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Conformational properties of *N*-acetyl-L-alanine *N*′,*N*′-dimethylamide★✪

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***Ab initio*/DFT analysis of the conformational properties of free Ac-Ala-NMe_2 (*N*-acetyl-L-alanine-*N*′,*N*′-dimethylamide) in terms of the N–H···O, N–H···N, C–H···O hydrogen bonds and $C^{\delta+}$ = $O^{\delta-}$ dipole attractions was performed. The Ala residue combined with the C-terminal tertiary amide prefers an extended conformation and that characteristic of the (*i* + 1)th position of the βVIb turn. These can be easily remodelled into a structure compatible with the (*i* + 1)th position of the βII/βVIa turn. The residue has also the potential to adopt the conformation accommodated at both central positions of the βIII/βIII′ turn or the (*i* + 1)th position of the βI/β′I turn.**

N-Alkylation of biologically active peptides can result in analogues with improved pharmacological properties, such as resistance to enzymatic degradation, receptor selectivity, enhanced potency and bioavailability, and sometimes in conversting an agonist into an antagonist (Gilon *et al.*, 2003). Although these structural modifications of the peptide backbone exert a powerful influence on peptide bioactivity, the consequences of the *N*-substitution for peptide conformation have not been thoroughly examined so far. The literature provides a systematic conformational theoretical analysis of only a few diamide and triamide models (Möehle & Hofmann, 1995; 1998). They show remarkable differences as

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compared with the corresponding standard peptide units, caused by the limited or lost capacity to form conventional hydrogen bonds. The authors notice the conformers stabilised with N–H···O hydrogen bonds, and the presence of those without such a bond is as a rule neglected. However, recent reports indicate weaker hydrogen bonds (Kim & Friesner, 1997; Malone *et al.*, 1997; Vargas *et al.*, 2000; 2001) and non-covalent attractions between two $C^{\delta+} = O^{\delta-}$ dipoles not mediated by a hydrogen atom (Maccallum *et al.*, 1995; Allen *et al.*, 1998) as important factors in stabilising the energy-minimised structures. The latest report on the most often studied peptide model Ac-Ala-NHMe (*N*-acetyl-L-alanine-*N*′-methylamide) (Vargas *et al.*, 2002) for the first time uses not only the N–H···O but also the weaker N–H···N and C–H···O hydrogen bonds as key to the energetics. In this work, we present a detailed *ab initio* and DFT study of the conformational properties of Ac-Ala-NMe_2 (*N*-acetyl-L-alanine-*N*′,*N*′-dimethylamide) in terms of all the contacts mentioned above and compare the results obtained with those on related molecules.

METHODS

The conformational properties were examined on a free molecule using the GAUSSIAN 98 package (Frisch *et al.*, 1998). To generate the (ϕ,ψ) potential energy surface, 576 structures calculated at the *ab initio* HF/6–31G*//HF/3–21G level were used. In each structure, all geometrical parameters were fully relaxed, except for the constrained torsion angles ϕ and ψ. Values of these angles were chosen by using a step size of 15°, within the range from –180° to 180° (Head-Gordon *et al.*, 1991). The minima observed on the surface were then subjected to full geometry optimisation at the DFT/B3LYP/6-31+G** level, which should enable correct prediction of the stability order of the minima calculated (Vargas *et al.*, 2002), followed by a second derivative analysis (frequency), which proved all of them to be minima. The geometrical parameters of the corresponding energy-minimised conformers were then further discussed.

The accessible conformational space of the molecule studied was assumed on the basis of the close resemblance between the Ramachandran contact map and the energy contours map within the limit of 5.0 kcal · mol^{-1} (Ramachandran & Sasisekharan, 1968), as is also applied elsewhere (Zimmerman *et al.*, 1977; Herzberg & Moult, 1991). The space was calculated by way of the Surfer 8 programme using the radial basis function as a gridding method.

As the overall conformational profiles of modified peptide models can differ from those of common peptide models, we describe the energy-minimised conformers of the investigated molecule by the general short hand letter notation introduced by Zimmerman (Zimmerman *et al.*, 1977).

RESULTS

Figure 1 depicts the landscape and contour Ramachandran plot for Ac-Ala-NMe_2, obtained at the HF/6–31G*//HF/3–21G level, accompanied by the conformers corresponding to seven minima fully re-optimised at the B3LYP/6–31+G** level. These conformers along with the energy information and selected geometric parameters are listed in Table 1. Figure 1 and Table 1 also present the comparative data for the Ac-L/DL-Ala-NMe_2 crystal conformers (Rzeszotarska *et al.*, 2002). Tables 2 and 3 collect the B3LYP/6–31+G** conformer geometric parameters, respectively, of the hydrogen bonds, based on Steiner's criteria (Steiner, 2002), and of dipole attractions, based on Allen's criteria (Allen *et al.*, 1998).

Calculations revealed two low-energy conformers E and C as well as five high-energy conformers G, A, A*, D* and F*. The lowest-energy conformer E (ϕ, ψ = –154°, 160°)

Table 1. Selected conformational parameters of the Ac-Ala-NMe$_2$ molecule in the solid state and in all energy-minimised conformers[a]

Methods	Conformer	Energy (Hartrees)	ΔE	ϕ	ψ	χ_C	χ_N	τ
Crystal structure [b]	H/F (L)	–	–	–81.6	157.3	–3.2	–1.3	178.8
	H/F (DL)	–	–	–66.4	158.1	–2.1	5.7	179.2
B3LYP/6–31+G**	E	–535.2027564	0.00	–153.7	159.6	–2.1	1.3	177.2
	C	–535.2006777	1.30	–101.5	112.7	–0.4	–1.3	174.6
	G	–535.1903810	7.76	–158.3	–47.9	–1.6	–20.5	170.3
	A	–535.1902603	7.84	–67.1	–35.8	1.5	–1.9	171.4
	A*	–535.1894708	8.33	50.1	53.4	–4.0	–3.7	–173.7
	D*	–535.1892693	8.46	114.1	–59.7	0.4	–1.5	177.7
	F*	–535.1885618	8.90	75.7	165.1	–0.1	–3.1	174.0

[a]Relative energy (ΔE) in kcal · mol^{-1}, ϕ, ψ, χ_C, χ_N, τ in degrees; [b](Rzeszotarska *et al.*, 2002)

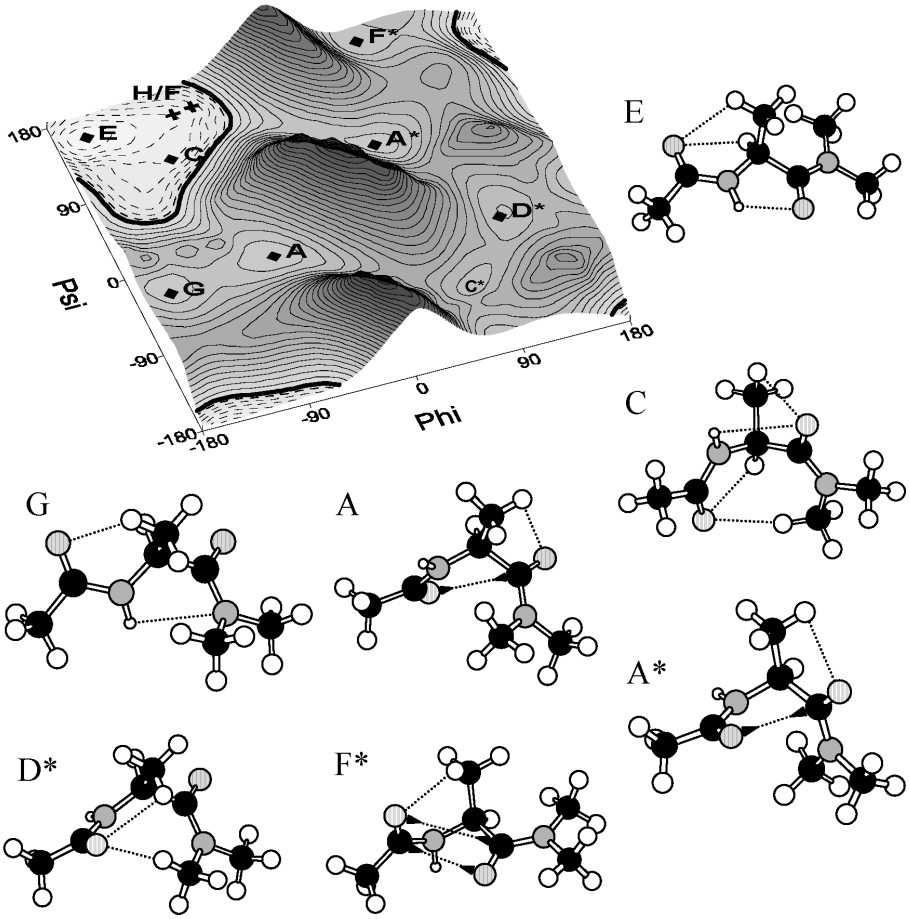

Figure 1. Landscape and contour representation of the ϕ, ψ potential energy surface of the Ac-Ala-NMe$_2$ molecule[a] together with its seven conformers[b] after optimisation at the B3LYP/6–31+G level of theory.**

[a]The map was calculated at the HF/6–31G*//HF/3–21G level of theory *in vacuo*. Geometry optimisations were performed on a 15° grid. The solid energy contours are drawn every 1 kcal · mol^{-1}. The dashed energy contours are drawn every 0.5 kcal · mol^{-1} to 5.0 kcal · mol^{-1} as the limit between the allowed and disallowed regions of Ramachandran diagram (Herzberg & Moult, 1991). ♦ DFT/B3LYP/6–31+G** minima. ✚ Solid state conformers. [b](·····) Hydrogen bond, (▶···◀) dipole attraction in the conformers. For geometric parameters of those interactions see Tables 2 and 3.

is stabilised by the conventional N–H···O hydrogen bond and by two weaker C–H···O bonds. The second lowest-energy conformer C (ϕ, ψ = –101°, 113°) contains also the conventional N–H···O hydrogen bond, however, with the geometry far away from the optimal parameters, and, in addition, three C–H···O bonds. The remaining conformers have much higher energy. Conformer G (ϕ, ψ = –158°, –47°), the third in the energy order is N–H···N and C–H···O hydrogen-bonded. Conformers A (ϕ, ψ = –67°, –36°) and A* (ϕ, ψ = 50°, 53°) are stabilised by the C–H···O hydrogen bond and the sheared parallel dipole C=O▶···◀C=O interaction. Conformer D* (ϕ, ψ = 114°, –60°) has two C–H···O hydrogen bonds. Finally, the highest-energy conformer F* (ϕ, ψ = 76°, 165°) is stabilised by the C–H···O hydrogen bond and the slightly sheared antiparallel motif involving a pair of dipole C=O▶···◀C=O interactions. The HF/6–31G*//HF/3–21G potential energy surface also shows minimum C*. However, it was not confirmed by the full optimisation and this region belongs to conformer D*.

Table 2. Structural parameters for the internal X–H···A interactions in the B3LYP/6–31+G conformers of the Ac-Ala-NMe_2 molecule[a]**

Parameters	Conformers						
	E	C [b]	G	A	A*	D*	F*
N–H···O							
H···O	2.150	–	–	–	–	–	–
N···O	2.626	–	–	–	–	–	–
∠N–H···O	106.7	–	–	–	–	–	–
∠C=O···H	87.7	–	–	–	–	–	–
N–H···N							
N–H···N	–	–	2.665	–	–	–	–
N···N	–	–	2.969	–	–	–	–
∠N–H···N	–	–	97.4	–	–	–	–
C–H···O							
H···O	*α*) 2.488 *β*) 2.869	*α*) 2.369 N) 2.382 *β*) 2.558	*β*) 2.539	*β*) 2.921	*β*) 2.892	N) 2.212 *β*) 2.553	*β*) 2.369
C···O	*α*) 2.799 *β*) 3.374	*α*) 2.824 N) 3.418 *β*) 2.863	*β*) 3.152	*β*) 3.175	β) 3.387	N) 3.260 *β*) 2.913	*β*) 2.910
∠C–H···O	*α*) 94.8 *β*) 108.2	*α*) 103.2 N) 158.2 *β*) 94.7	*β*) 114.5	*β*) 93.1	*β*) 107.6	N) 161.3 *β*) 98.1	*β*) 108.9
∠C=O···H	*α*) 79.9 *β*) 85.3	*α*) 82.3 N) 111.7 *β*) 83.3	*β*) 96.6	*β*) 67.1	*β*) 84.9	N) 90.2 *β*) 104.0	*β*) 105.0

[a]Data presented only for the X–H···A contacts (X = N, C; A = O, N) in which H···A ≤ 3.2 Å and ∠X–H···A > 90° acc. to Steiner (2002). Distances are given in Å. Angles are given in degrees. α, β and N denote C^{α}–H···O, C^{β}–H···O, and C^{N}–H···O hydrogen bonds, respectively. [b]The structural parameters for the N–H···O bond are: H···O = 2.809, N···O = 2.958, ∠N–H···O = 88.4, ∠C=O···H = 73.4

The departure from planarity of peptide bond is fully described by the twisting parameter τ and the out-of-plane parameters χ_C and χ_N (Winkler & Dunitz, 1971). The values of these parameters for the C-terminal tertiary amide in all conformers of Ac-Ala-NMe_2 essentially do not differ from the corresponding averages for the standard peptide bond (MacArthur & Thornton, 1996). However, the value of the parameter χ_N for conformer G and χ_C for conformer A* are outstanding, and seem to reflect some internal attractions. The high value of the former results from the

Table 3. Structural parameters for the internal C=O▶···◀C=O dipole interactions in the B3LYP/6-31+G conformers of the Ac-Ala-NMe_2 molecule[a]**

Conformer	Parameters								Type[b]
	$C^C\cdots O^N$	$C^N\cdots O^C$	C···C	O···O	$\angle(C{=}O)^N\cdots C^C$	$\angle O^N\cdots(C{=}O)^C$	$\angle C^N\cdots(O{=}C)^C$	$\angle O^C\cdots(C{=}O)^N$	
A	3.150	–	3.230	3.740	82.6	109.5	38.0	64.1	III
A*	2.883	–	3.070	3.359	86.8	101.9	42.5	57.2	III
F*	3.514	3.036	3.215	3.359	65.9	72.6	87.0	94.4	II

[a]Data presented only for the C=O▶···◀C=O contacts in which H···A ≤ 3.6 Å acc. to Allen *et al.* (1998). Distances are given in Å. Angles are given in degrees. [N,C]Denote the N-terminal and C-terminal carbonyl group. [b]As given by Allen *et al.* (1998).

N–H···N contact and the high value of the latter reveals the exceptionally short dipole C=O▶···◀C=O distance.

The accessible (ϕ,ψ) conformational space of the Ac-Ala-NMe_2 molecule, based on a 5.0 kcal · mol^{-1} cut-off (Zimmerman *et al.*, 1977; Herzberg & Moult, 1991), constitutes 18% of the total space, and is located almost entirely in the top-left corner of the map, in the flat region around conformers E and C. The conformer HF, found in the solid state of Ac-L/DL-Ala-NMe_2 (Rzeszotarska *et al.*, 2002), but not detected in the free molecule at the B3LYP/6–31+G** level, also falls in this shallow area.

DISCUSSION

The two lowest-energy conformers E and C of the Ac-Ala-NMe_2 molecule each contain the N–H···O hydrogen bond and the C^α–H···O one. The former bond is a conventional interaction commonly accepted to play the dominant role in determining the relative stability of peptide conformation. The latter has been recently shown to contribute considerably to the molecular energetics. For instance, when the hydrogen donor is made more acidic by the presence of electron-withdrawing groups, the C^α–H···O interaction can increase in strength to half of that of an N–H···O hydrogen bond (Vargas *et al.*, 2000). The Ac-Ala-NMe_2 molecule displays also five other conformers G, A, A*, D*, and F*, high in energy. The energetic gap between structure C and G is very large, *viz.* ΔE_{G-C} amounts to as much as 6.5 kcal · mol^{-1}. The conformers of this group are stabilised by neither N–H···O nor C^α–H···O hydrogen bonds. Nevertheless, they are clearly observable on the conformational map and exist due to C–H···O and N–H···N hydrogen bonds as well as dipole C=O▶···◀C=O interactions. The latter two contacts seem to be crucial for conformational stabilisation. However, because they are much weaker than the N–H···O or C^α–H···O hydrogen bonds, conformers G, A, A*, D*, and F* have much higher energy than conformers E and C, and are not so easily accessible for the Ac-Ala-NMe_2 molecule. Hence, one may conclude that the studied free molecule heavily prefers the extended conformation (conformer E) and the conformation typical of the (i + 1)th position of the βVIb turn (conformer C). Moreover, when the Ac-Ala-NMe_2 molecule is involved in a condensed phase, e.g. crystal (Rzeszotarska *et al.*, 2002), where some intermolecular forces operate, it readily adopts the conformation H/F located in a quite near neighbourhood of conformers E and C in the large flat region of the easily accessible conformational space (Fig. 1). The low-energy conformer H/F has been also found in the crystal of *t*BuCO-Ala-(Me)Ala-NHiPr and that of *t*BuCO-Ala-D-(Me)Ala-NHiPr, for their N-terminal Ala residue. Both these triamides assume a β-turn structure with this residue at its (i +1)th position. The former molecule adopts the

βVIa-turn (ϕ_1, ψ_1, ϕ_2, ψ_2 = −66°, 137°, −113°, 48°, respectively), and the latter adopts βII-turn (ϕ_1, ψ_1, ϕ_2, ψ_2 = −61°, 129°, 99°, −23°, respectively) (Vitoux *et al.*, 1986). The Ala residue combined with a C-terminal tertiary amide can be expected to accept also, although much less readily, a conformation (conformer A/A*) compatible with both central positions of the βIII/βIII′ turn or the (i + 1)th position of the βI/βI′ turn.

These results for Ac-Ala-NMe_2 compare well with the theoretical conformational profile of the triamide molecule Ac-Ala-NAla-NHMe (Möehle & Hofmann, 1998) (acc. to Gilon *et al.*, 2003, NAla = N-methylglycine). The ϕ, ψ torsion angles found for its N-terminal Ala residue in the peptide conformers correspond to those for the six conformers H/F, E, C, A*, A, and C* of the Ac-Ala-NMe_2 molecule, and there is a good agreement between the energetics of these two sets of conformers. Those conformers of Ac-Ala-NAla-NHMe molecule, which are the counterparts of the conformers H/F, E and C of the Ac-Ala-NMe_2 molecule are of low energy, and those related to conformers A*, A, and C* are of much higher energy. As seen, even the high-energy conformers found for the small diamide Ac-Ala-NMe_2 molecule can be adopted by the C-terminally methylated Ala residue in a larger system. So, the weak interactions within this residue can contribute to the energy of a given conformer stabilised by a strong N–H···O hydrogen bond(s) of a longer range.

Comparison of the conformational pattern of Ac-Ala-NMe_2 (E, C, G, A, A*, D*, F*, – in the given energy order) to that of the standard analogue, Ac-Ala-NHMe (C, E, C*, B, A*, G) (Vargas *et al.*, 2002), enables one to estimate the influence of the N'-methylation. Five conformers of the Ac-Ala-NMe_2 molecule, E, C, G, A*, and D*, have their counterparts among the Ac-Ala-NHMe conformers. Conformer A has no counterpart in Ac-Ala-NHMe that assumes conformer B instead. Conformer F*, although observed for Ac-Ala-NHMe in earlier reports (Head-Gordon *et al.*, 1991; Jalkanen & Suhai,, 1996), was not confirmed by higher level methods. The N'-methylation does not influence much the geometry of conformers E and G, which are stabilised by the C_5 type N–H···O and N–H···N hydrogen bond, respectively. However, N'-methylation markedly affects other conformers. Conformers C and D* are shifted toward top-left and bottom-right corners of the map with respect to the corresponding conformers of Ac-Ala-NHMe. Those of Ac-Ala-NHMe are stabilised mainly by the relatively strong C_7 type N–H···O hydrogen bond, whereas in those of Ac-Ala-NMe_2, this hydrogen bond cannot exist. In consequence, they are stabilised by various weaker hydrogen bonds including the weak C_5 hydrogen bond within conformer C. Similarly, the conformer A* of Ac-Ala-NHMe is stabilised mainly by the C_7 type N–H···O hydrogen bond whereas that of Ac-Ala-NMe_2 by the weaker dipole C=O▶···◀C=O interactions. All this features cause that Ac-Ala-NMe_2 suffers more restrictions of its conformational space than Ac-Ala-NHMe. The accessible conformational space is small (18%), and located almost entirely in the top-left corner of the map, in the flat region around the conformers E and C, which are N–H···O hydrogen-bonded. In contrast, all conformers of Ac-Ala-NHMe, except conformer G, are stabilised by the C_7 or C_5 type N –H···O hydrogen bond and therefore the conformational space accessible for this molecule is much greater (about 33%) (Ramachandran & Sasisekharan, 1968).

CONCLUSION

N-Alkylation of peptides is a powerful approach to modify their biological profiles. However, its conformational consequences are not sufficiently recognised. This work presents a detailed *ab initio* and DFT study of the conformational properties of the Ac-Ala-NMe_2 molecule (*N*-acetyl-L-alanine-N',N'-dimethyl-

amide) in terms of the conventional N–H···O hydrogen bond as well as the weaker N–H···N and C–H···O interactions and non-covalent $C^{\delta+} = O^{\delta-}$ dipole attractions.

The Ala residue combined with the C-terminal tertiary amide distinctly prefers the extended conformation as well as that characteristic of the (i + 1)th position of the βVIb turn. Both are stabilised first of all by the C_5 type N–H···O hydrogen bond and C^{α}–H···O one. They lie in the flat region of the potential energy surface and can easily remodel their torsion angles into ones compatible with the (i + 1)th position of the βII/βVIa turn, when influenced by other forces stabilising a larger system. This is documented by X-ray crystal structures (Vitoux *et al.*, 1986; Rzeszotarska *et al.* 2002) as well as suggested by the theoretical analysis on a triamide system (Möehle & Hofmann, 1998). The studied residue has the potential to adopt also the conformations typical of both central positions of the βIII/βIII′ turn or the (i + 1)th position of the βI/βI′ turn, however, at much higher energy cost. These conformations exist due to weak internal contacts. The stronger of the contacts manifest themselves in the departure from planarity of the tertiary amide bond.

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