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Interaction of LDS-751 with the drug-binding site of P-glycoprotein: A Trp fluorescence steady-state and lifetime study

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ABSTRACT

P-glycoprotein (ABCB1) is an ATP-driven efflux pump which binds drugs within a large flexible binding pocket. Intrinsic Trp fluorescence was used to probe the interactions of LDS-751 (2-[4-(4-[dimethylamino]phenyl)-1,3-butadienyl]-3-ethylbenzo-thiazolium perchlorate) with purified P-glycoprotein, using steady-state/lifetime measurements and collisional quenching. The fast decay component of P-glycoprotein intrinsic fluorescence (τ_1 = 0.97 ns) was unaffected by LDS-751 binding, while the slow decay component (τ_2 = 4.02 ns) was quenched by dynamic and static mechanisms. Both the wavelength-dependence of the decay kinetics, and the time-resolved emission spectra, suggested the existence of excited-state relaxation processes within the protein matrix on the nanosecond time-scale, which were altered by LDS-751 binding. The fast decay component, which is more solvent-exposed, can be attributed to cytosolic/extracellular Trp residues, while the slow decay component likely arises from more buried transmembrane Trp residues. Interaction of a drug with the binding pocket of P-glycoprotein thus affects its molecular structure and fast dynamics.

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Introduction

P-glycoprotein (Pgp; ABCB1),[1] a member of the ATP-binding cassette (ABC) superfamily [1], is a multidrug efflux pump for a large number of amphipathic chemotherapeutic drugs, peptides, and natural products, and is one cause of multidrug resistance (MDR) in human cancers [2–5]. Pgp is made up of two homologous halves, each comprising six transmembrane (TM) segments and one nucleotide-binding domain (NBD). The NBDs, located on the cytosolic side of the membrane, contain the consensus sequence Walker A and Walker B motifs, and the signature C motif that is characteristic of the ABC superfamily. Transport substrates are relatively hydrophobic, and interact with the TM regions of the protein in the cytoplasmic membrane leaflet [6–8]. Pgp is believed to act as a "vacuum cleaner" for amphipathic compounds that partition into the membrane interior [9] and may transport its substrates to the outer membrane leaflet, thus acting as a flippase [10,11]. A second class of compounds known as modulators can block drug efflux via Pgp [12], and appear to compete with substrates for binding and transport, although by an ill-defined mechanism.

Many compounds of diverse structural classes can interact with Pgp, which is a polyspecific transporter. By analogy to bacterial multidrug-binding transcriptional regulators [13], it was proposed that ligands are held in place within the substrate-binding pocket by extensive van der Waal's interactions, stacking interactions, and the hydrophobic effect [14]. Current models suggest the existence of a large flexible drug-binding region with several sub-sites, where drugs interact via multiple interactions by an induced fit type of mechanism [15,16]. The recent 3.8 Å X-ray crystal structure of mouse Pgp bound to two cyclic peptide inhibitors confirmed these ideas [17]. The large drug-binding cavity, which is located within the membrane regions of Pgp, has access to the inner leaflet. One structure shows a single molecule of the peptide RRR isomer (QZ59-RRR, cyclic-*tris*-(R)-valineselenazole) bound at a "middle" site in the center of the transporter. In contrast, two molecules of QZ59-SSS (cyclic-*tris*-(S)-valineselenazole) bind to Pgp, one at an "upper" site and one at a "lower" site, which both overlap with the middle site. Each drug molecule binds in a different orientation, and interacts with a different subset of aromatic and hydrophobic residues lining the cavity, which are contributed by the TM helices.

Intrinsic protein fluorescence, originating from the excitation of aromatic residues, has been an invaluable tool in structure–function studies, since it does not perturb the molecule under examination. Fluorescence spectroscopic methods can provide

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[1] *Abbreviations used:* ABC, ATP-binding cassette; CHAPS, 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate; FRET, Förster resonance energy transfer; GuHCl, guanidine hydrochloride; LDS-751, 2-[4-(4-[dimethylamino]phenyl)-1,3-butadienyl]-3-ethylbenzo-thiazolium perchlorate; MDR, multidrug resistant/resistance; NATA, *N*-acetyl-L-tryptophanamide; NBD, nucleotide-binding domain; Pgp, P-glycoprotein; QZ59-RRR, cyclic-*tris*-(*R*)-valineselenazole; QZ59-SSS, cyclic-*tris*-(*S*)-valineselenazole; TM, transmembrane; TRES, time-resolved emission spectrum/spectra.

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not only information about the structure of biomolecules (*e.g.* low resolution structural mapping, residue accessibility, etc.), but also insight into the environment and dynamics of the fluorescent probes being studied. Hamster Pgp (product of the *pgp1* gene) contains 11 Trp residues, whose location can be pinpointed using the X-ray crystal structure of the closely-related mouse protein. Fig. 1A shows the location of the Trp residues on a topological scheme of hamster Pgp, and Fig. 1B shows the exact location of the equivalent residues in the X-ray structure of mouse Pgp [17]. One residue is located in the cytoplasmic loop preceding TM1 (W44), two in intracellular loops (W159 and W800), one in TM2 (W133), one in TM4 (W229), one in TM5 (W312), two

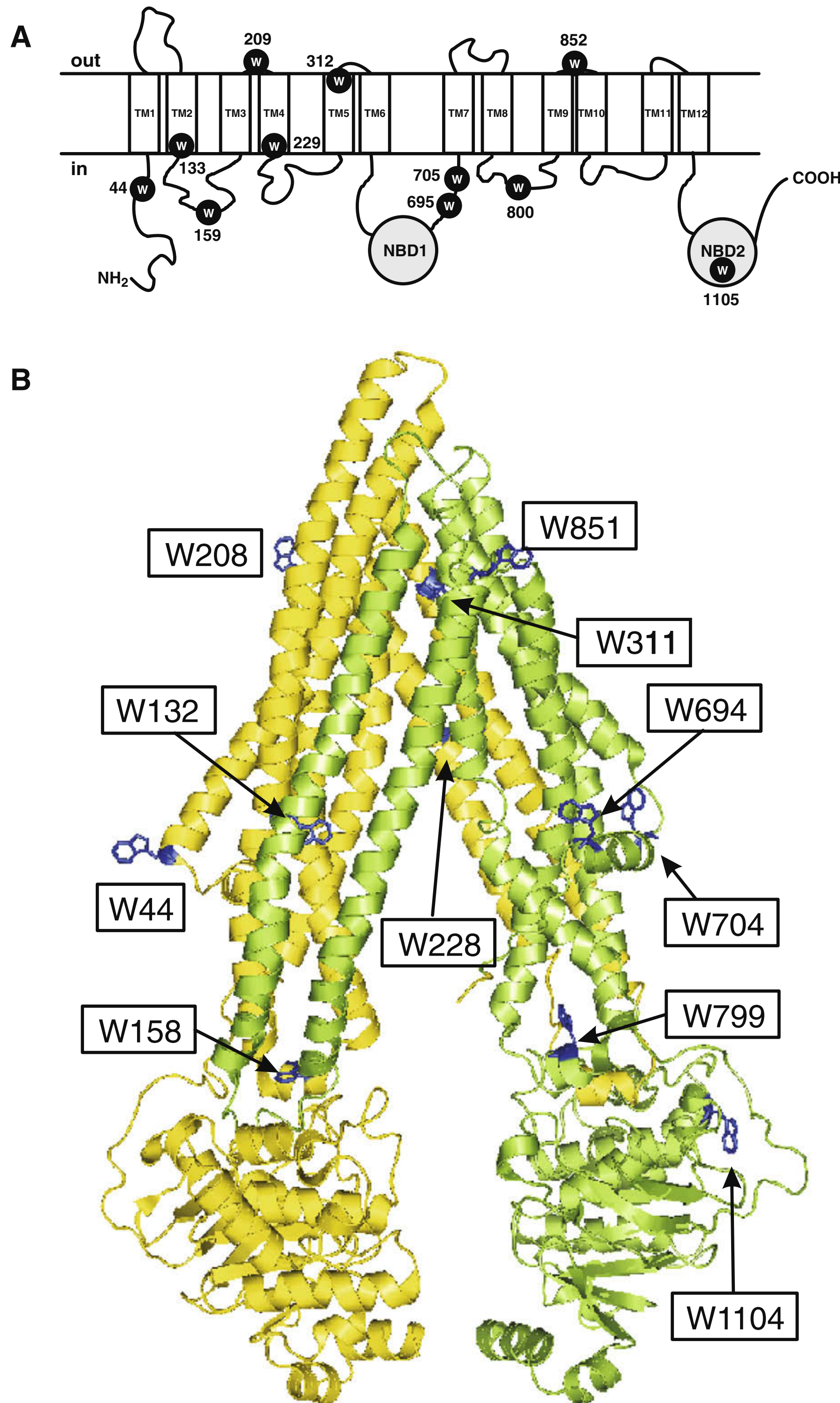

Fig. 1. (A) Topological model of hamster Pgp based on the mouse protein [17,50]. The 11 Trp residues are indicated, numbered according to the hamster amino acid sequence. (B) Side view of the X-ray structural model of mouse Pgp. The N-terminal NBD and TMD are shown in yellow, and the C-terminal NBD and TMD in green. The Trp residues are numbered and highlighted in blue. Structure was generated from pdb 3G5UB [17] using PyMOL. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

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