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Nucleotide dependence of the dimerization of ATP binding cassette nucleotide binding domains

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ABSTRACT

ATP-binding cassette proteins are ubiquitously present throughout all known genomes. Their basic functional unit possesses two transmembrane domains and two nucleotide-binding domains. The nucleotide-binding domains are responsible for ATP binding and hydrolysis, and their 3-dimensional structure is conserved across ATP-binding cassette proteins. Binding of ATP produces nucleotide-binding domain dimerization, a step necessary for hydrolysis. However, the possibility that nucleotide-binding domains bind and/or hydrolyze nucleotide triphosphates different from ATP has not been explored in detail. Here, we studied that possibility using *M. jannaschii* MJ0796, a prototypical ATP-binding cassette nucleotide-binding domain. We found that nucleotide-binding domain dimerization occurs as a result of binding to the natural nucleotide triphosphates ATP, GTP, CTP and UTP, and also to the analog ATP-γ-S. All the natural nucleotide triphosphates are hydrolyzed at similar rates, whereas ATP-γ-S is not hydrolyzed. We also found that the non-hydrolyzable ATP analog AMP-PNP, frequently assumed to produce the nucleotide-bound conformation, failed to elicit nucleotide-binding domain dimerization. Our results raise the possibility that not all the nucleotide binding sites of nucleotide-binding domains are occupied by ATP under physiological conditions, and that ATP is not always the nucleotide hydrolyzed to dissociate the nucleotide-binding domain dimers.

1. Introduction

ATP-binding cassette (ABC)[f] proteins comprise one of the largest superfamilies of proteins [1–3]. They are present throughout all known genomes, and most are directly involved in substrate transport across membranes [1–3]. Their basic functional unit has two transmembrane domains and two nucleotide-binding domains (NBDs) [1–3]. The NBDs are responsible for nucleotide binding and hydrolysis, and their structure is conserved, even though different ABC proteins perform very dissimilar functions [1,2].

MJ0796, a NBD from the *Methanocaldococcus jannaschii*, is a prototypical ABC NBD for which we have a wealth of structural and functional information [4–10]. MJ0796 is a monomer in the absence of ATP, but forms dimers in a head-to-tail arrangement upon ATP binding (Fig. 1A) [5,9,10]. In the dimer, two ATP molecules are sandwiched at the dimer interface (Fig. 1A) [4,10]. Each nucleotide-binding site (NBS) is formed by the Walker A and B motifs, A loop, H loop and Q loop of one NBD, and the D loop and signature motif of the other NBD (Fig. 1B) [4,10]. The Walker A motif is critical for binding of the nucleotide phosphates whereas the Walker B motif and Q loop are involved in Mg^{2+} and water coordination at the catalytic site [4,10]. The conserved aromatic residue of the A loop interacts with the adenine ring of ATP, whereas the signature motif and D and H loops coordinate the γ phosphate of ATP [4,10]. To form a stable dimer capable of hydrolysis both NBSs must be occupied by ATP [7], but dissociation of the dimer can proceed following the hydrolysis of ATP at only one of the NBSs [8].

Abbreviations: ABC, ATP-binding cassette; MJ, single-Cys, single-Trp MJ0796 mutant G14C/C53G/C128I/G174W; MJI, hydrolysis deficient MJ mutant E171Q; NBD, nucleotide-binding domain; NBS, nucleotide-binding site; NTP, nucleotide triphosphate; ATP-γ-S, adenosine 5′-[γ-thio]triphosphate; AMP-PNP, adenylyl-imidodiphosphate.

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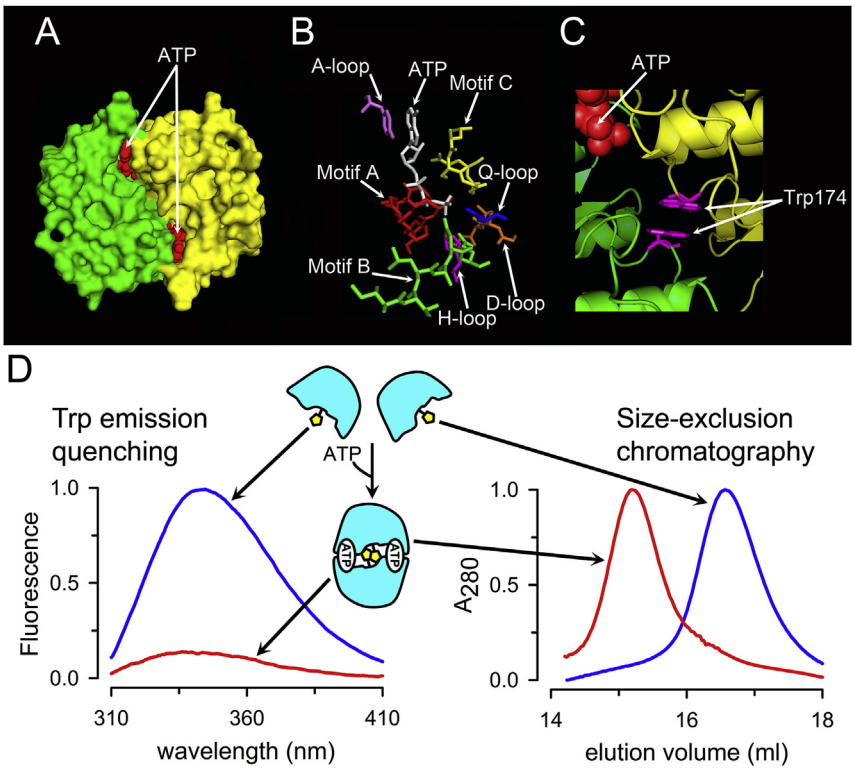

Fig. 1. Structure of the NBD dimer and effects of dimerization on the Gly174Trp mutants MJ and MJI. (A) Surface representation of the ATP-bound MJI dimer. Monomers are displayed in different colors (yellow and green) and ATP molecules are shown in red. (B) Zoomed view of one nucleotide-binding site. Stick representation showing only the ATP (white), Walker A (motif A, red), Walker B (motif B, green), signature sequence (motif C, yellow), A-loop (Tyr11, violet), H-loop (His204, magenta), Q-loop (Gln90, blue), and D-loop (orange). Other residues were removed to make viewing easier. (C) Zoomed view of the center of the dimer interface of the nucleotide-bound MJI dimer. The two Trp174 residues are shown (pink, sticks). (D) Effect of dimerization of MJI on Trp174 fluorescence emission spectra and hydrodynamic radius. Dimerization by ATP (representation in the top center) results in Trp emission quenching and increase in the hydrodynamic radius. The Trp emission quenching and size-exclusion chromatography records correspond to representative experiments showing the MJI responses to saturating ATP. A_{280}: absorbance measured at 280 nm, normalized to the peak value. The figures in panels A and B are based on PDB1L2T and that in panel C on PDB3TIF. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Although ATP has been used in the overwhelming majority of nucleotide triphosphate (NTP) binding and hydrolysis studies of ABC proteins, there are reports of binding and/or hydrolysis of GTP and other NTPs [11–17]. However, the possibility that many ABC proteins can bind and/or hydrolyze NTPs besides ATP, as well as binding of NTP analogs frequently assumed to bind to NBDs, has not been explored in detail. Here, we analyzed the effects of a variety of natural nucleotides and frequently used analogs for their ability to elicit NBD dimerization and determined which of the NTPs that produced NBD dimerization are actually hydrolyzed.

2. Material and methods

2.1. Protein expression and purification, and assessment of NBD dimerization

M. jannaschii MJ0796 mutants were expressed in BL21-CodonPlus (DE3)-RILP *E. coli* and purified by anion-exchange and size-exclusion chromatography as described [9,10]. The properties of single-Cys, single-Trp, MJ0796 mutants G14C/C53G/C128I/G174W (MJ) and G14C/C53G/C128I/E171Q/G174W (MJI) have also been published [7–10]. While MJ is catalytically active, the ATPase activity of MJI is negligible, but it still retains the ability to bind ATP with high affinity [7–10]. In both proteins, Gly174 was replaced with Trp for simple and efficient tracking of NBD dimerization by following Trp fluorescence emission [9,10]. Details on the evaluation of MJ and MJI dimerization by the quenching of Trp fluorescence (excitation at 295 nm and emission measured at 340 nm) and size-exclusion chromatography have been published [7–10]. Fluorescence was corrected by the quenching effect of the nucleotides determined experimentally using *N*-acetyl tryptophanamide as a Trp174 surrogate. Therefore, the empirical corrections include inner-filter effect (minimized by the short 3-mm pathlength) and effects due to direct interaction of the nucleotides with Trp. *N*-acetyl tryptophanamide quenching was very small and changed linearly with concentration to 0.5 mM ATP, UTP, ATP-γ-S and AMP-PNP (<10% mM^{-1}). The quenching to 100 μM GTP and CTP was also minimal (<10% mM^{-1}), but increased to 33 and 22% mM^{-1} between 100 μM and 1 mM, respectively (Supplementary Fig. 1). The corrections performed had negligible effect on the calculations because the K_d values for dimerization ranged from 1.8 to 53 μM (see Results and discussion). ATPase activity was measured through the release of Pi using the malachite green colorimetric assay, essentially as described [18].

2.2. Preparation of nucleotide solutions

ATP (product A2383), ADP (product A2754), GTP (product G8877), CTP (product C1506), UTP (product U6875), and ATP-γ-S (product A1388) were purchased from Sigma-Aldrich (St. Louis, MO). AMP-PNP was purchased from Sigma-Aldrich (product A2647) or Roche (product 10102547001). Nucleotide stocks were prepared before the experiments at a concentration of 100 mM in a solution containing 200 mM NaCl, 20 mM glucose, 1 mM EDTA, 10% glycerol, 1 μM hexokinase, and 50 mM Tris/HCl, pH 7.6. For most experiments, the stocks (other than ATP) were kept for 1 h at room