Structure and Conformational Behavior of Biopolymers by Density Functional Calculations Employing Periodic Boundary Conditions. I. The Case of Polyglycine, Polyalanine, and Poly-α-aminoisobutyric Acid in Vacuo

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Abstract: Fully quantum mechanical calculations exploiting periodic boundary conditions (PBC) have been applied to the study of four different regular structures (α- and 3_{10}-helix, fully extended and repeated γ-turns) of the infinite polypeptides of glycine, alanine, and α-aminoisobutyric acid (Aib) in vacuo. α-Helix is predicted to be the most stable conformer for polyalanine and polyglycine, being stabilized over the 3_{10}-helix mainly by more favorable dipole−dipole interactions. Contrary to previous suggestions, steric effects and hydrogen-bond strengths are comparable for both helix structures. 3_{10}-Helix is preferred for poly-Aib, since in this case α-helix is strongly distorted due to unfavorable intrachain repulsions. Extended structures and repeated γ-turns are much less stable than helix structures for all of the polypeptides examined, mainly due to the absence of favorable long-range interactions. The optimized geometries are in good agreement with the available experimental data and reveal a remarkable dependence on the nature of the residue forming the polypeptides; at the same time the electronic and structural parameters of each residue strongly depend on the secondary structure of the polypeptides.

1. Introduction

The elucidation of the factors influencing the stability of the protein secondary structures is one of the most important longstanding goals in biochemical research.[1−4] The complexity of proteins emphasized the role of synthetic homopolypeptides and block copolypeptides as models of general peptide and protein systems.[5,6] In this field the integration of experimental[5−10] and computational results[11−18] has been particularly fruitful, leading to considerable advances toward a reliable description of the geometry and the conformational equilibria of the most common secondary structures (e.g., α-helix, 3_{10}-helix, β-sheet),[19] and a better understanding of the factors responsible for their stability.[20−23] However, many questions are still open, even

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concerning the most thouroughly studied systems, like polyalanine and its parent compounds. These polypeptides have indeed been used the most to study the thermodynamics and the kinetics of protein folding and to determine the structural preferences of different amino acid residues.[12,13,16,24] Quite ironically, the relative propensity[25−27] of alanine to form α-helices has itself been one of the most challenging subjects of debate. On one hand alanine has been considered a very strong (if not the strongest) α-helix stabilizing residue.[28−34] On the other hand, alanine has been claimed to be "helix indifferent".[35−37] It has been also suggested that medium-size alanine homopolypeptides form 3_{10}-helices in solution.[38,39]

Molecular mechanics (MM)-based simulations agree in assigning to polyalanine a clear-cut preference for α-helix over 3_{10}-helix, but their results differ remarkably from the quantitative point of view. For example for the alanine decapeptide in vacuo Zhang and co-workers[15] suggest that the ΔE between α- and 3_{10}-helix is about 12 kcal/mol, whereas Kuczera and co-workers[12] predict a value of ∼18 kcal/mol. Among the derivatives of alanine, the peculiar structural features[40] and the biological importance of α-aminoisobutyric acid (Aib),[41] also known as methylalanine, spurred many experimental and computational investigations on the homopolypeptides of this residue.[12,14,17,40,42] The results of these studies are contradictory: according to Smythe at al.[14] 3_{10}- and α-helices are practically isoenergetic in vacuo for the Aib decapeptide ($(Aib)_{10}$), whereas several other papers[12,15] predict that α-helix is remarkably favored for homooligopeptides with more than six residues. These results are in disagreement with the experimental evidence: X-ray diffraction shows that $(Aib)_{10}$ adopts a 3_{10}-conformation in the solid state,[40a] and several vibrational studies of fibers of polyAib suggest that the preferred conformation is 3_{10}- and not α-helix.[43] The factors influencing the conformational equilibrium between α- and 3_{10}-helices in polyAib are also a matter of debate.[12]

Quantum mechanical methods could surely help to clarify these questions, provided that all of the interresidues, long-range interactions (hydrogen bonds, dipole−dipole interactions, etc.) are properly taken into account. Such interactions indeed critically influence the secondary structure of polypeptides and proteins, and cause the well-known failures[44] of the so-called "dipeptide approximation" (i.e., the modeling of a polypeptide by the residue capped with an acetyl group at the N-terminus and an acetamide group at the C-terminus). Furthermore, due to their cooperative nature, these effects are enhanced in repeating motifs extending over several units and are very important in stabilizing α-helices in proteins.[45,46] As a matter of fact, the average length of helix stretches in all α- proteins is 12.6 residues,[47] and stretches longer than 25 residues are not uncommon.

Unfortunately, severe computational requirements have restricted until now the use of accurate ab initio methods (but for very recent exceptions[48,49]) to the study of oligopeptides too small to fully display all of the long-range interactions,[50−54] thus remarkably limiting their usefulness.

Quantum mechanical methods rooted in the density functional theory (DFT) have emerged as the most effective tools to overcome these limitations, since they combine an accuracy comparable to those of the most refined post Hartree−Fock methods with a much lower computational cost, enabling the study of systems large enough to exhibit significant long-range interactions.

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However, the complete treatment of the latter effects requires a further step that can be accomplished by resorting to a method which exploits periodic boundary conditions (hereafter PBC) to treat infinite systems.[55,56] While PBC/DFT computations have a long history,[57] effective algorithms enabling geometry optimizations with large Gaussian basis sets have just recently been coded.[55,56] Here we report a complete DFT/PBC study of four representative regular structures (α- and 3_{10}-helices, fully extended and repeated γ-turn) of the infinite homopolypeptides of alanine (AIH) and Aib (AibIH) in vacuo, comparing them to the corresponding structures of the infinite homopolypeptide of glycine (GIH), whose preliminary analysis has been reported in a previous methodological paper.[58]

Besides their intrinsic interest, these results should provide a deeper insight on more general subjects, such as the factors influencing the relative stability of different secondary structures in polypeptides and in proteins. In this spirit, we present here: (i) a comparison between the behavior of the dipeptide analogue and the infinite polypeptide, that allows the discrimination and evaluation of interresidue and intraresidue interactions, (ii) an analysis of the effect (both steric and electronic) of multiple substitution at C^{α}.

A careful comparison between calculated and experimental equilibrium structures is also reported.

All of our results refer to isolated polypeptides and should therefore be compared to experimental results in low-polarity solvents. At the same time, in vacuo computations can constitute a useful premise for a forthcoming assessement of the role played by solvent effect.

2. Computational Details

Both nonperiodic and periodic DFT calculations were carried out with a development version of the Gaussian suite of programs.[59] The details of the periodic DFT algorithm for Gaussian orbitals as implemented in the Gaussian package were recently presented by two of us in ref 56. In each periodic calculation the number of **k** points employed was such to ensure the convergence of the energy and forces to 10^{-8} in conventional units: for α-helix 8 **k** points are sufficient, whereas for the other conformers 32 **k** points have been used (see also ref 58). From the variety of currently available density functionals we chose the PBE model[60] which provides good accuracy for a wide variety of systems including hydrogen bonds.[58,61] All of the calculations have been performed using the standard 6-31G(d) basis set. A benchmark study of GIH[58] has shown that extension of the basis set up to the 6-311++G(2d,2p) level and use of different density functionals does not change significantly the results obtained at the 6-31G(d) level.

Molecular geometry optimizations were carried out using the standard redundant internal coordinate algorithm available in the Gaussian package,[62] while the periodic optimizations employed the modified algorithm described in ref 63.

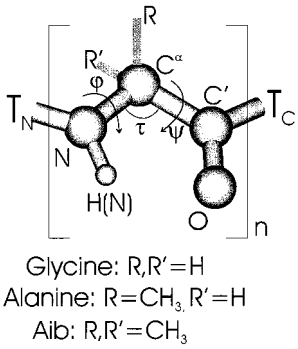

Figure 1. Schematic drawing of the amino acids examined in the present study together with atom labeling and ϕ,ψ dihedrals. When $n = 1$, T_N = acetyl group and T_C = *N*-methylamide, the drawing corresponds to the dipeptide analogue of the different amino acids.

3. Results

The atom labeling and the main geometric parameters of the amino acid residues studied are shown in Figure 1. The regular structures considered in the present study originate from the repetition of the four basic cycles closed by a hydrogen bond, shown in Figure 2, namely C5 ($\phi, \psi \approx 180°$), C7 ($\phi \approx \pm 90°$, $\psi \approx \mp 60°$), C10 ($\phi \approx \pm 60°$, $\psi \approx \pm 30°$), C13 ($\phi \approx \pm 55°$, $\psi \approx \pm 45°$). The first structure is related to β-sheets, whereas the other three structures lead to γ-turns, and 3_{10}- and α-helices, respectively. The H-bonds formed in the different situations (see Figure 2) involve a single residue (C5), or more or less distant pairs of residues ($i/i+2$, $i/i+3$, and $i/i+4$ for C7, C10, and C13 cycles, respectively). Note that for natural alanine residues equatorial C7 structures ($\phi < 0$, $\psi > 0$) and left-handed helices ($\phi < 0$, $\psi < 0$) are favored over the corresponding axial C7 structures ($\phi > 0$, $\psi < 0$) and right-handed helices ($\phi > 0$, $\psi > 0$). As a consequence we have considered only the former pair of structures in L-alanine infinite homopolypeptide (AIH). In the following we will use the label of the cycle also to denote the structure in which the cycle is regularly repeated.

As anticipated above, 3_{10}-helix has recently received considerable attention,[12,13,64] although only about 10% of protein helical residues adopts the C10 conformation, and the average length of 3_{10}-helices is usually less than 4 residues[47] (even if 3_{10}-helices spanning 7−12 residues have been reported). As a matter of fact, it has been suggested that this conformation is an intermediate in the coil−α-helix folding[13,65−68] and is

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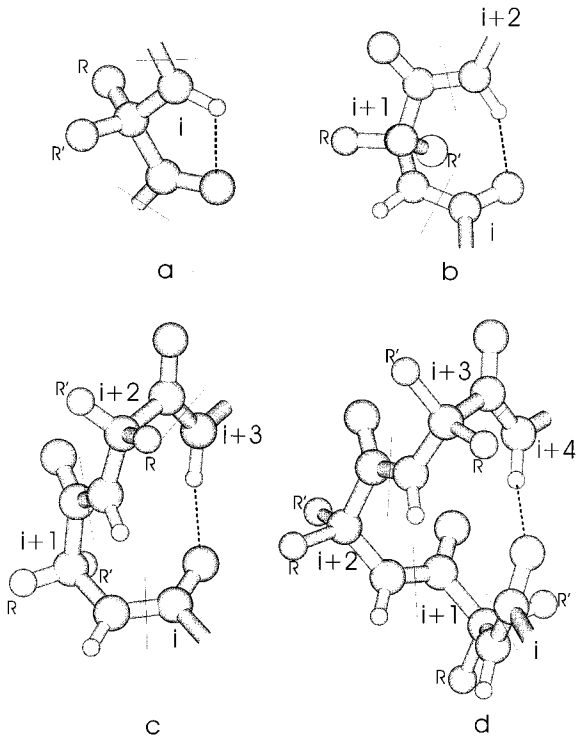

Figure 2. Schematic drawing of the four basic cycles closed by a H-bond from which originate the secondary structures examined in the present paper: (a) C5 (fully extended structure), (b) C7 (repeated γ-turns, (c) C10 (3_{10}-helix), (d) C13 (α-helix). H-bonds are represented by dotted lines.

involved in the ion channel formation by peptaibols. A 3_{10}-/α-helix transition can also be a crucial step in some enzymatic reactions.[69] Finally, 3_{10}-helix is the minimum energy conformation for several oligopeptides (often Aib-containing).[40] According to both experimental[70] and quantum mechanical[49,54] studies, C7 structure is the most stable for short oligopeptides in vacuo or in apolar solvents, but it is very uncommon in proteins. However, a molecular dynamics study suggests that this conformation could be an important intermediate in the transition from extended to folded structures.[66] Also the fully extended structure is relatively stable for oligopeptides,[71] but it is seldom found in proteins.

In the first part of this section we present the results relative to the dipeptide analogues of Ala and Aib (i.e., residue capped with an acetyl group at the N-terminus and an acetamide group at the C-terminus). Since C7 and C5 conformers exhibit a hydrogen bond already at the dipeptide level, the comparison between results of the PBE/6-31G(d) calculations and those of previous studies will validate the theoretical methodology for the study of hydrogen bonded systems. Furthermore, since helices do not form hydrogen bonds at the dipeptide level, these calculations can give useful insights on the importance of intraresidue steric interactions in determining the differential stability of α- and 3_{10}-helix. This also makes it easier to investigate the role played by the long-range interactions in determining the conformational equilibria in the infinite polypeptides. The second part is devoted to the analysis of the results of the DFT/PBC calculations on infinite homopolypeptides AIH, AibIH, and GIH.

First, we compare calculated and experimental structures: this step is crucial for determining the accuracy of our model and the reliability of the results that cannot be derived directly from the experiments. Second, the comparative analysis of the geometric and electronic structures of GIH, AIH, and AibIH will allow obtaining some insights on the effect that a systematic variation in the monomer (i.e., the addition of a methyl substituent on C^{α}) has on the conformation of a polypeptide chain.

From a complementary point of view, we then show that the secondary structure of the polypeptide has a remarkable influence on the intraresidue geometry, irrespective of the nature of the residue.

Finally, the PBC/DFT-optimized geometries will be used for some semiquantitative analyses, aimed to define which factors determine (i) the α-/3_{10}-helix equilibrium; (ii) the preference of the polypeptides for helix structures with respect to extended and repeated γ-turns structures; (iii) the stability of 3_{10}-helix for polyAib.

3.1. Dipeptide Analogues. In Tables 1 and 2 we report the results of PBE/6-31G(d) calculations on the Ala, Gly, and Aib dipeptide analogues (hereafter ADA, GDA, and AibDA, respectively). Since the conformations leading to α- and 3_{10}-helices are not energy minima for the isolated dipeptide analogues, the values reported in Tables 1 and 2 for these conformations refer to constrained optimizations where ϕ and ψ dihedrals have been frozen to their mean value in proteins.[47] We optimized at the PBE/6-31G(d) level also the geometry of a *N*-methylacetamide molecule (NMA) (which is obtained by joining the N-terminal and C-terminal cappings of the dipeptide). The difference between the energy of each dipeptide and the energy of NMA can indeed provide a "reference energy" for the monomer to be compared with the results of PBC calculations (see notes of Tables 1 and 2). Obviously, this comparison is really meaningful only for C5 and C7 conformations, which can form a hydrogen bond already at the dipeptide level.

Inspection of Table 1 shows that the geometries and the stability trend predicted at the PBE/6-31G(d) level compare well with the HF/MP2 and B3LYP results, confirming the reliability of the PBE functional for the study of peptides.[58,49] Helix conformers are remarkably less stable than C5 and C7 conformers, the latter corresponding to the absolute energy minimum for all of the residues; however, the C5 conformer is comparatively more stable in AibDA, where it is just 0.78 kcal/mol less stable than the C7 one. To establish an intraresidue H-bond, the $N-C^{\alpha}-C'$ valence angle (hereafter τ) is forced to a value about $6-7°$ smaller than the optimum value for a peptide residue. The $C^{\beta}-C^{\alpha}-C^{\beta'}$ angle in Aib is, of course, larger than the $C^{\beta}-C^{\alpha}-H^{\alpha}$ in alanine and $H^{\alpha}-C^{\alpha}-H^{\alpha'}$ in glycine. As a consequence, the τ angle is narrower in Aib than in the other residues, and this, in turn, stabilizes the C5 conformer. This effect is expected to grow by increasing the dimensions of the C^{α} substituents. As a matter of fact, oligopeptides based on symmetrically disubstitued $C^{\alpha,\alpha}$-dialkylglycines such as diethylglycine and di-*n*-propyl-glycine adopt an extended C5 conformation in the solid state.[71] Interestingly the τ bond angle in those peptides exhibits values as low as 103°,[71] in agreement with our results.

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Table 1. Relative Energy (in kcal/mol) and Main Geometric Parameters of the PBE/6-31G(d)-Optimized Structures for ADA[a]

	α-helix	3_{10}-helix	C7			C5		
				MP2[b]	B3LYP[c]		MP2[b]	B3LYP[c]
energy[d]	6.36	6.09	0.0	0.0	0.0	1.89	1.86	1.43
τ	111.7	114.0	111.0		111.0	106.5		106.8
ϕ	−62	−71	−82.1	−83.1	−81.9	−158.2	−158.4	−157.3
ψ	−41	−18	71.0	77.8	72.3	168.3	161.3	165.3

[a] Bond and dihedral angles in deg. [b] MP2/TZP//MP2/6-31G(d).[54] [c] B3LYP/6-31G(d).[53] [d] Energy for monomer (see text) (C7) Ala = −247.04151 au, (C5) Ala = −247.03849 au.

Table 2. Relative Energy (in kcal/mol) and Main Geometric Parameters of the PBE/6-31G(d)-Optimized Structures for GDA[58] and AibDA[a]

	α-helix	3_{10}-helix	GDA C7	GDA C5
energy[b]	5.62	4.52	0.0 (0.0)[c]	1.40 (1.99)[c]
τ	114.8	116.9	113.0 (112.9)[c]	108.2 (109.2)[c]
ϕ	±62	±71	∓80.9 (∓85.5)[c]	∓162.8 (−179.1)[c]
ψ	±41	±18	±71.7 (±72)[c]	±168.3 (−179.5)[c]
			AibDA	
energy	3.91	3.59	0.0	0.78 (0.2)[d]
τ	110.3	112.5	111.8	104.1
ϕ	±62	±71	∓72.9 (∓75.7)[d]	180.0
ψ	±41	±18	±57.2 (±58.0)[d]	180.0

[a] Bond and dihedral angles in deg. [b] Energy for monomer (see text) (C7) Gly = −207.78095 au, Aib = −286.29645 au; (C5) Gly = −207.77873 au, Aib = −286.29521. [c] MP2/TZP//HF/6-31G(d,p)[52f] calculations. [d] MP2/6-31G(d)//HF/6-31G(d)[52g] calculations.

Our computations predict that incipient helix structures are relatively more stable for AibDA, in agreement with the experimental finding that Aib has a strong helix-inducing power; this is the only residue for which 3_{10}-helix is a minimum of the potential energy surface ($\phi = -66.9°$, $\psi = -25.1°$). However, the lack of hydrogen bonds prevents those conformations becoming the most stable at the dipeptide level (still 3.51 kcal/mol less stable than C7 conformer).

For all of the amino acids examined 3_{10}-helix is more stable than α-helix. This clearly shows that the preference for the latter conformer cannot be traced back to the intraresidue steric repulsions. 3_{10}-Helix is relatively more stable for AibDA; however, the difference between AibDA and ADA is rather small (∼0.04 kcal/mol), suggesting that intraresidue interactions should play a minor role in determining the preference of Aib for 3_{10}-helix.

The electron inductive effect of the methyl substituents at C^α has been investigated on the helix conformers, which, due to the lack of hydrogen bonds, allow a more complete separation of the effects. Despite its limits, the Mulliken population analysis allows the analysis of general trends for closely related structures. In α-helix the bond order between N and C′ increases (from 0.242 to 0.250 au), and the C′−O one decreases (from 0.588 to 0.567 au) when going from GDA to AibDA. At the same time the atomic charges of N and O become more negative (from −0.570 to −0.587 au and from −0.447 to −0.455 au, respectively) when the number of methyl substituents on C^α increases. The inductive effect of the methyl group increases the electron density on C^α and, to a lower extent, on the nitrogen atom. This stabilizes the classic amidic resonance form, which involves a double N^+═C and a single C−O^- bond.

As already shown by Schäfer et al.,[51] the value of the τ angle depends strongly on the $\phi-\psi$ dihedrals: this result has been confirmed by a statistical survey of resolved protein structures.[72] However, ab initio calculations performed at different levels of theory and with different basis sets always overestimate the importance of this effect. This discrepancy (as already suggested by Schäfer and co-workers) should be mainly due to the lack in the computations of all of the long-range effects. Those results thus clearly show that calculations on dipeptide analogues can provide valuable information but, at the same time, point out the limitations of this approach.

3.2. PBC calculations. PBC calculations predict that all of the conformations we studied are minima on the potential energy surface (see Figure 3). Some test optimizations starting from a β-strand conformation ($\phi \approx -130°$, $\psi \approx 130°$) collapsed in the fully extended structure. This result could be expected since the β-conformation is strongly stabilized by H-bonds with the solvent or with adjacent polypeptide chains, both of which are lacking in our calculations. For C5 and C7 structures we used a repeating unit containing two residues, whereas for the 3_{10}-structure the repeating unit contains three residues (as in the standard 3_{10}-helix). For α-helix we used a repeating cell with 7 residues (3.5 × 2): this should be a good approximation, since the average pitch of an α-helix has been claimed to contain between 3.54 and 3.67 residues,[47,73,74] the former value referring to α-helices in globular proteins.[47] Moreover, geometry optimizations of GIH, using a repeating unit containing 18 residues (3.6 × 5), as it should be for an ideal α-helix, give very similar results, confirming the reliability of our assumption.[58]

3.2.1. Equilibrium Structures. The geometries predicted for helices of AIH (and GIH) are in good agreement with the experimental determinations for α-helix motifs in proteins (see Tables 4 and 6). There have been several estimates of the value of ϕ and ψ dihedral angles, differing on the number of proteins used for the statistical analysis: ϕ has been assigned the value of −62°[47] or, alternatively, of −65°,[3] whereas the determinations for ψ are centered around −41°.[3,47] The ϕ and ψ dihedrals predicted by PBC calculations for GIH and AIH are fully consistent with the experimental data, especially when they are compared with the average values per residues not exposed to the solvent: $\phi \approx -59°$ and $\psi \approx -44°$.[47]

The other experimental parameters reported in Table 4 have been estimated on the ground of the data reported by Karplus[72] from a statistical survey of 70 proteins refined at 1.75 Å or better (see electronic supplement of ref. 72), and although very meaningful, they cannot be considered completely refined from the statistical point of view. However, it is encouraging that the calculated and the experimental values are very close, with bond lengths and bond angles usually differing by less than 0.01 Å and 1°, respectively. The most remarkable discrepancy involves the N−C′ bond distances, whose PBC value is larger than its experimental counterpart by more than 0.02 Å. Even if it is nowadays accepted that the ω dihedral angle can assume

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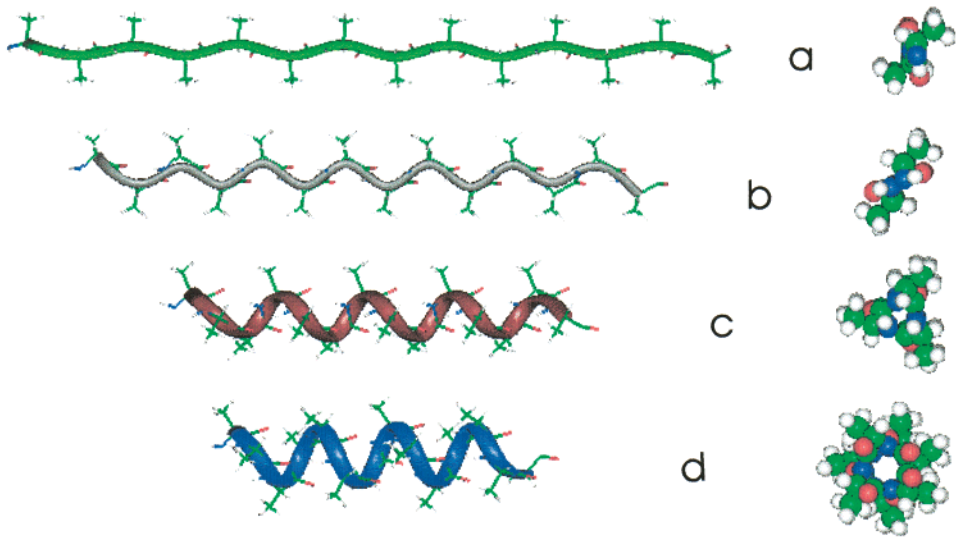

Figure 3. DFT/PBC-optimized structures for the infinite homopolypeptide of alanine. (a) fully extended structure (C5); (b) repeated γ-turns (C7); (c) 3_{10}-helix; (d) α-helix. (Left) View perpendicular to the translation vectors. (Right) View parallel to the translation vectors.

values quite far from the standard value of 180°, our calculations predict deviations too large in comparison to those of experimental results. This is probably due to the lack of solvent effects in our calculations; a polar solvent should indeed increase the tendence of the peptide bonds to assume a planar geometry.[76] Finally, it is worth noting that the statistical analysis of Chakrabarty and co-workers[30,31] gives further support to the reliability of our determination: it provides values of 116.3° ± 0.1° for the N−C′−C$^\alpha$ angle (calcd 116.8°) and of 123.5° ± 0.1° for the O−C′−N angle (calcd 123.0°).

The main geometric parameters describing the H-bond arrangement in α-helix are reported in Table 7. The N−O distances between two H-bonded residues is 2.94 Å, and the C′−O−N angle is 153.0°. The statistical survey of Thornton et al.[47] provides two different sets of values for the H-bonds in α-helices, one for residues exposed to the solvent and the other for residues in the buried side of helix. It is gratifying that the results of our gas-phase calculations compare well with the latter set of results concerning both N−O distances (2.91 ± 0.06 Å) and C′−O−N bond angles (157± 5°). The presence of the solvent distorts the α-helix leading to an average N−O bond length of 3.09 ± 0.13 Å and to an average C′−O−N angle of 148 ± 6°.

The computed AIH structure compares favorably with that of polyalanine fibers in helical conformation:[7] the pitch of the helix predicted by our calculations is close to the experimental one, while the ϕ and ψ dihedrals differ to some extent (ϕ = −57.4°, ψ = −47.5°). It is worth noting, however, that these experimental values involve several assumptions about the residue geometry (for example, perfect tetrahedral geometry at the C$^\alpha$).

The agreement with the experimental results is slightly worse for the 3_{10}-helix: whereas the calculated value of ψ (∼18°) is very close to the experimental average for protein residues in 3_{10}-helix (−18°,[47] −18.1 ± 19.7°,[77] −16.5 ± 34.7°[78]), there is a non-negligible discrepancy for the ϕ dihedrals: the calculated value ≈59° has to be compared with different experimental averages, −71°,[47] −69.3 ± 20.9°,[77] but also −62.8 ± 38.0°.[78] It is important to underline, however, that the spread of the experimental values is very high; moreover, the average length of 3_{10}-helices in proteins is less than four residues,[47] and thus our periodic calculations are expected to give different results, since they refer to infinite homopolypeptides. The comparison with the results of 32 polypeptides in 3_{10}-conformation (with a mean length of 4.9 residues) is indeed more favorable (ϕ = −57°, but ψ = −30°).[71]

Table 3. Stabilization Energy per Residue Obtained by 6-31G(d) PBC Calculations for the Four Conformations Examined[a]

amino acid	GIH	AIH	AibIH
α-helix	0.0	0.0	0.0
3_{10}-helix	0.87	1.35	−0.48
C7	3.43	3.56	2.94
C5	4.29	4.74	3.01

[a] All of the values (in kcal/mol) refer to the stabilization energy of α-helix.

The geometry of 3_{10}-helix predicted for AibIH is in good agreement with that predicted by X-ray diffraction studies of an Aib decapeptide (see Table 5) and the average of the values recorded for the whole series of Aib oligopeptides:[40] the differences in the bond length are never larger than 0.02 Å, and only the C′−N−C$^\alpha$ angle differs from the experimental average by more than 1°. There is also a good agreement between the calculated backbone dihedrals and the corresponding experimental averages. The comparison with the experimental results is even more favorable if the average is taken on the central residue of the decapeptide of ref 40 which is more representative of the preferred conformation of an Aib residue involved in a 3_{10}-helix: for example, the average value of ψ decreases from −31.2° to −28.0° (the calculated value is −25.2°). The comparison between our results and the average values of ϕ (−54°) and ψ (−28°) of Aib in 3_{10}-helical peptides[79] is also favorable.

3.2.2. Energetics. To compare the energy of structures whose repeating cell has a different number of residues it suffices to divide the total PBC energy by the number of residues, thus obtaining the energy per residue. Table 3 collects for each

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conformation the energy relative to a residue in α-helix. PBC calculations indicate that for both AIH and GIH the stability order is:

$$\alpha\text{-helix} > 3_{10}\text{-helix} \gg C7 > C5$$

At the 6-31G(d) level, the α-helix is more stable by ∼1 kcal/mol per residue than the 3_{10}-helix, whereas the differences with C7 (∼3.5 kcal/mol) and C5 (∼4.5 kcal/mol) are remarkably larger.

The PBC results are fully consistent with previous experimental and computational determinations: α-helix is the structure adopted more frequently by polypeptide chains in proteins.[47] Moreover, many experimental and computational results indicate that, for alanine-based polypeptides larger than 6−7 residues, the α-helix is the most stable structure.[12,13,15,29,39] The calculated energy difference between α- and 3_{10}-helices (about 1.3 kcal per residue in AIH) is also in line with the experimental results: it accounts for the clear-cut prevalence of α-helix, mostly for long polypeptide chains, but at the same time, it is not too high to rule out the possibility of the existence of 3_{10}-motifs in proteins. At the same time, C7- and C5-repeated structures are far less stable than helices, in line with their extreme rarity in proteins. Finally, alanine exhibits, from the energetic point of view, a propensity to form an α-helix similar to that of glycine. Also this result is in agreement with previous experimental determinations; it is entropy and not enthalpy that remarkably decreases the propensity of glycine toward the formation of α-helix. Actually, from the enthalpic point of view, glycine should exhibit a slightly larger tendency than alanine to adopt that conformation.[75b]

On the other hand PBC calculations for AibIH indicate that:

(i) the Aib residue has a very strong helix-inducing power: α-helix and 3_{10}-helix are more stable than the C7 structure by more than 3 kcal/mol per residue (the difference for alanine is 1 kcal smaller);

(ii) the 3_{10}-helix is slightly more stable than the α-helix (by about 0.5 kcal/mol);

(iii) the fully extended structure is relatively more stable than that for AIH and GIH.

These results are in full agreement with the conclusions drawn from the experiments: the Aib residue in peptide crystals always adopts right or left-handed helical conformations. There is a general consensus, besides, that 3_{10}-helix should be favored for Aib in low polarity solvents and for short polypeptides. Furthermore, our calculations predict that, at least in a nonpolar environment, 3_{10}-helix is more stable for a regular infinite homopolypeptide, and this result is confirmed by experiments.[43]

3.3. General Trends Derived by PBC Calculations. 3.3.1. Influence of the Nature of the Residue on the Secondary Structures. A comparison of the calculated equilibrium structures for AIH and AibIH with those of GIH[58] shows that the increase of the bulkiness of the substituents on C^α affects both the dihedral and the valence angles.

Starting our comparison from α-helix, replacement of one hydrogen atom by a methyl group has a small effect, at least for an L-amino acid in a right-handed helix, on the geometry of the α-helix. ϕ and ψ dihedrals are very similar in AIH and GIH (see Tables 4 and 6), and the two helices are almost perfectly superimposable (see Figure 4).

However, some small differences are present: glycine is predicted to favor smaller ψ and larger ϕ dihedrals, and also this feature seems to be confirmed by the experimental results (see supplementary electronic material of ref 72). The same behavior is found (even if to a lower extent) in 3_{10}-helices.

Table 4. Main Geometric Parameters of the PBC PBE/6-31G(d)-Optimized Structures for AIH[a]

	α-helix		3_{10}-helix	C7	C5
	exp[b]	calcd			
$N-C^\alpha$	1.465	1.460	1.458	1.468	1.455
$C^\alpha-C'$	1.525	1.550	1.547	1.553	1.547
$C'-N$	1.33	1.354	1.357	1.359	1.352
$C'-O$	1.24	1.250	1.249	1.248	1.245
N−H		1.033	1.033	1.036	1.028
$C^\alpha-C^\beta$	1.54	1.534	1.534	1.526	1.542
$C^\alpha-C'-N$	117.0	116.8	117.7	113.7	115.3
$C'-N-C^\alpha$	121.0	120.8	120.9	122.7	123.2
τ	110.5	111.5	113.0	110.5	105.3
ϕ	−62[c]	−61.4	−59.0	−80.9	−162.8
ψ	−41[d]	−39.8	−17.3	71.7	168.3
ω	179.6	174.7	172.3	−170.5	173.8
pitch	5.41	5.29	5.87	5.67	7.18
rise	1.495	1.51	1.96	2.83	3.59
T^d		10.58	5.87	5.67	7.19

[a] Distances in Å and angles in deg. [b] Derived from ref 72. [c] Reference 47. [d] Translation vector computed by DFT/PBC calculations.

Table 5. Main Geometric Parameters of the PBC PBE/6-31G(d)-Optimized Structures for AibIH[a]

	α-helix	3_{10}-helix		C7	C5
		exp[b]	calcd		
$N-C^\alpha$	1.473	1.470	1.472	1.484	1.467
$C^\alpha-C'$	1.558	1.54	1.559	1.568	1.566
$C'-N$	1.362	1.34	1.357	1.356	1.349
$C'-O$	1.247	1.23	1.249	1.251	1.248
N−H	1.027		1.032	1.040	1.030
$C^\alpha-C^\beta$	1.537	1.54	1.539	1.545	1.544
$C^\alpha-C^{\beta\prime}$	1.542	1.54	1.543	1.536	1.544
$C^\alpha-C'-N$	115.4	116.8	116.8	114.9	115.0
$C'-N-C^\alpha$	122.3	122.1	123.5	127.6	126.4
$N-C^\alpha-C^\beta$	110.4	110.7	110.7	111.2	110.6
$N-C^\alpha-C^{\beta\prime}$	108.3	107.0	107.9	106.2	110.5
τ	109.9	111.1	111.4	111.1	103.2
ϕ	−55.4	−54.0[c]	−51.3	−72.3	179.9
ψ	−43.8	−31.2[c]	−25.3	62.0	179.7
ω	175.6	176.1	177.5	−172.6	−179.6
pitch	6.02		6.00	5.65	7.24
rise	1.72		2.00	2.82	3.62
T^d	12.04		6.00	5.65	7.24

[a] Distances in Å and angles in deg. [b] Reference 11a. [c] Reference 40a. [d] Translation vector computed by DFT/PBC calculations.

Table 6. Main Geometric Parameters of the PBC PBE/6-31G(d)-Optimized Structures for GIH[58 a]

	α-helix	3_{10}-helix	C7	C5
$N-C^\alpha$	1.453	1.451	1.459	1.446
$C^\alpha-C'$	1.542	1.541	1.543	1.540
$C'-N$	1.354	1.358	1.359	1.351
$C'-O$	1.247	1.247	1.247	1.243
N−H	1.030	1.033	1.035	1.028
$C^\alpha-C'-N$	115.7	117.0	113.8	115.3
$C'-N-C^\alpha$	121.1	120.4	122.5	122.9
τ	113.0	114.5	113.8	106.7
ϕ	−56.8	−58.2	−77.5	180.0
ψ	−43.9	−18.0	70.0	180.0
ω	173.1	170.2	−173.3	180.0
pitch	5.26	5.81	5.72	7.24
rise	1.50	1.94	2.86	3.62
T^b	10.52	5.81	5.72	7.24

[a] Distances in Å and angles in deg. [b] Translation vector computed by DFT/PBC calculations.

Slightly smaller ψ dihedrals could indeed allow the minimization of the repulsion between the methyl and amide hydrogens; in GIH the absence of that interaction allows larger ψ dihedrals that can better reduce repulsions between backbone atoms.

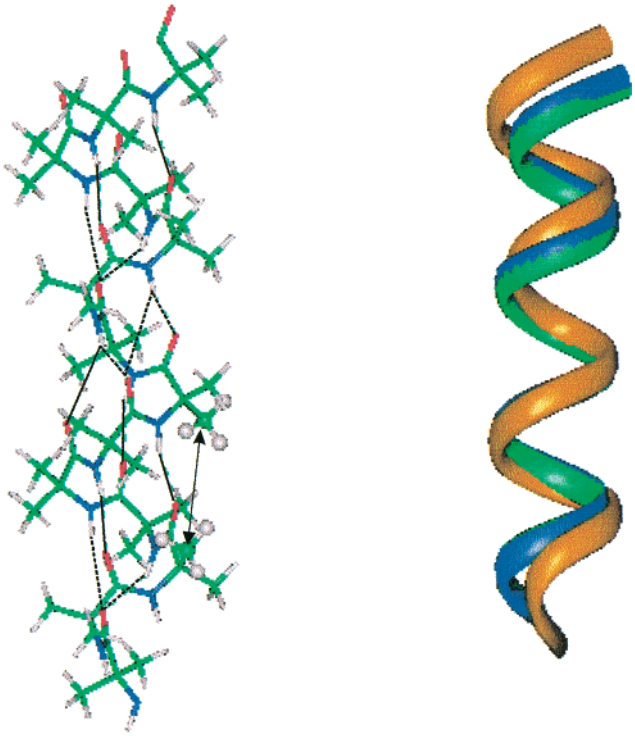

Figure 4. (Left) α-Helix of AibIH as predicted by DFT/PBC calculations. H-bonds are denoted by continuous lines when formed by residues in α-helix, by dashed lines when involving residues in a conformation intermediate between α- and 3_{10}-helix. The repulsion between the *i* and *i*+4 methyl groups is indicated by the double arrow. (Right) Superimposition of the ribbonlike visualization of α-helices of GIH (green), AIH (blue), and AibIH (yellow) obtained by DFT/PBC calculations.

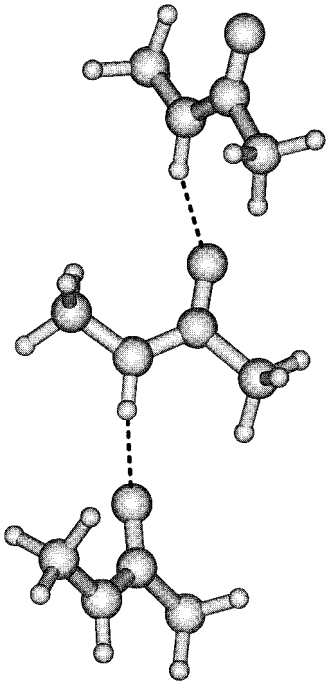

Figure 5. Trimer of *N*-methylacetamide molecules used to estimate the H-bond strengths in different secondary structures. All geometrical parameters except those involving methyl hydrogens are frozen to their values in the PBC/DFT minima.

Table 7. Hydrogen Bond Geometry in Four Different Conformations for AIH and AibIH, Calculated at the PBE/6-31G(d) Level by the PBC Method[a]

	α-helix	3_{10}- helix	C7	C5
		Ala		
O−H dist	1.97	1.92	1.88	2.04
N−O dist	2.94	2.94	2.81	2.60
N−H−O	155.1	168.1	147.8	112.1
H−O−C′	145.5	124.5	108.1	87.3
H−O−C′−Cα	−136.8	−112.5	−149.2	8.21
N−H−O−C′	155.0	162.2	5.42	−1.78
		Aib		
O−H dist	2.26	1.92	1.78	1.934
N−O dist	3.34	2.95	2.75	2.56
N−H−O	158.6	169.8	155.0	116.1
H−O−C′	138.8	129.4	106.4	88.7
H−O−C′−Cα	−131.4	−121.6	−150.4	0.12
N−H−O−C′	158.5	169.8	13.5	0.13

[a] Distances in Å and angles in deg.

When going from AIH to AibIH the geometry of α-helix is remarkably changed. PBC calculations predict for AibIH a strongly distorted structure (see Figure 5). Even if the average of the $\phi-\psi$ dihedrals (ϕ_{av}, ψ_{av}) is not strikingly different from the corresponding values of AIH α-helix, the ψ dihedrals of AibIH are spread within a range of 20° (between −31.3° and −51.8°), while the ϕ dihedrals range between −51.1° and −58.1°. As a result, the α-helix appears remarkably elongated (see Figure 4): its pitch is significantly longer than the pitch of α-helix AIH (6.02 vs 5.24 Å) and also the one of 3_{10}-helix AibIH. Furthermore many residues prefer a conformation intermediate between α- and 3_{10}-helices and are H-bonded both to the third and to the fourth next-nearest neighbor. As a matter of fact, the α- and 3_{10}-helices are much more similar in AibIH than in AIH and in GIH. The difference between the ψ_{av} is just 12.6° (in AIH it is 25.5°), and between the ϕ_{av} it is 1.4° (in AIH it is 2.4°). Actually, the possibility of a molten state intermediate between α- and 3_{10}-helices, has already been suggested on the ground of a molecular dynamics study.[14] The distortion of the structure obviously reflects in a weakening of the H-bonds (see Table 7) with an average H(N)-O distance 0.3 Å longer than in the AIH α-helix. It is important, however, to remember that the possibility of forming multiple H-bonds could increase the stability of that distorted conformation.

The distortion of α-helix is likely due to the steric hindrance of the extra methyl (see Figure 4) of Aib: if a polyAib chain has an ideal α-helix structure, two methyls, respectively on the *i* and on the *i*+4 residue (the ones involved in the hydrogen bonds) come too close. As a matter of fact, if AibIH α-helix had the same geometry as AIH, the distance between those methyls would be about 3 Å, and the distance between some of the methyl hydrogens would become shorter than 2 Å. In the distorted α-helix of AibIH, instead, the distance between the corresponding methyl groups is about 3.7 Å and that between the methyl hydrogens is longer than 2.6 Å. It is also possible that α-helix of AibIH is destabilized by larger repulsions between the extra methyl and the polypeptide backbone, even if the similarity of the stability of α-helix for ADA and AibDA suggests that this effect should play a minor role. However, only the study of the left-handed α-helix of AIH (already in progress) could give a definite answer to this question, since the repulsions between the methyl group of an L-amino acid and the backbone of a left-handed helix should be similar to that one suffered by the extra methyl of an Aib residue and a right-handed helix.

C7 and C5 conformers allow the highlighting of the importance of the symmetry of substitution at C^{α}. This feature is evident in the C5 structure where the symmetrically substituted GIH and AibIH exhibit ϕ and ψ dihedrals of 180°. In this

conformer, the repulsions between the C^{α} substituents on each residue and the oxygen atom of the preceding residue are minimized when the $O-C'-C^{\alpha}$ plane bisects the angle between the $C^{\beta}-C^{\alpha}-C'$ planes (or the $H^{\alpha}-C^{\alpha}-C'$ planes in GIH). In AIH the deviation of the peptide backbone from planarity decreases the repulsion between the methyl substituent and the carbonyl oxygen, although forcing H^{α} to get closer to the oxygen atom.

Analogously, in the C7 conformer the $\phi-\psi$ dihedrals of AIH are not intermediate between the ones of GIH and AibIH, but they are very similar to the ones of GIH. The extra methyl of Aib forces the ψ dihedrals to lower values in order to decrease the repulsions with the carbonyl oxygen of the preceding residue.

The nature of the C^{α} substituents affects also the intraresidue geometric parameters. In all of the conformations examined the τ angle is larger for GIH than for AIH and AibIH. The absence of bulky substituents leads indeed the $H-C^{\alpha}-N(C')$ bond angles to lower values, thus allowing the τ angle to slightly open in order to reduce the $N-C'$ repulsions. This result is confirmed by the analysis of 70 resolved protein structures[72] which shows that the average value of that angle is 110.4° if all 20 of the amino acids are considered and 112.1° if only glycines are taken into account. The same conclusions hold if AIH is compared with AibIH, the only exception being the C7 conformation where the τ angle is 110.5° for AIH and 111.1° for AibIH. In the latter conformation, however, the strong steric repulsions between the eclipsed methyl and the oxygen atom force the backbone to assume dihedrals remarkably different (more than 10°) from those predicted for AIH and GIH, thus making less meaningful the comparison of the τ angle. The increase of the steric hindrances with the bulkiness of the substituents possibly explains also the increase in the equilibrium $N-C^{\alpha}$ and $C^{\alpha}-C'$ bond lengths in going from GIH to AibIH. Also, this result is confirmed by the experiments which provide for $N-C^{\alpha}$ bond an average value of 1.464 Å for glycine and 1.467 for the other 20 natural amino acids, while the $C^{\alpha}-C'$ average bond distance increases from 1.523 Å to 1.526 Å.[72]

3.3.2. Tuning of Residue Properties by Secondary Structure. A careful comparison of the minimum energy structures for the different conformations of AIH (but also AibIH and GIH) shows that some geometric parameters exhibit a remarkable dependence on the secondary structure. This phenomenon has already been pointed out in the study of oligopeptides,[51] and it is now interesting to ascertain the role played by long-range effects. The most apparent trend concerns the dependence of the τ valence angle on the $\phi-\psi$ dihedrals. Our calculations predict that for AIH this angle opens up on going from C5 (105.3°) < C7 < α-helix < 3_{10}-helix (113.0°). These results compare nicely with the results of the statistical survey of Karplus[72] which suggests values for α-helix close to 111.4° and values 1−2° larger for the 3_{10}-helix. On the other hand, the average value in the C5 region is about 1−2° smaller than the average value of that angle in proteins (110.4°). Our results predict an angle even smaller for the fully extended structure, but it is important to underline that this angle tightens to maximize the intrachain H-bonds. When it is possible to form hydrogen bonds with the solvent or with different polypeptide chains, this effect should be less important and this could explain an average value that, even if remarkably smaller than the average, remains 2−3° wider than our estimate. A very recent ultrahigh-resolution X-ray determination of the structure of ribonuclease A[80] confirms the reliability of the estimates of PBC calculations. The values of τ angles are clustered around 111.8° for residues engaged in helix motifs, around 109.1° for residues participating in β-strands, and around 107.1° for residues adopting extended conformations outside β-strands. Moreover, the values of that angle increase when ψ approaches zero, in agreement with our prediction of τ angles being wider in 3_{10}-helix than in α-helix. By enlarging the valence angles τ and $C^{\alpha}-C'-N$ (vide infra) it is indeed possible to relieve the larger backbone repulsions associated with conformations with ψ close to zero.

Table 8. Mulliken Atomic Charges Calculated for Polyalanine at the PBE/6-31G(d) Level by the PBC Method

	α-helix	3_{10}-helix	C7	C5
O	−0.529	−0.528	−0.499	−0.510
C′	0.575	0.562	0.589	0.575
N	−0.595	−0.612	−0.574	−0.583
H	0.411	0.408	0.380	0.352

Several other geometrical parameters show a non-negligible dependence on the secondary structure (see Tables 4−6). For instance, $C^{\alpha}-C'-N$ valence angle is 117.7° in 3_{10}-helix and 116.8° in α-helix, whereas C5 conformation (115.3°) and, especially, C7 conformation (113.7°) exhibit remarkably smaller values. The experiments[72] suggest average $C^{\alpha}-C'-N$ angles of 118°, 117°, 116°, 114°, for residues engaged in 3_{10}-helices, α-helices, extended structures (C5), and γ -turns (C7), respectively.

It is also worth noting that our calculations predict positive ω deviation for C7, and negative ω deviations for helices and fully extended structures; that prediction is confirmed by the experimental results as well.[76] The trends of bond lengths (mostly $C'-O$ and $C'-N$) seem to be generally confirmed by the experiments; however, the predicted differences are very small, and an unbiased comparison would require an "ad hoc" statistical survey of high-resolution protein structures.

PBC calculations can give reliable insights also on the influence that the secondary structure has on the main electronic features of the polypeptide chain and of each residue. Our computations confirm that α-helix has the largest dipole moment among the conformations examined, whereas the 3_{10}-helix conformation, even if differing from the α-helix essentially for a smaller ψ dihedral (by ∼20°), has a significantly smaller dipole moment. Finally, the C7 and C5 conformers exhibit much smaller global dipole moments. The trend of dipole moments is mirrored by the atomic charges obtained by a Mulliken population analysis (see Table 8). For instance, the negative charge of the oxygen atom is reduced by 6% going from C13 to C7 structures, and a similar trend is found for the nitrogen atom; the variation computed for the positive charge of the amidic proton is even larger. These differences are probably due to the different strength of the dipolar interactions; the favorable dipole−dipole interactions experienced by the helix conformations lead to an increase of the dipole moment of each residue favoring more dipolar electronic structures. This could also enhance the stability of the H-bonds formed in those conformations.

The non-negligible dependence of the electronic properties of each residue on the polypeptide conformation deserves some comments. Several experimental studies suggest that the macrodipole constituted by an α-helix[46] or, following another point of view, the dipoles of the terminal residues in α-helix[81] could

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be important for the success of some enzymatic reactions.[81c] Our results are consistent with these results; moreover, they suggest that the secondary structure could affect the enzymatic catalysis, also influencing the properties of individual residues of the chain. However, it is important to underline that these considerations cannot be considered more than an appealing working hypothesis and, surely, need some purposely tailored study.

3.3.3. Some Insights on the Factors Influencing the Relative Stability of Different Secondary Structures. We start our analysis comparing α- and 3_{10}-helices. Many experimental and computational studies have been devoted to the analysis of the effects that make α-helix more stable than 3_{10}-helix, leading to somewhat contradictory conclusions. It has been suggested that steric effects strongly stabilize α- over 3_{10}-helix.[12,13] However, as mentioned above, the results obtained at the dipeptide level seem to rule out this possibility. Since none of these two conformations is able to form hydrogen bonds in a dipeptide analogue and the limited size of the system should reduce the influence of long-range effects, the preference for 3_{10}-helix over α-helix is likely due to different steric intraresidue repulsions. Also the analysis of the PBC geometries suggests that α- and 3_{10}-helices suffer similar intramolecular repulsions. According to several MM calculations, another factor favoring α-helix should be that hydrogen bonds are stronger than in 3_{10}-helix.[12] PBC geometry optimizations do not support this point of view; a comparison of the setup of H bonds in α- and in 3_{10}-helices does not show remarkable differences (see Table 7), suggesting a comparable stability for both H-bond networks. As a matter of fact, in the 3_{10}-helix, the H−O bond distance is shorter, the N−H−O bond angle closer to 180°, and the H−O−C′ bond angle closer to 120° (hydrogen atoms pointing toward the oxygen lone pair). On the other hand, in α-helix the hydrogen atom is closer to the peptide plane of the $i+4$ residue (which contains the nonbonding electrons of oxygen). To put these considerations on a semiquantitative basis, we have arranged three *N*-methylacetamide molecules in the same positions as the peptide groups in the 3_{10}-helix and in the α-helix, freezing all of the internal and the intermolecular degrees of freedom at the values they have in the helix and optimizing just the geometry of the methyl hydrogens (see Figure 5). The results of the single-point calculations performed at different levels of theory on this trimer and on the corresponding dimer are collected in Table 9. Our results confirm a significant cooperative effect of H-bond networks:[82] the stabilization of the trimer is always larger (>1 kcal/mol) than the double of that of the dimer. The stability of the α-helix-like system is very similar to that of the 3_{10}-helix (energy difference always smaller than 1 kcal/mol), confirming that the stabilization of the α-helix should not be due to the geometry of the H-bond network. However a word of caution is necessary; in a polypeptide chain, the strength of the H-bonds could depend on in addition to the local geometry arrangement, other long-range effects which could act synergistically (vide supra).

It is worth noting that the α-helix arrangement is favored over that of the 3_{10}-helix when going from the dimer to the trimer, α-helix being indeed strongly stabilized by dipole−dipole interactions. As reported above, the amido and the carbonyl group of residues involved in an α-helix exhibit a more enhanced dipole than in 3_{10}-helix, and this could increase the strength of the H-bonds they form. This can explain why PBC calculations indicate that α-helix has the longest average C−O bond length among the four conformations examined (see Table 4). This implies a larger dipole moment per residue and a greater energy stabilization. We exploited the PBC-optimized geometry to further check this conclusion and arranged six formaldehyde molecules in the positions occupied by the CO groups in α-helix, 3_{10}-helix, and C7 structure of AIH (see Figure 6). The α-helix arrangement is the most stable: about 1.2 kcal/mol more stable than that of the 3_{10}-helix and about 2.2 kcal/mol more than that of the C7 structure. This would imply that dipole−dipole interactions contribute at least 0.2 kcal/mol per residue to the stabilization of α-helix over 3_{10}-helix; moreover, this value is probably a lower bound for the value experienced in the polypeptide chain. The dipole moment of a peptide residue (not engaged in H-bonds) is about 3.7 D, whereas the dipole moment

Table 9. Total Energy (Relative to the α-Helix Arrangement) and Interaction Energy (Total Energy Minus the Sum of the Energies of the Monomers) for the *N*-Methylacetamide Dimer and Trimer (see Figure 5)

	PBE[b] 6-31G(d)	PBE[b] 6-31+G(d,p)	PBE0[b] 6-31+G(d,p)	MP2 6-31+G(d,p)
		α-helix geometry		
		dimer		
tot en.	0.0	0.0	0.0	0.0
int en	5.67	5.80	6.09	7.97
		trimer		
tot en.	0.0	0.0	0.0	0.0
int. en.	13.0	13.13	13.72	17.47
		3_{10}-helix geometry		
		dimer		
tot en.	−0.37	−0.18	−0.11	0.19
int en.	5.75	5.73	5.96	8.43
		trimer		
tot en.	0.26	0.09	0.24	−0.17
int en.	12.83	12.71	13.18	−18.33

[a] Energy in kcal/mol. Geometry optimized at the PBE/6-31G(d) level. [b] Corrected for BSSE. Without BSSE correction (in kcal/mol): (α-helix) Dimer PBE/6-31G(d) = 8.31, PBE/6-31+G(d,p) = 6.19, PBE0/6-31+G(d,p) = 6.46. Trimer PBE/6-31G(d) = 18.20, PBE/6-31+G(d,p) = 13.91, PBE0/6-31+G(d,p) = 14.49. (3_{10}-Helix) Dimer PBE/6-31G(d) = 7.90, PBE/6-31+G(d,p) = 6.15, PBE0/6-31+G(d,p) = 6.40. Trimer PBE/6-31G(d) = 17.12, PBE/6-31+G(d,p) = 13.57, PBE0/6-31+G(d,p) = 14.07.

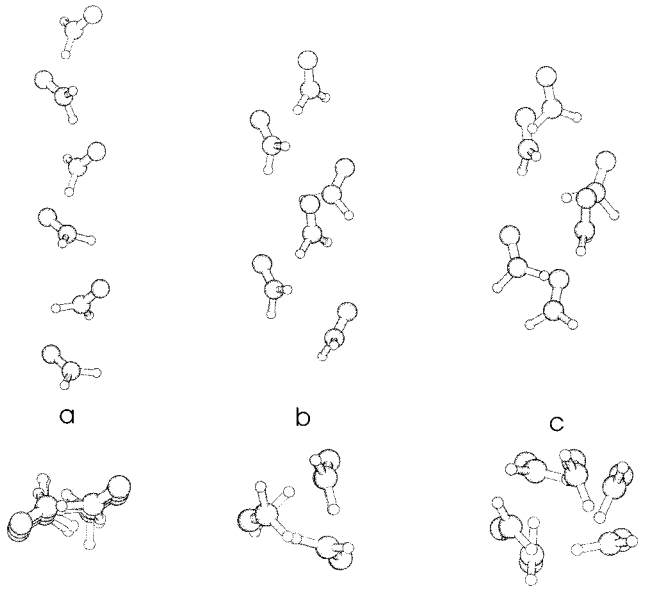

Figure 6. Six molecules of formaldehyde in the same geometrical arrangement as the carbonyl groups in (a) repeated γ-turns structure, (b) 3_{10}-helix, (c) α-helix. (Upper) View perpendicular to the translation vector of the polypeptide. (Lower) View parallel to the translation vector of the polypeptide.

(82) Akyama, M.; Torii, H. *Spectrochim. Acta* **2000**, *56(A)*, 137.

of a formaldehyde molecule is just about 2.2 D. Furthermore, we have considered six residues only, and due to the cooperative nature of the dipole−dipole interactions, the effect could be even more important when considering a larger number of residues.

Having already seen that for Aib α-helix is destabilized by methyl−methyl repulsion, it is worthy of ascertaining if 3_{10}-helix can be considered intrinsically more stable for AibIH than for AIH. As a matter of fact, a closer inspection of the H-bond network geometry suggests that the 3_{10}-helix geometry of AibIH could allow stronger H-bonds: the average N−H−O bond angle is indeed closer to 180° (170° vs 168° in AIH), and the amide hydrogens lie closer to the carbonyl plane. We have checked this hypothesis by comparing the energy of three *N*-methylacetamide molecules frozen in the same geometries of three H-bond-linked residues in 3_{10}-helices of AibIH and AIH, respectively. The former arrangement is about 1 kcal/mol more stable than the latter. This confirms that the preference of Aib for the 3_{10}-helix is due also to stronger H-bonds; the electron-donating power of the extra methyl groups, increasing the electron density at the nitrogen atom, could stabilize geometries that allow more stable H-bonds.

It is now interesting to understand why C5 and C7 conformations, which are the preferred ones for dipeptides, are much less stable than helix structures for longer polypeptide chains. Obviously, C5- and C7-repeating motifs form a number of H-bonds larger than helix structures (one more than 3_{10}- and two more than α-helix); however, the importance of this factor decreases when increasing the number of residues and is negligible for infinite polypeptides. For what concerns C7 conformation the strength of the H-bonds (see Table 7) should be comparable (if not larger) to that of the helix conformations. However, the C7 structure is destabilized by a close contact between the methyl substituent and the oxygen atom of each residue: the C^{β}−O distance is about 2.80 Å (in helix the same distance is longer than 3.1 Å). As a matter of fact, the optimization of a dipeptide forced to have the same dihedral angles as those characterizing the PBC-optimized C7 conformation (thus allowing the decrease of all of the internal strain coming from bond angles) is less stable than its helix analogues by about 2.5 kcal/mol. These considerations are confirmed by the fact that for Aib (which obviously suffers the most severe C^{β}−O repulsions) the C7 conformation is significantly less stable than for Ala and Gly (see Table 2). Another important disadvantage of the C7 conformation is the "alternate" arrangement of the peptide dipoles (see Figure 6a) that decreases substantially the energy gain due to dipole−dipole interactions (vide supra). Taking the dipeptide analogues as references, long-range interactions provide a stabilization of 0.16 kcal/mol per residue for AibIH, whereas their effect is negligible for GIH, and even destabilizing (by 0.2 kcal/mol per residue) for AIH.

Besides the already mentioned too-small τ angle, the non-optimal geometry for the H-bonds (see Table 7) is one of the main reasons for the relative instability of the C5 conformation. Also C5 conformation should not be stabilized to a significant extent by dipole−dipole interactions; however, its geometry and its higher dipole moment suggest that C5 should be more favored than C7 by long-range interactions. As a matter of fact the energy per residue calculated for the infinite polypeptide is increased by 0.5 kcal (AIH and GIH) and by 0.8 kcal (AibIH) with respect to the corresponding value in the dipeptide analogues.

4. Concluding Remarks

The results of the present study show that PBC/DFT calculations take into account properly all of the long-range effects influencing the geometry and the conformational behavior of polypeptides such as electrostatic interactions or network of H-bonds extending along several residues (also beyond the unitary cell considered). In agreement with previous experimental and computational determinations, α-helix is predicted to be the most stable conformer for the alanine infinite homopolypeptide. Moreover, the energy difference between α- and 3_{10}-helix is of the right order of magnitude (∼1 kcal/mol per residue) to explain why the 3_{10}-helix (which contains one additional H-bond with respect to the α-helix) is favored for polyalanine with less than seven residues. Our calculations suggest that α-helix is more stable than 3_{10}-helix mainly due to more favorable dipole−dipole interactions. On the other hand, steric repulsions and H-bonds should have similar strength in the two helices.

For what concerns the debate about the preferred conformations of Aib homopolypeptides, PBC/DFT calculations indicate (confirming previous experimental hints) that in apolar environments 3_{10}-helices are favored over α-helices, irrespective of the number of residues. The preference of AibIH for 3_{10}-helix over α-helix is mainly due to the severe distortion of the α-helix induced by methyl−methyl interresidue repulsions.

The optimized geometries of AIH and AibIH show a remarkable agreement with the avalaible crystallographic structures of α- and 3_{10}-helix motifs in proteins and in oligopeptides, concerning the main conformational parameters (e.g., ϕ and ψ dihedrals), valence angles, and bond lengths of the peptide backbone. Our method is also able to capture more subtle details, like the dependence of the backbone geometrical parameters on the adopted conformations, that only recently has been highlighted by the increasing resolution of X-ray spectra. From the structural point of view, PBC/DFT calculations could thus complement high-resolution X-ray diffraction in providing reliable target values both for molecular mechanics force fields and for X-ray refinement methods.[83]

From another point of view, PBC-optimized structures are sufficiently accurate to provide excellent starting points for useful a posteriori analysis, aimed at defining the main factors determining conformational equilibria of polypeptides. The availability of a correct geometry is surely crucial for the success of the latter analysis; just to make an example, trying to compare the strength of H-bonds in different conformations, without a good knowledge of their geometry, is, at least, hazardous.

PBC calculations can thus successfully handle polypeptide systems of a size comparable to the ones existing in vivo. It is possible to perform complete geometry optimizations (both of the internal and of the cell parameters) also using extended basis sets, and the accuracy of the calculations is increased by the use of methods rooted in the density functional theory, which can treat effectively the effects of electron correlation. These features confirm our expectation that the PBC/DFT approach could open new interesting possibilities to the quantum mechanical study of biological systems. As a matter of fact, even if infinite polypeptides do not exist in vivo, they can be a model of the environment of the central residues in an α-helix better than a medium-size (e.g., six to seven residues) oligopeptide, where edge effects can have an overwhelming influence. Infinite polypeptides can thus be considered upper limits for the "real" polypeptide chains, being useful to single out some effects and features which are present and effective also in proteins but can be hardly recognized, due the superposition of many small factors, often acting in different directions.

(83) Laskowsky, R. A.; Moss, D. S.; Thornton, J. M. *J. Mol. Biol.* **1993**, *231*, 1049.

In our opinion, a better understanding of such complex systems requires indeed an integrated approach, where the experimental results are compared with theoretical ones coming from model systems of all possible sizes: from the single residues, through oligopeptides, up to infinite polypeptides. The intrinsic periodic character of PBC calculations could also be an advantage in the study of several fundamental proteins which are close to periodicity (collagen, silk, etc.) as well as for a better characterization of the several periodic polypeptides possessing very interesting technological properties.

A final word of caution concerns the absence of solvent effects in our calculations; as a matter of fact, solvent can strongly influence both the geometry and the stability of different conformations. A separate study tackling this question is already in progress; we resorted to last-generation continuum models such as the polarizable continuum model (PCM),[84] that has already shown to treat effectively environmental effects. It is thus important to remember that the results hereby presented can mimic more closely the behavior of polypeptide chains in apolar environments such as cellular membranes, hydrophobic core of proteins, micelles, and so forth.

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JA003680E

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