Detection of novel radicals during HOBr-mediated protein oxidation

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Activated leukocytes generate the potent oxidants HOCI and HOBr via the formation of H_20_2 and the release of the enzymes myeloperoxidase and eosinophil peroxidase. HOCI and HOBr are potent bactericidal agents but excessive or misplaced production can cause tissue damage. While the reactions of HOCI have been studied extensively, relatively little is known about the reactions of HOBr, though reaction with protein amine groups to give bromarnines (RNHBr) is believed to be a major process. In previous studies we have shown that the related chloramines (RNHCI) decompose to give nitrogen-, and subsequently carbon-centered radicals. In this study we have investigated the formation and subsequent decomposition of bromamines to radicals using EPR spin trapping.

Bromamines formed on amino acids, peptides and proteins on reaction with HOBr decayed at much faster rates than the corresponding chloramines and gave rise to higher concentrations of radicals. Thus intense signals attributed to nitrogencentred radicals were observed from the a-amino group of lysine, whereas the corresponding chloramines yielded less intense, side-chain derived, nitrogen-centered radicals. With peptides nitrogen- centered radicals formed from the amide (peptide) bonds are detected. With proteins decay of chloramines yielded lysine-derived, nitrogen- centered radicals. In contrast, reaction with HOBr gave only weak nitrogen- centered radicals, but intense signals from carbon- centered species. The nature of these radicals has been investigated using DMPO and the newer nitrone traps DEPMPO, EMPO and BMPO. Some of these newer spin traps have major advantages over DMPO as they yield longer-lived adducts and more intense signals. These data suggest that the mechanism of oxidation of proteins by HOCI and HOBr are markedly different.