

Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention

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(Received 26 August, 2010; Accepted 10 September, 2010; Published online 28 December)

There is considerable interest in the role that mammalian heme peroxidase enzymes, primarily myeloperoxidase, eosinophil peroxidase and lactoperoxidase, may play in a wide range of human pathologies. This has been sparked by rapid developments in our understanding of the basic biochemistry of these enzymes, a greater understanding of the basic chemistry and biochemistry of the oxidants formed by these species, the development of biomarkers that can be used damage induced by these oxidants *in vivo*, and the recent identification of a number of compounds that show promise as inhibitors of these enzymes. Such compounds offer the possibility of modulating damage in a number of human pathologies. This reviews recent developments in our understanding of the biochemistry of myeloperoxidase, the oxidants that this enzyme generates, and the use of inhibitors to inhibit such damage.

Key Words: myeloperoxidase, hypochlorous acid, chloramines, protein oxidation, neutrophil

Overview of the Action of Myeloperoxidase and Other Heme Peroxidases

Activation of phagocytic leukocytes is a key process in the immune response to invading pathogens. Activation of these cells results in the assembly of a NADPH oxidase (NOX)-2 enzyme complex at the plasma membrane and a subsequent 'respiratory burst', in which O₂ is reduced, at the expense of NADPH, to superoxide radicals (O₂⁻).⁽¹⁾ This radical undergoes rapid spontaneous or catalysed (by superoxide dismutase) dismutation to give molecular oxygen and hydrogen peroxide (H₂O₂).⁽²⁾ High concentrations of H₂O₂ can be bactericidal or cytotoxic, but lower levels have limited effects. Although this species can be reduced, by trace iron and copper ions, to hydroxyl radicals (HO[•]), the availability of these metal ions is usually very limited, and hence HO[•] is unlikely to be a major oxidant produced by activated neutrophils (reviewed in Ref. 3).

The bactericidal properties of activated leukocytes have been attributed, at least in part, to the actions of myeloperoxidase (MPO), a heme enzyme released by activated neutrophils, from intracellular granules. This green enzyme is the most abundant protein in neutrophils, accounting for up to 5% of their dry mass.⁽⁴⁾ It is also present in monocytes, though at lower levels. The ability of monocytes to produce this protein decreases during maturation into macrophages, but evidence has been presented that macrophage-like cells, such as those detected in atherosclerotic lesions, have associated MPO.^(5,6) The biological significance of MPO is evident from studies on people with total or incomplete MPO deficiency. Neutrophils from such people usually phagocytose foreign material normally, and have a prolonged respiratory burst that produces H₂O₂ and O₂⁻ but MPO deficiency results in a greater risk of chronic infections.^(7,8)

MPO protein has little bactericidal effect per se, but enzymatic reaction with H₂O₂ and halide (Cl⁻, Br⁻, I⁻) or pseudohalide (SCN⁻) ions generates hypohalous acids: hypochlorous acid (HOCl), hypobromous acid (HOBr), hypoiodous acid (HOI), and hypothiocyanous acid (HOSCN).⁽⁹⁻¹¹⁾ These oxidants are widely believed to be responsible for much of the anti-bactericidal activity of neutrophils, although other oxidants (including nitric oxide (NO[•]), peroxyne (ONOOH) and H₂O₂) and enzymatic systems (e.g., peptides, proteases, lysozyme) clearly also play an important role.

Although the generation of oxidants by MPO is beneficial in terms of the immune response to invading pathogens, there is considerable evidence that inappropriate stimulation of oxidant formation by this enzyme (wrong place, wrong time, excessive levels) can result in host tissue damage. Thus marked damage to cells and other biological materials (extracellular matrix, biological fluids) has been detected at sites of inflammation (reviewed in^(10,12)). This damage has been linked with several human pathologies, and in at least some cases experimental and/or epidemiological evidence is available to suggest that oxidant generation by MPO (and/or other related heme peroxidases, such as eosinophil peroxidase (MPO)) is, at least partially, causal. This evidence for a role for MPO and its oxidants in human disease has been recently reviewed,^(10,12) and will not be covered further here.

Physical Properties and Structure of Myeloperoxidase and Other Heme Peroxidases

Mature MPO is a cationic, dimeric, protein with a mass of 146 kDa, consisting of two 73 kDa monomers linked via a cystine bridge at Cys153. Each monomer, which is identical and functionally independent, consists of two components: a 58.5 kDa, 467 amino acid, heavy chain and 14.5 kDa, 106 amino acid, light chain.⁽¹³⁾ The former is glycosylated and contains the modified iron protoporphyrin IX active site. The heme group sits at the bottom of a deep crevice,⁽¹⁴⁾ which hinders access of most materials: only H₂O₂ and small anions have ready access to the iron atom.⁽¹⁵⁾ Other materials that are oxidized by the enzyme (see below) bind in a hydrophobic pocket at the entrance to the distal heme cavity. The structures of human peroxidases have been recently reviewed (cf. Protein Data Base accession number 1exp for MPO).⁽¹⁶⁾

Although MPO is the most widely studied heme-peroxidase, related species also play a critical role in metabolism, and some major human pathologies. EPO is the major granule protein of eosinophils, which are specialized human phagocytic cells that eliminate parasites and related organisms.⁽¹⁷⁾ Unlike neutrophils, that phagocytose target organisms and subsequently release MPO into phagolysosomal compartments, eosinophils exocytose their granule contents on to the surface to which they are attached,

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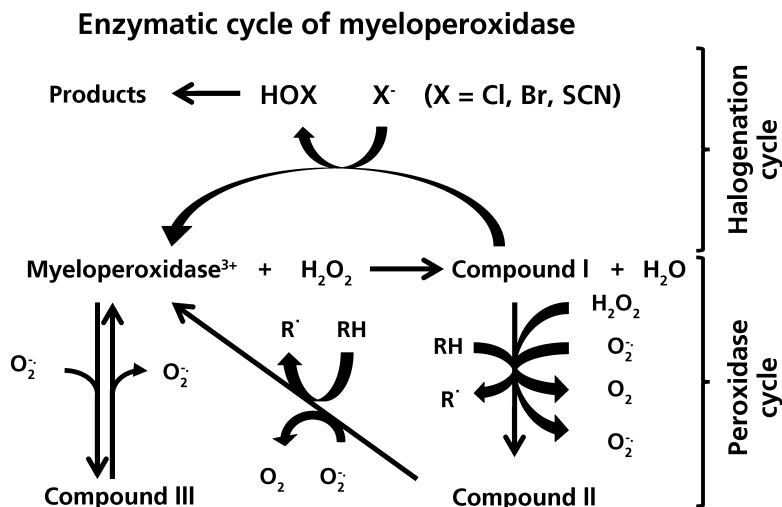


Fig. 1. The enzymatic cycles of myeloperoxidase. Initial oxidation of the resting iron (III) form of the enzyme by hydrogen peroxide gives rise to Compound I, which is formally an iron (V) species. This intermediate can then undergo either two electron reduction with halide or pseudohalide ions to form hypohalous acids (the halogenation cycle) or undergo two successive one-electron reductions, via Compound II, with consequent radical formation (the peroxidase cycle). The iron (III) form of the enzyme can also undergo one electron reduction with superoxide radicals to give Compound III. This latter reaction accounts for the SOD mimetic activity of myeloperoxidase.

as a result of the much larger size of most parasites. EPO shares a 70% amino acid homology with MPO and is also a cationic protein with a modified iron protoporphyrin IX heme active site.⁽¹⁸⁾ It is synthesized as a ~80 kDa single-chain precursor and subsequently processed in to a mature protein (69.8 kDa) consisting of a heavy (57.9 kDa) chain and light chains (11.9 kDa); these are analogous of MPO.⁽¹⁶⁾ Other related heme proteins include salivary peroxidase and lactoperoxidase (LPO); these are present in multiple human exocrine secretions including tears, milk, saliva and vaginal fluid. In each case their role appears to be as a defence against invading microorganisms.⁽¹⁹⁾

The genes for human MPO, EPO and LPO are adjacent to each other on chromosome 17 and have similar intron-exon structures, consistent with these arising via amplification from a common ancestor.⁽²⁰⁾ The cationic (pI ca. 10) nature of MPO and EPO (but not LPO) results in avid binding to negatively-charged structures including bacterial^(21–23) and endothelial cell surfaces,⁽²⁴⁾ cytokeratin 1,⁽²⁵⁾ extracellular matrix components (especially polyanionic glycosaminoglycan chains^(26–29)), albumin,⁽³⁰⁾ ceruloplasmin,⁽³¹⁾ α_1 -antitrypsin,⁽³²⁾ and apolipoproteins A-I and B-100.^(33,34)

Reactive Intermediates Generated by Heme Peroxidases

H_2O_2 reacts with the native, Fe^{3+} form of MPO to generate the two-electron oxidised species Compound I (an oxy-ferryl species, $\text{Fe}^{4+} = \text{O}$, with a porphyrin π -cation radical) and water. Compound I may be converted back to the ferric enzyme via two-electron reduction by (pseudo) halides (“the halogenation cycle”, Fig. 1), or via two sequential one-electron reduction reactions involving a second intermediate (Compound II, which retains the oxy-ferryl $\text{Fe}^{4+} = \text{O}$ center; “the peroxidase cycle”, Fig. 1). An additional intermediate, Compound III, can be generated via reaction of the Fe^{3+} form with $\text{O}_2^{\cdot-}$, or via one-electron reduction to the ferrous form and subsequent reaction with O_2 .

Due to the high reduction potentials of the Compound I/native enzyme (1.16 V), Compound I/Compound II (1.35 V) and Compound II/native enzyme (0.97) couples, MPO can oxidize multiple substrates via its halogenation and peroxidase cycles. The unusually high potential for MPO arises from heme distortion and a reduction in electron density induced by a covalent vinyl

sulfonium heme linkage.⁽³⁵⁾ These redox potentials, and hence the rate of substrate oxidation, are pH-dependent.⁽³⁶⁾ Compound III is unreactive towards most substrates, and is usually a catalytic ‘dead-end’; this species will however slowly oxidize ascorbate⁽³⁷⁾ and paracetamol (acetaminophen)⁽³⁸⁾ to radicals, and is implicated in $\text{O}_2^{\cdot-}$ -dependent catalytic activities of MPO.⁽³⁹⁾

The halogenation cycle. The ability of MPO to oxidize Cl^- is unique amongst peroxidases; Br^- and SCN^- are also oxidized at high rates. Each of these substrates donates two electrons to Compound I to regenerate the ferric enzyme, with concomitant formation of the corresponding (pseudo) hypohalous acids (HOX, X = Cl, Br, SCN). The second order rate constants reflect the ease of oxidation of each substrate ($\text{SCN}^- > \text{Br}^- > \text{Cl}^-$). However, the rate constants do not directly mirror the redox potentials as differences in topology of the active site, and binding sites exert a strong effect on substrate specificity.⁽¹⁶⁾ Thus the rates of Br^- and SCN^- oxidation by EPO are ~10-fold faster than for MPO,⁽⁴⁰⁾ even though the MPO reduction potential is higher (1.10 vs 1.16 V). At neutral pH and physiological concentrations of halide/pseudo-halide ions, MPO primarily generates HOCl and HOSCN⁽⁴¹⁾ with the specificity constants for Cl^- , Br^- and SCN^- being 1:60:730 respectively.⁽⁴¹⁾ The yield of each hypohalous acid is donor dependent, as smoking and diet can markedly elevate SCN^- levels and hence the extent of HOSCN formation from SCN^- . Yields of 5–10% and 40%, based on H_2O_2 consumed, have been reported for HOBr and HOSCN formation by MPO, using mean physiological ion concentrations; HOCl accounts for most of the remainder.^(41,42) In contrast, EPO primarily generates HOBr and HOSCN, and no HOCl;⁽⁴³⁾ LPO primarily generates HOSCN.⁽⁴⁴⁾ The yields of these materials is also modulated by secondary reactions; HOCl and HOBr can oxidize SCN^- , and HOCl can oxidize Br^- ,^(45,46) possibly via transhalogen species (e.g., BrCl), to the corresponding (pseudo) hypohalous acids.

The pK_a values for of HOCl, HOBr and HOSCN are 7.59, 8.7 and 4.85–5.3, respectively.^(47–49) Thus at physiological pHs, approximately equal concentration of HOCl and ^-OCl will be present, HOBr predominates over ^-OBr , and $^-\text{OSCN}$ predominates over HOSCN. The physiological mixtures of these species are referred to as HOCl, HOBr and HOSCN respectively, from hereon. The rates of formation of these species by Compound I are significantly greater at acidic pH,⁽⁴⁰⁾ with this believed to be

due to protonation of the distal histidine.^(16,50)

Whilst previous studies have disputed the formation of “free” HOCl by MPO/H₂O₂/Cl⁻,^(36,51) halogenation of large biological targets, which cannot enter the enzyme active site, occurs both rapidly and in near stoichiometric amounts (≥75% for heparan sulfate based on H₂O₂ supplied^(27,52)), consistent with the formation of diffusible oxidants.

The peroxidase cycle. In the peroxidase cycle, radicals are generated from substrates (both organic and inorganic) via one-electron oxidation by Compounds I and II. O₂⁻ and NO[•] are also oxidized. The catalytic activity of MPO is therefore partitioned between halogenation and peroxidation via competition between peroxidase substrates and (pseudo) halides for Compound I; this partitioning is relevant to inhibition of enzymatic activity (see below).

Physiologically- and pathologically-relevant peroxidase substrates for MPO, EPO and LPO include endogenous species (e.g., the amino acids tyrosine and tryptophan, thiols, ascorbate, steroid hormones and urate) as well as xenobiotics and drugs. MPO oxidizes a wider range of substrates than EPO and LPO due to its higher reduction potential for the Compound I/Compound II couple (1.35 vs 1.14 V for LPO^(53,54)). Substrates that react readily with Compound I, but not Compound II, i.e. species with reduction potentials between 1.35 and 0.97 V, are often termed ‘poor’ peroxidase substrates. In the absence of additional species that can recycle Compound II, metabolism of such substrates results in Compound II accumulation and arrest of the catalytic cycle (see Inhibition section below). Kinetic factors can also affect recycling of Compound II and even with peroxidase substrates such as Tyr, the rate constant for reaction with MPO Compound II is ~10 times slower than for Compound I.⁽⁵⁵⁾

The metabolism of Tyr by MPO and LPO is relatively insensitive to pH.^(55,56) With MPO, rate constants for reaction of Tyr with Compound I are maximal at basic pH values, but vary less than two-fold across physiologically-relevant pHs.⁽⁵⁵⁾ The predominance of the chlorinating activity of MPO at acidic pH, over its peroxidative metabolism, can be accounted for primarily by the greater pH-dependence of Cl⁻ oxidation.

Reactions of Hypohalous Acids

Hypochlorous acid. HOCl reacts rapidly with sulfur and nitrogen atoms (for rate constants see ⁽⁵⁷⁻⁵⁹⁾), including those present in thiols, thioethers, amines, and amides. Thus, Cys residues in proteins and glutathione (GSH) are key targets.^(57,60-63) Cys oxidation appears to yield a sulfenyl chloride (RS-Cl), which undergoes rapid reaction with excess thiol, to give the disulfide,⁽⁶⁰⁾ or with water to yield sulfenic (RSOH), sulfinic (RSO₂H), and sulfonic (cysteic acid, RSO₃H) acids [reviewed in ⁽⁶⁴⁾]. Disulfides (e.g., cystine) can be further oxidized to sulfonic acids via S-chlorinated and S-oxygenated intermediates [reviewed in ⁽⁶⁴⁾]. HOCl can also induce the formation of sulfenamide (RSNR[•]), sulfinamide [RS(O)NR[•]], and sulfonamide [RS(O)₂NR[•]] cross-links in peptides (e.g., GSH) and proteins,⁽⁶⁵⁻⁶⁷⁾ via nucleophilic attack of Lys or Arg side chains on RS-Cl, sulfenic or sulfinic acid intermediates. Glutathione sulfonamide (GSSG), formed from oxidation of GSH,⁽⁶⁸⁾ has been postulated as a potential marker for MPO-mediated damage in biological systems, as it is generated primarily by HOCl, and to a much lesser extent by other species such as HOBr and ONOO⁻/ONO[•].^(66,69)

The high susceptibility of Cys residues to oxidation has important biological implications as this can disrupt the cellular redox balance of cells by conversion of GSH to GSSG, and inactivate multiple cellular enzymes that contain active site Cys residues. Thus, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are rapidly inactivated by low levels of HOCl, with this correlating with thiol depletion.⁽⁷⁰⁾ Conversely, HOCl can activate the (inactive) pro- forms of matrix metalloproteinases

(MMP) (e.g., MMP-7) via conversion of a key Cys residue in the “cysteine switch” domain of pro-MMP-7 to a sulfinic acid.⁽⁷¹⁾ Thioethers such as Met side chains are also favored targets, with this resulting in sulfoxide formation⁽⁷²⁾ (and sulfones with very high HOCl excesses⁽⁷³⁾). These compounds are not useful as markers for HOCl damage, as they are also produced by other oxidants and can be repaired, intracellularly, by methionine sulfoxide reductases.⁽⁷⁴⁾ Met oxidation can lead to alterations in protein function by, for example, inactivating enzymes (e.g., lysozyme⁽⁷²⁾), protease inhibitors (such as α1-antitrypsin),^(72,75) proteinases,⁽⁷⁶⁾ growth factors (e.g., GroEl⁽⁷⁷⁾) and modulating signaling pathways (e.g., oxidation of IκB with resulting inhibition of NF-κB activity⁽⁷⁸⁾).

Amines, and to a lesser extent amides, are readily chlorinated by HOCl to give chloramines (RR'NCl) and chloramides (RC(O)N(R')Cl).^(57,79,80) Dichlorination also occurs at high HOCl excesses. Chloramines are formed on multiple biological targets including the nitrogen atoms on: the α-amino and side-chains of His, Lys and Arg of free amino acids and proteins; taurine; free nucleobases, nucleosides, nucleotides and DNA/RNA; amino sugars and glycosaminoglycans (e.g., hyaluronan, heparan sulfates, heparin); and on the amine-containing head groups of phospholipids (e.g., phosphatidylethanolamine, phosphatidylserine).^(58,59,64,81-85)

In addition to reaction with nucleophiles, HOCl undergoes addition to aromatic rings and double bonds, including some amino acids (Tyr and Trp), nucleobases, and fatty acid side chains [reviewed in ^(64,82)]. Addition to the phenolic ring of Tyr yields 3-chlorotyrosine (3-chloroTyr), which is widely used as a specific marker of HOCl generation.^(86,87) Secondary halogenation to give 3,5-dichlorotyrosine can also occur. Oxidation of Trp residues is relatively rapid with this resulting in the formation of multiple products [reviewed in Ref. 10, 64], including cyclized species when the Trp residue is present in specific peptide sequences.⁽⁸⁸⁾

Reaction of HOCl with nucleobases generates stable halogenated products, in addition to unstable chloramines, with these including 5-chlorocytosine, 5-chloro (2'-deoxy) cytidine, 5-chlorouracil, 8-chloroadenine, 8-chloro (2'-deoxy) adenosine, and 8-chloro (2'-deoxy) guanosine.⁽⁸⁹⁻⁹⁹⁾ Some of these products have been utilized as markers of HOCl generation.⁽⁹²⁻¹⁰⁰⁾ Hydroxylated and ring-opened species are also formed.^(94,96)

HOCl reacts with the double bonds of unsaturated lipids and cholesterol to give chlorohydrins (RCH(Cl)-CH(OH)R'); these can subsequently yield epoxides.^(36,101-107) Reaction with plasmalogen lipids, which contain a vinyl ether rather than an ester linkage, is particularly rapid⁽¹⁰⁸⁾ with this resulting in facile cleavage of the ether linkage to give an α-halogenated aldehyde and a lysophospholipid.^(109,110) Elevated levels of the former have been detected in human atherosclerotic lesions.⁽¹¹¹⁾

Hypobromous acid. HOBr is generally reactive and less discriminating than HOCl (for kinetic data see ^(58,112,113)), though many of the reactions are analogous to those of HOCl. It also targets thiol, thioether, disulfide and amine functions, with the last of these generating bromamines and bromamides. A major difference to HOCl is in the kinetics of reaction with aromatic rings and double bonds, with these being more rapid and important with HOBr.⁽⁵⁸⁾ Thus bromination of Tyr residues (to give 3-bromoTyr) is ~10,000-fold faster than chlorination by HOCl.⁽¹¹²⁾ This has important ramifications for the use of 3-bromoTyr and 3-chloroTyr as markers of damage.⁽¹¹²⁾ Similarly bromohydrin formation is more rapid than chlorohydrin generation by HOCl, and bromohydrins are more readily detected in biological systems.⁽¹¹⁴⁾

Hypothiocyanous acid. HOSCN has been postulated to be a significant product of MPO-mediated reactions, particularly in smokers.⁽¹¹⁵⁾ Elevated levels of SCN⁻ are present in such people as a result of hydrogen cyanide formation (from cigarette combustion) and subsequent metabolism. Non-smokers typically have plasma SCN⁻ concentrations <50 μM, whereas heavy smokers

have levels $\leq 250 \mu\text{M}$.^(116,117) This is believed to result in increased formation of HOSCN over other MPO-derived oxidants (see above and ⁽¹¹⁸⁾). Furthermore, reaction of HOCl and HOBr with SCN^- can enhance HOSCN formation.

Unlike HOCl and HOBr, which are relatively promiscuous oxidants, HOSCN is less reactive and highly selective. Thiols are the major site of reaction,^(49,119) though Trp residues are also oxidised when Cys residues are absent or depleted.⁽¹²⁰⁾ This observation is consistent with rapid reversible Cys oxidation, and low levels of irreversible incorporation of radiolabelled SCN^- into proteins.^(119–121) Thiol oxidation occurs via short-lived RS-SCN species which rapidly react with other thiols to give disulfides (and regenerate SCN^-), or water to give a sulfenic acid (RS-OH). The cellular effects of this oxidant are therefore, not surprisingly, linked to damage to enzymes containing critical Cys residues, including GAPDH⁽⁶³⁾ and protein tyrosine phosphatases;⁽¹²²⁾ inhibition of the latter enzymes results in increased levels of cellular protein tyrosine phosphorylation (i.e. hyperphosphorylation) and altered mitogen-activated protein kinase (MAPK) signalling. These alterations are believed to underlie the enhanced apoptosis observed with this oxidant in some cells.^(63,122)

There is little data available as to other direct reactions of HOSCN with biological targets, though it has been reported that a $\text{MPO}/\text{H}_2\text{O}_2/\text{SCN}^-$ system induces low-density lipoprotein (LDL) modification, with resulting formation of conjugated dienes and lipid hydroperoxides.⁽¹²³⁾ The detection of these products implies the occurrence of radical reactions, so this may arise from the peroxidation cycle of MPO rather than via HOSCN, though there is also evidence for the formation of radicals from MPO-catalysed oxidation of SCN^- .⁽¹¹⁵⁾

Radicals. A number of different types of radical can be generated by the peroxidase cycle of MPO i.e. as a result of one-electron oxidation by Compounds I and II. As the redox potential of Compound I is greater than that of Compound II, the former can oxidise a wider range of materials. Some substrates can be oxidised by Compound I, and not (or poorly) by Compound II resulting in “trapping”, at least *in vitro*, of MPO at this point in its enzymatic cycle. Whether such “trapping” occurs *in vivo* is unclear at present, as multiple reductants (e.g., ascorbate, O_2^-) that can convert Compound II back to the Fe^{3+} (native) form. Regardless of this, a greater availability of peroxidase substrates relative to halide ions is likely to diminish the yield of hypohalous acids (HOCl, HOBr and HOSCN) and result in higher radical yields. Whether this changes the overall extent of biological damage remains an open question, with this likely to depend, to a major extent, on the chemistry of the radicals formed (i.e. whether they are highly reactive and induce further damage, or are unreactive).

In some cases, termination reactions in the form of radical-radical dimerisation, appears to be a major fate. Thus dimers and higher polymers have been detected from phenols (e.g., dityrosine from Tyr oxidation) with the occurrence of these reactions minimizing further damage. Some radicals can also reduce native MPO to Fe^{2+} MPO, which generates Compound III upon reaction with O_2 . This occurs, for example, during MPO-mediated metabolism of hydroquinone,^(124,125) amsacrine,⁽¹²⁶⁾ hydrazines⁽¹²⁷⁾ and hydrazides.⁽¹²⁸⁾ Other fates of MPO-generated radicals include reaction with the parent proteins to generate protein-derived radicals⁽¹²⁹⁾ and covalent addition to heme.^(130,131) Radicals may also diffuse away from the MPO and damage other biomolecules including lipids^(132,133) and proteins.⁽¹³⁴⁾ Radicals formed on oxidation of (amino) phenols can undergo further one-electron oxidation or disproportionation to generate electrophilic quinones/quinimines that form covalent adducts with thiols (e.g., GSH) and other biomolecules.^(135,136) A number of drugs and xenobiotics induce adverse biological effects, including agranulocytosis, hepatotoxicity and cancer, which have been associated with their metabolism by heme peroxidases.^(136,137)

Reactions of Secondary Oxidation Products

The damaging actions of MPO persist for considerable periods after the cessation of initial oxidant (e.g., HOCl) production.⁽⁷⁹⁾ Much of the secondary damage is believed to arise from the reaction of long-lived chloramines/chloramides and/or bromamines/bromamides, formed via the reaction of HOCl/HOBr with amines and amides (see above). The longer lifetimes of these species allow diffusion away from the site of formation (e.g., through cellular membranes) and the initiation of oxidative damage at remote locations; thus extracellularly generated species may exert intracellular effects, with the extent of cell penetration being dependent on the structure of the halogenated species.^(138–141) Reactive aldehydes and radicals may also play a significant role in inducing secondary damage (see above and below).

Chloramines and bromamines. Chloramines (RNHCl) and bromamines (RNHBr), and the corresponding amide species [$\text{RC}(\text{O})\text{NCIR}'$; $\text{RC}(\text{O})\text{NBrR}'$] retain the oxidizing equivalents of the parent HOCl/HOBr and can induce further reactions.^(81,142,143) Some of these processes regenerate the parent amine (which may result in an underestimate of the extent of damage) as a result of halogen transfer (e.g., Ref. 144, 145) or radical reactions (e.g., Ref. 146, 147), whereas others result in conversion of the amine group (e.g., via hydrolysis, probably via an imine) to an aldehyde and ammonia.^(64,148–150) Aldehyde formation from bromamines occurs more readily than from chloramines.^(72,151,152) The resulting carbonyls can react with protein or lipid amine groups to generate Schiff base imines, which can ultimately yield advanced glycation end products (AGEs); the latter have been linked to vascular disease.⁽¹⁵³⁾

Halogenated amines and amides can decompose to give nitrogen-centred radicals and subsequently carbon-centred radicals by rearrangement reactions; both may initiate further damage. Radical formation is promoted by low-valent redox-active metal ions (Fe^{2+} , Cu^+) and O_2^- .^(84,146,147,154,155) Halamines oxidize thiols and thioethers (e.g., Cys and Met, respectively) though at slower rates than HOCl and HOBr.^(58,140,156) The lower reactivity of these species results in more selective damage, and a more limited range of products. Low pK_a Cys residues are particularly susceptible to oxidation, with this resulting in selective inactivation of some enzymes.⁽¹⁴¹⁾ Thiols are primarily converted to disulfides and sulfenic/sulfonic acids (and not sulfonamides as observed with GSH⁽⁶⁶⁾). These processes can result in the induction of apoptosis and necrosis.^(141,142,157)

Activation of phagocytes has been reported to result in ~15% conversion of the HOCl formed to chloramines,⁽⁷⁹⁾ whilst reaction of HOCl or an MPO system with *Escherichia coli* results in up to 50% of the HOCl being recovered as bacteria-derived chloramines;⁽¹⁵⁸⁾ some of these materials have lifetimes of many hours in buffer or complete media. Experiments with PMA-activated neutrophils indicate that these chloramines are formed on both low- and high-molecular mass materials (as determined by mass fractionation).⁽¹⁵⁸⁾ The former appears to be primarily taurine chloramine (which is consistent with the high concentration of this material in neutrophils), but this result may be distorted by the discovery of rapid chlorine transfer between amine groups,^(139,144,145) and the greater stability of this chloramine over other species. Many of the chloramines have been reported to be hydrophilic,⁽¹⁵⁸⁾ but this conclusion may also be perturbed by the more rapid cellular penetration of hydrophobic species, and hence greater rate of loss of these materials via subsequent intracellular reactions, compared to those that remained external to the cells.

Hydroperoxides. Recent studies have shown that O_2^- can react with some of the radicals generated by the peroxidase activity of MPO. Thus phenols can be oxidised to organic hydroperoxides via phenoxyl radicals that subsequently undergo radical-radical termination reactions with O_2^- .^(159,160) Such reactions occur with both the free amino acid tyrosine (and various derivatives)

and Tyr residues present on peptides and proteins.^(159–163) Some of the factors that control the hydroperoxide yields relative to other products (e.g., dimeric species such as di-tyrosine) have been elucidated.⁽¹⁶⁰⁾ Similar $O_2^{\cdot-}$ -dependent reactions appear to occur with other (long-lived) radicals generated by MPO, including those formed on urate, indoles and other related species (e.g., melatonin and serotonin^(164,165)). The hydroperoxides formed by these reactions may undergo further reactions resulting in the oxidation of thiols (by two-electron oxidation reactions) and/or radicals (as a result of one-electron reduction by trace transition metal ions). Such reactions have been characterised with a range of other amino acid-, peptide- and protein-derived hydroperoxides.^(166–169) These reactions may contribute to the secondary damage induced at sites of inflammation and may rationalize the detection of hydroxylated products from MPO-catalysed reactions.

Reactive aldehydes. Chloramines and bromamines can decompose to yield aldehydes and ammonia with this reaction being particularly facile with species formed on the (N-terminal) amine groups of free amino acids and peptides.^(150,170) Those formed on side-chain amines (e.g., the ϵ -amino group of Lys side chains) decompose via this route to only a minor extent,⁽¹⁷¹⁾ though this site, and others including phospholipid head groups, can be modified via further reactions of carbonyls formed at different sites, via Schiff-base formation.^(149,172) Some of these materials, such as p-hydroxyphenylacetaldehyde derived from the oxidation of free Tyr residues, have been detected at elevated levels in diseased tissues, such as atherosclerotic lesions.⁽¹⁷²⁾ Oxidation of other free amino acids, such as Ser and Thr, can generate highly reactive aldehydes, such as glycoaldehyde and 2-hydroxypropanol (and the highly reactive α , β -unsaturated material acrolein by dehydration of 2-hydroxypropanol), via chloramines.⁽¹⁷¹⁾ These materials react rapidly with amine (e.g., Lys) and guanidine groups (e.g., Arg residues) to products similar to the AGEs detected in people with diabetes. Many of these materials are potent protein cross-linking agents due to their bifunctional nature. These compounds also form adducts with phospholipids and DNA bases.⁽¹⁷³⁾ Although reactive aldehyde formation occurs in both simple, and cellular, systems the quantitative significance of such reactions with free amino acids (present in plasma at \leq low millimolar levels⁽¹⁷⁴⁾) may be limited due to the much higher concentrations of proteins *in vivo* which have similar reactive sites.^(118,168)

Cyanate. Cyanate (OCN^-) is a downstream product of SCN^- oxidation by MPO, with this arising predominantly from HOSCN.⁽¹⁷⁵⁾ Cyanate reacts with amines under physiological conditions, with this resulting in carbamylation of N-terminal amine groups and the Lys side chain; the latter results in homocitrulline formation.⁽¹¹⁷⁾ Plasma protein-bound homocitrulline concentrations have been reported to be an independent predictor of cardiovascular disease (CVD), future myocardial infarction, stroke, and death, suggesting a potential role for HOSCN and SCN^- -derived products in atherosclerosis.⁽¹¹⁷⁾

Modulation of Myeloperoxidase Catalytic Activity by Other Oxidants

Both endogenous and exogenous compounds can modify the activity of MPO, and thereby modulate the nature and extent of oxidant formation and biological damage induced by this enzyme.

Hydrogen peroxide. Although H_2O_2 initiates the catalytic cycles (halogenation and peroxidase) of MPO, it can also inhibit MPO by acting as a competitive substrate for Compound I, by reacting with Compound II to generate (catalytically-inactive) Compound III, and by irreversibly inactivating the enzyme.^(11,16) In the absence of other substrates (an unlikely scenario *in vivo*) MPO displays significant catalase activity, due to direct two-electron reduction of Compound I by H_2O_2 to the native enzyme and to a slower, competing process initiated via one-electron reduction of

Compound I by H_2O_2 to Compound II;⁽¹⁷⁶⁾ the latter process has been proposed to occur via formation of the ferrous enzyme.⁽¹⁷⁷⁾

Superoxide radical anion. $O_2^{\cdot-}$ undergoes rapid one-electron transfer reactions with Compounds I, II and III of MPO.⁽³⁹⁾ $O_2^{\cdot-}$ rapidly converts ferric MPO to Compound III [formally an Fe(II) species with a bound oxygen molecule] which can be subsequently recycled to the ferric form. MPO can therefore act as a superoxide dismutase, with these reactions modulating halogenation and peroxidase activity.⁽³⁹⁾ Computational modelling of reactions within the neutrophil phagosome, where MPO concentrations are high (ca. 1 mM), indicates that most of the $O_2^{\cdot-}$ produced by the NADPH complex is consumed via the superoxide dismutase activity of MPO, and that efficient recycling of Compound III by $O_2^{\cdot-}$ ensures that phagosomal HOCl production is not constrained.⁽¹⁷⁸⁾ Under other situations however (e.g., when the MPO concentrations and fluxes of H_2O_2 are low and turnover of the ferric enzyme is rate limiting), Compound III formation can inhibit HOCl production (reviewed in Ref. 16, 39).

At high H_2O_2 fluxes, where reaction of Compound I with H_2O_2 results in Compound II accumulation, $O_2^{\cdot-}$ can maintain MPO-mediated chlorination by recycling Compound II to the ferric enzyme.^(16,39,179) This activity is also important in maintaining MPO activity during the oxidation of poor peroxidase substrates (see also below).^(39,180,181)

Interaction of MPO with $O_2^{\cdot-}$ can promote catalytic activities that appear to be independent of the halogenation and peroxidase cycles. MPO has been shown to hydroxylate aromatic substrates, such as phenols⁽¹⁸²⁾ and salicylate,⁽¹⁸³⁾ and oxidize melatonin via $O_2^{\cdot-}$ -dependent processes.⁽¹⁶⁴⁾ These reactions may occur via reaction of ferric MPO with $O_2^{\cdot-}$ to form Compound III, and involve a reactive intermediate such as singlet oxygen (1O_2).⁽¹⁶⁴⁾ Aromatic hydroxylation is also observed during the metabolism of the hydrazide derivative isoniazid by MPO where Compound III is generated.⁽¹²⁷⁾ As discussed above, it is now established that $O_2^{\cdot-}$ can react with some of the radicals generated by MPO, to yield hydroperoxides, and subsequent decomposition of these species may account for the formation of some of these hydroxylated products.

Nitric oxide. NO^{\cdot} reacts rapidly with Compounds I and II of MPO via one-electron transfer.⁽¹⁸⁴⁾ The initial product of these reactions, the nitrosonium ion (NO^+), is a short-lived species that reacts with water to yield NO_2^- which may ultimately result in the formation of nitrosylated products.⁽¹⁸⁵⁾ The interaction of NO^{\cdot} with MPO may therefore alter the distribution of redox intermediates during steady state catalysis, and the balance between halogenation and peroxidase cycles.^(186–188) Although the reactions of NO^{\cdot} with MPO Compounds I and II are facile, MPO-dependent consumption of NO^{\cdot} in human plasma at physiologically-relevant fluxes (steady state \ll 1 μ M) is likely to predominantly occur via reaction with radicals generated by the peroxidase cycle (e.g., tyrosyl and ascorbate radicals).⁽¹⁸⁹⁾

Nitrite. NO_2^- is a major decomposition product of NO^{\cdot} and is generated by autoxidation (via the formation of N_2O_3) or from metabolism by heme proteins such as hemoglobin⁽¹⁹⁰⁾ or peroxidases (see above). NO_2^- reacts with Compound I and Compound II of MPO^(191,192) to generate NO_2^{\cdot} . With MPO a small but significant fraction of NO_2^{\cdot} is oxidized to a species that can induce hydroxylation as well as nitration, a property shared by peroxyxynitrite ($ONOO^-/ONOOH$). In contrast to free peroxyxynitrite, MPO- H_2O_2 - NO_2^- induces aromatic hydroxylation only at acidic pH, and CO_2 does not enhance aromatic nitration, consistent with an enzyme-bound intermediate.⁽¹⁹³⁾ Analogous experiments with LPO indicate that this intermediate may be a complex of $ONOO^-$ with MPO.⁽¹⁹⁴⁾

Peroxyxynitrite. Whilst NO^{\cdot} and $O_2^{\cdot-}$ have important, independent interactions with MPO the radical-radical termination product of these species, $ONOOH$ also reacts. Thus ferric MPO can promote aromatic nitration by peroxyxynitrite.⁽¹⁹⁵⁾ Ferric MPO appears to be directly converted to Compound II, consistent with

the generation of NO₂[•].^(196,197) Reaction with ferric MPO is faster at acidic pHs, with the pH dependency consistent with the pK_a of ONOOH/ONOO⁻, suggesting that ONOOH is the reactant,⁽¹⁹⁶⁾ and that Compound II formation occurs via dissociation of an intermediate oxygen-coordinated complex of ONOO⁻ (see also preceding section).⁽¹⁹⁷⁾ Peroxynitrite also rapidly converts Compound I and Compound III, to Compound II. Compound II does not appear to oxidize peroxynitrite, despite this process being thermodynamically favorable.⁽¹⁹⁷⁾

Inhibition of Myeloperoxidase Activity

In the light of the data supporting a role for MPO in a range of human diseases (reviewed in ^(10,12)), there is considerable interest in the development of MPO inhibitors. A number of different strategies to limit oxidant formation and damage have been investigated; these are summarised below.

Limiting the availability of substrates for oxidant production. MPO-mediated oxidant generation can obviously be limited by decreasing the availability of H₂O₂. This can be achieved by inhibiting the membrane-bound NOX of phagocytes, and related NOXs present in other cells (e.g., endothelial and smooth muscle cells^(198,199)). A number of such compounds have been identified,^(200,201) with the most extensively studied being diphenylene iodonium chloride (DPI).^(202,203) This compound also inhibits other sources of O₂⁻ formation (and hence H₂O₂ levels) including mitochondrial activities.⁽²⁰⁴⁾ Despite the undoubted utility of these compounds in isolated systems, the plethora of sources of O₂⁻ and H₂O₂ *in vivo*, may limit the usefulness of this strategy in some cases. Furthermore, low levels of H₂O₂ appear to be required for cell signalling, and hence limiting O₂⁻ formation may not be always beneficial.

NO[•] can suppress NOX activity in endothelial cells via S-nitrosylation of the p47phox subunit⁽²⁰⁵⁾ and thus may limit MPO-mediated damage by decreasing vascular H₂O₂ concentrations, in addition to its direct effects on MPO activity (see above). Whether sufficient NO[•] is present *in vivo* to have a major modulatory effect on MPO activity, in addition to its multitude of other effects, remains an open question. Oxyhemoglobin oxidises NO[•] and NO₂⁻ to the inactive product NO₃⁻ and thus may be an important intravascular modulator of peroxidase-dependent and -independent nitration.⁽²⁰⁶⁾

Whilst modulation of Cl⁻ levels is clearly not readily achievable in most situations, the levels of Br⁻, I⁻ and SCN⁻ can be modulated by dietary or other means (e.g., cessation of smoking for SCN⁻). Whilst lower levels of these ions can modulate the nature of the oxidants formed, the overall yield may not be markedly affected, as HOCl formation may then predominate.

Heme poisons. Small anions, including azide and cyanide, can bind competitively to the heme centre of MPO in place of its typical halogenation substrates, thereby inhibiting hypohalous acids formation. The use of these agents is, however, limited by their multitude of interactions with other heme centres and subsequent toxicity.

MPO binding agents. Caeruloplasmin, a copper-containing plasma protein, binds MPO avidly⁽³¹⁾ and inhibits its peroxidase and halogenation activities.^(31,207) This protein may therefore limit the activity of MPO in plasma. Anti-MPO antibodies can reverse this inhibition.⁽²⁰⁸⁾ The nature of some of these caeruloplasmin-MPO complexes has been investigated.⁽²⁰⁹⁾ Polyanionic glycosaminoglycans, such as the anticoagulant heparin, bind (cationic) MPO electrostatically,⁽²⁸⁾ and can liberate MPO sequestered in the artery wall.⁽²¹⁰⁾ This interaction with MPO may exacerbate damage to heparin, or other glycosaminoglycans to which it is bound,^(84,155) but divert oxidation from other critical sites. Modulation of MPO activity in this fashion could be an important, additional function of heparin *in vivo*. However in situations where both protein and glycosaminoglycans are present (e.g., on proteoglycans, such as

the basement membrane species perlecan), damage appears to be primarily localised to the protein, with this resulting in changes to proteoglycan function.⁽²⁷⁾

Phenols and other poor peroxidase substrates. Substrates that react readily with Compound I, thereby competitively inhibiting halide/pseudohalide ion oxidation, are a potentially attractive mechanism of inhibiting hypohalous acid formation. If such materials are poorly oxidised by Compound II, then these materials may “trap” the enzyme and prevent completion of the catalytic cycle (though see the caveats discussed above, regarding *in vivo* significance). The potential of this approach is however, limited by the high drug concentrations required to compete with halide oxidation. Some plasma components may exhibit this behaviour, with both ascorbate and urate oxidised by MPO;^(211,212) the quantitative significance of these reactions *in vivo* remains to be fully established.

A number of non-steroidal anti-inflammatory drugs have been shown to inhibit the formation of HOCl (and other hypohalous acids) by MPO by acting as poor peroxidase substrates for the enzyme.^(211,213) The most effective agents are likely to be those with redox potentials between 1.35 and 1.1 V, which results in significant rates of oxidation by Compound I, but not Compound II.^(211,214) As O₂⁻ can reduce Compound II to the ferric state, these compounds would be expected to be most effective (i.e. have lower IC₅₀ values, the concentration of drug required to produce 50% inhibition) in the presence of SOD *in vitro*.⁽²¹⁵⁾ This is unlikely to happen within the neutrophil phagosome however, where the SOD concentration is limited.⁽¹¹⁾

The common anti-inflammatory drug paracetamol (acetaminophen) reaches some of the highest plasma concentrations of any exogenous compound. Thus standard pharmacological doses (1 g) give peak plasma levels of up to 130 μM.⁽¹³⁵⁾ Such levels result in marked inhibition of HOCl and HOBr generation by MPO-H₂O₂-halide systems (IC₅₀ 77 μM for HOCl formation, 19 μM for HOBr formation, and 92 μM for both ⁽⁵²⁾). Similar inhibition of hypohalous acid formation was detected with activated neutrophils (IC₅₀ ~ 100 μM), without perturbation of O₂⁻ formation.⁽⁵²⁾ This inhibition is accompanied by paracetamol dimer formation as a result of oxidation of the drug to the corresponding phenoxyl radical and subsequent dimerisation;⁽⁵²⁾ these dimers may act as a useful marker of MPO inhibition by this drug.⁽⁵²⁾ Further trials of paracetamol in humans are under way.

Other phenols may behave in a similar manner, but whether these compounds achieve sufficiently high plasma concentrations to be effective *in vivo* is unclear. Recent studies have reported that flavanoids may act as MPO substrates, with this resulting in irreversible inactivation, and oxidation of the flavonoid to a radical and subsequent dimerization. In the presence of GSH, hydroquinone formation was detected and subsequent GSH conjugation.⁽²¹⁶⁾ Despite this interaction, the yield of chlorinating oxidants was not markedly affected, and therefore it was concluded that these materials were unlikely to be pharmacologically useful.⁽²¹⁶⁾ In contrast, quercetin, and some analogues and metabolites, has been shown to be an effective inhibitor (IC₅₀ ~ 1 μM for quercetin) of neutrophil-mediated LDL modification, with this ascribed to inhibition of MPO-mediated oxidant formation by quercetin.⁽²¹⁷⁾ The effect of the flavonoids and related materials may therefore be complex and structure dependent. As many of these materials have low biological availability (i.e. peak plasma concentrations being nM or low μM⁽²¹⁸⁾) very low μM IC₅₀ values for MPO inhibition would be required for any biological effect.

As might be expected on the basis of the above data, anilines and indoles (including the amino acid tryptophan⁽²¹⁹⁻²²¹⁾ and analogues⁽²²²⁾) are readily oxidized by MPO Compound I, and act as poor peroxidase substrates (i.e. result in the accumulation of Compound II, and in cases Compound III⁽²¹⁹⁾). As with phenols, these compounds show lower IC₅₀ values in the presence of SOD, as a result of the removal of O₂⁻.⁽²¹⁹⁾ The efficiencies and

mechanisms of inhibition by different tryptophan analogues has been examined.⁽²²²⁾ These studies indicate that structural changes, particularly those that increase hydrophobicity, increase the effectiveness of these materials.

An alternative approach has also been examined: rather than providing a substrate that can be metabolized to a radical by MPO (which might be damaging), stable radicals that might be metabolized to non-radical products were examined.⁽²¹⁵⁾ Thus long-lived nitroxide radicals, that do not give rise to overt toxicology in extended feeding studies,⁽²²³⁾ have been examined as MPO inhibitors. A number of these compounds are potent inhibitors of MPO-mediated oxidant formation, from both isolated MPO and activated neutrophils, with IC₅₀ values in the low μM range.⁽²¹⁵⁾ Evidence has been presented for Compound II accumulation with some nitroxides, but others appear to induce heme destruction.⁽²¹⁵⁾ The IC₅₀ values show a marked structure dependence with positively-charged species being much more effective than negatively-charged; this has been ascribed to an interaction between the nitroxide and negatively-charged groups on the enzyme. *In vivo* studies are required to elucidate the true therapeutic potential of these compounds.

A number of other drugs, including hydroquinone⁽¹²⁴⁾ and amsacrine,⁽¹²⁶⁾ can also divert MPO from HOCl production via an alternative mechanism by promoting Compound III formation; again O₂⁻ would be expected to antagonise this inhibition, by recycling this species to the native enzyme.

Hydrazines and hydrazides. The most “effective” (i.e. lowest IC₅₀ values) inhibitors of MPO are irreversible enzyme inhibitors (‘suicide’ substrates); these include hydrazines (RNHNH₂) and hydrazides (RCONHNH₂).^(224–226) Despite their low IC₅₀ values these compounds may not however be the most applicable *in vivo*. Benzoic acid hydrazides, including the most potent MPO inhibitor identified to date, 4-aminobenzoic acid hydrazide (ABAH),⁽¹²⁸⁾ are believed to act via heme destruction arising from generation of ferrous MPO,⁽²²⁴⁾ however the precise mechanism is uncertain. Irreversible inhibition of MPO by the hydrazide derivative isoniazid, an anti-tuberculosis drug, has also been proposed to involve heme modification via a mechanism initiated by Compound III formation.⁽²²⁷⁾

Conclusions

There is now considerable evidence that MPO and other heme peroxidases are enzymes that have both beneficial and damaging actions. These species have a clear beneficial function in terms of killing invading pathogens, but this same enzymatic activity

can result in host tissue damage and play a role in a number of important human pathologies. Information and an understanding of the enzymology and mechanisms of action of these enzymes is clearly therefore of major importance, both in terms of enhancing their positive actions in terms of disinfection and improved hygiene, and diminishing their deleterious effects. Recent studies have provided valuable information on the targets and actions of the oxidants generated by these enzymes and their mechanisms of biological damage. Ongoing studies on the control of the halogenation and peroxidase cycles are providing valuable data on new therapeutic strategies to limit oxidant damage to human tissues and it is possible that selective inhibition of these enzymes will have major clinical benefits.

Acknowledgments

The author is grateful for financial support of his research by Australian Research Council under the ARC Centres of Excellence (CE0561607) and Discovery (DP0988311) programs, the National Health and Medical Research Council (570829) and the National Heart Foundation (Grants in Aid: G09S4313 and G08S3769).

Abbreviations

NOX	NADPH oxidase
O ₂ ⁻	superoxide radicals
H ₂ O ₂	hydrogen peroxide
HO [•]	hydroxyl radicals
MPO	myeloperoxidase
NO [•]	nitric oxide
ONOOH	peroxynitrite
EPO	eosinophil peroxidase
LPO	lactoperoxidase
GSH	glutathione
GSSG	glutathione disulfide
MMP	matrix metalloproteinases
3-chloroTyr	3-chlorotyrosine
MAPK	mitogen-activated protein kinase
AGEs	advanced glycation end products
CVD	cardiovascular disease
¹ O ₂	singlet oxygen
NO ⁺	nitrosonium ion
DPI	diphenylene iodonium chloride
ABAH	4-aminobenzoic acid hydrazide

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