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Structural insight into substrate and inhibitor discrimination by human P-glycoprotein

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Abstract

ABCB1/P-glycoprotein actively extrudes xenobiotic compounds across the plasma membrane of diverse cells, which contributes to cellular drug resistance and interferes with therapeutic drug delivery. We determined the 3.5 Å cryo-EM structure of substrate-bound human ABCB1 reconstituted in lipidic nanodiscs, revealing a single molecule of the chemotherapeutic compound paclitaxel bound in a central, occluded pocket. A second structure of inhibited, human-mouse chimeric ABCB1 revealed two molecules of zosuquidar occupying the same drug-binding pocket. Minor structural differences between substrate- and inhibitor-bound ABCB1 sites are amplified towards the NBDs, revealing how the plasticity of the drug-binding site controls the dynamics of the ATP-hydrolyzing NBDs. Ordered cholesterol and phospholipid molecules suggest how the membrane modulates the conformational changes associated with drug binding and transport.

ABCB1/P-glycoprotein is an ATP binding Cassette (ABC) transporter of significant physiological and clinical relevance. Its nucleotide binding domains (NBDs) harness the energy of ATP hydrolysis to generate conformational changes in the transmembrane domains (TMDs) that facilitate the shuttling of chemically diverse compounds across many blood-organ barriers (1–4). Consequently, ABCB1 activity can confer multidrug resistance to cancer cells and prevent drugs from reaching therapeutic concentrations in target cells or organs, complicating chemotherapy and/or the treatment of certain neurological disorders. Despite showing promise in model systems (5–7), chemo-sensitization of multidrug-resistant cells through the simultaneous delivery of ABCB1 inhibitors (e.g. the third-generation

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Author contributions:

A.A. carried out all experimental procedures related to protein expression and purification and EM sample preparation. A.A. prepared cryo-EM grids and A.A. and J.K. collected and processed EM data. E.B. and I.B.R. provided UIC2-producing hybridoma cells and helped analyze data. K.P.L. and A.A. conceived the project and planned the experiments. A.A. and K.P.L. wrote the manuscript with input from all authors.

Competing interests:

The authors declare no competing interests.

Data and Materials availability:

All data is available within the main text or supplementary materials section. Cryo-EM maps and refined coordinates for deposited models are available at the Electron Microscopy Data Bank with accession codes EMD-4539, EMD-4540, and EMD-4541 (maps 1, 2, and 3 of Taxol-bound ABCB1, respectively) and EMD-4536 (zosuquidar-bound ABCB1). Coordinates for deposited models are available at the Protein Data Bank with IDs 6QEX (Taxol-bound ABCB1) and 6QEE (zosuquidar-bound ABCB1).

inhibitor zosuquidar) and chemotherapeutic drugs (e.g. taxol/paclitaxel) has so far been clinically unsuccessful(8, 9). To understand its interaction with small-molecule compounds, rationalize its substrate specificity and the discrimination of substrates and inhibitors, and to facilitate the development of more specific or potent inhibitors for clinical use, structural insight into drug and inhibitor binding to ABCB1 is essential. No structures of ABCB1 bound to transport substrates are available at present, while inhibitor-bound and apo structures are only available for detergent-solubilized ABCB1 and remain controversial because proper ABCB1 function is strongly dependent on the membrane.

We reconstituted ABCB1 in nanodiscs comprising a mixture of brain polar lipids and cholesterol and determined near-atomic resolution cryo-EM structures in complex with taxol (3.6Å resolution) or zosuquidar (3.9Å resolution). In both cases, the antigen binding fragment (Fab) of the inhibitory antibody UIC2(10), shown to be compatible with inward-open and occluded conformations(11), was added (complex mass ~200kDa) to facilitate higher resolution structure determination.

Nanodisc-reconstituted wild-type human ABCB1 (ABCB1_H) displayed ATPase activity in the range of 200–400nMol ATP mg⁻¹min⁻¹, which was mildly stimulated by taxol and inhibited by zosuquidar (Fig. 1A), in agreement with earlier observations(12, 13). This suggested that at 10uM, the taxol concentration chosen for structural studies, a sufficiently large fraction of ABCB1_H molecules should contain bound drug. We observed two main conformations in our single particle cryo-EM analysis (Fig. S1). The highest-resolution structure (Fig. 1B) revealed an occluded conformation with density covering a single taxol molecule (Fig. 1C) in a central cavity formed by the closing of a gate region consisting of TM4 and TM10 (Fig. 1D). The NBDs were closer together than in previously determined, inward-open apo structures of mouse ABCB1(11, 14–16) and more closely resembled those of disulfide-trapped ABCB1_{hm} structures(11), despite the absence of nucleotides or disulfide crosslinking. The second conformation revealed a slightly larger separation of the NBDs, and poorly ordered TM4 and TM10 segments. In this conformation, the cytoplasmic gate to the drug-binding cavity is open. Our results demonstrate that binding of taxol to ABCB1 induces an occluded conformation and a concomitant closure of the inter-NBD gap, in line with earlier mutagenesis and biochemical work (17, 18). The central pocket of taxol-bound ABCB1 is lined by amino acid residues from all 12 TM helices. While the density for interacting residues was well defined, that of the taxol molecule was less clear, suggesting the possibility of multiple binding modes. The orientation of taxol shown in Fig. 1C and Fig. 1E had the strongest density assigned to the tetracyclic/baccatin III core with the cyclooctane ring in a crown conformation. The peripheral moieties displayed conformational heterogeneity and their placement was guided by fitting the Y shaped “tail” of the molecule to avoid steric clashes with neighboring side chains. Given its volume, only one taxol molecule can bind to the central cavity of ABCB1 and occlusion of the drug-binding pocket is triggered irrespective of which binding mode the molecule adopts. The drug-binding cavity of ABCB1 is globular in shape, in contrast to the flatter, slit-like drug-binding pocket previously visualized in the human multidrug transporter ABCG2(19, 20). This is in line with the finding that taxol cannot bind to ABCG2 or modulate its activity (21, 22). A comparison of the substrate/inhibitor bound structures of these two key human multidrug exporters therefore allows us to rationalize their divergent substrate specificities.

For the zosuquidar-bound structure, we used a hydrolysis-deficient EQ variant of chimeric ABCB1_{HM} (ABCB1_{HM-EQ}) and added ATP to ensure that zosuquidar had indeed trapped ABCB1 in an inhibited state, given that in the presence of ATP/Mg²⁺, this ABCB1 variant had been shown to adopt a closed NBD conformation coupled to a closed TMD conformation with a collapsed translocation pathway (23). Our zosuquidar-inhibited ABCB1_{HM-EQ} structure (Fig. 2A, Fig. S3–S5) revealed a similar overall conformation as taxol-bound ABCB1, with the TMDs forming an analogous occluded cavity, but containing two bound zosuquidar molecules. We again observed two main ABCB1 conformations, characterized by a distinct degree of NBD opening. However, unlike for taxol, both conformations revealed bound zosuquidar molecules. The density for zosuquidar was better defined than that for taxol and allowed unambiguous placement in a unique binding mode (Fig. S4, S5B–C). This likely stems from the increased contact area (buried surface) between the two zosuquidar molecules and ABCB1 (~1000 Å²), compared to taxol (~800 Å²) as well as the inter-molecular contact between the two zosuquidar molecules themselves (interface surface area of ~190 Å²). The orientation and binding interactions of the zosuquidar molecules are similar to those previously observed in the disulfide-trapped, detergent-solubilized ABCB1_{HM} structure, suggesting that the opposite effect of zosuquidar has on the ATPase rate of ABCB1 in lipid bilayers (reduction of ATPase activity) or detergent solution (stimulation) is not attributable to distinct binding sites of zosuquidar in these lipidic environments.

Zosuquidar and taxol binding to the same pocket raises the key question of how ABCB1 distinguishes transport substrates from inhibitors and how these compounds exert opposite effects on ATPase activity. To explain the polyspecificity of ABCB1, plasticity of the drug binding pocket in terms of side chain and backbone rearrangements to accommodate distinct substrates has often been invoked (24, 25). We therefore superimposed taxol-bound and zosuquidar-bound ABCB1 and found that the main chain and side chain conformations of the residues surrounding bound drugs are largely similar (Fig. 2B–D and Fig. S6). However, there were a number of small but significant structural differences localized primarily in TMD2 and NBD2. As seen in Fig. 2B, the subtle changes originate at the drug-binding site and are transmitted and amplified via the helix pair TM7–TM8 and TM12 to NBD2 (Fig. 2C–D). They lead to an outward shift of the intracellular helix 2 (coupling helix 1) in zosuquidar-bound ABCB1, increasing the distance between the NBDs and offering a plausible explanation for the reduced ATPase activity in the presence of zosuquidar. Thus, our results demonstrate that plasticity occurring within the confines of the occluded drug binding pocket can be linked to NBD movement and ATPase activity of ABCB1.

The function of ABCB1 is known to be modulated by the lipidic membrane. As was observed in the structures of nanodisc-reconstituted ABCG2 (19), we found ordered cholesterol and phospholipid molecules bound to the transmembrane region of ABCB1 (Fig. 3, Fig. S2). At the level of the outer membrane leaflet, a ring of ordered cholesterol molecules is bound to surface grooves on ABCB1, and specific interactions include hydrogen bonds with the hydroxyl group of cholesterol and stacking interactions to aromatic side chains, as observed for other cholesterol/protein interactions (26). At the level of the inner leaflet, density compatible with a bound phospholipid and cholesterol was observed in a membrane-exposed pocket near TM3, TM4, and TM6 (Fig. 3, left panel) and at the

pseudosymmetrically related site near TM9, TM10, and TM12 (Fig. 3, right panel). Because the phospholipid and cholesterol-binding sites are formed by the kinking of TM4 and TM10 in response to drug and inhibitor binding, our observation suggests a direct mechanism of ABCB1 modulation by inner leaflet lipids.

When combined with the previously reported structure of ATP-bound ABCB1_{EQ} (23), our results offer a structural mechanism both for drug extrusion in a transport cycle and competitive inhibition of this reaction by small-molecule inhibitors. Formation of the closed ATP-bound NBD dimer triggers conformational changes in TM4 and TM6 from TMD1 as well as the symmetrically related TM10 and TM12 from TMD2, generating a steric clash with bound drugs (Fig. 4A–B). This suggests that a peristaltic mechanism contributes to the extrusion of bound substrate during the transport cycle (Fig. 4C). Competitive inhibitors like zosuquidar likely function by arresting the transporter in an occluded conformation, thus 1) restricting access to the substrate binding site, 2) preventing NBD closure and consequently inhibiting ATPase activity, and 3) preventing a transition to an outward open state. Our data is in line with proposed mechanisms of relaying long range structural changes upon substrate/inhibitor binding to the NBDs(17) as well as subtle induced-fit type rearrangements of a dynamic binding pocket (18, 24). In contrast to transport substrates, where multiple holo and apo forms of ABCB1 likely co-exist, inhibitors have fewer or a single binding mode, more completely fill the drug-binding cavity, and form a larger number of contacts with ABCB1. This suggests that in a continuum of substrates and inhibitors, 3D shape-complementarity and the strength of the contacts rather than distinct binding sites are key determinants distinguishing substrates from inhibitors. While the functional modulation of ABCB1 by cholesterol and lipids has previously been demonstrated (12, 27–29) our results visualize specific interactions and binding sites in cholesterol-containing lipid bilayers.

Finally, given that the structures taxol- and zosuquidar-bound ABCB1 reveal critical interactions in a key state of the transporter, our results may allow medicinal chemists and computational biologists to exploit structural insight into ABCB1 to guide the design of drugs or inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

In vitro function and structure of nanodisc-reconstituted ABCB1. **A** Taxol- and zosuquidar-modulated ATPase activity ($n=3$, error bars indicate SD). **B** Ribbon diagram of human ABCB1 bound to taxol (green spheres). The N- and C-terminal halves of ABCB1 are colored yellow and orange, respectively with the UIC2 Fab shown in blue. **C** Close up of binding site showing side chains of residues within 5 Å of bound taxol (green sticks), viewed parallel to the membrane plane. EM density is shown as a blue mesh, contoured at 6 σ . **D** Ribbon representation of TM4 (yellow) and TM10 (orange) adopting a kinked conformation, with taxol located in the center of the occluded cavity. EM density after nanodisc subtraction is contoured at 8 σ . **E** Interactions between taxol and ABCB1 side chains. Non-bonded interactions are represented by spoked arcs and hydrogen bonds are indicated by dashed black lines.

Figure 2.

Comparison of zosuquidar- and taxol-bound ABCB1. **A** Cartoon of zosuquidar-bound ABCB1_{HM-EQ} structure with zosuquidar molecules shown as yellow and magenta spheres. The N- and C-terminal halves of ABCB1 are colored pink and blue. **B-D** Superposition of taxol-bound human ABCB1 (green ribbon) and zosuquidar-bound ABCB1_{HM-EQ} (magenta ribbon) with intracellular helices interacting with NBDs shown as cylinders. Zosuquidar molecules are shown in sphere representation.

Figure 3.

Phospholipids and cholesterol bound to ABCB1. Center panel: Surface representation of taxol-bound human ABCB1 showing bound lipid (PE, magenta spheres) and cholesterol molecules (purple spheres). Zoom in panels show details of binding sites of phospholipid (left panel, magenta sticks) and cholesterol (right panel, purple sticks) at the level of the inner leaflet, near the kinking TM helices TM4 and TM10. EM density (blue mesh) is contoured at 6σ .

Figure 4.

Proposed mechanism of P-glycoprotein / ABCB1 substrate transport and small-molecule inhibition. **A** Side view of select TM helices of ABCB1 after superposition of the drug-bound, occluded conformation (green and magenta ribbons for taxol-and zosuquidar-bound structures) with the previously reported, ATP-bound post-translocation state (dark grey ribbon). Taxol and zosuquidar molecules are shown as green and red spheres, respectively. **B** Same as **A** but viewed from the cytoplasm. **C** Schematic of proposed ABCB1 transport cycle in the presence of substrate (taxol, green star) and inhibitor (zosuquidar, red L-shape). ATP and ADP are indicated by T and D, respectively. Dashed lines in models represent ATP binding elements required for NBD dimerization and ATP hydrolysis. Major conformational states are represented by circled numbers. State 1: Apo state (pdb IDs 4M1M & 4QNH, among others); States 2 and 2': Drug-and inhibitor-bound, this study. State 3: Proposed outward-facing conformation based on Sav1866 structure(30). State 4: Collapsed post-translocation state (pdb ID 6C0V).