ABSTRACT

During inflammatory diseases the levels of hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) are markedly increased in cells. Heme proteins can use these components to produce nitrating agents. These reactions are of great interest because the presence of nitrated proteins is often observed under pathophysiological conditions. A few potential nitrating agents have been recognized *in vivo*, but the delineation of other biochemical pathways responsible for biological nitration and the identification of specific protein targets for nitration are essential for a full understanding of the mechanisms of NO_2-derived pathologies.

In this work, the ability of lactoperoxidase (LPO) to nitrate tyrosine residues, in the presence of hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) has been examined. The ability of peroxidases to promote these reactions is well known. As role model we used myoglobin (Mb) which is a known nitrating agent of both exogenous and endogenous tyrosine residues, although no mechanistic detail concerning the active species involved in these reactions is known.

In the Mb/NO_2^-/H_2O_2 system, both endogenous and exogenous nitration of tyrosine residues were observed in a reaction mechanism dependent on the reactant concentrations.

In the LPO/NO_2^-/H_2O_2 system, only exogenous nitration of tyrosine residues was observed in a reaction similar to that of myoglobin. The reaction mechanism is totally dependent upon reactant concentrations and pH.

Two competing paths are possible responsible for the observed reactions. In the first, lactoperoxidase or myoglobin reacts according to a peroxidase-like cycle forming two active intermediates, which can induce one-electron oxidation of the substrates. The LPO/$MbFe^{IV}$=O intermediate oxidizes nitrite to nitrogen dioxide, which, after reaction with the phenol or with a phenoxy radical, yields the nitrophenol. In the second mechanism, hydrogen peroxide reacts with iron-bound nitrite to produce an active nitrating species, which we assume to be a protein-bound peroxynitrite species, LPO/$MbFe^{III}$-N(O)OO.