The role of the mitochondrial membrane system in apoptosis:

The influence of oxidative stress on membranes and their interactions with apoptosis-regulating Bcl-2 proteins

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Abstract
Apoptosis is a crucial process in multicellular organisms in sculpting them, especially during embryogenesis. In addition, apoptosis is responsible for the clearance of harmful or damaged cells which can otherwise be detrimental to the organism. The Bcl-2 family proteins are key players in the regulation of the intrinsic pathway of the apoptotic machinery. This family consists of three subfamilies with B-cell CLL/lymphoma 2 (Bcl-2) protein itself representing anti-apoptotic members, the Bcl-2-associated X protein (Bax), and pro-apoptotic BH3-only signaling proteins. The interplay between pro- and anti-apoptotic proteins on the mitochondrial membranes is central to the balance between the life and death decision of whether the membrane should be permeabilized or not. The cytosolic Bax protein can upon cellular stress translocate to the mitochondrial membrane where it can either carry out its action of forming homo-oligomers that cause outer membrane permeabilization or be inhibited there by the anti-apoptotic membrane protein Bcl-2. Upon mitochondrial outer membrane permeabilization (MOMP) apoptogenic factors leak out from the intermembrane space (IMS) of the mitochondria, leading to caspase activation and ultimately cell death. A common stress signal initiating apoptosis is an increased formation of reactive oxygen species (ROS in the mitochondria, who can cause oxidative damage to lipid membranes. This membrane damage presumably influences the lipid landscape and the membrane features and hence the interactions of the Bcl-2 family proteins with each other and the mitochondrial outer membrane (MOM). To investigate the significance of membrane oxidation on the behavior of the Bcl-2 family proteins, especially Bax, synthetically produced oxidized phospholipids (OxPls) were incorporated in MOM-mimicking vesicles. Differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) spectroscopy and circular dichroism (CD) spectroscopy revealed a major perturbation in membrane organization in the presence of OxPls. These changes in membrane properties increase the affinity of Bax to its target membrane and enable its partial penetration and formation of pores, as fluorescence leakage assays confirmed. However, in the absence of BH3-only proteins these pores are not sufficiently large for the release of apoptotic factors such as cytochrome C (CytC). To understand the inhibition of Bax by the full-length Bcl-2 protein, suitable detergent solubilizing conditions were carefully chosen to enable the measurement of their direct binding to each other outside the membrane, by an antimycin A; fluorescence assay. The observed protein-protein interaction was confirmed by surface plasmon resonance (SPR). An established protocol for the reconstitution of Bcl-2 into stable proteoliposomes now paves the way for structural studies of this key protein, in its membrane environment near physiological conditions; information essential for understanding its function, on a molecular level, and its potential as a cancer drug target.

Keywords
Bax, Bc-2, apoptosis, mitochondria, membranes, oxidized lipids, NMR, calorimetry, circular dichroism,