BamA Alone Accelerates Outer Membrane Protein Folding In Vitro through a Catalytic Mechanism

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Supporting Information

ABSTRACT: β-Barrel assembly machinery protein A (BamA) plays a critical role in the biogenesis of outer membrane proteins (OMPs); however, a mechanistic understanding of its function is lacking. Here, we report an in vitro assay that investigates whether the mechanism of BamA-catalyzed OMP folding is stoichiometric or catalytic. We found that BamA accelerates the folding of OMPs in vitro via a catalytic mechanism, similar to the activity of the full multiprotein β-barrel assembly machinery (BAM) complex in vivo. As BamA alone can repeatedly facilitate the folding of OMPs, we suggest the additional BAM components accelerate this basal activity to biologically relevant time scales.

The β-barrel assembly machinery (BAM) protein complex is essential for the folding and assembly of β-barrel outer membrane proteins (OMPs) in Escherichia coli. The efficient and correct assembly of OMPs is critical to several cellular processes, including transport of vital nutrients, secretion, and signaling. In E. coli, the BAM complex is composed of five proteins (BamABCDE). β-Barrel assembly machinery protein A (BamA) is itself a β-barrel OMP that facilitates the folding of client OMPs into the surrounding lipid bilayer both in vivo and in vitro. BamA associates with four lipoproteins in vivo (BamBCDE). BamA and BamD are required for cell viability. The other nonessential BAM proteins have been shown to play a role in outer membrane integrity. It is not known how the entire BAM complex works together to accelerate OMP folding; however, it has been suggested that BamA functions in vivo via a catalytic mechanism involving recycling of BamA by BamD and BamE. In vitro studies indicate that BamA accelerates OMP folding in a concentration-dependent manner, but the details of the catalytic mechanism of BamA have not been definitively established. The observation that BamA alone accelerates OMP folding in vitro raises the following questions: Can BamA alone repeatedly catalyze the folding of OMPs? Or, is BamA irreversibly consumed by a stoichiometric interaction with OMPs and subsequently requires regeneration by the additional BAM subunits?

To address these questions, we developed an experimental assay to determine if BamA is consumed during the acceleration of OMP folding. Our approach allows for differentiation between a stoichiometric and a catalytic mechanism of BamA function. Briefly, BamA is folded to completion into large unilamellar vesicles (LUVs) composed of synthetic lipids. The folded BamA is then presented with a high concentration of an OMP client of interest. A stoichiometric mechanism would be characterized by consumption of BamA upon interaction with this client OMP; therefore, the maximal amount of client OMP that could be folded by BamA is limited to the amount of BamA folded. In contrast, if the ability of BamA to accelerate the folding of a client OMP involves a catalytic mechanism, BamA would not be consumed by interacting with the OMP client and could productively interact with a greater amount of client OMP than the amount of folded BamA.

To quantify the amount of client OMP that interacts with BamA, we analyze OMP folding kinetics by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, as OMPs exhibit characteristic shifts in apparent molecular weight between the folded and unfolded species. The client OMP utilized in this study is outer membrane protein A (OmpA) because it has previously been shown to undergo BamA-accelerated folding in vitro. Additionally, OmpA folds slowly through the always accessible intrinsic folding (i.e., BamA-independent) pathway under certain conditions. The unique folding profile of OmpA—limited intrinsic folding and known acceleration of folding by BamA—makes this client OMP ideal for this experimental setup.

The quantity of BamA-catalyzed folded OmpA is defined as the difference at a given time point between the concentration of OmpA folded in the presence of BamA and the concentration of OmpA folded through the intrinsic pathway (Supplementary Figures 1–3). Because the volumes of all experiments are identical, the concentrations of all species can serve as a proxy for their amounts. Figure 1 indicates that the concentration of OmpA folded by BamA into LUVs composed of 20% 1,2-didecanoyl-sn-glycerol-3-phosphoethanolamine and 80% 1,2-didecanoyl-sn-glycerol-3-phosphocholine reaches 1.5 μM after 1 h. Densitometric analyses reveal that the total amount of folded BamA under these conditions is approximately 0.9 μM. Therefore, the concentration of OmpA that interacts productively with BamA is greater than the concentration of folded BamA. This result suggests that BamA alone is able to repeatedly interact with client OMPs in vitro to accelerate their folding via a catalytic mechanism.

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Here we have presented findings that BamA acts catalytically in vitro to independently and repetitively accelerate the folding of OMPs. These findings agree with the catalytic mechanism of BamA in vivo\textsuperscript{11} and further validate in vitro OMP folding studies. Interestingly, these complementary studies utilize different OMP clients, suggesting the ability of BamA to turnover is independent of OMP client identity. Conserved architecture and structural motifs between E. coli BamA and homologues in both prokaryotes\textsuperscript{18} and eukaryotes\textsuperscript{19} hint that OMP folding mechanisms of these BamA homologues may also be catalytic. The molecular basis of this catalytic mechanism of BamA warrants further experimental investigation. Understanding the BamA catalytic mechanism of OMP folding aids in a more complete understanding of OMP biogenesis and provides insight into the function of the BAM complex.

ASSOCIATED CONTENT

Supporting Information

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Detailed methods (PDF)

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