Journal of Theoretical Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Theoretical Biology

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

Protein cold adaptation: Role of physico-chemical parameters in adaptation of proteins to low temperatures

Soheila Shokrollahzade [a], Fatemeh Sharifi [b], Akbar Vaseghi [c], Maryam Faridounnia [d], Samad Jahandideh [e,*]

[a] Department of Medicinal Biotechnology, Iran University of Medical Sciences, Tehran, Iran
[b] Department of Computer Science, Shahid Beheshti University, G.C., Tehran, Iran
[c] Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[d] Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands
[e] Bioinformatics and Systems Biology Program, Sanford-Burnham Medical Research Institute, 10901 N Torrey Pines Rd, La Jolla, CA 92307, United States

HIGHLIGHTS

- Psychrophilicity rules are not merely the inverse rules of thermostability.
- Psychrophilic/mesophilic protein pairs are different in terms of physico-chemical properties.
- Contact orders of psychrophilic/mesophilic protein pairs are not different.

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form
21 June 2015
Accepted 16 July 2015

Keywords:
Structural features
Thermostability
Systematics analysis
Packing density
Surface accessibility

ABSTRACT

During years 2007 and 2008, we published three papers (Jahandideh, 2007a, JTB, 246, 159-166; Jahandideh, 2007b, JTB, 248, 721-726; Jahandideh, 2008, JTB, 255, 113-118) investigating sequence and structural parameters in adaptation of proteins to low temperatures. Our studies revealed important features in cold-adaptation of proteins. Here, we calculate values of a new set of physico-chemical parameters and perform a comparative systematic analysis on a more comprehensive database of psychrophilic–mesophilic homologous protein pairs. Our obtained results confirm that psychrophilicity rules are not merely the inverse rules of thermostability; for instance, although contact order is reported as a key feature in thermostability, our results have shown no significant difference between contact orders of psychrophilic proteins compared to mesophilic proteins. We are optimistic that these findings would help future efforts to propose a strategy for designing cold-adapted proteins.

1. Introduction

Living organisms, based on the temperature they endure, are categorized into psychrophiles, mesophiles and thermophiles. Psychrophiles and thermophiles are those that are adapted to regions with extreme temperature, where only few organisms could survive (Baldasseroni and Pascarella, 2009). By using physiological strategies, psychrophiles grow in 5 °C in the depth of oceans, in glaciers and mountains, and maintain their own function in these temperatures (Gianese et al., 2002). Near three-quarters of the Earth's surface is owned by cold regions (Feller, 2013). Psychrophilic proteins are found in prokaryotic and eukaryotic organisms including bacteria, archaea, algae, and yeasts as well as glaciers ice worms, plants and animals (Buzzini et al., 2012; Cameron et al., 2012; Cavicchioli, 2006). From the first time in 1975 that Morita introduced psychrophilic bacteria, molecular bases of cold adaptation have attracted the interest of researchers (Cipolla et al., 2012; Morita, 1975).

From the industrial and biotechnological point of view, psychrophilic proteins are endowed with many considerable merits, which make them applicable. Psychrophilic proteins save energy due to decrease in the free energy of activation $\Delta G^{\#}$. Consequently, less energy is required to inactivate a psychrophilic enzyme and therefore it can increase the reaction speed (Baldasseroni and Pascarella, 2009; D'Amico et al., 2002). This makes psychrophilic proteins economically beneficial for the processes in which they are involved. Examples of such industries include detergent, food, textile manufacturing, pharmaceutical, biofuels, and energy production (Feller, 2013; Jahandideh et al., 2007a).

Structural and sequential properties of cold-adapted proteins are subjected to many studies either as small datasets of individual

* Corresponding author.
E-mail address: sjahandideh@burnham.org (S. Jahandideh).

http://dx.doi.org/10.1016/j.jtbi.2015.07.013
0022-5193/

proteins or large ones such as genomics, metagenomics and proteomics studies (Goodchild et al., 2004; Grzymski et al., 2006; Lauro et al., 2009; Metpally and Reddy, 2009). To address some of those important studies, comparative genomics studies for amino acid composition in some cold adapted organisms have shown higher content of noncharged polar amino acids and a lower content of hydrophobic amino acids as well as more propensity in solvent accessible area for some hydrophobic residues (Casanueva et al., 2010; Saunders et al., 2003). In addition, many researches have reported the difference in occurrence of some amino acid substitutions in psychrophilic proteins as a likely adaptation strategy by psychrophiles (Jahandideh et al., 2008; Methe et al., 2005). As for structural characterization of psychrophilic proteins and their pertinent adaptation process, cavities and clefts are indispensable factors to consider in cold-adaptation (Paredes et al., 2011). Studies in cavities, clefts and void volumes by many methods have suggested larger cavities in psychrophilic proteins (Collins et al., 2002; Feller, 2013; Liang et al., 1998; Paredes et al., 2011).

Up to now, many efforts have been done by previous investigators in using physico-chemical parameters and properties although for different biomacromolecules and purposes (Chen et al., 2013, 2014; Feng et al., 2013; Guo et al., 2014; Kandaswamy et al., 2011; Lin et al., 2014; Liu et al., 2015a, 2015b; Qiu and Xiao, 2014; Xiao and Wang, 2012, 2013; Xiao and Lin, 2013; Xiao et al., 2013a, 2013b; Wu and Xiao, 2010). In this work, we have investigated physico-chemical properties of mesophilic and psychrophilic protein pairs. For this purpose, we have created a dataset of 30 pairs of mesophilic and psychrophilic proteins. To investigate the differences, we compared several structural properties; molecular weight (MW) per residues, radius of gyration (ROG), hydrogen bond (HB), packing density (PD), accessible surface area (ASA), buried surface area (BSA), and number of cavities. These global parameters are assigned to general structures of psychrophilic/mesophilic pairs, while in our previous studies, local parameters attributed to specific locations of proteins have been discussed (Jahandideh et al., 2007a, 2007b, 2008). The results suggest some possible general rules for protein design experiments aimed to produce enzymes catalytically more effective at low temperatures.

2. Materials and methods

2.1. Database

We followed some steps to provide a psychrophilic–meshophilic homologus database of proteins. Initially, keywords concerning cold adaptation were used to search for psychrophilic proteins in protein data bank (PDB) (Berman et al., 2000). To ensure that psychrophilic proteins are correctly chosen, related research articles reporting the structures were reviewed. Then, non-wild-type proteins, i.e. mutant proteins, and those composed of abnormal residues, such as Asx or Glx, were eliminated from the database. This provided us with a list of 30 mesophilic proteins. Finally, protein–protein BLAST (blastp) was used, by entering each single psychrophilic protein's PDB ID as a query, to find its best homologous mesophilic protein pair with more than 25% identity. Therefore, in the blast result, subjects with the most identity were selected and their accession number was checked in protein data bank. To make sure the selected protein is mesophilic, molecular description including its classification, organism as well as habitat was checked. All of the selected homologous proteins were in the same protein and enzyme classification as their psychrophilic homologs. The database contains 30 pairs of psychrophilic–meshophilic homologous proteins, all of which are X-ray structures determined with a resolution of at least 3 Å. PDB IDs, species and resolution of these protein structures are shown in Table 1.

2.2. Data analysis

2.2.1. Packing density

One of the structural features denoting the intrinsic compressibility of proteins is *packing density* (PD). Packing density provides information associated with stability and flexibility of proteins. A distinct example of this correlation is hydrophobic interactions, which, by bunching hydrophobic residues tightly together, make the protein more thermostable (Criswell et al., 2003).

Structural analysis has shown that adaptation of hyper-thermophiles is strongly related to increase in *packing density* in the surface of protein due to decrease in the number of cavities (Baldasseroni and Pascarella, 2009). Pack et al. have shown different packing density in exposed surface of thermophilic proteins (Pack and Yoo, 2005). To address the question of whether or not the differences in cold adaptation of psychrophilic proteins is due to the *packing density*, we calculated *packing density* for each particular pair of PDB structures by Voronoia software. Voronoia calculate radius of atoms for amino acids of intended proteins. The atomic packing density is defined as

$$\mathrm{PD} = \frac{V(\mathrm{VdW})}{[V(\mathrm{VdW}) + V(\mathrm{SE})]}$$

where V(Vdw) is volume of van der Waals radius and V(SE) is surface excluded volume voronoia attribute van der Waals radii for neighboring atoms in the proximity of a specific residue. It then splits the solvent excluded protein by a polyhedron space, called voronoia volume (Goede et al., 1997).

2.2.2. Cavity

A cavity is often a buried region in protein without opening to outside (Fig. 1). It acts as ligand-binding site or any putative internal water and plays important roles in binding and flexibility of proteins (Liang et al., 1998). Some studies have reported larger cavities in psychrophilic proteins. Larger cavities, by adopting more water molecules within themselves, make it possible to act in low temperature (Paredes et al., 2011). Solvent probes with often a specific size are used to measure cavity volume and once the cavity is filled the probe cannot measure the cavity. In addition to volume of cavities, numbers of cavities in proteins are important. We used the web interface of Voronoia program to examine cavity volumes for each pair of proteins. This program (available at http://bioinformatics.charite.de/voronoia/) uses a 1.4 Å solvent probe to examine cavities (Goede et al., 1997).

2.2.3. Surface area

The total surface area of a biomolecule that is approachable by a solvent is called accessible surface area (ASA). In 1971, it was introduced as the area of the region bounded by tracing the locus of solvent probe, as it is rolling over the van der Waals surface (Fig. 1) by Lee and Richard introduced this concept as the area of the region bounded by tracing the locus of solvent probe, as it is rolling over the van der Waals surface (Fig. 1) (Lee and Richards, 1971). This boundary is somehow equivalent to extending the surfaces of atoms with a radius equivalent to solvent probe radius. These expanded spheres might intersect; the surface exterior to the union of these overlapping spheres is considered (Richmond, 1984). Shrake–Rupley algorithm, a common and simple algorithm, uses a numerical method, and a solvent probe radius of 1.4 Å to calculate ASA. Approximately equal to the radius of water molecule, 1.4 Å is a typical value for probe radius, however, different algorithms may consider different probe radii (Shrake and Rupley, 1973).

ASA values impact many characteristics of a protein, e.g. thermostability and flexibility. Therefore, they have been used in different articles to facilitate the process of solving hydrophobicity effect problem, and to estimate free energy, heat capacity of hydration, and enthalpy (Marsh, 2013a, 2013b; Ooi et al., 1987; Richmond, 1984; Stellwagen and Wilgus, 1978). By using PDBePISA online server

Please cite this article as: Shokrollahzade, S., et al., Protein cold adaptation: Role of physico-chemical parameters in adaptation of proteins to low temperatures. J. Theor. Biol. (2015), http://dx.doi.org/10.1016/j.jtbi.2015.07.013