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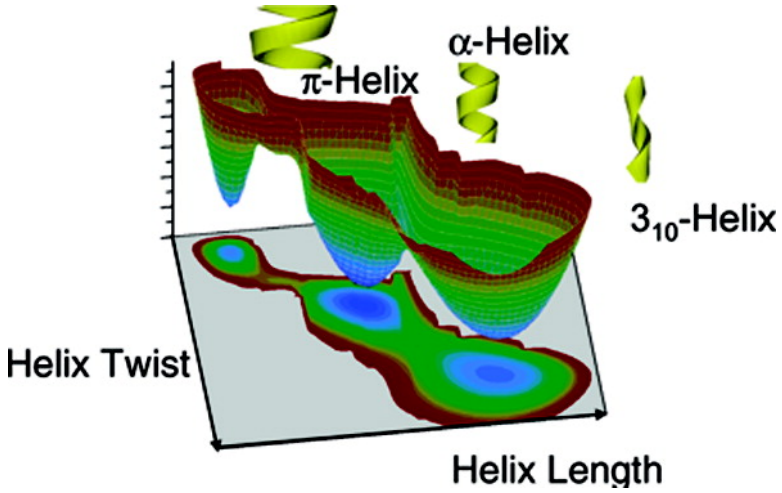

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Structural Transitions in the Polyalanine α-Helix under Uniaxial Strain

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Abstract: We analyzed the response to strain of an infinite polyalanine chain in the α-helical conformation using density functional theory. Under compressive strain the α-helix is found to undergo structural transitions to a π-helix when the length of the helix is reduced by more than 10%. Under tensile strain the structure changes into a 3_{10}-helix when the length is stretched by more than 10%. Our analysis of these transitions shows that they proceed essentially in two steps: At first there is mainly a length change, and only with some delay the helix twist adjusts.

Introduction

Under common physiological conditions proteins in cells undergo constant motions and structural changes. Mechanical forces generated in the cell during, e.g., protein folding and unfolding, and diffusion through membranes, will compress or stretch the protein structure.[1] Therefore, knowledge of the mechanical properties of proteins is necessary to understand its biological function or possible malfunction. Protein behavior under tensile strain has been studied by single-molecule experiments with atomic-force microscopes.[1] And static high-pressure[2] and fast-shock experiments[3] were used to study their mechanical response under compression. In the folded state, the protein mechanical properties are largely ruled by van der Waals interactions and hydrogen bonds (hbs). The latter is the main stabilizing force of the secondary structure of proteins, e.g., helices and β-sheets. In helices hbs are formed between the N−H group of the residue i and the oxygen atom in the C═O group of the residue $i + n$, where $n = 3$, 4, or 5. These three numbers correspond to three very different helical conformations, labeled as the 3_{10}, i.e., 3 residues per turn and 10 atoms in the ring formed by the hb, the 3.6_{13}-helix or α-helix, and the 4.4_{16}-helix or π-helix. In all these structures hbs are nearly parallel to the helical axis. They strongly affect the mechanical response of a helix to uniaxial strain. For example, abrupt changes in the length of helical polypeptides upon stretching by an atomic force microscope are associated with the rupture of hbs.[4] However, though the helical conformation is the most ubiquitous secondary structure of proteins, understanding of its response to strain and the detailed role of hbs is still shallow. Theoretical works on pulling or compressing helices has so far mainly evoked elasticity theory[5] and force-field models.[6] Or, in statistical-mechanics studies, the employed interaction models were just qualitative.[7] A study considering the full role of the self-consistent electronic structure was so far missing, and this is in fact what we are reporting in this work.

Our below reported work describes density functional theory (DFT) calculations of the response of a polypeptide helix to uniaxial strain. Besides the potential energy surface (PES), the interactions with solvents and the dynamics of the nuclei along the PES, i.e., finite-temperature effects, dictate the response of materials to strain. However, all these effects together tend to obscure the role and weight of each individual contribution to the response. In the present work, our foremost focus is the PES. As we will show, the PES determines the basic structural features of those helical conformations relevant in nature and illuminates in greatest clarity the action of hydrogen bonding. We decided to study an infinitely long chain, which enables us to focus on the properties of the center (bulk) of a long helix, and to properly describe and analyze the cooperativity of hbs. The latter refers to the important aspect that in a helix the alignment of hbs leads to a cooperative strengthening of each individual hb, an effect that is crucial to stabilize the bulk of a helical conformation.[8−13] Infinite-chain models have shown to

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properly describe the structure of polyalanine in helical[11,13] and β-sheet structures,[14] as well as their vibrational properties.[15] Our DFT results reveal the existence of three minima of the PES corresponding to three qualitatively different conformations, namely the α-, 3_{10}-, and π-helix structures. While the α-helix is confirmed to be the most stable one at 0 K, transitions to the other structures are predicted here when the helix is strained. Alanine is a hydrophobic amino acid that forms helices in solvent-free environments,[16] e.g., in membrane proteins. However, water molecules may interact with the helix by hydrogen bonding with the backbone. The helix−water interaction is neglected in the present study. Nevertheless, we will discuss the possible influence that water may have on the helical structure. The results are also discussed with respect to the standing controversy between experimental and some theoretical results on the occurrence of π- and 3_{10}-helical conformations in protein structures and in the folding/unfolding event of an α-helix.

Method

We employ DFT and ab initio pseudopotentials[17] as it is incorporated in the parallel version of the fhi98md code.[18] The exchange-correlation functional was treated in the generalized gradient approximation of Perdew, Burke, and Ernzerhof.[19] The energy cutoff of the plane-wave basis set is 70 Ry, and the sampling of the Brillouin zone is replaced by the Γ-point. Both choices are indeed appropriate and provide a highly accurate description for the studied systems. Further details can be found in ref 13. For the questions of interest the accuracy of our study is comparable to that of second-order Møller−Plesset perturbation theory (MP2) or coupled cluster methods.[20] However, as we had found earlier, when hbs are strongly bent the error may increase to as much as 1.5 kcal/mol with respect to MP2 results.

An infinite helix can be described as a one-dimensional crystal with lattice vector $\mathbf{R}_n = r\cos(n\,\theta)\,\mathbf{e}_x + r\sin(n\,\theta)\,\mathbf{e}_y + n\,L\,\mathbf{e}_z$. As indicated in Figure 1, L is the helix length per residue (measured along the helix axis, chosen here as the z axis, e.g., from one oxygen atom to the next one). r is the helix radius as measured from the helix center to an oxygen atom, and θ is the helix twist angle. Typically the geometry of helical conformations is given in terms of the torsion angles ϕ and ψ that measure the relative position of a residue with respect to the previous one. These are not directly given by L, r, and θ; i.e., information of other internal parameters is required, e.g., bond lengths and bond angles. As we describe the system within the supercell approach, where periodic boundary conditions are assumed, the helix twist is $\theta = 360°\ m/N$, where N is the number of residues (i.e., lattice sites) per supercell, and m is the number of helix turns per supercell. We define the chemical potential of this helix: $\mu^{\infty}(\theta,L) = E^{\infty}_{helix}(\theta,L)/N$, where $E^{\infty}_{helix}(\theta,L)$ is the total energy of a helix consisting of N residues per supercell. Thus, for sampling the PES of the infinite chain we have calculated $\mu^{\infty}(\theta,L)$ for 13 different θ values in the range from 72° to 132°. For each θ, the L values were sampled with at least 6 points. Since m must be an integer number inside the supercell, different N values were used to model helices with different twists; e.g., for a helix with $\theta = 96°$ we used $N = 15$, and for a helix with $\theta = 120°$ we use $N = 3$. A total of 208 structures were calculated, fully relaxing all internal coordinates. It is convenient to set the energy zero as the total energy per residue of the unstrained infinite chain in the fully extended structure (FES), because this reference system lacks hbs. The length per residue of the unstrained FES is calculated as $L = 3.57$ Å. The quantity $\Delta E = N(\mu^{\infty}(\theta,L) - \mu^{\infty}_{FES}$, with $N = 1$), is thus a measure of the stability of a residue in an infinite helical conformation with respect to the unstrained FES at 0 K.[13] The 208 calculated geometries and energies enable us to plot the full PES, using a cubic spline interpolation, also covering twist angles that could not be calculated directly by our DFT approach.

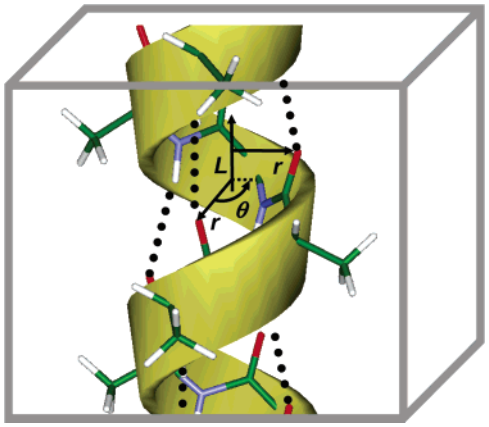

Figure 1. Geometry of polyalanine in helical conformation inside the employed supercell. Dotted lines mark the hydrogen bonds. Nitrogen atoms are shown in blue, oxygen atoms, in red, carbon atoms, in green, and hydrogen atoms, in white. L, r, θ are the helix geometry parameters (length, radius, twist angle), as also described in the text.

Results

The PES in the L, θ region where the helical conformation is more stable than the unstrained FES, i.e., where $\Delta E < 0$, is shown in Figure 2. This reveals three minima which correspond to the following helical parameters: (i) $\theta_\pi = 80°$ (4.5 residues per turn) and $L_\pi = 1.17$ Å, (ii) $\theta_\alpha = 98.2°$ (3.66 residues per turn) and $L_\alpha = 1.50$ Å, (iii) $\theta_{3_{10}} = 115.5°$ (3.12 residuess per turn) and $L_{3_{10}} = 1.95$ Å. As indicated by the indices these geometries correspond to the π-, α-, and 3_{10}-helix conformations. The corresponding torsion angles are ($\phi_\pi = -76°$, $\psi_\pi = -53°$), ($\phi_\alpha = -63°$, $\psi_\alpha = -42°$), and ($\phi_{3_{10}} = -60°$, $\psi_{3_{10}} = -20°$). These values agree with those reported in the literature.[11,13] The minimum energy pathway connecting these minima is plotted in Figure 3. The minimum of the α-helix is lower in energy by ∼ 0.5 kcal/mol with respect to the π- and 3_{10}-helix minima; i.e., α-helix conformation will be preferred over the other two at T = 0 K. According to Figure 3, an α-helix undergoes a structural transition to a π- or 3_{10}-helix both under uniaxial compressive and under tensile strain, if the length of the helix is changed by more than 10%. Thus, an α-helix supports a large uniaxial load without yielding. The calculated energy barriers for the transition from the α- to the π-helix and from the α- to the 3_{10}-helix are 2 and 1.3 kcal/mol, respectively. These barriers correspond to situations where the compressive or the tensile force is applied sufficiently slowly so that the

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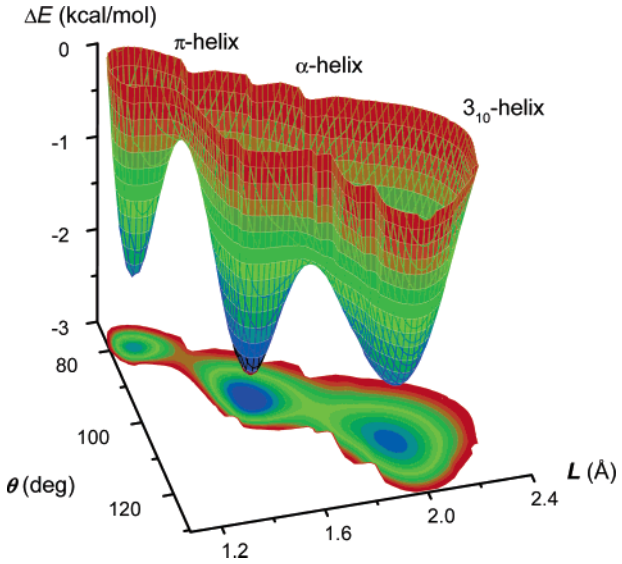

Figure 2. Calculated potential-energy surface as function of the helix length L and twist angle θ of an infinite polyalanine helix. The helix radius is fully relaxed, as are all other internal parameters of the 10 atoms per residue.

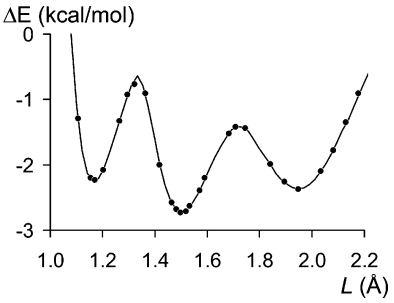

Figure 3. Minimum energy pathway along Figure 2. The dots mark points where actual DFT calculations were performed.

helix will always remain in the ground state of the strained geometry. We expect, however, that these energy barriers are slightly overestimated by DFT because in the transition states the hbs deviate noticeably from a linear arrangement. We will come back to this point below.

The values for the $d_n^{O\cdots H}$ distance, measured between the O atom in residue i and H atom in residue $i + n$, along the minimum energy pathway are presented in Figure 4a. The smallest $d_n^{O\cdots H}$ value for a given L is considered as the hb length. The values for L where $d_n^{O\cdots H} = d_{n+1}^{O\cdots H}$ correspond to helical conformations in the transition states. This reflects the presence of bifurcate hbs in the transition state structures, i.e., each C═O group is trying to form two hbs. The N—H bond length may be considered as a measure of the hb strength, i.e., the longer the N—H bond the stronger the hb. The value for the N—H bond length, d^{N-H}, along the minimum energy pathway is shown in Figure 4b. The tendency depicted there shows that helical conformations in the transition state have the shortest N—H bond length; hence the hb strength is weaker in the transition state structures than in the unstrained helical conformations. The hbs are highly bent in the transition state structures (Figure 4c); i.e., the angle $\sigma_n^{N-H\cdots O}$ formed by N—H···O atoms (along the i, $i + n$ residues) strongly deviates from 180°. Usually $\sigma_n^{N-H\cdots O}$ is in the range from 140° to 180°, and typically smaller $\sigma_n^{N-H\cdots O}$ values go together with a weaker hb strength. To quantify the hb strength it is useful to write the energy of our infinite helical conformations as $\Delta E = E_{rep} + N_{hb} \times E_{hb}$, where N_{hb} is the number of hbs per C═O group, and E_{rep} is the repulsive energy between nearest neighbor residues. Thus the hb strength in an infinite chain, E_{hb}, can be evaluated once E_{rep} is known. The E_{rep} term is estimated by calculating finite chains which are sufficiently short to avoid the formation of (i, $i + n$) hbs.[13] The resulting E_{hb} values for helices with different helical parameters are given in Table 1. For the transition states we assume bifurcated hbs, i.e., $N_{hb} = 2$. These results show that hbs in the transition state structures are indeed weaker than hbs in the unstrained helices by more than 5 kcal/mol. The θ against the L curve (Figure 4d) presents plateaus for values of L corresponding to low-strain helices,

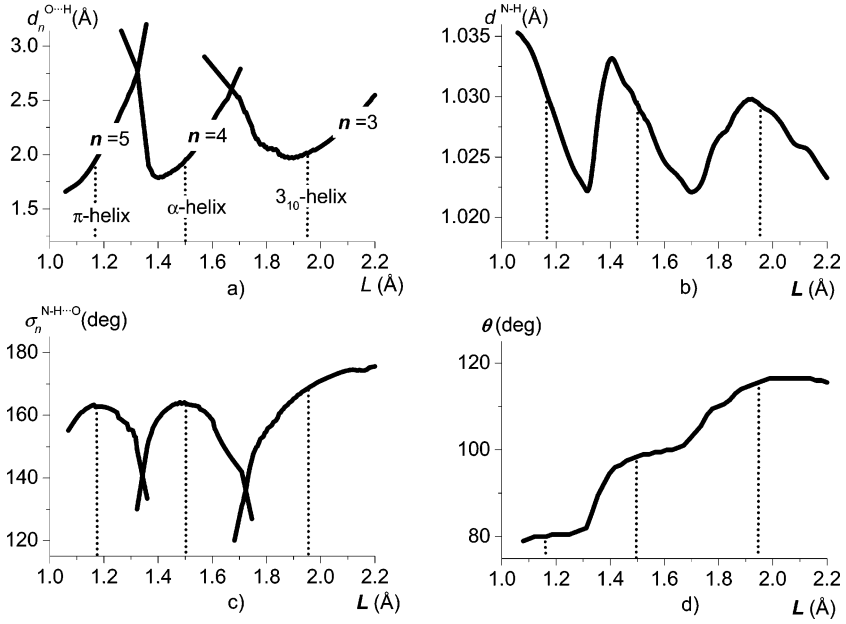

Figure 4. Geometrical parameters along the minimum energy pathway of a strained polyalanine helix: (a) $d_n^{O\cdots H}$ distance measured as the distance between the H atom in residue i and the O atom in residue $i + n$; (b) N—H bond length; (c) angle formed by N—H···O atoms (along the i, $i + n$ residues); (d) change of the helical twist along the minimum energy pathway. The dotted lines give the positions of the PES minima of Figure 2b.

Table 1. Hydrogen Bond Strength, E_{hb}, in Infinite Helices with Different (L, θ)[a] Parameters

L	θ	N_{hb}[b]	E_{hb}
1.17	80.0	1	−10.4
1.32	83.1	2	−3.9
1.50	98.2	1	−8.6
1.71	102.9	2	−3.3
1.95	120.0	1	−7.7

[a] The length per residue, L, and the twist, θ. [b] N_{hb} stands for the number of hydrogen bonds formed per C═O group.

i.e., geometries close to the PES minima. In highly strained helices, i.e., those close to the transition state, θ largely changes under small variations in L. Consequently the structural transitions described above proceed essentially in two steps: Upon external strain, there is at first mainly a length change, and only with some delay the helix twist adjusts.

Discussion

Under realistic conditions solvent effects, temperature, helix endings, and the tertiary structure of proteins will affect protein stability and their mechanical properties. Ending effects may selectively stabilize the residues at the furthermost part of the helix in one of the three helical conformations. As a result, the conformation of the endings may differ from that of the bulk if enough stabilization energy is provided. The influence of solvents on the structure and stability of the helical conformation has been recognized long since.[21] Nevertheless, studying helices in a vacuum permits the isolation of effects intrinsic to the peptide structure otherwise hidden by solvents. To illustrate this statement let us consider the following: evidence from experimental data[22−24] suggests that the hydration of the helix backbone tilts the C═O groups outward, toward the solvent. Though the N−H and C═O groups are nearly parallel to the axis of a helix in a vacuum, we observe a noticeable deviation from such orientation. The N−H group tends to point toward the center of the helix, and the C═O group, in the opposite direction. Both tensile and compressive strain increases the tilting with respect to that in the unstrained helices; i.e., tilting of C═O groups is not specific to the hydration process, it is also an intrinsic response of the helix to strain.

Several theoretical studies at an ab initio level have shown that hbs and their cooperativity stabilize the α- and 3_{10}-helix with respect to the FES.[8−13] However studies on structural transitions between the different helical conformers at this level of theory are scarce.[25] Our results show that an α-helix (in a vacuum and a 0 K) undergoes structural transitions under uniaxial strain. We consider it likely that free energy contributions from the solvent and temperature are similar for different helical conformations. Hence we do not expect that the well pronounced minima revealed in the PES vanish under realistic conditions. Thus, these results suggest that the unfolding of an α-helix by stretching will transit throught a 3_{10}-helical conformation. Although the mechanism behind unfolding by an external force, assumed in our work, may differ from a thermally induced or solvent-induced unfolding, experimental observations suggest indeed that the 3_{10}-helix is an intermediate along the thermodynamic folding pathway of helices.[26,27]

Further, our results suggest that the occurrence of π-helices in protein crystals will be scarce; e.g., assuming the strength of hbs in the infinite chains, a π-helix must consist of at least 20 residues to be stable with respect to FES. But helices in protein crystals consist of 10 residues on average.[28] However, under the same assuption made above an α-helix must consist of at least 11 residues, and a 3_{10}-helix of at least 8 residues, to be stable with respect to FES. Therefore they have more probability to occur in protein crystals than π-helices. In fact, in experimental observations α- and 3_{10}-helices are often found, but the π-helix is rarely observed and only stabilized under exceptional circumstances.[29] In contrast, molecular dynamics simulations on short polypeptides with empirical force fields have found a significant presence of the π-helical conformation,[30] even higher than the presence of the 3_{10}-helical conformation. It has been pointed out that this result is a force-field artifact.[31] Our results suggest that an α- to π-helix transition is less favorable than an α- to 3_{10}-helix transition; e.g., for a helix formed by 11 residues the barrier of the α- to π-helix transition is about 7 kcal/mol larger than the barrier for the α- to 3_{10}-helix transition. Thus according to our DFT results, in a molecular dynamics simulation of an α-helix the occurrence of 3_{10}-helices will be favored over the occurrence of π-helical conformations, except if solvent and temperature contributions to the stability largely favor the π-helix over the 3_{10}-helical conformation. A study fully including the solvent and temperature effects is beyond the scope of this report. This subject deserves further investigations.

Conclusions

In conclusion, we have performed a systematic study of the mechanical and energetic response of an α-helix to strain on an atomic level including the full electronic structure. Our analysis provides fundamental insight into the response of an α-helix on uniaxial strain and the detailed role of hbs on it. The structural transitions identified here such as the α- to π-helix and the α- to 3_{10}-helix transitions induced by compressing or stretching the helix are intrinsic features of the helicity. We therefore expect a qualitatively similar behavior also for proteins with high helicity content, e.g., membrane proteins, or for helices inside a protein structure when forces are applied along the helix axis.

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