Role of glycine-534 and glycine-1179 of human multidrug resistance protein (MDR1) in drug-mediated control of ATP hydrolysis

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The human multidrug resistance protein (MDR1) (P-glycoprotein), a member of the ATP-binding cassette (ABC) family, causes multidrug resistance by an active transport mechanism, which keeps the intracellular level of hydrophobic compounds below a cell-killing threshold. Human MDR1 variants with mutations affecting a conserved glycine residue within the ABC signature of either or both ABC units (G534D, G534V, G1179D and G534D/G1179D) were expressed and characterized in *Spodoptera frugiperda* (Sf9) cell membranes. These mutations caused a loss of measurable ATPase activity but still allowed ATP binding and the formation of a transition-state intermediate (nucleotide trapping). In contrast with the wild-type protein, in which substrate drugs accelerate nucleotide trapping, in the ABC

signature mutants nucleotide trapping was inhibited by MDR1-substrate drugs, suggesting a miscommunication between the drug-binding site(s) and the catalytic domains. Equivalent mutations of the two catalytic sites resulted in a similar effect, indicating the functional equivalence of the two sites. On the basis of these results and recent structural information on an ABC-ABC dimer [Hopfner, Karcher, Shin, Craig, Arthur, Carney and Tainer (2000) *Cell* **101**, 789–800], we propose a key role of these glycine residues in the interdomain communication regulating drug-induced ATP hydrolysis.

Key words: ABC signature, ATP-binding cassette, allosteric effect, nucleotide trapping, P-glycoprotein.

INTRODUCTION

The human multidrug resistance protein (MDR1) (P-glycoprotein) is a primary active transporter for hydrophobic drugs and causes multidrug resistance in cancer cells by maintaining the level of cytotoxic agents below a cell-killing threshold. MDR1 belongs to the superfamily of ATP-binding cassette (ABC) transporters [1–3]; it contains a tandem duplication of transmembrane domains ('TMD') with six transmembrane helices and ATP-binding (ABC) domains. The ABC domain harbours two short conserved peptide motifs (Walker A and Walker B) and a third conserved sequence called the 'ABC signature' motif, diagnostic for the entire superfamily. MDR1 exhibits a basal ATPase activity that is stimulated by the transported drug substrates [4]. The transport and ATPase cycle of MDR1 is inhibited by vanadate, which stabilizes a transition-state intermediate of the ATPase cycle, with an occluded nucleotide in the catalytic sites within the ABCs [5,6]. Beryllium fluoride (BeF_x) and AlF_4^- were also shown to inhibit the MDR1 ATPase catalytic cycle by trapping a nucleotide in the catalytic centre [7,8]. Similarly to the ATPase activity of MDR1, the rate of transition-state formation in the presence of the above anions is greatly accelerated by the transported drug substrates [9–11].

The mechanism by which the transported drugs enhance the catalytic activity is currently unknown. The site or sites interacting with the drug substrates are presumably encoded in the transmembrane domains [12,13], whereas the catalytic centres, which energize the transport, are localized in the ABC domains. Thus allosteric control of the drugs on the ATPase activity requires intramolecular interaction between the two regions of

the protein. Such intramolecular connections cannot be examined directly at present, because no high-resolution three-dimensional structure is available for any of the mammalian ABC transporters or for their separate transmembrane or ABC domains. A structure of MDR1, determined by electron microscopy and single-particle image analysis, has been published [14]. Because the resolution of this structure is only approx. 2.5 nm, details of intramolecular interactions cannot be analysed. As a major advance in the field, the crystal structures of two bacterial ABC subunits, those of the ribose transporter system (RbsA-N) [15] and the enterobacterial histidine permease (HisP) [16], have been solved. These structures permitted the identification of several peptide motifs and residues that interact directly with ATP or Mg^{2+} .

ABC transporters show co-operative ATP hydrolysis: in MDR1 both catalytic sites are active and their interaction is essential for the drug-stimulated ATPase and drug-transport activity [17]. A major limitation of both bacterial structures is that the functional co-operativity between the two ABC subunits cannot be studied: RbsA-N was crystallized as a monomer, whereas the ATP-interacting sites (active sites) in the HisP dimer are in an exposed peripheral location, practically excluding direct interaction between the two sites. Furthermore, the nucleotide-binding sites in both structures appear as a shallow, more or less open groove, which would form a rather atypical active site.

The three-dimensional structure of another bacterial ABC domain has also been solved recently [18]. Rad50 has a key role in double-stranded DNA repair; its catalytic ABC ATPase domain (Rad50cd) harbours the three peptide motifs conserved

Abbreviations used: ABC, ATP-binding cassette; BeF_x , beryllium fluoride (the exact composition of beryllium fluoride is not known); HisP, histidine permease (ATPase subunit of the maltose-transport system of enterobacteria); MDR1, human multidrug resistance protein; Rad50cd, catalytic domain (a dimer of the ATPase subunits) of Rad50; RbsA, ATPase subunit of the ribose-transport system of enterobacteria; Sf9 cells, *Spodoptera frugiperda* ovarian cells.

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within the family. The ATP-hydrolysing sites (catalytic sites) in this dimer are completely buried, resembling the nucleotide-binding sites of other enzymes. The major breakthrough in this work is that the dimer structure represents two functionally interacting ABC subunits, in which each of the two ABC domains complements the other's active site. The structure also shows that two highly conserved residues of the ABC signature motif [LSGG (single-letter amino acid codes)], with a serine residue in the second position and a glycine residue in the fourth, interact with the oxygen of the γ -phosphate of the ATP bound in the active site(s). The conservation of the glycine residue in the fourth position of the ABC signature region is almost universal in the family.

To study the role of the glycine residue in the identical position of the MDR1 signature region in each ABC domain (Gly-534 and Gly-1179), we have generated MDR1 variants containing a valine or aspartic residue at these positions. We found that these mutations abolished the ATPase activity of the protein, but still permitted the formation of a transition-state intermediate of the ATP hydrolysis cycle. In these mutants, in contrast with that in the wild-type MDR1, the transported drug substrates inhibited this partial reaction. These results suggest a key role for the conserved glycine residue in the drug-induced allosteric control of the ATP hydrolysis by MDR1.

MATERIALS AND METHODS

Construction of the recombinant transfer vectors

The recombinant baculovirus transfer vector carrying the G534D-MDR1 variant was constructed previously [19]. Additional constructs were prepared as follows. For convenient cloning of various cDNA constructs, the baculovirus transfer vector pAcUW21 was modified. A linker (L, 5'-AGATCCTCTAGAAGATCTGCGGCCGAGCTCAAATTC-3') was ligated to the *Bgl*II-*Eco*RI site of pAcUW21. pAcUW21-LMDR1 was constructed by removing the full-length human MDR1 cDNA from pVL941MDR1 by digestion with *Bam*HI and ligating the resulting fragment to the *Bgl*II site of pAcUW21-L. Mutagenesis was performed by overlap-extension PCR [20] with pAcUW21-L as template. Two internal complementary primers were used, each containing the specific mutation (G1179D, 5'-TCTGGTGATCAGAAACAACGCAT-3'). The internal primers were used in conjunction with two outer primers: mls1122 (containing the unique restriction site for *Pst*I, coding strand, 5'-TGTTTGACTGCAGCATTGCT-3') and pacuw (non-coding strand of pAcUW21-L, 5'-CCAACTGGTAATGTAGCGA-3'). Reaction I contained 1 μ M of mls1122 and the non-coding strand mutagenic primer. Reaction II contained 1 μ M of pacuw and the coding mutagenic primer. The PCR reaction was performed with Vent *Taq* Polymerase (Biolabs) on 0.5 μ g of template DNA. The reaction was started by the addition of 2 units of Vent *Taq* polymerase at 94 °C (hot start). After 20 cycles (94 °C for 40 s, 56 °C for 1 min, 72 °C for 1 min) the samples were incubated at 72 °C for 10 min. Equimolar amounts of products from reactions I and II served as templates in reaction III, which contained the outer primers (mls1122 and pacuw). The PCR reaction was performed as described above. The product and the pAcUW21-LMDR1 vector were digested with *Not*I and *Pst*I, and the product was ligated into the vector, resulting in pAcUW21-L-G1179D. The mutation was confirmed by sequencing the *Pst*I-STOP fragment from the recombinant baculovirus as described [19]. The pAcUW21-G534D/G1179D construct was engineered by replacing the 1177–3372 *Eco*RI-*Pst*I fragment of pAcUW21-G534D with that of pAcUW21-L-G1179D.

Generation of recombinant baculoviruses

Recombinant baculoviruses carrying different MDR1 cDNA species were generated with the BaculoGold Transfection Kit (PharMingen), in accordance with the manufacturer's protocol. Sf9 (*Spodoptera frugiperda* ovarian) cells were infected and cultured as described [19]. Individual clones expressing a high level of the MDR1 variants were obtained by endpoint dilution and subsequent amplification.

Membrane preparation, ATPase activity and labelling with [α - ^{32} P]8-azido-ATP

Virus-infected Sf9 cells were harvested, their membranes were isolated and the membrane protein concentrations were determined as described [19]. Membranes were kept at –80 °C and were used within 6 months of preparation. Drug-stimulated ATPase activity and [α - ^{32} P]8-azido-ATP binding to MDR1 in the isolated membranes were measured as described [11]. In the nucleotide trapping experiments, isolated Sf9 cell membranes were incubated for 2–10 min at 37 °C in a reaction buffer with or without a trapping agent (Na_3VO_4 , BeF_x or AlF_4^-) and 0.1–0.2 MBq of [α - ^{32} P]8-azido-ATP (666 GBq/mM; ICN Biomedicals), as described [9,11]. The reaction was stopped by the addition of 500 μ l of ice-cold buffer containing 10 mM MgATP; the membranes were washed twice in the same buffer and then irradiated with UV at 4 °C. The labelled samples were run on 7.5% Laemmli-type SDS-containing gels, the proteins were electroblotted to PVDF membranes, and the blots were dried and subjected to quantitative autoradiography in a Phosphor-Imager (Bio-Rad). The identity of the ^{32} P-azido-nucleotide-labelled bands was confirmed by immunostaining of the same blot. Immunoblotting of MDR1, with 4077 as a first antibody and peroxidase-conjugated anti-rabbit IgG (Jackson ImmunoResearch) as a secondary antibody, was performed as described [19]. The experiments were conducted in triplicate.

Limited digestion with trypsin

Crude membrane preparations containing MDR1 and its mutants were subjected to mild digestion with trypsin after [α - ^{32} P]8-azido-ATP labelling. The reaction was performed in a buffer containing 40 mM Mops/Tris, pH 7.0, 50 mM KCl, 0.5 mM EGTA and 2 mM dithiothreitol. The reaction was started with the addition of 20 μ g/ml trypsin. The samples were kept on ice for 10 min, at which point the reaction was stopped by the addition of excess soybean trypsin inhibitor. The membranes were washed in the above buffer, collected in electrophoresis buffer and subjected to electrophoresis and immunoblotting. The identities of the N- and C-terminal fragments were confirmed by immunostaining with antibodies 4077 and 4007 respectively.

RESULTS

We have shown previously that several MDR1 variants, containing mutations in the ABC signature motif of the N-proximal half of human MDR1, can be expressed in Sf9 cells [19]. In the same study we found that the MDR1 variant G534D was expressed at a level comparable with the wild-type protein, whereas the expression of a similar variant (G534V-MDR1) was not detected. In the present study, with careful selection and subsequent amplification of individual clones, we were able to express high levels of the G534V mutant as well (Figure 1). Figure 1 also shows that both the G1179D mutant (affecting the equipositional glycine in the C-terminal ABC unit) and the variant containing aspartic residues at both sides (G534D/

Figure 1 Expression of the MDR1 signature mutants

Isolated Sf9 cell membranes (10 μ g) expressing G534D-MDR1 (lane 1), G1179D-MDR1 (lane 2), G534D/G1179D-MDR1 (lane 3), G534V-MDR1 (lane 4) or wild-type MDR1 (lane 5) were run on SDS/7.5% (w/v) PAGE gels. Immunoblotting of MDR1, with 4077 as a first antibody, was performed as described in [19]. The arrow indicates the position of human MDR1 on the blot. The positions of molecular-mass markers are indicated (in kDa) at the right.

G1179D) could be expressed efficiently in Sf9 cells. All the mutants analysed in this study were found to be defective in ATP hydrolysis. We found that neither the basal nor the drug-stimulated ATPase activity of any of the mutants was significantly greater than background, represented by control membranes expressing β -galactosidase (results not shown).

To examine whether the loss of ATPase activity was due to an impaired binding of ATP, we performed photoaffinity-labelling experiments with [α - ^{32} P]8-azido-ATP (Figure 2). Isolated Sf9 membranes containing different MDR1 variants were incubated on ice in the presence of 5–20 μ M [α - ^{32} P]8-azido-ATP and 2 mM MgCl_2 . After cross-linking with UV, followed by gel electrophoresis and immunoblotting, radioactive bands corresponding to the MDR1 variants were observed in all mutants. Quantification of the radioactive signal associated with the individual bands showed that the amount of radioactivity incorporated was similar in the mutants and in the wild-type MDR1 protein. The radioactive label of the bound ATP was eliminated by pretreatment with 1 mM unlabelled MgATP (Figure 2, lane 4) and was absent from control membranes expressing β -galactosidase (Figure 2, lane 5). The presence of transported drug substrates did not have any effect on ATP binding (results not shown).

In the next set of experiments we addressed the question of whether transition-state complexes can be formed by these mutants when vanadate, AlF_4^- or BeF_x is present as a stabilizing anion. Trapping was performed under conditions that ensured labelling of the wild-type MDR1 below saturation (2 min incubation at 37 $^\circ\text{C}$ in the presence of vanadate; 10 min incubation at 37 $^\circ\text{C}$ in the presence of AlF_4^- or BeF_x at 20 μ M [α - ^{32} P]8-azido-Mg-ATP). We observed nucleotide trapping in mutants G534D,

Figure 2 Binding of [α - ^{32} P]8-azido-ATP by the MDR1 signature mutants

ATP-binding was performed with isolated Sf9 cell membranes (100 μ g) expressing G534V-MDR1 (lane 1), wild-type MDR1 (lanes 2 and 8), G534D-MDR1 (lanes 3 and 4), β -galactosidase (lane 5), G534D/G1179D-MDR1 (lane 6) or G1179D-MDR1 (lane 7). Lane 4 shows the effect of pretreatment with 1 mM unlabelled ATP. The arrow indicates the position of human MDR1 on the immunoblot. The positions of molecular-mass markers are indicated (in kDa) at the right.

Figure 3 Transition-state formation (nucleotide trapping) in the ABC signature mutants

Autoradiogram of the isolated Sf9 membranes labelled with [α - ^{32} P]8-azido-Mg-ATP. Membranes expressing G534D/G1179D-MDR1 (lanes 1 and 2), G534V-MDR1 (lanes 3–5), G534D-MDR1 (lanes 6–8) or G1179D-MDR1 (lanes 9–11) were incubated at 37 $^\circ\text{C}$ for 10 min in the presence of 20 μ M [α - ^{32} P]8-azido-Mg-ATP as described in the Materials and methods section. Additional reagents were vanadate (lane 1) AlF_4^- (lanes 2–11), 500 μ M *N*-ethylmaleimide (lanes 4, 7 and 10) and 2 mM EDTA (lanes 5, 8 and 11). The arrow indicates the position of human MDR1 on the immunoblot. The positions of molecular-mass markers are indicated (in kDa) at the right.

G534V and G1179D in the presence of AlF_4^- (see Figure 3) or BeF_x (results not shown). Labelling of the ABC signature mutants was weaker than that of the wild-type MDR1 (40–60% as determined by quantitative autoradiography; results not shown) but, similarly to the wild-type, labelling was not affected by washing the membranes with 10 mM MgATP after incubation with 8-azido-MgATP. The reactions required the presence of Mg^{2+} and were eliminated by pretreatment with 500 μ M *N*-ethylmaleimide, indicating that these mutants are engaged in a genuine MDR1-like catalytic reaction (Figure 3). Interestingly, neither G534D-MDR1 nor G1179D-MDR1 was labelled in the presence of vanadate, not even at higher azido-ATP concentrations and longer incubation times (up to 100 μ M and 10 min respectively), whereas G534V showed vanadate-induced nucleotide trapping activity (results not shown). The double mutant (G534D/G1179D-MDR1) showed no nucleotide trapping activity in the presence of either vanadate or AlF_4^- (Figure 3).

Similarly to the ATPase activity of MDR1, the rate of the vanadate-dependent [9,10] and AlF_4^- - or BeF_x -dependent [11] nucleotide occlusion in the wild-type protein is greatly accelerated by the transported drug substrates. We found that the key difference between the wild-type MDR1 and the ABC signature mutant proteins was that in these latter cases the MDR1 substrates had a negative effect on the formation of the transition-state intermediates, irrespective of the stabilizing anion used. The addition of 36 μ M verapamil (a strong stimulator of the MDR1 ATPase activity) significantly inhibited the rate of formation of the catalytic intermediates in the ABC signature mutants (Figure 4). Figure 4 shows that, for the wild-type MDR1, the rate of nucleotide trapping was accelerated approx. 4-fold. Conversely, the same reaction with the ABC signature mutants G534D (ratio 0.32), G534V (ratio 0.41) and G1179D (ratio 0.48) was inhibited by verapamil (36 μ M). The ratio of incorporated radioactivity was determined by quantitative autoradiography with a PhosphorImager in trapping experiments ($n = 5$ –8) performed in the presence or in the absence of 36 μ M verapamil.

Inhibition persisted over a wide range of azido-ATP concentration (5–50 μ M) and was observed in the presence of other drugs (calcein-AM, vincristine, cyclosporin A; results not

Figure 4 Effect of verapamil on transition-state formation (nucleotide trapping) in the presence of AlF_4^-

Labelling was performed with isolated Sf9 cell membranes (100 µg) expressing wild-type MDR1 (lanes 1 and 2), G534V-MDR1 (lanes 3 and 4), G534D-MDR1 (lanes 5 and 6) or G1179D-MDR1 (lanes 7 and 8) as described in the Materials and methods section. In some experiments the incubation medium contained 36 µM verapamil (lanes 2, 4, 6 and 8). The arrow indicates the position of human MDR1 on the immunoblot. The positions of molecular-mass markers are indicated (in kDa) at the right.

Figure 5 Limited proteolysis of the ABC signature mutants after nucleotide trapping in the presence of AlF_4^-

Labelling was performed with isolated Sf9 cell membranes (200 µg) expressing wild-type MDR1 (lane 1), G534D-MDR1 (lane 2), G534V-MDR1 (lane 3), G1179D-MDR1 (lane 4) or β -galactosidase (lane 5). Nucleotide trapping, photaffinity labelling and treatment with trypsin were performed as described in the Materials and methods section. Immunostaining of the same blot revealed no difference between the three MDR1 mutants and the wild type in sensitivity to trypsin. The arrows indicate the position of human MDR1 and the tryptic fragments containing the N-terminal and C-terminal halves. The positions of molecular-mass markers are indicated (in kDa) at the left.

shown). 5-Fluorouracil, a cytotoxic drug that is not transported by MDR1, had no effect on this reaction.

To assess the participation of the individual halves of the mutant MDR1 variants, we performed digestion with trypsin after nucleotide trapping and UV cross-linking of the labelled nucleotide to the MDR1 protein (see the Materials and methods section). Limited trypsin digestion of human MDR1 cleaves the protein into two major products, corresponding to the N- and C-terminal halves, each containing an intact ABC domain [21]. In our hands, distribution of the ^{32}P label after nucleotide occlusion by the wild-type MDR1 was nearly symmetrical, with a slight C-terminal preference, over a wide range of azido-ATP concentrations (2.5–50 µM), regardless of the choice of stabilizing anion [11]. It should be noted that the radioactivity associated with the bands corresponding to the N- and C-termini might not be a quantitative measure of the actual site occupancy, owing to possible artifacts during manipulations [8]. Nevertheless, as shown in Figure 5, catalytic intermediates stabilized by AlF_4^- were formed in both ABC domains of the G534D, G534V and the G1179D mutants (BeF₃⁻ gave similar results; results not shown). The characteristic C-terminal preference of the radioactive label seen in the wild-type MDR1 [8,11] was more

pronounced in the ABC signature mutants. However, labelled N-halves were always seen in the ABC signature mutants (Figure 5, lanes 1–4), whereas no radioactive band was observed in the corresponding position in control membranes expressing β -galactosidase (Figure 5, lane 5).

DISCUSSION

The ABC signature region is a short peptide motif conserved exclusively in ABC proteins. In the present study we have analysed the role of a particularly well conserved glycine residue at the N-terminal LSGGQ segment (shown here in **bold**) of the ABC signature region. Mutations at this position have been shown to impair the function of several ABC transporters seriously [22,23]. The MDR1 ABC signature variants could be expressed at high levels in Sf9 cells, but showed a complete loss of drug-stimulated ATPase activity.

Here we show that the ATPase-negative MDR1 variants can nevertheless enter the MDR1 hydrolysis cycle and are able to form certain transition-state intermediates, indicating that the mutated residues are not required for catalysis.

The analysis of the formation of the transition-state complexes allowed us to investigate the role of the conserved glycine residue in the catalytic activity of the transporter. In wild-type MDR1 the initial rate of formation of a catalytic intermediate (i.e. the transition state with an occluded adenine nucleotide in the catalytic centre) is accelerated on the binding of drugs [9–11], as with stimulation of the ATPase activity by drugs [4]. The accelerating effect of drugs on the rate of transition-state formation is preserved even in the ATPase-negative Walker A mutants, which suggests that the intramolecular communication network between the site(s) interacting with the transported drug and the catalytic site(s) is not affected by the Walker A residues [11].

We show here that ABC signature mutations (at either ABC domain) alter the effect of MDR1 drug substrates on the formation of the transition state (Figure 4). In MDR1 the drug-binding sites were shown to be localized in the transmembrane regions [12]; it has also been shown that the nucleotide-binding (ABC) domains are not required for the interaction of MDR1 with its drug substrates [13]. We found that the inhibitory effect of drugs on nucleotide trapping in the ABC signature mutants is not due to a decreased binding of ATP (Figure 2). In the light of these observations, it seems unlikely that there is any direct effect of the mutations analysed in this study on drug binding.

The fact that unilateral mutations (directly or indirectly) perturb ATP hydrolysis at both catalytic sites both supports the model in which the two ABC domains form a tightly coupled catalytic structure and indicates that the functional interaction between the two catalytic sites is preserved in the ABC signature mutants.

However, our results suggest a miscommunication between the drug-binding site(s) and the catalytic apparatus of MDR1 in the ABC signature mutants.

The three-dimensional structures of the ABC domain of the two bacterial transporters HisP [16] and RbsA [15] do not provide a structural basis for understanding the above results. In both of these structures the evolutionarily conserved ABC signature region was found to be located relatively far from the catalytic site within the same subunit, at a distance of more than 15 Å. No direct interaction between the elements of the ABC signature and the bound nucleotide was observed.

At present there are two models for the dimeric arrangement of the ABC domains in MDR1. One predicts that the MDR1-ABCs adopt the dimeric arrangement found in the HisP crystal

structure, with the monomers arranged in a back-to-back position. The other model, proposed on the basis of the crystallographic study of an alternative HisP dimer and sequence analysis [24], suggests that the ABCs dimerize in such a way that each signature sequence binds to the ATP in the opposite monomer.

This latter model is strongly supported by the dimeric structure of Rad50cd, which represents two functionally interacting ABC subunits [18]. In the ATP-bound form, both subunits bind one MgATP^{2-} molecule and the two ATP molecules are buried in the dimer interface. In this dimeric structure each of the two ABC subunits complements each other's active site: the Walker A segment (P-loop) of one Rad50cd subunit and the ABC signature motif of the opposite Rad50cd subunit are involved in the formation of a single ATP-binding site. The ATP-hydrolysing sites (catalytic sites) in this dimer are completely buried, resembling the nucleotide-binding sites of other ATPases/GTPases. Within this structure the two highly conserved residues at the N-terminal part of the signature motif, a serine residue and a glycine residue in the second and fourth positions in the LSGG segment, interact with the oxygen of the ATP γ -phosphate. Moreover, helix F of the Rad50cd structure (which holds a large portion of the signature motif and is directly connected to the LSGG segment) makes a 30° rotation, resulting in a $9 \text{ \AA}$ displacement of the ATP-interacting residues of the LSGG motif towards the bound ATP molecule within the opposite subunit. This movement, which might be a general feature in ABC proteins, provides the structural requirement for the completion of the catalytic site.

The loss of ATPase activity from the mutants analysed in the present study supports the notion that the signature regions participate in the formation of the catalytic sites of MDR1. Our results also show that mutations of the conserved glycine residue in the fourth position of the LSGG motifs of either ABC domain resulted in an error in the allosteric control of the ATPase activity on drug binding. A similar role of the ABC signature motif in the allosteric activation of the MalK ATPase, the ABC subunit of the bacterial maltose transport system, has recently been proposed [25]. One possible explanation of our finding is that the characteristic rotation of the ABC signature motif found in the Rad50cd dimer is an element of the drug-induced allosteric control of MDR1-ATPase activity. The resulting conformational change ensures the precise geometry of the active catalytic site(s) in the wild-type protein, but further decreases the possibility of effective interaction with the ATP substrate when the crucial glycine residue is mutated. We suggest that the conserved glycine residue of the ABC signature region is a endpoint of an intramolecular communication system within the MDR1 transporter and that this might be a general feature of action of the members of the ABC protein family.

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