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Nucleotide-induced conformational change in the catalytic subunit of the phosphate-specific transporter from *M. tuberculosis*: Implications for the ATPase structure

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Abstract

The nucleotide binding subunit of the phosphate-specific transporter (PstB) from *Mycobacterium tuberculosis* is a member of the ABC family of permeases, which provides energy for transport through ATP hydrolysis. We utilized the intrinsic fluorescence of the single tryptophan containing protein to study the structural and conformational changes that occur upon nucleotide binding. ATP binding appeared to lead to a conformation in which the tryptophan residue had a higher degree of solvent exposure and fluorescence quenching. Substantial alteration in the proteolysis profile of PstB owing to nucleotide binding was used to decipher conformational change in the protein. In limited proteolysis experiments, we found that ATP or its nonhydrolyzable analog provided significant protection of the native protein, indicating that the effect of nucleotide on PstB conformation is directly associated with nucleotide binding. Taken together, these results indicate that nucleotide binding to PstB is accompanied by a global conformational change of the protein, which involves the helical domain from Arg^{137} to Trp^{150}. Results reported here provide evidence that the putative movement of the α-helical sub-domain relative to the core sub-domain, until now only inferred from X-ray structures and modeling, is indeed a physiological phenomenon and is nucleotide dependent.
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Keywords: ABC ATPase; Conformational change; Nucleotide binding subunit of the mycobacterial phosphate specific-transporter; Nucleotide binding; Phosphate-specific transporter; Tryptophan fluorescence

1. Introduction

Phosphate uptake is of fundamental importance in the cell physiology of bacteria because phosphate is required as a nutrient. The phosphate acquisition system is best understood in *Escherichia coli*, which has evolved several gene clusters allowing the assimilation of phosphate via a variety of systems. As phosphate is often a limiting nutrient, its import in bacteria is accomplished through several parallel transport systems [1,2]. The Pst (phosphate-specific transporter) system is a tightly regulated high-affinity system, and the expression is operon controlled. Interestingly, three putative *pst* operons have been identified from the genome sequence of *Mycobacterium tuberculosis* [3,4], suggesting that many copies of the same phosphate transporter in mycobacteria might be involved in subtle biochemical adaptations of this microorganism for its growth and survival under highly varying (e.g. phosphate limiting) conditions during infectious cycle [2]. Apart from transporting phosphate, the Pst system plays an important role in the regulation of a number of coordinately regulated genes collectively referred to as the phosphate (pho) regulon [5].

The Pst system of *E. coli* comprises four distinct subunits encoded by the *pstS*, *pstA*, *pstB* and *pstC* genes [5,6]. The PstS protein is a phosphate-binding protein located in the periplasmic space. PstA and PstC are hydrophobic and they form the transmembrane portion of the Pst system. PstB is the catalytic subunit, which couples the energy of ATP hydrolysis to the import of phosphate across cellular

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membranes through the Pst system, often referred as ABC-protein [7]. In *M. tuberculosis*, the presence of several copies of all the components of the operon except for PstB has been reported [3,4]. PstB belongs to one of the largest superfamilies of proteins characterized by a highly conserved adenosine triphosphate (ATP) binding cassette (ABC), which is also a nucleotide binding domain (NBD). The unifying feature of ABC-transporters is their molecular organization, consisting minimally of two membrane-spanning domains that form the translocation pathway, and two cytosolic ABC-ATPase domains energizing transport through their ATPase activity. The ATPase subunit remains as a conserved and essential structural module of all ABC-transporters. Their primary structures include a characteristic set of motifs, including Walker A, Walker B and ABC Signature sequence (see Fig. 1). The Walker A (or P loop) and B sites are the two short peptide motifs involved in the chemistry of nucleotide binding and/or hydrolysis [8]. The Signature motif, strictly specific to ABC-ATPases, is involved in the binding of the pyrophosphate moiety of the bound nucleotide in the NBD dimer [9,10]. Mutants in this motif have been shown to be defective in ATP hydrolysis [11,12].

Given the biomedical importance of ABC-transporters in diseases such as cystic fibrosis or cancer, in recent years, they have been subject of intense scrutiny. Crystallographic studies of the ABC-ATPases, HisP from *Salmonella typhimurium* [13], MalK from *Thermococcus litoralis* [14], LivG and LolD from *Methanococcus jannaschii* [15,16] and the nucleotide binding domain of human TAP1 [17], reveal that ABC-ATPases share a common three-dimensional architecture and a nucleotide binding fold. Based on high cooperativity of ATP hydrolysis by ABC-ATPases, it is suggested to function as a dimer [18,19]. A number of recent studies have confirmed the dimeric arrangement of ATP binding cassettes within an intact transporter [9,20–22]. It has been proposed that physiologically relevant dimers undergo cycles of association and dissociation during transport and thereby allowing conversion of chemical energy to mechanical stroke [23–26].

Bacterial ABC-ATPases have also been implicated in diverse biological functions [27,28]. It has been shown that PstB is over-expressed as well as amplified in a fluoroquinolone-resistant colony of *M. smegmatis*, suggesting a novel role of this subunit in addition to its involvement in the process of phosphate import [29–31]. Furthermore, genomic and biochemical investigations have identified *pstB* throughout all the prokaryotic genome sequences available so far indicating the essentiality of this gene in microorganisms. PstB protein from *M. tuberculosis* has been purified and characterized in an active soluble form [32]. Recently, it was shown to be an unorthodox ATPase since the enzyme activity remained unaltered at 80 °C and the ATP hydrolyzing ability was found to be rather Mg^{++} independent and resistant to known ATPase inhibitors [32]. Although the structural data of NBD from a number of family members have improved our understanding on the functioning of the ATPase domain, a number of aspects of PstB ATPase action remain unclear. To understand the mechanistic basis for this activity, the structural changes during nucleotide turnover must be elucidated.

In this work, we have used the analysis of intrinsic tryptophan fluorescence and limited proteolysis by trypsin as means to investigate conformational change in the protein that occur upon nucleotide binding. The results show that the conformational change of the protein incurred upon nucleotide binding is the result of a specific ligand–protein interaction event where ATP or its nonhydrolyzable analog recognizes the specific structural determinant of the nucleotide-binding domain of PstB. Moreover, a comparison of structures of other transport ABC-ATPases shows that the conformational difference(s) in the presence of the nucleotide may be of relevance to understand catalysis at the active site of the transport ATPase. We consider how these results might influence models and experiments pertaining to the large family of transport ATPases.

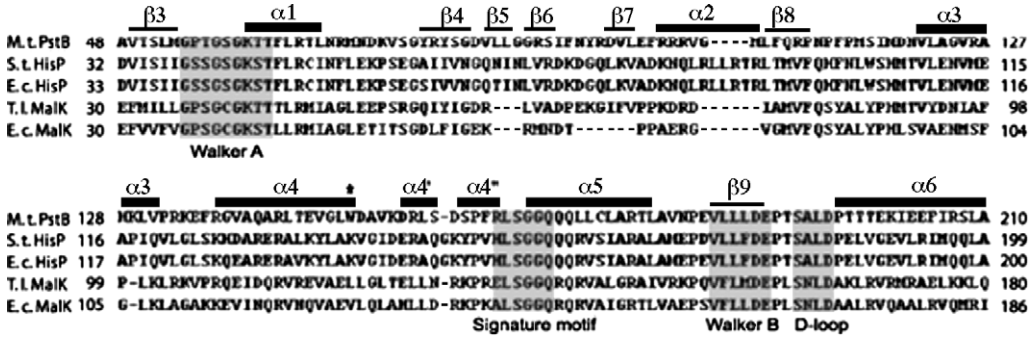

Fig. 1. Conservation of sequence in PstB-like family of ABC-ATPases. *M. tuberculosis* PstB from amino acid residues 48–210 is aligned with the putative nucleotide binding domains of the related family members: *S. typhimurium* HisP, *E. coli* HisP, *T. litoralis* MalK and *E. coli* MalK. Secondary structural elements of PstB are numbered as in Hung et al. [13]. α-helices are indicated by thick overbars and β-strands, by thin overbars. Key regions conserved in all five sequences as discussed in the text are indicated and highlighted in shaded boxes. The location of the unique tryptophan in PstB is indicated by an asterisk. Dashes represent gaps introduced to improve sequence alignment.

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