NMR Studies of Structure and Interactions of Human VDAC1

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Much is known about apoptotic signaling.

What happens at the mitochondrial membrane?
Death signals lead to cellular events that cause formation of a pore in the outer mitochondrial membrane.

Cytochrome c and other factors are released initiating cellular destruction.

What happens in the OMM during apoptosis?

Apoptosis, Bcl-2, VDAC

Apoptosis

Apoptosome (Apaf-1, casp9, cyt c)

VDAC

Bcl-xI

Bax

caspase-8

tNBid tCBid

tCBid

‘BH3 only’

ATP

ADP
• Bcl-2-type proteins are crucial for apoptotic events in cytoplasm and in OMM
• Protein family with different functions.
  • **Antiapoptotic**: Bcl-xl, Bcl-2. Bind to BH3 domains, protect from cell death.
  • **Multidomain, Proapoptotic**: Bax, Bak.
  • **BH3-only, proapoptotic**: Bid, Bad, Bim.
  • Death signals.
• Interact with VDAC

The voltage-dependent anion channel (VDAC) transports metabolites across OMM, regulated by Bcl-2 proteins.
VDAC is the most abundant protein of the outer mitochondrial membrane.

VDAC belongs to the eukaryotic porin family of membrane proteins.

It provides the primary pathway for metabolite transport between the mitochondrion and the cytoplasm.

VDAC forms an aqueous channel through which anionic metabolites, such as ATP and ADP, can pass.

There are three known isoforms of VDAC, we are mainly concerned with VDAC1.
VDAC in apoptosis

- VDAC also plays a central role in apoptosis through interactions with pro- and antiapoptotic Bcl-2 family proteins (Bax, Bim, t_cBid, and Bcl-xl).

- We are interested in the role of an interaction between Bcl-2 proteins and VDAC in regulating cellular fate and metabolite availability.

- A clear understanding of mitochondrial-mediated cell death at the molecular level is hampered by the occurrence of major apoptotic events in a membrane environment.

- We are investigating the solution structure of VDAC in micelles and its interaction with Bcl-2 proteins by NMR spectroscopy.
VDAC properties

- VDAC is **conserved throughout all eukaryotes**, unlike Bcl-2 proteins.
- hVDAC1 is a **283 residue** mitochondrial outer membrane protein predicted to form a **12-16 strand β-barrel**:

Two models for Bcl-xL regulation of VDAC:

1) Model 1: Bcl-xL closes VDAC and prevents the loss of membrane polarization.

2) Model 2: Bcl-xL stabilizes VDAC’s open conformation. When Bcl-xL is bound, metabolites can be freely transported across the membrane.

- VDAC also interacts with tCBid, Bax, Bim.
- Interaction of VDAC with hexokinase for easy access to ATP.
Sample Preparation

• VDAC expresses in inclusion bodies at very high yield, with and without a C-terminal Hist6-tag: ~50mg/L culture.

• VDAC is purified by Ni-NTA chromatography followed by refolding in LDAOO micelles (and His-tag removal).

• LDAO was found the best detergent so far.

• VDAC is a 32kD polypeptide and is ~90 kD in LDAO micelles.

• NMR samples are prepared at concentrations between 5μM and 1 mM VDAC.
Deuterated VDAC exhibits excellent TROSY-HSQC spectra

Preparation produces a folded polypeptide with defined conformation

\[ ^{15}\text{N-VDAC1}, \text{ fully protonated.} \]

\[ ^{2}\text{H}, \, ^{15}\text{N-VDAC1}. \]

- ~80% of expected peaks: 250/290.
- Dispersion indicates β-barrel structure.

Deuteration greatly improves the TROSY-HSQC spectrum.
Apparent exchange broadening effect make spectroscopy difficult.
Spectroscopic properties of a 90 kDa system
Is the prepared VDAC1 functional?

• Does it bind ATP?
• Interaction with the substrate NAD?
• Interaction with β-NADH, a reported VDAC inhibitor?
• Is there single-channel conductance?
Excess ATP causes specific changes in the VDAC spectrum

Black: 0.5 mM VDAC1
Red: add 15 mM ATP

Specific but weak interaction with ATP
Physiological Substrate NAD induces specific shifts in the VDAC spectrum
ATP, NAD and β-NADH affect the same subset of residues indicating a common binding site

Physiological inhibitor β-NADH induces shifts in VDAC1 spectrum
Backbone assignment of VDAC1 in micelles

Assignment of CA resonances from TROSY-HNCA/HN(CO)CA.

Success depends on whether resonances are visible.

Broadening of resonances due to exchange effects may interrupt assignment connections.
NOEs defining $\beta$ sheets
NOEs defining β sheets
Previous models for the VDAC fold are inconsistent with NOE data.
The structure determination is still in progress.
Is VDAC1 a monomer or an oligomer?

Cross-linking experiments indicate trimerization above 10 μM VDAC concentration.
Is VDAC1 a monomer or an oligomer?

At 5 μM VDAC concentration new peaks appear (black) that are broadened at 0.5 mM (red) due to monomer-trimer exchange.
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VDAC exhibits a dynamic monomer - oligomer exchange > exchange broadening of peaks.
Interaction with Bcl-xL?
Bcl-xl directly interacts with VDAC

- Addition of Bcl-xl causes large changes in the HSQC spectrum of $^2$H,$^{15}$N-VDAC.
- Arrows highlight the major spectral changes.
- Shows that this VDAC preparation is functional with respect to Bcl-xL binding.
- Confirms the relevance of VDAC regulation by Bcl-xL!
Bcl-xl undergoes a major conformational change upon transfer into LDAO micelles.

HSQC reveals dramatic structural change of Bcl-xl in the presence of detergent micelles.

Proposed model of Bcl-xl in micelles. Helices 5 and 6 insert into membranes.
• $^{15}\text{N-Bcl-xL}$ prepared in LDAO.
• Addition of VDAC causes specific changes in the Bcl-xL HSQC spectrum.
• This verifies the functional significance of the Bcl-xL:VDAC interaction.
• Bcl-xL retains function within LDAO micelles.
Assignment of Bcl-xl in LDAO micelles

Assignment of HNCA stretch for region between helix 6 and 7.
Shifts observed in HSQC spectrum of $^{15}$N-Bcl-xl after addition of VDAC1.

Shifted peaks (red) assigned to region including helix 6-7.
Summary of VDAC contacts in Bcl-xL

Red, green and blue residues experience VDAC1 contacts in one or several NMR mapping experiments
Model for VDAC1-Bcl-xL interaction

Side chains shown indicate residues interacting with VDAC
Red: Significant chemical shift changes
Green: transferred cross saturation
Blue: significant effects in both experiments
VDAC1-Bcl-xL crosslinking
VDAC isoform 2 plays a key role in apoptosis

VDAC2 Inhibits BAK Activation and Mitochondrial Apoptosis

Emily H.-Y. Cheng, Tatiana V. Sheiko, Jill K. Fisher, William J. Craigie, Stanley J. Korsmeyer

Lack of VDAC2:
- enhanced apoptosis
- BH3-only proteins (tBid) displace Bak from VDAC2

VDAC1/VDAC2 Alignment: 75% identity.

- Lack of VDAC2: enhanced apoptosis
- BH3-only proteins (tBid) displace Bak from VDAC2
VDAC2 forms aggregates when prepared with the same strategy as VDAC1. Possible sources of poor behavior: 9 Cysteines, N-terminal extension. Mutational analysis will be performed to identify aggregation-mediating elements of VDAC2.
Summary

• Expression and purification method to produce an NMR-suitable form of VDAC1
• Recombinant VDAC1 binds ATP, NAD and β-NADH in LDAO micelles
• Trimerization (~ 20 μM)
• VDAC1 binds Bcl-xL, and interaction is manifested in spectra of both proteins
• NMR assignments of VDAC1 and Bcl-xL in LDAO micelles are in progress but are hampered by dynamic oligomerization
• VDAC2 isoform is directly involved in apoptosis but exhibits difficult NMR spectra
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