Abstract

The existence of locally regular secondary structure motifs, such as helices, sheets, or turns, plays a central role for the biological function of proteins. However, open questions remain the thermodynamic stability of the secondary structure motifs. In particular, the *intrinsic stability* , i.e., the stability in the absence of any environmental effects, has – on the basis of experimental studies – not been acquired yet, even for the most abundant secondary structure motif, the helix. A detailed understanding of the intrinsic stability is in turn fundamental for a systematic theory of protein folding. Accurate theoretical studies of model systems are therefore highly desirable.

Density functional theory (DFT) is a powerful electronic structure method which meets the high requirements on accuracy demanded by these systems. However, existing DFT studies on helices focus on the static stability at the absolute temperature zero point and do not account for the strong thermal vibrations which occur at the biologically relevant temperature range. In this project we have therefore faced the challenge to employ DFT to determine the *temperature dependence* of the intrinsic helix stability.

The study includes all three experimentally observed helix types, i.e, the α-, the π- and the 3_{10}-helix. Further it includes several unfolded conformations, which serve as reference for the stability analysis. A key quantity to address the helix stability is the free energy. In the present study the free energy has been determined from the harmonic phonon spectrum, which in turn is determined from the dynamical matrix. In order to achieve the extreme high numerical accuracy required for this project, we had to extend the established standard methodology for calculating the dynamical matrix by a novel method, consisting of a three-stages refinement scheme. To further refine the results, we then extended the study by explicitly calculating anharmonic effects. We therefore have implemented the thermodynamic integration approach and combined it with an efficient stochastic Langevin dynamics scheme, which shows a dramatic increase in the computational efficiency as compared to common deterministic molecular dynamics schemes.

Employing this novel approach on the poly-L-alanine chain we are able to demonstrate that vibrational entropy plays a key role for the stability of the helix in the biologically relevant temperature range, since it strongly reduces the phase stability of the helices compared to the unfolded states. Nonetheless, we find that the enthalpic contributions arising from the cooperative hydrogen bond network of the α-helix are still sufficiently strong to make it the most stable bulk phase at room temperature, and also stable against unfolding. These results provide a very fundamental conclusion: The α-helix is *intrinsically* stable at room temperature, without the need of environmental effects, such as solvent or pressure. Furthermore, our results reveal trends on the temperature dependence of the *relative* stability between the three helix types. Most important, the π-helix exhibits a significant entropic "penalty" with respect to the two other helix types. By carefully mapping our DFT

data on an analytical model, we show that this trend is almost exclusively driven by the geometric peculiarities of the π-helix as compared to the α-helix and 3_{10}-helix. Since these peculiarities are roughly independent of the specific amino acid sequence and of the environment, they rationalize why the π-helix is in *general* the least common of the three helix types in proteins.

Based on these insights we have studied the impact of the side chain of the amino acids, which constitute the basic building blocks of the proteins, on the helix stability by performing a detailed comparative analysis between chains composed of two different amino acids, glycine and L-alanine. According to the experimental results, glycine is a very weak helix former, whereas L-alanine is a very strong helix former. The origin of this difference has not been clarified yet. By means of our approach we can show that vibrational free energy contributions siginificatly lower the glycine helical propensities compared to L-alanine, which also verifies that helical propensities of the amino acids already exist in the absence of *any* environmental effects.