Biochimica et Biophysica Acta 1797 (2010) 1292–1299

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Structure and evolution of mitochondrial outer membrane proteins of β-barrel topology

Kornelius Zeth *

Max Planck Institute of Developmental Biology, Department Protein Evolution, Spemannstr. 35, D-72076 Tübingen, Germany

ARTICLE INFO

Article history:
Received 15 December 2009
Received in revised form 25 April 2010
Accepted 27 April 2010
Available online 5 May 2010

Keywords:
VDAC
Tom40
Sam50
Membrane protein structure

ABSTRACT

Gram-negative bacteria are the ancestors of mitochondrial organelles. Consequently, both entities contain two surrounding lipid bilayers known as the inner and outer membranes. While protein synthesis in bacteria is accomplished in the cytoplasm, mitochondria import 90–99% of their protein ensemble from the cytosol in the opposite direction. Three protein families including Sam50, VDAC and Tom40 together with Mdm10 compose the set of integral β-barrel proteins embedded in the mitochondrial outer membrane in *S. cerevisiae* (MOM). The 16-stranded Sam50 protein forms part of the sorting and assembly machinery (SAM) and shows a clear evolutionary relationship to members of the bacterial Omp85 family. By contrast, the evolution of VDAC and Tom40, both adopting the same fold cannot be traced to any bacterial precursor. This finding is in agreement with the specific function of Tom40 in the TOM complex not existent in the enslaved bacterial precursor cell. Models of Tom40 and Sam50 have been developed using X-ray structures of related proteins. These models are analyzed with respect to properties such as conservation and charge distribution yielding features related to their individual functions.

1. Introduction

The mitochondrial outer membrane originates from the uptake of an endosymbiotic precursor bacterium from the α-proteobacterial subgroup into a eukaryotic precursor cell [1]. During the course of evolution the lipid composition of both membranes has changed significantly due to the different environmental requirements but the protein representatives carrying important functions remained partly the same. In yeast five integral outer membrane proteins of β-barrel topology were identified, three of which, scVDAC1, scVDAC2 and scTom40 belong to the 19-stranded β-barrel group. Sam50 displays a high sequence similarity to the bacterial precursor protein Omp85 and very probably belongs to a second 16-stranded β-barrel subgroup [2,3]. Another protein Mdm10 involved in the maturation of MOM protein complexes that is predicted to contain β-sheet structure is not present in higher eukaryotes and has also no sequence homologs among bacteria [4]. Together, this ensemble of β-barrel proteins in complex with adjacent factors (TOM and SAM associated proteins) allows the communication and exchange between mitochondria and the surrounding cytosol and forms the platform for a variety of protein–protein interactions.

In the early 70's and 80's the mitochondrial porin has been discovered as a channel structurally and functionally resembling many bacterial porins [5,6]. At the same time, in 1976, the discovery of the switch between an anion- to a cation-selective state of lower conductance at low voltages in planar lipid membranes led to the term of the voltage dependent anion channel, VDAC [7]. These investigations and selected following experiments represented the hallmark characteristics of VDAC family proteins and constrained the view onto the channel for about two decades. In 2008, the 3D structure of VDAC has been determined independently by three research groups using either high resolution NMR spectroscopy, X-ray crystallography or a hybrid approach combining information of both techniques [8–11]. All three structure models basically show the same architecture of a 19-stranded β-barrel with the N-terminal helix enclosed. This structure investigation was not only a breakthrough in the understanding of MOM structure biology but also forms the reliable basis for further biochemical and modeling experiments.

From sequence alignments it became indicative that Tom40 shares the same overall architecture as determined for VDAC proteins [9]. The channel forms the integral part of the TOM complex which in *S. cerevisiae* is assembled by three preprotein receptor molecules Tom20, Tom22 and Tom70 and a number of small Tom proteins (Tom5, Tom6, Tom7) [12]. Tom40 is the core component of the 400–500 kDa pore-like structure which allows preprotein passage into the intermembrane space where proteins are further targeted into the four compartments [13]. Electron microscopy data provided the first structural insights into the entire (containing the Tom70 component) or core TOM complex with a dynamic equilibrium of two or three Tom40 subunits present in the protein ensemble [14,15]. The TOM reconstructions acquired so far

Abbreviations: MOM, mitochondrial outer membrane; TOM, translocase of the outer membrane; SAM, sorting and assembly machinery

* Tel.: +49 7071 601 323; fax: +49 7071 601 349.
E-mail address: Kornelius.zeth@tuebingen.mpg.de.

0005-2728/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2010.04.019

represent only fungi–mitochondrial complexes (from *S. cerevisiae* and *N. crassa*) at medium resolution but the pore structures agree well with the Tom40 model we generated on the basis of VDAC structures, both in dimensions of vertical and horizontal size (our unpublished data). The major function of the Tom40 protein is to enable the passage and lateral release of partially folded or unfolded secondary structure elements [14,16]. From the dimensions of this pore model, a folded α-helix may easily pass through without previous unfolding but a β-hairpin structure would have to be unfolded before passage. This model of one pore forming protein per barrel wall is also supportive for the idea of e.g. Rapaport that mitochondrial outer membrane proteins comprising one C-terminal α-helix may be inserted into a rim of several Tom40 β-barrels and subsequently released laterally into the outer membrane [17].

Notably, the composition of proteins assembling the TOM and SAM complexes (see next paragraph) is not consistent between mitochondria of different phylogenetic lineages (for review see: [18,19]). For TOM a subset of three proteins, Tom40, Tom22 and Tom7 are ubiquitously found in all eukaryotes [20].

The third protein of barrel topology, Sam50, (which is also known as the Tob55 protein of the TOB complex: *t*opogenesis of mitochondrial *o*uter membrane *b*eta-barrel proteins) belongs to the sorting and assembly machinery, SAM, which consists in yeast of three proteins: the integral Sam50 pore and two Sam35 and Sam37 accessory proteins [21,22]. Sam50 and also the plant homolog Toc75 from chloroplasts both show a clear sequence similarity to the Omp85 evolutionary precursor protein from Gram-negative bacteria [19,23,24]. By contrast to the bacterial Omp85 homologs which N-terminally harbor five POTRA (*Po*lypeptide *T*ranslocation *A*ssociated) domains, the Sam50 protein has evolved differently and contains only one of these scaffold domains [24]. POTRA domains together with the integral membrane portion form the typical structural fingerprint of proteins belonging to the Omp85 or related membrane insertase families. Functionally, SAM complexes can recognize the β-signal stretch which consists of a four residue motif encoded with high similarity in the very last β-strand of any MOM protein [25]. This arrangement is somewhat reminiscent to a sequence similarly observed in outer membrane proteins of bacteria where a handful of residues are strongly discriminative for functions including (I) insertion into the outer membrane and (II) binding to DegS and induction of the σE-dependent stress response [26,27]. This led to the conclusion of the C-termini of OMPs and β-barrel MOMs harboring specific motifs important for their stability.

Crystal structures of mammalian VDAC and the bacterial FhaC protein provide a solid basis to model related MOM proteins of β-barrel topology of sufficient similarity such as the human Tom40 (similar to VDAC) and Sam50 (on the basis of FhaC protein) [8–10,28]. In this article the relationship between these proteins in sequence and structure are discussed.

2. Structural and functional basis of VDAC proteins

VDAC has long been implicated in functions as a mediator between the cytoplasm and the mitochondrion (for review [29–32]). In mammalian mitochondria three isoforms (hVDAC1, hVDAC2 and hVDAC3) have been identified and were studied to unravel their distinct roles in cell life and death [33–36]. Their specific role in the exchange of small metabolites including the energy equivalents ATP and ADP is undisputed, however, the possible role of VDAC proteins in cell death is still under an ongoing discussion [33–36]. In cell life under physiologically stable conditions VDAC1 is likely to participate in the binding of hexokinase isoforms I and II at the mitochondrial interface and thereby provide direct access of mitochondrially produced ATP to the metabolic pathways of the cell (see Fig. 1) [37–39].

In the cell death scenario two principle pathways including VDAC isoforms may lead to MOM permeabilization and subsequent exit of pro-apoptotic factors such as CytC into the cytoplasm. (I) The mitochondrial permeability transition pore complex (PTPC; consisting of VDAC, ANT and hexokinase I/II) may be regulated from the cytoplasm to cause the breakdown of the inner membrane potential [39,40]. This process and the precise composition is however an area debated more controversially and would require the indirect influence of the VDAC onto the inner membrane stability possibly by channel closure. (II) VDAC channels have also been reported to form complexes with members of the Bcl-2 protein family (Bax, tBid, Bak) [33–36]. The multi-BH domain proteins Bax and Bak are the major pro-apoptotic proteins know to permeabilize the MOM after oligomerization [37]. Collectively, there is compelling evidence that VDAC isoforms may function as receptors for pro-apoptotic proteins to tether and keep them in proximity to the MOM (see Fig. 1).

Early structure work on the VDAC channels embedded in native membranes using electron microscopy has provided insights into the arrangement and approximate dimensions of the channel [5,6,41]. Recently, these investigations were complemented by atomic force microscopy studies of native mitochondrial outer membranes from tomato and yeast [42,43]. In summary these studies at about 2 nm resolution provided a picture of VDAC as a channel with barrel dimensions of ~3 nm (lateral diameter) and ~5 nm in height [11].

High resolution structures of the voltage dependent anion channel have been determined by three independent methodologies after production of the protein in *E. coli* and refolding into a functional form [8–11]. As the most reliable basis for the current structure analysis and modeling approach of Tom40 the high resolution X-ray structure has been selected (see Fig. 2A and next paragraph). This structure is in good agreement with the data derived by our hybrid approach however the localization and orientation of the helix has been modeled more precisely due to resolution constraints [10]. The number of β-strands being debated for many years and yielded models of VDAC predicted to consist of 12 up to 19 strands depending on the approach used [44–46]. Notably, the very first model published in 1987 based on pure secondary structure analysis had already predicted the correct number and localization of 19 β-strands [47]. While this number of strands has now been confirmed by the 3D structures, the length of β-strands deviates from the original model with an average of 10 residues [10]. Another detail of the structure in question was the localization of the N-terminal helix. This helix was in most of the models predicted to be part of the barrel or exposed towards the outside and remained so far very speculative [44–46]. The 3D structures now clearly demonstrate the helix to be localized midway inside the barrel pore, changing the pore diameter by approx. 40% relative to the non-occluded form (see Fig. 2A) [9,10]. This particular structural feature is somewhat reminiscent to the long loop L3 observed in the specific and unspecific bacterial porins most significantly narrowing the bacterial pores. Loops on opposite sides of the barrel are almost equal in length with a slightly longer extension on the cytoplasmic side [47,48]. This feature is in particular contrast to all bacterial outer membrane protein structures which show a strong asymmetry with long loops at the extraplasmic side and short turns facing the periplasm. Aside the uneven number of β-strands this feature is the most obvious structurally diverging element compared to their bacterial homologs [9].

3. A model of Tom40 based on the VDAC structure

In our recent paper on the structure analysis of human VDAC1 we traced a significant sequence similarity between this channel and the Tom40 family (15–25% identical/40–60% similar residues depending on sequences used) proteins which led to our proposal of an evolutionary relationship between these two protein families [9]. In our analysis here we improved this picture by the alignment of the two sequences, assigning a secondary structure to both using secondary structure prediction tools and finally compared these features with the recent 3D structure (see Fig. 2B). From both