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BamE Modulates the *Escherichia coli* Beta-Barrel Assembly Machine Component BamA

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Biogenesis of the outer membrane (OM) is an essential process in Gram-negative bacteria. One of the key steps of OM biogenesis is the assembly of integral outer membrane beta-barrel proteins (OMPs) by a protein machine called the Bam complex. In *Escherichia coli*, the Bam complex is composed of the essential proteins BamA and BamD and three nonessential lipoproteins, BamB, BamC, and BamE. Both BamC and BamE are important for stabilizing the interaction between BamA and BamD. We used comprehensive genetic analysis to clarify the interplay between BamA and the BamCDE subcomplex. Combining a Δ*bamE* allele with mutations in genes that encode other OMP assembly factors leads to severe synthetic phenotypes, suggesting a critical function for BamE. These synthetic phenotypes are not nearly as severe in a Δ*bamC* background, suggesting that the functions of BamC and BamE are not completely overlapping. This unique function of BamE is related to the conformational state of BamA. In wild-type cells, BamA is sensitive to externally added proteinase K. Strikingly, when Δ*bamE* mutant cells are treated with proteinase K, BamA is degraded beyond detection. Taken together, our findings suggest that BamE modulates the conformation of BamA, likely through its interactions with BamD.

The outer membrane (OM) is an essential organelle of Gram-negative bacteria such as *Escherichia coli* (26). A key function of the OM is to serve as a barrier preventing access of toxic molecules from the extracellular environment into the bacterial cell (4, 5, 8, 26). Unlike the inner membrane (IM), the OM is an asymmetric bilayer with lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner leaflet (5, 26). LPS repels hydrophobic compounds, including some antibiotics, and is a well-characterized antagonist of the human innate immune system. The OM also contains many proteins, including integral outer membrane beta-barrel proteins (OMPs) and lipoproteins which are tethered to the OM by amino-terminal lipid moieties (28). OMPs are involved in a variety of physiological processes, including nutrient acquisition and efflux of small molecules such as antibiotics (18, 20, 21).

OMPs are assembled by a machine localized in the OM called the Bam complex (10, 14, 26). In *E. coli*, this multisubunit complex is composed of the OMP BamA and four associated OM lipoproteins, BamBCDE (13, 21). Depletion of either BamA or BamD results in severe OMP assembly defects, and both proteins are essential for growth (16, 19, 30). In contrast, null mutations in *bamB*, *bamC*, or *bamE* cause minor OMP assembly defects (27, 30).

Genetic and biochemical studies have begun to show how the subunits of the Bam complex interact with one another (13, 21). For example, it is now known that BamCDE form a stable subcomplex that interacts with BamA and that the BamA-BamCDE interaction occurs independently of the interaction between BamA and BamB (12, 27). *In vitro* reconstitution studies have demonstrated that to achieve optimal assembly of OmpT, all five members of the Bam complex (and the periplasmic chaperone SurA) must be present in the reaction mixture (11, 12). However, the full extent of the interactions between Bam complex members and their contribution to OMP assembly remains poorly defined.

Very little is known about the underlying mechanism by which the Bam complex folds and inserts OMPs into the OM. In this report, we describe efforts to better define the role of the BamCDE subcomplex during OMP assembly. In particular, we wanted to understand the function of BamC and BamE and their relationship to BamA and BamD. Biochemical data show that BamC and BamE share a function in that both are important for stabilizing the interaction between BamA and BamD. By comparing the phenotypes conferred by and the genetic interactions observed between Δ*bamC* and Δ*bamE* and genes for other proteins involved in OMP biogenesis and by exploiting the accessibility of BamA to externally added protease, we have uncovered an additional novel function for BamE: BamE functions to control the conformation of BamA, likely through its interactions with BamD.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. All strains and plasmids used in this study are listed in Table 1. The *bamC*::*kan* and *bamE*::*kan* alleles, obtained from the Keio collection (2), were cured as described previously to generate the Δ*bamC* and Δ*bamE* alleles. The BamD overexpression plasmid contains a mutation in pBamD (27) that increases plasmid copy number and was isolated in a screen for *bamD* mutants (N. W. Rigel and T. J. Silhavy, unpublished). Cultures were grown in Luria-Bertani (LB) medium at 30°C or 37°C as indicated. Where appropriate, antibiotics were included in the growth medium at the following concentrations: ampicillin, 125 μg/ml; kanamycin, 25 μg/ml; tetracycline, 25 μg/ml; and chloramphenicol, 20 μg/ml.

Growth curves. Cultures of each strain were grown overnight in LB broth at 30°C. The next day, strains were diluted 1:1,000 to give a starting optical density at 600 nm (OD_{600}) of approximately 0.05. All strains were then incubated with vigorous shaking at 37°C, and growth was monitored by measuring OD_{600} every hour.

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TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Description	Reference
Strains		
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 thi*	6
JAS16	MC4100 Δ*surA*	This study
JAS111	MC4100 Δ*skp* Δ*degP*	This study
JAS185	MC4100 *bamB*::*kan*	This study
JAS189	MC4100 Δ*bamE* Δ*skp* Δ*degP*	This study
JAS192	MC4100 Δ*bamC*	This study
JAS384	MC4100 Δ*bamC* Δ*surA*	This study
JAS386	MC4100 Δ*bamC* Δ*skp* Δ*degP*	This study
JAS387	MC4100 Δ*bamC* Δ*bamE*	This study
JAS394	MC4100 Δ*bamE* Δ*surA*	This study
JAS544	MC4100 Δ*bamE*	This study
JAS609	MC4100 Δ*bamC bamB*::*kan*	This study
JCM290	MC4100 Ara$^{r/-}$ Δ*bamD* Δ(λ*att-lom*)::*bla* P_{BAD} *bamD araC*	16
JCM375	MC4100 *bamE*::*cam*	27
NR698	MC4100 *lptD4213*	23
NWR513	MC4100 *bamE*::*cam*/pZS21	This study
NWR523	MC4100 *bamE*::*cam*/pBamD O/E	This study
NWR543	NWR523/*bamA101*	This study
Plasmids		
pBamD	Kan^r	27
pBamD O/E	Kan^r	N. W. Rigel and T. J. Silhavy

Antibiotic sensitivity assays. Sensitivity to antibiotics was determined by disc diffusion assays. One hundred microliters of an overnight culture was used to inoculate 3 ml of fresh LB broth. This culture was mixed with 3 ml of molten LB top agar and poured on top of an LB agar plate. Once the top agar solidified, sterile 6-mm filter discs (BBL) impregnated with antibiotics were placed on top. After the plates were incubated overnight at 37°C, zones of growth inhibition around the discs were measured. Representative data from one of three independent experiments are shown.

Immunoblot analysis. To monitor the assembly of OMPs in each mutant, we performed immunoblotting on total protein extracts from late-exponential-phase cultures. Cell pellets from 1-ml aliquots of each culture were harvested by centrifugation and then resuspended in SDS-PAGE sample buffer in a volume equal to the OD_{600} divided by 5. Equal volumes of each extract were resolved by SDS-PAGE according to standard methods. Proteins were transferred to nitrocellulose membranes and probed with polyclonal rabbit antibodies raised against LamB and OmpA (1:15,000), LptD (1:5,000), DegP (1:30,000), BamA (1:20,000), BamB (1:10,000), BamC (1:10,000), BamD (1:20,000), BamE (1:10,000), RpoD (1:20,000), SurA (1:8,000), and maltose binding protein (MBP) (1:15,000). Donkey anti-rabbit secondary antibody conjugated to HRP was used at a concentration of 1:10,000. Blots were developed with ECL (Amersham) and visualized using X-ray film (Denville). The SurA antibody was a gift from Roberto Kolter. The RpoD antibody was a gift from Richard Burgess. The BamB and BamD antibodies were a gift from Dan Kahne.

Protease sensitivity assays. The topology of the BamA barrel domain was predicted using PRED-TMBB (3). Sensitivity of BamA to protease treatment was tested based on a protocol published elsewhere (15). A cell pellet from 0.1 ml of overnight culture was collected by centrifugation and washed 3 times in an equal volume of reaction buffer composed of 20 mM Tris HCl (pH 7.4) and 0.1 M NaCl. When appropriate, proteinase K was added to a final concentration of 0.5 mg/ml, and each reaction mixture was incubated at 37°C for 30 min. Proteinase K was inactivated by the addition of phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 5 mM, followed by boiling for 10 min. Samples were resuspended in SDS-PAGE buffer and analyzed by immunoblotting. This experiment was also performed using actively growing cells in exponential phase with similar results.

Protein pulldowns. To simplify purification of the Bam complex, a plasmid encoding BamA with an N-terminal His_6 tag was transformed into wild-type and Δ*bamC* and Δ*bamE* mutant strains. The resulting transformants were cultured in 100 ml LB until they reached OD_{600} of 2.0. Cells were harvested by centrifugation and frozen overnight at −80°C. The frozen cell pellet was then thawed at room temperature and lysed using 4 ml of BugBuster (Novagen) supplemented with 1 mM PMSF according to the manufacturer's protocol. The resulting lysate was centrifuged at 20,000 × *g* to remove unbroken cells. The clarified lysate was incubated with 0.1 ml of Ni-nitrilotriacetic acid (NTA) resin (Qiagen) for 2 h at 4°C. This mixture was loaded onto a column and washed with 8 ml wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 20 mM imidazole, pH 8.0). Purified BamA-His and any interacting proteins were eluted using 0.5 ml elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 250 mM imidazole, pH 8.0).

RESULTS

OMP assembly defects are similar in Δ*bamC* and Δ*bamE* mutants. As has been reported previously, both Δ*bamC* and Δ*bamE* single mutants grow as well as wild-type *E. coli* in liquid media and on solid media under standard laboratory conditions, as does a mutant lacking the other nonessential lipoprotein, BamB (Fig. 1) (27, 30). Defects in OMP assembly are reflected by decreased OMP levels, since misfolded, mistargeted OMPs are rapidly degraded in the periplasm (17). We monitored the levels of several different kinds of OMPs, including trimeric LamB, monomeric OmpA, and a difficult-to-assemble Bam substrate, LptD (7, 22). We did not notice any appreciable difference in OMP levels between the Δ*bamC* and Δ*bamE* mutants (Fig. 2). In fact, both mutants showed only slight OMP assembly defects compared to wild-type *E. coli*. This is in contrast to the OMP assembly defects conferred by a *bamB*::*kan* mutation. In the absence of BamB, OMP assembly defects are more pronounced (Fig. 2). We conclude that the function(s) of BamC and BamE is not required for OMP assembly under standard laboratory growth conditions.

OM permeability defects are similar in Δ*bamC* and Δ*bamE* mutants. We tested the Δ*bamC* and Δ*bamE* mutants for sensitivity to several different antibiotics as a way to measure integrity of the OM. Regardless of the antibiotic used, the Δ*bamC* and Δ*bamE* mutants displayed sensitivity that was only slightly different from

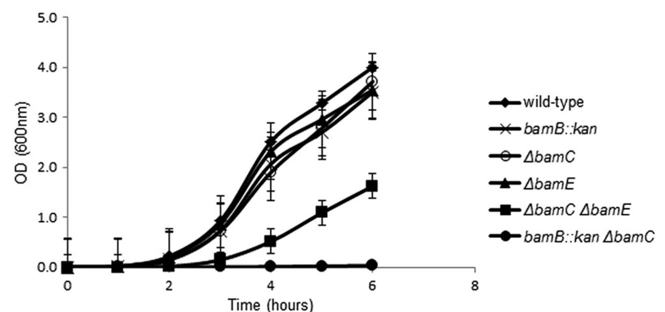

FIG 1 Bam complex double mutants have growth defects in liquid culture at 37°C. Each strain was initially grown in LB broth overnight at 30°C and then subcultured into fresh LB and grown at 37°C. Growth was monitored by measuring the OD_{600}.

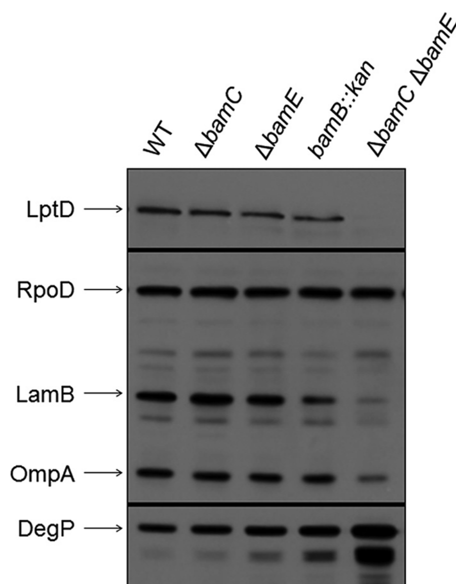

FIG 2 OMP assembly is impaired in some *bam* mutants. Whole-cell protein extracts were prepared from wild-type (WT) and *bam* mutant strains grown at 37°C. The extracts were then separated by SDS-PAGE and analyzed by immunoblotting. To monitor steady-state OMP levels, anti-LamB, -OmpA, and -LptD antibodies were used. Anti-DegP antibodies were used to monitor induction of the envelope stress response. Anti-RpoD antibodies were used as a loading control.

that of the wild-type parent strain (Table 2). Again, this phenotype is in contrast to that of strains lacking BamB. The *bamB*::*kan* mutant is more sensitive to antibiotics than wild-type *E. coli*, indicating that the barrier function of the OM has been compromised to a greater extent in this mutant (Table 2). As expected from the lack of an OMP assembly defect, the barrier function of the OM is not as strongly affected by the loss of BamC or BamE.

BamC and BamE both stabilize the Bam complex. In wild-type *E. coli*, all five members of the Bam complex are stably associated such that the entire five-member complex can be purified in the absence of a chemical cross-linker (21). From previous studies, we know that BamA directly interacts with BamB, as well as BamD (16, 30). Both of these interactions occur independently of each other. However, in the absence of BamC or BamE, the BamA-BamD interaction is diminished (27). The degree to which the BamA-BamD interaction is destabilized is similar in both the Δ*bamC* and Δ*bamE* mutants (Fig. 3A). Notably, the absence of BamC or BamE does not weaken the interaction between BamA and BamB. It is also important to note that even though the Bam complex is destabilized by the loss of BamC or BamE, this destabilization has no impact on the steady-state levels of any other Bam complex member (Fig. 3B). Thus, BamC and BamE share a function: they stabilize the interaction between the two essential Bam proteins BamA and BamD.

Synthetic phenotypes are more pronounced in Δ*bamE* mutants than in Δ*bamC* mutants. In marked contrast to the phenotypic and biochemical similarities described in the previous sections, differences between BamC and BamE are readily apparent when genetic interactions are examined. These genetic interactions were revealed by combining the Δ*bamC* and Δ*bamE* alleles with other OMP assembly-defective mutations. In particular, we systematically paired the Δ*bamC* and Δ*bamE* alleles with mutations in difficult OMP substrates (*lptD4213*), periplasmic chaperones (*surA* and *skp degP*), and the Bam machine itself (*bamB*::*kan* and *bamA101*) (Table 3).

LptD is an essential OMP that contains intramolecular disulfide bonds and must associate with its lipoprotein partner LptE in order to be correctly assembled in the OM (7, 22, 24). The *lptD4213* allele was identified in a screen for mutants with increased OM permeability (25). LptD4213 contains a 23-amino-acid deletion that destabilizes the protein and impairs assembly of this essential OMP. When we combined the assembly-defective *lptD4213* mutation with Δ*bamC*, the resulting strain was able to grow just as well at 37°C as when *lptD4213* was introduced into an otherwise wild-type strain. However, we could not construct a Δ*bamE lptD4213* double mutant under these conditions, suggesting that BamE is critical for the efficient assembly of this mutated essential protein.

To compromise OMP assembly at a different step, we attempted to construct Δ*bamC* or Δ*bamE* mutants that also were deficient for periplasmic chaperones. In *E. coli*, parallel chaperone pathways deliver OMPs to the Bam complex (27, 29). SurA is important for assembling the major OMPs efficiently, especially the essential protein LptD. Skp and DegP can also aid in OMP assembly and are particularly important in the absence of SurA. Both Δ*bamC surA* and Δ*bamC skp degP* mutants were viable at 37°C, but they exhibited growth defects compared to *surA* and *skp degP* mutants, respectively. In contrast to the *bamC* mutant phenotypes, however, the Δ*bamE surA* mutant strain grew poorly, and we were unable to construct a Δ*bamE skp degP* mutant at 37°C.

As noted above, mutants lacking BamB exhibit greater defects than mutants lacking either BamC or BamE. Figure 2 and Table 2 summarize the phenotypes of *bamB*::*kan*, Δ*bamC*, and Δ*bamE* mutants. The phenotypes of a Δ*bamC* Δ*bamE* double mutant are clearly more severe than the defects displayed by the *bamB*::*kan* mutant. However, the Δ*bamC* Δ*bamE* double mutant is the least defective of all the double mutants. Simultaneous deletion of *bamB* and *bamC* results in a temperature-sensitive phenotype. As reported previously and confirmed here, a Δ*bamB* Δ*bamE* double mutant is not viable on LB at 37°C.

Lastly, we combined the Δ*bamC* and Δ*bamE* alleles with a mutation that affects the central component of the Bam complex, BamA. We chose the previously isolated *bamA101* allele, which reduces production of BamA protein 5-fold. In a *bamA101* strain,

TABLE 2 Antibiotic sensitivity profiles of Bam complex mutants

Strain	Zone of inhibition (mm) with[a]:				
	Bac	Erm	Nov	Rif	Vanc
Wild type	9	9	9	9.5	9
bamB::*kan* mutant	10.5	11.5	10.5	16.5	15
Δ*bamC* mutant	8.5	8.5	8.5	9	9
Δ*bamE* mutant	9.5	10	9	9	9.5
Δ*bamC* Δ*bamE* mutant	13.5	12.5	11	16.5	19

[a] One hundred microliters of overnight culture of the indicated strain was mixed with molten top agar and overlaid onto LB agar plates. Filter discs impregnated with the indicated antibiotics were placed onto the solidified agar. After overnight growth at 37°C, the diameters of the zones of growth inhibition (including the 6-mm disc) were measured and recorded in mm. Bac, bacitracin; Erm, erythromycin; Nov, novobiocin; Rif, rifampin; Vanc, vancomycin. The results shown are from a representative experiment.

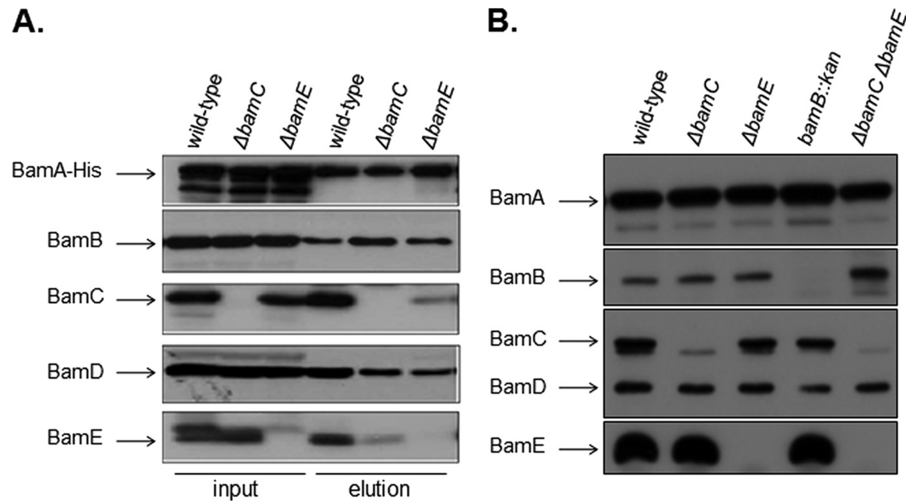

FIG 3 The Bam complex is destabilized in the absence of BamC or BamE. (A) Lysates were prepared from wild-type or Δ*bamC* or Δ*bamE* mutant strains carrying a plasmid encoding His-tagged BamA. After purification over an Ni-NTA column, proteins were eluted with SDS-PAGE sample buffer and analyzed by SDS-PAGE and immunoblotting. (B) Whole-cell lysates of the indicated strains were prepared from exponential-phase cultures and analyzed by SDS-PAGE and immunoblotting with antibodies directed against each protein in the Bam complex.

assembly of the OMPs LamB and OmpA is decreased, but the level of BamA protein is still sufficient to support cell growth (1). The Δ*bamC bamA101* mutant was viable, but it displayed growth defects at 37°C. Despite several attempts, we could not construct a Δ*bamE bamA101* double mutant under any condition. The Δ*bamE bamA101* mutations are synthetically lethal.

The results of our genetic analysis are striking. While the phenotypes of Δ*bamC* and Δ*bamE* single mutants are basically indistinguishable, the phenotypes of Δ*bamC* and Δ*bamE* double mutants are quite different. In every case that we tested, the phenotype of a Δ*bamE* double mutant is significantly worse than that of a Δ*bamC* double mutant. Our findings argue that although both BamC and BamE share a function in stabilizing the interaction between the essential proteins BamA and BamD, BamE must have an additional function not shared by BamC. This function is critically important for cell survival when any stress is introduced to the OMP assembly pathway, whether it is a defect in the substrate, a chaperone, or the Bam machine itself.

BamA is surface exposed. Although the structure of the beta-barrel domain of BamA is not known, the predicted topology reveals 8 potential extracellular loops. Using immunofluorescence microscopy, BamA was shown to be surface exposed in a previous study (1). Experiments on the BamA homologs in *Borrelia burgdorferi* and *Treponema pallidum* confirmed that a portion of the barrel domain is indeed surface exposed (9, 15). We performed similar experiments to determine if *E. coli* BamA was surface exposed. Whole cells were harvested from an overnight culture of wild-type *E. coli* and treated with proteinase K. Upon inactivation of the protease, the cells were resuspended in sample buffer, and the proteins were resolved by SDS-PAGE and analyzed by immunoblotting. By using antibodies raised against the periplasmic portion of BamA, we observed that BamA was cleaved upon addition of proteinase K, yielding a fragment of approximately 70 kDa (Fig. 4A). Based on the predicted topology and the known molecular weight of BamA, generation of the observed fragment would correspond to cleavage in the sixth extracellular loop. As a control, we blotted for the periplasmic proteins MBP and SurA to show that the proteinase K did not enter the cell (Fig. 4B). We also blotted for BamB and BamD, both of which are predicted to be anchored to the inner leaflet of the OM (Fig. 4C). Indeed, it appears that the protease remained outside the cell. Our results confirm that *E. coli* BamA is exposed to the extracellular environment.

TABLE 3 Comparison of Δ*bamC* and Δ*bamE* double mutant phenotypes

	Growth[a] with added allele:		
Strain	Wild type	Δ*bamC*	Δ*bamE*
Wild type	+++	+++	+++
lptD4213 mutant	++	++	−
surA mutant	+++	++	+
skp degP mutant	+++	++	−
bamA101 mutant	+++	+	−
bamB::*kan* mutant[b]	+++	+	−

[a] The growth of each strain was scored after incubation at 37°C overnight on LB plates. Strains were scored on the following scale: +++, normal growth; ++, intermediate growth; +, weak growth; −, no growth. The results shown are from a representative experiment.

[b] Strains carrying the *bamB*::*kan* allele were analyzed at 30°C.

The protease sensitivity of BamA is dramatically increased in the absence of BamE. One possible function of the nonessential Bam lipoproteins is to modulate the conformation of the essential proteins BamA and BamD. The protease sensitivity assay described in the previous section provides a convenient way to monitor the conformation of BamA. Whole cells of *bamB*::*kan*, Δ*bamC*, and Δ*bamE* mutants were treated with proteinase K and analyzed as described above (Fig. 4A). In both the *bamB* and Δ*bamC* mutants, the pattern of BamA proteolysis was similar to the result observed with wild-type cells. However, treating the Δ*bamE* mutant with protease had far different effects on BamA. Strikingly, we were unable to detect any BamA fragments by immunoblotting following proteolysis of the Δ*bamE* mutant. To confirm that the control proteins in the periplasm are susceptible to proteinase K, we intentionally permeabilized cells with SDS and then added the protease. As expected, MBP is degraded when the

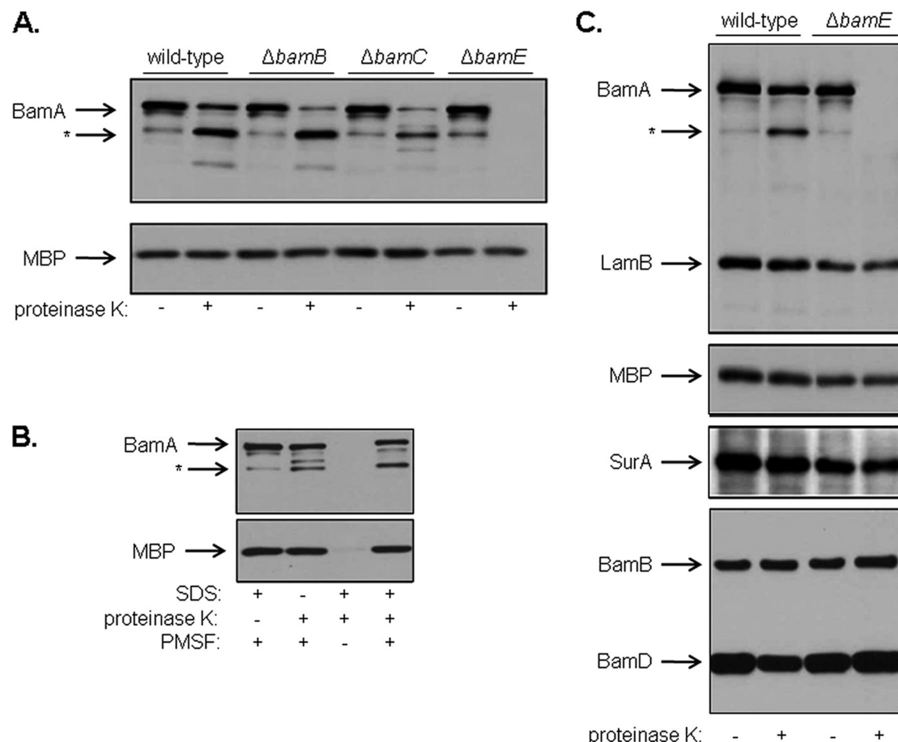

FIG 4 BamA is sensitive to externally added protease, especially in a Δ*bamE* mutant. (A) Cells from overnight cultures of wild-type and *bam* mutant strains were resuspended in reaction buffer and treated with proteinase K where indicated. PMSF was added to inactivate the protease, cells were lysed in SDS-PAGE sample buffer, and the extracts were analyzed by immunoblotting using the indicated antibodies. MBP served as a lysis control. (B) MBP is degraded in cells intentionally permeabilized with SDS prior to addition of proteinase K. (C) Enhanced proteolysis is specific to BamA. Wild-type and Δ*bamE* mutant cells were treated with proteinase K as for panel A. LamB, MBP, SurA, BamA, BamB, and BamD levels were monitored using antibodies raised against each protein. The N-terminal fragment of BamA is indicated by an asterisk.

OM is disrupted (Fig. 4B). We examined several periplasmic proteins (MBP, SurA, BamB, and BamD) and found that they were not degraded in the Δ*bamE* mutant, suggesting that the barrier function of the outer membrane was not compromised by the Δ*bamE* mutation and that the cells remained intact during the experiment (Fig. 4C). Taken together, these results show that in wild-type cells, portions of BamA are surface exposed. In the absence of BamE, the protease sensitivity of BamA increases dramatically. These results suggest that BamE, either directly or indirectly, modulates the conformation of BamA.

Overproducing BamD can suppress the synthetic lethality of a *bamA101* Δ*bamE* double mutant. BamD is known to interact with both BamA and BamE (16, 27). However, there is no evidence that BamE interacts directly with BamA. We suggest that BamE modulates the conformation of BamA indirectly by controlling the activity of BamD. The following genetic experiment addresses this issue.

Recall that in our earlier genetic analysis, we showed that *bamA101* and Δ*bamE* are a synthetically lethal pair. We have discovered that overproducing BamD suppresses this synthetic lethality. By introducing a plasmid carrying *bamD* and a mutation that increases copy number, we overexpressed *bamD* in wild-type and *bamA101* and Δ*bamE* mutant backgrounds (Table 4). In all three strains, overexpressing *bamD* had no impact on viability or OMP assembly under standard laboratory conditions. However, when *bamD* was overexpressed in the Δ*bamE* mutant, we were able to introduce the *bamA101* allele by generalized transduction. This shows that overproduction of BamD can bypass the requirement for BamE when BamA levels are reduced. As described below, we argue that this supports the view that BamE modulates the conformation of BamA indirectly through BamD.

DISCUSSION

In *E. coli*, the five-member Bam complex is composed of two stable subcomplexes, BamAB and BamCDE, each of which contains an essential component, i.e., the OMP BamA or the lipoprotein BamD (21). The remaining three lipoproteins, BamBCE, are all nonessential. Indeed, mutants lacking any one of these three proteins have only minor defects in OMP assembly and OM integrity under laboratory conditions. It is likely that the nonessential lipoproteins function to increase the overall efficiency of the OMP assembly process, a role that becomes critically important in the

TABLE 4 Growth of strains that overproduce BamD

	Growth[a] with:	
Strain	Vector	pBamD O/E
Wild type	+++	+++
Δ*bamE* mutant	+++	+++
Δ*bamA101* mutant	+++	+++
Δ*bamE bamA101* mutant	−	+++

[a] The growth of each strain was scored after incubation at 37°C overnight on LB plates. Strains were scored on the following scale: +++, normal growth; ++, intermediate growth; +, weak growth; −, no growth.

normally stressful environment that the bacterium inhabits. If so, then mutants lacking any one of the nonessential lipoproteins may exhibit assembly defects that provide insight into the mechanism of OMP assembly, provided that we can find a way to reveal them. We focused on BamC and BamE because they are both members of the same subcomplex and presumably affect the activity of BamD.

As has been reported previously, the interaction between BamA and BamD is altered by removing either BamC or BamE (27). Under the conditions we used here, the BamA-BamD interaction is weakened to a similar extent. Thus, BamC and BamE share a function to increase the stability of the BamA-BamD interaction. This is gratifying because the stability of the individual Bam proteins is not affected by the loss of BamC or BamE, and the phenotypes of mutants lacking either lipoprotein are indistinguishable. Under laboratory conditions, this shared function is not terribly important. This is not surprising either. It is known that the levels of BamA can be reduced 10-fold or more without significantly affecting growth *in vitro* (1). This also reduces the total number of five-member Bam complexes in the cell.

To impose stress on the OMP assembly process in the laboratory environment, we used mutation. In particular, we used assembly-defective substrates (LptD4213), chaperone defects (*surA*, *skp*, and *degP* mutations), and mutations that affect the Bam complex directly (*bamB*::*kan* and *bamA101*). In each and every case, taking away BamE exacerbates the defect in the double mutants far more than does taking away BamC. The simplest explanation for this difference in double mutants is that BamE has an additional function not shared by BamC and that this second function is critically important under stress conditions.

Hints into the nature of this additional function for BamE came from the analysis of BamA surface exposure. As expected from studies with other organisms, BamA is surface exposed in *E. coli* (9, 15). We discovered that taking away BamE, but not BamB or BamC, dramatically increases the protease sensitivity of BamA. We do not yet understand why no periplasmic domains survive proteinase K treatment in *bamE* mutants. It seems likely that these domains are degraded by periplasmic proteases when released from the OM by externally added protease, but further work is required to characterize how BamA is removed from the membrane and degraded. In any event, we conclude that BamE alters the conformation of BamA and that this is a function not shared with BamB or BamC. Some of the experimental conditions we tested are stressful, and in these cases, BamE function becomes essential for the cell. Parsimony would argue that it is this second function of BamE, the ability to alter BamA conformation, which is critically important under stress conditions.

Although we have no direct evidence, we suspect that the protease-sensitive form of BamA that is observed in Δ*bamE* mutants represents an active form of the protein. Recall that under laboratory conditions, Δ*bamE* mutants have no growth defects and relatively minor OM biogenesis defects. However, virtually all of the BamA present is protease sensitive. If this were an inactive form of the protein, a more dramatic phenotype might be expected when BamE is absent. We think it likely that during the OMP assembly reaction, BamA assumes different conformations. The protease-sensitive and largely protease-resistant forms we see may represent two of these conformations. Indeed, during the assembly reaction, BamA may cycle from one conformation to the other.

Finally, it seems likely that BamE exerts its affects on BamA conformation indirectly through interactions with BamD. As noted above, when BamA levels are strongly reduced as they are in *bamA101* strains, BamE becomes essential. The requirement for BamE can be suppressed by increasing the levels of BamD. This is simply explained if BamE controls the activity of BamD, which in turn affects the conformation of BamA. If BamE controls the activity of BamD, then it is not surprising that increasing BamD activity by increasing the amount of the protein bypasses the need for BamE.

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