Structural intermediates in the assembly landscape of the human mitochondrial anion channel

Radhakrishnan Mahalakshmi*

Molecular Biophysics Laboratory, Department of Biological Sciences, Indian Institute of Science Education and Research, Bhopal, India.
E-mail: maha@iiserb.ac.in

Abstract

Human voltage-dependent anion channels (VDACs) are metabolite transporters of the mitochondrial outer membrane. Due to its anti-apoptotic nature, VDAC isoform 2 is particularly of pharmacological interest as a therapeutic target for cancer. In addition to ATP/ADP and NADH flux, as well as calcium homeostasis, VDAC2 is selectively upregulated in cancer. However, we know surprisingly little about the folding and assembly pathway(s) of VDAC2, thermodynamic regulators of its 19-stranded β-barrel structure, and functional bioenergetics of this anion channel. Here, we deduce the atomic folding pathway of VDAC2 in phosphocholine membranes and identify molecular regulators of barrel stability, channel gating, and metabolite transport. We show that early events in the assembly of the 19-stranded structure involve parallel pathways. The kinetically faster pathway, although more populated, rapidly accumulates an off-pathway intermediate that subsequently undergoes slow rearrangement to give rise to the native VDAC2 structure. The slow folding intermediate forms several non-native interactions across the sequence involving both the lumen– and lipid–facing residues. The C-terminal strands β15–β19 impede folding by accumulating non-native interactions in both pathways. Interestingly, however, the C-terminal strands are thermodynamically essential as post-folding membrane anchors for the energetically–compromised structure of VDAC2. Additionally, we demonstrate that residues vital for the folding and directed assembly of VDAC2 also regulate voltage-gated channel opening, ATP flux, and in vivo survival. Thus, we obtain a direct synergy between molecular regulators of human VDAC2 folding and function. Our findings provide the first insight into the molecular folding pathway of this essential protein, and demonstrate why in vivo assembly of this essential β-barrel is chaperone–assisted.