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# Comparative structural studies of psychrophilic and mesophilic protein homologues by molecular dynamics simulation

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ABSTRACT

Comparative molecular dynamics simulations of psychrophilic type III antifreeze protein from the North-Atlantic ocean-pout *Macrozoarces americanus* and its corresponding mesophilic counterpart, the antifreeze-like domain of human sialic acid synthase, have been performed for 10 ns each at five different temperatures. Analyses of trajectories in terms of secondary structure content, solvent accessibility, intramolecular hydrogen bonds and protein–solvent interactions indicate distinct differences in these two proteins. The two proteins also follow dissimilar unfolding pathways. The overall flexibility calculated by the trace of the diagonalized covariance matrix displays similar flexibility of both the proteins near their growth temperatures. However at higher temperatures psychrophilic protein shows increased overall flexibility than its mesophilic counterpart. Principal component analysis also indicates that the essential subspaces explored by the simulations of two proteins at different temperatures are non-overlapping and they show significantly different directions of motion. However, there are significant overlaps within the trajectories and similar directions of motion of each protein especially at 298 K, 310 K and 373 K. Overall, the psychrophilic protein leads to increased conformational sampling of the phase space than its mesophilic counterpart.

Our study may help in elucidating the molecular basis of thermostability of homologous proteins from two organisms living at different temperature conditions. Such an understanding is required for designing efficient proteins with characteristics for a particular application at desired working temperatures.



## 1. Introduction

There has been a growing interest in understanding the molecular basis of thermostability of proteins from the organisms living at different temperature conditions. Such an understanding is critical for designing efficient enzymes with characteristics for a particular application. Homologous proteins can be found in organisms that live in very different environments. Such homologous proteins may be highly similar in their sequences, structures but drastically different in the temperature dependencies of their activity and stability. Psychrophilic organisms live at low temperature, where most other organisms cannot grow. Type III antifreeze proteins (AFP III) are very small (~65 amino acids long) psychrophilic proteins that are seasonally found at high concentrations in the blood of some fishes living in polar or high latitude temperate seas [1]. AFPs depress the freezing point of blood and body fluids below that of the surrounding seawater by binding to and inhibiting the growth of seed ice crystals [2,3]. Because of their lack of enzymatic activity, study of AFPs may allow to differentiate between features of cold-adapted proteins that may have arisen as consequence of structural features, such as flexibility, that are needed for catalysis [1]. The mesophilic homologue of AFP III is the C-terminal domain of human sialic acid synthase, called the antifreeze like (AFL) domain [4], since it is similar to those observed in a variety of functional type III antifreeze proteins. It has been proposed that the AFL domain is also involved in sugar binding [4], but the details of its function have remained elusive. Recently the crystal structure of AFP III containing 66 residues from *Macrozoarces americanus* [5] has been solved. The AFL domain of human sialic acid synthase consisting of 79 residues has also been determined by NMR spectroscopy [4]. These two proteins show 38% sequence identity (Fig. 1A) and the root-mean-square deviation of Cα atoms of superimposed structures is 2.0 Å. Their overall fold contains one α-helix (residues 37–40), two $3_{10}$ helices (residues 19–21 and 57–59) and two β-strands (residues 4–7 and 22–25) (Fig. 1B). In addition the AFP III

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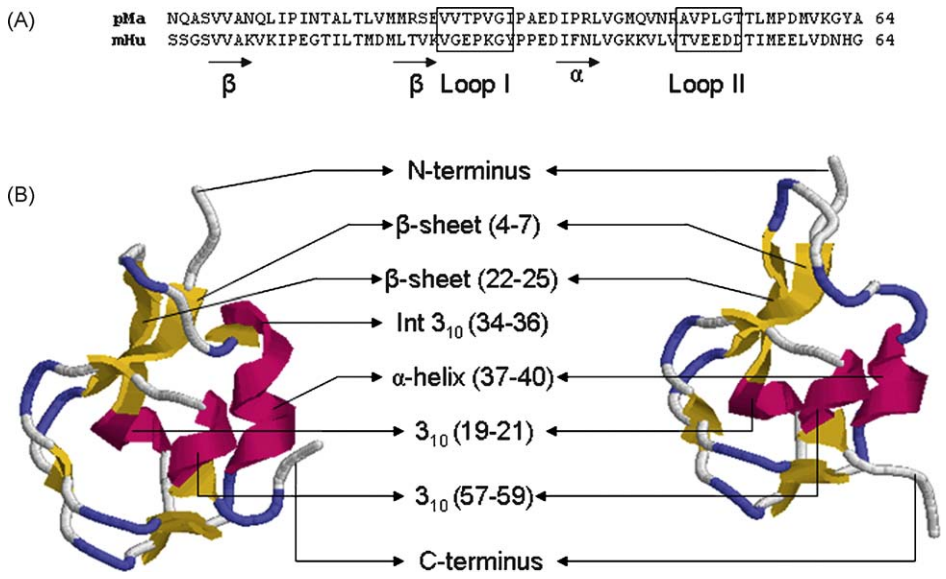


**Fig. 1.** (A) Pairwise sequence alignment of pMa and mHu. Sequences of loops I and II are shown in box. (B) The native structure of (I) pMa and (II) mHu. Helices are shown as magenta ribbons, β-strands as yellow arrows and the rest are shown as loops.

has an intermediate $3_{10}$ helix (residues 34–36). The rest of the structures comprise mostly of β-bridges and loops. Despite of very similar structure, they have very different temperature dependencies in stability and activity. The working temperature of AFP III protein is around 273 K (0 °C) while the working temperature of mesophilic proteins is in the range of 293–310 K (20–37 °C) [1]. Psychrophilic proteins have been found to be much more temperature liable than mesophilic counterpart [6] indicating that psychrophilic proteins are generally less stable compared to mesophilic homologue [6]. Until recently, the absence of available crystal structures of cold-adapted proteins limited the comparison to their homologues from meso and thermophilic organisms. Molecular dynamics (MD) simulation is a suitable tool to evaluate the comparative basis of protein thermostability between homologous psychrophilic and mesophilic proteins. However only a few comparative molecular dynamics studies aimed at evaluating temperature dependencies of the dynamics of psychrophilic and mesophilic proteins at different temperatures have been done [6–10]. In fact to our knowledge no comparative molecular dynamics study dealing with wide range of temperature has been reported. In this paper for the first time we have performed molecular dynamics simulation of AFP III and its mesophilic homologue at five different temperatures namely 298 K, 310 K, 373 K, 423 K and 473 K. In addition to five temperatures we have carried out 277 K (4 °C) simulation for AFP III, which is close to the growth temperature of this protein. The dynamic properties of two proteins at different temperatures have been compared in terms of secondary structure content, molecular flexibility, intramolecular hydrogen bonds and protein–solvent interactions. The thermal unfolding pathways of two proteins have also been investigated. Comparisons of essential conformational subspaces of these two proteins at different temperatures have been monitored by principal component analysis.

## 2. Methodology

### 2.1. Molecular dynamics simulation

All MD simulations were performed using the GROMACS 3.3.1 [11,12] package and GROMOS96 [13] 43a1 force field implemented on LINUX architecture. The coordinates for starting configurations AFP III and its mesophilic homologue were obtained from the Protein Data Bank (PDB entry codes 9AME [5] and 1WVO [4]), which consisted of 66 and 79 residues, respectively. Pairwise sequence alignment by ClustalW [14] was performed to find out the correspondence between residues of two proteins. In order to make equal number of residues for both the proteins four residues from the N-terminus and eleven residues from the C-terminus of 1WVO and one residue each from N and C termini of 9AME were deleted in such a way that original fold retained in both the structures. The starting structure of MD simulation of both the proteins thus contained 64 residues. We abbreviated the truncated 9AME as pMa and 1WVO as mHu throughout the text. Crystallographic water molecules and heteroatoms were removed from the systems. All starting structures were immersed in a triclinic box of SPC water molecules [15]. Box dimensions for pMa and mHu were 5.503 nm × 4.821 nm × 4.554 nm with 3652 SPC water molecules and 4.781 nm × 4.923 nm × 4.783 nm with 3407 SPC water molecules, respectively. All protein atoms were at a distance equal to 1.0 nm from the box edges. No counter ions were added to pMa because the system was already neutral, whereas 6 $NA^+$ ions were added to neutralize the charge of mHu. Each system was subjected to energy minimization for 2000 steps by steepest descents. The minimized systems for both mHu and pMa were equilibrated for 50 ps each at five different temperatures namely 298 K, 310 K, 373 K, 423 K and 473 K by position restrained molecular dynamics simulation in order to relax the solvent. The equilibrated systems were then subjected to molecular dynamics simulations for 10 ns each at five different temperatures. An additional MD simulation was also performed at 277 K for pMa only. In sum, trajectories of 110 ns (60 ns for pMa, 50 ns for mHu) were collected for two proteins investigated. Periodic boundary conditions combined with minimum image convention were used under isothermal, isobaric conditions using Berendsen coupling algorithm [16] with relaxation times of 0.1 ps and 0.5 ps, respectively. The LINCS algorithm [17] was used to constrain bond lengths using a time step of 2 fs for all calculations. Electrostatic interactions were calculated using the Particle Mesh Ewald (PME) [18,19] summation scheme. van der Waals and Coulomb interactions were truncated at 0.9 nm. The non-bonded pair list was updated every 10 steps and conformations were stored every 2 ps. Secondary structure analysis was performed