Interaction of cytochrome *c* with phospholipid model membranes probed by SANS

Alexander Johs and Liyuan Liang

Environmental Sciences Division, Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37830. Phone: 865-574-7444

Mitochondrial cytochrome *c* is a monoheme electron shuttle found between the inner and outer mitochondrial membrane and is essential for the last step in aerobic respiration delivering electrons from cytochrome *c* reductase to cytochrome *c* oxidase. Its molecular configuration is highly conserved across a wide range of eukaryotes, such as yeast, plant cells and mammals. Interaction of mitochondrial cytochrome *c* with acidic phospholipids is relevant in several processes. Examples are the association between cytochrome c and cytochrome c oxidase or activation of caspases during apoptosis by association of cytochrome c with acidic phospholipids in the mitochondrial membrane. It was shown that this protein-lipid association involves electrostatic and hydrophobic interactions resulting in changes in protein conformation as well as bilayer structure. The primary electrostatic interaction leads to a partial unfolding of cytochrome *c* exposing non-polar residues to hydrocarbon chains of the lipids. However, little is known about the impact on phospholipid bilayer organization.

This study was undertaken to investigate the effects of cytochrome *c* binding to phospholipid model membranes by small angle neutron scattering (SANS). The goal was to observe changes to bilayer organization and structural implications of the protein interaction with negatively charged phospholipid membranes. Highly monodisperse suspensions of large unilamellar vesicles consisting of the neutral phosphatidylcholine (PC) and negatively charged phosphatidylglycerol (PG) were used as a model membrane system to investigate the interaction with cytochrome *c*. SANS data was collected at multiple contrast conditions and fitted using a form factor for a spherical multishell particle model separately accounting for contributions from the two phospholipid headgroup layers, the lipid hydrocarbon layer and protein. Additional corrections for vesicle polydispersity and instrument resolution were included in the model calculations. Results show a change in the mode of interaction as a function of concentration of the acidic phospholipid. Model-dependent fits to the experimental data indicate the formation of a dense protein layer and perturbation of the underlying phospholipid headgroup layer in membranes containing PG while no such effects were observed for neutral membranes. Implications on the lateral organization of phospholipids in the membrane bilayer and the function of cytochrome *c* will be discussed.