

Effect of Oxidized Phospholipids on the Interaction of Cytochrome *c* With Mitochondrial Mimetic Liposomes

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Phospholipid oxidation in the biological membranes occurs due to high production of excited and radical species in cells. Regarding the inner mitochondrial membrane (IMM), the study of the effects of lipid oxidation on the binding and activity of cytochrome *c* is particularly interesting due to the role played by this protein in the cell respiration and apoptosis. We investigated the interaction of cytc with IMM mimetic liposomes containing the oxidized phosphatidylcholine PazePC (1-O-Hexadecyl-2-Azelaoyl-sn-Glycero-3-Phosphocholine) at pH 7.4 and 6.2. The binding to the bilayers was assessed by fluorescence quenching promoted by resonance energy transfer pair from the pyrene (fluorescent probe) containing phospholipid incorporated into liposomes to cytc heme group. The binding isotherms show cooperative profiles and were treated using a model based in Hill plots, in which there are two binding sites (K_D^1 and K_D^2) and coefficient m which represents the degree of cooperativity. There was no effect of both pH and presence of PazePC in the value of m , which was always around 2. We observed a decrease in the binding affinity by decreasing pH from 7.4 to 6.2, i.e. K_D^1 decreased from 87.3 to 1070 nM. We expected that this effect is due to the protonation of the CL. In both pHs there was an increase in the binding constant of the high affinity binding site with the inclusion of PazePC, for example K_D^2 decrease from 23 to 1.5 nM. Therefore, we observed that lipid oxidation cause an increase in cytc affinity which may represent a mechanic of cellular protection against oxidation misbalance.

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