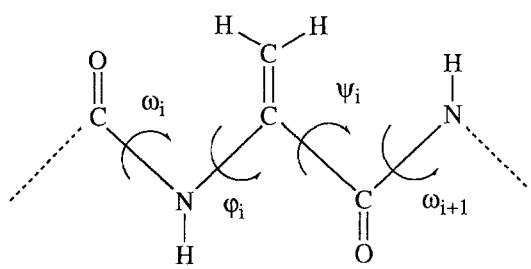
difficulties in predicting backbone folding patterns from primary sequence information [2,3] are major obstacles in the path of de novo protein design. One important area of current peptide and protein research attempts to exploit the novel structural properties of unusual nonnaturally occurring amino acids in designing oligopeptide conformations [1,4].

In recent years, considerable efforts have been made in the study of the conformational preferences of residues modified at the C^{α} atom [5–13]. In particular, we are interested in the conformational properties of the dehydroalanine residue (ΔAla), an α,β-unsaturated amino acid (see Scheme 1) commonly found in a number of naturally occurring peptide antibiotics of bacterial origin [14–17]. Due to the double bond of the side chain, this residue exhibits very interesting chemical features. Indeed, ΔAla has been found to play an active role in the catalytic activity of some yeast systems [18] and in bacterial enzymes [19], probably due to the nucleophilic character of the side chain. On the other hand, the residue exhibits a very well defined conformational behaviour.

Recently, the conformational preferences of ΔAla dipeptide have been studied by several authors [12,13]. In early work, Palmer et al. [12], using the semiempirical method AM1, found the extended conformation, also known as C_5 (five-membered hydrogen-bonded system), as the most preferred. The authors also pointed out the unusual ability of the residue to induce an inverse γ-turn conformation, also known as C_7 (seven-membered hydrogen-bonded system), in the preceding residue of the polypeptide chain. More recently, the present authors, using force-field and quantum-mechanical calculations, have confirmed the preference for an extended conformation [13]. Furthermore, this conformational behaviour has been observed experimentally in structures of short peptides containing a small number of ΔAla residues, determined by both NMR spectroscopy [20–22] and X-ray crystallography [12,23–26].

In the light of these results, the assessment of the conformational preferences of a polypeptide chain of ΔAla residues appears challenging. Indeed, an extended conformation must be expected to be the most stable structure, due to the fact that the extended conformation in the ΔAla dipeptide is the lowest energy minimum and the presence of ΔAla tends to induce this conformation in short peptides. On the other hand, since ΔAla induces an inverse γ-turn in the preceding residue and this conformation is also a local minimum for the dehydroalanine dipeptide [12,13], a 2_7-ribbon structure is also possible. Surprisingly, force-field calculations predicted the 3_{10}-helix as the most favourable energy minimum for the ΔAla homopolypeptide [13]. Thus, when a chain of $(\Delta Ala)_{19}$ is considered, the extended and 2_7-ribbon conformations are considerably higher in energy than the 3_{10}-helix [13]. Consequently, it appears that the number of residues is critical for the conformation found. However, to date no systematic investigations have been carried out on the factors responsible for the extended–3_{10}-helix transition in ΔAla peptides.



Scheme 1. Structure of ΔAla.

In order to study the conformational conversion from extended and 2_7-ribbon to 3_{10}-helix in ΔAla homopolypeptides, ab initio and semiempirical SCF–MO calculations of the ΔAla dipeptide and of five ΔAla oligopeptides were carried out. The present paper addresses the influence of the number of residues on the stability of the different conformations and the effects that determine these stabilities. The results show changes in the energy ordering of the different accessible conformations when the number of residues is larger than a threshold number. Furthermore, large cooperative energy effects were found for the 3_{10}-helix conformation.

METHODS

To address the problem outlined in the introduction, conformational studies of ΔAla dipeptide (hereafter referred to as AΔAlaN) and of the oligopeptides shown in Scheme 2 (where n varies from 2–6), were carried out, using ab initio and semiempirical quantum-mechanical calculations. Comparison with 6-31G* [27] calculations for AΔAlaN allowed us to establish the suitability of the 4-31G [28] basis set for the conformational analysis of ΔAla oligomers. Consequently, ab initio calculations for ΔAla di-, tri- ($A\Delta Ala_2N$), tetra- ($A\Delta Ala_3N$) and pentapeptide ($A\Delta Ala_4N$) were performed with the 4-31G basis set. For semiempirical calculations, the AM1 method [29] was selected. AM1 is a well-known method which yields molecular geometries very similar to those resulting from ab initio calculations [29–31]. In addition, the AM1 method provides an acceptable description of the hydrogen-bonding interactions [32–34] which are of enormous importance for the present investigation. Thus, although in some cases AM1 tends to give three-centered hydrogen bonds, it provides a good description of the intramolecular hydrogen bonds in helical structures [34]. The reliability of the AM1 method in describing the conformational behaviour of ΔAla dipeptide was ensured also by comparison with the results of 6-31G* calculations. Thus, conformational energy calculations for all the oligomers studied, i.e. from the dipeptide up to the heptapeptide, were performed with the AM1 method.

In the present work all the geometries were fully optimized using AM1. The Davidson–Fletcher–Powell (DFP) optimization procedure [35,36] was used in all cases. Every minimum was characterized as such by determining and diagonalizing the Hessian matrix, and ensuring that all the eigenvalues were positive [37].

All polypeptides were blocked with an acetyl group at the N-terminus and with an *N'*-methylamide group at the C-terminus; the peptide bonds were taken to be in the trans configuration. The conformations used in the investigation were not the result of a complete conformational search. We selected three conformations which were characterized as minima on the fully relaxed energy potential surface of ΔAla dipeptide [12,13]: extended (I), C_7 (II) and helix (III). Due to the sp^2 nature of the α-carbon in the ΔAla residue, these minima are twofold degenerate: $\phi,\psi = -\phi,-\psi$. Consequently, equivalent structures with a $-\phi,-\psi$ backbone torsion angle combination were not

$CH_3CO\text{-}NHC(CH_2)CO\text{-}NHCH_3$	$A\Delta AlaN$	
$CH_3CO\text{-}\{NHC(CH_2)CO\}_n\text{-}NHCH_3$	$A\Delta Ala_nN$	$n=2,..,6$

Scheme 2. The compounds under investigation.

TABLE 1
AM1 DIHEDRAL ANGLES (DEGREES) AND RELATIVE ENERGIES (KCAL/MOL) AT THREE DIFFERENT CALCULATION LEVELS FOR THE C_5, C_7 AND HELIX MINIMA OF ΔAla DIPEPTIDE

	ω_1	ϕ	ψ	ω_2	$\Delta E_{6\text{-}31G^*}$	ΔE_{AM1}	$\Delta E_{4\text{-}31G}$
C_5	175.7	175.0	−138.7	−177.7	0.0	0.0	0.0
C_7	176.4	78.0	−62.1	−178.3	4.2	1.4	4.1
Helix	−167.5	−43.7	−30.2	175.1	4.9	2.8	7.2

considered in our study. For each ΔAla oligopeptide the three structures were built by repeating forms of these conformations, i.e. assigning to each residue of the chain the same ϕ,ψ values.

Ab initio calculations were performed with the HONDO 7.0 program [38]. Semiempirical calculations were performed with a locally modified [39] version of the MOPAC program [40], using the standard parameters [29]. Calculations were run on IBM RISC-6000 and 3090 computers.

RESULTS AND DISCUSSION

To ascertain the ability of the 4-31G basis set and the AM1 method to accurately determine the conformational stabilities of the oligopeptides investigated, test calculations were performed on AΔAlaN. Calculated 6-31G* relative energies were taken as reference values. Table 1 lists the ab initio 6-31G* and 4-31G, and semiempirical AM1 relative energies for the three minima studied. Conformational parameters obtained from AM1 geometry optimizations are also shown. The results for the three conformations are seen to agree within a few kcal/mol, independently of the computational procedure. The most stable conformation is predicted to be the extended

TABLE 2
DIHEDRAL ANGLES (DEGREES) AND RELATIVE ENERGIES (SEMIEMPIRICAL AM1 AND AB INITIO 4-31G, KCAL/MOL) FOR THE REPEATING C_5, C_7 AND HELIX CONFORMATIONS OF $A\Delta Ala_nN$, WHERE n VARIES FROM 2 TO 6

n		ω_1	ϕ_1	ψ_1	ω_2	ϕ_2	ψ_2	ω_3	ϕ_3	ψ_3
2	C_5	−176.7	−173.8	138.4	179.8	−168.2	139.5	179.0	–	–
	C_7	178.2	64.5	−87.9	174.8	78.5	−58.2	−177.5	–	–
	Helix	−170.9	−24.3	−67.5	−176.1	−32.6	−40.7	174.5	–	–
3	C_5	−176.6	−172.6	137.9	179.8	−169.9	138.8	179.6	−169.4	138.7
	C_7	177.4	68.3	−85.4	177.7	58.7	−91.4	−174.1	78.2	−65.8
	Helix	−169.7	−25.0	−61.7	177.8	−24.1	−53.4	180.0	−32.9	−40.2
4	C_5	−176.0	−173.9	137.9	−179.8	−169.3	137.7	−179.9	−169.6	138.8
	C_7	178.2	64.1	−87.5	176.8	65.1	−88.4	177.0	61.2	−92.0
	Helix	−170.2	−30.4	−57.0	179.8	−29.6	−45.2	174.9	−28.5	−48.4
5	C_5	−176.8	−173.0	137.8	−179.3	−171.7	137.5	−179.2	−171.6	138.2
	C_7	177.0	68.8	−85.0	176.8	63.7	−88.0	−176.5	66.0	−87.4
	Helix	−170.1	−28.0	−59.6	179.6	−28.1	−47.8	176.7	−30.2	−44.0
6	C_5	−176.1	−172.9	138.9	−179.8	−170.0	137.9	179.7	−167.5	138.9
	C_7	−178.1	63.3	−85.5	−176.0	64.9	−87.0	−177.8	65.4	−87.4
	Helix	−169.0	−30.9	−57.6	179.2	−24.5	−47.2	176.5	−30.4	−45.2

form, which is characterized by a five-centered hydrogen-bond ring. The next minimum, at higher energy, occurs at $\phi,\psi = 78.0°,-62.1°$, i.e. the C_7 conformation. Finally, a minimum is located in the helical region of the Ramachandran map, with torsion angles $\phi,\psi = -43.7°,-30.2°$. Thus, although AM1 seems to overestimate the stability of C_7 and helix conformations, the results indicate the reliability of both AM1 and ab initio 4-31G calculations in predicting the correct ranking of the different minima of the ΔAla oligomers.

Next, we evaluated the energy of the oligopeptides $A\Delta Ala_2N$, $A\Delta Ala_3N$ and $A\Delta Ala_4N$, using both AM1 and 4-31G calculations. For this purpose, molecular geometries were fully optimized using the AM1 method. Table 2 shows the relative energies and backbone torsion angles of the three minima, characterized for all three oligomers. Comparison with Table 1 indicates that the fully optimized ϕ,ψ angles are similar to those obtained on the AΔAlaN potential energy surface [12,13]. Computed energies for ΔAla oligopeptides seem to suggest that the AM1 potential energy surfaces are flatter than those obtained with ab initio 4-31G calculations.

In agreement with the AΔAlaN results, the extended conformation is the lowest energy form in all cases. There are stabilizing hydrogen bonds, in each case involving the carbonyl oxygen and the amide hydrogen in the same residue. For $A\Delta Ala_2N$, Table 2 shows that the repeat helix form is less stable than the repeat C_7 form at both AM1 and 4-31G levels. Thus, the C_7 structure is stabilized by hydrogen bonds between the carbonyl oxygen in residue i and the amide hydrogen in residue i+2. In contrast to this energy pattern, for $A\Delta Ala_3N$ at the ab initio 4-31G level, the C_7 conformation is found 4.3 kcal/mol higher in energy than the helix, whereas with AM1 the C_7 conformation is still 1 kcal/mol more stable than the helix. However, for $A\Delta Ala_4N$ the C_7 structure is less stable than the helix at both levels of theory. Thus, a conformational transition from 2_7-ribbon to 3_{10}-helix is predicted by both computational procedures. This occurs when the number of ΔAla residues is three (ab initio 4-31G) or four (AM1), depending on the computational procedure.

Owing to the size of the hexa- ($A\Delta Ala_5N$) and heptapeptide ($A\Delta Ala_6N$) of ΔAla, ab initio calculations on these compounds were not computationally feasible. Thus, calculations were

TABLE 2 (continued)

ω_4	ϕ_4	ψ_4	ω_5	ϕ_5	ψ_5	ω_6	ϕ_6	ψ_6	ω_7	$\Delta E_{AM1\ (4\text{-}31G)}$
–	–	–	–	–	–	–	–	–	–	0.0 (0.0)
–	–	–	–	–	–	–	–	–	–	2.3 (9.1)
–	–	–	–	–	–	–	–	–	–	3.4 (10.1)
176.4	–	–	–	–	–	–	–	–	–	0.0 (0.0)
179.1	–	–	–	–	–	–	–	–	–	2.8 (14.2)
173.9	–	–	–	–	–	–	–	–	–	3.8 (9.9)
180.0	−169.1	138.5	176.3	–	–	–	–	–	–	0.0 (0.0)
175.0	−76.1	−70.9	178.5	–	–	–	–	–	–	3.3 (19.3)
178.6	−33.4	−39.5	173.8	–	–	–	–	–	–	3.3 (6.9)
−179.4	−172.2	138.2	−179.1	−171.5	138.6	177.3	–	–	–	0.0
176.7	62.7	−90.6	174.6	77.0	−71.3	178.1	–	–	–	3.7
174.1	−27.7	−48.7	178.8	−33.3	−40.0	173.6	–	–	–	2.7
179.4	−166.8	139.3	180.0	−170.5	139.1	−179.5	−170.1	138.7	176.1	0.0
176.2	65.7	−87.7	176.5	63.6	−88.9	175.6	74.9	−72.8	177.3	4.0
175.7	−29.9	−43.7	−174.1	−27.7	−49.9	−178.5	−33.1	−39.8	173.8	1.8

performed at the semiempirical level only, which yielded qualitatively similar results to those of ab initio calculations, as was demonstrated in the above results. Table 2 shows AM1 results for both oligopeptides. As can be noted, the helix conformation is stabilized with respect to the C_7 conformation, whereas the lowest energy structure corresponds to the extended conformation in all cases. However, an important trend must be noted. The relative energy difference between the extended and helix conformations decreases when the number of ΔAla residues in the oligopeptide increases. Thus, the present results permit us to predict a conformational transition from extended to 3_{10}-helix when the number of ΔAla residues in the polypeptide chain increases considerably.

Figure 1 shows the variation of the calculated energy with chain length obtained by AM1 and ab initio 4-31G calculations. The figure shows that for a small number of residues, the C_7 conformations are more stable than the helix. For chains with three (ab initio 4-31G) or four (AM1) residues, a conformational transition from 2_7-ribbon to 3_{10}-helix is observed. Moreover, if a linear behaviour is assumed for larger chains, the helix tends to be more stable than the extended conformation, with the number of residues for which this transition occurs varying according to the computational procedure. From Fig. 1 we predict that for oligopeptides with six (ab initio 4-31G) or eight (AM1) residues, the 3_{10}-helix is the lowest energy conformation. This is in excellent agreement with our previous force-field calculations on the $(\Delta Ala)_{19}$ polypeptide [13].

The fact that oligopeptides with a large number of ΔAla residues tend to adopt a helical structure can be explained in terms of cooperative effects. Thus, the present results permit investigation of the cooperative energy effects in ΔAla oligopeptides. For this purpose, following the procedure described by Schafer et al. [41], the difference between the energy of the blocked dipeptide AΔAlaN and the tripeptide $A\Delta Ala_2N$ in a characteristic conformation can be associated with the residue energy increment (REI) that results when a single-residue fragment is inserted into a peptide chain in the same conformation. The REI values at the AM1 (ab initio 4-31G) level are: −11.8 (−153 297.8) kcal/mol for the extended conformation; −11.0 (−153 292.7) kcal/mol for the C_7 conformation; and −11.1 (−153 294.8) kcal/mol for the helix conformation. In an n-peptide, n−1 ΔAla residues are included in the chain. If the total energy, for a defined conforma-

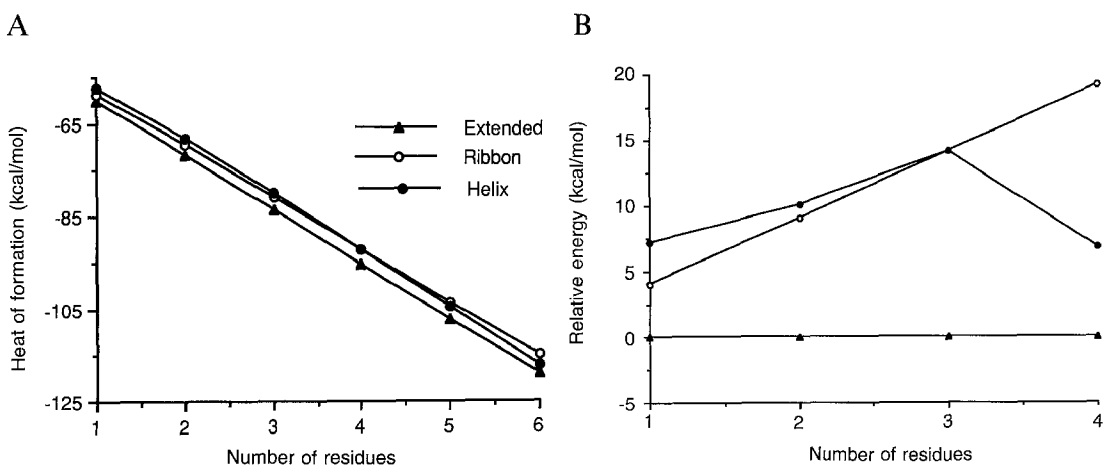


Fig. 1. Variation of (A) the AM1 heat of formation and (B) the ab initio 4–31G relative energy vs. chain length for the extended, 2_7-ribbon and 3_{10}-helix conformations.

TABLE 3
AM1 AND 4-31G COOPERATIVE ENERGY DIFFERENCES (KCAL/MOL) FOR AΔAla$_n$N OLIGOPEPTIDES, WHERE n VARIES FROM 3 TO 6 (SEE TEXT)

	n			
	3	4	5	6
C_5	0.0 (−0.2)	0.0 (−0.4)	−0.1	−0.1
C_7	2.5 (−0.3)	0.6 (−0.6)	0.9	1.3
Helix	0.3 (2.9)	1.5 (8.5)	2.7	4.2

4-31G energies are given in parentheses.

tion, does not contain any cooperative effects, then the AM1 heat of formation and the ab initio 4-31G energy can be predicted with the following equations:

$$\Delta H_f^{predicted}\text{ (n-peptide)} = (n-1) * REI + \Delta H_f^{SCF}(A\Delta AlaN)$$

$$E^{predicted}\text{ (n-peptide)} = (n-1) * REI + E^{SCF}(A\Delta AlaN)$$

In particular, for the heptapeptide the predicted heats of formation are −119.0, −113.5 and −112.9 kcal/mol for the extended, C_7 and helix conformations, respectively, whereas the calculated SCF–MO values are −118.9, −114.9 and −117.1 kcal/mol. Thus, the cooperative energy effects in the heptapeptide are 0.1, −1.4 and −4.2 kcal/mol for the extended, C_7 and helix conformers.

Table 3 shows AM1 and 4-31G cooperative energy differences for all the oligomers investigated. In general, large cooperative effects are present for the helical forms of ΔAla oligopeptides, especially at the ab initio 4-31G level, whereas smaller effects are obtained for the C_7 forms. On the other hand, no cooperativity was observed for the extended conformations. These terms permit us to explain the stabilization of the 3_{10}-helix with respect to the extended and C_7 conformations when the number of residues in the polypeptide chain increases. Similar cooperative effects have been predicted recently by other authors for alanine and glycine oligopeptides [41,42].

No solvent effect was included in our calculations, although this is likely to be significant. Indeed, the stabilization of the helix conformation is favored in vacuo, due to the fact that the electrostatic interactions were not screened. We are currently working on the evaluation of the solvent effects in the structures of ΔAla oligopeptides, using force-field-derived techniques.

CONCLUSIONS

In summary, the results reported in the present work rationalize the different structural tendencies shown by strands of $(\Delta Ala)_n$, depending on the number of residues n. Specifically, the calculations show the changes produced in the energy ordering of the C_5, C_7 and 3_{10}-helix minima when the number of residues increases. Extended and 2_7-ribbon conformations are the more stable forms for a small number of residues, but the helical form is predicted to be the most stable when this number increases.

ACKNOWLEDGEMENTS

The authors are indebted to Prof. F. Illas, Dr. J. Rubio and Dr. F.J. Luque who provided computational facilities.

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