Expanding the Scope of Tyr-Cys Cross-Linked, Protein Derived Cofactors

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To date, over twenty crosslinked protein derived cofactors (CPDC) have been identified. Mechanistic studies of cross-link biogenesis has only been performed in two of these proteins; galactose oxidase and methylamine dehydrogenase. These studies of CPDC formation are necessary to uncover the role of ineffective crosslink formation in human disease. Using a bioinformatics approach and oxidative chemistry, our group is examining the scope of cross-linked, protein derived cofactor biogenesis under oxidative stress conditions, where over 300 proteins were identified with pre-attack geometries for cross-link formation. The tyrosine-cysteine (Tyr-Cys) cross-link from galactose oxidase has been selected to test the hypothesis that the number of amino acid side-chain cross-links increases under oxidative stress conditions. One of the predicted Tyr-Cys cross-links is in hemoglobin (Hb) between Tyr-β145 and Cys-β93. Upon treatment of excess H_2O_2, the UV-visible and EPR spectra changed and were consistent with a compound I mediated formation of tyrosyl radical(s). Formation of the Tyr-Cys cross-link was identified by MALDI analysis of chymotryptic digested Hb before and after H_2O_2 treatment. The indirect identification of Tyr-Cys crosslink formation is detected by *p*-chloromercuricybenzoate assay where and number of free thiols is decreased upon peroxidation. However treatment with 2,3- bisphosphoglycerate caused no peroxidative decrease in free thiol concentration. These results suggest that Tyr-Cys crosslinking is significant and only occurs in the R-state. Human cytochrome *c* has been generated to address the intermolecular electron transfer methodology, as opposed to the intramolecular electron transfer process in Hb. Tyrosyl radical genesis in human cytochrome *c* is being used to form the Tyr-Cys cross-link in hemoglobin and in another predicted Tyr-Cys crosslink forming protein, α-actin. The Tyr-Cys crosslink in α-actin is between Tyr169 and Cys374, which may link H_2O_2 inhibition of F-actin formation. Peroxidative crosslinking studies of α-actin are being performed both presence and absence of cytochrome *c*. Results of this experiments will be discussed, along with the potential for expanding this class of crosslink/cofactors.