Journal of Molecular Liquids 227 (2017) 66–75

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Influence of an ionic liquid on the conformational sampling of Xaa-Pro dipeptides

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ARTICLE INFO

Article history:
Received 15 August 2016
Accepted 28 November 2016
Available online 01 December 2016

ABSTRACT

Room temperature ionic liquids have demonstrated promise for the selective control of protein structure and function, but fundamental aspects of ionic liquid effects on peptides and proteins remain unclear. In this study molecular dynamics simulations are used to understand the effect of the room temperature ionic liquid 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl) imide ($[C_4mpy][Tf_2N]$) on the behavior of Xaa-Pro dipeptides, where Xaa is any of the common amino acids, at several temperatures and compared to water, octanol, and vacuum. Generally, compared to water and octanol, the room temperature ionic liquid is found to restrict peptide conformational sampling, which results in narrower radius of gyration distributions and area visited in Ramachandran space. Despite the restricting effects of $[C_4mpy][Tf_2N]$, it is found to result in isomerization of the Trp-Pro dipeptide bond to the cis state at 298 K, suggesting that this ionic liquid can be used to stabilize otherwise infrequently observed dipeptide conformations. Our simulations suggest that stacking of the Trp ring and Pro ring in the cis state versus the trans state contribute to this effect for the Trp-Pro dipeptide. When the temperature of the ionic liquid system is increased, the viscosity of the solvent decreases and the area sampled by the dipeptide in Ramachandran space becomes more similar to water, octanol, and vacuum environments.

1. Introduction

Room temperature ionic liquids (ILs) have received attention for the possibility of controlling protein structure and function [1]. The promise of ionic liquids is greatly related to the large number of possible combinations of cation and anion components, which means they can often be tuned to achieve specific physical properties. It is interesting to consider selecting ionic liquids so that the behavior of proteins is specifically affected. There have been numerous studies of ionic liquids in biologically relevant systems, but the effects of ILs remain difficult to generalize.

There are a great number of studies establishing the promise of ILs for a wide variety of applications in biologically-relevant systems. A brief overview includes applications in the dissolution and separation of cellulose and other chemicals [2,3], carbon capture and sequestration [4,5], selective targeting of bacterial or mammalian cells [6], pharmaceutical preparation, storage and delivery [7,8], and peptide synthesis [9–13]. While these demonstrate the great opportunities for the use of these materials across a broad spectrum of fields, a detailed elucidation of the relevant molecular mechanisms of IL action is still emerging.

There have been significant efforts exploring biomolecular systems in ionic liquids and this is an area in which theory and simulation can play a central role in deepening our understanding of IL-biomolecular interactions. For example, Pfaendtner and coworkers have shown that some enzymes can retain their bioactive structure in IL [14,15], but effects are specific to the enzyme considered [16]. While many of the conclusions are restricted to the specific systems considered, there are tantalizing trends emerging. For example, surface charge of the enzyme plays an important role in enzyme-IL interactions [17], and enzymes with more negatively charged surfaces are more likely to retain their bioactive secondary structure in ionic liquids [16]. The effects of ILs have often been categorized within the larger scheme of the Hofmeister series with limited success, but a recent study of protein unfolding rates in ILs using the infrequent metadynamics method suggests that IL effects on the protein may be better understood in terms of ion affinity for hydrophobic residues [18]. There has been a lot of work put into cellulose dissolution and preparation for various biomaterials applications [19,20]. Such simulations of biomass processing have revealed the important role of cellulose-IL hydrogen-bond formation in cellulose dissolution and how anti-solvents can disrupt these hydrogen-bonds for cellulose reconstitution [19]. The large system sizes and long time-scales involved have made resolving atomistic details of biomass preparation in ILs challenging and a wide survey of ILs prohibitive. Therefore, it is useful to consider smaller biomolecular systems, where more exhaustive simulations can resolve many of these features in order to build up an understanding of the utility

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http://dx.doi.org/10.1016/j.molliq.2016.11.127
0167-7322/

of ILs. For example, studies of small proteins have revealed that their structure and dynamics are greatly affected by ILs [12,21,22]. Even in the case of the fast folding protein Trp-cage, it is challenging to study the influence that ionic liquids have on protein dynamics in a systematic way [21,23]. When starting from an unfolded state, aqueous Trp-cage folds to a stable structure in less than 500 ns, but simulations of Trp-cage in an ionic liquid at room temperature do not reach a folded state over 500 ns of simulation [21]. In essence, the high viscosity of ionic liquids compounds the sampling problems that already exist in molecular dynamics simulations [21,24], making exploring protein dynamics in these liquids a formidable challenge.

Despite generally greatly slowing protein dynamics when compared to water, the ionic liquid 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl) imide ([C_4mpy][Tf_2N]) is found to facilitate cis/trans isomerizations of omega angles containing proline in Trp-cage [21]. The influence of ionic liquids on the cis/trans isomerization of peptide bonds near proline has also been previously reported in the experimental literature [23,25]. Bunagan and coworkers examined folding of small proteins with circular dichroism and suggested that cis/trans isomerization could be induced by the ionic liquid [C_4mpy][Tf_2N] and frustrate folding of the protein [23,26]. Wehofsky et al. looked at the effect of a buffer containing the IL 1,3-dimethylimidazolium dimethylphosphate on cis/trans isomerization in peptides and protein fragments with solvent jump experiments and found the cis/trans equilibrium is affected, stabilizing the trans state of Xaa-Pro peptide bonds by 2.7 to 3.8 kJ/mol [25]. The propensity of amino acids with the motif Xaa-Pro to undergo cis/trans isomerization is well-documented, where Xaa is any of the standard amino acids [27–29] so it is not surprising to see peptide bonds involving proline implicated in isomerization events. These isomerization events are more than a biochemical curiosity, with proline isomerization being one of the rate determining steps in protein folding [29,30]. The presence of Pro is correlated with intrinsic disorder in proteins and proline is the most "disorder-promoting" amino acid, leading to a dramatic increase in cis-isoforms [31]. In addition to greatly influencing the folding kinetics and structure of many proteins, proline cis/trans isomerization is proposed as a mechanism for native state function control [32,33]. This suggests that the control of cis or trans state populations with ionic liquids could be a powerful tool to direct protein activity. However, characterizing the effects of ILs on peptide bond cis/trans state is difficult, which makes the selection of an IL to specifically affect the cis/trans state populations challenging.

Therefore, in this work, we assess the effect of solvation in IL, water, octanol, and vacuum at several temperatures on cis/trans isomerizations around the peptide bond in a minimal representative system. We have selected the 20 possible Xaa-Pro dipeptides, where Xaa represents any of the 20 standard amino acids. We choose the common IL [C_4mpy][Tf_2N] because it has been used previously in experiments studying several fast-folding proteins using circular dichroism spectroscopy [23] and it is the IL that we studied in our simulations of the Trp-cage protein [21]. Elevated temperatures are considered to resolve effects arising from the large viscosity of this IL at 298 K. The behavior of the dipeptides in IL is contrasted with water, octanol, and vacuum to provide additional information about the effect of the environment. Octanol is selected because it is distinctly different from water and it is a frequently used approximation of a lipid membrane environment in partitioning experiments [34].

In this work we observe that the free energy difference between cis and trans conformations of the peptide bond is often different between aqueous solution, octanol, water, and vacuum, and is influenced by the system temperature as well as the nature of the Xaa amino acid in the Xaa-Pro dipeptides. In particular, all Xaa-Pro dipeptides exhibit a preference for the trans state of the peptide bond in all environments tested, except for the Trp-Pro dipeptide, which has a more favorable cis state compared to the trans state in IL at 298 K. It is suggested from our trajectories of Trp-Pro in the cis and trans conformations that this increased stabilization of the Trp-Pro dipeptide in the cis state could arise from preferential packing that can occur between the proline ring and the very bulky side chain of tryptophan in the cis state of the system. We observe in general that IL at 298 K tends to increase the barrier to cis/trans isomerization, however, compared to water.

2. Methods

2.1. Simulation details

2.1.1. Initial system setup and unbiased simulations

The dipeptide structures were generated using the leap program in AmberTools. We consider all twenty dipeptides of the form Xaa-Pro, where the N-terminus is acetylated and the C-terminus is methylated. The dipeptide interactions are modeled using the FF14SB parameterization of the Amber force field [35], the water with TIP3P [36], octanol with the general amber force field [37], and the RTIL with the force field developed by Xing et al. [38] following the implementation described in Baker et al. [21]. These structures were neutralized and solvated with water, RTIL, or octanol using Packmol [39]. Simulations were performed using the GPU-implementation of Amber 14 pmemd [40]. The system temperature was regulated with the Langevin thermostat with a collision frequency of 1.0 ps^{-1}. The IL, water, octanol, and vacuum systems were simulated at 298 K, and the IL was simulated at 365 K and 500 K as well. Bonds involving hydrogen were constrained with the SHAKE algorithm in all simulations. These configurations were equilibrated following a three step process: 10,000 steps minimization, 1 ns NVT simulation, and 2 ns NPT simulation. This was followed by 100 ns of NPT production. This constitutes a total of 12 μs of production simulation for the 20 dipeptides. The equilibrium densities for each dipeptide system were determined from each production simulation, and the configuration most closely matching the average density was selected for the umbrella sampling simulations.

2.1.2. Umbrella sampling simulations

We explore the energetics of rotating the omega angle of the peptide bond between amino acids using umbrella sampling. Harmonic biasing potentials are imposed at omega angles of 0°, 20°, 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 140°, 160°, 180°, 200°, 220°, 240°, 260°, 270°, 280°, 290°, 300°, 320°, 340°, and 360° with a force constant of 50 kcal/mol/rad^2. We collected 20 ns of NVT simulation in each window for each system studied. Umbrella sampling simulations were carried out in IL (at 298, 365, and 500 K) as well as water and octanol (both at 298 K), and in total 50 μs of umbrella sampling simulation were accumulated for analysis. The potential of mean force of rotating around the dipeptide bond was constructed using the weighted histogram analysis method (WHAM) [41]. Additionally, due to the slower dynamics arising from the increased viscosity of the ionic liquid, we also simulated the ionic liquid at 365 and 500 K. The entropy change of the trans-to-cis transition was estimated according to the thermodynamic relation $\Delta S = -(dA/dT)_V$ from the slope of a linear fit to $\Delta A_{isomerization}$, which is the difference between the trans and cis minima in the potential of mean force, and the temperature. Invoking this relationship requires the assumption that ΔS and ΔU for the isomerization are constant over the temperature range considered.

2.2. Analysis

2.2.1. General analysis

The AmberTools 14 version of cpptraj [40,42] was used to calculate the peptide backbone dihedral angles, radius of gyration, and radial distribution functions.

2.2.2. Association free energy and distance

The association free energy was determined from the radial distribution function between the center of mass of the peptide and the atoms in the indicated solvent. Each radial distribution function was analyzed to determine the radius with the maximum density. The association free