
FOR THE RECORD

Intrinsic contributions of polar amino acid residues toward thermal stability of an ABC-ATPase of mesophilic origin

JYOTI SARIN, GAJENDRA P.S. RAGHAVA, AND PRADIP K. CHAKRABORTI

Institute of Microbial Technology, Chandigarh 160 036, India

(RECEIVED March 23, 2003; FINAL REVISION May 13, 2003; ACCEPTED May 16, 2003)

Abstract

The nucleotide-binding subunit of phosphate-specific transporter (PstB) from mesophilic bacterium, *Mycobacterium tuberculosis*, is a unique ATP-binding cassette (ABC) ATPase because of its unusual ability to hydrolyze ATP at high temperature. In an attempt to define the basis of thermostability, we took a theoretical approach and compared amino acid composition of this protein to that of other PstBs from available bacterial genomes. Interestingly, based on the content of polar amino acids, this protein clustered with the thermophiles.

Keywords: ATP-binding cassette ATPase; polar amino acid; PstB; thermostability

Supplemental material: See www.proteinscience.org.

Proteins from thermophilic organisms usually exhibit intrinsic stability to high temperatures compared with that of their mesophilic counterparts. Interestingly, irrespective of the temperature at which organisms live, the sequence of any protein is derived from the same 20 essential amino acids. Therefore, in this postgenomic era, the comparison of sequences and structures of homologs from thermophiles, as well as from mesophiles, has formed the basis of theoretical efforts to elucidate the mechanisms underlying the thermostability (Gromiha et al. 1999; Szilagy and Zavodsky 2000; Fukuchi and Nishikawa 2001). Although complex factors governing protein thermostability has become a tenet for intensive investigations (Chakrabarty and Varadarajan 2000; Kumar and Nussinov 2001; Kumar et al. 2001), this aspect is only partially understood.

We have recently reported that the B-subunit of the phosphate-specific transporter (PstB) from *Mycobacterium tuberculosis* is a thermostable ATPase (Sarin et al. 2001).

Because a mesophilic bacteria contains a thermostable protein, we thought this will lend us an opportunity to define the basis of unusual stability of this ATP-binding cassette (ABC) ATPase to high temperatures. Furthermore, this approach has novelty in the sense that the theoretical approaches to resolve this issue were confined to sequence comparison between the homologs from mesophiles and thermophiles only.

BLAST search (Altschul et al. 1997) using *M. tuberculosis* protein as a search probe in nonredundant protein sequence databases exhibited significant homology with different PstB and ABC-ATPases (expect value, e^{-144} to $6e^{-20}$). Among them, the first 39 hits were different bacterial PstBs of either mesophilic (31 sequences) or thermophilic (8 sequences) origin (least expect value, e^{-48}). All of them, except hypothetical/probable/putative sequences (eight mesophilic and two thermophilic), were considered for further analysis.

Multiple sequence alignments (see Supplemental Material) with these retrieved PstB sequences, using the CLUSTAL W 1.74 program (Thompson et al. 1994), revealed the presence of highly conserved nucleotide-binding motifs, NB1 and NB2, which is a characteristic feature of all ABC transporters (Higgins 1992). We further constructed a rooted phylogenetic tree from the multiple sequence align-

Reprint requests to: Pradip K. Chakrabarti, Institute of Microbial Technology, Sector 39A, Chandigarh 160 036, India; e-mail: pradip@imtech.res.in; fax: 91-172-690585.

Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.0397603>.

ment data by using the CLUSTAL W program (random seed number, 111; bootstrap value, 1000). The *M. tuberculosis* protein showed a clustering with PstBs of mesophilic bacteria, such as *M. intracellulare*, *Mesorhizobium loti*, and *Xylella fastidiosa* (Fig. 1). Interestingly, the protein from the thermophiles did not show any distinct pattern; rather they were randomly distributed throughout the phylogram (Fig. 1). Furthermore, this analysis did not seem to be an artifact in sequence alignment or in phylogenetic tree construction (Fig. 1; Supplemental Material). Conversely, based on simple statistical tests with stabilizing factors (e.g., protein size, number of residues involved in hydrogen bonding, β -strand content, helix stabilization through ion pairs, relative amount of hydrophobic β -branched amino acids) and secondary structure analysis, the *M. tuberculosis* PstB protein confirmed many of the properties characteristic to thermostable proteins (data not shown).

To resolve this ambiguity, we analyzed the amino acid composition, which is known to be one of the important

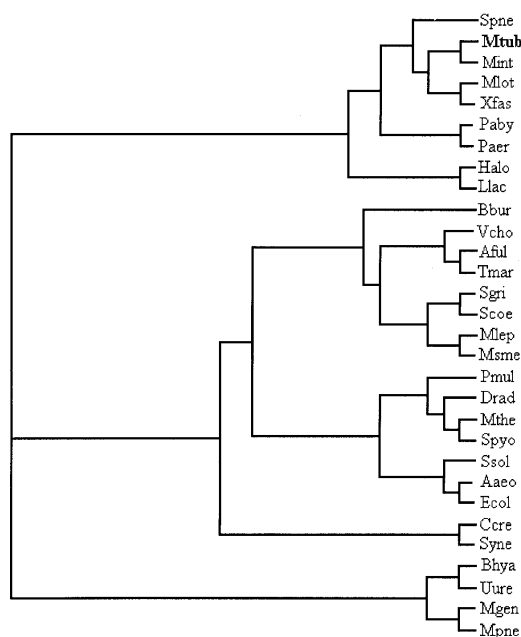


Figure 1. Phylogenetic placement of *M. tuberculosis* PstB. Aaeo indicates *Aquifex aeolicus*; Aful, *Archaeoglobus fulgidus*; Bbur, *Borellia burgdorferi*; Bhya, *Bacillus hyalodurans*; Ccre, *Caulobacter crescentus*; Drad, *Deinococcus radiodurans*; Ecol, *Escherichia coli*; Halo, *Halobacterium* sp.; Llac, *Lactococcus lactis*; Mlot, *Mesorhizobium loti*; Mthe, *Methanobacterium thermoautotrophicus*; Mint, *Mycobacterium intracellulare*; Mlep, *Mycobacterium leprae*; Msme, *Mycobacterium smegmatis*; Mtub, *Mycobacterium tuberculosis*; Mgen, *Mycoplasma genitalium*; Mpne, *Mycoplasma pneumoniae*; Paby, *Pyrococcus abyssi*; Paer, *Pseudomonas aeruginosa*; Pmul, *Pasteurella multocida*; Spne, *Streptococcus pneumoniae*; Spyo, *Streptococcus pyogenes*; Ssol, *Sulfolobus solfataricus*; Scoe, *Streptomyces coelicolor*; Sgri, *Streptomyces griseus*; Syne, *Synechocystis*; Tmar, *Thermotoga maritima*; Uure, *Ureaplasma urealyticum*; Vcho, *Vibrio cholerae*; and Xfas, *Xylella fastidiosa*.

parameters in determining protein thermostability. Thermostable proteins contain an increased proportion of charged residues at the expense of uncharged polar amino acids, conferring them rigidity and stability by minimizing deamidation and backbone cleavages (Fukuchi and Nishikawa 2001). We therefore determined the contribution of charge and polarity on all the PstB sequences, including that of *M. tuberculosis*. Two separate dendrograms based on these results were generated (data not shown) by using OC software (Barton 1993). The dendrogram based on the percentage of charged amino acid residues in each PstB showed two major clusterings, and *M. tuberculosis* was grouped with mesophiles only. On the other hand, in a tree created on the basis of polarity of different PstBs, thermophiles were found to be distinct from mesophiles. Interestingly, PstB from *M. tuberculosis* is placed with thermophiles, which is in conformity with our experimental evidence (Sarin et al. 2001). Furthermore, this is apparent when the data is plotted as the function of percentage of either charged or polar amino acid residues (placement of *M. tuberculosis*; Fig. 2, cf. A and B). Such an observation is not only *M. tuberculosis*-specific because other pathogenic mycobacteria (*M. intracellulare*, *M. leprae*) showed a higher propensity toward thermophilic characteristics compared with the nonpathogen (*M. smegmatis*). Our analysis also predicts that PstBs from *Pseudomonas aeruginosa* and *Halobacterium* sp. are thermostable. However, it needs experimental validation, and of course, the rationale behind such thermostability of PstB protein in some mesophiles remains to be elucidated.

Although charge–charge interactions increase the number of salt bridges and are associated with enhanced stability in thermophiles, one must take into consideration that these interactions are also important for the functionality of a protein. Thus, it can be expected that in cases in which the increase in electrostatic interactions interferes with the enzyme activity, other mechanisms for thermal adaptation might be used. For example, the dodecameric state of ornithine carbamoyl transferase from *Pyrococcus furiosus* is stabilized by hydrophobic interactions at the trimeric catalytic motif interface (Villeret et al. 1998), whereas glutamate dehydrogenase from the same organism is stabilized by electrostatic interactions (Karshinkoff and Landenstein 2001). Therefore, it is possible that the maintenance of lower charge by PstB may be best suited to the mesophilic living conditions of mycobacteria.

Finally, the principle of protein thermostability not only is of academic interest but also has practical and technical implications (Karshinkoff and Landenstein 2001). Therefore, in designing thermostable enzymes (Sanchez-Ruiz and Makhatadze 2001), there would definitely be a need to envisage the status of polar amino acid residues, in addition to optimization of charge–charge interactions on the surface of a protein.

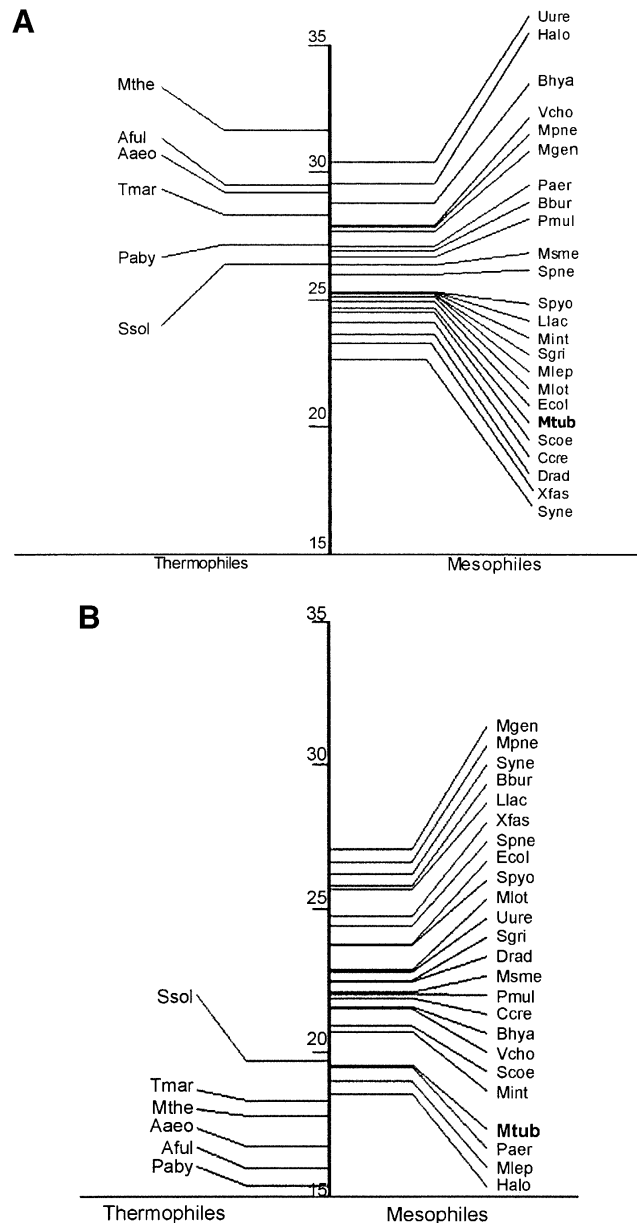


Figure 2. Placement of *M. tuberculosis* PstB on the basis of charged (A) and polar (B) amino acid residues. After BLAST search using *M. tuberculosis* PstB as a probe, the percentages of charged and polar residues of 29 retrieved sequences were calculated. The figures are graphical representations of the positions of different PstBs from both mesophiles and thermophiles as the function of percentage of charged or polar residues (denoted by numbers in X-axis). Abbreviations used are same as in Figure 1.

Acknowledgments

We thank Dr. A. Ghosh, Director, Institute of Microbial Technology, for providing us with excellent laboratory facilities. We are grateful to Dr. Dibyendu Sarkar for critical reading of the manuscript. We also acknowledge the Council of Scientific and Industrial Research (CSIR) and the Department of Biotechnology (DBT) for financial support.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search program. *Nucleic Acids Res.* **25**: 3389–3402.
- Barton, G.J. 1993. OC: A clustal analysis program: OC, version 1.0. <http://www.compbio.dundee.ac.uk/software/oc/oc.html>.
- Chakrabarty, S. and Varadarajan, R. 2000. Elucidation of determinants of protein stability through genome sequence analysis. *FEBS Lett.* **470**: 65–69.
- Fukuchi, S. and Nishikawa, K. 2001. Protein surface amino acid composition distinctively differ between thermophilic and mesophilic bacteria. *J. Mol. Biol.* **309**: 835–843.
- Gromiha, M.M., Oobatake, M., and Sarai, A. 1999. Important amino acid properties for enhanced thermostability from mesophilic to thermophilic proteins. *Biophys. Chem.* **82**: 51–67.
- Higgins, C.F. 1992. ABC transporters: From microorganisms to man. *Annu. Rev. Cell. Biol.* **8**: 67–113.
- Karshinkoff, A. and Landestien, R. 2001. Ion pairs and the thermotolerance of proteins from hyperthermophiles: A traffic rule for hot roads. *Trends Biochem. Sci.* **26**: 550–556.
- Kumar, S. and Nussinov, R. 2001. How do thermophiles deal with heat? *Cell. Mol. Life Sci.* **58**: 1216–1233.
- Kumar, S., Tsai, C.J., and Nussinov, R. 2001. Thermodynamic differences among homologous thermophilic and mesophilic proteins. *Biochemistry* **40**: 14152–14165.
- Sanchez-Ruiz, J.M. and Makhatadze, G.I. 2001. To charge or not to charge? *Trends Biotechnol.* **19**: 132–135.
- Sarin, J., Aggarwal, S., Chaba, R., Varshney, G.C., and Chakraborti, P.K. 2001. B-subunit of phosphate transporter from *Mycobacterium tuberculosis* is a thermostable ATPase. *J. Biol. Chem.* **276**: 44590–44597.
- Szilagyi, A. and Zavodsky, P. 2000. Structural differences between mesophilic, moderately thermophilic and extremely thermophilic protein subunits: Results of a comprehensive survey. *Structure* **8**: 493–504.
- Thompson, L.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.
- Villeret, V., Clantin, B., Tricot, C., Legrain, C., Roovers, M., Stalon, V., Glansdorff, N., and Van Beeumen, J. 1998. The crystal structure of *Pyrococcus furiosus* ornithine carbamoyltransferase reveals a key role for oligomerization in enzyme stability at extreme high temperatures. *Proc. Natl. Acad. Sci.* **95**: 2801–2806.