FOR THE RECORD

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

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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.
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 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 deamination 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 omithine 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.
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References


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.

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