FEBS Letters 587 (2013) 3522–3528

FEBS Letters

journal homepage: www.FEBSLetters.org

# NMR structure and MD simulations of the AAA protease intermembrane space domain indicates peripheral membrane localization within the hexaoligomer

Theresa A. Ramelot [a,*], Yunhuang Yang [a], Indra D. Sahu [a], Hsiau-Wei Lee [b], Rong Xiao [c,d], Gary A. Lorigan [a], Gaetano T. Montelione [c,d], Michael A. Kennedy [a,*]

[a] *Department of Chemistry and Biochemistry, Northeast Structural Genomics Consortium, Miami University, Oxford, OH 45056, USA*
[b] *Complex Carbohydrate Research Center, Northeast Structural Genomics Consortium, University of Georgia, Athens, GA 30602, USA*
[c] *Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, Northeast Structural Genomics Consortium, Rutgers, University, Piscataway, NJ 08854, USA*
[d] *Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA*

ARTICLE INFO

*Article history:*
Received 16 July 2013
Revised 21 August 2013
Accepted 6 September 2013
Available online 18 September 2013

Edited by Maurice Montal

*Keywords:*
*m*-AAA protease
Molecular dynamics
NMR structure

ABSTRACT

**We have determined the solution NMR structure of the intermembrane space domain (IMSD) of the human mitochondrial ATPase associated with various activities (AAA) protease known as AFG3-like protein 2 (AFG3L2). Our structural analysis and molecular dynamics results indicate that the IMSD is peripherally bound to the membrane surface. This is a modification to the location of the six IMSDs in a model of the full length yeast hexaoligomeric homolog of AFG3L2 determined at low resolution by electron cryomicroscopy [1]. The predicted protein–protein interaction surface, located on the side furthest from the membrane, may mediate binding to substrates as well as prohibitins.**
**© 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.**

## 1. Introduction

Here we report the solution NMR structure of the intermembrane space domain (IMSD, residues) of human mitochondrial ATPase associated with various activities (AAA) protease AFG3-like protein 2 (AFG3L2). In humans, AFG3L2 forms homo- or hetero-oligomeric complexes with paraplegin (49% identical) in the mitochondrial inner membrane (IM). They are named *m*-AAA proteases, meaning that their catalytic domains are located in the mitochondrial *m*atrix. The catalytic domains of both AFG3L2 and paraplegin are made up of two subunits, the ATPase associated AAA domain and the metallopeptidase domain (PD) responsible for substrate proteolysis. A single chain of AFG3L2 or paraplegin has two transmembrane (TM) helices, so that the topology in the IM results in the short N-terminus and large catalytic domain on the matrix side and a small ~70-residue IMSD on the lumen side. Established and putative functions for *m*-AAA proteases have been extensively characterized and several recent reviews are available [2–4]. Although the crucial functions of *m*-AAA proteases are proteolytic processing and degradation of both non-membrane and membrane-embedded substrates, the role of IMSDs remains to be determined.

AFG3L2 and paraplegin have mammalian, plant, and fungal homologs, including yeast Yta10 and Yta12 (59% identical to AFG3L2) in addition to the well-studied bacterial homolog, FtsH (filamentous temperature sensitive H, 45% sequence identity), which forms only homo-oligomers, and is essential for survival in *Escherichia coli*. The IMSD of AFG3L2 belongs to a conserved protein family (Pfam), Pfam PF06480.

Prior to the NMR structure reported here, no atomic resolution information was available for domains in this diverse Pfam. However, the overall architecture of a *m*-AAA protease was recently determined in a 12 Å-resolution cryo-electron tomography (CET) map of yeast Yta10/Yta12 hetero-oligomer solubilized in a detergent micelle [1]. The authors observed symmetrical hexamers that rely on residues in PD for oligomerization. In their model, the six IMSDs splayed-out in a hexagon around 12 clustered TM helices and have no contact with IMSDs on other subunits [1]. Prediction of the IM position based on the location of the TM helices resulted in the IMSDs being half buried in the membrane. Our results, however, are inconsistent with this aspect of the model, rather, our data suggests that this domain has only peripheral contacts with

* Corresponding authors. Fax: +1 513 529 5715.
*E-mail addresses:* theresa.ramelot@miamiOH.edu (T.A. Ramelot), kennedm4@miamiOH.edu (M.A. Kennedy).

0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.09.009

the IM. We propose a modification to the previous model that is consistent with our structural data and molecular dynamics (MD) simulations.

## 2. Materials and methods

### 2.1. Cloning, expression and purification

The selected IMSD fragment of the *afg3l2* gene was cloned into a pET15 expression vector (NESG Clone ID HR6741A-15.1) as described elsewhere [5,6]. The IMSD, residues 164–251 of AFG3L2, included 11 non-native N-terminal residues (MGHHHHHHSHM). Expression and purification were conducted following standard protocols of the Northeast Structural Genomics Consortium (NESG) to prepare [U-$^{13}$C, $^{15}$N]- and U-$^{15}$N, 5% biosynthetically-directed $^{13}$C (NC5) samples [5,6] and details can be found in Supplemental Procedures. The final NMR samples were 0.7–1.0 mM in the NMR buffer: 20 mM MES, 100 mM NaCl, 5 mM $CaCl_2$, 10 mM dithiothreitol, and 0.02% $NaN_3$ at pH 6.5. The protein was monomeric under the conditions used in the NMR experiments based on analytical static light scattering in-line with gel filtration chromatography and rotational correlation time estimates from $^{15}$N relaxation data ($\tau_c$ 7.1 ± 0.5 ns, Fig. S1).

### 2.2. NMR and structure determination

NMR data were collected at 298 K on NC and NC5 samples of ~300 μl in 5 mm Shigemi NMR tubes on 600 MHz Varian Inova spectrometer with a 5-mm HCN cold probe and 850 MHz Bruker Avance III spectrometer equipped with a conventional 5-mm HCN probe. A description of NMR experiments and methods for structure determination and refinement can be found in Supplemental Procedures. The assigned $^{1}$H–$^{15}$N HSQC spectrum is provided as Fig. S2. Chemical shifts, NOESY peak lists, and raw FIDS were deposited in the BioMagResBank with ID 18156). The final ensemble of 20 models and NMR resonance assignments were deposited to the Protein Data Bank with ID 2LNA.

### 2.3. MD simulations

MD studies of the lowest energy structure from the NMR ensemble (residues 16–95) with a 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) membrane were performed using NAMD version 2.9 [7] with the CHARMM27 [8] force field. The Visual Molecular Dynamics software, VMD version 1.8.7 [9], was used for simulation setup and MD trajectory analysis. A pre-equilibrated POPC bilayer with an 80 Å$^2$ surface was generated with the VMD membrane builder plugin. The IMSD was positioned >8 Å above the membrane and the system was solvated into a water box and neutralized with NaCl. Equilibration and simulations were performed similar to those reported in the KcsA tutorial for VMD and NAMD [10] and is described in detail in the Supplemental Procedures.

## 3. Results

### 3.1. Solution NMR structure

The human AFG3L2 IMSD features an uncommon mixed α + β fold comprised of two α-helices (α1, 20–30; α2, 67–81) and a five-stranded β-sheet (β1, 16–17; β2, 33–39; β3, 43–48; β4, 60–63; β5, 91–93;) arranged in a βαβββαβ topology (Fig. 1A–C). The first helix (α1) has a break at Tyr27 resulting in a bend in the helix. The β-sheet has an up–up–down–up–up topology with the order β1–β4–β3–β2–β5 and packs against the two helices to form a compact structure, whereas a poorly-defined loop is located between β3 and β4 (loop 3–4; Thr49-Gln58). Structural statistics for the NMR ensemble are presented in Supplemental Table S1.

### 3.2. Homologs have the same predicted secondary structure

IMSDs of human paraplegin, yeast Yta10 and Yta12, share 36%, 40%, and 43% sequence identity with that of human AFG3L2. However, these ~85-residue domains were all predicted to have the same order of secondary structural elements by PSIPRED 3.0 [11] (Fig. 1D). The prediction for the human AFG3L2 IMSD was in good agreement with the NMR structure with the exception that the first short β-strand was slightly shifted from the PSIPRED prediction (Fig. 1D). Similarly, the ~70-residue *E. coli* FtsH periplasmic domain had an equivalent secondary structure prediction although it is ~14 residues shorter (Fig. 1D). This result suggests that the IMSD structure will be conserved within Pfam PF06480, including bacterial homologs.

### 3.3. Structural alignment comparison and protein–protein interaction prediction

Structural alignment analysis of AFG3L2 IMSD by Dali [12] found no protein structures with RMSD <2.5 Å or sequences with >20% identity. Dali identified the 60S eukaryotic ribosome subunit protein L38 (PDB ID 3U5E:k and 4A18:p, Z-scores 4–5) as a similar topology protein. L38 is part of a large complex of ribosomal proteins. According to predictions of protein–protein interactions by PredUS [13], residues near α1 (Asn 26) and part of the α2-loop-β5 (Gly82-Glu86, with the highest score for Gly85) are likely to interact with other proteins (Fig. 3C and D). The prediction is primarily due to structural alignment with the N-terminal domain of the molecular chaperone heat shock protein Hsp90 (Fig. S4). This domain contains an ATP binding site and binds co-chaperones (reviewed in [14]). In human cells, Hsp90 binds to more than 10 known co-chaperones that together with Hsp90 regulate the stability and activation of other proteins, many of which are involved in cellular signaling pathways. For example, the co-chaperone Cdc37 regulates Hsp90 interactions with kinases and the stability of the respective kinases determined the extent of association with Hsp90, with unstable kinases having increased affinity [15]. Taken together, the structural alignment results indicate a role in the assembly of protein–protein complexes that could regulate substrate recognition.

### 3.4. Membrane interaction surface

In AFG3L2, the interaction of the IMSD with the membrane is governed, in part, by the tethering to the TM helices at its N- and C-termini. Analysis of the IMSD surface electrostatic surface potential [16] revealed a patch of basic and hydrophobic residues at one end of the protein that constitutes a putative membrane interaction surface (Fig. 2A–D). It is primarily made up of loops between secondary structural elements, including loop 2–3 and loop 3–4 (Fig. 2A–D). Solvent exposed hydrophobic residues, as well as basic residues from this surface are shown in Fig. 2A and B. It is well known that Trp and Tyr residues have a preferred localization at membrane interfaces and that Arg and Lys residues can interact with phospholipid headgroups.

ConSurf [17] analysis revealed that conserved residues across the eukaryotic homologs are located on one side and that the putative membrane binding surface has few conserved residues. The conserved surface patch has an acidic surface resulting primarily from residues Glu71 and Glu75 (Fig. 2C and E), but the function of this patch is unknown. On the putative membrane-binding

Download English Version:

https://daneshyari.com/en/article/10871077

Download Persian Version:

https://daneshyari.com/article/10871077

Daneshyari.com