Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

# Folding of β-barrel membrane proteins in lipid bilayers — Unassisted and assisted folding and insertion☆

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ARTICLE INFO

*Article history:*
Received 22 March 2015
Received in revised form 6 May 2015
Accepted 7 May 2015
Available online xxxx

*Keywords:*
β-barrel membrane protein
Membrane protein folding
Kinetics
Outer membrane
Periplasmic chaperone
BAM complex

ABSTRACT

In cells, β-barrel membrane proteins are transported in unfolded form to an outer membrane into which they fold and insert. Model systems have been established to investigate the mechanisms of insertion and folding of these versatile proteins into detergent micelles, lipid bilayers and even synthetic amphipathic polymers. In these experiments, insertion into lipid membranes is initiated from unfolded forms that do not display residual β-sheet secondary structure. These studies therefore have allowed the investigation of membrane protein folding and insertion in great detail. Folding of β-barrel membrane proteins into lipid bilayers has been monitored from unfolded forms by dilution of chaotropic denaturants that keep the protein unfolded as well as from unfolded forms present in complexes with molecular chaperones from cells. This review is aimed to provide an overview of the principles and mechanisms observed for the folding of β-barrel transmembrane proteins into lipid bilayers, the importance of lipid–protein interactions and the function of molecular chaperones and folding assistants. This article is part of a Special Issue entitled: Lipid–protein interactions.



**Contents**



*Abbreviations:* C, cysteine; CAC, critical association concentration; CD, circular dichroism; *di*$C_{10:0}$PC, 1,2-dicapryl-*sn*-glycero-3-phosphocholine; *di*$C_{12:0}$PC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; *di*$C_{12:0}$PE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; *di*$C_{12:0}$PG, 1,2-lauroyl-*sn*-glycero-3-phosphoglycerol; *di*$C_{14:0}$PC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; *di*$C_{18:1}$PC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; *di*$C_{18:1}$PE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; *di*$C_{18:1}$PG, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol; F, phenylalanine; FomA, major outer membrane protein A from *Fusobacterium nucleatum*; hVDAC1, voltage-dependent anion-selective channel, human isoform 1; KTSE, Kinetics of Tertiary Structure Formation by Electrophoresis; LPS, lipopolysaccharide; LUVs, large unilamellar vesicles; OM, outer membrane; OmpA, OmpC, OmpF, OmpG, OmpT, OmpW, and OmpX, outer membrane proteins A, C, F, G, T, W, and X from *Escherichia coli* (see also Table 1); OMP, outer membrane protein; PAGE, polyacrylamide gel electrophoresis; PagP, lipid A palmitoyltransferase from *E. coli*; SDS, sodium dodecylsulfate; Skp, seventeen kilodalton protein; SurA, survival factor A; SUVs, small unilamellar vesicles; TM, transmembrane; TMP, transmembrane protein; TMD, transmembrane domain; VDAC, voltage-dependent anion-selective channel; W, tryptophan; wt, wild-type; Y, tyrosine

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http://dx.doi.org/10.1016/j.bbamem.2015.05.004
0005-2736/

Please cite this article as: J.H. Kleinschmidt, Folding of β-barrel membrane proteins in lipid bilayers — Unassisted and assisted folding and insertion, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.05.004

## 1. Introduction

Biological membranes are essential structuring components of all living cells. The core elements of membranes are lipid bilayers and integral and peripheral proteins. While a lipid bilayer constitutes the hydrophobic barrier of a membrane and prevents the arbitrary exchange of solutes, transmembrane proteins (TMPs)[a] allow the regulated exchange of solutes across the lipid bilayer or they transduce signals from one side of the membrane to the other. Many membrane proteins perform enzymatic reactions, which take place at the membrane–water interface. Specific lipid–protein interactions are important for the stable integration and activity of integral and peripheral membrane proteins (see, *e.g.* [1–3]). The unique structure of the lipid bilayer requires specific surface properties of integral and peripheral proteins that are necessary for their function. Protein surfaces exposed to the fatty core of the membrane are typically hydrophobic, while protein surfaces exposed to the aqueous space usually are composed of polar amino acid residues. These properties of membrane proteins have raised strong interest to examine the physical principles, how integral proteins fold and insert into membranes (see, *e.g.*, [4–7]). TMPs can be subdivided into two classes according to their transmembrane (TM) structure, namely α-helical TMPs and TMPs with β-sheet secondary structure in the lipid bilayer. While TMPs with a single TM helix are common [8], all TMPs with β-sheet secondary structure known to date form closed β-barrels with at least 8 antiparallel TM β-strands. In these β-barrels, all β-strands are connected to their next neighbors through hydrogen bonds between the amide-protons and the carbonyl groups of the polypeptide backbone. The β-barrel structure is closed through hydrogen bonds formed between the amino-terminal and the carboxy-terminal membrane-spanning β-strands. The strands of β-barrel TMPs are connected by short periplasmic β-turns and by long loops facing the polysaccharide region and the space outside the cell or cell organelle. The geometry of the β-strands and the necessity to form hydrogen bonds between polar amide and carbonyl groups of the polypeptide chain within the hydrophobic core of the membrane exclude that individual β-strands can exist in a lipid bilayer. In the TM region of a β-barrel, polar and apolar residues alternate with one another: the β-strands are amphipathic. The hydrophobic residues face the apolar lipid phase, while polar residues face the barrel lumen, across which nutrients in aqueous solution are transported. Therefore in comparison to α-helical TMPs, the average hydrophobicity of TM β-barrels is low. With the exception of the capsule transporter Wza [9], all currently known outer membrane proteins (OMPs) from bacteria form β-barrel TM structure. β-barrel TMPs are also found in the outer membranes (OMs) of mitochondria and chloroplasts. Some representative examples are shown in Fig. 1. β-barrel TMPs are characterized by the number of antiparallel β-strands and by the shear number, which is a measure for the inclination angle of the β-strands against the barrel axis [10]. The OMPs of known crystal structure from bacteria form TM β-barrels with even numbers of β-strands ranging from 8, like in the TM domain (TMD) of outer membrane protein A (OmpA) from *Escherichia coli*, to 26 in the lipopolysaccharide (LPS) channel LptD. The voltage-dependent anion-selective channel, human isoform 1 (hVDAC1) from mitochondria forms a β-barrel with 19 TM strands. OMPs exist as monomers (for example OmpA, BtuB), dimers (OmPlA) or trimers (ScrY, LamB). OMPs can be grouped into at least 10 different categories regarding their function [7,11]. They may serve as structural proteins (for example OmpA), as toxin binding proteins (OmpX), as passive unspecific diffusion porines (OmpF, OmpC), as specific porines (LamB, ScrY, FadL, Tsx), as active transporters (BtuB, FhuA), as proteases (OmpT), lipases (OmPlA), or acyltransferases (PagP), as adhesion proteins (NspA, OpcA), as insertion machines (BamA), as pilus assembly plattforms (PapC, FimD), as export channels (TolC), *etc.* Some OMPs of known crystal structure of their β-barrel domains are listed in Table 1, together with their molecular weights, pI, number of TM β-strands in the β-barrel domain, number of amino acid residues, oligomeric state and function. In this review, we provide an overview of the observations and biophysical principles of folding and membrane insertion of β-barrel membrane proteins into lipid bilayers of defined composition.

## 2. Denaturation and refolding of β-barrels

OmpA is the first TMP for which successful refolding was shown. In 1978, Schweizer et al. [12] demonstrated that more than 90% of heat-denatured OmpA (Omp II [13]) regained its native structure in the presence of LPS and Triton-X-100. Later, Dornmair et al. [14] established that OmpA can refold in micelles of octylglucoside in the absence of LPS after dilution of the denaturants, sodium dodecyl sulfate (SDS) or urea. Further experiments demonstrated that urea-denatured OmpA can be folded both in lipids and in a wide range of detergents, provided that the concentration is above the CAC [15]. OmpA folds even from certain fragments in micelles of octylglucoside [16,17], an observation first made *in vivo* for separately expressed fragments [18]. Later, very large β-barrel TMPs like the 22-stranded iron transporter FepA, which contains an additional domain in the barrel lumen, have been refolded successfully from their denatured forms in chaotropic denaturants into detergent micelles upon denaturant dilution [19]. The list of successfully refolded β-barrels also includes the 19-stranded human VDAC from the OM of mitochondria [20–23]. Interestingly, circular dichroism (CD) spectra indicated that human VDAC, isoform 1, develops slightly different secondary structure in LDAO detergent micelles than in lipid bilayers of various phospholipids [20]. In LDAO/$di C_{12:0}$PC mixtures, the β-sheet content decreased from ~37% in pure $di C_{12:0}$PC bilayers to ~32% in pure LDAO micelles, whereas the content of α-helical secondary structure increased from ~11% to ~16%. A change of 5% in secondary structure corresponds to about 14 residues out of 282 residues in VDAC. This is not a very large fraction, but could be enough to form an additional transmembrane β-strand at the expense of some of the α-helical structure obtained for the detergent solubilized form. Expression of β-barrel TMPs in form of cytosolic inclusion bodies and subsequent refolding has in fact become a major strategy for the isolation of many β-barrel TMPs (for a recent review, see *e.g.*, Ref. [24]). A



Please cite this article as: J.H. Kleinschmidt, Folding of β-barrel membrane proteins in lipid bilayers — Unassisted and assisted folding and insertion, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.05.004