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Differential dynamics and direct interaction of bound ligands with lipids in multidrug transporter ABCG2

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ABCG2 is an ATP-binding cassette (ABC) transporter that extrudes a wide range of xenobiotics and drugs from the cell and contributes to multidrug resistance in cancer cells. Following our recent structural characterization of topotecan-bound ABCG2, here, we present cryo-EM structures of ABCG2 under turnover conditions in complex with a special modulator and slow substrate, tariquidar, in nanodiscs. The structures reveal that similar to topotecan, tariquidar induces two distinct ABCG2 conformations under turnover conditions (turnover-1 and turnover-2). μs-scale molecular dynamics simulations of drug-bound and apo ABCG2 in native-like lipid bilayers, in both topotecan- and tariquidar-bound states, characterize the ligand size as a major determinant of its binding stability. The simulations highlight direct lipid-drug interactions for the smaller topotecan, which exhibits a highly dynamic binding mode. In contrast, the larger tariquidar occupies most of the available volume in the binding pocket, thus leaving little space for lipids to enter the cavity and interact with it. Similarly, when simulating ABCG2 in the apo inward-open state, we also observe spontaneous penetration of phospholipids into the binding cavity. The captured phospholipid diffusion pathway into ABCG2 offers a putative general path to recruit any hydrophobic/amphiphilic substrates directly from the membrane. Our simulations also reveal that ABCG2 rejects cholesterol as a substrate, which is omnipresent in plasma membranes that contain ABCG2. At the same time, cholesterol is found to prohibit the penetration of phospholipids into ABCG2. These molecular findings have direct functional ramifications on ABCG2's function as a transporter.

ABC transporters | cryo-EM | molecular dynamics | lipids | membrane proteins

Significance

ABC transporters constitute a major class of multidrug resistance proteins, e.g., in cancer cells. Membrane lipids have been long known to influence The newly solved structures and functional dynamics of these proteins. Here, using cryo-EM studies of ABCG2, a prominent member of the ABC family, in its ligand-bound form and in its native turnover state, combined with extended molecular simulations, we show that lipids as well as the ligand size can impact the drug-binding mode of ABC transporters. Not only do we demonstrate that phospholipids from the cellular membrane can penetrate into the protein and directly interact with bound drugs, we also provide evidence for differential behavior of cholesterol which is not transported by ABCG2 but ironically needed for its proper transport function.

ABCG2 is a human ATP-binding cassette (ABC) transporter expressed in a variety of tissues and with broad substrate specificity. It exports a wide range of endobiotics and xenobiotics from the cell (1–3) and, thereby, plays a major role in the traffic and distribution of diverse compounds between different compartments of the human body. The ability of ABCG2 to extrude a wide spectrum of substrates is exploited by some cancer cells, which overexpress the transporter and thereby actively pump out various chemotherapeutic agents, a phenomenon generally referred to as multidrug resistance (MDR) (4–6).

ABCG2 functions as a homodimer, and its transport activity is mediated by large-scale conformational changes between the inward-facing (IF) and outward-facing (OF) states, in which the substrate-binding site is alternatively exposed to the cytoplasmic and extracellular sides of the membrane (7, 8). Structurally, ABCG2 is composed of two transmembrane domains (TMDs), which form the substrate-binding cavity and carry on the actual transport process, as well as two highly conserved nucleotide-binding domains (NBDs), which act as the engine for the transporter and drive the conformational changes required for active transport by binding to and hydrolyzing ATP (9–12).

High-resolution structures of ABCG2 have been resolved by cryoelectron microscopy (cryo-EM), shedding light on the protein's functional conformations arising during the transport cycle (13–17). The IF conformation reveals a slit-like binding cavity, acting as the binding site for both substrates and inhibitors (13, 14). The binding cavity accommodates flat, polycyclic, hydrophobic small molecules that stack in between two prominent phenylalanine side chains (5, 18, 19). Available structures of ABCG2 suggest that while substrates bind as a single copy, the size of the inhibitors might affect their binding stoichiometry; relatively small inhibitors (e.g., MZ29) bind in pairs, whereas larger inhibitors (e.g., MB136) occupy the binding pocket as a single copy (8, 14, 15, 17). Variable binding stoichiometry has also been observed for a few ligands of other ABCG transporters (20, 21).

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