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Investigation of Membrane Curvature Dependency on Cytochrome c Binding to Cardiolipin

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Abstract
Cytochrome c (Cyt c), an efficient electron transport protein in cellular respiration that makes biochemical energy ATP, is recently found to take part in initiating apoptosis (programmed cell death) through first existing a lipid called cardiolipin, and then dissociating from the inner membrane of mitochondria to trigger the apoptosis cascade. If cell apoptosis is inhibited, it can cause cancer. Regulation of Cyt c in cardiolipin binding on the mitochondrial membranes potentially enables regulation of the intrinsic pathway of apoptosis. Cardiolipin has four hydrocarbon chains and a negatively charged head group which can interact with anionic site A on Cyt c. It is believed that the electrostatic interactions between anionic site A and Cyt c on the inner membrane of a mitochondria lead to protein binding and partial unfolding. In this charged lysine amino acids. It is believed that the electrostatic interactions between anionic site A and trapped Cyt c mitochondrial inner membrane. Circular dichroism spectroscopy is used to monitor the amount of negatively charged head group which can interact with anionic site A on Cyt c. The regulation of Cyt c in mitochondria to trigger the apoptosis cascade. If cell apoptosis is inhibited, it can cause cancer.

Experimental Design

Protein preparation
- Separate proteins with High-performance liquid chromatography (HPLC).
- Measure protein concentration with UV-Vis spectroscopy.
- Protein oxidation with Ferriyanide.
- Gel filter chromatography separation (Fig 3).
- High protein concentration 100 mg/ml.
- Prepare varying concentrations of high salt and high protein concentration.

Lipid & Vesicles Preparation
- Prepare Non-Actin-CL solution.
- Dry CL w/nonactin using nitrogen gas.
- Mix dried lipid containing nonactin with proteins.
- Freeze- warm water bath-mix cycle.
- Standardize vesicle size to 100 nm with extrusion.
- Exchange high salt buffer to no salt buffer to allow binding.
- Concentrate protein filled vesicles.

Nonactin (Ionomophore)
- No UV-Vis signals.
- No CD signals.
- Open pores on the lipid membrane.
- Allow salt in and out of vesicles.
- Used to remove high salt and permit protein binding.

Protein & Lipid Concentration Determination
- Ultraviolet-Visible spectroscopy (UV-Vis) protein and vesicles scattering.
- Calculate vesicle/lipid concentration and protein concentration.

Using Conformational Change to Detect Lipid Binding Events

Figure 3: G-25 gel chromatography separation

Figure 4: Nonactin opens pores on vesicles membrane to allow salt ion exchange.

Figure 5: Nonactin structure

Figure 7 Left panel: Exposed Cyt-CL binding ratio on the inside of vesicles. The arrow points in the direction of Trp-59 fluorescent signal increase corresponding to increase in lipid concentration. 0 U M lipid corresponds to the black spectrum and the red spectrum is the max lipid concentration. Right panel: Soret region circular dichroism spectra for each Cyt c for 100 nm CL lipid vesicle titrations. Black arrow indicates direction of signal shift during titrations. 0 M exposed lipid concentration corresponds to black spectrum and the red spectrum is the max lipid concentration.

Figure 8 Left panel: Cyt c Trp-59 fluorescent amplitude as a function of exposed Lipid/Protein Ratio. Right panel: Cyt c Soret CD signal as a function of exposed Lipid/Protein Ratio. All titrations were carried out at pH 8 and 25 °C using 100 nm lipid vesicles with no salt buffer as determined with a conductivity meter.

Summary
- Able to load protein into vesicles.
- Able to induce binding (figure 7).
- Initial results show that titration of protein into vesicles using this technique are possible (figure 8).
- Rough titration curves reveal shift from unbound to bound protein.