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The crystal structure of BamB suggests interactions with BamA and its role within the BAM complex

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Abstract

E. coli BamB is the largest of four lipoproteins in the β -barrel assembly machinery (BAM) complex. It interacts with the periplasmic domain of BamA, an integral outer membrane protein essential for outer membrane protein biogenesis. Although BamB is not essential, it serves an important function in the BAM complex, significantly increasing the folding efficiency of some OMPs *in vivo* and *in vitro*. To learn more about the BAM complex, we solved structures of BamB in three different crystal forms. BamB crystallized in space groups P2₁3, I222, and P2₁2₁2₁, with one molecule per asymmetric unit in each case. Crystals from the space group I222 diffracted to 1.65 Å resolution. BamB forms an 8-bladed β -propeller with a central pore and is shaped like a doughnut. A DALI search revealed that BamB shares structural homology to several eukaryotic proteins containing WD40 repeat domains, which commonly have β -propeller folds and often serve as scaffolding proteins within larger multi-protein complexes that carry out signal transduction, cell division, and chemotaxis. Using mutagenesis data from previous studies, we docked BamB onto a BamA structural model and assessed known and possible interactions between these two proteins. Our data suggest that BamB serves as a scaffolding protein within the BAM complex by optimally orienting the flexible periplasmic domain of BamA for interaction with other BAM components and chaperones. This may facilitate integration of newly synthesized outer membrane proteins into the outer membrane.

Introduction

Gram-negative bacteria, mitochondria, and chloroplasts contain both an inner and outer membrane. The outer membrane contains numerous β -barrel proteins commonly called **outer membrane proteins** (OMPs), which serve essential functions in cargo transport and signaling and are also vital for membrane biogenesis. In Gram-negative bacteria, OMPs are synthesized in the cytoplasm and then transported across the inner membrane and into the periplasm by the Sec translocon¹. Once in the periplasm, chaperones such as SurA, Skp, and DegP guide nascent OMPs across the periplasm and peptidoglycan layer to the inner surface of the outer membrane². Here, the nascent OMPs are recognized by a five component complex known as the β -barrel **assembly machinery** (BAM) complex which folds and inserts the new OMPs into the outer membrane³. The *E. coli* BAM complex consists of five

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Accession Numbers

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subunits named BamA (formerly YaeT), BamB (YfgL), BamC (NlpB), BamD (YfiO), and BamE (SmpA)⁴. Although we do not yet understand how the BAM complex functions in detail, studies have shown that BamA and BamD are essential for cell viability and OMP biogenesis⁵. Similar mechanisms for OMP biogenesis exist for both mitochondria and chloroplasts, providing further evidence of the evolutionary relationships of these organelles^{6,7}. Structures of large portions of the BamA periplasmic domain were solved recently by X-ray crystallography^{8–10} and NMR¹¹ which provided insight into how BamA recognizes BamB and possibly even nascent OMPs. Still, structures of additional BAM components (and eventually of the intact assembly) are needed in order to fully understand how the BAM complex takes nascent OMPs and then folds and inserts them into the outer membrane.

In *E. coli*, the BAM complex consists of at least 5 components: BamA (88 kDa), BamB (40 kDa), BamC (34 kDa), BamD (26 kDa) and BamE (10 kDa). BamA is a β -barrel protein itself and comprises two domains, an N-terminal periplasmic domain and a C-terminal transmembrane β -barrel domain. The periplasmic domain can be further divided into five subdomains called (polypeptide transport-associated) POTRA domains, numbered 1–5 from N- to C-terminus, with POTRA 5 located closest to the β -barrel domain. BamB, BamC, BamD, and BamE are all lipoproteins bound to the inner leaflet of the outer membrane and attached either directly or indirectly to BamA (Supplementary Figure S1). Recent studies have shown that BamD interacts with BamA through POTRA 5 and that these two components are necessary for cell viability and may form the core of the complex^{5,10}. BamB has been shown to interact directly with BamA through a β -bulge in POTRA 3 and also with POTRA 2, 4, and 5¹⁰. BamC interacts only indirectly with BamA, using the C-terminal portion of BamD to associate with the BAM complex⁵. Finally, BamE is reported to enhance the association of BamD with BamA². The recent X-ray and NMR structures of BamA POTRA subdomains^{8–11} exhibit differing spatial arrangements with significant conformational flexibility between POTRA 2 and POTRA 3 that results in the observed extended or compacted structures. Since the POTRA domains consist primarily of β -strands, a likely mode of association with nascent OMPs could be through β -strand augmentation¹⁰. While the native oligomeric state of the BAM complex is still unknown, some studies indicate that it may function as a dimer or tetramer¹². However, the five components can be assembled *in vitro* and the complex behaves as a monomer with a presumed stoichiometry of 1:1:1:1:1. This complex can fold and insert a β -barrel protein into liposomes in a reaction that requires no energy source as long as a soluble chaperone, SurA, is present¹³.

When BamB is absent from the *in vitro* assembly, OMP folding rates are greatly reduced¹³. Hagan *et al.* concluded that BamB, while non-essential, plays an important role in the assembly of OMPs that are delivered by SurA. In order to better understand the BamA-BamB interaction and how this might facilitate OMP folding and insertion, we solved the structure of BamB in three crystal forms and determined the X-ray crystal structure at 1.65 Å resolution. BamB is an eight-bladed β -propeller that shows homology to other WD40-repeat domain proteins. Residues previously identified by mutagenesis as important in BamA-BamB interactions were used to guide docking of the BamB structure onto a BamA structural model. We propose that BamB acts as a scaffold to optimally orient the flexible POTRA regions of BamA for interaction with other BAM components, chaperones, and nascent OMPs.

Results

The structure of *E. coli* BamB

The structure of BamB was solved in three different space groups, one native (P2₁3) and two with selenomethionine substitution (I222, P2₁2₁2₁) (Table 1, Supplemental Figure S2).

Phases were calculated from a three wavelength MAD experiment in space group I222 using PHENIX¹⁴. An initial model was built by AutoBuild¹⁴ containing ~65% of the total residues and was finished by manual model building using COOT¹⁵. The other structures were subsequently solved by molecular replacement using the I222 crystal structure as a search model. The best crystals diffracted to 1.65 Å resolution. All crystal forms contained one molecule per asymmetric unit suggesting that BamB is a monomer *in vitro*, which was further confirmed by light scattering experiments (discussed below). The native BamB structure contains residues 21–392 arranged as an eight-bladed β-propeller with an N-terminus that extends away from the core of the structure (Figure 1A and 1B). The N-terminus was found disordered in the other crystal forms, indicating that the order observed in our P2₁3 crystal form can be attributed to its interaction with a symmetry mate within the crystal lattice. Each blade is connected by an interconnecting loop (IL), where blade 1 is composed of both N-terminal residues and the C-terminus. Sequence analyses of BamB from *Alphaproteobacteria* predicted the 8-bladed β-propeller motif correctly¹⁶. A surface representation of BamB shows it to have an overall doughnut shape with a hole through the center having a minimal diameter of 2.6 Å as determined by the program HOLE¹⁷ (Figure 1C and 1D). An electrostatic surface potential representation shows that BamB is strongly electronegative (Figure 2A, 2B, and 2C), particularly along the center hole on each side due to clustering of strongly electronegative residues found in these regions (E197, D246, D248, D288, D303, E370). This appears to be a unique property of BamB since charge distributions vary among other β-propeller structures, indicating that the net negative charge may be present for specific functional purposes such as binding to BamA. Mapping hydrophobic residues on the side of BamB with the interconnecting loops shows only a few scattered hydrophobic patches (Figure 2D). All interconnecting loops were well ordered except for IL4 (residues 188–199) and IL5 (residues 233–248), where IL4 is mostly ordered while IL5 contains several completely disordered residues as depicted by a B-factor putty representation (Figure 3A). When comparing the different space groups for BamB, IL4 is ordered in P2₁3 and P2₁2₁2₁, but not in I222, while IL5 is largely disordered in all three space groups reported here (Supplemental Figure S4). Interestingly, conserved residues that were previously reported to be important for interacting with BamA were mapped to IL4 and IL5 (Figure 3B). Another noteworthy feature is IL2 (residues 97–113), which is the largest interconnecting loop. IL2 extends away from core of the molecule and could represent another functional region not previously explored. IL2 is also partially disordered in the I222 space group, whereas it is ordered in the others (Supplemental Figure S4).

A sequence alignment of BamB proteins from *Escherichia coli* (EcBamB), *Pseudomonas aeruginosa* (PaBamB), and *Vibrio cholerae* (VcBamB) has previously been used to investigate conserved regions important for function¹⁸. Using this alignment (Figure 4A), we mapped all residues having a conservation score of 7.5 or greater (max is 11) onto our BamB structure (Figure 4B). Conserved residues were found throughout the entire length of the molecule with the most conserved clusters found in IL4 and IL5 (containing residues L192, L194, R195, D246, and D248 identified by mutagenesis¹⁸), providing further evidence that these two loops are involved in binding BamA. IL2 is the most divergent portion of the entire BamB sequence, suggesting that it may be involved in strain specific interactions. Another possibility that cannot be excluded is that IL2 may serve no function in the BAM complex. More studies are needed to determine what role, if any, IL2 serves.

BamB is a monomer in solution and in the crystal structures

We determined the molecular mass of BamB in solution using size exclusion chromatography coupled to light scattering, UV, and refractive index detectors (SEC-LS/UV/RI). Light scattering measured by 18 fixed angle detectors depends on the molecular weight of the protein, the protein concentration, and the dependence of refractive index on

protein concentration, dn/dc ¹⁹. We used the standard dn/dc value of 0.187 for soluble proteins and normalized the data to BSA. In this system BamB runs as a monomer with a calculated mass of about 44 kDa, which is consistent with its predicted molecular mass from sequence of 40.2 kDa (Supplemental Figure S5). Likewise, BamB occurs as a monomer in our three crystal forms, with one molecule per asymmetric unit in all cases.

BamB shows structural similarity to proteins containing WD40 repeat-like domains

To look for clues about the role of BamB in the BAM complex, a DALI search was performed which showed structural similarity to several other proteins with β -propeller folds, including Fbw7 (F-box/WD repeat-containing protein 7)²⁰, Sif2p (SIR4-interacting protein)²¹, AHD-IIG (quinoxemoprotein alcohol dehydrogenase IIG)²², and cytochrome cd1 nitrite reductase²³ (Supplemental Figure S6). Further investigation revealed all these proteins contain WD40 repeat or WD40 repeat-like domains which are typically about 40 residues long and are characterized by containing the terminating W-D dipeptide sequence^{24–26} (Figure 5A–D). WD40 repeat domains are known to fold into β -propeller structures usually with seven or eight blades, but can range from four to sixteen^{24–26}. Proteins containing WD40 repeat domains often serve as scaffolds that regulate specific protein-protein interactions. One example is RACK1, which is a conserved eukaryotic scaffold protein involved in various signaling pathways including cell stress, translation, and development; RACK1 helps to stabilize protein kinase C signaling complexes²⁷. Exemplifying its role as a mediator of protein-protein complexes, RACK1 has been postulated to interact with more than 80 different proteins. Another example is Han11, which functions as a scaffold protein in the regulation of the kinases HIPK2 and MEKK1²⁸. By binding MEKK1, Han11 is able to couple interactions with HIPK2 and another kinase DYRK1 for the regulation of gene transcription. WD40 repeat and WD40 repeat-like domain containing proteins were initially thought to be found only in eukaryotes²⁵. However, it was later discovered that these proteins are also present in prokaryotes and that they have retained their evolutionarily conserved function as scaffolds involved in the assembly of multi-protein complexes^{26,29}.

Modeling the BamA-BamB complex

To model the interactions between BamB and BamA, we used data from previously reported studies showing that BamB interacts with BamA through residues L192, L194, R195, D246, and D248¹⁸. Our crystal structures revealed that these residues are found in IL4 (L192, L194, R195) and IL5 (D246, D248), two loops having an inherent flexibility that may relate to their function. This observation, coupled with the fact that these loops are slightly longer than the other interconnecting loops (with the exception of IL2) and contain highly conserved sequences, further supports the idea of their involvement in binding to BamA. To explore the possible interaction between BamB and BamA, we used the Zdock³⁰ server to dock BamB onto one of the previously reported BamA-POTRA 1-4 crystal structures (PDB code 3EFC), including a forced interacting interface at D241 of BamA¹⁰ and at residues L192, L194, R195, D246, and D248 of BamB¹⁸ (Figure 6A and 6B). After visually inspecting the top ten docked structures, the most favored complex was found to agree best with previously reported literature and made significant interactions between BamB and BamA-POTRA 2-4, which was not the case for the other docking solutions, although each did have a range of various plausible interactions. In addition, upon closer inspection of the docked complex, R195¹⁸ from BamB was in an ideal position to form a salt bridge with D241¹⁰ of POTRA 3, so this was included in our model (Figure 6D) and is supported by studies showing that shifting the position of D241 reduces or eliminates interaction with BamB¹⁰. We expanded the POTRA 1-4 model using the recently reported POTRA 4-5 crystal structure⁹ and then included the modeled β -barrel domain of BamA by using the previously reported FhaC crystal structure³¹, which has been proposed to contain a

prototypical BamA fold. This allowed us to model the BamA-BamB complex, offering a first impression of what this complex may look like in the outer membrane of Gram-negative bacteria and offering clues to BamB's role in the BAM complex (Figure 7). In our model, BamB residues in IL4 bind to BamA-POTRA 3 through β -strand augmentation. A salt bridge between BamA D241 and BamB R195 could strengthen the interaction. Electrostatics may also play an important role in complex formation; the surface of BamA-POTRA 3 that BamB binds to is highly electropositive (Figure 6C) and none of the other POTRA domains show a similar charge distribution. Residues D246 and D248 of IL5 may derive their functional importance from contributions to the overall charge of POTRA3. Recall that BamB is highly electronegative, especially near the center of the β -propeller.

Discussion

The BAM complex is responsible for recognizing nascent OMPs and then folding and inserting them into the outer membrane of Gram negative bacteria³. The *E. coli* BAM complex contains at least five components consisting of BamA (an OMP itself), BamB, BamC, BamD, and BamE⁴. However, we don't yet understand how these components work together. To better comprehend how the BAM complex functions, we solved crystal structures of BamB at high resolution in three different space groups. Our structures of BamB combined with previously reported crystal structures of BamA-POTRA 1-4^{8,10}, BamA-POTRA 4-5⁹, and FhaC³¹, allowed us to model what the BamA-BamB complex may look like at the outer membrane. Our BamA-BamB model incorporates previously reported bioinformatics, mutagenesis, and functional assays^{10,18} that place constraints on binding interfaces for both BamB and BamA, which we used with Zdock in order to dock the two protein molecules. The docked complex agrees well with previously reported literature which indicates that BamB interacts not only with POTRA 3 and 4, but also POTRA 2¹⁰. In our model, POTRA 3 interacts with BamB loops IL4 and IL5, contributing the largest interface of the POTRA domains and interacting across BamB blades 1-5. POTRA 2 sits primarily along the outer edge of BamB blade 4, while POTRA 4 interacts with the opposite edge of BamB, primarily along blade 1. An interesting observation of the model is that IL2, which is the longest interconnecting loop (found extending away from the core of the BamB structure), appears to cooperate in binding POTRA 3 by acting as a molecular arm to possibly help stabilize the BamA-BamB interaction. Given that this loop is the most divergent in sequence, this result suggests that IL2 may play a role in strain specific interactions with either BamA or with other effector proteins. To our knowledge, no studies have directly looked at IL2 and its role in mediating the BamA-BamB interaction. Mapping the electrostatics of the POTRA domains to the surface shows various patches of charge scattered over the entire molecule (Figure 6C). Closer inspection of the proposed binding interface shows that there is a large region of electropositive charge observed on the surface of POTRA 3 which would complement the strong electronegative charge observed on BamB, suggesting that the electrostatics may play a substantial role in the recognition of BamA.

While preparing this manuscript for publication, coordinates for BamB (PDB code 3P1L) were deposited into the Protein Data Bank. In this structure, IL4 and IL5 are well ordered³², in contrast to our data that suggest an inherent flexibility in these regions. However, closer inspection of the crystal packing (space group P4₃2₁2) shows that these loops make primary crystal contacts with a symmetry related molecule, explaining their observed stabilized conformation. In all three space groups reported here for BamB, IL4 and IL5 are either partially or completely disordered, suggesting that their flexibility is a physiological feature of BamB and may help to mediate interaction with BamA.

Using the BamB structure to look for clues about its role in the BAM complex, a DALI search revealed that BamB contains WD40 repeat-like domains and may therefore act as a scaffolding protein to assist in coordinating the POTRA domains for nascent OMP recognition and subsequent insertion. While it is known that BamB is important, particularly in folding some OMPs, it serves a non-essential function since cells survive without its expression. However, recently it has been shown using an *in vitro* folding assay that BamB may be more important than initially thought since its absence led to a significant decrease in the folding efficiency of OmpT¹³. It has also been shown that the POTRA domains of BamA are inherently flexible and may assume different conformations⁹, making it difficult to understand their role within the mechanism of the BAM complex. However, given that the presence of BamB leads to a significant increase in OMP folding efficiency observed both *in vivo*^{33–35} and *in vitro*¹³, and given its relation to proteins containing WD40 repeat-like domains, BamB may act primarily as a scaffold in the BAM complex to stabilize the POTRA domains of BamA in a conformation that is optimal for nascent OMP recognition and folding. Note that the most flexibility in BamA is observed between POTRA domains 2 and 3⁹, precisely where BamB is predicted to bind. In addition, BamB may also serve to regulate other effector proteins and in some instances, participate in direct binding or delivery of nascent OMPs. This notion is supported by a study showing that BamB mutants and SurA mutants resulted in the same phenotype, leading to a significant reduction in the efficiency of LamB folding at the outer membrane³⁶.

Another recent report using an *in vitro* folding assay showed that when both BamB and SurA were present, folding efficiency of nascent OmpT was significantly higher compared to when one of the proteins (BamB or SurA) was absent¹³, suggesting a direct interaction. If this is the case, BamB would likely act as a scaffold to mediate the transfer of substrate from SurA to BamA by interacting with both concurrently. Although mutations in BamB IL4 led to reduced binding of BamA and SurA (giving rise to the suggestion that BamB does not bind SurA directly)¹⁸, it is also possible that those mutations destabilize a BamA-BamB-SurA triple complex. We suggest that in order for SurA to deliver a nascent OMP, it would have to interact with BamA, and BamB may help mediate this interaction. Although it is unlikely that BamB directly transfers the nascent OMP to BamA since it has no demonstrated enzymatic activity (and other WD40 domains don't either), it could mediate the stable association between BamA and SurA. Without BamB, this interaction still occurs, since BamB is not essential for viability, however, with BamB the process is much more efficient. Our docking study supports and illustrates this notion of BamB being a scaffolding protein within the BAM complex, since in order for it to simultaneously interact with POTRA 2-4 and possibly POTRA 5 as well¹⁰, a significant restriction on the conformational freedom of the POTRA domains would be imposed, leading to a conformation that would be optimal for nascent OMP delivery and subsequent folding and insertion into the outer membrane.

Materials and Methods

Cloning and expression

The *E. coli* BamB coding sequence starting at residue S21 was amplified from genomic DNA prepared from BL21(DE3) cells using the following primers which add an N-terminal NcoI site and a C-terminal XhoI site for cloning: (1) 5'-GATATCCATGGGATCGCTGTTTAACAGCGAAGA-3' and (2) 5'-CTATGCTCGAGTTAACGTGTAATAGAGTAC-3'. The insert was then digested and cloned into the pHis2-parallel vector (Novagen) and the sequence verified by sequencing analysis (NIH/FDA facility). This plasmid was then transformed into T7 Express cells (New England Biolabs) and plated and incubated on LB-carbenicillin agar plates overnight. A single colony was used to inoculate a 5-mL LB-carbenicillin culture and allowed to grow to

OD₆₀₀ ~0.6 and then 0.75 mL was added to six 2-L baffled flasks containing 750 mL 2xYT media supplemented with carbenicillin (100 µg/mL). Cultures were allowed to grow to OD₆₀₀ between 0.7 and 1.0 and then induced with 0.5 mM IPTG. The temperature was then reduced to 20°C and cells were harvested after 4–8 hours. For selenomethionine substituted protein, the BamB expression plasmid was transformed into B834 cells (Novagen) and plated and incubated on LB-carb agar plates overnight. A single colony was used to inoculate a 25 mL LB-carb culture and allowed to grow to OD₆₀₀ ~0.8–1.0. The cells were then centrifuged, washed 3 times with minimal media lacking methionine, resuspended in 6 mL wash media and then 1 mL added to six 2-L baffled flasks containing 750 mL minimal media supplemented with 40 mg/L selenomethionine and carbenicillin (100 µg/mL). These cultures were allowed to grow 24 hours at 37 °C or until OD₆₀₀ was above 0.6, then induced with 0.5 mM IPTG and allowed to grow an additional 24 hours at 37 °C before harvesting.

Protein purification and crystallization

For wild-type protein, cells were resuspended in lysis buffer (3 mL lysis buffer per gram of cells) containing 1X PBS (154 mM NaCl, 0.8 mM KH₂PO₄, and 5.6 mM Na₂HPO₄) supplemented with DNaseI and protease inhibitors. Cells were lysed at 18,000 psi by two passes through a French Press instrument and the lysates centrifuged at 18,000 rpm for 45 minutes using an SS34 Sorvall rotor. The supernatants were then filtered and applied to a pre-equilibrated 5 mL Ni-NTA resin column. The column was washed with at least 10 column volumes of 1X PBS, a pre-elution performed using 1X PBS with 20 mM imidazole, and a final elution done with 1X PBS containing 250 mM imidazole. To remove the N-terminal His-tag, TEV protease was added to the final protein sample along with 0.5 mM DTT and dialyzed overnight at 4°C in 1X PBS. The dialyzed sample was then again applied to a 2 mL pre-equilibrated Ni-NTA resin column. The filtrate was collected, concentrated and then applied to a S-100 sephacryl gel filtration column as a final purification step. Selenomethionine substituted protein was purified using the same protocol as with wild-type except 2 mM β-ME was added to all buffers during the purification (except for gel filtration) to prevent oxidation of the selenium atoms. For crystallization, the protein samples were concentrated to 10 mg/mL and then screened using sparse crystallization matrices on a TTP LabTech Mosquito crystallization robot. Lead conditions were further optimized when needed. Wild-type BamB (P2₁3) was crystallized from 1.0 M lithium sulfate and 2% PEG 8000 and selenomethionine substituted BamB crystals grew from 0.18 M tri-ammonium citrate and 20% PEG 3350 in two other unique space groups (I222 and P2₁2₁2₁).

Data collection and structure determination

Crystals for both wild-type and selenomethionine substituted protein were harvested directly from their drops and flash frozen in liquid nitrogen. Crystals were typically pre-screened using our in-house X-ray instrument (Rigaku MicroMax-007 HF microfocus X-ray generator, Raxis IV++ detector) and final datasets for wild-type BamB collected at SERCAT beamline 22ID at the Advanced Photon Source, Argonne National Laboratory. Final datasets for selenomethionine substituted crystals were collected at GM/CA CAT beamline 23ID at the Advanced Photon Source, Argonne National Laboratory. All data were processed using HKL2000³⁷. For MAD phasing, the I222 datasets (peak, high energy remote, edge, 2.0 Å) were used within PHENIX¹⁴ to locate the selenium sites (found 5 of 6 Se) followed by AutoBuild which was able to quick-build ~65% of the molecule. The electron density was of excellent quality and the structure was finished by manual building within COOT¹⁵ (Supplemental Figure S2). Molecular replacement was used to solve the native structure (P2₁3) as well as the other selenomethionine substituted structures using PHASER³⁸ and refinement was performed for all structures using PHENIX¹⁴. Data collection and refinement statistics are summarized in Table 1. All figures were made using PyMOL

(Schrödinger), including hydrophobic (VaSCo plugin³⁹) and electrostatic potential calculations (APBS2 plugin⁴⁰) and visualizations.

Molecular mass determination by SEC-LS/UV/RI

A 200 μ L aliquot of BamB (2.0 mg/ml) was centrifuged for 15 minutes (4°C) at 60,000 rpm using a TLA 120.1 rotor in a Beckman Optima MAX ultracentrifuge and then passed through a 0.2 μ M filter. This sample (50 μ L) was loaded into an Agilent Technologies 1200 Series Quaternary HPLC instrument equipped with a Shodex KW-803 gel filtration column (50–150,000 MW Range) coupled to a Wyatt Dawn Heleos multi-angle light scattering detector and a Wyatt Optilab T-rEX refractive index detector. The sample was eluted at 0.3 mL/min for 60 minutes in 1X PBS and analyzed using the Wyatt Astra software. Results were normalized to BSA which was used as a standard (data not shown).

Sequence alignment

A sequence alignment of BamB homologs from *Escherichia coli* (EcBamB), *Pseudomonas aeruginosa* (PaBamB), and *Vibrio cholerae* (VcBamB) was performed based on a previous study¹⁸ using the program STRAP⁴¹ and JalView⁴². Those residues having a conservation score of 7.5 or greater were color coded from yellow (7.5) to blue (11, max) and mapped onto the surface of the BamB structure. The BamB secondary structure was then mapped onto the sequence alignment and annotated based on the elements within the crystal structure. Those residues that have previously been reported to be involved in binding BamA are indicated by red asterisks (Figure 4).

Docking and Modeling Studies

To determine how BamB might interact with the POTRA domains of BamA, we set out to model the complex. Initially we examined the deposited coordinates for POTRA 1-4 in two different conformations (PDB codes 2QDF and 3EFC), both of which likely represent physiologically relevant states. However, while manually docking BamB onto each structure individually we observed that the coordinates 3EFC were in a more optimal conformation for interacting with BamB while preserving the known interactions. Subsequent docking of the BamB and POTRA 1-4 (PDB code 3EFC) crystal structures was performed using the Zdock server³⁰. Based on previous studies, residues L192, L194, R195, D246, and D248 of BamB¹⁸ were forced into the interaction interface, along with residue D241 of POTRA 1-4¹⁰. The top ten docked solutions were manually inspected with the top solution agreeing the best with published literature. The POTRA 1-5/BamB model was formed by aligning the recently published POTRA 4-5 crystal structure (PDB code 3OG5) to our docked complex. For modeling the β -barrel domain of BamA, POTRA 5 of the POTRA 1-5 model was aligned to POTRA 2 of FhaC (PDB code 2QDZ). The BamA model was formed by merging the β -barrel domain of FhaC with the POTRA 1-5 model. The BamA model was then superimposed onto the docked complex structure of POTRA 1-4:BamB to form the modeled BamA:BamB complex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overall structure of BamB. A, BamB has an eight bladed β -propeller fold with each blade shown in a different color (N-terminal residues 21–40 have been removed for clarity) and is rotated 90° about the x-axis in panel B. C, Space-filling representation of BamB rendered transparent and rotated 90° about the x-axis in panel D.

Figure 2.

Surface characteristics of BamB. A, Electrostatic surface potential representation of BamB shows that the core region is strongly electronegative on both sides (B and C, rotated +90° and -90° about x-axis compared to panel A, respectively, to illustrate both sides). D. Surface hydrophobicity of BamB highlighting the most hydrophobic residues in yellow. The locations of IL2, IL4, and IL5 are indicated for reference.

Figure 3.

BamB IL4 and IL5 are partially disordered and contain residues important for binding BamA. A, B-factor putty representation of BamB (P2₁₃) shows that the structure is mostly ordered with only N-terminal residues 21–40, IL4, and IL5 showing significant flexibility. B, Zoomed view indicating residues in BamB that were previously shown to lie within the binding interface with BamA. BamB is color coded according to Figure 1, important residues are shown in stick representation, and the disordered residues in IL5 are represented by a dashed line.

Figure 4. Sequence alignment of BamB homologs from *Escherichia coli* (EcBamB), *Pseudomonas aeruginosa* (PaBamB), and *Vibrio cholerae* (VcBamB). A, Residues having a conservation score of 7.5 or greater are color coded from yellow (7.5) to blue (11, max) and mapped onto the surface of the BamB structure. The BamB secondary structure is shown above the sequence alignment. Residues that have previously been reported to be involved in binding BamA are indicated by red asterisks. B, Conserved residues (blue) having a conservation score of 7.5 or greater were mapped onto the BamB crystal structure reported here.

Figure 5.

BamB shows structural similarity to proteins containing WD40 repeat-like domains. A, β -propeller blade 2 from the BamB crystal structure (stick model) showing the electron density (blue mesh) from a σ -A weighted 2Fo-Fc map contoured at 1.0 σ . B, Ribbon representation of blade 2 illustrating its WD40 repeat-like domain structure, showing tryptophan and aspartate residues in stick representation. C, Ribbon representation of a WD40 repeat domain from Cdc41, showing tryptophan and aspartate residues in stick representation. D, Superposition of the WD40 domain from Cdc41 and the WD40 repeat-like domain from BamB.

Figure 6.

Docking the BamA-BamB complex. A, Docked structure of BamB (green) onto POTRA 1-5, with each of the POTRA domains shown in a different color and in panel B, rotated 90° along the y-axis. C, Electrostatic charge distributions for POTRA 1-4, with BamB shown in green. D, Zoomed view of docked structure along POTRA 2-4, highlighting residues in IL4 (blue) and IL5 (pink) important for binding and a potential salt bridge formed between D241 (BamA) and R195 (BamB). As predicted from previous reports and obtained as a result in our docked model, strand $\beta 2$ from POTRA 3 appears to interact with BamB IL4 (blue) by β -strand augmentation.

Figure 7. Model depicting how BamB interacts with BamA at the outer membrane. This model is based upon previously reported studies, homology to known structures, and our docking studies with the BamB structures.

Table 1

Data collection and structure refinement summary.

	Native	SeMet-p	SeMet-hr	SeMet-e	SeMet	SeMet
Data Collection						
Space group	P2 ₁ 3	I222	I222	I222	I222	P2 ₁ 2 ₁ 2 ₁
Mol/ASU	1	1	1	1	1	1
a (Å)	103.145	50.117	50.113	50.173	50.318	49.004
b (Å)	103.145	122.663	122.746	122.848	121.437	69.991
c (Å)	103.145	134.134	134.260	134.448	134.291	117.630
α (°)	90	90	90	90	90	90
β (°)	90	90	90	90	90	90
γ (°)	90	90	90	90	90	90
Resolution (Å)	2.1	2.0	2.0	2.0	1.65	1.8
γ (Å)	1.00	0.97944	0.94944	0.97960	1.00	0.97957
Completeness (%) *	99.7 (98.9)	99.7 (100)	99.7 (100)	99.7 (100)	99.9 (99.9)	86.8 (99.0)
R_{sym} *	0.14 (0.61)	0.09 (0.58)	0.05 (0.31)	0.06 (0.47)	0.08 (0.88)	0.09 (0.75)
I/σ (I) *	12.0 (1.8)	19.8 (2.1)	30.5 (4.5)	28.5 (2.9)	24.1 (1.6)	19.1 (1.8)
Refinement						
R/Rfree	0.21/0.26				0.16/0.19	0.19/0.23
Bonds (Å)	0.009				0.006	0.006
Angles (°)	1.265				1.065	1.066
Protein atoms	2715				2541	2639
Waters	127				331	360
B-factors						
Protein	25.70				25.21	21.00
Waters	25.40				38.03	32.33
Ramachandran Analysis						
Core (%)	85.1				87.6	87.8
Allowed (%)	13.7				12.0	11.5

	Native	SeMet-p	SeMet-hr	SeMet-e	SeMet	SeMet
Generously Allowed (%)	1.3				0.3	0.7
Disallowed (%)	0				0	0

* highest resolution shell shown in parentheses