Abstract:

Experimental and theoretical research on peptides and small model proteins has a crucial meaning in understanding of the folding mechanism of proteins. One of the methods of learning about the mentioned mechanism is to design and examine artificial amino acid sequences which possess the ability to self-organize quickly and spontaneously into a unique three-dimensional structure without the tendency to oligomerize. In this work, the conformational characterization of TRP-CAGE mini-protein and its three variants was attempted. The analysis of three-dimensional structures and conformational dynamics of those mini-proteins was performed by using the nuclear magnetic resonance (NMR) spectroscopy. Experiments were carried out in a wide range of temperatures: 278 – 313 K (with a particular consideration of melting temperatures of investigated systems), and next, the molecular dynamics (MD) simulations were executed. The results of performed research allowed to fully characterize the conformational states of chosen peptides at various temperatures and, in the case of one analyzed sequence, to fully characterize the folded state, as well as the unfolded state (the knowledge of which still remains limited). Experiments demonstrated that at low temperatures ensembles of tightly-packed structures (so called native structures) which exhibit large amounts of long-range contacts dominate, and the increase of temperature causes a decrease in the amount of these connectivities, which leads to higher conformational dynamics of the system and its lower structurization. However, even at high temperatures (above the melting temperature) connectivities that were present at low temperatures are still visible. Therefore, investigated mini-proteins seem to be an example of folding according to the downhill model, because in the folding/unfolding process at their melting temperatures the collection of polypeptide chain conformations is not a mixture of fully folded and fully unfolded conformations (which is implied in earlier accepted for these peptides two-state model), but rather homogenous ensembles of conformations including structural properties related to native states of the proteins. Moreover, experiments conducted in this work showed that the problem of interpretation of data concerning thermodynamic stability of single point mutants is more complex than it was earlier assumed. So far, the influence of single point mutations was examined only in the context of change of the amino acid residue side chains and their exposition into the solvent or establishment of hydrogen bonds. Presented results indicate that not only the type of amino acid residue is relevant, but also its position and order in the amino acid sequence, because some combinations of amino acid residues can lead to significant differences in local flexibility/rigidity of a polypeptide chain.