

VDAC

Structural model confirmed by automated lipid bilayer experiments

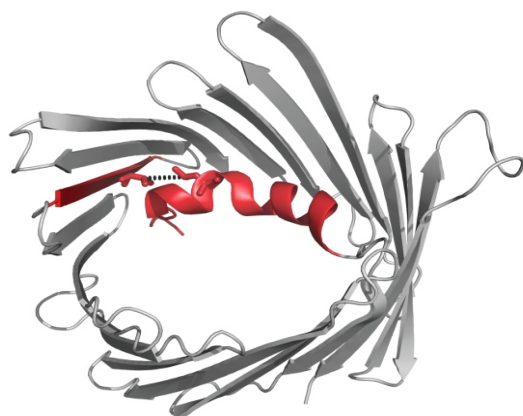


Introduction

The voltage-dependent anion channel (VDAC), also known as mitochondrial porin, is the most abundant protein in the mitochondrial outer membrane (ca. 10.000 copies per mitochondrion). It regulates mitochondrial function and regulates homeostasis by constituting the major pathway for the transport of ADP, ATP, and other metabolites. VDAC is a critical player in the release of apoptogenic factors from mitochondria of mammalian cells and it is discussed as an anti-cancer target. In carcinoma cells VDAC is highly expressed and can form a complex with hexokinase and Bcl2 proteins making the cell resistant towards chemotherapeutic agents by supporting anti-apoptotic behaviour. By blocking VDAC function with erastin, cancer cells can be destroyed selectively.

VDAC structure has been studied intensively and there is a general consensus that VDAC has a 19-stranded β -barrel architecture with an N-terminal α -helix positioned horizontally midway within the pore (1). The N-terminus is involved in the voltage-dependent gating process of the channel and may adopt different conformations depending on external factors.

In order to verify the structural hypothesis, measurements at an artificial membrane with the **Ionovation** technology are the method of choice. Here protein-protein interactions, lipid-protein interactions (2), and electrophysiological properties of various mutants of VDAC can be examined easily, fast and routinely. This is of specific interest, as VDAC is regulated by the mitochondrial lipid composition as well as protein interaction partners.



Native conformation of the human VDAC1. View of the NMR crystal structure with N-terminus and β -strand 9 coloured red (3).

Several diseases are discussed to be related to VDAC1 malfunction:

Alzheimer's disease

In Alzheimer's disease the main components of the mitochondria are altered. Complex IV of the respiratory chain is reduced; complex V which metabolizes ADP to form ATP is oxidatively damaged and functionally altered; and VDAC is damaged as a result of oxidative stress.

Mitochondrial Encephalomyopathies in skeletal muscle

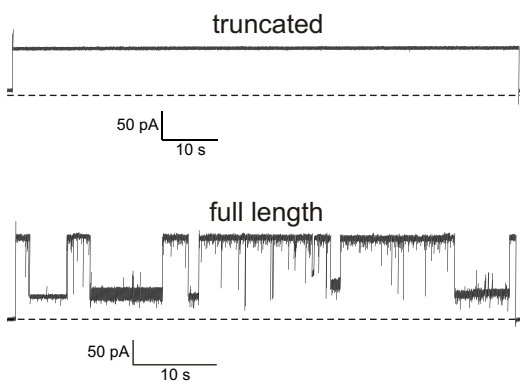
Tissue-specific VDAC isoform 1 deficiency in human skeletal muscle is responsible for a rare mitochondrial encephalomyopathy, fatal in childhood.

Cancer

Cancer cells are frequently glycolytic and overexpress hexokinase II. In cancer cells, the majority of hexokinase II is localized to the mitochondria through interaction with VDAC.

Example hVDAC1; N-terminal truncation results in lower conductances

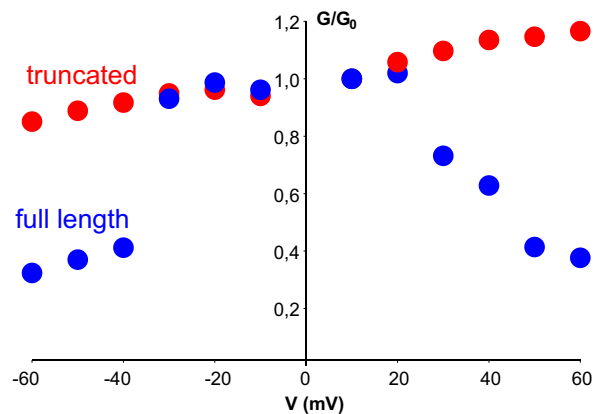
Truncated preparations of hVDAC1 were created to study the location of different domains. Functionally, hVDAC1 mutants were confirmed by electrophysiological measurements in automated lipid bilayer recordings with the **Ionovation Compact** after reconstitution of the detergent solubilized full length and truncated variant, respectively. In particular, N-terminally truncated hVDAC1 were found to exhibit lower conductances than the full-length channel and showed no gating. This has been taken as an indication that the N-terminal helix may not lie inside the pore but forms part of the barrel wall.



Electrophysiological experiments on hVDAC1 reconstituted into asolectin lipid bilayers and automatically measured with the **Ionovation Compact**.

Representative current traces of full-length hVDAC1 (bottom) and N-terminally truncated $\Delta(1-20)$ -hVDAC1 (top) in lipid bilayer experiments.

Depolarisation step from a holding potential of 0 mV to +40 mV sampled at 5 kHz and filtered at 2 kHz



Conductance profile (relative conductance G/G_0 vs. voltage V) of purified hVDAC1, showing characteristic voltage-dependent behaviour of full-length hVDAC1 but not of N-terminally truncated $\Delta(1-20)$ -hVDAC1.

G refers to the dominant conductance at the respective applied voltage V , and G_0 to the dominant conductance at +10 mV (4).

Conclusion

- It is shown that the N-terminus of functional hVDAC1 in a lipid environment assumes a well-defined rigid conformation in agreement with the crystal structure of hVDAC1.
- Changes in the beta barrel upon truncation of the N-terminus explains why N-terminally truncated hVDAC1 exhibits lower conductances than the full-length channel.

References

- (1) Bayrhuber et al., Structure of the human voltage-dependent anion channel, PNAS 2008, 105(40):15370-15375
- (2) Betanelli et al., The Role of Lipids in VDAC Oligomerization, Biophys J 2012, 102:523-531
- (3) Data by kind permission of A. Lange, Department for Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen
- (4) Schneider et al., The native conformation of the human VDAC1 N-terminus, Angew Chem Int Ed Engl. 2010, 49(10):1882-5