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Biochemical studies on the structure–function relationship of major drug transporters in the ATP-binding cassette family and solute carrier family☆

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ABSTRACT

Human drug transporters often play key roles in determining drug accumulation within cells. Their activities are often directly related to therapeutic efficacy, drug toxicity as well as drug–drug interactions. However, the progress for interpretation of their crystal structures is relatively slow. Hence, conventional biochemical studies together with computer modeling became useful manners to reveal essential structures of these membrane proteins. Over the years, quite a few structure–function relationship information had been obtained for members of the two major transporter families: the ATP-binding cassette family and the solute carrier family. Critical structural features of drug transporters include transmembrane domains, post-translational modification sites and domains for cell surface assembly and protein–protein interactions. Alterations at these important sites may affect protein stability, trafficking to the plasma membrane and/or ability of transporters to interact with substrates.

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Abbreviations: ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; BODIPY-prazosin, boron-dipyrromethene-Prazosin; BSP, bromsulphthalein; CCK8, cholecystokinin octapeptide; CsA, cyclosporine A; DEPC, diethyl pyrocarbonate; DOG, *sn*-1,2-dioctanoylglycerol; $E_2$17βG, 17-β-(D-glucuronide); ES, estrone-3-sulfate; FSBA, 5′-fluorosulfonylbenzoyl 5′-adenosine; Gly-Sar, glycylsarcosin; IKEPP, intestinal and kidney-enriched PDZ protein; LTC_4, leukotriene C4; MDR, multidrug resistance; MPP, methyl-4-phenylpyridinium; MRP, multidrug resistance associated protein; MSD, membrane spanning domain; MTSES, sodium (2-sulfonatoethyl) methanethiosulfonate; MTSET, (2(trimethylammonium)-ethyl) methane-thiosulfonate bromide; MTX, methotrexate; MX, mitoxantrone; NBD, nucleotide-binding domains; NHERF, Na^+/H^+ exchanger regulatory factor; PAH, *p*-aminohippurate; PDZ, postsynaptic density 95/disc-large/zona occludens; P-gp, P-glycoprotein; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; SGK1, serum and glucocorticoid inducible kinase 1; SLC, solute carriers; TEA, tetraethylammonium; TMD, transmembrane domains; TM, transmembrane segments.

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1. Introduction

Drug transporters are membrane proteins that mediate the efflux and uptake of wide ranges of therapeutic agents. Over the years, it has been more and more realized that these proteins are important in the adsorption, distribution, metabolism and excretion (ADME) of various clinical drugs. Drug transporters are key determinants of drug accumulation within cells and their activities are often directly related with therapeutic efficacy, drug toxicity and drug–drug interactions. Drug transporters are also considered as novel targets for drug design and important factors for interpretation of inter-individual difference in response to drugs [1].

Generally drug transporters can be molecularly and mechanistically classified as two families: the ATP-binding cassette (ABC) family and the solute carrier (SLC) family. ABC transporters are primary active transporters that require ATP hydrolysis for substrate transport and they are mainly efflux transporters, mediating the transfer of drugs out of the cells; while the SLC family members utilize an electrochemical potential difference or an ion gradient generated by primary active transporters for the transport of substrates and are thus categorized as facilitated transporters or secondary active transporters [2].

Although many high resolution crystal structures of soluble proteins have been revealed in recent years, the progress for interpreting the crystal structures of membrane proteins is relatively slow. This may be due to several reasons: the amphipathic nature of their surface structure, low concentrations presented in tissues and their inherent conformational flexibility that make it difficult to obtain stable crystals of the transporter proteins [3], hence homology modeling that relies on homologous proteins with known structures (mostly bacterial membrane proteins), computer-based hydropathy analysis to identify putative transmembrane domains, conventional biochemical studies such as cysteine or alanine scanning, in which individual or multiple amino acid residues are replaced with cysteine or alanine and the transport function and/or protein expression of the corresponding mutants are analyzed, construction of chimeric proteins with homologous recombination in order to identify discrete regions involved in substrate specificity of transporters in the same family have been utilized for revealing the critical structures of these membrane proteins and quite a few structure–function relationship information had been obtained. Critical structural features of transporters include transmembrane domains, post-translational modification sites and domains for cell surface assembly and protein–protein interactions. Changes at these essential sites may alter stability of the transporter proteins, their trafficking to the cell membrane and/or their ability to interact with substrates.

2. Functionally important amino acid residues in transmembrane segments of drug transporters

Transmembrane segments (TMs) are crucial structural features of membrane proteins. It has been demonstrated by various reports that TMs may be critical for substrate binding and correct processing of the transporter protein. Studies on single nucleotide polymorphism (SNP) also pointed out that mutants located within TMs often result in functional changes [4].

2.1. ABC family members

ABC family members including P-glycoprotein (P-gp/ABCB1), multidrug resistance associated protein 1 (MRP1/ABCC1) and breast cancer resistance protein (BCRP/ABCG2) have been identified as key determinants for the pharmacokinetics and pharmacodynamics of various drugs [5]. It has been repeatedly shown in the drug-selected model cell lines that over-expression of P-gp, MRP1 and/or BCRP is one of the major mechanisms responsible for multi-drug resistance (MDR) [6,7]. Typical ABC transporters contain three peptide motifs: *Walker* A and *Walker* B sequences and the so-called ABC-signature sequence (i.e. C motif, "LSGGQ") [8]. A full transporter is composed of two nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). Each TMD contains 6 transmembrane (TM) α-helices, which are mainly involved in substrate recognition, binding and transport; while the NBDs are responsible for the ATP binding and hydrolysis that energize transport of the substrates [9].The overall sequence identity among ABC proteins is low, especially in the transmembrane domains. This is consistent with the notion that TMs participate in the varied functions of these transporters. The NBDs, on the other hand, are more structurally conserved [10]. Drug translocation at the TMDs and ATP hydrolysis at the NBD must work in concert for active efflux, thus interdomain communication is critical for proper function of the transporter protein [11]. Here only residues in or near TMs that are essential for proper transporter functions are discussed. Crucial amino acids of NBDs have been adequately reviewed elsewhere [10,12].

2.1.1. P-glycoportein (P-gp/ABCB1)

The residues located in transmembrane segments of P-gp are important for their proper functions, for labeling studies with photoactive drug analogues suggest that these regions are involved in drug-bindings [13]. Since first identified as the multidrug efflux pump in Chinese hamster ovary cells in 1976 [14], P-gp has been extensively studied for 40 years. It consists of two TMDs and two NBDs, with a molecular weight of 170 kDa. One of the most remarkable features of this transporter protein is that it binds and transports hundreds of structurally and functionally diverse substrates [15]. In 2009, Aller et al. first reported the X-ray structure of mouse P-gp at a resolution of 3.8–4.4 Å [16]. More recently, crystal structure of the *Caenorhabditis elegans* P-gp at 3.4 Å resolution [17] and refined structures of mouse P-gp [18] were reported. All X-ray structures of mouse P-gp show two portals open to the membrane inner leaflet, delineated by the transmembrane domains 3 and 4 on one side, and transmembrane domains 9 and 10 on the other side [18,19]. Both portals are created upon TM4 and 5 (and TM10 and 11) crossovers to make extensive contacts with the α-helical bundle of the opposite domain. Drugs may enter the central cavity of the transporter protein through these particular gates, though further experimental data is needed to rule out the possibility that drugs can enter via other pathways [15]. The drug-binding sites of P-gp are believed to be within the transmembrane domains because a deletion mutant consisting of only the TMDs (with the NBDs deleted) retained the ability of cell surface expression and drug substrate binding [20]. One of the distinct features of P-gp is that there are no positively or negatively charged residues located in the drug-binding pocket. Hence the major interactions between substrates and the protein amino acid residues are hydrogen-bonding, van der Waals, and hydrophobic interactions [15]. Functionally important amino acid residues had been identified in all P-gp TMs and most of them are hydrophobic or polar residues (Table 1).

A useful manner to identify critical amino acid residues for drug interaction is cysteine-scanning mutagenesis and reaction with thiol-reactive chemical agents [15]. Loo and Clarke generated a cysless P-gp with all endogenous cysteines substituted with alanine. The mutant, which was designated as P-glycoprotein-A52, retained most of the ability to confer resistance to vinblastine, colchicine, doxorubicin, and actinomycin D when compared with the wild-type

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