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Myeloperoxidase

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Abbreviations

MPO	Myeloperoxidase
NOS	Nitric oxide synthase
PMN	Polymorphonuclear
RNS	Reactive nitrogen species
ROS	Reactive oxygen species

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Abstract

Myeloperoxidase (MPO) is a lysosomal hemoprotein located in the azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. It uses hydrogen peroxide generated by the neutrophil oxidative burst to produce hypochlorous acid and other reactive intermediates including reactive oxygen species (ROS), reactive nitrogen species (RNS) and other radicals. These oxidative species and radicals are important component of the neutrophil's antimicrobial armory. However, they also promote tissue damage in numerous inflammatory diseases, especially atherosclerosis, in which LDL oxidized by these oxidants generated by MPO.

Introduction

Myeloperoxidase (MPO) is a lysosomal hemoprotein located in the azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. MPO is a dimeric protein and each dimer contains a 60-kDa heavy unit and a 12-kDa light subunit. The primary translation product is a single 80-kDa protein that undergoes cotranslational glycosylation, proteolytic processing, and lysosomal targeting during the promyelocytic stage of myeloid development [1]. MPO is a member of the homologous mammalian peroxidase family (superfamily II) also including eosinophil peroxidase and thyroid peroxidase. Typically, phagocyte activation and MPO secretion are accompanied by an oxidative burst where superoxide ($O_2^{\bullet -}$) and its dismutation product, hydrogen peroxide (H_2O_2), are formed by the NADPH oxidase complex [2]. MPO amplifies the oxidizing potential of H_2O_2 by using it as co-substrate to generate a host of reactive oxidants and diffusible radical species through a classic peroxidase cycle [3].

Machanism

Figure 1 shows a working kinetic model for MPO in which MPO is a complex heme protein that possesses multiple intermediate states [4]. All states are influenced by the availability of reduced oxygen species such as $O_2^{\bullet -}$ and H_2O_2, and nitric oxide (NO, nitrogen monoxide) [5]. At ground state, MPO exists in the ferric (Fe(III)) form. Upon addition of H_2O_2, the heme group of MPO is oxidized two e^- equivalents forming a reactive ferryl π-cation radical intermediate termed Compound I. In the presence of halides such as Cl^-, Br^-, and I^-, and the psuedohalide thiocyanate (SCN^-), Compound I is readily reduced in a single two e^- step, regenerating MPO-Fe(III) and the corresponding hypohalous acid (HOX). At plasma levels of halides and thiocyanate (100 mM Cl^-,

100 μM Br^-; 50 μM SCN^-, 100 nM I^-), chloride is a preferred substrate and hypochlorous acid (HOCl), a potent chlorinating oxidant, is formed (Reaction 1) [6].

$$Cl^- + H_2O_2 + H^+ \xrightarrow{MPO} HOCl + H_2O \quad (1)$$

The ability of MPO to generate chlorinating oxidants Compound I can also oxidize numerous organic substrates while the heme undergoes two sequential one-electron reduction steps, generating compound II and MPO-Fe(III), respectively (Figure 1). In addition to halides and SCN^-, some of the naturally occurring substrates for MPO include nitrite (NO_2^-), tyrosine, ascorbate, urate, catecholamines, estrogens, and serotonin [7, 8]. The products they form, and the reactions they initiate may play a significant biological function. MPO-Fe(III) can also be reduced to an inactive ferrous form, MPO-Fe(II). MPO-Fe(III) and MPO-Fe(II) bind to $O_2^{\bullet-}$, and O_2, respectively, forming a ferrous dioxy intermediate, compound III (MPO-Fe(II)-O_2) (Figure 1). Spectral studies demonstrate that addition of H_2O_2 to Compound III ultimately forms compound II. Thus, compound III may indirectly promote one-electron peroxidation reactions.

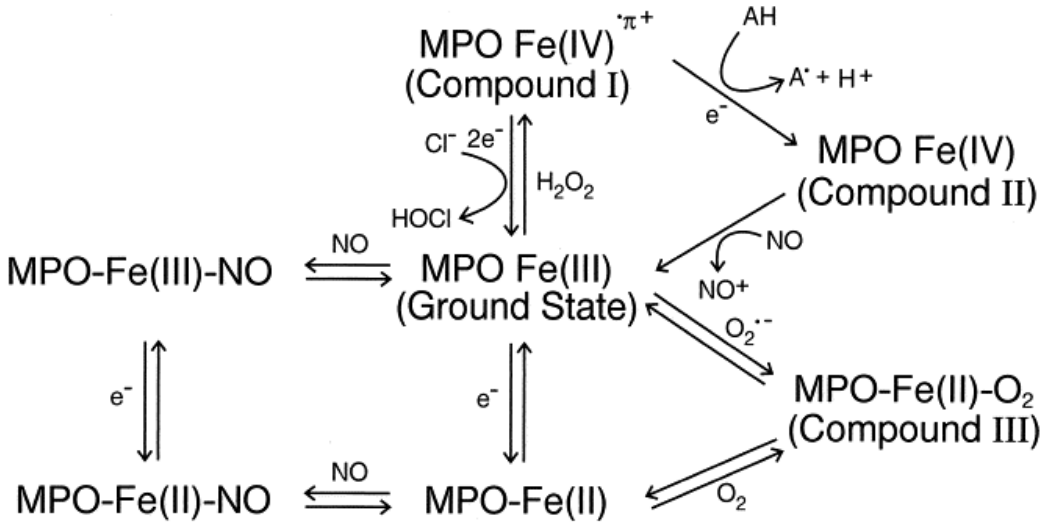

Figure 1. Model for myeloperoxidase reactions [4]

Of the oxidized intermediates of MPO, compound I is the only species that takes part in both the peroxidatic cycle and the chlorination activity. Compound I of MPO has some unusual features. An excess of hydrogen peroxide is necessary for its formation, it is very unstable and decays to its one-electron reduction product, compound II. Some study showed that H_2O_2 and other organic hydroperoxides can function as one-electron donors of compound I. [9].

Regulation of MPO

The regulation of MPO activity is typically thought to primarily rely upon the rate of O_2^- production, the availability of H_2O_2 and other cosubstrates, or the abundance of antioxidant species such as ascorbate or methionine [3]. However, recent studies identify a role for NO, a relatively long-lived free radical generated by nitric oxide synthase (NOS), in modulating MPO peroxidase activity [5]. NO plays important bioregulatory roles in many physiological processes, and is implicated in the regulation of many hemoproteins. MPO and the inducible isoform of NOS are colocalized in the primary granule of leukocytes. Rapid kinetics studies demonstrate that at low levels of NO, the initial rate of MPO-catalyzed peroxidation of substrates is enhanced. The mechanism is through acceleration of the rate-limiting step in MPO catalysis, reduction of compound II to MPO-Fe(III) (Figure 1) [5]. At higher levels of NO, reversible inhibition of MPO occurs through formation of a spectroscopically distinguishable nitrosyl complex, MPO-Fe(III)-NO [5]. NO also can serve as a substrate for MPO compound I, resulting in its reduction to Compound II [13]. Furthermore, in the presence of NO, the overall turnover rate of MPO through the peroxidase cycle is enhanced nearly 1000-fold [10]. The net result is that MPO can serve as a catalytic sink for NO, limiting its bioavailability. Finally, NO also reversibly binds to MPO-Fe(II) forming the corresponding MPO-Fe(II)-NO intermediate, which is in equilibrium

with MPO-Fe(II) and MPO-Fe(III)-NO (Figure 1) [5, 10]. The regulation of MPO *in vivo* is thus complex since the enzyme performs its functions in a wide variety of environments with differing pH and levels of NO, H_2O_2, $O_2^{\bullet-}$, O_2, inorganic and organic substrates, and reducing agents.

Chlorinating Oxidants

HOCl is a potent chlorinating oxidant generated only by MPO in vivo. The reactivity of HOCl against biological targets is well established and includes chlorination of amines and unsaturated lipids, oxidation of thiols and thiol esters, and oxidative bleaching of heme groups and iron sulfur centers [11]. Cysteine and methionine residues react rapidly with HOCl to give oxyacids and cystine (from Cys) and sulphoxides (from Met)[12]. The treatment of isolated proteins with HOCl is known to result in the alteration of amino acid side chains, protein fragmentation [13] and cross-linking/aggregation [14]. The main biological chlorination reactions of HOCl are with amine groups to give chloramines. The free amino groups of lysine residues are known to form chloramine (RNHCl) intermediates readily on reaction with HOCl [15]. HOCl also reacts with tyrosine residues to give ring chlorinated products, including 3-chlorotyrosine and 3,5-dichlorotyrosine[Figure 3]. 3-Chlorotyrosine is a nonphysiologic oxidation product of tyrosine which is both acid stable and not readily formed by artificial mechanisms—characteristics which make it well suited to serve as a specific molecular marker for MPO-catalyzed oxidation [16]. Besides the chlorination of amino acids, HOCl reacts with unsaturated lipids and cholesterol to give chlorohydrins (Figure 2) and with nucleic acids to give ring chlorination of cytosine residues.

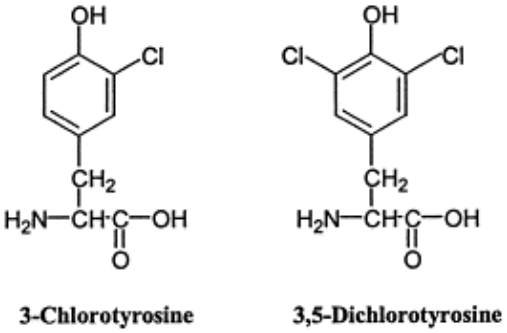

Figure 2. Chlorinated tyrosines formed form the reaction of HOCl with tyrosine residues in proteins [14].

Apart from the above reaction, interaction of HOCl with H_2O_2 and $O_2^{\bullet-}$ is reported to form singlet oxygen and hydroxyl radical, respectively [17].

$$O_2^{\bullet-} + HOCl \rightarrow {}^{\bullet}OH + Cl^- + O_2 \quad (2)$$

The chlorinating intermediate generated by MPO-catalyzed oxidation of Cl^- include not only HOCl or its conjugate base, hypochlorite (ClO^-; pK_a 7.4 [18]), but also chlorine gas (Cl_2). Cl_2 was reported to serves as the proximate chlorinating intermediate in chlorotyrosine formation from the free amino acid [19]. HOCl is in equilibrium with Cl_2 via a reaction that requires Cl^- and H^+. The formation of Cl_2 is thus favored by acidic pH and the presence of Cl^-, suggesting that under acidic conditions such as in the phagolysosome, Cl_2 could potentially execute oxidation/halogenation reactions normally ascribed to HOCl/ClO^- [19].

$$HOCl + Cl^- + H^+ \rightarrow Cl_2 + H_2O \quad (3)$$

MPO and RNS

A variety of reactive nitrogen species derived from ${}^{\bullet}NO$ are powerful oxidants, and they may contribute to oxidative damage. At least 3 distinct pathways for generating reactive nitrogen species have been suggested. The first involves formation of $ONOO^-$ through the interaction of ${}^{\bullet}NO$ and $O_2^{\bullet-}$.The formation of $ONOO^-$ is shown in the following equation:

$$^{\bullet}NO + O_2^{\bullet-} \rightarrow ONOO^- \ (k = 3.7 \times 10^7\ M^{-1}s^{-1}\ [20]) \quad (4)$$

Two alternative pathways for generating reactive nitrogen species involve MPO The first pathway involves MPO-dependent oxidation of nitrite (NO_2^-), a stable end-product of NO metabolism, forming a reactive nitrogen species, presumably nitrogen dioxide (NO_2) [21].

$$2NO_2^- + H_2O_2 + 2H^+ \xrightarrow{MPO} 2NO_2^{\bullet} + 2H_2O \ (k = 4.2\times10^7\ M^{-1}S^{-1}) \quad (5)$$

The second pathway involves secondary oxidation of NO_2^- by MPO-generated HOCl [21]. HOCl can react with nitrite to form nitryl chloride (NO_2Cl, reaction 6), which can chlorinate and nitrate tyrosine residues as well as oxidize LDL lipids and antioxidants.

$$HOCl + NO_2^- + H^+ \rightarrow NO_2Cl + H_2O \quad (6)$$

Tyrosyl radical generation by MPO

MPO-dependent formation of tyrosyl radical in vitro has been demonstrated through electron spin resonance spectroscopy and structural identification of dityrosine and other tyrosyl radical addition products. It was found that tyrosyl radical generated by MPO through the following pathway [22].

$$MPO + H_2O_2 \rightarrow \text{Compound I} + H_2O \ (k=1.4\text{-}1.8\times10^7\ M^{-1}s^{-1}\ [23]) \quad (7)$$

$$\text{Compound I} + \text{L-tyrosine} \rightarrow \text{Compound II} + \text{tyrosyl radical} \quad (8)$$

Tyrosyl radical generated might serve as a diffusible catalyst for the formation of o,o'-dityrosine cross-links proteins. In addition, tyrosyl radical can also promote lipid peroxidation (Figure 3) [24].

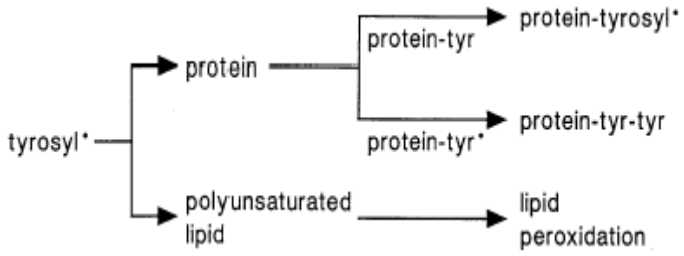

Figure 3. Proteins and lipid oxidation by tyrosyl radical

Summary

Myeloperoxidase (MPO) is a major neutrophil protein and is also present in monocytes. It is a heme enzyme that uses hydrogen peroxide generated by the neutrophil oxidative burst to produce hypochlorous acid and other reactive intermediates. On one hand, the reactive intermediates including reactive oxygen species (ROS), reactive nitrogen species (RNS) play important role in MPO's antibacterial function. On the other hand, the oxidative products also promote tissue damage in numerous inflammatory diseases, especially atherosclerosis, in which LDL oxidized by these oxidants generated by MPO.

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