The role of cytochrome c site L and A on association with liposomes and lipid oxidation

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The interaction of cytochrome c (cytc) with inner mitochondrial membrane (IMM) is crucial to determine its activity as electron carrier or promote the apoptosis process when released from the membrane. Cytc exhibits two positively charged sites responsible to associate the protein with IMM: site A and L. The site L controls a pH-dependent binding which exhibits $pK_a \approx 7.0$, while site A presents high $pK_a$. However the mechanism of cytc release through the membrane is not clear. In this work we investigated the role of cytc sites A and L to promote lipid oxidation when associated with PCPECL vesicles. The measurements were assessed by electronic spectrometry and lipid oxidation by thiobarbituric acid (TBARS) dosage. The association of cytc to the membranes at pH 7.4 permits the protein to bind via site A and the TBARS content is higher than found at pH 6.2. This last condition favors the binding via site L or A. The results showed the interaction of cytc with liposomes composed by PCPECL in acidic pH disfavor the ROS production. These results indicate that the interaction of cytc with membranes by site L, is not responsible to produce ROS. A recombinant cytc (substitution of histidines 26 and 33 residues by asparagines presented on site L) promoted the production of TBARS in a pH-independent manner, similar as observed for native protein at pH 7.4. We can conclude the association of cytc via site A with membrane favors the ROS production.

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