

How Do Thermophilic Proteins Deal With Heat?

Sandeep Kumar* and Ruth Nussinov††

*LECB, NCI-Frederick, Frederick, MD 21702

†IRSP, SAIC Frederick, NCI-Frederick, Frederick, MD 21702

††Sackler Institute of Molecular Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Abstract

We have compared macroscopic thermodynamic properties of 19 two-state folders in five families containing homologous thermophilic and mesophilic proteins. The stability curves of the thermophilic proteins are up-shifted and broader as compared to those of the mesophilic proteins. Thermophilic proteins have greater maximal stabilities as compared to their mesophilic homologues. The thermophilic proteins achieve greater thermodynamic stability by forming additional specific interactions. Various sequence and structural properties were compared in 18 families containing homologous thermophilic and mesophilic proteins. Increased occurrence of salt bridges and side chain side chain hydrogen bonds is the most consistent trend shown by the thermophilic proteins. Salt bridges and their networks in *Pyrococcus furiosus* glutamate dehydrogenase have highly stabilizing electrostatic free energy contributions. These observations provide a better understanding of the molecular basis of protein thermostability.

Introduction

Thermodynamic stability of a protein varies with temperature. For a protein which follows simple two-state folding process (Native (N) \leftrightarrow Denatured (D)), is stable over a certain temperature range and has constant (> 0) heat capacity change (ΔC_p) in this range, the Gibbs-Helmholtz equation can be used to plot its stability curve:

$$\Delta G(T) = \Delta H_c (1-T/T_c) - \Delta C_p [(T_c - T) + T \ln (T/T_c)]$$

Where, $\Delta G(T)$ is Gibbs free energy change between folded (N) and unfolded (D) states of the protein. ΔH_c is enthalpy change at melting temperature (T_c). Shape of the protein stability curve is skewed parabola. Spectroscopic (CD and fluorescence) and differential scanning calorimetry (DSC) experiments can be used to study protein thermal denaturation and determine the values of these parameters. Here, we compare thermophilic and mesophilic protein stability curves and interpret differences in terms of microscopic properties of the proteins.

Results and Discussion

Figure 1 compares protein stability curves for 19 thermophilic and mesophilic proteins in five families. The stability curves of the thermophilic proteins are up-shifted and broader than the stability curves of the mesophilic proteins. In case of cold shock protein from *Thermotoga maritima*, the protein stability curve is both up- and right-shifted. From protein stability curve of a protein, one can calculate its maximal stability ($\Delta G(T_s)$). Thermophilic proteins have higher maximal stabilities than the mesophilic proteins. Figure 2 shows that (i) maximal protein stability ($\Delta G(T_s)$) is correlated with melting temperature, T_c , (ii) enthalpy change at melting temperature (ΔH_c) is correlated with maximal protein stability and (iii) h_c (residue specific enthalpy change at T_c) is correlated with the melting temperature. These observations indicate that formation of specific interactions may be responsible for greater stability for the thermophilic proteins.

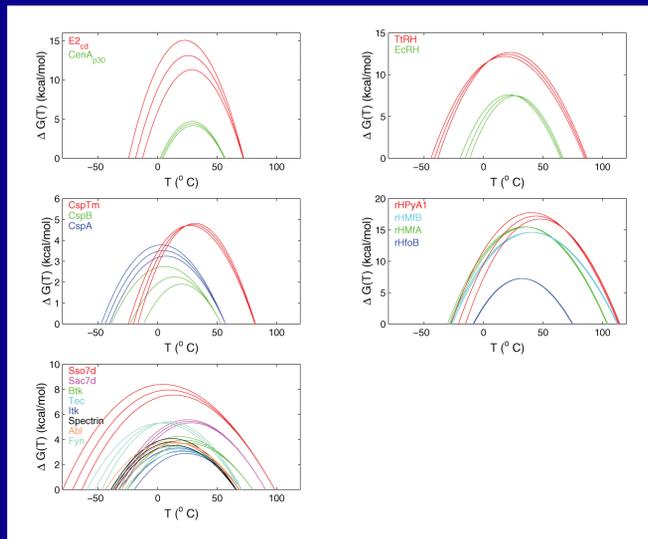


Figure 1. Protein stability curves for homologous thermophilic and mesophilic proteins in five families. These curves are plotted using Gibbs-Helmholtz equation.

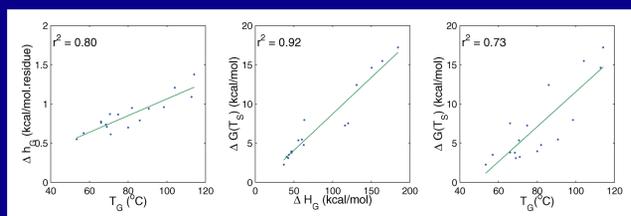


Figure 2. Plots showing linear correlation between residue specific enthalpy change (Δh_c) and melting temperature (T_c), maximal protein stability ($\Delta G(T_s)$) and enthalpy change at melting temperature (ΔH_c), and maximal protein stability ($\Delta G(T_s)$) and melting temperature (T_c). The observations indicate the role of additional specific interactions in greater thermodynamic stability of the thermophilic proteins.

The nature of the specific interactions can be gleaned from microscopic sequence and structural comparison of homologous thermophilic and mesophilic proteins. Recently, we have analyzed sequence composition, sequence insertion and deletions, proline substitution in loops, α -helical content, α -helix geometry, hydrophobicity, compactness, polar and nonpolar surface areas buried and exposed to water, oligomerization, hydrogen bonds and salt bridges in 18 non-redundant families of homologous thermophilic and mesophilic proteins. Among these, close range electrostatic interactions such as side chain side chain hydrogen bonds and salt bridges show the most consistent trend. These interactions increase in the majority of thermophilic proteins both within the sub-units and at the interfaces (Figure 3).

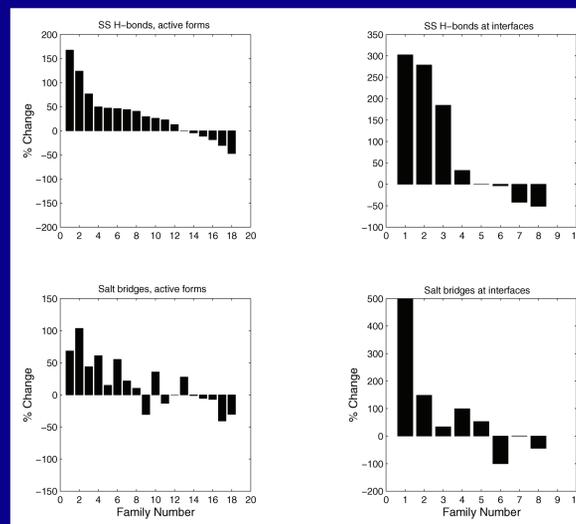


Figure 3. Bar diagrams showing the changes in side chain side chain hydrogen bonds and salt bridges between homologous thermophilic and mesophilic proteins both within and across sub-units.

One of the 18 families in this study contains homologous Glutamate dehydrogenases from *Pyrococcus furiosus* (PfGDH) and *Clostridium symbiosum* (CsGDH). PfGDH and CsGDH share 34% sequence identity but show ~ 60 difference in their melting temperatures. Figures 4 and 5 show the location of charged residues that form salt bridges in PfGDH and CsGDH respectively. Increased occurrence of salt bridges and their networks is responsible for greater thermostability of PfGDH. Using a continuum electrostatics based methodology, we have computed electrostatic strengths of 29 salt bridges within a PfGDH monomer and 17 salt bridges in the corresponding CsGDH monomer. Our results indicate that the salt bridges and their networks in PfGDH are highly stabilizing, while those in CsGDH are only marginally stabilizing.

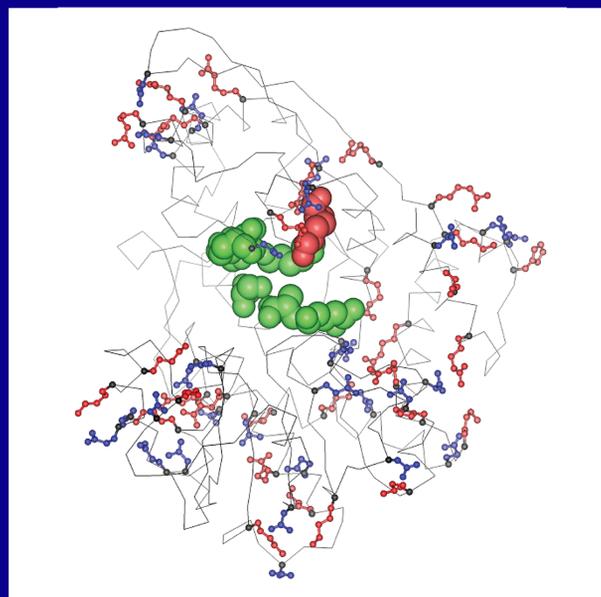


Figure 4. Location of charged residues forming salt bridges and their networks in a monomer of *Pyrococcus furiosus* glutamate dehydrogenase. Active site residues are shown in CPK and the charged residues are shown in ball and sticks.

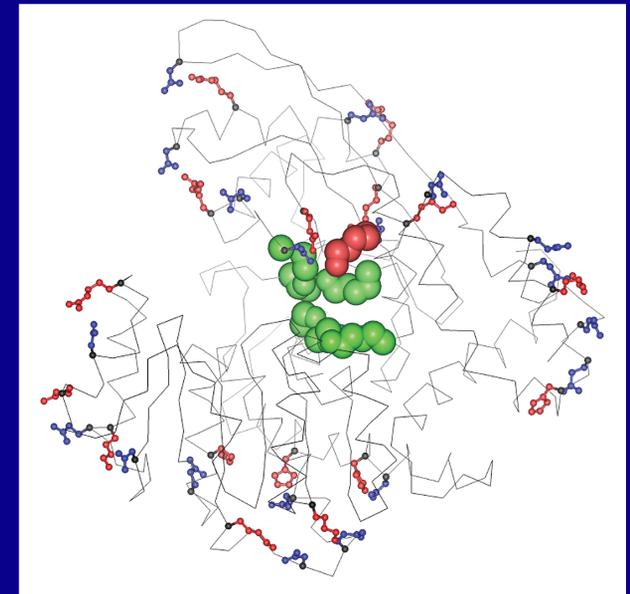


Figure 5. Location of charged residues forming salt bridges and their networks in a monomer of *Clostridium symbiosum* glutamate dehydrogenase. Active site residues are shown in CPK and the charged residues are shown in ball and sticks.

Salt bridges may be stabilizing or destabilizing towards the protein. We have carried out a statistical analysis of salt bridges and their electrostatic strengths in a database of 222 salt bridges from 36 non-homologous protein monomers with high resolution (1.6 Å or better) crystal structures. Most ($\approx 86\%$) of the salt bridges have stabilizing electrostatic free energy contributions towards protein stability. Overall electrostatic strength of a salt bridge depends upon its location in the protein globule and geometrical orientation of the side chain charged groups with respect to each other as well as with respect to the other changes in rest of the protein.

Conclusions

The goal of the studies presented here was to correlate both macroscopic (thermodynamic) and microscopic (sequence and structural) differences among thermophilic and mesophilic proteins. The analysis of macroscopic parameters shows that thermophilic proteins have greater maximal protein stabilities as compared to their mesophilic homologues. The thermophilic proteins acquire higher thermodynamic stabilities by increasing the enthalpic contributions. This indicates formation of additional specific, such as close range electrostatic, interactions in the thermophilic proteins. Comparison of sequence as well as structural properties in the families of homologous thermophilic and mesophilic proteins indicates that close range electrostatic interactions are the most consistent factors enhancing protein thermostability. Increased occurrence of charged residues, formation of salt bridges and co-operative effect of the formation of their networks results in a more favorable electrostatic environment in the thermophilic proteins as compared to their mesophilic homologues.

References

- I. Kumar, S., Tsai, C. J. and Nussinov, R. (2000). Factors enhancing protein thermostability. *Protein Engineering*, 3, 179 – 191.
- II. Kumar, S., Ma, B., Tsai, C. J., and Nussinov, R. (2000). Electrostatic strengths of salt bridges in thermophilic and mesophilic glutamate dehydrogenase monomers. *Proteins: Structure, Function and Genetics*, 38, 368 – 383.
- III. Kumar, S. and Nussinov, R. (1999). Salt bridge stability in monomeric proteins. *Journal of Molecular Biology*, 293, 1241 – 1255.
- IV. Kumar, S., Tsai, C. J. and Nussinov, R. (2001). Factors enhancing protein thermostability. II. Analysis of thermodynamic parameters and microscopic interpretations. Submitted.
- V. Kumar, S. and Nussinov, R. (2001). How do thermophilic proteins deal with heat? *Cellular and Molecular Life Sciences*, in press.

Reprints of these papers are available at www-lecb.ncifcrf.gov/~kumarsan.