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Gas-phase compaction of helical polymers

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ABSTRACT

To complement the ion mobility mass spectrometry data from the gas-phase experiments on polymers and proteins we have investigated conformations of helical polypeptides by molecular dynamic simulations. Due to strong residue attraction and hydrogen bonding, not mitigated by solvent molecules, a large content of helices, turns and bends was found to persist in long alanine polypeptides $(Ala)_n$ even at very high temperatures. As a result, the unsolvated chain dimensions are significantly contracted relative to the random-coil state and the compact structures such as crumpled coils, melted and nematic globules arise at enhanced temperatures. The compactness exponents determined from the power-law plots of the radius of gyration *vs* lengths suggest that the scaling theory underestimates the polypeptide compaction in the gas phase. At room temperature the polyalanine chains are organized into a variety of helical bundles of antiparallel helical fragments linked by short coils.

1. Introduction

A variety of polymers, synthetic or biological, can adopt stable helical conformations in solution and solid state. In synthetic macromolecules like isotactic polypropylene or polyisocyanates the helix is mostly a consequence of steric hindrance between side-groups. However, even simple polymers composed of backbone atoms only, such as poly(oxymethylene) can favor the helical conformation. Biopolymers DNA and proteins possess unique and specific ordered structures like a right-handed double helix or a single α-helix. The helical structure plays an essential role in functioning of the biomacromolecules, for example at interaction and recognition of other molecules. The importance of the helical structure in nature has prompted the design of the numerous synthetic helical macromolecules mimicking the structures and functions of biological helices [1].

Polypeptides are macromolecules in which the repeat units are based on amino acids. As a simpler analog of proteins, they provide an opportunity to understand the complex behavior of native proteins in living systems. The formation of helices in polypeptides is driven by local interactions and particularly by a formation of a hydrogen bond network along the chain backbone. Thus the α-helix is characterized by a repeating pattern of hydrogen bonds between *i*th and $i+4$th residue. The 3_{10} helix and other types of polypeptide helices are also stabilized by intra-chain hydrogen-bonds. The α-helix is the most abundant structural motif in protein architecture. As a rule, a number of other secondary structures such as β-sheets, turns, and bends coexist in polypeptides and proteins with helices. Understanding stabilities of α-helices and other secondary structural elements is central to the protein-folding problem. The helical conformations in synthetic and biological macromolecules are stable only under certain conditions (temperature, medium) and get disordered to globular and random-coil conformations when these conditions are changed. The competition in transitions between the helix, coil and globule conformation may result in a rich physical behavior of the biomacromolecules [2].

The exploration and assignment of preferred conformations of polymers have traditionally been restricted only to the condensed phases. In recent years the advanced experimental techniques become available for the investigation of the gas-phase structure of polymers [3,4]. Prominently, the mass spectrometry, long-time popular in determination of the structure of small organic molecules, has become revolutionized by introduction of the gentle vaporization techniques such as matrix assisted laser desorption/ionization (MALDI) and electrospray ionization that can produce ions of very large polymers [4,5].To obtain the gas-phase conformational data on macromolecules, mass spectroscopy is frequently combined with the ion mobility method [6,7]. It is presumed that the mobility of polymer ions drifting through a buffer gas is determined by their collision cross-section. Using such techniques the stable conformations of a variety of macromolecules such as

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poly(ethylene)oxide and related polymers [6], polypeptides [7,8], and proteins [9] once thought to be impossible outside the solution, have been experimentally studied in the gas phase.

Stabilization of α-helices in solution is controlled by the complex mixture of intra and intermolecular interactions. The experimental studies in the gas state provide an insight into inherent preferences of helix folding in the absence of peptide–solvent interactions. Jarrold [8] in series of papers examined the intrinsic molecular interactions responsible for conformations of helix forming polypeptides large enough to generate secondary structures in the gas phase. By combining both theory and experiment, they confirmed [8,10] a remarkable temperature stability of α-helices, surviving in the gas phase at temperatures of at least 725 K. Moreover, a strong evidence is now accumulated [4,5,9] that if vaporization is done under mild conditions, desolvation of proteins during the phase transition from solution to the gas in some cases does not affect their structure and stability. Thus, rather surprisingly, protein structure remains quite stable in the gas phase despite the absence of the hydrophobic effect. In other way around, the gas phase ensembles can be used in such cases to accurately model the solution structure. The gas phase with its low dielectric constant ($\varepsilon \sim 1$) may serve as some equivalent of a hydrophobic environment like that found in lipid membranes where the effective constant ε is about 2.

Computational studies in vacuo complement the experimental effort in elucidating the structure and stability of helical polymers in solvent-free environment. Very recent insightful review [11] covers all key aspects of the gas-phase structure of proteins using both experimental and computational sources of information. In simulations of polypeptides by Monte Carlo (MC) and molecular dynamics (MD) methods the alanine-rich molecules are used as convenient models. A small and neutral residue alanine (Ala) is generally viewed as the most α-helix-stabilizing amino acid residue. Hydrophobic peptides with high Ala content are known for their inclination to form the helical structures in aqueous solution. The all-atom gas-phase simulations [12–16] of short $(Ala)_n$ peptides up to the length $n = 20$ explored the transitions between α-helix, β-sheet and random coil. The results [12–16] corroborate a strong dominance of helical conformations in polyalanines in the gas phase at low temperatures. At elevated temperatures the high-temperature phase of disordered chains is formed and this single-step process can be portrayed by a smooth helix-coil transition curve [12–14]. Alternatively, the helix melting by a double-step process was invoked [15,16] in short $(Ala)_n$ peptides that involves an intermediate β-sheet structure in-between the helix and coil conformations.

In the recent extensive MD simulations [17–19] the low-temperature structure of unsolvated alanine peptides of size much exceeding $n = 20$ was explored. We found that the prevailing helical structure of $(Ala)_n$ chains at room temperature was markedly affected by the polypeptide length. The straight α-helices became fully absent in long unsolvated $(Ala)_n$ molecules above the critical length of about 55 residues [19]. Instead, the α-helices were fragmented into shorter pieces that organized into regular bundles of antiparallel helices. The computed average length of about 28 residues in α-helices in bundles excellently concurred with the experimental data on the span of the transmembrane helices of membrane proteins.

In the present paper we explore the gas-phase structure of long helical polypeptides (up to $n = 300$) similar in size to typical proteins. From extensive all-atom MD simulations in vacuum we compute the chain dimensions and the amount of the secondary structures in $(Ala)_n$ molecules in a wide range of temperature. A large occurrence of helices, turns and bends was found in chains even at very high temperatures. As a result, the unsolvated chain dimensions are significantly contracted, and the compact structures such as “crumpled” coils and condensed globule arise at elevated temperatures. This explanation is also supported by the scaling exponents determined from the power-law plots of chain dimensions *vs* lengths. On cooling to room temperature the helicity of $(Ala)_n$ increases to 90%; the α-helical pieces self-organize into discrete bundles of antiparallel helical fragments linked by short coils.

2. Computational method

The methodology used follows that described in our previous studies [17–19]. Single molecules of poly(alanine) of the lengths n from 20 to 300 Ala residues in the L-form were assumed. A wide range of chain lengths allowed to explore the development in the structural behavior from the short $(Ala)_n$ peptides to long polypeptides analogous to proteins in the membrane complex typically counting up to 200 amino acid residues [20]. The $(Ala)_n$ peptides were capped at N- and C-ends by acetyl and methyl amide (NHMe) groups to ensure the charge neutrality.

The MD simulations with all atoms treated explicitly were carried out in a canonical NVT ensemble without solvent. The Gromacs [21] program package implemented on a parallel computing cluster was employed. The popular, third-generation force field Amber03 [22] was chosen to account for all interactions in a molecule. The force field version Amber03, as well as the Amber99φ ones used in our previous studies [17–19], builds on many years of Amber force field improvements to mend an alleged tendency of some older Amber versions to over-stabilize the helical structure. The recent force fields assessment [23] yielded a realistic prediction of helicity in short polyalanine peptides by both Amber99φ and Amber03 alternatives. A switch function is used for van der Waals interactions. The Lennard-Jones potential is standard out to 1.2 nm, after which it is switched off to reach zero at 1.4 nm. Both the potential and force functions are continuously smooth. The cut-off distance for the short-range neighbor list was 1.4 nm. The reaction-field-zero electrostatics method with Coulomb cut-off 1.4 nm with dielectric constant set to infinity beyond the cut-off was used. It is computationally more expensive than normal reaction-field method. It makes the potential zero beyond the cut-off avoiding bad energy conservation.

The leaping-frog integration algorithm was applied for integrating Newton’s equations of motion. The temperature was maintained close to the intended value by temperature coupling using velocity rescaling with a stochastic term. The time constant for coupling τ of 0.1 ps was used. This thermostat is similar to Berendsen coupling, with the same scaling using τ, but the stochastic term ensures that a proper canonical ensemble is generated. The time step applied in integration was 2 fs at temperatures below 900 K and 1 fs for simulations at high temperatures. The velocities were generated according to a Maxwell distribution at initial temperature 1500 K. No constraints were applied for bonds in simulations above 900 K. The bonds with H-atoms were converted to constraints for simulations below 900 K. The P-LINCS [24] constraint algorithm was applied.

For each of the chain lengths sixteen independent MD simulation runs were performed starting with a random coil-like structure of $(Ala)_n$ prepared by initial 200 ps simulation at high temperature ($\sim$1500 K). The initial structures of $(Ala)_n$ contained no helical structures. Subsequently, a disordered initial structure of $(Ala)_n$ served as a starting point for MD simulation subjected to cooling to 300 K by pre-defined series of simulation annealing. Depending on chain length three different temperature protocols of the time run from 175 to 575 ns were applied. The total simulation time of all 160 simulation runs with ten different chain lengths of $(Ala)_n$ was 64.8 μs.

The secondary structure elements in $(Ala)_n$ molecules were assigned according to the DSSP classification [25] which has gained general acceptance among the protein computation community.