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Mini-review

The power of the pump: Mechanisms of action of P-glycoprotein (ABCB1)

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ABSTRACT

Members of the superfamily of ATP-binding cassette (ABC) transporters mediate the movement of a variety of substrates including simple ions, complex lipids and xenobiotics. At least 18 ABC transport proteins are associated with disease conditions. P-glycoprotein (Pgp, ABCB1) is the archetypical mammalian ABC transport protein and its mechanism of action has received considerable attention. There is strong biochemical evidence that Pgp moves molecular cargo against a concentration gradient using the energy of ATP hydrolysis. However, the molecular details of how the energy of ATP hydrolysis is coupled to transport remain in dispute and it has not been possible to reconcile the data from various laboratories into a single model. The functional unit of Pgp consists of two nucleotide binding domains (NBDs) and two trans-membrane domains which are involved in the transport of drug substrates. Considerable progress has been made in recent years in characterizing these functionally and spatially distinct domains of Pgp. In addition, our understanding of the domains has been augmented by the resolution of structures of several non-mammalian ABC proteins. This review considers: (i) the role of specific conserved amino acids in ATP hydrolysis mediated by Pgp; (ii) emerging insights into the dimensions of the drug binding pocket and the interactions between Pgp and the transport substrates and (iii) our current understanding of the mechanisms of coupling between energy derived from ATP binding and/or hydrolysis and efflux of drug substrates.

1. Introduction

The ATP-binding cassette (ABC) family of transport proteins represents one of the largest families of proteins in living organisms (Dean and Annilo, 2005; Dean et al., 2001; Gottesman and Ambudkar, 2001) and members of this family play a central role in cellular physiology. Mutations in ABC transporters have been linked to several human diseases including cystic fibrosis, persistant hyperinsulinemic hypoglycemia of infincay, the Dubin-Johnson syndrome, Stargardt disease and Tangier disease (Gottesman and Ambudkar, 2001). The unit of an ABC transporter is a nucleotide binding domain (NBD) and one trans-membrane domain containing six helices (Ambudkar et al., 1999). A functional ABC protein consists of two units as a single polypeptide chain or composed of either a homo- or hetero-dimer. Each NBD includes the highly conserved Walker A and B motifs, the ABC signature domain and the Q-, D- and H-loops. The sites of drug transport appear to be formed by both trans-membrane domains within the lipid bilayer (Ambudkar et al., 1999; Dey et al., 1997; Sauna et al., 2001).

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P-glycoprotein (Pgp) is the most extensively studied mammalian ABC transporter and is often regarded as the prototype for understanding the biochemical mechanisms (Gottesman et al., 1996, 2002). The clinical importance of Pgp stems from the fact that this molecular pump is implicated in multidrug resistance (MDR), the phenomenon by which tumor cells simultaneously exhibit intrinsic or acquired cross-resistance to diverse chemotherapeutic agents, resulting in the failure of chemotherapy for many cancers (Ambudkar et al., 1999). Systemic chemotherapy is the treatment of choice in cancers that are metastatic (approximately 50% of all cancers) and no more than 10% of patients are cured by chemotherapy. Moreover, several other ABC transporters have been associated with MDR in fungal and protozoan infections (Kontoyiannis and Lewis, 2002; Sanglard and Odds, 2002). This family of proteins also plays an important role in absorption, disposition, metabolism and excretion (ADME) of a variety of drugs and thus has enormous clinical significance and understanding the mechanistic basis of action of these transporters remains an important goal.

There is strong evidence in the literature that Pgp obtains the energy required for the vectorial transport of drug-substrates across the membrane via the hydrolysis of ATP (for reviews see (Davidson, 2002; Gottesman and Pastan, 1993; Higgins and Linton, 2004; Sauna et al., 2001; Senior et al., 1995)). The mechanistic basis of the transport of substrates by ABC transport proteins has been intensively studied for over 15 years. These studies have focused on (i) the characterization of the ATP hydrolysis and transport of a variety of drug-substrates and (ii) understanding how these two activities are coupled. The transport cycle of Pgp has been the most extensively studied and serves as a prototype for studies with other ABC transport proteins. Moreover, structural information has been obtained from several prokaryotic ABC transporters in recent years providing a context for the plethora of data obtained from genetic and biochemical studies. In this review, we present the emerging view of the NBDs and transport substrate sites of Pgp based on biochemical data as well as X-ray crystallographic studies of related proteins. We also discuss competing models for the molecular description of the transport cycle and the data in the literature that support these models.

2. The nucleotide binding sites

A functional unit of an ABC transporter requires two NBDs or ABCs which are composed of several conserved sequence motifs, the A-loop (an aromatic residue 25 amino acids upstream of the Walker A), the Walker A, the Walker B, the signature motif (LSGGQ motif, linker peptide or C motif) and the D, H and Q-loops. Crystal structures of the ABC domains of several ABC transporters indicate that a functional ATP site is formed by the interaction of residues from both halves of the protein (Chang and Roth, 2001; Hopfner et al., 2000; Locher, 2004; Locher et al., 2002; Reyes and Chang, 2005). Moreover, the structure of NBDs of several ABC and closely related proteins show that the two NBDs form a 'nucleotide-sandwich dimer' with ATP bound along the dimer interface, flanked by the Walker A and B motifs of one subunit and the signature motif and D-loop of the other (Hopfner et al., 2000; Smith et al., 2002). In addition, the adenine ring of ATP interacts with an aromatic residue (A-loop) upstream of Walker A and the three phosphate and magnesium moieties interact with the Walker A (P-loop) and B motifs, and possibly the H-loop (Hung et al., 1998; Smith et al., 2002; Zaitseva et al., 2005). The crystal structures of the NBDs of several ABC transport proteins suggest specific roles for several highly conserved amino acid residues within the ABC (see (Davidson and Chen, 2004; Smith et al., 2002; Zaitseva et al., 2005) for comprehensive maps of these residues). The biochemical function of the key residues in the NBDs of Pgp are summarized in Table 1.

The Walker A motif of both NBDs of Pgp contains a highly conserved lysine residue (K433 and K1076) thought to interact directly with the phosphate groups of the ATP molecule. It has been demonstrated that a K → M mutation in either of the two NBDs abolishes the drug-stimulated ATPase activity, however the binding of [α-^{32}P]8azidoATP was retained. A double mutant where the lysines in both NBDs were simultaneously replaced with methionines, however, could neither bind [α-^{32}P]8azidoATP nor hydrolyze ATP (Muller et al., 1996). Comparable studies with mouse Pgp in which the conserved lysines were mutated to arginines gave similar results (Azzaria et al., 1989). The residues homologous to the pairs D555/D1200 and E556/E1201 in human Pgp are also highly conserved among ABC transporters and have been demonstrated to play a critical role in ATP hydrolysis. Mutations in these residues results in a loss of function (Hrycyna et al., 1999; Sauna et al., 2002). Experimental data demonstrates that in human Pgp the D555/D1200 pair is involved in the coordination of Mg^{2+} (Hrycyna et al., 1999). The residues that correspond to the pairs E556/E1201 in the Walker B region and Q475/1118 in the Q-loop of human Pgp ATP sites, on the other hand, are postulated to form hydrogen bonds with water molecules that interact with the γ-phosphate (Hung et al., 1998). Hydrolysis of ATP occurs via the attack of a water molecule on the γ-phosphate and it is often the side chain of an amino acid that activates the attacking water molecule by deprotonation. Senior and coworkers have demonstrated in mouse Mdr3 that the residues equivalent to Q475 and Q1118 of human Pgp are involved neither in the activation of the attacking water for ATP hydrolysis, nor in the coordination of the essential Mg^{2+} cofactor in the Mg^{2+}-nucleotide (Urbatsch et al., 2000a). Of the E556 and E1201 mutants, the double mutant that can trap nucleotide even in the absence of orthovanadate (Vi) is of considerable interest (see (Delannoy et al., 2005; Sauna et al., 2002; Tombline et al., 2004b, 2005; Urbatsch et al., 2000b) for a complete biochemical characterization of these residues in mouse and human Pgp). A comparable mutant (E171Q) in MJ0796 was used to obtain the structure of the nucleotide sandwich dimer (Smith et al., 2002) suggesting that this mutant may be useful in understanding the dimerization of the two NBDs in Pgp. Studies with Pgp, however, show a stoichiometry of one nucleotide occluded per Pgp molecule rather than the two expected (Tombline et al., 2004a,b, 2005). Moreover, the trapped transition state of the mutant shows characteristics similar to the Vi-trapped transition state in wild-type Pgp (Sauna et al., 2002).

Between the Walker A and B sequences is found a linker peptide with the sequence LSGGQ, also known as the C-region or ABC signature sequence, as it is the hallmark of