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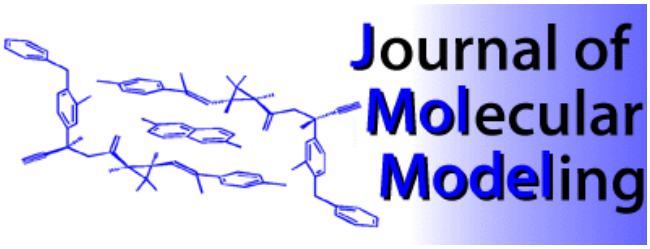

FULL PAPER

Stability Order of Basic Peptide Conformations Reflected by Density Functional Theory

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Abstract *Ab initio* density functional methods at the B3LYP/6-311+G(d, p) and 6-31G(d) levels were performed on several basic peptide conformations representing typical elements of secondary structure (β-sheets, β- and γ-turns). The results are compared with those from Hartree-Fock and MP2 correlation energy calculations. Whereas the geometries of the structures are well described at all approximation levels, there are considerable discrepancies of the stability orders. Contrary to the Hartree-Fock calculations, the correlation energy methods provide the more compact structures with intramolecular hydrogen bonds distinctly favoured over extended conformations when comparing the energy differences. However, due to considerable compensation of correlation energy and entropy contributions, the stability order at the Gibbs free energy level closely corresponds to that at the Hartree-Fock level.

Keywords Peptide secondary structure, Ab initio, Density functional theory (DFT), Hydrogen bonds, Correlation energy, Gibbs free energy

Introduction

The description of the typical secondary structure elements of peptides such as helices, β-sheets and reverse turns by means of quantum chemical methods is of special interest for the understanding of the folding process of peptides and proteins and the development of accurate force fields for calculations on larger peptides and proteins. The energy differences between the essential structure alternatives in peptides are sometimes very small and, therefore, their stability order might strongly be affected by the choice of the level of theory. Geometry optimisation at higher levels of *ab initio* MO theory is rather tedious even for smaller peptide units. Thus, most of the calculations performed so far are limited to several diamide and triamide structures neglecting correlation energy, zero-point vibration energy and entropy effects because of the time- and storage-consuming procedure necessary for their estimation [1-13]. In a recent study [14], we examined the influence of the basis set size at the Hartree Fock (HF) and MP2 correlation energy levels and also the effect of zero-point vibration energy (ZPVE) and entropy contributions on the stability of various peptide structures. It could be shown that the increase of the basis

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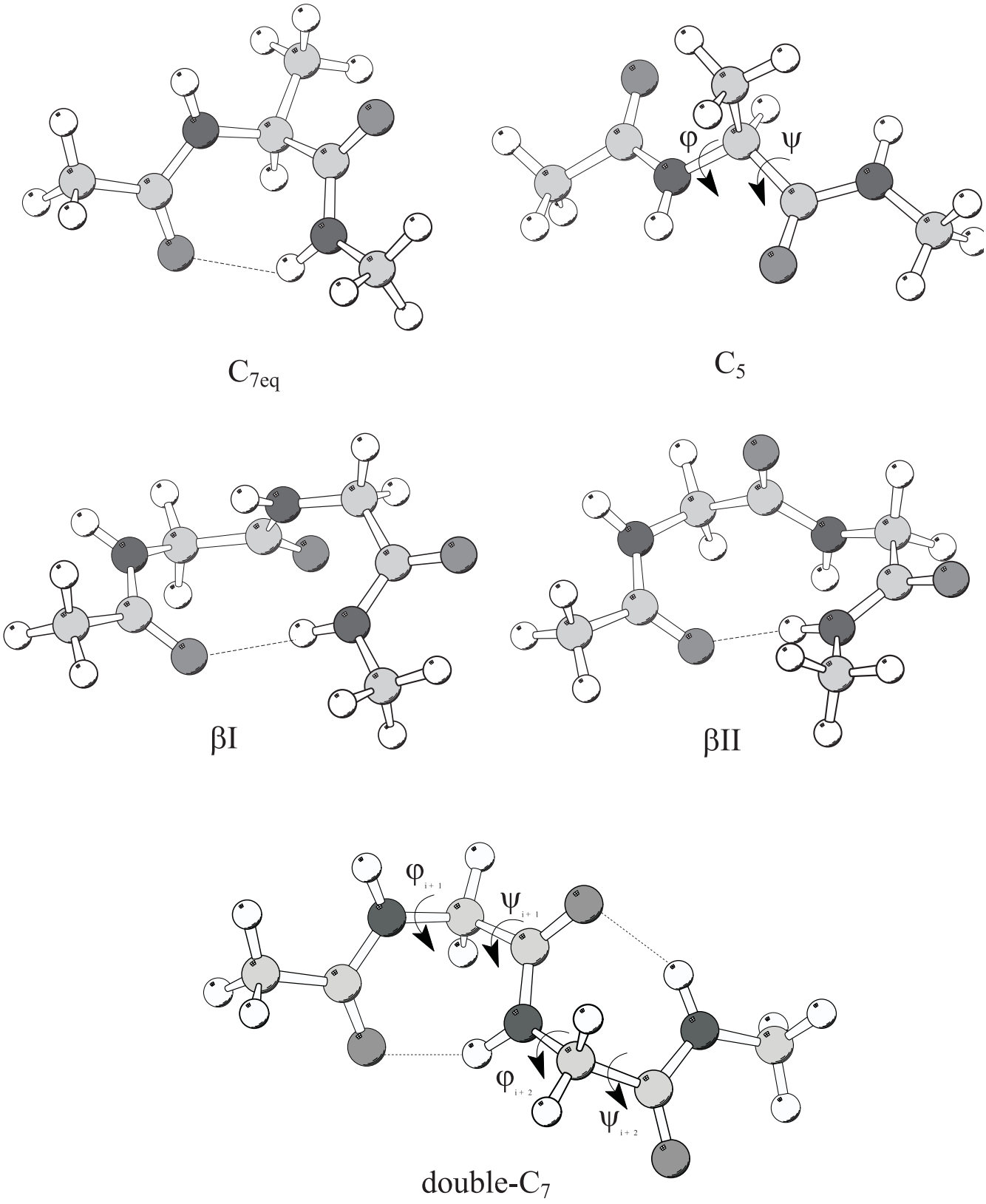

Figure 1 *Sketch of the selected conformations of the model compounds* ***1*** *(*C_{7eq}*,* C_5*) and* ***2*** *(βI, βII, double-*C_7*)*

Table 1 *Energy relations and structural data of the minimum conformations of **1** at the DFT and HF approximation levels [a]*

		C_{7eq}	C_5	C_{7ax}	α_L	β_2	α'
HF/6-31G(d)	ΔE	**0.0** [b]	1.7	12.1	19.7	10.8	21.7
	φ	-85.4	-157.4	75.9	66.8	-132.5	-165.5
	ψ	79.4	158.8	-58.9	30.7	22.2	-39.8
B3LYP/6-31G(d)	ΔE	**0.0** [c]	5.9	10.9	24.1	13.0	28.7
	φ	-82.9	-158.1	73.6	68.1	-126.6	-169.4
	ψ	72.8	164.1	-57.7	26.9	20.9	-39.3

[a] Angles in degrees, relative energies in kJ/mol
[b] E_T = -492.861542 a.u.
[c] E_T = -495.855138 a.u.

set size beyond the 6-31G(d) and 6-31G(d, p) split-valence basis sets affects only insignificantly the energetic relations between the conformers both at the HF and the MP2 levels. However, the HF and MP2 stability orders may be completely reversed if secondary structure elements of different type are compared, in particular those with and without or with a different number of intramolecular hydrogen bonds. Correlation energy generally supports the hydrogen-bonded conformations. However, considering zero-point vibration energies and entropies of the various conformers at the MP2 correlation energy level, the stabilisation of the hydrogen-bonded structures is considerably compensated due to entropy effects and the stability order originally estimated at the HF energy level results again. Thus, a stability comparison of peptide conformations of different type on the basis of MP2 energy differences may be misleading and the stability orders at the HF level employing sufficiently large basis sets agree quite well with the Gibbs free energy differences obtained at the correlation energy level due to error compensation between the correlation energy and entropy parts.

In the search for more efficient methods to calculate peptide structures, the application of *ab initio* density functional theory (DFT) seems to be promising because it is less extensive than other *ab initio* methods including correlation energy and may be, therefore, better capable to treat larger molecules [15-17]. However, some of the above-mentioned problems may also be expected in DFT calculations. In order to test the efficiency of the DFT methods for these purposes, we performed calculations on basic peptide conformations which represent typical elements of secondary structure and compared the obtained results with those of the corresponding MP2 and HF calculations [14].

Methods

The di- and triamides *N*-acetylalanyl-*N'*-methylamide (Ac-L-Ala-NHMe) **1** and *N*-acetylglycylglycine-*N'*-methylamide (Ac-Gly-Gly-NHMe) **2** have been selected as model compounds. Several peptide secondary structures as for instance the basic structures of β-sheets, β- and γ-turns can be generated from these compounds (Figure 1). The DFT geometry optimisations were performed employing the Becke 3LYP functional [18] and the 6-31G(d) and 6-311+G(d, p) basis sets, respectively. The frequencies calculated at these levels can be used without scaling for the estimation of the zero-point vibration energies and entropy contributions [19]. The Gaussian 94 program package was used for all calculations [20].

Results and discussion

HF/6-31+G(d) calculations on For-L-Ala-NH_2 [1] provide six minimum conformations on the potential energy hypersurface (C_{7eq}, C_5, C_{7ax}, α_L, β_2, α'), which are also confirmed for Ac-L-Ala-NHMe **1** at the HF/6-31G(d) level (Table 1, see also Refs. 2-9,11,13). The first four of them show some relations to peptide secondary structures. The so-called C_{7eq} form representing the global minimum on the gas phase potential energy hypersurface is realised by a seven-membered ring closed by a hydrogen bond (Figure 1). It is the simplest model for a γ-turn in peptide structures which reverses a peptide chain via three amino acids. The less stable C_{7ax} conformer corresponds to the approximate mirror image of the C_{7eq} peptide backbone (γ' or γ_i) with the L-Ala side-chain in pseudo-axial position to the seven-membered ring. Much more important

Table 2 *Structural data of the peptide backbone of* ***1****. Bond lengths in Å, bond and torsion angles in degrees*

	HF/6-31G(d)		B3LYP/6-31G(d)	
	C_{7eq}	C_5	C_{7eq}	C_5
Bond lengths				
C_1C_2	1.512	1.513	1.519	1.522
C_2N_3	1.349	1.348	1.360	1.362
N_3C_4	1.457	1.442	1.468	1.449
C_4C_5	1.535	1.526	1.552	1.538
C_5N_6	1.345	1.345	1.359	1.360
N_6C_7	1.446	1.448	1.451	1.453
Bond angles				
$C_1C_2N_3$	116.2	115.8	116.2	115.5
$C_2N_3C_4$	123.0	122.2	123.0	122.0
$N_3C_4C_5$	109.8	107.4	110.8	106.9
$C_4C_5N_6$	114.6	115.6	113.4	115.0
$C_5N_6C_7$	121.2	121.7	122.3	123.0
Torsion angles				
$C_1C_2N_3C_4$ (ω_1)	179.6	-179.9	-177.5	176.7
$C_2N_3C_4C_5$ (φ)	-85.4	-157.4	-82.9	-158.1
$N_3C_4C_5N_6$ (ψ)	79.4	158.8	72.8	164.1
$C_4C_5N_6C_7$ (ω_2)	-174.0	179.4	-175.7	177.5

is the C_5 form (Figure 1) that represents the parent conformer for the β-sheet conformation in peptides and proteins. The α_L conformation is the basic structure for left-handed helices. Although it appears as a minimum conformation for **1**, left-handed helices were not found in longer sequences of natural peptides. Interestingly, the basic conformation for right-handed helices (α_R), which frequently occurs in peptides and proteins, is not indicated for **1** and appears only when considering the influence of polar solvents [5, 8, 21]. The DFT calculations completely confirm this general picture of the conformation of **1**. The structure and energy data for the six conformers are given in Table 1 for comparison. Again, all attempts to localise the basic conformation for right-handed helices failed. There is a fair agreement between the important backbone rotation angles φ and ψ (Figure 1) given by the various methods. The peptide backbone bond lengths, bond angles and torsion angles for the most important C_{7eq} and C_5 conformers, which might be useful as reference data, are presented in Table 2. Considering the energetic relations

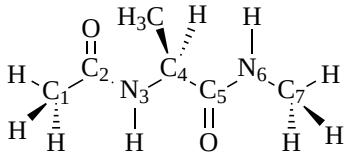

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Table 3 *Structural, energetic and thermochemical data obtained at various levels of ab initio theory for selected minimum conformations of **1** [a]*

	HF/6-31G(d)		Becke 3LYP/6-31G(d)		Becke 3LYP/6-311+G(d,p)		MP2/6-31G(d)	
	C_{7eq}	C_5	C_{7eq}	C_5	C_{7eq}	C_5	C_{7eq}	C_5
φ	-85.4	-157.4	-82.9	-158.1	-83.5	-154.8	-82.9	-158.6
ψ	79.4	158.8	72.8	164.1	76.1	159.0	77.9	161.1
ΔE	**0.0**[b]	1.7	**0.0**[c]	5.9	**0.0**[d]	3.4	**0.0**[e]	7.2
ΔG	0.9	**0.0**[f]	**0.0**[g]	2.7	0.9	**0.0**[h]	**0.0**[i]	3.5
ZPVE [j]	0.200765	0.200229	0.187269	0.186831	0.185506	0.184976	0.190620	0.190136
S [k]	457.9	464.3	458.5	467.2	459.0	473.9	463.6	473.9

[a] Angles in degrees, relative energies in kJ/mol
[b] E_T = -492.861542 a.u.
[c] E_T = -495.855138 a.u.
[d] E_T = -496.0112363 a.u.
[e] E_T = -494.310898 a.u.
[f] G = -492.700161 a.u.
[g] G = -495.706874 a.u.
[h] G = -495.865071 a.u.
[i] G = -494.159913 a.u.
[j] In a.u.
[k] In J/mol·K

between these two basic peptide conformers, the DFT results show the same stability increase for the hydrogen-bonded C_{7eq} conformer relative to the extended C_5 conformer as already found in the MP2 calculations [14], whereas both conformations are still of comparable stability at the HF level (Table 3). Obviously, correlation energy supports the more compact peptide conformers. Again as is at the MP2 approximation level, the DFT stability order is changed at the Gibbs free energy level when considering zero-point vibration energies and entropies. It can be seen that the DFT as well as the MP2 free enthalpy differences agree well with the corresponding energy differences obtained at the HF level (Table 3). This close correspondence of the HF energy to the DFT and MP2 free enthalpy data is caused by a considerable compensation of correlation energy and entropy effects. Increasing the basis set from 6-31G(d) to 6-311+G(d, p) in the DFT calculations does not change this situation.

Compound **2** may serve as another example to characterise these aspects. The conformation of this triamide model is characterised by the backbone torsion angles φ_{i+1}, ψ_{i+1}, φ_{i+2}, and ψ_{i+2} (Figure 1). Most important are the various β-turns that reverse a peptide chain via four amino acids as for instance the βI (common turn) and the βII (glycine turn) conformations (Figure 1), which are frequently found in peptides and proteins [22-24]. Both turns are stabilised by a hydrogen bond between the first and third peptide bonds. The peptide backbone geometry parameters for these two important turns in Table 4 obtained with the DFT calculations show again good agreement with the HF/6-31G(d) data. For the discussion of the stability relations, the extended conformation, which corresponds to the β sheet structure in peptides and proteins and does not exhibit hydrogen bonds, may serve as a reference conformation. Referring the stability of the turn conformations to this structure provides an idea on the folding tendency of peptide chains. Additionally, the so-called double-C_7 conformation with two hydrogen bonds (Figure 1) corresponding to two consecutive γ-turns in a peptide chain was considered. According to quantum chemical calculations [10], this conformer belongs to the most stable conformations on the potential energy hypersurface of **2**, although experimental hints for its existence in condensed phase are missing. The comparison of the energetic relations between the conformers at the HF and DFT levels shows the expected differences (Table 5). Whereas the extended conformer is most stable at the HF level, the double-C_7 conformer with the two hydrogen bonds is preferred according to the DFT calculations. Comparing the stabilities of the two β-turns, βII is more stable than βI as it is typical for β-turns with the amino acid glycine in the third position [23, 24]. Since the two conformations are of the same type, i.e. both have one hydrogen bond, their stability order does not change at the various approximation levels. This can also be seen when comparing the HF and MP2 results [14], whereas the stability of the extended form relatively to the turn conformations is completely reversed at the two different levels. Calculating the Gibbs free energies at the DFT level provides the stability order extended > double-C_7 > βII > βI which shows the extended conformer again more stable than the hydrogen-bonded structures as it is estimated at the HF energy level. The higher order in the more compact structures reflected by lower en-

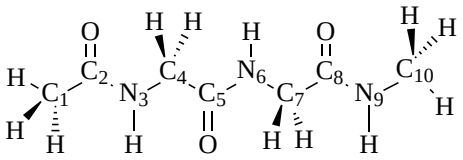
Table 4 *Structural data of the peptide backbone of* ***2****. Bond lengths in Å, bond and torsion angles in degrees*

	HF/6-31G(d)		B3LYP/6-31G(d)	
	βI	βII	βI	βII
Bond lengths				
C_1C_2	1.511	1.508	1.518	1.516
C_2N_3	1.360	1.355	1.371	1.364
N_3C_4	1.449	1.442	1.459	1.454
C_4C_5	1.524	1.527	1.537	1.540
C_5N_6	1.348	1.350	1.362	1.365
N_6C_7	1.443	1.444	1.451	1.453
C_7C_8	1.526	1.527	1.539	1.539
C_8N_9	1.341	1.341	1.357	1.356
N_9C_{10}	1.446	1.447	1.452	1.453
Bond angles				
$C_1C_2N_3$	116.1	116.3	116.2	116.4
$C_2N_3C_4$	120.6	118.7	120.9	119.6
$N_3C_4C_5$	116.2	111.1	116.5	110.9
$C_4C_5N_6$	117.0	115.5	116.2	114.9
$C_5N_6C_7$	122.9	122.7	122.5	122.3
$N_6C_7C_8$	116.1	116.3	115.7	115.5
$C_7C_8N_9$	117.6	117.8	116.4	116.6
$C_8C_9C_{10}$	120.9	120.7	121.3	120.6
Torsion angles				
$C_1C_2N_3C_4$ (ω_1)	-165.9	169.6	-168.6	172.4
$C_2N_3C_4C_5$ (φ_1)	-73.3	-60.9	-73.4	-62.3
$N_3C_4C_5N_6$ (ψ_1)	-17.7	136.4	-14.1	130.3
$C_4C_5N_6C_7$ (ω_2)	175.6	-174.6	170.5	-170.5
$C_5N_6C_7C_8$(φ_2)	-101.9	95.5	-105.3	102.5
$N_6C_7C_8N_9$(ψ_2)	11.9	-11.7	16.4	-16.7
$C_7C_8N_9C_{10}$(ω_3)	175.9	-176.3	176.6	-176.7

Table 5 *Structural, energetic and thermochemical data obtained at various levels of ab initio theory for selected minimum conformations of* ***2*** *[a]*

	HF/6-31G(d)				Becke 3LYP/6-31G(d)			
	extended	βI	βII	double-C_7 [b]	extended	βI	βII	double-C_7 [b]
φ_{i+1}	-179.9	-73.3	-60.9	-85.8	179.4	-73.4	-62.3	-81.2
ψ_{i+1}	-179.7	-17.7	136.4	65.4	179.8	-14.1	130.3	63.7
φ_{i+2}	-179.7	-101.9	95.5	-86.1	180.0	-105.3	102.5	-82.7
ψ_{i+2}	-179.7	11.9	-11.7	60.9	-179.9	16.4	-16.7	64.7
ΔE	**0.0**[c]	5.9	1.2	2.7	5.7	8.8	5.2	**0.0**[d]
ΔG	**0.0**[f]	20.2	16.6	14.7	**0.0**[g]	17.0	12.5	9.0
ZPVE [i]	0.229991	0.231450	0.231671	0.231804	0.213901	0.215348	0.215420	0.215862
S [j]	558.6	516.2	513.5	527.8	564.6	524.3	528.4	525.1

	MP2/6-31G(d)			
	extended	βI	βII	double-C_7 [b]
φ_{i+1}	-171.2	-72.1	-58.6	-83.2
ψ_{i+1}	-176.9	-21.2	139.8	66.4
φ_{i+2}	-179.8	-99.6	92.7	-85.2
ψ_{i+2}	-179.8	15.3	-14.0	67.7
ΔE	14.6	3.4	**0.0**[e]	0.3
ΔG	**0.0**[h]	7.7	5.4	3.4
ZPVE [i]	0.217266	0.218868	0.219081	0.21933
S [j]	580.4	522.5	520.7	530.8



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[a] Angles in degrees, relative energies in kJ/mol
[b] Chair conformation
[c] E_T = -660.641395 a.u.
[d] E_T = -664.550692 a.u.
[e] E_T = -662.547719 a.u.
[f] G = -660.458731 a.u.
[g] G = -664.382142 a.u.
[h] G = -662.374123 a.u.
[i] in a.u.
[j] in J/mol·K

tropy values is responsible for their destabilisation in comparison to the β-sheet structure. Even if the correlation energy methods (DFT, MP2) provide the same general tendency of the stability orders of the various peptide conformers, the quantitative agreement is not perfect. Interestingly, the entropy values for the two β-turns and the double-C_7 conformer are rather similar despite the different number of hydrogen bonds. Obviously, the β-turn conformations with only one hydrogen bond and the double-C_7 conformer with its two γ-turns are of comparable order.

Conclusions

The results of this study show considerable discrepancies of the stability order of basic peptide conformations dependent on the employed approximation level in the calculations. Remembering the sometimes small energy differences between structure alternatives of peptides, errors found for shorter peptide sequences, even if they are small, may lead to incorrect descriptions of larger systems and may be misleading in our general understanding of structure formation in peptides and proteins. Most important is the different de-

scription of the stabilities of hydrogen-bonded conformations at the Hartree-Fock and correlation energy levels. In order to get reasonable stability orders for peptide conformers of different type by means of density functional theory or other correlation energy methods, it is necessary to consider zero-point vibration energies and entropies, whereas HF energy differences obtained with more extended basis sets may be sufficient for the same purpose due to considerable compensation of the correlation energy and entropy contributions. The effects described here demonstrate some serious problems for a reasonable description of peptide and protein structures by means of theoretical methods and make a reliable extension of these formalisms to the condensed phase difficult. These aspects might also be important for the development of empirical force fields on the basis of different approximation levels of *ab initio* MO theory.

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References

1. Head-Gordon, T.; Head-Gordon, M. ; Frisch, M. J.; Brooks III, C. L.; Pople, J. A. *J. Am. Chem. Soc*. **1991,** *113* , 5989-5997.
2. Böhm, H.-J.; Brode, S. *J. Am. Chem. Soc*. **1991**, *113*, 7129-7135.
3. Gould, I. R.; Kollman, P. A. *J. Phys. Chem.* **1992**, *96* , 9255-9258.
4. Frey, R. F.; Coffin, J.; Newton, S. Q.; Ramek, M.; Cheng, V. K. W.; Momany, F. A.; Schäfer, L. *J. Am. Chem. Soc*. **1992**, *114* , 5369-5377.
5. Rommel-Möhle, K.; Hofmann, H.-J. *J. Mol. Struct. (THEOCHEM)* **1993**, *285*, 211-219.
6. Gould, I. R.; Cornell, W. D.; Hillier, I. H. *J. Am. Chem. Soc.* **1994**, *116*, 9250-9256.
7. Endredi, G.; Perczel, A.; Farkas, O.; McAllister, M. A.; Csonka, G. I.; Ladik, J.; Csizmadia, I. G. *J. Mol. Struct. (THEOCHEM)* **1997**, *391*, 15-26.
8. Brooks III, C. L.; Case, D. A. *Chem. Rev* **1993**, *93*, 2487-2502.
9. Perczel, A.; McAllister, M. A.; Csaszar, P.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1993**, *115*, 4849-4858.
10. Böhm, H.-J. *J. Am. Chem. Soc.* **1993**, *115*, 6152-6158.
11. Schäfer, L.; Newton, S. Q.; Cao, M.; Peeters, A.; Van Alsenoy, C.; Wolinski, K.; Momany, F. A. *J. Am. Chem. Soc.* **1993**, *115*, 272-280.
12. Möhle, K.; Gußmann, M.; Hofmann, H.-J. *J. Comput. Chem.* **1997**, *18*, 1415-1430.
13. Beachy, M. D.; Chasman, D.; Murphy, R. B.; Halgren, T. A.; Friesner, R. A. *J. Am. Chem. Soc.* **1997**, *119*, 5908-5920.
14. Möhle, K.; Gußmann, M.; Rost, A.; Cimiraglia, R.; Hofmann, H.-J. *J. Phys. Chem. A* **1997**, *101*, 8571-8574.
15. Parr, R. G.; Yang, W. *Density-Functional Theory of Atoms and Molecules*; Oxford University: New York, 1989.
16. *Density Functional Methods in Chemistry*; Labanowski, J. K.; Andzelm, J. W., Eds.; Springer: New York, 1991.
17. *Modern Density Functional Theory – A Tool for Chemistry*; Seminario, J. M.; Politzer, P., Eds.; Elsevier Science: Amsterdam, 1995.
18. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372-1377.
19. Jalkanen, K. J.; Suhai, S. *Chem. Phys.* **1996**, *208*, 81-116.
20. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Peterson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challcombe, V.; Peng, C.Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94 (Revision D.4)*, Gaussian Inc., Pittsburgh, PA, 1996.
21. Shang, H. S.; Head-Gordon, T. *J. Am. Chem. Soc.* **1994**, *116*, 1528-1532.
22. Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425-1436.
23. Richardson, J. *Adv. Protein Chem.* **1981**, *34*, 167-339.
24. Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135-175.