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Research article

Molecular dynamics studies show solvation structure of type III antifreeze protein is disrupted at low pH

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ABSTRACT

Antifreeze proteins are a class of biological molecules of interest in many research and industrial applications due to their highly specialized function, but there is little information of their stability and properties under varied pH derived from computational studies. To gain novel insights in this area, we conducted molecular dynamics (MD) simulations with the antifreeze protein 1KDF at varied temperatures and pH. Water solvation and H-bond formation around specific residues – ASN14, THR18 and GLN44 – involved in its antifreeze activity were extensively studied. We found that at pH1 there was a disruption in water solvation around the basal and the ice binding surfaces of the molecule. This was induced by a small change in the secondary structure propensities of some titrable residues, particularly GLU35. This change explains the experimentally observed reduction in antifreeze activity previously reported for this protein at pH1. We also found that THR18 showed extremely low H-bond formation, and that the three antifreeze residues all had very low average H-bond lifetimes. Our results confirm long-standing assumptions that these small, compact molecules can maintain their antifreeze activity in a wide range of pH, while demonstrating the mechanism that may reduce antifreeze activity at low pH. This aspect is useful when considering industrial and commercial use of antifreeze proteins subject to extreme pH environments, in particular in food industrial applications.

1. Introduction

Freezing damages, and many times is lethal to, cellular organisms. To limit this damage, many species of insects (Wu et al., 1991), plants and fish (Griffith et al., 2005; Fletcher et al., 2001), bacteria (Gilbert et al., 2005) and fungi (Lee et al., 2012) have developed methods to survive freezing temperatures using antifreeze proteins or glycoproteins (AFPs). Many applications have been described for antifreeze proteins including increasing freeze tolerance of plants to extend the agricultural season; in farm fish production; extending shelf life of frozen foods (Zhang et al., 2007; Venketesh and Dayananda, 2008), or cryopreservation of cells, organs and tissues (Amir et al., 2003; Inglis et al., 2006), among others. Some limitations have prevented them from being developed commercially, including difficulty in purification and synthesis, and chemical instability in solutions (Leclere et al., 2011; Medina and Mastai, 2011). For many of these applications, additional studies are required to understand and to characterize their behavior in varied chemical and physical conditions. This is because depending on their mode of application and use, antifreeze proteins are subject to environments where temperature and pH can vary widely, degrading or changing their mechanism of action, or elimination (Cziko et al., 2014).

Antifreeze proteins interact with ice planes, inhibiting recrystallization. The mechanisms have been in the past associated to H-bond formation and hydrophobicity, but there are other proposed factors involved, for example unusual properties of the water shell and location of specific residues in the ice binding surface (Meister et al., 2014; Kuffel et al., 2014; Kuiper et al., 2015; Sharp, 2011) or effect of exposure to very low temperatures (Kar and Bhunia, 2015). Computational MD simulation methods have been used to study antifreeze proteins (Kundu and Roy, 2008; Cheng and Merz, 1997; Calvaressi et al., 2012), but basic computational studies of selected properties at varied pH have been rarely reported. Most of the studies of changes in pH and T in antifreeze activities have been experimental, centered in the analysis of variations of thermal hysteresis (Friis et al., 2014; Kawahara et al., 2007; Marshall et al., 2005; Qiu et al., 2010; Leiter et al., 2016).

The properties of AFPs are dependent on T and pH and affect their utilization in biotechnological applications. For this reason, we carried out MD simulations with a type III antifreeze protein, the ocean pout 1KDF at different temperatures and pH (1, 7 and 12), and assessed their stability at varied pH using conventional and

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accelerated molecular dynamics simulations. This protein was experimentally well characterized by Chao and coworkers (Chao et al., 1993, 1994), with detailed description of residue contribution to the antifreeze activity. They demonstrated that residues THR18, ASN14 and GLN44 were mostly responsible for its antifreeze activity, as measured by thermal hysteresis. Mutations N14S or T18N reduced the activity to 10% and 25% from the original wild type. Their pH studies failed to identify any role for antifreeze activity due to the ionization state of the residues and showed that pH had no influence in that activity between pH 2 and pH 11, but decreased 20% at pH1. Additional mutations to probe the effect of pH, like N14D and Q44E at pH3 also failed to notice any decrease in activity. At the time, the authors did not compare experimental results with computer simulations. We decided to identify the reasons behind the drop in antifreeze activity at pH1 using computational methods in a supercooled water environment using the TIP3P water model.

Variations in pH are simulated in computational studies by changing residue's protonation state based on the calculated ionization states of the protein, as described in the Methods section below. As we note later, in 1KDF and other antifreeze proteins titrable residues that change protonation states are not the ones that are involved in antifreeze related activities or even located within the ice-binding surface. Overall, we found that physical and chemical properties that have normally been associated with antifreeze activity – H-bond, secondary structure, water solvation- changed at pH1 but remained essentially unaffected at other pH. The restructuring of the water solvation shell around the protein and, in particular, near selected residues, is the most likely explanation of the experimental results found by Chao and coworkers.

In addition to standard MD methods, we also used accelerated Molecular Dynamics (aMD) (Pierce et al., 2012; Hamelberg et al., 2004), a technique proposed to accelerate the study of biomolecular processes in biomolecular simulations. We have described this implementation previously (Peramo, 2016). Our intention here was to assess whether there are *differential* changes by T and pH in fluctuations, hydrogen bond or secondary structure using aMD techniques that can be applicable to analysis of molecular properties related to antifreeze activity.

2. Methods

2.1. Execution environment

Computations were performed with an Asus Quantum TXR-500-032R (EXXACT Corp) workstation, with an Intel Core i7–3930 K Processor (12 M Cache, 3.20 GHz) and 32GB DDR3 1600 MHz memory. The workstation had an NVIDIA GeForce GTX 780 available for GPU computations with the CUDA toolkit version 5 installed. The OS was CentOS 6.5 and MD simulations ran with AMBER 12, compiled using gcc-4.4.7. At the start of our work, we had access only to AMBER12, which was upgraded to AMBER14 by the developers during the period of time we were running our calculations. Details are provided in Supplementary information Table S1. AMBER was compiled to use the *pmemd.cuda* with mixed precision (SPDP).

2.2. Computational methods

Simulations were run at three pH (1, 7 and 12), at 248 K, 268 K, 310 K and 373 K, using *pmemd. cuda* as we described previously (Peramo, 2016). Details are included in Supplementary information S2 section and Table S2. The water model was TIP3P with the calculations performed with the NPT ensemble. We have previously shown that using AMBER with *pmemd.cuda* is equivalent to using *sander*, and for this reason we used only CUDA GPU generated trajectories for our analysis. The 100 ns calculations were performed at pH 7 and four temperatures (248 K, 268 K, 310 K and 373 K) with the same water model, following the procedure described elsewhere (Pierce et al., 2012) and described in the Supplementary material S3. Data was extracted from trajectories using the AmberTools 13 (Case et al., 2016) and Amber 14 versions of CPPTRAJ (Roe, 2013). Molecular graphics were generated using VMD version 1.9.1 (Humphrey et al., 1996) or the free version of the molecular package Discovery Studio 4. Plots were generated using the utility Grace version 5.1.22 in CentOS.

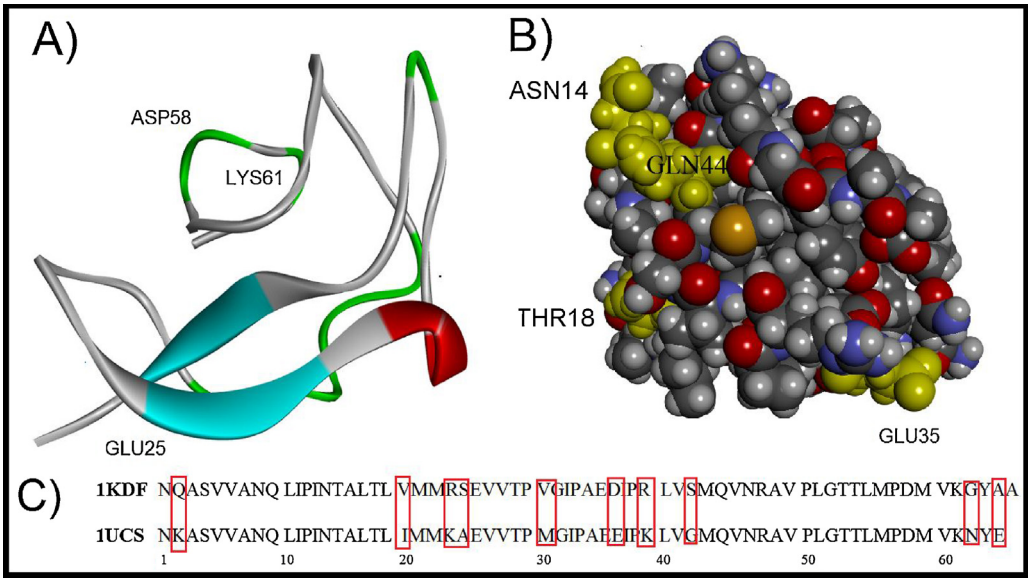

Fig. 1. Protein Data Bank 1KDF structure as downloaded from rcsb.org (A). The three main residues involved in antifreeze activities – ASN14, GLN44 and THR18 – are shown, located on the ice-binding surface (B). The names of some of the residues that change their protonation states (GLU25, GLU35, ASP58 and LYS61) and their locations are also shown (A and B). AFPs type III show highly conserved sequences with few residue changes, as seen comparing the sequences of 1KDF with the sequence of 1UCS from Antarctic eel pout (C).