J Korean Soc Appl Biol Chem (2015) 58(2):209–212
DOI 10.1007/s13765-015-0033-y

Online ISSN 2234-344X
Print ISSN 1738-2203

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Molecular dynamics simulation of a psychrophilic adenylate kinase

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Received: 11 November 2014 / Accepted: 6 January 2015 / Published online: 30 January 2015

Abstract Proteins from psychrophilic organisms can function at low temperatures and have shown potential for industrial applications. In the present study, thermal denaturation of a psychrophilic adenylate kinase (AKpsychro) from *Bacillus globisporus* was measured using circular dichroism spectroscopy and its molecular dynamics (MD) simulations were performed. The results from the unfolding experiment and MD simulations of AKpsychro were compared with those of its mesophilic and thermophilic homologues to study the relationship between dynamic motion and the cold adaptation of proteins.

Keywords Adenylate kinase · Enzyme · Molecular dynamics simulation · Protein · Psychrophile

Introduction

In recent years, there has been an increase in the discovery and characterization of organisms living at low temperatures (Margesin and Miteva 2011). To maintain the metabolic rates required to sustain life in such cold environments, proteins from psychrophilic organisms remain functional even at very low temperatures (Feller 2007, 2010; Reed et al. 2013). Therefore, psychrophilic proteins are desirable for use in many research and industrial settings that require biological activity at low temperatures (Feller and Gerday 2003; Feller 2013).

One means of characterising the molecular mechanisms of cold adaptation in psychrophilic proteins is to compare and contrast them with homologous proteins from organisms living at moderate and high temperatures, or mesophiles and thermophiles, respectively (D'Amico et al. 2003; Feller 2010; Reed et al. 2013). In many such comparisons, psychrophilic proteins contained fewer intramolecular non-covalent interactions such as hydrogen bonding, electrostatic interactions and hydrophobic contacts than their mesophilic and thermophilic homologues (Feller 2010; Reed et al. 2013). It would, therefore, seem that there is a correlation between the number of intramolecular interactions and the operating temperature of the source organism. This is thought to be the result of the adaptations that allow for the flexibility required to maintain biological function at different environmental temperatures (Somero 1978).

We have previously reported the crystal structures of adenylate kinases (AKs) from a psychrophile, *Bacillus globisporus* (AKpsychro, Protein Data Bank code 1S3G), a mesophile, *Bacillus subtilis* (AKmeso, Protein Data Bank code 1P3J) and a thermophile *Geobacillus stearothermophilus* (AKthermo, Protein Data Bank code 1ZIO) (Berry and Phillips 1998; Bae and Phillips 2004). Structural analysis indicated that AKpsychro has the fewest non-covalent intramolecular interactions among the three homologous proteins (Bae and Phillips 2004). The thermal denaturation midpoint (T_m) of AKpsychro was lower than those of its

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mesophilic and thermophilic homologues. The T_m values for AKpsycrho, AKmeso and AKthermo have been determined previously by differential scanning calorimetry (DSC) to be 43.3, 47.6 and 74.5 °C, respectively (Glaser et al. 1992; Bae and Phillips 2004). We have also performed molecular dynamics (MD) simulations of AKmeso and AKthermo and compared their relative dynamic motions (Bae and Phillips 2005). In the current study, we measured the T_m of AKpsychro using circular dichroism (CD) spectroscopy to confirm its temperature preference and performed MD simulations to study the relationship between dynamic motion and cold adaptation.

Materials and methods

T_m measurement

To determine its T_m value using CD spectroscopy, AKpsychro overexpressed in *Escherichia coli* was purified by a two-step procedure involving affinity and size-exclusion chromatography as described previously (Bae and Phillips 2004). CD traces of the purified protein (0.5 mg/mL in 10 mM potassium phosphate pH 7.0) were measured at 220 nm using the Chirascan-plus CD Spectrometer (Applied Photophysics, UK) at a scanning rate of 1 °C/min. CD data were then analysed to determine T_m based on a protocol developed previously (John and Weeks 2000).

MD simulations

The crystal structure of AKpsychro (Protein Data Bank code 1S3G) was used as a starting model for MD simulations (Bae and Phillips 2004). In this structure, a magnesium ion, which is essential for catalysis, and its four coordinating water molecules are not present due to the crystallisation condition. To generate these missing molecules, the AKpsychro structure was structurally aligned with that of AKmeso (Bae and Phillips 2004), from which the magnesium ion and the four water molecules were added to complete the starting model.

The MD simulations of AKpsychro were performed at 300 and 330 K as described previously for those of AKmeso and AKthermo (Bae and Phillips 2005). Briefly, the CHARMM27 force field was used for the simulations with NAMD software (MacKerell et al. 1998; Kalé et al. 1999). The system was minimised for 1,000 steps and equilibrated at 300 K for 20 ps with the fixed crystallographic atoms. Additional minimization for 2,000 steps and equilibration at 300 K for 100 ps was carried out with all atoms free to move. For the simulation at 330 K, the system was further equilibrated at 330 K for 100 ps. MD simulations at each temperature were then performed for 1.2 ns and the coordinates were saved at every 500 steps. All analyses were performed for residues 1–212, excluding the highly disordered C-terminal region during the last 1 ns of the simulations.

Results

Determination of T_m value for AKpsychro using CD spectroscopy

In the thermal denaturation experiment monitored by CD spectroscopy (Fig. 1), AKpsychro undergoes a single major unfolding transition with a T_m of 42.7 °C, which is similar to the value (43.3 °C) determined by the previous DSC measurement (Bae and Phillips 2004). The T_m value of AKmeso was previously determined to be 46.4 and 47.6 °C using CD spectroscopy and DSC, respectively (Bae and Phillips 2004; Moon et al. 2014), indicating that AKpsychro yielded lower T_m values than its mesophilic homologue regardless of the experimental techniques to monitor thermal denaturation. CD spectroscopy can directly observe structural changes of proteins, while DSC measures heat transfer involved in the denaturation process. It was previously noted that T_m values of AKs could vary substantially (up to 8.5 °C) depending on the instruments and the experimental settings (Moon et al. 2014). Our result confirms the relatively unstable nature of AKpsychro, which is characteristic of psychrophilic proteins.

Stability of trajectories from the MD simulations of AKpsychro

The root-mean-square deviation (RMSD) between the crystal structure used as the starting model and the final structure after minimization was only 0.45 Å, indicating that the force field used in this study is sufficiently accurate. RMSD values for the

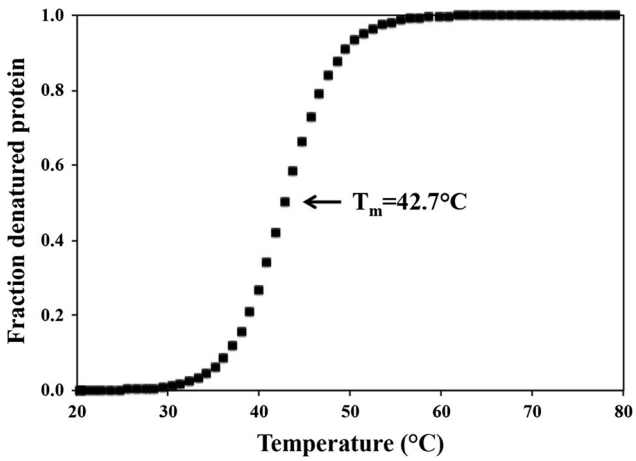

Fig. 1 Thermal denaturation curve of AKpsychro measured by CD spectroscopy

total and backbone atoms between the final minimised AKpsychro structure and the coordinates saved at 500 step intervals during equilibration (0.1 ns) and simulation (1.2 ns) are plotted in Fig. 2. The observed increase of the RMSD value at the onset of the 300 K equilibration was due to heating of the system from 0 to 300 K. This initial RMSD increase was reduced for equilibration at 330 K because the starting model for this process was the final structure resulting from the equilibration at 300 K.

At both temperatures, the system seemed to reach equilibrium prior to the last 1.0 ns of the simulation, for which the trajectories were analysed. The stability of the trajectories in the simulations can also be judged by calculating the radius of gyration. Average radii of gyration during simulations at 300 and 330 K were both 16.8 Å with standard deviations well below 0.1 Å. This suggests that the overall dimension of the AKpsychro structure was stable during simulation. The geometry of the zinc and magnesium binding sites was also well preserved during simulations indicating that the configurations around these sites were fairly accurate.

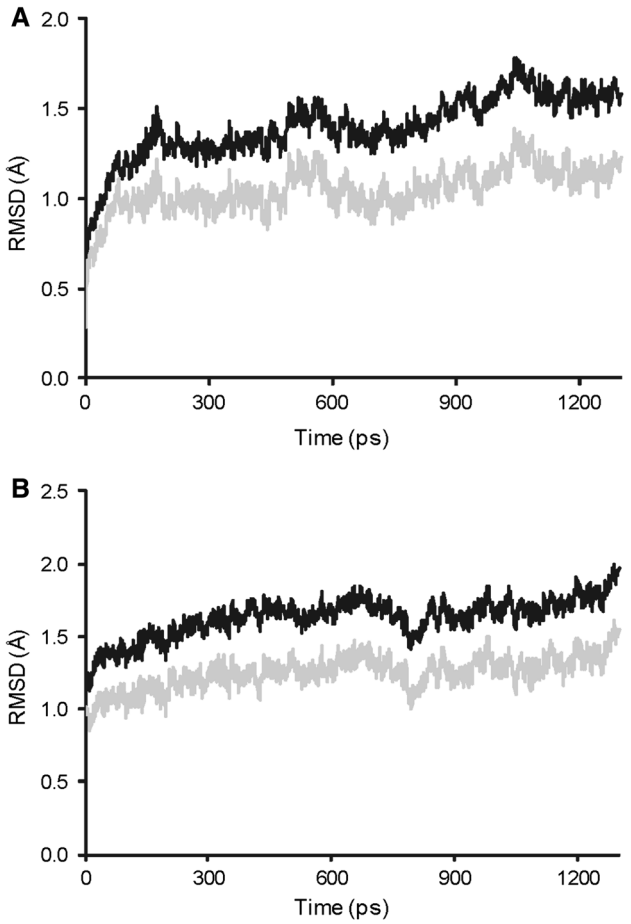

Fig. 2 RMSD values of AKpsychro structure during MD simulations. Total and backbone RMSDs from the final minimised structure are shown in *black* and *grey*, respectively, for MD simulations at 300 K (**a**) and 330 K (**b**)

Flexibilities of AKpsychro during the MD simulations

To estimate overall flexibility during MD simulations, root mean square fluctuation (RMSF) was calculated for the simulated AKpsychro structure. The RMSF was defined as the RMSD between each instantaneous structure saved at each 500 step interval during simulation and a reference structure that was obtained by aligning and averaging all of the instantaneous structures. Thus, this average RMSF is a measure of a protein's overall flexibility during the simulation. With the AKpsychro simulations performed in this study, the calculated average total and backbone RMSF values are listed in Table 1 alongside those from previous MD simulations with AKmeso and AKthermo (Bae and Phillips 2005).

The simulation of AKpsychro at 330 K resulted in higher average RMSF values for the total and backbone atoms than did the simulation performed at 300 K, indicating that AKpsychro is more flexible at higher temperatures. At either temperature, the average backbone RMSF value of AKpsychro was smaller than those computed for the total atoms, which confirms the relative rigidity of the backbone atoms. The same trend, increased flexibility at higher temperatures and less flexible backbone atoms, was observed previously in the simulations with AKmeso and AKthermo (Table 1) (Bae and Phillips 2005).

Discussion

It has been suggested that the overall flexibility and operating temperature of proteins are closely related. This 'corresponding state' hypothesis was first proposed by Somero and postulates that homologous proteins display similar dynamic properties at their physiologically relevant temperatures where they exhibit their optimal catalytic activities (Somero 1978). Based on this hypothesis, psychrophilic proteins are expected to be more flexible than their mesophilic and thermophilic homologues at the same temperature. However, the comparison of the average RMSF values between AK homologues in the present study reveals unexpected results regarding flexibility.

Table 1 Average RMSF values (Å) of AKpsychro and its homologues in the MD simulations

	AKpsychro		AKmeso[a]		AKthermo[a]	
	300 K	330 K	300 K	330 K	300 K	330 K
Total	0.84	0.91	0.83	0.99	0.84	0.95
Backbone	0.66	0.68	0.63	0.75	0.63	0.71

[a] From a previous study (Bae and Phillips 2005)

In the MD simulations, AKpsychro did not show a clear increase in overall flexibility relative to AKmeso and AKthermo (Table 1). In simulations at 300 K, the three AK homologues displayed very similar total RMSF values. For the backbone atoms, the RMSF of AKpsychro was slightly higher than those of AKmeso and AKthermo. In the simulations at 330 K, both total and backbone RMSF values of AKpsychro were lower than those of the mesophilic and thermophilic AKs, indicating that AKpsychro was less flexible than AKmeso and AKthermo in simulations at the higher temperature. Therefore, no definitive correlations were identified between the overall flexibility as calculated from MD simulations and experimentally determined temperature-related properties such as operating temperature and T_m value.

These unexpected results can be attributed to several reasons. The 1-ns simulation time may not be sufficient, and longer timescales such as μs and ms might be more appropriate to describe the overall flexibility of the proteins. In addition, the force field used for the simulations may not be sufficiently accurate for a relative comparison of flexibility between homologous proteins that are similar in both sequence and structure. In fact, several previous MD simulations, in which homologous proteins were compared with respect to optimal temperature and overall flexibility, did not show a clear correlation between flexibility and temperature-related properties (Grottesi et al. 2002; Wintrode et al. 2003; Papaleo et al. 2008; Kundu and Roy 2009).

It is also possible that overall flexibility may not be tightly related to the temperature preference in some proteins. Functionally, important dynamic motions can be local and uncoupled from overall flexibility or stability (Haney et al. 1999; Bae and Phillips 2006), and adaptation to extreme temperatures may require modulating flexibility only in specific area(s) of the protein (Feller 2013). Consequently, it is still unclear whether there is a direct connection between the overall flexibility and the operating temperature of a given protein. Additional experimental and/or computational studies are needed to obtain more conclusive results.

Acknowledgments This work was supported by the Seoul National University Research Grant and the Cooperative Research Programme for Agricultural Science & Technology Development funded by Rural Development Administration (PJ009781).

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