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# Importance of main-chain hydrophobic free energy to the stability of thermophilic proteins

K. Saraboji[a], M. Michael Gromiha[b], M.N. Ponnuswamy[a,*]

[a] *Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai 600025, India*
[b] *Computational Biology Research Center (CBRC), National Institute of Advanced Industrial Science and Technology (AIST), AIST Tokyo Waterfront Bio-IT Research Building, 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan*

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## Abstract

Living organisms are found in the most unexpected places, including deep-sea vents at 100 °C and several hundred bars pressure, in hot springs. Needless to say, the proteins found in thermophilic species are much more stable than their mesophilic counterparts. There are no obvious reasons to say that one would be more stable than others. Even examination of the amino acids and comparison of structural features of thermophiles with mesophilies cannot bring satisfactory explanation for the thermal stability of such proteins. In order to bring out the hidden information behind the thermal stabilization of such proteins in terms of energy factors and their combinations, analysis were made on good resolution structures of thermophilic and their mesophilic homologous from 23 different families. From the structural coordinates, free energy contributions due to hydrophobic, electrostatic, hydrogen bonding, disulfide bonding and van der Waals interactions are computed. In this analysis, a vast majority of thermophilic proteins adopt slightly lower free energy contribution in each energy terms than its mesophilic counterparts. The major observation noted from this study is the lower hydrophobic free energy contribution due to carbon atoms and main-chain nitrogen atoms in all the thermophilic proteins. The possible combination of different free energy terms shows majority of the thermophilic proteins have lower free energy strategy than their mesophilic homologous. The derived results show that the hydrophobic free energy due to carbon and nitrogen atoms and such combinations of free energy components play a vital role in the thermostablisation of such proteins.

*Keywords:* Protein stability; Hydrophobic free energy; Three-dimensional structure

## 1. Introduction

Several organisms, mainly archaea live under extreme environmental temperature conditions. Proteins from thermophilic organisms usually exhibit substantially higher intrinsic thermal stabilities than their counter parts from mesophilic organisms. Identifying and understanding the factors contributing to the stability of proteins from organisms living under extreme conditions stand out to be a long-standing problem. Although the molecular bases of protein thermostablisation have been the focus of many theoretical and experimental research efforts, this subject is only partially understood. Studies of thermostability can be divided into three categories: (i) by examining a single thermophilic protein and comparing its structure at atomic level with one or more mesophilic homologues, (ii) systematic approach on the analysis based on sequence and structural information for a group of proteins in order to reach general conclusions and (iii) large scale comparison between thermophilic and mesophilic genome sequences. A number of examples can be quoted for the comparison of structures of mesophilic homologues but systematic studies are very limited. The recent progress in genome sequence projects enables one to make a comparative study of these thermophilic and mesophilic organisms [1].

There has been a growing interest in understanding the mechanism of stabilization of thermophilic proteins from these organisms. Understanding the physiochemical principles of thermostability will, no doubt, aid in the comprehension of protein folding and protein interaction mechanisms. Theoretical and experimental approaches

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* Corresponding author. Tel.: +91 44 22351367; fax: +91 44 22300122.
*E-mail address:* mnpsy2004@yahoo.com (M.N. Ponnuswamy).

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have been undertaken to examine the stability of proteins. Comparison of the sequences and tertiary structures of homologous proteins from thermophiles, mesophilies and thermophobes has formed the basis of theoretical efforts [2,3]. Indeed, one review revealed many different physical and chemical reasons such as hydrogen bonding, hydrophobic packing, secondary structure propensity and helix dipole stabilization wherein the researchers reported the enhanced thermostablisation [4].

In recent years, several works have been carried out theoretically and experimentally to trace the secrets of thermostablisation through mutation studies [5–9] and also based on the analysis of amino acid composition. Fukuchi and Nishikawa [10] showed the amino acid composition on protein surface and interior of thermophilic and mesophilic bacteria. They observed the reduction in the number of charged residues and rich in polar residues in mesophilic bacteria and concluded that the bias of amino acid composition of thermophilic protein is due to the residues on protein surfaces, which may be due to extreme environment. Akke and Forsen [11] showed that the electrostatic interactions between charges on the surface of a protein could have significant effects on protein stability.

With regard to helix stabilizing factors and stabilization of thermophilic proteins, Facchiano et al. [12] made the analysis on 13 thermophilic proteins and showed that the helices of thermophilic proteins are more stable than mesophilic homologues. Gromiha et al. [13] studied the relationship between stability changes caused by buried mutations and changes in 48 amino acid properties; this provides the correlation of hydrophobicity with the stability of proteins.

The intramolecular interactions, namely hydrophobic, electrostatic, van der Waals and hydrogen bonds play an important role in the stability of protein structures [14–16,24]. Several investigations have been carried out to understand the mechanism for the thermostability of proteins. Gromiha et al. [17] made a comparative analysis on the relation between thermostability and amino acid properties for a family of meso and thermophilic proteins wherein the Gibbs free energy change of hydration and shape play a dominant role in thermostability of proteins. The mutational study by Hasegawa et al. [18] agreed with the results of Gromiha et al.; they analysed the increased stability of mesophilic cytochrome c through five substitutions and observed that the $-G_{\mathrm{hN}}$ may contribute to the stability.

Szilagyi and Zavodsky [19] made a systematic study on 25 protein families consisting of 64 mesophilic and 29 thermophilic proteins and concluded that different protein families adapt to higher temperatures utilizing different sets of structural devices and the number of ion pairs increased with the increase in growth temperature. Querol et al. [4] found the relationship between thermal stability and conformational characteristics of proteins. The thermostability of 16 different families of mesophilic and thermophilic proteins has been examined by Vogt et al. [20] and a good correlation evinced between the thermostability of the familial members and the number of hydrogen bonds, as well in the fractional polar surface. The statistical analysis on 18 families of thermophilic and mesophilic proteins by Kumar et al. [21] showed the increase of the salt bridges and side-chain–side-chain hydrogen bonds in majority of the thermophilic proteins; the occurrence of residues Arg and Tyr are more frequent in thermophilic proteins. Kumar and Nussinov [22] made the analysis on fluctuations, ion pair contributions and stabilities in NMR conformer ensembles and found that the overall stabilizing contribution of ion pair is conformer population dependent. Recently, Gromiha [23] analyzed the medium and long-range contacts in mesophilic and thermophilic proteins of 16 different families and explained the fact that thermophiles prefer to have contacts between residues through hydrogen bonds; apart from hydrophobic contacts and also between polar and non-polar residues in thermophiles than mesophilies. Ponnuswamy and Gromiha [24] made the investigations on the conformational stability of folded proteins where the hydrophobic force drives the polypeptide chain to the folded state overcoming the entropic factor, while the other factors, especially hydrogen bonds and van del Waals attraction, define the shape and keep it from falling apart.

Recently, Yano and Poulos [25] compiled the factors that are reported to be important for increased protein stability. It has been mentioned that electrostatic interactions, cation–pi interactions, aromatic and hydrophobic interactions and other factors would enhance the stability [26,27]. From this diverse collection of studies, it is difficult to come to a general conclusion about the structural features underlying the increased thermal stability of proteins from thermophilic microorganism. The contradictions and the limited understanding are the consequences of the limited data available and the non-uniform approach of the contributing researchers. Though the proteins can be engineered or engineer themselves in vivo to achieve greater stability by utilizing one or more of these strategies, it is clear that no single and preferred mode has yet to be established.

The aim of present work is to combine the different free energy components of a set of thermophilic and mesophilic proteins to assess the contributions from different stability factors into a unified model. We compute the major free energy components of hydrophobic, electrostatic, hydrogen bonding, van der Waals and disulfide bonding interactions of the folded state of proteins, and also the conformational entropy of the unfolded state of the corresponding proteins. Here an in depth statistical analysis of parameters was made and investigated the importance of each interaction towards protein thermostability.

## 2. Materials and methods

### 2.1. Data set

Recently, Kumar et al. [21] constructed the data set of 36 thermophilic and mesophilic proteins from 18 different