L-Trp as a Target of Radical Species Generated from Triplet Acetone

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Excited compounds of molecules are known to cause oxidative damage to biomolecules and have been detected in biological events such as lipid peroxidation. Several studies concerning singlet molecular oxygen (1O2) adducts have been performed but the role of triplet carbonyl is not well established. The aim of this work is to evaluate the effect of triplet acetone products generated by the oxidation of isobutanal (IBAL)/horseradish peroxidase (HRP) in aerated medium system over target amino acids (L-Cys, L-Trp, L-His). Suppression of excited compounds were analyzed through O2 consumption and chemiluminescence assays. The results show that target amino acids can suppress light emission of excited species with kq of 6.1x10^{-2} M^{-1} (L-His), 5.2x10^{-2} M^{-1} (Cistine) and 6.4x10^{-5} M^{-1} (L-Trp). EPR experiments with MNP spin trap demonstrated the formation of acetyl radical (aN 0.81 mT) and a tertiary IBAL radical (aN 1.59 mT). Amino acids adducts were separated by HPLC and capillary electrophoresis and identified by electrospray mass spectrometry. Adducts of IBAL radical with α-amino group of Cistine and lateral chain of L-Trp were observed when those amino acids are incubated with IBAL/HRP system under flow of O2. There is also a product with m/z 247 that indicates the presence of acetyl adduct. Although experiments using TEMP spin trap indicates the presence of 1O2, no adducts of this excited specie were observed so far. These results show the generation of acetyl and tertiary radical from excited compounds that could lead to protein modification through L-Trp oxidation.

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