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Structural and dynamic insights on the EmrE protein with TPP^+ and related substrates through molecular dynamics simulations

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ABSTRACT

EmrE is a bacterial transporter protein that forms an anti-parallel homodimer with four transmembrane helices in each monomer. EmrE transports positively charged aromatic compounds, such as TPP^+ and its derivatives. We performed molecular dynamics (MD) simulations of EmrE in complex with TPP^+, $MeTPP^+$, and $MBTPP^+$ embedded in a membrane. The detailed molecular properties and interactions were analysed for all EmrE-ligand complexes. Our MD results identified that Lys22, Tyr40, Phe44, Trp45, and Trp63 formed potential π interactions with all three ligands and further confirmed the essential role of Glu14. Moreover, distance analysis and structural changes in the EmrE translocation pathway suggest that ligand recognition and protein conformational changes depend on the structural properties of the substrate. Analysis of the movement of the ligand in the protein binding site and rotation of the ligand's aromatic rings confirm that substrates with aromatic moieties, such as $MBTPP^+$, exhibit relatively stable binding to EmrE. Interestingly, the aromatic rings of Tyr40, Phe44, Trp45, and Trp63 underwent parallel movements with the aromatic rings of TPP^+. Based on the MD results, we propose that π interactions, as well as the mutual rotation of the aromatic rings in the protein and ligand, can be regarded as sources of ligand movement, and thus, the whole complex may work as a "molecular propeller".

1. Introduction

EmrE is an *E. coli* multidrug transporter protein and the most extensively characterized protein from the small multidrug resistance (SMR) family (Gottschalk et al., 2004; Bay et al., 2008; Yerushalmi et al., 1995). The functional unit of EmrE is an anti-parallel homodimer, wherein each monomer consists of four hydrophobic transmembrane helices (TMs) (Cho et al., 2014; Tate et al., 2001; Amadi et al., 2010; Chen et al., 2007; Morrison et al., 2012; Lehner et al., 2008). TM1, TM2, and TM3 form a substrate binding pocket, whereas TM4 is involved in contacts that stabilize the EmrE dimer (Schuldiner et al., 2001; Fleishman et al., 2006; Ubarretxena-Belandia et al., 2003; Tate, 2006; Korkhov and Tate, 2009). EmrE is proposed to function via an alternating access model in which transporters are inherently dynamic proteins, converting between two different (inward- and outward- facing) conformations to move substrates across a membrane barrier (Morrison et al., 2012; Fleishman et al., 2006; Gayen et al., 2013; Lloris-Garcera et al., 2013; Lloris-Garcera et al., 2012). Energetically, the binding of substrate must reduce the barrier for conformational exchange.

The tight coupling between the substrate binding and protein conformational exchange is important for secondary active transporters, which use a proton gradient to drive transport (Morrison and Henzler-Wildman, 2014). Although it is known that a membrane-embedded conserved residue (Glu14) in TM1 plays a key role in the transport process (Yerushalmi and Schuldiner, 2000; Schuldiner, 2009), the molecular details of the EmrE antiport mechanism (ligand recognition, protein conformation change, and ligand release) have not been elucidated. Moreover, it is not clear how different ligands are recognized by this protein. One may expect that the ligand enters the transporter from the cytoplasm and subsequently is expelled outside of the cell, but another model (known as vacuum cleaner model) in which the transporter recognizes and catches the ligand when it is still in the membrane may also be possible. This concept is partly supported by results that show that a monomeric, detergent-solubilized form of EmrE is capable of forming multimeric complexes that are enhanced by chemically diverse quaternary cation compounds (Bay and Turner, 2012). By analogy, monomeric forms of EmrE may similarly recognize ligands in the membrane, which may lead to the formation of functional dimer complexes.

EmrE imports two protons across the inner membrane of *E. coli* to export polyaromatic cation substrates, thus conferring resistance to a wide range of molecules that match this chemical description (Morrison and Henzler-Wildman, 2014; Schuldiner, 2009). Cryo-EM studies

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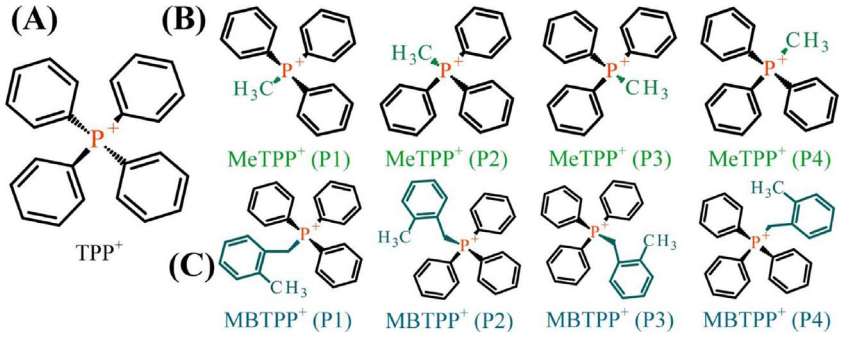

Fig. 1. Chemical structure of ligand molecules. (A) TPP^+, (B) and (C) Structure of $MeTPP^+$ and $MBTPP^+$, with their four (P1–P4) different possible topologies in the EmrE binding site. In case of $MeTPP^+$ and $MBTPP^+$, the position of ligands in the protein binding site were defined with regard to the position of TPP^+ ligand. Conformation of protein and residues surrounding ligand in starting model remain the same and only conformation (P1-P4) of ligand is different.

suggest that EmrE alters its structure when it binds to planar or tetrahedral substrates (Morrison and Henzler-Wildman, 2014; Korkhov and Tate, 2008; Wong et al., 2014). Thus, the flexibility of the EmrE protein structure is expected to be important for multidrug recognition; this type of strategy has been adopted by transporter proteins of different sizes (Bay et al., 2008). The main objective of our work was to analyse the behaviour of the EmrE protein in the presence of TPP^+ and related compounds (Fig. 1) that exhibit different affinities for this transporter (Morrison and Henzler-Wildman, 2014). The molecular details obtained from our study will be useful to understand essential elements of EmrE that are important for ligand recognition, binding, and transport.

Commonly identified substrates of SMR proteins involve quaternary ammonium compounds, such as methyl viologen (MV), tetraphenylphosphonium$^+$ (TPP^+), benzalkonium (Bz), cetyltrimethyl-ammonium bromide (CTAB), and cetylpyridinium chloride (CTPC), and intercalating dyes, such as ethidium bromide (Et), acriflavin (Ac)/proflavin (Pro), crystal violet (CV), pyronine Y (PY), and safranin O (SO) (Bay et al., 2008). Morrison et al. studied a series of tetrahedral (tetraphenylphosphonium$^+$ (TPP^+), methyltriphenyl-phosphonium$^+$ ($MeTPP^+$), 2-methylbenzyltriphenyl-phosphonium$^+$ ($MBTPP^+$), ethyltriphenylphosphonium$^+$ ($EtTPP^+$), 2,5-diethoxyphenyltriphenyl-phosphonium$^+$ ($DPhTPP^+$)) as well as planar (ethidium$^+$, propidium^{2+}, and dequalinium^{2+}) substrates for EmrE (Morrison and Henzler-Wildman, 2014). They observed a correlation between ligand hydrophobicity and binding affinity within the tetrahedral substrate series, which confirms the importance of this property for substrate interactions with the hydrophobic binding pocket of EmrE (Morrison and Henzler-Wildman, 2014).

The only structure available in the PDB database for the ligand bound EmrE protein is in complex with ligand TPP^+ (Chen et al., 2007). Considering this, substrates sharing a similar molecular geometry (tetrahedral) (Morrison and Henzler-Wildman, 2014) were selected for our study to analyse how the EmrE protein responds to ligand molecules with small differences in their structure (ligand recognition specificity). In addition to TPP^+, the ligands $MeTPP^+$ and $MBTPP^+$ were also selected for study. These ligands have one less or one different aromatic ring compared to TPP^+ (Fig. 1) and exhibit substantial differences in binding to EmrE (Lloris-Garcera et al., 2013). The dynamic behaviour of EmrE-ligand complexes, which includes the different topologies of the selected ligands inside the protein binding pocket, was studied using a molecular dynamics (MD) simulation approach (Fig. 1).

MD simulations are often used to study membrane-protein systems because they help to explore conformational dynamics of proteins at the molecular level, and this approach can also complement crystallographic or other experimental analyses (Becker et al., 2010). Our MD results characterize the functional residues of EmrE for ligand recognition, exhibiting both polar as well as hydrophobic interactions. Moreover, interactions between the aromatic rings of ligands and protein residues (π-π/cation/sigma interactions) were also observed. A possible mechanism of aromatic ligand transport is proposed and is called a “molecular propeller”. The residues of EmrE that are able to make specific interactions with membrane components and the aqueous environment were identified. Our results were compared with experimental data concerning the mechanistic properties of the system and indicated important positions of certain residues that were identified in mutagenic and other experimental studies (Lebendiker and Schuldiner, 1996; Ulmschneider and Sansom, 2001; Rotem et al., 2006; Wang et al., 2014; Melchior et al., 2016; Brill et al., 2015; Banigan et al., 2015; Bay and Turner, 2012).

2. Materials and methods

2.1. Protein structure preparation

In a previous study (Padariya et al., 2015), we prepared an all-atom model structure of EmrE (PDB ID: 3B5D (Chen et al., 2007)) using Discovery Studio Client 3.1 program (BIOVIA – former Accelrys, San Diego, USA). For our current work with ligand bound systems, as a starting point we considered two structures of EmrE: (i) all-atom model generated from a native X-ray structure (Chen et al., 2007) and (ii) structure obtained from 1000 ns simulation of the apo-EmrE system (Padariya et al., 2015). Superimposing these two structures, the simulated structure showed well-defined helix regions compared to the initially generated model based on Cα coordinates (Chen et al., 2007). Therefore, we used the simulated apo-EmrE dimer in this study. To prepare ligand bound complexes, the position of TPP^+ in the X-ray structure was used as a template. Because $MeTPP^+$ and $MBTPP^+$ are not symmetrical, and their topology inside the protein is unknown, we generated four possible topologies for each ligand inside the protein (Fig. 1). The TPP^+ ligand was modified to prepare systems of $MeTPP^+$ and $MBTPP^+$ in complex with EmrE. The two monomers of the EmrE dimer were named Chain P and Chain U.

Nine different systems of the EmrE anti-parallel dimer containing ligand were generated: (i) TPP^+, (ii) $MeTPP^+$ (P1), (iii) $MeTPP^+$ (P2), (iv) $MeTPP^+$ (P3), (v) $MeTPP^+$ (P4), (vi) $MBTPP^+$ (P1), (vii) $MBTPP^+$ (P2), (viii) $MBTPP^+$ (P3), and (ix) $MBTPP^+$ (P4) (Fig. 1 and Table S1 in the Supporting Material). Discovery Studio Client 3.1 was used to prepare and optimize the structure of the protein-ligand complexes. To avoid abnormal behaviour of the ligand during the initial steps of structure optimization, water molecules were added within 6 Å distance from each monomer (near ligand) (Fig. S1). While preserving the protein coordinates, only the ligand and water molecules were energy-minimized in all systems. Energy minimization was performed using the CHARMM forcefield and ‘smart minimizer’ algorithm (1000 steps). Non-bonded list radius was set to 14.0 Å (nonbonded higher cut-off distance 12 Å and lower cut-off distance 10 Å) and RMS gradient was set to 0.1. The resultant structures were used to prepare membrane-embedded protein systems.

2.2. System setup

The initial position of EmrE protein in the membrane was calculated using PPM web server (Lomize et al., 2012), which uses the 3D structure of a protein as an input and calculates rotational and translational