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Minireview

Molecular insights into the mechanism of ATP-hydrolysis by the NBD of the ABC-transporter HlyB

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Abstract The ABC-transporter HlyB is a central element of the Type I protein secretion machinery, dedicated to export the *E. coli* toxin HlyA in a single step across the two membranes of the cell envelope. Here, we discuss recent insights into the structure and the mechanism of ATP-hydrolysis by the NBD of HlyB. Combining structural and biochemical data, we have suggested that substrate-assisted catalysis (SAC), but not general base catalysis, is responsible for ATP-hydrolysis in this NBD and might also operate in other NBDs. Finally, the implications and advantages of SAC are discussed in the context of ATP-induced dimerization of the NBDs.

Keywords: ABC-transporters; Dimerization; Nucleotide-binding domain; Substrate-assisted catalysis; Crystal structures

1. Type I protein secretion – the ABC transporter pathway

Type I protein secretion in bacteria, also called the ABC-pathway [1,2], is a rather promiscuous, Sec-independent process used to translocate polypeptide toxins, hydrolytic enzymes, and surface bound proteins in one step across the double membrane of *E. coli* [3]. All information necessary and sufficient for this transport process is encoded as a targeting signal in the C-terminal part of Type I substrates. This secretion signal is not cleaved after translocation, another feature distinguishing this mechanism from the well-understood Sec-apparatus [4]. The paradigm of the Type I system is the haemolysin A (HlyA) machinery, which was discovered in the early 1980s in certain uropathogenic *E. coli* strains [5]. This system is composed of three indispensable elements, the ABC-transporter HlyB, the membrane-fusion protein HlyD, both residing in the inner membrane, and the outer membrane protein TolC [6]. It is now commonly accepted that these three membrane proteins form a continuous channel across the cell envelope [7] following engagement by the *allocrite* HlyA, our preferred term for a transported substrate [8], thereby preventing the appearance of periplasmic intermediates.

2. ABC-transporters

HlyB is a member of the continuously growing family of ABC- (ATP-binding cassette) transporters [9], that are found in all three kingdoms of life. These ATP-dependent channels or pumps ultimately use the energy of ATP-hydrolysis to achieve translocation of an astonishing variety of allocrites, ranging from small ions such as chloride (CFTR), to nutrients such as amino acids or sugars (histidine and maltose 'permeases'), up to large proteins (HlyB) [10]. Despite this diversity, including both importers and exporters, all these transporters share the same organization: two nucleotide-binding domains (NBD) and two transmembrane domains (TMD) to form a functional unit. In eukaryotes, all four domains are found in a single polypeptides, whilst in the importer systems in prokaryotes, the ABC–NBD and the membrane domain are usually found as separate proteins (e.g., HisP). On the other hand, exporters such as HlyB, or the peptide transporter TAP, in eukaryotes, are so called half-size transporters with the NBD and the membrane domain in a single polypeptide. Since the first report of an ABC-transporter in 1982 [11], we have seen tremendous achievements. However, we are still a long way from a functional understanding. For example, it is not clear how the energy of ATP is coupled to allocrite transport or how molecular signals are sent back and forth between the motor domains (the NBDs) and the TMDs [9,12].

Membrane protein research is still hampered by a significant degree by the fact that overexpression and purification of a functional protein is certainly not a straightforward task. Furthermore, transporters require reconstitution in lipsosomes in order to study vectorial translocation of an allocrite in vitro. In the case of an ABC-transporter, we took the view that it should be possible to analyze the many details of the mechanism of ATP-hydrolysis and the associated conformational changes with the isolated NBDs alone, in addition to longer-term studies with the intact protein. This approach we argued, would provide an opportunity, which should in principle more easily allow a detailed analysis of the catalytic mechanism of the NBD. However, one always has to keep in mind that in

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Abbreviations: ABC, ATP-binding cassette; Hly, haemolysin; NBD, nucleotide-binding domain; SAC, substrate-assisted catalysis; TMD, transmembrane domain

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the absence of the TMD, the tight interaction between NBD and TMD is lost, which consequently results in a free diffusion of the isolated NBD in solution, thereby increasing its mobility. Furthermore, the important communication between NBD and TMD is completely absent. Nevertheless and without question, isolated NBDs are proven valid model systems in relation to certain questions. For example, detailed mechanistic insights have been obtained from the isolated NBD of the yeast transporter Mdl1p [13] or the NBD of HlyB [14–16]. In fact, whilst it is quite feasible that the underlying kinetics will be affected by the presence or absence of the TMD, it is unlikely that a TMD will change the fundamental thermodynamics of the system. Thus, isolated NBDs are in our opinion suitable systems to derive insights into how chemical energy stored within ATP, is transformed into mechanical energy and how the NBDs, or motor domains, might fuel allocrite translocation.

3. The nucleotide-binding domain of HlyB

The first crystal structure of an NBD, HisP, was described in 1998 [17]. Since that time several structures of isolated NBDs and full-length ABC-transporters have been reported [17–30]. All ABC-NBDs are L-shaped molecules (Fig. 1) with a two domain architecture. The catalytic domain harbors the RecA-like [31] nucleotide binding site and contains various conserved motifs; the Walker A and B motifs [32], also present in other ATPases and GTPases [33], and the D- and H-loops. The second and smaller helical domain is built up entirely of α-helices and contains the C-loop or signature motif, the diagnostic feature of ABC-transporters. Both domains of the NBD are connected via the Q-loop and the Pro-loop [25]. Over the years,

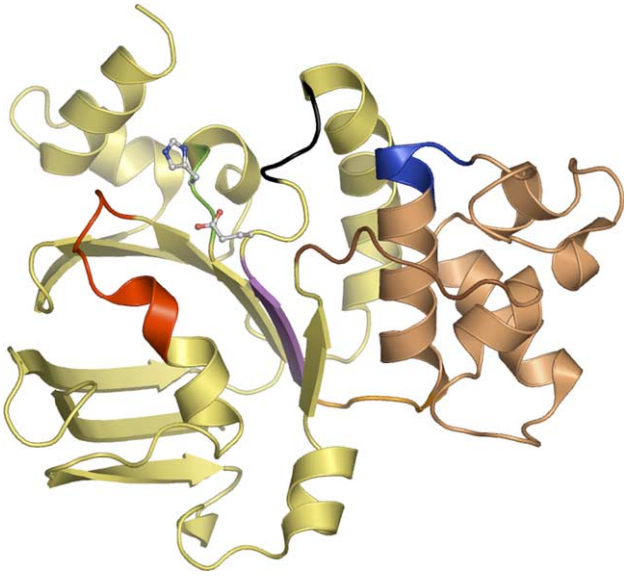

Fig. 1. Overall structure of monomeric, wildtype HlyB-NBD (pdb entry 1MT0). The catalytic and helical domains are shown in light yellow and light tan, respectively. Conserved motifs are colored in red (Walker A, residues 502–510), brown (Q-loop, residues 550–556), C-loop (blue, residues 606–610), Pro-loop (orange, residues 622–625), Walker B (magenta, residues 626–630), D-loop (black, residues 634–637), and H-loop (green, residues 661–663). The catalytic dyad (E631 and H662) is shown in ball-and-stick representation.

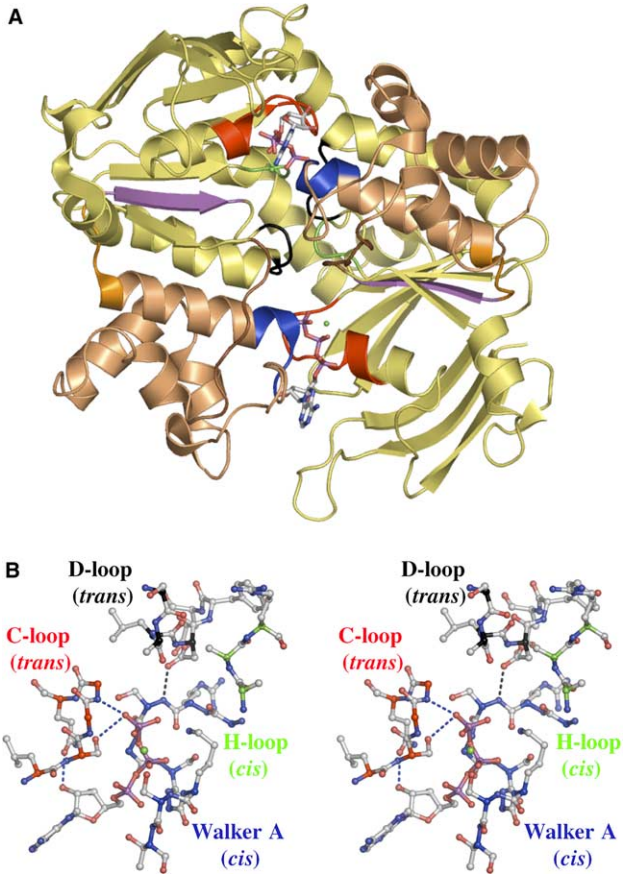

Fig. 2. (A) Cartoon representation of the crystal structure of the dimeric ATP/Mg^{2+}-bound form of HlyB-NBD H662A (pdb entry 1XEF). Color-coding is identical to Fig. 1. ATP is shown in ball-and-stick representation and the cofactor Mg^{2+} as a green sphere. (B) Stereoview of the architecture of the composite ATP/Mg^{2+}-binding site in the HlyB-NBD H662A. For simplicity only the interactions of the C-loop amino acids of the *trans* monomer and the D-loop of the *cis*-monomer are shown. To visualize conserved motifs, only Ca-atoms of the motifs have been color-coded according to Fig. 1.

structural work with the isolated NBDs demonstrated that ATP binding to the catalytic domain, induces a rigid body motion of the helical domain of roughly 20° [16,20,23,30] and consequently dimerization.

The architecture of the NBD-dimer, although initially controversial, is now solidly established due to recent biochemical and structural data [16,20,23,30,34]. Thus, in the dimer, ATP acting as a molecular glue, is sandwiched between the Walker A motif of the *cis* monomer and the C-loop motif of the opposing *trans* monomer (Fig. 2A). However, with the exception of a single interaction with the hydroxyl moiety of the ribose, all the other interactions of the C-loop of the *trans* monomer with the bound ATP are directed towards the γ-phosphate (Fig. 2B). This explains why this composite dimer is only stable in the presence of ATP but not ADP.

4. ATP-induced dimerization and the mechanism of ATP-hydrolysis

Recent studies have indicated that even in the absence of nucleotides, the two NBDs are in close proximity [35], and