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Regulation of the ATPase activity of ABCE1 from *Pyrococcus abyssi* by Fe–S cluster status and Mg^{2+}: Implication for ribosomal function

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ABSTRACT

Ribosomal function is dependent on multiple proteins. The ABCE1 ATPase, a unique ABC superfamily member that bears two Fe_4S_4 clusters, is crucial for ribosomal biogenesis and recycling. Here, the ATPase activity of the *Pyrococcus abyssi* ABCE1 (*Pab*ABCE1) was studied using both *apo*- (without reconstituted Fe–S clusters) and *holo*- (with full complement of Fe–S clusters reconstituted post-purification) forms, and is shown to be jointly regulated by the status of Fe–S clusters and Mg^{2+}. Typically ATPases require Mg^{2+}, as is true for *Pab*ABCE1, but Mg^{2+} also acts as a negative allosteric effector that modulates ATP affinity of *Pab*ABCE1. Physiological [Mg^{2+}] inhibits the *Pab*ABCE1 ATPase (K_i of ~1 μM) for both *apo*- and *holo*-*Pab*ABCE1. Comparative kinetic analysis of Mg^{2+} inhibition shows differences in degree of allosteric regulation between the *apo*- and *holo*-*Pab*ABCE1 where the apparent ATP K_m of *apo*-*Pab*ABCE1 increases >30-fold from ~30 μM to over 1 mM with Mg^{2+}. This effect would significantly convert the ATPase activity of *Pab*ABCE1 from being independent of cellular energy charge (φ) to being dependent on φ with cellular [Mg^{2+}]. These findings uncover intricate overlapping effects by both [Mg^{2+}] and the status of Fe–S clusters that regulate ABCE1's ATPase activity with implications to ribosomal function.

Introduction

Biogenesis and the functions of the ribosome are supported by numerous proteins at various stages. Among these proteins, ABCE1 is essential for ribosome function and for cellular viability in eukaryotic systems. ABCE1 is the lone member of class E in the wide family of ATP[1] Binding Cassette (ABC) proteins [1–5]. While most members of the ABC protein family have trans-membrane domains (TMDs) and function as transporters of various metabolites, ABCE1 has no TMDs [6]. Instead, ABCE1 has two nucleotide binding domains that are connected by a hinge region and uniquely has a domain containing two Fe_4S_4 clusters (Fig. 1). The exact function of these Fe_4S_4 clusters has been somewhat enigmatic.

ABCE1 was initially identified as the RNAseL Inhibitor (RLI) that inhibited the binding of RNA and the anti-viral nuclease activity of RNAse L, which is involved in the pathway for interferon mediated antiviral defense [7]. Later, ABCE1 was also found to be a modulator of a protein–RNA interaction in relation to HIV [8,9]. The expression of ABCE1 appears to be upregulated upon HIV infection, which increases the virulence of HIV by decreasing the 2′–5′ oligoA/RNAse activity [7,10]. Later, ABCE1 has also been shown to be required for late stage capsid assembly as part of the HIV life cycle [11].

The critical importance of ABCE1 in Eukaryotes was first observed when it was found to be one of 356 genes in *S. cerevisiae* that cause a lethal phenotype when deleted as part of a systematic genome-wide deletion analysis [12]. A later study showed the specific *in vivo* essentiality of the Fe–S clusters of ABCE1 where the cytosolic Fe–S cluster assembly system was disrupted and led to stalling of ribosome maturation [3]. In these studies in cells with disrupted Fe–S cluster assembly, the Fe–S clusterless form of ABCE1 (*apo*-ABCE1) accumulated *in vivo* and was found bound to nucleolar non-functional immature ribosomes and also to cytoplasmic polysomes. Furthermore, tetrad analysis using strains with heterozygous mutations of the Fe–S cluster ligating cysteine residues further showed the specific importance of the Fe–S clusters toward the function of ABCE1 where the homozygous mutated progeny did not grow [3]. These studies showed the essential nature of ABCE1 and its Fe–S clusters in ribosome assembly and maturation.

Recent cell biological studies have shown that ABCE1 and its ATPase activity have roles in translation termination and ribosome recycling. First, it was demonstrated with *Hs*ABCE1 that ATP

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[1] *Abbreviations used:* ADP, adenosine di-phosphate; ATP, adenosine tri-phosphate; *Pab*ABCE1, *Pyrococcus abyssi* ABCE1; TMD, trans-membrane domain; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid.

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hydrolysis is required for ribosome dissociation [13]. Studies with the ABCE1 from *Sulfolobus solfataricus* showed that the Fe–S domain is required for association with ribosomes [14]. In a more recent study, the role of *S. cerevisiae* ABCE1 and other recycling factors, Dom34 and Hbs1, in ribosome recycling was investigated and results show the ATPase activity is significantly increased in the presence of Hbs1 and/or Dom34. Additionally, *Sc*ABCE1 can promote recycling prior to the release of the peptide [15].

The structures of ABCE1 from two *Pyrococci* have been determined with (*P. abyssi*) and without (*P. furiosus*) the Fe–S cluster domain [16,17]. A recent cryo-electron microscopy reconstruction of mixed complexes of *S. cerevisiae* ribosome recycling factors and *P. furiosus* ABCE1 shows interactions with Dom34/Pelota near the peptidyl transferase center on the ribosomes [18]. The structures of these thermophilic ABCE1s are a great starting point for biochemical understanding of this important protein. Much remains to be elucidated about the biochemical roles and mechanism of this protein in relation to ribosome biogenesis, translation and possibly with viral lifecycles. Among the key questions are: how is the energy derived from ATP hydrolysis utilized, what is the role of the Fe–S clusters, and how does it interact with immature and mature ribosomes? Toward the goal of better biochemical understanding for some of these aspects, the hydrolysis of MgATP by the *Pyrococcus abyssi* ABCE1 (*Pab*ABCE1) was studied in the present work. Somewhat unexpectedly, the ATP hydrolysis activity was found to be very sensitive to the concentration of Mg^{2+}, which may possibly be relevant to a mode of regulation *in vivo*.

Materials and methods

Expression and purification of PabABCE1

Recombinant protein was expressed in *E. coli*, Rosetta 2 BL21(DE3)pLysS transformed with plasmid pET28-N-strep-*Pab*ABCE1Δ4 (gift from K.P. Hopfner). Briefly, cells were grown to an optical density of 0.8 followed by induction with 0.5 mM IPTG for 4 h at 37 °C. Purification was performed as described [17] with one modification: Q Sepharose was used for ion exchange chromatography. Following Streptactin affinity chromatography, protein was concentrated to 35 μM using a Vivaspin 20 concentrator (GE Healthcare). Protein concentration was determined by standard BCA assay with TCA precipitation of purified protein in order to remove reductants [19].

Fe–S reconstitution

The as purified *Pab*ABCE1, designated the *apo-Pab*ABCE1, typically contained ~0.9 Fe per protein. The *holo-Pab*ABCE1 was prepared by reconstituting the Fe–S cluster after purification. The Fe–S reconstitution procedure was conducted in an anaerobic glove box containing at least 5% H_2 and less than 1 ppm O_2. The buffer for the concentrated protein was exchanged to 50 mM Tris, pH 8.0, 200 mM NaCl, 5 mM DTT simultaneously with the Fe–S cluster reconstitution by dialysis overnight at 4 °C. All buffers, protein, and other solutions were degassed and buffers were stored in the glove box for at least 6 h prior to beginning Fe–S reconstitution. Dialysis was performed using a Slide-A-Lyzer MINI dialysis device, 10 K MWCO (Thermo Scientific). $FeCl_2$ was added anaerobically to degassed protein (15:1 Fe:protein ratio) for 5 min prior to dialysis. The protein supplemented with Fe^{2+} was placed into dialysis and equilibrated against 500 fold greater volume of buffer containing 20 μM sodium sulfide. Dialysis/ Fe–S reconstitution proceeded overnight at 4 °C. The buffer was then thoroughly exchanged to dialysis buffer containing no sodium sulfide. The protein was then removed from dialysis and aliquots in crimp-sealed vials were prepared, frozen on liquid N_2, and stored at −80 °C until ready for use. Concentration of bound iron was determined by a colorimetric assay [20] with an additional procedure of modifying DTT by incubating samples with 20 mM iodoacetamide at 37 °C for 1 h, prior to the colorimetic detection of Fe. The reconstituted *holo-Pab*ABCE1 contained 8.5 ± 0.6 Fe per protein.

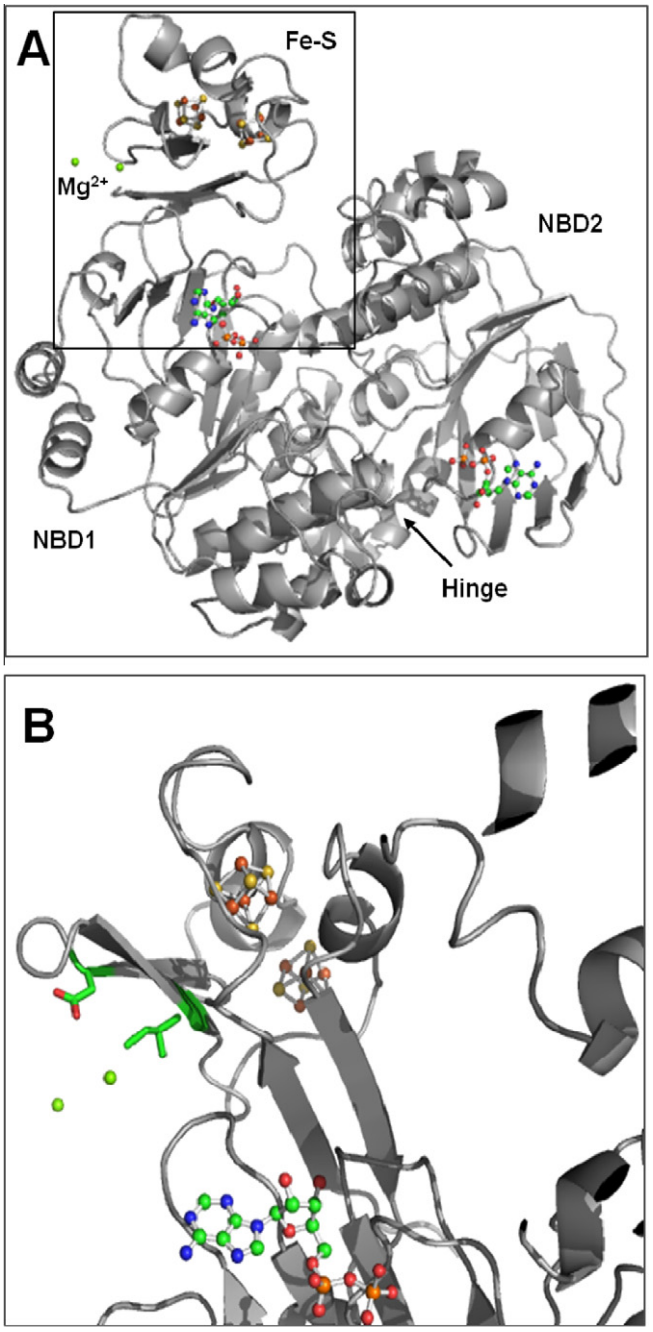

Fig. 1. Structure of *Pab*ABCE1, ligands and cofactors. Shown is the complete crystal structure (A) and Fe–S domain (B) of *Pab*ABCE1 (PDB 3BK7, (17)). The two Fe–S clusters and both ADP molecules located with each of the nucleotide binding domains are shown in colored ball-and-stick models and green spheres represent two Mg^{2+} ions. Labels refer to different parts of the proteins as described in the Introduction. A box is shown in Panel (A) to indicate the section shown in Panel (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)