Peroxidase Activity Induced by Cardiolipin Hydroperoxides in Cytochrome c-Cardiolipin Complex

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INTRODUCTION: Cytochrome c (cyt c) is a heme protein highly conserved in eukaryotic cells that acts as an electron carrier between complexes III and IV in the respiratory chain. As a peripheral protein, cyt c is anchored on cardiolipin (CL), an anionic phospholipid found in the inner membrane by a combination of electrostatic and hydrophobic interactions, forming a cytochrome c-cardiolipin complex with peroxidase activity. This work investigated the effect of cardiolipin mono, di and tri hydroperoxides \([\text{CL(OOH)}_n] \quad n=1,2,3\) on cyt c-CL binding and peroxidase activity.

MATERIAL AND METHODS: The \([\text{CL(OOH)}_n] \quad n\) were synthesized by photooxidation and purified by HPLC. Liposomes composed of dioleoyl phosphatidylcholine (DOPC), CL and increasing amounts of \([\text{CL(OOH)}_n] \quad n\) were prepared by extrusion in buffer containing 100\(\mu\)M DTPA. The peroxidase activity was determined with Amplex Red, by measuring resorufin formation at \(\lambda\) 571 nm. The binding was determined by measuring the amount of free cyt c in the absence and presence of KCl 250 mM after ultracentrifugation at 110000 \(g\) for 4h at 4°C.

RESULTS AND DISCUSSION: Peroxidase activity of cyt c-CL complex induced by \([\text{CL(OOH)}_n] \quad n\) decreased in this order: mono>di>tri hydroperoxide, and were 10 times greater than the activity induced by hydrogen peroxide. The binding assay showed that membranes containing either CL or \([\text{CL(OOH)}_n] \quad n\) are both capable to keep the electrostatic interaction between cyt c and CL. CONCLUSIONS: The significant difference between the peroxidase activity of cyt c induced by \([\text{CL(OOH)}_n] \quad n\) and hydrogen peroxide may be attributed to the anchoring of cyt c on CL and peroxide proximity to the heme group. Moreover, contrary to previous findings, our data shows that \([\text{CL(OOH)}_n] \quad n\) can still bind to cyt c and serve as substrate for the peroxidase activity. Investigations are undergoing to characterize this binding and their effect on cyt c function.

Keywords: peroxide activity, cytochrome c, cardiolipin
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