Apoptosis: Mitochondria – Oxidized Lipids

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Biological Chemistry

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Programmed Cell Death: Development

Elimination of useless and/or dangerous cells

Life time of erythrocyte: 120 days

dysfunction = pathology (cancer, Alzheimer….)
Apoptosis: Mitochondrion / Cardiolipin

Collaboration E. Dufourc, CNRS Bordeaux, F.
Life or Death Decision at Mitochondrion

Machinery

Mitochondrial Pathway
Life or Death Decision at Mitochondrion

Principle

![Diagram showing balance between "Yang" and "Yin" proteins and their effects on cell death signals, leading to life or death decisions.]

- "Yang" proteins (e.g., Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A-1) with "Yang" in excess block cell death signals, leading to Life.
- "Yin" proteins (e.g., Bax, Bak, Bik, Bad, Bid) with "Yin" in excess promote cell death signals, leading to Death.
Cancer Treatment

- Drug induced cell death
- Tumor cells become resistant against drugs
- Cells develop strategies to block death signals

Main Player: Bcl2 family
Amyotrophic Lateral Sclerosis (ALS) – Lou Gehrig Disease

Cu/Zn superoxide dismutase (SOD1)

\[
2O_2^- + 2H^+ \leftrightarrow O_2 + H_2O_2
\]

Famous persons with ALS
- Mao Tse Tung (1893-1976)
- Lou Gehrig (1905-1941)
- Immendorff (died 2007)
- Stephen Hawkings (still alive)

Familiar ALS (FALS) associated with mutations in the SOD1 gene

Protein folding disease

? Neurotoxic Action ?
? Are Membranes perhaps Involved?

native

(partly) unfolded

A104F

net charge increase

aggregated

Survival time (years)

Survival time (years)

net charge decrease

-5

-4

-3

-2

ΔΔG (kcal/mol)

A4V

Lindberg et al. PNAS | July 12, 2005 | vol. 102 | no. 28 | 9754-9759

Survival time = f (protein stability)

membrane
Experimental Setup

Reducing conditions:
0.5 mM TCEP
APO state:
10mM EDTA

Lipid Membranes

Neutral Surface
eukaryotic cells: outside

Charged Surface
mitochondrial membranes
cancer cells: outside
prokaryotes
Circular Dichroism (CD): Structural Changes

- Charged membranes required for association
- apo-SOD1 (native fold) undergoes structural changes
Membrane-Association: WT versus Mutants

Apo-state 37C

- Charged membranes required for association
- Membrane association increases with stability ($\Delta G$)
- Unfolded protein population does not bind
Crowding on Membrane Surfaces: NMR-Tools for Lipids

$^{31}$P MAS NMR

negative part of dipole

$^{14}$N MAS NMR

positive part of dipole

Lindström, Williamson, Gröbner JACS (2005)
$^{14}\text{N}/^{31}\text{P}$ NMR: Surface Electrostatics

$^{14}\text{N}$ NMR

DMPC

$\Delta n_Q$ and $\sigma_i$: both report on surface potential
Electrostatic Membrane Association of Amyloid Peptide

DMPC/DMPG (2:1)

\[ \text{A} \beta(1-40) \text{ Peptide} \]

\[ ^{31}\text{P CP MAS NMR} \]

(20ms contact time)

PC

PG

no peptide

60:1 L/P

30:1 L/P

JACS 2005
Alzheimer’s Disease: Membrane Involvement

$^{31}$P MAS NMR: SOD1 and Mutants

- **NMR:** no simple charge compensation mechanism
- **most aggressive mutant** – less interactions
How does SOD1 bind?

**Holo-SOD1**

90 Debye

**apo-SOD1**

180 Debye

**A**

**B**

*Electric dipole moments, calculated according to Felder et al. (negative, red sphere; positive, blue cone)*

**not binding**

**binding**
SOD1’s Interplay with Membranes

I. Electrostatic Absorption
II. Conformational Changes
III. Hydrophobic Membrane Action

apo-state monomer
conformation
unstable stable

I.

II.

III.

Leakage -> Toxicity ?

Equilibrium <-> Aβ-protein
Mitochondrial Membranes: Potential SOD1 Target Sites

M-WT dimer

neutral

M aggregating or M₂

partially charged

highly charged

apo wtSOD1 affinity to charged membranes
What Happens at the Mitochondrion?

Collaboration E. Dufourc, CNRS Bordeaux, F.
Model System: Bcl-2 Segements and Mitochondrial Model Membranes

BH4 and α1 Peptides

Main Player: Bcl2 family
BH4 domain prefers cardiolipin

31P CP MAS NMR Buildup Curves: Lipid Dynamics

**Principle**

Peptide Induced Changes

**Behaviour of BH4 versus Bax-α1**

<table>
<thead>
<tr>
<th></th>
<th>BH4 Ri50</th>
<th>Bax-α1 Ri50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Time (ms)</td>
<td>0, 2, 4, 6, 8, 10</td>
<td>0, 2, 4, 6, 8, 10</td>
</tr>
<tr>
<td>Intensities normalized on DMPC higher value</td>
<td>0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0</td>
<td>0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0</td>
</tr>
</tbody>
</table>

- **MitoCT PC/PE/CL (28:22:20)**
- **BH4 Ri50**
- **Bax-α1 Ri50**

**by Murray**
Bax-α1: Membrane insertion or not?

2H NMR

PC/PE

PC/PE/CL

35°C

Hydrophobic core

5% mol Bax-α1
2% mol Bax-α1
pure

31P NMR

PC/PE/CL

PC/PE

35°C, 6kHz spinning

Headgroup region
**Bax-α1/BH4: Membrane Interaction Models**

**Bax-α1 apoptotic action**

- BH4 interacts with negatively charged vesicles
- Increases membrane order and therefore their stiffness

This behaviour could interfere with the anchoring of the pro-apoptotic Bax protein into the outer mitochondrial membrane system.

**BH4 anti-apoptotic action**


Complex Machinery

How do intact mitochondria respond to external death signals

Challenge is to apply high resolution solid state NMR \textit{Ex Vivo}

4 steps:
- Grinding
- Centrifugation/Washing (5000g)
- Centrifugation/isolation (30000g)
  - by gradient at 28\% Percoll
- Centrifugation/Washing (5000g)

Results/Yield:

2kg potatoes = 400\mu L of pure mitochondria
About 70mg/mL of proteins = 15-30mg/mL of lipids

About 1-2mg of lipids loaded per sample
Mitochondria contain ions/paramagnetic agents

*ex vivo* $^{31}$P NMR on Mitochondria

**A)** $^{31}$P Static NMR on Mitochondria

**B)** $^{31}$P MAS NMR

Lamellar phase

CL-Cytochrome c complex

! Lipids can monitor mitochondrias’ integrity!
Mitochondrial Respiration

(Oxygen Consumption)

![Diagram of Mitochondrial Respiration](image)

<table>
<thead>
<tr>
<th></th>
<th>Fresh mitochondria</th>
<th>Mitochondria after NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rates</td>
<td>41 ± 3</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>(nmolO₂.min⁻¹.mg⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCR</td>
<td>3.0 ± 0.2</td>
<td>1.8 ± 0.2***</td>
</tr>
</tbody>
</table>

Mitochondria survived
31P NMR Study: Degradation of Mitochondria

- narrow lineshapes
- downfield shifts
- small vesicle formation
- changes in lipid headgroup packing
Cardiolipin-Cytochrome c Complex

paramagnetic
Cardiolipin

apoptotic stimuli

Dissociation driving force behind CL NMR signal recovery
31P NMR: Ca\textsuperscript{2+} Effect on Mitochondria

Ca\textsuperscript{2+} ions – apoptotic stimuli
induce swelling of mitochondria and cytochrome C release

NMR shows: lamellar phase destruction and no evident hexagonal formation
Respiration upon Ca$^{2+}$ Overload

**A** Mal/Glu → ADP → RCR: 2.0 → 48nmolO$_2$.min$^{-1}$.mg$^{-1}$prot → ADP → RCR: 2.4 → 56nmolO$_2$.min$^{-1}$.mg$^{-1}$prot → ADP → RCR: 2.3

**B** Mal/Glu → ADP → RCR: 1.7 → 40nmolO$_2$.min$^{-1}$.mg$^{-1}$prot → ADP → RCR: 2.0 → 45nmolO$_2$.min$^{-1}$.mg$^{-1}$prot → ADP → RCR: 1.7

**1mM Ca$^{2+}$**

**Outer Membrane Misfunctioning**

Oxidation decoupled from Phosphorylation

Intact mitochondria:

OM swelling

f([Ca$^{2+}$])
Solid State NMR based ex vivo assay

- NMR based *intracellular* toxicity assays: SOD1 mutants
- Drug screening on mitochondria from resistant tumors
- ex vivo NMR on various tissue mitochondria
- Dissecting the effects of different apoptotic proteins (Bcl2/Bax) on mitochondrial survival.

A kind of in cell (in mitochondria) NMR possible

- Lipid composition of mitochondria from tumour tissues and upon drug treatment
- Role of oxidized lipids: Apoptosis Onset, Membrane Biophysics
**Novel 2D Solution NMR Technique**

- Proton Detection via cryo probe – highly sensitive
- separating lipids overlapping in direct 31P NMR Spectra

Role of Oxidized Lipids

* Oxidized lipids by reactions of unsaturated lipids with oxygen species.
* They interact with bilayer: impact on mitochondrial membrane organization
Phase Behaviour of DMPC Membranes by Oxidized Lipids

DMPC Bilayer: sharp transition

Sharp and broad components: OxLi-poor and OxLi-rich lipids domains
$^{31}$P NMR: DMPC/Paze 9:1 Bilayers

- Two Phases
- CSA increases with T
\[ ^{31}\text{P NMR: DMPC/Poxno 9:1 Bilayers} \]

- Two Phases
- CSA increases with T
- different to Paze
Oxidized Lipids: Future

- Impact on Membrane Structure and Dynamics
- Specific Interactions with Bcl-2 Proteins
- Modulation of Membrane-mediated Misfolding
- Occurrence in Mitochondria during Apoptosis

not much known
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