A structural basis for the inhibitory effect of cholesterol on the insertion of the pore-forming pro-apoptotic protein Bax into lipid bilayers

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The permeabilization of the mitochondrial outer membrane, MOM, by Bcl-2 family proteins is an important and irreversible step in apoptosis[1,2], whose dysregulation is often associated with tumor formation and resistance to anti-cancer treatments. Addition of cholesterol to mitochondrial-like membranes has been shown to inhibit the pore formation process.[3,4] The fact that the enantiomer of cholesterol also has this inhibitory effect[3] provides support for an inhibition mechanism based on structural changes in the membrane as opposed to one based on direct interaction between the proteins and cholesterol. Structural investigations of phospholipid membranes consistently show that cholesterol increases both the thickness[5] and rigidity[6] of lipid bilayers. However, available studies were done for membranes containing, at most, three lipid components. We thus asked the question of whether cholesterol affects more complex membranes, such as the mitochondria-like membrane, in the same way. This complex membrane is composed of five different lipids (PC, PE, PI, DOPS and TOCL) and has been shown to support Bcl-2 mediated pore formation.

We used neutron reflectometry at four D_2O contrast concentrations and 2,2,3,4,4,6-D_6 labeled cholesterol, to establish the position and orientation of cholesterol in the mitochondria-like membrane. It was found that cholesterol sits vertically in the membrane with its polar hydroxyl group located in the membrane head group region as seen in Figure1. Cholesterol's effects on the membrane were determined by investigating the membrane and water neutron scattering length distributions (NSLD). The membrane was thickened by cholesterol as seen in both the membrane and water profiles in Figure 2 a) and b). The hydrophobic thickness, *t*, defined as the distance between the two membrane peaks, increased as a function of cholesterol. The water penetration, defined as the position of the logistic fit to the water profile, decreased as a function of cholesterol. However this decrease is of the same magnitude as the membrane thickening observed, indicating that it is simply the result of the increase in the membrane thickness, and not of a decrease in membrane permeability. From this we conclude that cholesterol increases the overall membrane thickness by straightening membrane hydrocarbon chains thus increasing the thickness of the membrane hydrophobic region and membrane order. As volume must be conserved, the thickening effect must be accompanied by an area condensation effect, characteristic of cholesterol in simple model membranes.

These structural changes to the mitochondria-like membrane, although small (1-2Å), are expected to be sufficient to inhibit pore formation by Bax. Membrane elasticity is known to be important for the conformational changes proteins undergo while integrating into the membrane[7,8]. It is hypothesized that thickening the membrane reduces the chance of Bax becoming transmembrane, while the corresponding area condensation increases the membrane order thus reducing the elasticity of the membrane. Such a reduction would make defects in the membrane less likely and inhibit the conformational change required for Bax to insert into the membrane. Further

experiments directly investigating lipid order in the membrane as a function of cholesterol would help to give a complete picture of cholesterol's effects on a mitochondria-like membrane.

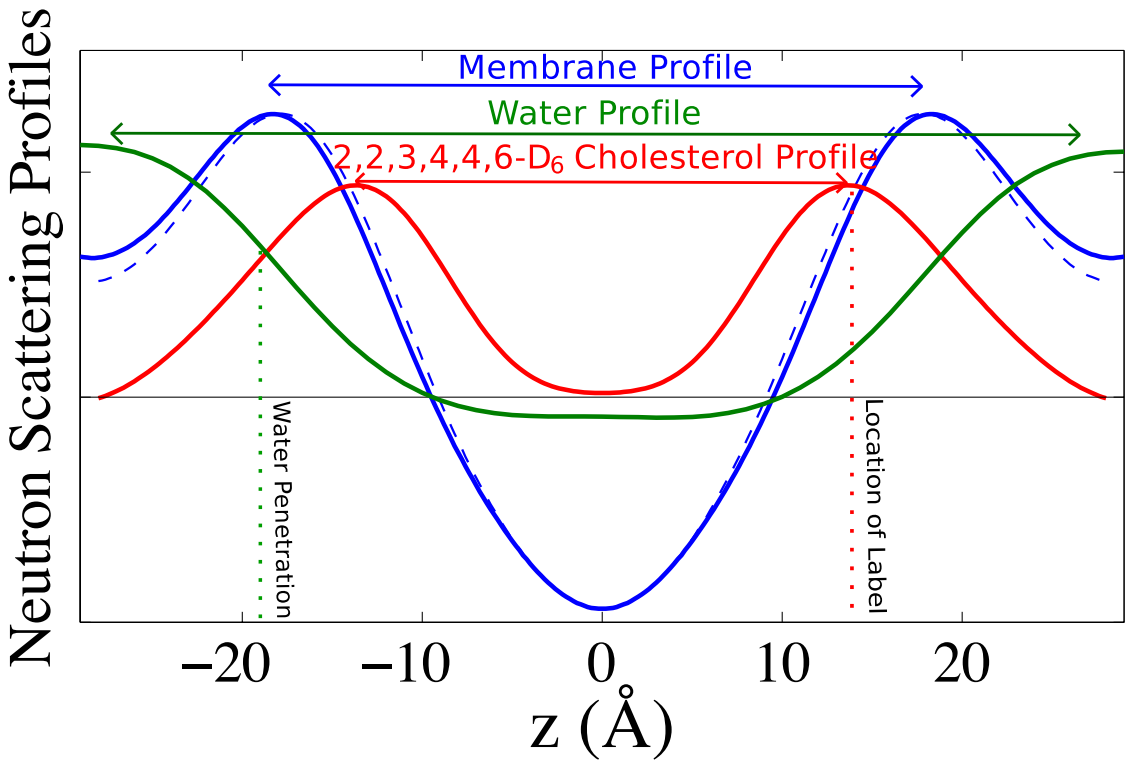

Figure 1: Neutron Scattering Length Density Profiles from a Mitochondria-like sample with 10 mol % Cholesterol. The blue curve represents the membrane profile of the sample containing the unlabeled cholesterol obtained directly from the scattering data in 8% D_2O, while the dashed blue curve represents the membrane profile of the sample containing labeled cholesterol. The red curve represents the 2,2,3,4,4,6-D_6 cholesterol label profile, obtained from subtracting the scattering length density of the unlabeled sample from the labeled sample, leaving the scattering from the six deuterium atoms. The center of the red peak gives the location of the label, and thus the location of cholesterol's head group. The green curve represents the water profile, and the center of the logistic like curve gives a quantitative measure of the water penetration into the membrane.

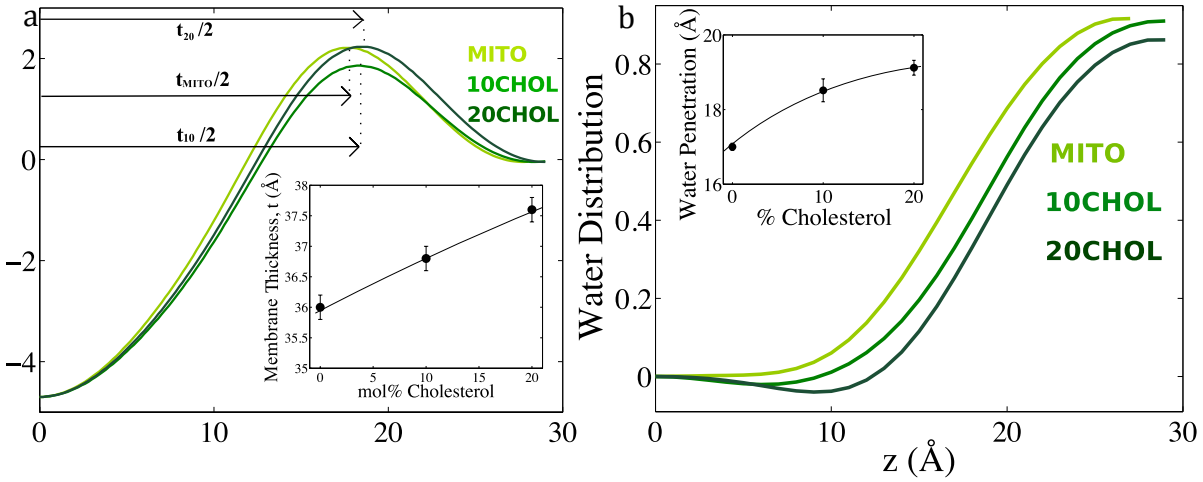

Figure 2: Cholesterol Increases Membrane Thickness, t, and Increases Water Penetration in Mitochondria-like Membranes. a) NSLD profiles for a monolayer of the mitochondria-like, 10 % cholesterol and 20% cholesterol samples. The distance from the center of the membrane, *z=0*, and the center of the profile peak gives a value for half the membrane thickness. The membrane thickness is plotted as a function of cholesterol content in the inset. b) Water distribution in the mitochondria-like, 10% cholesterol and 20% cholesterol samples, obtained from averaging the difference in the SLDs of each of the contrasts. The profiles can be fit with a logistic function whose position determines the penetration of water into the membrane. Water penetration defined from the center of the membrane as a function of cholesterol is plotted in the inset.

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