Dissecting the folding mechanism of the outer membrane protein PagP

Gerard Huysmans, Lindsay McMorran, Robert Schiffrin, Steven Baldwin,
Sheena Radford and David Brockwell

Introduction

The *E.coli* outer membrane (OM) is densely packed with outer membrane proteins (OMPs) that carry out a diverse range of functions that include (non)specific transport of small and large ligands, proteolytic and synthetic reactions and cellular recognition and adhesion. However, despite their importance, understanding the biogenesis of OMPs and their folding and insertion into membranes is a formidable challenge (Figure 1). For soluble proteins, excellent progress towards answering how the information inherent in the amino acid sequence of a protein enables it to adopt a native, three-dimensional structure has been made by integrating experimental folding studies on small model proteins with computer simulations. By contrast, progress in understanding the folding mechanisms of membrane proteins has been much more limited, in part because of the complexity added to delineating the mechanisms of folding by the membrane environment in which the protein resides.

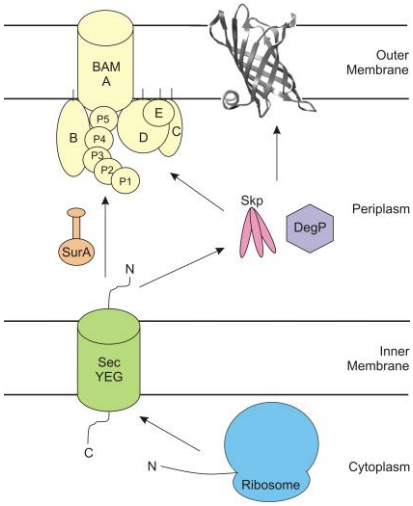

Figure 1: schematic of biogenesis of outer membrane proteins (OMPs) in *E. coli.* OMPs are synthesised on the ribosome before post-translational translocation across the inner membrane by the SecYEG translocon. Unfolded OMPs are then chaperoned across the periplasm to the β-barrel assembly (BAM) complex, which aids folding and insertion into the outer membrane. BAM complex proteins are labelled A-E, and the periplasmic polypeptide transport-associated (POTRA) domains of BamA are labelled P1-5.

Biological membranes comprise a complex two-dimensional fluid with a heterogeneous lipid composition that largely determines the physicochemical properties of the membrane. The organization of the lipid bilayer (a hydrophobic core flanked by often asymmetrical polar interfaces) poses significant spatial restrictions on the folding process, which are difficult to mimic *in vitro*. In addition, membrane curvature imposes stresses on the bilayer that may be alleviated or exacerbated by protein insertion and hence can also modulate the rate, or efficiency, of folding. In order to understand the native structures of membrane proteins, it is thus necessary to investigate how the lipid membrane contributes to, and/or limits, protein folding, stability and conformational dynamics.

Results

To address this question we have investigated the effects of lipid composition on the folding mechanism of the bacterial outer membrane protein PagP. Under a defined range of protein, lipid and denaturant concentrations we have previously shown that PagP displays single exponential folding kinetics, inserting into zwitterionic *di*$C_{12:0}$PC liposomes *via* a polarized transition state from a liposome-associated unfolded state. Using mutational analysis the C-terminal β-strands were found to be highly structured, while the N-terminal α-helix and β-

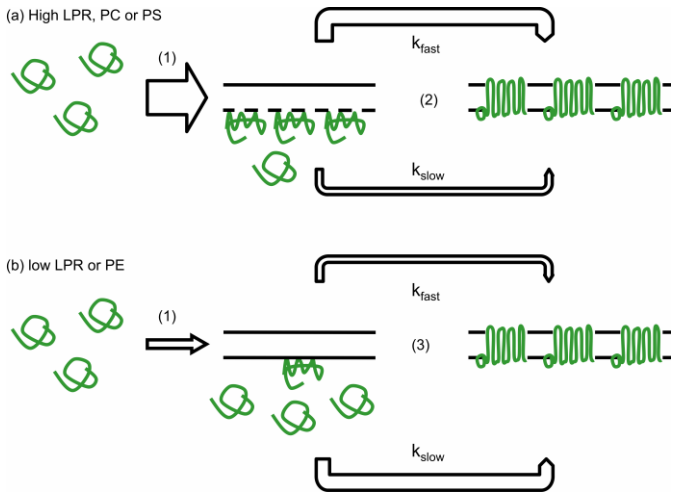

Figure 2: schematic representation of the folding mechanism of PagP. Folding is initiated by rapid adsorption to the membrane (1). The amplitude of this process is increased with increasing lipid-to-protein ratios (LPR) in (a) and decreased by decreasing LPR or by incorporation of *di*$C_{12:0}$PE in (b). Folding proceeds predominantly by a fast (2) or a slow folding route (3), modulated by the bilayer properties determined by the LPR and *di*$C_{12:0}$PE fraction in the liposome as indicated. The dotted line in (a) represents higher flexibility in the membrane compared to (b). Including *di*$C_{12:0}$PS in the liposome does not change the preferred pathway but does influence the rate of insertion. The thickness of the arrows indicates the amplitude of the phases.

strands remain largely disordered. By decreasing the denaturant concentration and the ratio of lipid to protein, PagP folds from a completely unfolded and membrane dissociated state, revealing additional kinetic refolding phases. Application of an interrupted folding assay definitively demonstrated that this complexity reflected the existence of parallel folding pathways (Figure 2). The kinetic partitioning between these pathways was found to be modulated by the elastic properties of the membrane. For example, folding into mixed *di*$C_{12:0}$PE:*di*$C_{12:0}$PC liposomes resulted in a decrease of PagP adsorption to the liposomes and a switch to a slower folding pathway. By contrast, inclusion of *di*$C_{12:0}$PS in *di*$C_{12:0}$PC liposomes only resulted in a decrease of the folding rate of the fast pathway. Our results were used to refine the kinetic folding mechanism of PagP and contribute to delineating the role of the membrane in membrane protein folding.

Current work

Our laboratory is also employing kinetic folding and spectroscopic techniques to look at refolding of PagP in low concentrations of urea, to allow investigation of how the chaperone proteins found in the periplasm of *E.coli* (Skp, SurA and the POTRA domains of BamA, Figure 1) can influence folding *in vitro*. By elucidating the mechanism of action of these proteins, which are essential for the correct trafficking, folding and insertion of OMPs *in vivo*, it is hoped to gain insights into the mechanisms of OMP folding in the cell.

Publications

Huysmans, G., Radford, S., Baldwin, S. & Brockwell, D. (2012) Malleability of the folding mechanism of the outer membrane protein pagp: Parallel pathways and the effect of membrane elasticity. *J. Mol. Biol.* **416:** 453-464.

Funding

We thank Nasir Khan for technical support and the Wellcome Trust and BBSRC for funding.