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Nucleotide dissociation from NBD1 promotes solute transport by MRP1☆

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Abstract

MRP1 transports glutathione-S-conjugated solutes in an ATP-dependent manner by utilizing its two NBDs to bind and hydrolyze ATP. We have found that ATP binding to NBD1 plays a regulatory role whereas ATP hydrolysis at NBD2 plays a dominant role in ATP-dependent LTC4 transport. However, whether ATP hydrolysis at NBD1 is required for the transport was not clear. We now report that ATP hydrolysis at NBD1 may not be essential for transport, but that the dissociation of the NBD1-bound nucleotide facilitates ATP-dependent LTC4 transport. These conclusions are supported by the following results. The substitution of the putative catalytic E1455 with a non-acidic residue in NBD2 greatly decreases the ATPase activity of NBD2 and the ATP-dependent LTC4 transport, indicating that E1455 participates in ATP hydrolysis. The mutation of the corresponding D793 residue in NBD1 to a different acidic residue has little effect on ATP-dependent LTC4 transport. The replacement of D793 with a non-acidic residue, such as D793L or D793N, increases the rate of ATP-dependent LTC4 transport. Along with their higher transport activities, their Michaelis constant K_ms (ATP) are also higher than that of wild-type. Coincident with their higher K_ms (ATP), their K_ds derived from ATP binding are also higher than that of wild-type, implying that the rate of dissociation of the bound nucleotide from the mutated NBD1 is faster than that of wild-type. Therefore, regardless of whether the bound ATP at NBD1 is hydrolyzed or not, the release of the bound nucleotide from NBD1 may bring the molecule back to its original conformation and facilitate the protein to start a new cycle of ATP-dependent solute transport.

Keywords: Multidrug resistance-associated protein 1; Nucleotide binding domain; ATP binding; Dissociation of the bound ATP; ATP hydrolysis; ATP-dependent LTC4 transport

1. Introduction

The over-expression of either P-glycoprotein (ABCB1 or P-gp), breast cancer resistant protein (ABCG2 or BCRP) and/or multidrug resistance-associated protein 1 (ABCC1 or MRP1) confers cancer cells with resistance to a broad range of anticancer drugs. Although these ATP-binding cassette (ABC) transporters transport anti-cancer drugs out of cells in an ATP-dependent manner by utilizing their membrane-spanning domains and nucleotide binding domains (NBDs) [1–16], the means by which they pump drugs out of cells are different, i.e. for example, P-gp transports hydrophobic compounds directly [1–3], whereas MRP1 transports anionic conjugates, such as glutathione-S-conjugates [4–12]. Therefore it is reasonable to ask whether they share the same mechanism of coupling ATP binding/hydrolysis to anti-cancer drug transport.

In the extensively studied P-gp, its two NBDs have been shown to be functionally equivalent with identical ATP hydrolysis steps occurring alternately at each NBD [17,18]. If one NBD enters the transition-state conformation, the other site is prohibited from doing so [17,19,20]. The mutation of an essential residue in the Walker A motif in either NBD

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Abbreviations: P-gp, P-glycoprotein; MRP1, multidrug resistance-associated protein 1; NBD, nucleotide binding domain; LTC4, leukotriene C4; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; 8-N_3ATP, 8-azidoadenosine 5′ -triphosphate; Sf21, *Spodoptera frugiperda* 21

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completely abolishes ATP-dependent transport activity [21,22]. Similarly, the covalent modification of either site completely blocks the turnover of ATP [17,23]. Although some reports indicated that the two NBDs of P-gp were essential for its function but not entirely symmetric [24,25], most NBD1 segments could be replaced by homologous segments of NBD2 without a loss of P-gp function [26]. It has been clearly demonstrated that the two NBDs of MRP1 have different properties and functions [27–30]. Thus, P-gp and MRP1 may not share the same mechanism to couple ATP binding/hydrolysis to anti-cancer drug transport.

It has been reported that there are two independent ATP hydrolysis events in a single drug transport cycle by P-gp, one associated with drug transport and the other causing conformational resetting to the original state of the molecule [31,32]. In addition, these two NBD sites, with equal affinity for ATP, are recruited randomly [31,32]. The two ATP binding/hydrolysis sites of MRP1 seem not to be recruited in a random manner because photolabeling by the non-hydrolyzable [α-^{32}P]-8-N_3AMP–PNP occurred predominately at NBD1 in the absence of other nucleotides and was shifted to NBD2 by a low concentration of 8-N_3ATP, implying that NBD1 may have a higher affinity for nucleotide than NBD2 and the binding of 8-N_3ATP at NBD1 induces a conformational change of the molecule and enhances AMP–PNP binding at NBD2 [33]. Indeed, NBD1 of MRP1 has slightly higher affinity for ATP than NBD2 [34]. A similar conclusion can also be reached from experiments in which the N-proximal half and C-proximal half of MRP1 are expressed simultaneously in Sf21 cells and labeled with either [α-^{32}P]-8-N_3ATP or [γ-^{32}P]-8-N_3ATP on ice [27]. It is also clear that the two NBDs of other ABC proteins, including the sulfonylurea receptor (ABCC8 or SUR1) [35] and cystic fibrosis transmembrane conductance regulator (ABCC7 or CFTR) [36–38], have very distinctive properties.

The functioning of the acidic amino acid directly adjacent to the aspartic acid in Walker B motif as a catalytic

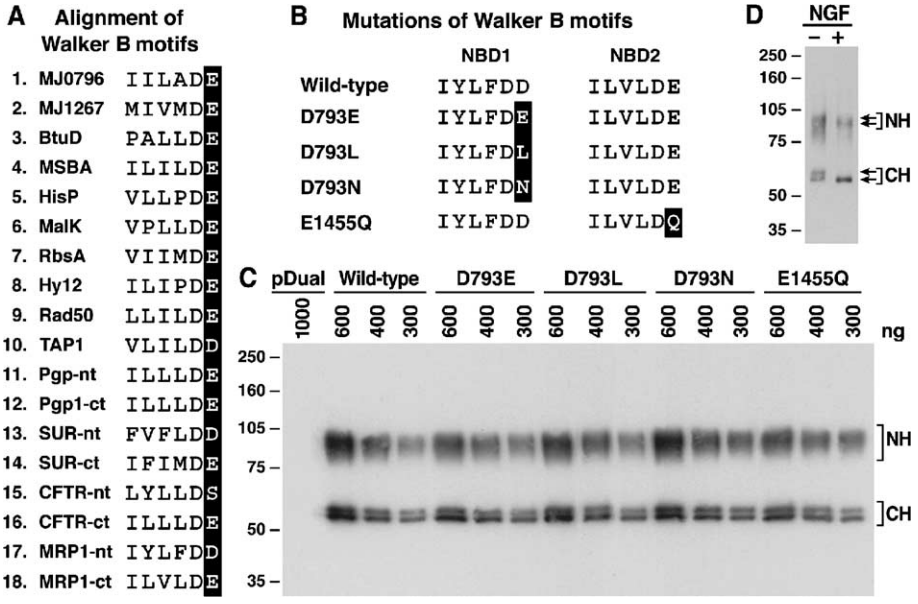

Fig. 1. Expression of wild-type and putative catalytic base mutant MRP1s in Sf21 insect cells. (A) Sequence alignment of Walker B motifs, including the acidic amino acid directly adjacent to the highly conserved D residue in Walker B motif, from some of the ABC transporters. The highlighted letters indicate the putative catalytic base interacting with the water molecule [39,40]. The terms nt and ct indicate N-proximal (NBD1) and C-proximal (NBD2) Walker B motifs. (B) Mutations of the putative catalytic bases in human MRP1 protein. The highlighted letters indicate the D to E, L and N mutations in NBD1 and E to Q mutation in NBD2. The definition of D793E means that the D793E mutated N-half is co-expressed with wild-type C-half and E1455Q, the wild-type N-half co-expressed with E1455Q mutated C-half. (C) Expression of wild-type and mutant variants of human MRP1 protein in Sf21 cells. Membrane vesicles were prepared from Sf21 cells infected with viral particles expressing pDual without MRP1 cDNA insertion (lane 1), wild-type N-half+wild-type C-half (Wild-type, lanes 2–4), D793E mutated N-half+wild-type C-half (D793E, lanes 5–7), D793L mutated N-half+wild-type C-half (D793L, lanes 8–10), D793N mutated N-half+wild-type C-half (D793N, lanes 11–13) and wild-type N-half+E1455Q mutated C-half (E1455Q, lanes 14–16). The amounts (ng) of membrane vesicle proteins loaded in the gel are indicated on top of the gel. The membrane proteins were resolved on a 7% SDS-PAGE, electroblotted to a nitrocellulose membrane and probed with MRP1-specific mAb 42.4 and 897.2 [28,29]. The molecular weight markers are indicated on the left. NH and CH on the right indicated the N-proximal half and C-proximal half of the MRP1 proteins. The intensities of the N-half and C-half bands were measured by a scanning densitometer. The ratios of the band intensities in the same amount of total membrane proteins, for example, 300 ng of wild-type N-half (co-expressed with wild-type C-half) versus 300 ng of the D793E mutated N-half (co-expressed with wild-type C-half), were determined, considering the amount of wild-type N-half (or C-half) as 1.000. Since the ratio of N-half, for example, D793E mutated N-half, is similar to that of the C-half co-expressed with D793E mutated N-half, the mean ratios of the protein expressions including N-half and C-half are: 0.993±0.168 (D793E), 0.991±0.073 (D793L), 1.151±0.186 (D793N) and 0.921±0.108 (E1455Q). (D) The lower mobility band of C-half is glycosylated whereas the higher mobility band of C-half is not. Membrane vesicles containing wild-type N-half and C-half were incubated in the absence (−) or presence (+) of *N*-glycosidase F and resolved (500 ng) on a 7% SDS-PAGE. The upper arrow in NH or CH indicates the glycosylated N-half or C-half, whereas the lower arrow in NH or CH indicates the de-glycosylated (or un-glycosylated) N-half or C-half.