The role of the periplasmic domain of BamA in folding and insertion of the outer membrane protein A

Esther Talmon — Department of Biology, University of Konstanz

Abstract

In *Escherichia coli*, folding and insertion of β-barrel outer membrane proteins (OMPs) are mediated by the β-barrel assembly machinery (Bam), which is composed of the β-barrel protein BamA and the four lipoproteins BamB, BamC, BamD and BamE. BamA consists of a transmembrane β-barrel and a soluble periplasmic domain (PPD-BamA), which itself is comprised of five polypeptide transport-associated (POTRA) domains. Although it is known that the PPD is necessary for the activity of the Bam complex, the mechanism how it functions is not understood. To examine whether the PPD-BamA acts like a chaperone and promotes folding and insertion of OMPs into lipid membranes, we expressed and isolated it from *E. coli*. The secondary structure of PPD-BamA was determined by circular dichroism spectroscopy and was composed of 36% α-helix, 15% β-strand, 20% turns, and 29% random coil. For OMP folding experiments, we isolated outer membrane protein A (OmpA) in its urea-unfolded state. In order to investigate the effect of PPD-BamA on the kinetics of OmpA folding into the model membranes four different sets of experiments were performed. In parallel experiments, namely in the absence and presence of PPD-BamA, the dependence of OmpA refolding on the pre-incubation time, temperature, lipid concentration and lipid composition were examined by the KTSE-assay (Kinetics of tertiary structure formation by electrophoresis) [1]. PPD-BamA was found to promote folding of OmpA into lipid bilayers when the negatively charged phosphatidylglycerol (PG) was present in the membrane. No effect on the folding of OmpA could be observed in the absence of PG. Fluorescence spectroscopy showed that PPD-BamA binds to lipid bilayers, but only when these contain PG.

References

[1] Kleinschmidt, J.H. (2003) Membrane protein folding on the example of outer membrane protein A of *Escherichia coli*. *CMLS, Cell. Mol. Life Sci.* **60** 1547–1558