New and Notable

Unraveling Membrane-Mediated Substrate-Transporter Interactions

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Using molecular dynamics (MD) simulations and homology modeling, Omote and Al-Shawi in this issue of *Biophysical Journal* bring new insights into the complex field of membrane-mediated substrate-transporter interactions. Most well-known transporters, such as peptide or sugar transporters, move either just one substrate or a single class of substrates across the membrane, whereby substrate binding and release occurs in the aqueous phase. P-glycoprotein (MDR1, ABCB1) differs by taking care of a broad range of chemically diverse substrates and moving them from the inner to the outer membrane leaflet. Substrate binding and most likely also release, thus take place in the lipid phase. Direct measurement of substrate-transporter recognition in the lipid phase has not yet been possible. Visualization of substrate-transporter complexes by means of x-ray crystallography (e.g., Yu et al. (1)) must also be considered with great caution because the essential element for Pgp activity, the lipid membrane, is missing.

In comparison to the aqueous milieu the anisotropic lipid environment changes many physical-chemical parameters. Among others, it partially or totally strips off the hydration shell of the partitioning molecule, induces preferential molecular orientation, stabilizes secondary structural elements such as α-helix and β-sheet, in the case of peptides, and increases the rate of translational diffusion to the protein target. It also enhances electrostatic interactions, including H-bonding interactions, due to its lower dielectric constant. Membrane-mediated processes have been discussed in the field of substrate-receptor interactions (2,3). However, only a few publications have addressed the field of membrane-mediated substrate-transporter interactions.

Some aspects of membrane mediation have been outlined for P-glycoprotein (4). The binding of a substrate from water to the activating binding region of P-glycoprotein has been shown to occur in two steps, a partitioning step from water to the lipid membrane, characterized by the lipid-water partition coefficient, K_{lw}, and a binding step from the lipid membrane to the transporter, characterized by the transporter-lipid binding constant, K_{tl}. The transporter-water binding constant, K_{tw}, can thus be expressed as product of two individual binding constants K_{lw} and K_{tl}, and the free energy of binding, ΔG^0_{tw}, as sum of two corresponding free energies, ΔG^0_{lw} and ΔG^0_{tl}. The free energies, ΔG^0_{tw} and ΔG^0_{lw}, were determined experimentally, which allowed quantitative estimation of the free energy of substrate binding from the lipid membrane to the transporter ΔG^0_{tl}. To address the most intriguing question as to the nature of the substrate-transporter interactions an educated guess is still required. Based on the observation that all Pgp substrates carry H-bond acceptor groups and that the putative transmembrane sequences of P-glycoprotein are rich in H-bond donor groups (5) a modular recognition process based on H-bond formation was proposed (6). The challenging field of membrane mediated substrate transporter interactions thus still awaits new insight.

Omote and Al-Shawi approached the question as to how substrates are recognized by P-glycoprotein in the lipid membrane by a new and original combination of three independent steps. First, they carried out MD simulations of different P-glycoprotein substrates diffusing into a dipalmitoyl phosphatidylcholine bilayer, monitoring a substrate molecule embedded in a system of 128 lipid molecules and >4000 water molecules for a period of 10–20 ns. To characterize different substrates and to obtain information on their H-bonding potential after diffusion into the lipid bilayer they measured the frequency with which the molecules formed H-bonds with the phosphatidylcholine headgroups and the water molecules in the interfacial region of the membrane. In a second step, they compared the frequency of H-bond formation of the different substrates with experimental kinetic data, obtained by stimulating P-glycoprotein with the different substrates. They found a linear correlation between the logarithm of the time-averaged number of H-bonds formed with the lipid-water interface (which can be interpreted as the free energy of H-bond formation) and the logarithm of the intrinsic rate of P-glycoprotein transport. This result suggests that the H-bonding capacity of a substrate parallels its binding affinity to P-glycoprotein and thus influences the intrinsic rate of transport.

In a third step, Omote and Al-Shawi built a new P-glycoprotein homology model using as templates the crystal structure coordinates of the *Vibrio cholerae* lipid A transporter, MsbA, for the transmembrane domains and those of the *Salmonella typhimurium* histidine permease, HisP, and the human TAP1 for the nucleotide binding domains. The order of the transmembrane helices obtained was the same as that seen in the recent x-ray crystal structure of *S. typhimurium* MsbA. The energy-minimized homology model, moreover, recapitulated the ''nucleotide sandwich'' dimer interface seen in *Escherichia coli* BtuCD, although this structure was not used in modeling. The BtuCD nucleotide interface is believed to represent a physiologically relevant form. The homology model also conforms to the cross-linking data

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and other biochemical data available. They then carefully screened the structure for potential substrate interaction sites in the transmembrane sequences of P-glycoprotein and specifically pointed out the numerous H-bonding residues concentrated in P-gp sequences crossing the cytosolic membrane leaflet. They therefore propose that substrates that encounter P-glycoprotein via lateral diffusion in the cytosolic leaflet exchange their H-bonds with water molecules for H-bonds with the transporter. H-bonding of substrates with P-glycoprotein can be energetically more favorable than H-bonding with the lipid-water interface due to the specific matching with H-bond acceptor groups embedded in the hydrophobic core region of the membrane. This solvation exchange mechanism proposed by Omote and Al-Shawi seems very plausible and is moreover supported by a previous simpler static approach demonstrating an exponential decrease of the rate of intrinsic transport with the potential free energy of H-bonding between substrate and transporter (reviewed in Seelig and Gatlik-Landwojtowicz (4)).

Omote and Al-Shawi thus beautifully illustrate the physical-chemical consequences of moving a drug interaction site from the aqueous phase into the lipid phase. Using MD simulations they predict important parameters (H-bond interaction energies) that are not directly measurable and provide a compelling link between the rate of transport and the H-bonding potential of the substrates. This model goes beyond descriptive and provides new mechanistic insight into drug transport. Even though, the transport model proper is still relatively crude, it is based on physical-chemical principles and provides a well-founded basis for further discussion.

P-glycoprotein, which is so far the best-investigated transporter functioning on the basis of membrane-mediated substrate recognition, will serve as a model for other less investigated transporters of hydrophobic molecules, such as the MXR and the MRP1 transporter or analogous bacterial transporters that all play an important role in the development of multidrug resistance.

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