

Characterization of Dityrosine Crosslinking of Cytochrome c Induced by Cholesterol 7 α -hydroperoxide

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Introduction: Cytochrome c (Cyt c, 12 kDa) is a small protein that has an important function in physiological processes as electron carrier in the respiratory chain and also in apoptosis pathway. Several reports have demonstrated that cytochrome c can undergo homo- and hetero-oligomerization. However, this process is not completely elucidated and is not known which amino acids residues are involved. A study recently published by our group showed the detection of protein-bound tyrosil radicals in the reaction of Cyt c with cholesterol-derived hydroperoxides (Ch-OOH). Here we hypothesized that Cyt c oligomerization is driven by the formation of dityrosine cross-links. **Objectives:** The aim of this study was to characterize dityrosine cross-links in cholesterol hydroperoxide (ChOOH) induced Cyt c oligomers. **Material and Methods:** Cyt c was initially mixed with SDS micelles and then incubated with Ch-OOH (mixture of 4 isomers) or cholesterol-7 α -hydroperoxide (Ch-7 α -OOH) for 1 h. Oligomerization was checked by SDS-PAGE and dimers were sequenced by nano-LC-Q-TOF-MS/MS after in gel digestion of the dimeric bands. **Results and Discussion:** SDS-PAGE analysis showed that ChOOH or Ch-7 α -OOH efficiently converted most of Cyt c monomeric species into dimeric (80 %) and trimeric forms (20 %). LC-MS/MS analysis detected 3 major cross-linked peptides involving Y48-Y48, Y48-Y74 and Y48-Y97. **Conclusions:** Our study clearly identified the presence of dityrosine cross-links in Cyt c dimers formed in the presence of Ch-OOH. We hypothesize that this cross-linking mechanism can impact Cyt c function in energy production and apoptosis.

Keywords: Dityrosine cross-link, Cytochrome c dimer, Cholesterol hydroperoxide