X-ray Structure of the EmrE Multidrug Transporter in Complex with a Substrate

Owen Pornillos, Yen-Ju Chen, Andy P. Chen, Geoffrey Chang*

EmrE is a prototype of the Small Multidrug Resistance family of efflux transporters and actively expels positively charged hydrophobic drugs across the inner membrane of *Escherichia coli*. Here, we report the x-ray crystal structure, at 3.7 Ångstrom resolution, of one conformational state of the EmrE transporter in complex with a translocation substrate, tetraphenylphosphonium. Two EmrE polypeptides form a homodimeric transporter that binds substrate at the dimerization interface. The two subunits have opposite orientations in the membrane and adopt slightly different folds, forming an asymmetric antiparallel dimer. This unusual architecture likely confers unidirectionality to transport by creating an asymmetric substrate translocation pathway. On the basis of available structural data, we propose a model for the proton-dependent drug efflux mechanism of EmrE.

A major obstacle to effective treatment of bacterial infections is the emergence of strains that are resistant to available antibiotics. Of particular concern are multidrug-resistant strains that cause common diseases such as tuberculosis, gonorrhea, and hospital-acquired staphylococcal infections (1). Multidrug resistance arises, in part, through the action of integral membrane proteins called multidrug transporters (1, 2). Each of these transporters can actively expel a wide variety of drugs and toxic compounds from the cell. There are two broad classes of transporters: ATP-binding cassette (ABC) proteins directly couple drug efflux to adenosine 5'-triphosphate (ATP) hydrolysis, whereas secondary transporters use energy derived from proton or cation electrochemical gradients across the lipid bilayer.

EmrE is a proton-dependent secondary transporter from *Escherichia coli* and is a prototype of the Small Multidrug Resistance (SMR) family (3, 4). SMRs represent the smallest transporters in nature; each polypeptide has only 105 to 120 amino acid residues and four transmembrane helices, and forms homo- or heterodimers (3). EmrE is well documented to function as a homooligomer (3–9) and confers resistance to positively charged hydrophobic antibiotics, such as tetracycline, ethidium, and tetraphenylphosphonium (TPP) (3, 4). EmrE exchanges two or more protons per drug molecule through a “hydrophobic” translocation pathway (10, 11).

The general model for multidrug efflux by EmrE and other secondary transporters is the alternating access mechanism (12, 13). In this model, the EmrE transporter has at least two conformational states, inward-facing and outward-facing, with the drug-binding site accessible to the cytoplasm or periplasm, respectively. Interconversion between the two conformations is promoted by drug and/or proton binding. Here, we describe the x-ray crystal structure of one conformation of the EmrE transporter in complex with the drug TPP. The structure was determined to 3.7 Å resolution by anomalous dispersion methods, using the arsonium analog of TPP and selenomethionine (SeMet)–substituted proteins (Fig. 1A) (14).

SeMet-labeled proteins used for this study were produced in a cell-free system, because SeMet-EmrE did not express well in vivo. Briefly, EmrE was expressed by use of the T7 promoter in *E. coli* lysates supplemented with nucleotide triphosphates, T7 polymerase, and appropriate amino acids (14). Experimental maps derived from Se and As data are very well correlated, indicating that in vitro and in vivo–expressed EmrE proteins adopt a similar structure. Our work shows that cell-free methods are a viable alternative to traditional large-scale protein expression systems.

Consistent with biochemical studies showing that EmrE is primarily a dimer in detergent and binds drugs with a 2:1 protein/drug ratio (9, 15), the asymmetric unit of the EmrE-TPP crystal is composed of two molecules of EmrE and one molecule of TPP (Fig. 1). The minimally functional unit of EmrE is therefore...
Fig. 1. Structure of the EmrE transporter in complex with TPP.  

A) Stereoview cartoon representation of the asymmetric unit, composed of two EmrE subunits (subunit A in yellow and subunit B in green), and one bound TPP (red). Anomalous difference density shows the position of As (blue, contoured at 1σ), derived from SeMet-EmrE-TPP crystals. Methionine side chains are shown explicitly and labeled; corresponding residues in subunit B and subunit A are distinguished by the asterisks. The coloring scheme in this panel is maintained throughout Figs. 1 to 3.  

B) Stereoview of the EmrE homodimer. The N and C termini of the two subunits are indicated. The boundaries of the lipid bilayer, deduced from the SeMet-EmrE-TPP crystals, are shown (green). Methionine positions are indicated by Se atoms (magenta, 4σ).  

C) Top view of the dimer, with the four transmembrane helices in each subunit labeled. The short helix connecting helices A2 and A3 is indicated by an asterisk. This view clearly shows that TPP is bound at the dimerization interface. The two Glu-14 residues are shown in red.  

D) Best-fit superposition of subunits A (yellow) and B (green) (root mean square deviation of 3.5 Å over equivalent Cα positions). The first three helices form a left-handed three-helix bundle, whereas the fourth helices are positioned differently.  

E) Crystal packing of EmrE-TPP. The lattice is stabilized by side-by-side transmembrane contacts and loop interactions, reminiscent of type I and 2D membrane protein crystals. A potential dimer of dimers is colored as above; the symmetry-related elements are colored gray. The unit cell is boxed in black. This view is perpendicular to the bc plane.
connecting helices A2 and A3, is absent in subunit B (asterisk in Fig. 1C).

Viewed along the transmembrane axis, the two three-helix bundles are on opposite sides of the dimer, flanked by helices A4 and B4 (Fig. 1C). Helices A1 and B1 are located in the middle of the dimer and form part of the TPP-binding site, in accord with their essential role in drug transport (Fig. 1, B and C). These two helices form an approximate V shape with a ~30° crossing angle, consistent with predictions from electron paramagnetic resonance (EPR) spin-labeling studies of unbound EmrE (23). The relative positions of the first and fourth transmembrane helices are also in general agreement with predictions from cross-linking experiments (7).

Biochemical studies also suggest that EmrE can form a dimer of dimers (8), and examination of packing interactions in the EmrE-TPP crystal suggests a possible tetramerization interface. Helices A4, B1, and B2 from one dimer pack against their symmetry-related mates in another dimer, burying a total surface area of ~1600 Å² (Fig. 1E). Although it remains to be confirmed by mutagenesis and other biophysical methods, EmrE tetramerization could, in principle, increase the efficiency of drug recognition and efflux through avidity effects.

A possible drug translocation pathway is evident in the structure, consisting of two cone-shaped pockets that open to opposite sides of the lipid bilayer (shown by arrows in Fig. 2A). The two pockets are demarcated by helices A1 and B1. Consistent with the symmetric nature of the EmrE dimer, this putative translocation pathway is also asymmetric. In addition to A1 and B1, the larger pocket is surrounded by helices A2, B3, and B4, whereas the smaller one is lined by helix A2. TPP appears bound at the bottom of the larger pocket, wedged between helices A1, A2, and B1 (Fig. 2B). We presume that these three helices act as a “gate,” controlling passage of the drug into the smaller pocket. Extensive mutagenic studies of EmrE by Schuldiner and co-workers have previously identified residues required for drug binding and translocation (10–12, 24–28). These residues nicely map to the proposed translocation pathway (Fig. 2A).

The volume of the binding pocket is considerably larger than TPP (Fig. 2B) and can accommodate a wide range of substrate sizes and shapes, explaining EmrE’s polyspecificity. The binding site complements the positively charged hydrophobic nature of TPP and other EmrE substrates (3, 4). Hydrophobic residues in the pocket are well positioned to participate in van der Waals contacts with the four phenyl rings of TPP (Fig. 2B). The electrostatic component is provided by a membrane-embedded acidic residue located at the bottom of the pocket, Glu-14 in helix A1 (colored red in Fig. 2B). Mutagenesis indicates that this glutamate is absolutely required for proper EmrE function (24–26), although such studies cannot readily distinguish between the two Glu-14 residues in the homodimer. In this EmrE conformation, the second Glu-14 in helix B1 does not appear to contact TPP (Glu14* in Fig. 2B).

Its carboxylate group is located ~16 Å away from the TPP phosphate atom and appears to face the smaller pocket. Thus, the two glutamates do not necessarily contact the bound drug simultaneously, as previously proposed (4), and we suggest that instead they bind TPP sequentially (see below).

A structure of the EmrE-TPP complex has also been reported by electron microscopy (EM) of two-dimensional (2D) crystals to resolutions of 7.5 Å in-plane and 16 Å perpendicular to the lipid bilayer (13). The EM model also shows EmrE as an asymmetric dimer with the drug-binding site located between the two monomers, and also suggests an antiparallel arrangement. The x-ray and EM structures appear to have captured two different conformations of drug-bound EmrE transporter (Fig. 3). Independent superposition of the two EmrE subunits in the x-ray structure with the EM model allowed us to define the subunit boundaries and propose helical assignments for the EM structure (Fig. 3A). This
Fig. 4. A potential mechanism for proton-dependent drug translocation by EmrE. For clarity, only the three putative gating helices (A1, A2, and B1) and two membrane-embedded Glu-14 side chains are shown explicitly. Drug substrates and protons are represented by the yellow sphere and red balls, respectively.

unique match was obtained because the dimer is asymmetric, and with the constraint that the helix closest to the putative TPP density in the EM map is helix 1 (13). The transformation between the two structures can be approximated by a relative twist of ~30° between the two subunits, although helical tilt angles also change within individual monomers. Curiously, the binding pockets open to the same side in both models.

There are several interesting differences between the x-ray and EM models of EmrE-TPP. (i) TPP appears bound to subunit B in the EM structure, and not subunit A (Fig. 3B). Although this remains to be confirmed by other methods, it suggests that the two subunits may alternately bind drug during the transport cycle and explains the requirement for both membrane-embedded glutamate in the dimer. (ii) The drug-binding pocket in the EM model appears larger and more open, surrounded by six helices (Fig. 3B) (13). In the x-ray model, the binding site is more closed, surrounded by only five helices (Fig. 3C). We speculate that these differences reflect conformational changes that occur upon transfer of TPP between subunits A and B. (iii) In the EM model, the second pocket on the opposite side of the drug translocation pathway is absent. This is because the three gating helices, A1, A2, and B1, are in different positions. Helices A1 and B1 in the EM model are more parallel and ~20 Å apart, whereas helix A2 is tilted differently (Fig. 3A). Conformational mobility in these helices is evident from both EPR and nuclear magnetic resonance data (27, 29), and we speculate that helix-coil conversion in the A2-A3 loop may help facilitate movement. An attractive model is that the x-ray and EM structures represent the two EmrE conformations deduced from the EPR studies: one where helices A1 and B1 form a V-shaped configuration and another where they are >20 Å apart (23). We speculate that interconversion of the EmrE transporter between these two (and perhaps other) conformations may drive drug transport.

On the basis of the above observations, we propose the following outline for proton-dependent drug transport by EmrE and other SMR transporters (Fig. 4). With its large, open binding site, we suggest that the EM structure represents an inward-facing conformation of EmrE. With two potential entry points to the binding site (13), this EmrE conformation could act as an efficient “hydrophobic vacuum cleaner” (30), filtering both the cytoplasm and the inner leaflet of the lipid bilayer for substrates. We envision that TPP first binds to Glu-14 in subunit B in exchange for one proton. As a next step, the bound drug is transferred to subunit A (that is, the translocation pathway) in exchange for the second proton. Conformational changes in the dimer could serve to bring the two Glu-14 side chains in proximity, facilitating the exchange. In support of this idea, the Glu-14→Asp EmrE mutant forms a transporter that binds TPP with high affinity but with impaired transport function (24), likely because the Asp side chain is too short. The x-ray structure may represent the postexchange conformation in which the drug is bound to subunit A, and Glu-14 in subunit B is oriented toward the outer pocket and reprotonated. Rearrangement of helices A1, A2, and B1 in the putative translocation pathway could transfer TPP to this pocket, where it would rapidly dissociate owing to unfavorable proton exchange at the periplasmic space.

In the proposed mechanism, the two subunits in the EmrE dimer are structurally and functionally nonequivalent, an arrangement that would favor unidirectional transport. Indeed, this is a recurring theme in structural studies of the different transporter families (12, 22, 31). The large conformational changes that appear to occur during drug translocation are consistent with the low turnover number for this transporter (32).

References and Notes
14. Materials and methods and structure statistics are available as supporting material on Science Online.
22. For a recent example, see C. Hunte et al., Nature 435, 1197 (2005).
33. We thank C. Ma for invaluable contributions to EmrE-TPP crystallization; T. Kudicki and J. Fletcher (Invi- trogen) for in vitro translation reagents; S. Lieu for TPP crystallization; T. Kudlicki and J. Fletcher (Invi- trogen) for in vitro translation reagents; S. Lieu for general lab support; and the staff at Stanford Synchrotron Radiation Laboratory, Advanced Light Source, and Advanced Photon Source for assistance with data collection. We thank C.G. Tate (MRC Laboratory of Molecular Biology) for providing the EM map of EmrE-TPP, and S. H. White, P. E. Wright, H. J. Dyson, R. A. Milligan, C. L. Reyes, and Y. Yin for critical reading of the manuscript. This study was supported by grants from the NIH (GM67644 and GM073197) and NASA (NASA8-1834) to C.G. O.P. is supported by an NIH postdoctoral fellowship. Coordinates have been deposited in the Protein Data Bank (PDB code: 2f2m).

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5756/1950/DC1
Materials and Methods Table S1
References and Notes
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We wish to retract our Research Article “Structure of MsbA from \textit{E. coli}: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters” and both of our Reports “Structure of the ABC transporter MsbA in complex with ADP-vanadate and lipopolysaccharide” and “X-ray structure of the EmrE multidrug transporter in complex with a substrate” (1–3).

The recently reported structure of Sav1866 (4) indicated that our MsbA structures (1, 2, 5) were incorrect in both the hand of the structure and the topology. Thus, our biological interpretations based on these inverted models for MsbA are invalid.

An in-house data reduction program introduced a change in sign for anomalous differences. This program, which was not part of a conventional data processing package, converted the anomalous pairs ($I^+$ and $I^-$) to ($F^-$ and $F^+$), thereby introducing a sign change. As the diffraction data collected for each set of MsbA crystals and for the EmrE crystals were processed with the same program, the structures reported in (1–3, 5, 6) had the wrong hand.

The error in the topology of the original MsbA structure was a consequence of the low resolution of the data as well as breaks in the electron density for the connecting loop regions. Unfortunately, the use of the multicopy refinement procedure still allowed us to obtain reasonable refinement values for the wrong structures.

The Protein Data Bank (PDB) files 1JSQ, 1PF4, and 1Z2R for MsbA and 1S7B and 2F2M for EmrE have been moved to the archive of obsolete PDB entries. The MsbA and EmrE structures will be recalculated from the original data using the proper sign for the anomalous differences, and the new C\textalpha coordinates and structure factors will be deposited.

We very sincerely regret the confusion that these papers have caused and, in particular, for subsequent research efforts that were unproductive as a result of our original findings.

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References
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