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Nucleotide Dependent Packing Differences in Helical Crystals of the ABC Transporter MsbA

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Abstract

Bacterial ATP binding cassette (ABC) exporters fulfill a wide variety of transmembrane transport roles and are homologous to the human multidrug resistance P-glycoprotein. Recent x-ray structures of the exporters MsbA and Sav1866 have begun to describe the conformational changes that accompany the ABC transport cycle. Here we present cryo-electron microscopy structures of MsbA reconstituted into a lipid bilayer. Using ATPase inhibitors, we captured three nucleotide transition states of the transporter that were subsequently reconstituted into helical arrays. The enzyme-substrate complex (trapped by ADP-aluminum fluoride or AMPPNP) crystallized in a different helical lattice than the enzyme-product complex (trapped by ADP-vanadate). ~20Å resolution maps were calculated for each state and revealed MsbA to be a dimer with a large channel between the membrane spanning domains, similar to the outward facing crystal structures of MsbA and Sav1866. This suggests that while there are likely structural differences between the nucleotide transition states, membrane embedded MsbA remains in an outward facing conformation while nucleotide is bound.

Keywords

ABC Transporter; MsbA; cryo-electron microscopy; structure; helical crystal

Introduction

The ABC transporter superfamily, named for the conserved ATP Binding Cassette (ABC) motif, is expressed ubiquitously from bacteria to mammals. All ABC transporters are integral membrane proteins minimally composed of two transmembrane domains (TMD) that provide a conduit through the membrane, and two nucleotide binding domains (NBD) which contain the conserved ABC motifs. The ABC core couples the energy of ATP hydrolysis to distant conformational changes in the TMDs, facilitating transport of a wide spectrum of compounds (peptides, lipids, antibiotics, chemotherapeutics, etc.) across cellular membranes (Higgins, 1992; Paulsen, et al., 1998; Schneider and Hunke, 1998; Holland and Blight, 1999; Hopfner, et al., 2000; Davidson, 2002).

MsbA is a member of the ABC transporter superfamily that is found in gram-negative bacteria (Doerrler, et al., 2001). Its native function is to transport core lipid A, the precursor to lipopolysaccharide (LPS), from the inner leaflet to the outer leaflet of the inner bacterial membrane (Doerrler, et al., 2001; Doerrler and Raetz, 2002; Doerrler, et al., 2004). MsbA is

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also structural and functional homologue of the mammalian P-glycoprotein (Pgp) (Reuter, et al., 2003), which is well known for its role in multidrug resistance in human tumor cells (Dean and Annilo, 2005). In two different studies MsbA has been shown to confer resistance to the antibiotic erythromycin and interact with the chemotherapeutic agents daunomycin and vinblastine (Reuter, et al., 2003; Woebking, et al., 2005). Both MsbA and Pgp contain 12 transmembrane helices per complete transporter with MsbA arranged as a symmetric homodimer while Pgp is expressed as a single polypeptide with pseudo-twofold symmetry. MsbA from *E. coli* is 32% and 36% identical to the C-terminal and N-terminal halves of human Pgp, respectively. The overlapping substrate specificity and topology suggest that the transport mechanism is conserved amongst the ABC transporter superfamily.

Because of its important biomedical role Pgp is the most well studied ABC transporter. A wealth of structural and biochemical data exists describing a series of nucleotide dependent conformational changes in the transporter, though no high-resolution structure has been solved. Using mutations and transition state inhibitors, Sauna, et al. (2006; 2007) correlated reaction intermediates of the ATPase cycle with changes in substrate affinity in the TMDs of Pgp. In the study they defined the beryllium fluoride trapped state as the enzyme-substrate (ES) complex and the vanadate trapped state as the enzyme-product (EP) complex. A different trapping study by Russell and Sharom (2006) supported these conclusions and derived a similar mechanism reinforcing the difference between pre-hydrolysis (ES) and post-hydrolysis (EP) nucleotide bound states. Both studies also concluded that the transition states differed based on whether they were forward (ATP added and converted to ADP) or reverse (ADP added directly) trapped, suggesting that the energy from ATP hydrolysis created a distinct intermediate.

Low and moderate-resolution ($\sim 8\text{--}20\text{\AA}$) two-dimensional projection structures of Pgp in a lipid bilayer, derived from electron microscopy (EM) data, have also shown a variety of nucleotide dependent conformations (Rosenberg, et al., 2001; Rosenberg, et al., 2003; Lee, et al., 2008). The low-resolution projection structures of Pgp (Rosenberg, et al., 2001) suggest that there are structural differences between AMPPNP and reverse ADP-vanadate trapped states. In contrast, x-ray structures of MsbA (Ward, et al., 2007) show no significant differences between the AMPPNP and forward trapped ADP-vanadate state. Crystal structures of nucleotide bound MsbA are also nearly identical to structures of Sav1866 (Dawson and Locher, 2006; Dawson and Locher, 2007), a gram-positive homologue of MsbA, that were crystallized with ADP and AMPPNP. These structures, which represent both the pre- and post-hydrolysis states, also suggest that the transporter undergoes little or no conformational change during the catalysis of ATP to ADP and phosphate.

Here we present three $\sim 20\text{\AA}$ cryo-electron microscopy structures of MsbA trapped in different nucleotide transition states. By using cryo-EM we were able to investigate the structures of MsbA in a reconstituted lipid bilayer environment. Further, because MsbA was crystallized into helical arrays we were able to calculate three-dimensional maps of each state without tilting the specimen. The resultant electron density maps represent low-resolution models of MsbA in AMPPNP, ATP-AlFx, and ADP-vanadate states. All three states resemble the MsbA-AMPPNP crystal structure (pdb id: 3b5x) with tightly associated NBDs and a large channel between the TMDs, but the data suggest that there are structural differences between the ES and EP states.

Materials and Methods

Expression and Purification of MsbA

MsbA from *Vibrio cholerae* (VC) and *Salmonella typhimurium* (ST) was expressed and purified as previously described (Ward, et al., 2007). Briefly, MsbA was cloned into the

pET19b expression vector (Novagen, Madison, WI) and expressed in *E. coli* BL21 (DE3) (Novagen, Madison, WI) in a 100 L batch fermentor at 37°C using 2 mM IPTG (Anatrace, Maumie, OH) as the inducer. MsbA was extracted from *E. coli* by agitation in the presence of 1–2% (w/v) Cymal-7 (C7) for VC MsbA or 1–2% (w/v) undecyl- β -D-maltoside (β -UDM) for ST MsbA at 4°C. Extracted MsbA was purified in the presence of 20 mM Tris-HCL (pH 8.0), 20 mM NaCl, and 0.04–0.1% C7 or β -UDM in the presence of 10% glycerol using nickel-chelate and ion-exchange chromatography. Purity was assayed using SDS-PAGE and coomassie staining.

Reconstitution and Crystallization of *V cholerae* MsbA

Dioleoyl phosphatidylserine (DOPS) and dimyristoyl phosphatidylcholine (DMPC) lipids (1:1) in a chloroform solution (Avanti Polar Lipids) were dried down under argon gas and resuspended in crystallization buffer (20 mM citrate buffer pH 5–6 and 50mM NaCl). Cymal-7 solubilized *V cholerae* MsbA was then added to the buffer at a final concentration of 0.6 mg/ml, with a lipid to protein ratio (LPR) equal to 1:1. Lastly, nucleotide was added to the crystallization mixture. For the vanadate co-crystals, sodium orthovanadate was boiled immediately before use and added to a final concentration of 5mM with 5mM MgCl₂ and 5mM ATP. For the AlFx co-crystals, AlCl₃ and NaF were added to a final concentration of 4mM with 5mM MgCl₂ and 5mM ATP. All experiments were incubated at room temperature for 1 hr before ~1mg BioBeads SM2 (BioRad) were added to adsorb the detergent. Biobeads were prepared as indicated by Rigaud et al. (1997). MsbA was subsequently reconstituted into bilayers and formed helical crystals (Table 1). Crystals were harvested between 12 and 36 hours after the addition of BioBeads. For the vanadate co-crystals, 500 μ M LPS (Ra mutant, Sigma Aldrich) were added.

Reconstitution and Crystallization of *S typhimurium* MsbA

Dioleoyl phosphatidylserine (DOPS) and dimyristoyl phosphatidylcholine (DMPC) lipids in a chloroform solution (Avanti Polar Lipids) were dried down under argon gas and resuspended in crystallization buffer (20 mM glycine buffer pH 9 and 50mM NaCl). B-UDM solubilized *S typhimurium* MsbA was then added to the buffer, yielding final concentrations of 0.6mg/ml protein, 0.6mg/ml lipid (50% DOPS, 50% DMPC). Lastly, 5mM MgCl₂ and 5mM AMPPNP were added to the crystallization mixture. The crystallization solution was incubated at room temperature for 1 hr before ~1mg BioBeads SM2 (BioRad) were added to adsorb the detergent. MsbA reconstituted into bilayers and formed helical crystals. Crystals were harvested between 12 and 36 hours after the addition of BioBeads.

Cryo-imaging

4 μ l of the solution containing the helical tubes was placed on a freshly glow discharged Quantifoil (SPI Supplies) holey carbon grid, blotted, and freeze plunged into liquid ethane and subsequently stored in liquid nitrogen. Grids were loaded into a Gatan cryo-transfer specimen holder and imaged at liquid nitrogen temperature using a Philips CM120 electron microscope at an accelerating voltage of 120 kV. All images were recorded on Kodak SO135 film under low dose conditions at a nominal magnification of 35,000x and a defocus range of 0.8–1.4 μ m under focus. The helical tubes varied in length from 0.1 μ m to 3 μ m and tube diameter was relatively uniform within each nucleotide prep; AlFx and AMPPNP (32–34nm) and vanadate (24–27nm).

Image Processing and Analysis

Images of crystals with visible diffraction to ~20Å were selected using an optical diffractometer. The selected crystals were digitized using a Perkin Elmer microdensitometer with spot and step sizes equivalent to 5.8Å at the specimen. The Windex graphical interface

(Ward, et al., 2003) was used to sort images and determine layer line and Bessel orders. Subsequent image processing and averaging was done with the helical processing software, PHOELIX (Whittaker, et al., 1995). The contrast transfer function (CTF) was not corrected in these low-resolution ($\sim 20\text{\AA}$) structures because all of the diffraction data was inside the first zero. Final, averaged maps were calculated from 5–8 images of helices, 1–2 μm in length. Averaged maps had very little noise and at this resolution adding more images did not yield any benefit so only the very best images were included in the final average. Averaged layer lines with strong phase overlap were included in the final reconstruction and the resolution was limited to $20\text{\AA}$ to reduce noise (Table 2 summarizes the data that went into the final average). The x-ray structure of MsbA with the bound nucleotide AMPPNP (pdb id: 3b5x) was used for fitting into the EM density maps. Fitting of the x-ray structure was done manually in MOLOC (Gerber Molecular Design) and PyMol (Delano Scientific) as a rigid body.

Results

Overview

Two different MsbA orthologues, from *Salmonella typhimurium* (ST) and *Vibrio cholerae* (VC) that have 67% sequence identity, were used in this study. Both orthologues were trapped with nucleotide and formed membrane embedded helical arrays in the presence of lipids. Each orthologue crystallized as a single predominant helical family depending on the transition state inhibitor. The ES state (trapped by AMPPNP or AlFx) packed into a different helical lattice than the EP state (trapped by ADP-Vanadate).

Crystallization

MsbA was initially reconstituted into membranes in its no nucleotide (apo) form under a variety of crystallization conditions. In many cases densely packed proteoliposomes were obtained and some contained small crystalline areas (Figure 1A). The crystalline areas never grew larger than 50–100nm on a side so the protein was rescreened in the presence of nucleotide.

When MsbA was rescreened in the presence of 5mM Mg^{2+} ATP helical crystals grew out of densely packed proteoliposomes 6–12hrs after detergent removal was initiated (Figure 1B, 1C). Lipids alone, subjected to the same conditions did not form tube-like structures. Addition of the diphosphohydrolase, apyrase, to the Mg^{2+} ATP co-crystals led to disorder in the crystal lattice and eventual destruction of the helical arrays. Over periods longer than 15 minutes the crystals became irrecoverably disordered, confirming that nucleotide was required in order to form and maintain helical crystals.

In the presence of Mg^{2+} ATP the crystalline order of the helices, as measured by optical diffraction of cryo-EM images, but did not exceed $\sim 35\text{\AA}$ resolution. In addition, the helices visually decayed over the course of several days, suggesting the crystals were unstable in the presence of Mg^{2+} ATP. We subsequently chose to forward trap MsbA with the transition state inhibitors AMPPNP, ADP-AlFx, and ADP-vanadate. Trapping was done under similar conditions that have been published for Pgp (Sauna, et al., 2006; Russell and Sharom, 2006; Sauna, et al., 2007). Each complex inhibited ATPase activity (data not shown), likely stabilizing MsbA in conformations that yielded well-ordered helical crystals (Figure 2A, 2C, 2E) that diffracted to $\sim 20\text{--}24\text{\AA}$ resolution (Figure 2B, 2D, 2F). The helical crystals were stable for several days at room temperature under these conditions. In the presence of AMPPNP and ADP-AlFx MsbA crystallized in identical helical lattices (Figure 2G-2H). In the presence of ADP-vanadate MsbA crystallized in a lattice unique from the other inhibitors (Figure 2I).

3D EM Maps

EM maps were calculated for all three conditions using Fourier Bessel inversion techniques (Table 2). All three EM maps (truncated to $\sim 20\text{\AA}$ resolution) displayed discrete knobby subunits arranged in a helical fashion (Figure 3A-C) extending outward orthogonal to the helix axis. In all maps each subunit of the reconstructed helices revealed a two-fold symmetric arrangement of density with a globular region on the outside of the helix connected to density that extended in toward the middle of the helix (Figure 4). The inner radius of the helix is composed of two elongated densities that create a large channel that is continuous with the inside of the tube (Figure 4). Measuring from the inner wall of the helix this gap extends $\sim 50\text{\AA}$ into the density map and is $\sim 15\text{\AA}$ at its widest point (Figure 4). In the AIFx and AMPPNP crystals there are strong crystal contacts between the outer globular densities (Figure 3A-3B), and weaker contacts between the elongated domains toward the inside of the tubes. In the vanadate crystal the only crystal contacts are located toward the inside of the helix between the elongated domains (3C).

Fitting the X-ray Structure into the EM Density

Initial examination of the EM reconstructions revealed that both crystal forms (AIFx/AMPPNP and vanadate) were arranged such that the TMDs are located on the inside and the NBDs on the outside of the helix. Each subunit in the helix is equal to the volume of an intact dimer of MsbA and we were able to fit the MsbA-AMPPNP crystal structure (pdb id: 3b5x) into that density (Figure 3D-3I). Due to the resolution limits of the EM maps the MsbA dimer was fit as a single rigid body by hand without further refinement. The globular density on the outside of the helix accommodates the NBD sandwich dimer (Figure 3D-3I) while the TMDs fit into the apposed elongated densities perpendicular to the wall of the tube (Figure 3D-3F). When viewed perpendicular to the helix access (down the two-fold axis of the MsbA dimer) the vanadate MsbA dimer is rotated $\sim 30^\circ$ clockwise relative to the AIFx/AMPPNP MsbA dimer (Figure 3D-3F). Each dimer in the AIFx and AMPPNP crystal makes 2 crystal contacts with neighboring NBDs in one direction and 2 crystal contacts with the neighboring TMDs of dimers in the orthogonal direction (Figure 3A-3B, 3D-3E, 3G-3H). Each dimer in the vanadate state only makes lattice contacts with 2 other dimers, and those contacts occur in the TMD region (Figure 3C, 3F, 3I).

Discussion

The bacterial ABC exporter MsbA has been crystallized under a variety of different conditions for x-ray crystallography in a detergent micellar environment (Ward, et al., 2007). In those structures the biggest conformational change occurs upon nucleotide binding, which converts MsbA from an inward to an outward facing state. In contrast, there is no difference between the various outward facing nucleotide bound structures ($<2.5\text{\AA}$ rmsd between the Ca positions). Here we have studied MsbA in a membrane embedded environment for comparison to the x-ray structures of detergent solubilized MsbA.

Structural Description

Initial attempts to crystallize membrane reconstituted MsbA yielded small two-dimensional crystals. These crystals were too small for image processing but suggest that in the absence of nucleotide there were somewhat homogenous populations of MsbA stable enough to form a crystal lattice. The lack of long-range order, however, was likely due to an overall conformational heterogeneity in the sample, which was consistent with the x-ray structures of MsbA with no nucleotide (Ward, et al., 2007).

Addition of Mg^{2+} ATP to the crystallization matrix resulted in the formation of helical arrays of MsbA. From this result we concluded that MsbA, in a nucleotide bound state, formed

protein-protein contacts that drove the deformation of the membrane and subsequent crystal packing into tube-like structures. The different behavior of MsbA in the presence or absence of nucleotide is also consistent with the conclusions of the x-ray structural studies (Ward, et al., 2007). The lack of stability of these initial crystals was likely due to a diminishing supply of nucleotide as MsbA underwent ATP hydrolysis. The necessity of nucleotide and the NBD outside orientation of MsbA in the tubes was supported by the apyrase dependent destruction of the helical lattice. Because approximately half of the tubes were visibly closed at the ends, the inside of the helical array must have been isolated from the surrounding environment (Figure 1C). If the NBDs were facing the inside of the helix, apyrase would not have been able to access internal ATP and disrupt the equilibrium of ATP binding to the NBDs. Because of these issues we employed known transition state inhibitors that resulted in highly ordered helical crystals and in turn also allowed us to examine the structures of discrete intermediates along the ATPase cycle.

Cymal-7 solubilized *Vibrio cholerae* (VC) MsbA was crystallized using two different transition state inhibitors, AIFx and vandate, which trap the enzyme-substrate (ES) and enzyme-product (EP) complexes, respectively. Each inhibitor resulted in a unique helical lattice of MsbA under the same crystallization conditions (Figure 3A, 3C). The inhibitor, therefore, is the sole variable responsible for the packing differences between MsbA dimers. This suggests that each state has different moieties contributing to lattice contact formation. The greater twist angle between adjacent subunits in the vanadate crystal form prohibits lattice contacts between the NBDs (Figure 3). Interestingly, the vanadate co-crystals presented in this study improved in resolution from ~35Å to ~20Å upon addition of LPS (Ra mutant) but did not change the crystal packing. Resolution improvement was also seen in the x-ray crystals of MsbA when LPS (Re mutant) was added (Reyes and Chang, 2005). LPS cannot be resolved in the EM reconstructions but may bind to MsbA and increase the stability of the protein or lattice contacts. If LPS (~2kD) were bound in the middle of the TMDs we would expect to see additional density in the channel of the EM map but there is none. Therefore, it is likely bound to the outside of the protein, where it is indistinguishable from protein-protein interactions and the lipid bilayer.

β-UDM solubilized *Salmonella typhimurium* (ST) MsbA was crystallized using the ES state inhibitor AMPPNP. In the presence of AMPPNP, ST MsbA crystallized in the same helical lattice as VC MsbA with AIFx. Each orthologue was purified in a different detergent and the crystallization conditions differed by ~3.5 pH units (Figure 3A-3B). This suggests that the two orthologues, trapped in an ES state, are in the same conformation with similar moieties contributing to lattice contact formation.

Previous biochemical studies suggested that the ABC transporter Pgp exhibited differences in substrate affinity between the ES and EP states (Sauna, et al., 2006; Russell and Sharom, 2006; Sauna, et al., 2007). Here we show that MsbA crystallization is dependent on the transition state and that there is an inherent difference in the way in which each state crystallizes. This is in contrast to the x-ray structural data that showed nucleotide bound MsbA (AMPPNP, ADP-Vanadate) crystals were isomorphous. This suggests that there may be important differences between micellar and membrane embedded MsbA, consistent with biochemical studies of reconstituted Pgp (Sauna, et al., 2006; Russell and Sharom, 2006; Sauna, et al., 2007). The resolution here was not good enough to examine these differences and the difference in symmetry of the helical crystals prevented rigorous difference mapping. We therefore can only conclude that there are likely structural differences between the ES and EP states of MsbA reconstituted in a bilayer. We can however, confirm that MsbA remains in an outward facing conformation in both the ES and EP states. In addition, the nucleotide binding domains remain tightly associated and the MsbA dimer appears to be symmetric at this resolution.

Comparison to Pgp Structures

Projection structures of Pgp suggest that there is a large conformational change between the AMPPNP and reverse trapped ADP-vanadate state (Rosenberg, et al., 2001) and examination of the EM maps reveals that the ADP-vanadate state closely resembles the nucleotide free state. Here we see that MsbA-AMPPNP and forward trapped MsbA-vandate closely resemble each other, both in an outward facing conformation (Figure 3). This reinforces the hypothesis that there are important differences between the forward and reverse trapped states of ABC transporters.

Another important difference between the MsbA and Pgp structures is that data was collected from helical versus two-dimensional crystals, respectively. The three-dimensional structures of Pgp (Rosenberg, et al. 2003; Rosenberg et al., 2005; Lee, et al., 2007; were calculated from tilted two-dimensional crystals and the resulting maps have artifacts and a missing cone of information that make interpretation difficult. Helical crystals of MsbA, on the other hand, give all possible views of the transporter and do not require tilting to obtain a three-dimensional structure. In addition, the EM maps are isotropic and at $\sim 20\text{\AA}$ resolution the maps closely resemble the overall shape of the x-ray structures (Figure 3).

Comparison to X-ray Structures

In contrast to biochemical studies, all structural studies of full-length ABC transporters with a variety of nucleotide mimetics have resulted in very similar structures. In x-ray crystallographic studies of Sav1866, ADP was used to trap the outward facing state and the authors concluded that the even though ADP was present, the crystal was trapped in the ES transition state (Dawson and Locher, 2006). A subsequent study by the same group showed that the ADP could be exchanged to AMPPNP by soaking pre-formed ADP co-crystals in AMPPNP solution for several days (Dawson and Locher, 2007). This exchange did not produce any change in the crystal structure except for the nucleotide identity. This is not surprising given that the protein was fixed in a three-dimensional lattice before the exchange took place. In x-ray crystallographic studies of MsbA all nucleotide mimetics (AMP-PNP, ATP- γ -S, ADP-AlFx, and ADP-Vi) crystallized in the same space group with nearly identical cell dimensions (unpublished observations). In the present study we trapped MsbA using different nucleotide mimetics while in the micellar state, as was done in the x-ray crystallographic studies (Ward, et al., 2007). At the same time we removed the detergent and reconstituted the protein into lipid bilayers wherein they were free to diffuse and create lattice contacts and crystallize. It is well known that detergents inhibit crystallization by preventing lattice contacts in membrane proteins. Our data suggest that the micelle and three-dimensional lattice may also mask changes in the protein. The three-dimensional lattice then builds up using the exposed soluble domains in lattice formation and can thereby alter the nature of the protein in the process. Here we show that the ES states (AMPPNP and ATP-AlFx) form one set of lattice contacts while the EP state (ATP-Vi) form a different set of lattice contacts. This result agrees with biochemical data that suggest that these two states should be discrete (Sauna, et al., 2006; Russell and Sharom, 2006; Sauna, et al., 2007). While we can only indirectly observe these differences due to resolution, our electron microscopy reconstructions show that the protein is in a similar overall outward facing conformation in both states. In the reconstituted bilayer environment, the protein may be less constrained and able to crystallize with subtle differences as compared to the micellar three-dimensional crystal used for x-ray crystallography. Small changes in the protein may not be observable by x-ray crystallography because the detergent micelle may mask them.

Summary

MsbA reconstitution and crystallization appears to be detergent/orthologue independent (Table 1). The AMPPNP/AlFx form crystallized in a detergent independent manner and both β -UDM

and C7 detergents were suitable for stabilization and crystallization. We have shown that helical arrays of membrane reconstituted MsbA are sensitive to subtle conformational changes induced by various transition state inhibitors. The strength of these analyses lies in the use of a lipid bilayer environment to study these protein states rather than a detergent micelle system. Though we cannot currently observe those changes directly this work establishes a potential system to examine the three dimensional nucleotide dependent changes of an ABC transporter in a membrane. These efforts, combined with x-ray and biochemical data will allow us to delineate the stepwise progression of MsbA through its catalytic/transport cycle.

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Figure 1.

Negative stain electron micrographs of membrane reconstituted MsbA crystals. (A) Small two-dimensional crystal. (B) Helical crystal growing out of a reconstituted proteoliposome. (C) Illustration of uranyl acetate stain penetration (1) and exclusion (2) by helical crystals, suggesting that the inside of some helices are open to the environment while others remain isolated.

Figure 2.

Cryo-electron micrographs and diffraction patterns from helical crystals with highest visible layer line indicated. (A,B) *V. cholera* MsbA-AIFx (VMsbA-AIFx), (C,D) *S. typhimurium* MsbA-AMPPNP (SMsbA-AMPPNP), (E,F) VMsbA-vanadate. Reciprocal space lattices of helical crystals. (G) VMsbA-AIFx, (H) SMsbA-AMPPNP, (I) VMsbA-vanadate. Note that G and H have the same lattice.

Figure 3.

EM maps of MsbA. Longitudinal views of (A) VMsbA-AIF_x, (B) SMsbA-AMPPNP, (C) VMsbA-vanadate EM helical density maps (gray surface, contoured at 1 sigma). The stars denote individual MsbA dimers. In the (A) VMsbA-AIF_x and (B) SMsbA-AMPPNP crystal forms lattice contacts (blue line) are made between the NBDs of dimers with a red star. Additional lattice contacts are made between the TMDs of different dimers (indicated by the black and lower red star). In VMsbA-vanadate (C) lattice contacts are only made in the TMDs of the dimers (indicated by the black and lower red star). Longitudinal views of (D) VMsbA-AIF_x, (E) SMsbA-AMPPNP, (F) VMsbA-vanadate EM helical density maps (blue mesh, contoured at 1 sigma) with x-ray structure of SMsbA-AMPPNP fit into the density (red and black ribbons). The two-fold axis is labeled with the yellow symbol, illustrating that the VMsbA-vanadate dimer is rotated ~30° clockwise relative to the VMsbA-AIF_x/SMsbA-AMPPNP dimer. Cross sectional views of (G) VMsbA-AIF_x, (H) SMsbA-AMPPNP, (I) VMsbA-vanadate EM helical density maps (blue mesh, contoured at 1 sigma) with x-ray structure of SMsbA-AMPPNP fit into the density (red ribbons). The putative location of the bilayer is denoted by the black lines.

Figure 4.

Longitudinal section through EM map showing one dimer of VMsbA-AIFx. The globular domain is on the outside of the helix while the elongated domains are toward the inside of the helix. A long (~50Å) channel that is continuous with the inside of the helix is formed between the elongated domains.

Table 1

Protein/Crystal Information.

	ALFx	AMPPNP	Vanadate
MsbA orthologue	<i>V cholerae</i>	<i>S typhimurium</i>	<i>V cholerae</i>
pH	5–6	9	5–6
Detergent	Cymal-7	β-UDM	Cymal-7
Helical Repeat (Å)	570	570	740
Diameter (Å)	330	330	250
Symmetry ($n_{1,0}$, $n_{0,1}$) [*]	(13, –8)	(13, –8)	(11, –5)

^{*} Refers to the Bessel order of the layer lines from the (1,0) and (0,1) directions of the underlying 2D lattice.

Table 2

Crystal averaging statistics.

	AlFx	AMPPNP	Vanadate
Number of images	8	5	6
Number of helical repeats	60	48	40
Number of layer lines included in final average	11	11	9