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Comprehensive Invited Review

Mammalian Heme Peroxidases: From Molecular Mechanisms to Health Implications

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ABSTRACT

A marked increase in interest has occurred over the last few years in the role that mammalian heme peroxidase enzymes, primarily myeloperoxidase, eosinophil peroxidase, and lactoperoxidase, may play in both disease prevention and human pathologies. This increased interest has been sparked by developments in our understanding of polymorphisms that control the levels of these enzymes, a greater understanding of the basic chemistry and biochemistry of the oxidants formed by these species, the development of specific biomarkers that can be used *in vivo* to detect damage induced by these oxidants, the detection of active forms of these peroxidases at most, if not all, sites of inflammation, and a correlation between the levels of these enzymes and a number of major human pathologies. This article reviews recent developments in our understanding of the enzymology, chemistry, biochemistry and biologic roles of mammalian peroxidases and the oxidants that they generate, the potential role of these oxidants in human disease, and the use of the levels of these enzymes in disease prognosis. *Antioxid. Redox Signal.* 10, 1199–1234.

I. INTRODUCTION

It is now widely accepted that two major superfamilies of heme peroxidase enzymes exist in biologic systems, in addition to a large number of other peroxidases with alternative catalytic sites (*e.g.*, the glutathione and thioredoxin peroxidase families). This review is focused entirely on the heme peroxidases. The first of the two major superfamilies of heme peroxidases comprises those found in plants, fungi, and archae bacteria. This superfamily appears to have arisen from gene duplication of a single ancestral gene, with three classes recognized within this family on the basis of their sequence alignment and biologic origin (130, 290). Crystallographic studies of members of each class have shown that these have the same helical folds and relatively minor differences in their structures.

The second superfamily consists of the mammalian heme peroxidases, which differ dramatically from the plant/fungi/bacteria family in multiple ways. Thus, they have different primary (sequence) and tertiary structures, as well as different prosthetic groups. The designation of this group of peroxidases as mammalian peroxidases is now known to be not strictly correct, as it has been shown that similar genes, and protein products with significant sequence similarities, are present in a range of other species, including arthropods, molluscs, *Caenorhabditis elegans* worms, and *Drosophila*. This mammalian/animal family of peroxidases is dramatically different from the first superfamily and appears to be the result of convergent evolution, with the two families of proteins arriving at a "common" function *via* different pathways. This review is concerned primarily with three major mammalian heme peroxidases: myeloperoxidase (MPO), eosinophil peroxidase (EPO), and lactoperoxidase (LPO). The last of these appears to be very similar or identical to the peroxidase present in saliva (189). Thus, human salivary peroxidase is very similar both structurally and catalytically to

bovine LPO, but whether human milk LPO is identical to human salivary peroxidase is not completely resolved; they are, however, coded by the same gene. The origin, structure, and biologic activity of these human saliva enzymes are reviewed elsewhere (189). Other peroxidases are also present in mammals, including thyroid peroxidase, which plays a key role in thyroid function, in which it catalyzes the iodination of Tyr residues to form mono- and diiodotyrosines as well as the coupling of the monoiodotyrosine residue in the protein thyroglobulin to give thyroxine and triiodothyronine, which are important human hormones. This specialized membrane-associated peroxidase is not discussed further here; further details on this enzyme can be found elsewhere (261, 274, 290).

II. CELLULAR ORIGINS OF MYELOPEROXIDASE, EOSINOPHIL PEROXIDASE AND LACTOPEROXIDASE

A. Myeloperoxidase

Myeloperoxidase (MPO) is a key component of the antimicrobial armory of neutrophils, the major effector cell of the innate immune system. These cells provide the front line of defense against invading microbes and are primed to generate or release a battery of materials that enable the rapid killing of such species. This is accomplished by engulfing and ingestion of foreign organisms, with formation of a phagolysosomal compartment into which materials stored in intracellular granules of the neutrophil are released. Neutrophils possess at least three types of intracellular granules; one of which–the azurophil or primary granules–stains positively for peroxidase activity. The other two major forms of granules do not appear to contain significant peroxidase activity. The lysosome-like azurophil granules, which are manufactured by the promyelocyte, contain multiple components including serine proteases, lysosomal hydrolyases, and MPO. The last of these is a major component and comprises ~5% of the dry mass of neutrophil cells. These proteins are tightly packed inside the granule and are associated with a matrix-like material consisting of polyanionic glycosaminoglycans. The highly cationic granule proteins are believed to bind to this matrix material in a conformation or state that renders them inactive. During neutrophil activation, the azurophilic granules are secreted in to the phagosomal compartment, with subsequent release of MPO. Thus, most of the MPO remains within the phagolysosome, although some is also released extracellularly. Coincident with the secretion of the azurophilic granules into the phagolysosomal compartment, an NADPH oxidase is assembled on the internal membrane surface, with this acting as a source of superoxide radicals ($O_2^{\bullet -}$) and H_2O_2 (probably *via* spontaneous or catalyzed dismutation of the $O_2^{\bullet -}$). The latter acts as an essential cofactor for the generation of oxidants by MPO.

In addition to the synthesis of MPO by promyelocytes that become neutrophils, monocyte precursor cells also synthesize this enzyme during their maturation in the bone marrow; this synthesis usually ceases after this stage of cell development, so circulating monocytes do not actively synthesize the enzyme. The subsequent maturation of monocyte cells within tissues into macrophages is likewise associated with a loss of MPO, so fully matured macrophages contain little or no active enzyme, although evidence has been presented for the re-initiation of MPO synthesis from quiescent macrophage cells under some circumstances, such as in the brain plaques present in Alzheimer's disease (347). Whether a similar reinitiation of enzyme formation occurs in tissue macrophages in other pathologies is unclear, but this may occur in, for example, atherosclerotic lesions, in which abundant evidence exists for MPO, but few if any neutrophils. It has been suggested that such aberrant MPO synthesis, with incorrect cellular trafficking and processing, may give rise to the significant amounts of MPO detected extracellularly in atherosclerotic lesions (152). The biosynthesis, processing, and sorting of human MPO has been recently reviewed in depth; the reader is referred to an excellent summary for further details of the intracellular events that result in the formation and subsequent release of MPO (152).

Mature MPO is a highly cationic, dimeric protein with a mass of 146 kDa. It consists of two monomer units (73 kDa each) joined by a cystine (disulfide) bridge at Cys 153. Each monomer consists of a heavy (58.5 kDa, 467 amino acids) and light chain (14.5 kDa, 106 amino acids) (115), with the former chain glycosylated. The heavy chains contain the active site modified iron protoporphyrin IX derivative. This is located at the bottom of a deep crevice (462), which hinders access of most materials to the iron atom, with this being restricted to H_2O_2 and small anions (47). Other substrates that are oxidized by the enzyme (see later) bind in a hydrophobic pocket at the entrance to the distal heme cavity. The two dimers are identical and functionally independent. The structure of the human peroxidases was recently reviewed (130).

B. Eosinophil peroxidase

Eosinophil peroxidase (EPO) is the major granule protein of eosinophils, which are specialized human phagocytic cells that eliminate parasites and related organisms. Unlike neutrophils, which phagocytose their target organisms and subsequently release MPO primarily in to the phagolysosomal compartment, eosinophils are forced by the larger size of their parasitic targets to exocytose their granule contents on to the parasite surface to which they are attached. The major contents that are released from the eosinophil granules are EPO (which constitutes *ca*. 40% by mass), major basic protein, eosinophil cationic basic protein, and eosinophil-derived neurotoxin (1). Like MPO, with which it shares a 70% amino acid homology (397), EPO is a highly cationic protein with a modified iron protoporphyrin IX prosthetic group. It is synthesized as a *ca*. 80-kDa single-chain precursor, which is subsequently processed in to a mature protein of 69.8 kDa, consisting of a 57.9-kDa heavy chain and a 11.9-kDa light chain, which has close analogies to the arrangement of the MPO monomer (130). The H_2O_2 that this enzyme requires for its catalytic action is believed to arise primarily from $O_2^{-\bullet}$ generated by an NADPH oxidase system related to that of neutrophils (101). This superoxide-generating enzyme appears to be particularly robust and capable of generating very large amounts of $O_2^{-\bullet}$ and H_2O_2, with the levels of these materials estimated to be threefold to 10-fold higher than those generated by the neutrophil system (101).

C. Lactoperoxidase

Salivary peroxidase or lactoperoxidase (LPO) is found in multiple human exocrine secretions including tears, milk, saliva, and vaginal fluid. In each case, its primary role appears to be as a first line of defense against invading microorganisms that might enter the human body (189). The human LPO and salivary peroxidase gene codes for a protein of 712 amino acids, with 51% sequence identity to MPO (290) and an analogous heme group and binding site (189). The gene product is subsequently processed to give a single-chain glycoprotein of *ca*. 80 kDa (130). As with the other human peroxidases, lacto/salivary peroxidase binds avidly to many surfaces and binds to the enamel of human teeth in an active form.

III. ENZYMOLOGY OF MYELOPEROXIDASE, EOSINOPHIL PEROXIDASE, AND LACTOPEROXIDASE

A. General overview

The genes for human MPO, EPO, and LPO are adjacent to each other on chromosome 17 and have similar intron–exon structures, consistent with each being generated *via* amplification from a common ancestral gene at this locus (417). MPO and EPO are both highly cationic (pI, *ca*. 10), which enables them to bind avidly to a wide range of negatively charged structures, including bacterial cell surfaces (265, 336, 369), endothelial cell surfaces (32), cytokeratin 1 (22), extracellular matrix (235), perlecan (Rees *et al*., unpublished data), heparin (95), hyaluronan (144), albumin (411), ceruloplasmin (366), α_1-antitrypsin (51), apolipoprotein A-I (470), and low-density lipoproteins (76). In contrast, LPO has a pI of *ca*. 7.5.

B. Active site and substrate binding

The crystal structures of multiple forms of both MPO and LPO have been determined and can be readily examined *via* the free software available at the Protein Data Base (http://www.rcsb.org/pdb/results/results.do; accession numbers 1cxp for MPO and 2pt3 and 2nqx for LPO). The nature of the active sites of each of these enzymes was reviewed recently (130). The proximal histidine ligand in MPO, EPO, and LPO is a key regulator of the redox properties of the heme iron and is hydrogen-bonded to an asparagine residue, which assists in stabilizing the ferric form of the enzymes by acting as a hydrogen-bond acceptor (130). The redox properties of MPO are significantly affected by a covalent sulfonium ion linkage between Met-243 and the heme group (461), which distorts the heme from planarity and withdraws electron density because of its positive charge. The distal histidine ligand of these enzymes is believed to act as an acid/base catalyst, accepting a proton from H_2O_2 to initiate compound I formation and donating a proton on cleavage of the oxygen–oxygen bond to form water; a nearby arginine residue may assist in cleavage of H_2O_2 by polarizing the oxygen–oxygen bond (130). In MPO, the distal ligand is hydrogen-bonded *via* a buried water molecule to an asparagine residue and a side-chain histidine residue and also to a chain of four water molecules leading to the surface of the molecule (115); conservation of the side-chain asparagine and histidine residues in EPO and LPO indicates the importance of the previously mentioned hydrogen bonding network in catalysis (130). Each enzyme has a conserved asparagine residue adjacent to the distal histidine ligand that appears to play a role in correctly aligning this histidine ligand *via* its coordination with a bound calcium ion (130).

The x-ray crystal structure of the MPO–bromide complex shows that halides bind within the distal cavity in close proximity (3.5 Å) to the N^{ε} atom of the distal histidine ligand (46, 115). Halide binding by MPO is favored at acidic pH (190), consistent with protonation of the distal histidine. Evidence has also been presented for an additional, lower-affinity, halide binding site within the distal cavity (316). The site of thiocyanate (SCN^-) binding is close enough to the heme iron to affect its reduction potential and its affinity for H_2O_2 (392); a weak interaction of SCN^- with the distal histidine has also been observed in MPO (46). With the nitrite (NO_2^-) complex of MPO, spectroscopic changes on binding suggest a direct interaction with the heme iron (60), with the affinity for nitrite increased at acidic pH, which is attributed to protonation of the distal histidine ligand (60). MPO, EPO, and LPO have a conserved hydrophobic region at the entrance to the distal cavity where aromatic substrates bind (183).

C. Generation of catalytic intermediates

H_2O_2 reacts with the native, ferric forms of MPO, EPO, and LPO to generate compound I, which consists of an oxy-ferryl ($Fe^{IV}=O$) heme center and a porphyrin π-cation radical. In this reaction, oxidation of the ferric heme center and the porphyrin ring yields two electrons that are used to reduce H_2O_2 to water. Compound I may be converted back to the ferric enzyme *via* direct, two-electron reduction by (pseudo)halides ("the halogenation cycle"), or *via* two sequential one-electron reduction reac-

Table 1. Standard Reduction Potentials (at pH 7) for the Redox Couples of Species Involved in the Peroxidase Cycle of Human Heme Peroxidases

	Standard reduction potentials (V)		
	MPO	*EPO*	*LPO*
Compound I/ferric enzyme	1.16[a]	1.10[a]	1.09[c]
Compound I/compound II	1.35[b]	—	1.14[c]
Compound II/ferric enzyme	0.97[b]	—	1.04[c]

[a]From Arnhold J, Furtmuller PG, Regelsberger G, and Obinger C. Redox properties of the couple compound I/native enzyme of myeloperoxidase and eosinophil peroxidase. *Eur J Biochem* 268: 5142–5148, 2001, with permission.

[b]From Furtmuller PG, Arnhold J, Jantschko W, Pichler H, and Obinger C. Redox properties of the couples compound I/compound II and compound II/native enzyme of human myeloperoxidase. *Biochem Biophys Res Commun* 301: 551–557, 2003, with permission.

[c]From Furtmuller PG, Arnhold J, Jantschko W, Zederbauer M, Jakopitsch C, and Obinger C. Standard reduction potentials of all couples of the peroxidase cycle of lactoperoxidase. *J. Inorg Biochem* 99: 1220–1229, 2005, with permission.

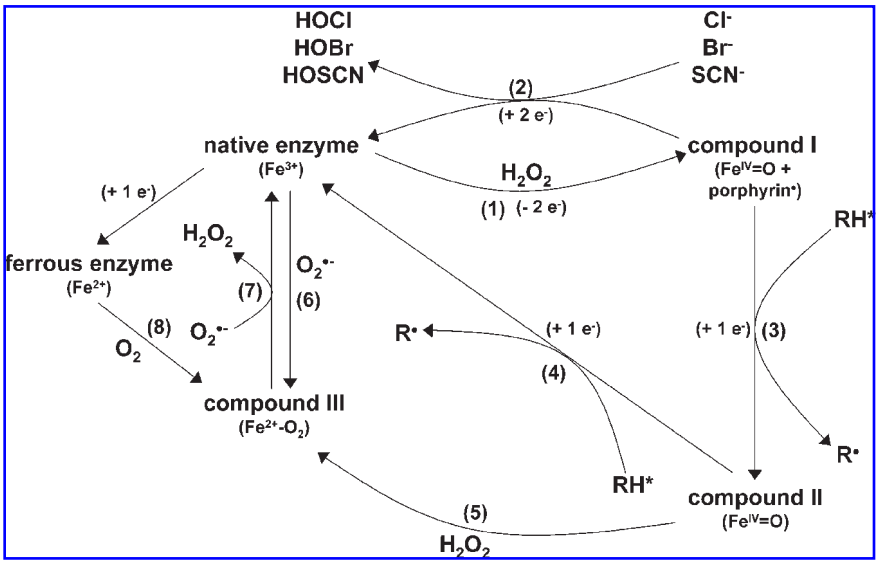

FIG. 1. Generation and reactions of redox intermediates of MPO, EPO, and LPO. *RH denotes a classic organic peroxidase substrate; other species also undergo one-electron oxidation by compound I and compound II (see text).

tions involving an intermediate called compound II, which retains the oxy-ferryl ($Fe^{IV}=O$) heme center ("the peroxidase cycle"). Reduction potentials for these processes are given in Table 1. An additional intermediate, compound III, is generated *via* reaction of the ferric enzymes with $O_2^{\bullet-}$, or *via* one-electron reduction to their ferrous forms and subsequent reaction with O_2. These processes are summarized in Fig. 1, with selected apparent second-order rate constants for these reactions shown in Table 2.

Because of the high reduction potentials of their compound I/native enzyme and the compound I/compound II couples, MPO, EPO, and LPO can oxidize a wide range of substrates *via* their halogenation and peroxidase cycles. The unusually high values of these reduction potentials for MPO can be rationalized by the heme distortion and reduction in heme electron density induced by the covalent vinyl sulfonium heme linkage (461). The potentials of the redox couples vary with pH,

TABLE 2. SELECTED APPARENT SECOND-ORDER RATE CONSTANTS (/*M*/SEC) FOR THE REACTIONS LISTED IN THE HALOGENATION AND PEROXIDASE CYCLES OF MPO, EPO, AND LPO SHOWN IN FIG. 1

Reaction no.	*Reaction description*	*MPO*	*EPO*	*LPO*
1	Native enzyme + H_2O_2 → Compound I	1.4×10^{7}[a]	4.3×10^{7}[a]	1.1×10^{7}[a]
2	Compound I + Cl^- → Native enzyme + HOCl	2.5×10^{4}[a]	3.1×10^{3}[a]	—
2	Compound I + Br^- → Native enzyme + HOBr	1.1×10^{6}[a]	1.9×10^{7}[a]	4.1×10^{4}[a]
2	Compound I + SCN^- → Native enzyme + HOSCN	9.6×10^{6}[a]	1.0×10^{8}[a]	2.0×10^{8}[a]
3	Compound I + RH* → Compound II + R/$^{\bullet}$	Tyr, 7.7×10^{5}[b] Trp, 4.5×10^{5}[b] NO_2^-, 2.2×10^{6}[e]	Tyr, 3.5×10^{5}[c] NO_2^-, $\sim 3 \times 10^{6}$[d]	Tyr, 1.1×10^{5}[b] Trp, 2.4×10^{4}[b] NO_2^-, 2.2×10^{7}[e]
3	Compound I + $O_2^{\bullet-}$ → Compound II + O_2	5×10^{6}[f]		
3	Compound I + H_2O_2 → Compound II + $O_2^{\bullet-}$	7×10^{4}[f]	—	
4	Compound II + RH* → Native enzyme + R*	Tyr, 1.6×10^{4}[b] Trp, 6.9[b] NO_2^-, 550[e]	Tyr, 2.7×10^{4}[c] NO_2^-, 5.6×10^{3}[d]	Tyr, 1.0×10^{4}[b] Trp, 84[b] NO_2^-, 3.5×10^{5}[e]
4	Compound II + $O_2^{\bullet-}$ → Native enzyme + O_2 + H_2O	1×10^{6}[f]		
5	Compound II + H_2O_2 → Compound III + H_2O	50[f]	—	220[g]
6	Native enzyme + $O_2^{\bullet-}$ → Compound III	2×10^{6}[f]		
7	Compound III + $O_2^{\bullet-}$ → [Native enzyme + H_2O_2] + O_2	1×10^{5}[f]		
8	Ferrous enzyme + O_2 → Compound III	1.1×10^{4}[h]		1.8×10^{5}[g]

[a]Measured at pH 7.0 and 15°C [reviewed in (130)].
[b]Measured at pH 7.0 and 25°C (195).
[c]Measured at pH 7.0 and 15°C (125).
[d]Measured at pH 7.4 and 21°C (421).
[e]MPO and LPO values measured at pH 7.0 and 7.2, respectively [reviewed in (19)];.
[f]From Winterbourn CC, Hampton MB, Livesey JH, and Kettle AJ. Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. *J Biol Chem* 281: 39860–39869, 2006, with permission.
[g]Measured at pH 7.0 and 25°C (194).
[h]Measured at pH 7.0 and 25°C (192).

and this may impose pH-dependent thermodynamic thresholds on substrate oxidation (381). Unlike compounds I and II, compound III is unreactive toward most potential substrates and is essentially a catalytic "dead end." However, MPO compound III can induce slow, one-electron oxidation of ascorbate (253) and paracetamol (acetaminophen) (254) and is implicated in $O_2^{\bullet -}$-dependent catalytic activities of MPO (221).

Whereas H_2O_2 is responsible for initiating the halogenation and peroxidase cycles by generating compound I, it can also inhibit these processes by acting as a competitive substrate for compound I, by reacting with compound II to generate the catalytically inactive compound III and by inducing irreversible enzyme inactivation (130, 215). In the absence of other substrates, MPO displays significant catalase activity, because of 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 (217); the latter process has been proposed to occur *via* formation of the ferrous enzyme (193).

D. The halogenation cycle

The ability of MPO, EPO, and LPO to oxidize Cl^-, Br^-, and the pseudohalide SCN^- at high rates is unique among peroxidases. All of these substrates donate two electrons to compound I to generate the ferric form of the enzyme and are converted to the corresponding (pseudo)hypohalous acids (HOX, X=Cl, Br, SCN). With each enzyme, the second-order rate constants for these reactions reflect the ease of oxidation of each substrate ($SCN^- > Br^- > Cl^-$; Table 3). However, the relative rate constants do not always mirror the redox potentials, as differences in active-site topology and binding sites between the enzymes exert strong effects on substrate specificity (130). For example, the rates for Br^- and SCN^- oxidation by EPO are *ca.* 10-fold faster than those for MPO (19), even though the reduction potential for the compound I/native enzyme couple is higher for MPO (cf. data in Table 1). At neutral pH and physiologic concentrations of Cl^-, Br^-, and SCN^-, MPO primarily generates HOCl and HOSCN (422), EPO primarily generates HOBr and HOSCN (420), and LPO primarily generates HOSCN (128). Specificity constants and K_m for these anions have been reported (420, 422). Above pH 7, production of HOBr by MPO is significantly enhanced, with this product reported to account for 40% of the H_2O_2 consumed at pH 7.8 in the presence of physiologic concentrations of Cl^- and Br^- (370). The mechanistic basis for this phenomenon is uncertain and cannot be accounted for by the relative rates of reaction of Br^- and Cl^- with compound I (370); thermodynamic thresholds on Cl^- oxidation could be imposed at basic pH because of changes in the redox potential of the compound I/native enzyme couple (381). The rate of (pseudo)halide oxidation by compound I of MPO and EPO is significantly enhanced at acidic pH (19), which is attributable to an increased affinity for halides resulting from protonation of the distal histidine (190); Cl^- shows the most dramatic increase in its rate of oxidation at low pH, indicating that protonation of the distal histidine may also enable Cl^-, but not the larger (pseudo)halides, directly to access the oxy-ferryl heme center (130).

Debate exists about the nature of the species generated *via* the interaction of halides with compound I. It has been argued that no experimental evidence exists for the production of free HOCl by the MPO-H_2O_2-Cl^- system (381). The basis of this argument is that assays of HOCl production have generally used only small substrates as targets for chlorination, which may react directly with enzyme-bound intermediates. Kinetic evidence has been obtained that taurine undergoes direct chlorination by an enzyme-bound species rather than by free HOCl at low pH (255). Kinetic modeling of the oxidation of Cl^- by MPO compound I indicates formation of an intermediate compound I-Cl^- complex before the release of free HOCl with a rate constant of 5.2×10^4/sec; this enzyme-bound species is likely to be responsible for oxidation of taurine at low pH. We have shown that the MPO-H_2O_2-Cl^- system efficiently chlorinates free amine groups (RNH_2) of extracellular matrix polysaccharides such as heparan sulfate, with the concentration of chloramines detected accounting for 75% or more of the H_2O_2 consumed (Mallak, Rees, Davies; unpublished data). As access of this bulky polysaccharide to the distal cavity will be limited, these data implicate free HOCl as the chlorinating agent. Overall, it is evident that free HOCl is produced by MPO, but this process is not concerted and involves the generation of an intermediate compound I–halide complex capable of directly oxidizing small substrates.

The final distribution of species generated by peroxidase-H_2O_2-(pseudo)halide systems may be modulated by reaction of the initial products with other (pseudo)halides. HOCl and HOBr can oxidize SCN^-, and HOCl can oxidize Br^- (cf. Table 3). These reactions generate transhalogen species (*e.g.*, oxidation of Br^- by HOCl yields ClBr) and, ultimately, the corresponding (pseudo)hypohalous acids. Evidence has been obtained for HOCl-dependent production of brominating species by the MPO-H_2O_2-Cl^-/Br^- system *in vitro* at acidic pH, by using deoxycytidine as a target (178). However, above pH 7, HOCl does not induce bromination of deoxycytidine (178) or protein Tyr residues (370) in the presence of physiologic concentrations of Br^-, which limits the potential *in vivo* significance of transhalogenation in the production of brominating oxidants.

An interesting aspect of (pseudo)halide oxidation by MPO, EPO, and LPO is the resistance of their heme moieties to modification by the (pseudo)hypohalous acids generated during catalysis, a property that appears to derive from steric barriers to reaction associated with the covalent heme linkages in these enzymes (186). Heme modification by (pseudo)hypohalous acids is likely to be a major route to the irreversible enzyme inactivation during catalysis (258, 284).

TABLE 3. TWO-ELECTRON REDUCTION POTENTIALS OF (PSEUDO)HYPOHALOUS ACIDS

Standard reduction potential (V) at pH in 7 water		
$HOCl/Cl^-$	$HOBr/Br^-$	$HOSCN/SCN^-$
1.28[a]	1.13[a]	0.56[a]

[a]From Arnhold J, Monzani E, Furtmuller PG, Zederbauer M, Casella L, and Obinger C. Kinetics and thermodynamics of halide and nitrite oxidation by mammalian heme peroxidases. *Eur J Inorg Chem* 3801–3811, 2006, with permission.

E. The peroxidase cycle

In the classic peroxidase cycle, organic substrates are converted to radicals *via* one-electron oxidation by compounds I and II (Fig. 1). Inorganic compounds such as nitrite, and radicals such as $O_2^{\bullet-}$ and nitric oxide ($NO^{\bullet}$), can also undergo one-electron oxidation by compounds I and II. The catalytic potential of MPO, EPO, and LPO is partitioned between their peroxidase and halogenation cycles *via* competition between peroxidase substrates and (pseudo)halides for reaction with compound I.

Physiologically relevant organic peroxidase substrates for MPO, EPO, and LPO include a range of endogenous compounds (*e.g.*, Tyr, ascorbate, steroidal hormones, and urate) as well as xenobiotics and drugs. MPO can oxidize the widest range of substrates because of the unusually large reduction potential of its compound I/compound II couple (see Table 1). Substrates that react readily with compound I, but not compound II, are often termed "poor" peroxidase substrates. In the absence of species that can recycle compound II, metabolism of poor peroxidase substrates results in the accumulation of this intermediate and arrest of the catalytic cycle. Thermodynamically, each enzyme has a unique subset of potential poor-peroxidase substrates whose one-electron oxidation potentials lie between the reduction potentials for the compound I/compound II couple and the compound II/ferric enzyme couple; with MPO, this range of redox potentials is unusually large (see Table 1). Kinetic factors can also affect recycling of compound II, and even with a "good" peroxidase substrate such as Tyr, the rates of reaction with MPO compound II are 10 times slower than those with compound I (256).

The metabolism of Tyr by MPO and LPO is relatively insensitive to pH (37, 256). With MPO, rate constants for reaction of Tyr with compound I are maximal at basic pH values, but vary less than twofold within physiologically relevant pH values (256). The predominance of the chlorinating activity of MPO at acidic pH over its peroxidative metabolism of Tyr (173), trimethyl-benzene (TMB) (433), and indole and tryptamine derivatives (196) can be accounted for primarily by the pH dependence of Cl^- oxidation.

The radicals generated *via* the metabolism of peroxidase substrates by MPO, EPO, and LPO can have a variety of fates. Termination reactions of these radicals can yield dimers and higher polymers. Some radicals can reduce ferric MPO to ferrous MPO, which generates compound III on reaction with O_2. Compound III formation *via* this process occurs during its metabolism of hydroquinone (61, 213), amsacrine (222), hydrazines (426), and hydrazides (219). Reaction with the enzyme may generate protein-derived radicals, *via* hydrogen atom abstraction or addition to aromatic amino acid residues (376). Covalent addition to the heme moiety may also occur (86, 87). Alternatively, radicals can diffuse away and damage other biomolecules such as lipids (210, 360) and proteins (174). Metabolism of amino/phenol derivatives can generate radicals that can undergo further one-electron oxidation or disproportionation to generate electrophilic imino/quinone species capable of forming covalent adducts with thiol derivatives (*e.g.*, glutathione) and other biomolecules (42, 290). A number of drugs and xenobiotics induce adverse biologic effects, including agranulocytosis, hepatotoxicity, and cancer, which have been associated with their metabolism by peroxidase enzymes such as cytochrome P450 (290, 391); MPO, EPO, and LPO are capable of participating in drug and xenobiotic bioactivation; however, their contributions are presently unclear.

F. Modulation of catalytic activities by superoxide

$O_2^{\bullet-}$ rapidly converts ferric MPO to compound III and undergoes rapid one-electron transfer to compounds I, II, and III (221). Reduction of compound III by $O_2^{\bullet-}$ regenerates ferric MPO and produces H_2O_2 and O_2; thus, MPO can act as a superoxide dismutase. As a result, $O_2^{\bullet-}$ can be a key modulator of the halogenation and peroxidase activities of this enzyme. Computational modeling predicts that within the neutrophil phagosome, where MPO concentrations are high (*ca.* 1 m*M*), most of the $O_2^{\bullet-}$ produced by the NADPH complex is consumed *via* the superoxide dismutase activity of MPO, and that efficient recycling of compound III by $O_2^{\bullet-}$ ensures that phagosomal HOCl production is not constrained (449). However, compound III formation can inhibit HOCl production where MPO concentrations and fluxes of H_2O_2 are low and turnover of the ferric enzyme is rate limiting [reviewed in (130, 215)]. Extracellular HOCl production by neutrophils *in vitro* can be inhibited by $O_2^{\bullet-}$ and thus appears to occur under these conditions (215). At high H_2O_2 fluxes, at which reaction of compound I with H_2O_2 results in compound II accumulation, $O_2^{\bullet-}$ can maintain the chlorinating activity of the enzyme by recycling compound II to the ferric enzyme (130, 215). The ability of $O_2^{\bullet-}$ to recycle MPO compound II to the native enzyme also is important in maintaining enzymatic activity during the oxidation of poor peroxidase substrates (212, 218).

Interaction of MPO with $O_2^{\bullet-}$ can promote catalytic activities that are independent of the halogenation and peroxidase cycles. MPO can induce hydroxylation of aromatic substrates, such as phenol (387) and salicylate (214), and oxidize melatonin to N^1-acetyl-N^2-formyl-5-methoxykynuramine in $O_2^{\bullet-}$-dependent processes. These reactions are believed to be initiated by reaction of ferric MPO with $O_2^{\bullet-}$ to form compound III and to involve a common reactive intermediate with the properties of singlet oxygen ($^1O_2$) (456). Aromatic hydroxylation is also observed during the metabolism of the hydrazide derivative isoniazid by MPO, in which compound III is generated (426) and may involve common reactive intermediates.

G. Interactions with nitric oxide, nitrite, and peroxynitrite

$NO^{\bullet}$ reacts rapidly with compounds I and II of MPO, EPO, and LPO *via* one-electron transfer (3). The initial product of these reactions, the nitrosonium ion (NO^+), is a short-lived species that reacts with water to yield NO_2^-; this species can nitrosylate biomolecules (238). The ability of $NO^{\bullet}$ to act as a peroxidase substrate and thereby alter the distribution of redox intermediates during steady-state catalysis enables it to modulate the metabolism of other substrates *via* the halogenation and peroxidase cycles (2, 132, 133). Although the reactions of $NO^{\bullet}$ with MPO compounds I and II are facile, MPO-dependent consumption of $NO^{\bullet}$ in human plasma at physiologically relevant fluxes (steady state <1 μM) is likely to occur predominantly *via* its reaction with radicals generated *via* the peroxidase cycle (*e.g.*, tyrosyl and ascorbate radicals) (109).

NO_2^- is a major decomposition product of $NO^\bullet$ and is generated by autoxidation (*via* the formation of N_2O_3) or from metabolism by heme proteins such as hemoglobin (148) or peroxidases (see earlier). NO_2^- reacts with compound I and compound II of MPO (60, 423), EPO, (421) and LPO (58) to generate $NO_2^\bullet$. With MPO and EPO, a small, but significant fraction of NO_2^- is oxidized to a species that can induce hydroxylation as well as nitration, a property shared by peroxynitrite ($ONOO^-$/ONOOH). In contrast to free peroxynitrite, the MPO-H_2O_2-NO_2^- and EPO-H_2O_2-NO_2^- systems induce aromatic hydroxylation only at acidic pH, and CO_2 does not enhance their ability to induce aromatic nitration, consistent with the active intermediate being enzyme bound (52). Evidence for the production of an equivalent enzyme-bound species by LPO-H_2O_2-NO_2^- has been obtained, but this was significant only at supraphysiologic concentrations of NO_2^- (268). The similarity of the spectroscopic features of the ferric LPO-NO_2^- complex and the intermediate species generated by the LPO-H_2O_2-NO_2^- system suggests that the latter is a nitrogen-coordinated complex of $ONOO^-$ (268).

Although $NO^\bullet$ and $O_2^{\bullet-}$ have important, independent interactions with MPO, EPO, and LPO, their reaction product peroxynitrite also reacts with peroxidases. The potential importance of such reactions is highlighted by the observation that ferric MPO can promote aromatic nitration by peroxynitrite (356). Ferric MPO and LPO are converted directly to compound II, consistent with the generation of $NO_2^\bullet$ (117, 131). Reaction with ferric MPO is faster at acidic pH values, with the pH dependence consistent with the pK_a of ONOOH/$ONOO^-$, suggesting that ONOOH is the reactive species (117). Kinetic studies indicate that compound II formation occurs *via* dissociation of an intermediate oxygen-coordinated complex of $ONOO^-$ (131). Peroxynitrite also rapidly converts MPO compound I and compound III to compound II. Compound II does not appear to oxidize peroxynitrite, although this process is thermodynamically favorable (131).

IV. OXIDANTS GENERATED BY MYELOPEROXIDASE, EOSINOPHIL PEROXIDASE, AND LACTOPEROXIDASE

A. Hypohalous acids (HOCl, HOBr) and N-halogenated amines (halamines)

As outlined previously, HOCl and HOBr are generated by MPO and EPO *via* H_2O_2-catalyzed oxidation of Cl^- and Br^- (229, 378, 406, 420, 422). HOCl and HOBr are both strong oxidants and also are capable of halogenation reactions. At a physiologic pH of 7.4, both HOCl and HOBr exist in equilibrium with their conjugate bases, hypochlorite ($^-$OCl) and hypobromite ($^-$OBr). The pK_a of HOCl is 7.59 (271), thus at pH 7.4 both HOCl and $^-$OCl are present at approximately equimolar concentrations, whereas for HOBr, the pK_a is 8.7 (325), resulting in HOBr being the predominant species present at pH 7.4. Both HOCl and HOBr exhibit moderate absorption bands in the UV region of the spectrum, allowing the concentrations of these species to be monitored in reagent solutions. The conjugate base, $^-$OCl, exhibits a band with λ_{max} 292 nm and ε_{292} 350/*M*/cm (271), whereas for $^-$OBr, the peaks are shifted to longer wavelength, with λ_{max}($^-$OBr) 329 nm and ε_{329} 332/*M*/cm (237).

In addition to the equilibria described, at acidic pH values in the presence of excess halide ions, HOCl and HOBr are also in equilibrium with molecular chlorine (Cl_2) and bromine (Br_2). These species have been reported to contribute to peroxidase-mediated damage under acidic conditions, as might be found in phagosomes (167, 381). Furthermore, it has been suggested that Cl_2 and Br_2, and not HOCl and HOBr, are produced directly by MPO (381). It has also been demonstrated that the interhalogen gas, bromine chloride (BrCl), can be generated *via* equilibration reactions between HOCl and Br^-, or HOBr and Cl^- (381). BrCl, generated *via* these reactions, has been implicated as a reactive species *in vivo* (178, 381). Some debate also exists, as outlined earlier, as to whether "free" halogenating species are formed at all, with enzyme-bound intermediates postulated as the key species, particularly in the case of MPO (255, 381). Although enzyme-bound species are undoubtedly formed in some cases, reagent HOCl and HOBr, and the oxidants generated by the enzymatic systems, have similar reactivities with a wide range of potential targets [*e.g.*, (447)]; it is therefore assumed in the following discussion that HOCl/HOBr are the reactive species involved, unless otherwise stated.

HOCl and HOBr react avidly with nucleophiles, especially those containing sulfur or nitrogen atoms, such as thiols, thioethers, amines, and amides. Thus, Cys residues in proteins and in reduced glutathione (GSH) are key targets for both HOCl and HOBr (70, 99, 185). Oxidation of these residues by HOCl yields a sulfenyl chloride (RS-Cl), which undergoes rapid reaction with excess thiol, to give the disulfide (70), or with water to yield sulfenic acid (RSOH), sulfinic acid (RSO_2H), and ultimately, the sulfonic acid, cysteic acid (RSO_3H) [reviewed in (160)]. The disulfides, can be further oxidized to sulfonic acids *via* S-chlorinated and S-oxygenated intermediates [reviewed in (160)]. HOCl can also induce the formation of sulfenamide (RSNR'), sulfinamide [RS(O)NR'], and sulfonamide [$RS(O)_2NR'$] crosslinks in peptides (*e.g.*, GSH) and proteins (122, 153, 332), *via* nucleophilic attack of Lys or Arg side chains on RS-Cl, sulfenic or sulfinic acid intermediates. Glutathione sulfonamide, formed from oxidation of GSH, has been postulated as a potential marker for MPO-mediated damage in biologic systems, as it is generated primarily by HOCl, and to a much lesser extent by HOBr, $ONOO^-$/ONOOH, or other oxidants (153, 448).

The high susceptibility of Cys residues to oxidation by hypohalous acids has important implications for cells. Thus, disruption of the cellular redox balance by conversion of GSH to oxidized glutathione (GSSG) is likely to impinge on a wide range of redox reactions, and a number of key cellular enzymes contain free Cys residues in their active sites. Thus, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are readily inactivated by HOCl, with the loss of activity correlating with thiol depletion (312, 329). Conversely, HOCl can activate the (inactive) pro- forms of matrix metalloproteinases (*e.g.*, MMP-7) *via* conversion of a key Cys residue in the cysteine switch domain of pro-MMP-7 to a sulfinic acid derivative (123).

Thioethers such as Met side chains are another favored target for hypohalous acids, with this resulting in conversion of Met to Met sulfoxide (and with very large excesses of oxidant

to Met sulfone) (156, 304). These compounds have little applicability as markers for MPO-mediated reactions, as they are readily produced by other oxidants. Oxidation of Met residues can lead to impairment of protein function, for example, inactivating enzymes (*e.g.*, lysozyme) and protease inhibitors such as a_1-antitrypsin (156, 158, 258, 428). This is not universally true, as inactivation of soybean trypsin inhibitor does not correlate with Met oxidation, even though this protein has a related structure and similar active site to a_1-antitrypsin (156, 158).

Nitrogen-containing functional groups, particularly amines and to a lesser extent amides, are readily converted to *N*-chlorinated and *N*-brominated species by HOCl/HOBr. The amine-derived species are generically known as halamines (chloramines, RR′NCl; bromamines, RR′NBr), whereas those species formed from amides are halamides (chloramides, RC(O)N(R′)Cl; bromamides, RC(O)N(R')Br). For primary halamines (where R′ = H), further reaction with HOCl and HOBr yields dichloramines ($RNCl_2$) or dibromamines ($RNBr_2$). With chloramines, dichloramine formation occurs to an appreciable extent only when a large excess of HOCl over amine is found (406). However, with bromamines, rapid equilibration reactions can occur that result in dibromamine formation, even when the amine is present in large excess (406).

All of the halamines exhibit moderate absorption bands in the UV region of the spectrum, with chloramines typically displaying a single peak at 252 nm [ε_{252} 350–450/*M*/cm; *e.g.*, (10, 402, 406)] and bromamines, a single absorbance at 288 nm with similar extinction coefficients to the chloramine peaks (406, 434). The absorbance spectra for the dihalogenated derivatives typically display two absorbance peaks that are either side of the absorbance for the monohalamines. Thus, dichloramines have a major absorbance maximum at 205 nm with a smaller peak at 300 nm [ε_{205} *ca.* 2,500 *M*/cm and ε_{300} *ca.* 350–400/*M*/cm (341, 402, 406)], whereas for dibromamines, the major absorption is at 241 nm, with a minor peak at 336 nm, with both displaying intensities similar to those observed for the dichloramines (406). The UV/visible absorption spectra of halamides are less distinct than those for the halamines, and typically feature a UV absorbance that increases in intensity from *ca.* 240 nm down to 200 nm (298, 299, 340, 342).

Halamines and halamides can be generated on a wide variety of biologic substrates. Free amino acids yield halamines on the α-amino group, and in cases such as Lys, His, and Arg in which the side chain features a nucleophilic nitrogen center, halamines are also generated at these sites (10, 15, 160, 298, 299, 402, 406). Incorporation of amino acids into proteins results in derivatization of the α-amino group, with halamine formation on proteins therefore restricted primarily to His, Lys, and Arg side chains, together with the *N*-terminal amino group. Halamide formation can occur at backbone peptide (amide) bonds with high excesses of hypohalous acids, and the amide-containing side chains of Gln and Asn also can be converted to halamides (160).

A range of other small molecules that contain an amino residue and are abundant *in vivo* can also form halamines. A prime example of this is the sulfonated β-amino acid, taurine, which has been postulated to act as a cellular defense mechanism against hypohalous acid production by reacting rapidly with HOCl and HOBr to give long-lived intermediates [reviewed in (364)]. However, taurine halamines are also capable of inducing further oxidation and hence detrimental cellular effects (312, 313, 364); thus, the protective role of taurine remains debatable.

Halamine formation also occurs on free nucleobases, nucleosides, nucleotides, and with DNA and RNA (141, 155, 305). The purine and pyrimidine bases contain two structurally distinct classes of nucleophilic nitrogen centers that yield halamines. Primary amine substituents that are exocyclic to the ring structures of the bases are present in cytosine, adenosine, and guanosine, whereas secondary amines that are part of the heterocyclic rings yield halamines in thymidine, uridine, and guanosine.

The amine head groups of phospholipids (phosphatidylethanolamine and phosphatidyl-serine) also react readily with HOCl/HOBr, with chloramines and bromamines detected on these sites (72, 163, 429); similar species are also generated by MPO/EPO in the presence of H_2O_2 and halide ions.

Hypohalous acids react with the amine groups of amino sugars (either free or derivatized to amides or sulfonamides) in glycosaminoglycans; this has been shown to occur with both free glycosaminoglycans (*e.g.*, hyaluronan) and those present on proteoglycans, which are an important component of the extracellular matrix. These reactions result in the generation of mono- and dihalamines, halamides (*e.g.*, $RN(Cl)C(O)CH_3$), and *N*-halosulfonamides (*e.g.*, $RN(Cl)SO_3^-$) (340–342). Subsequent decomposition of these species can result in release of polysaccharide fragments from both isolated glycosaminoglycans and extracellular matrix (342, 451).

It is clear from the data reviewed that halamines and halamides are key products of HOCl/HOBr reactions with a wide range of biologic molecules. These halamines/amides retain the oxidizing capacity of the parent oxidant and can induce further reactions (402, 403, 406), some of which regenerate the parent amine as a result of halogen transfer or radical reactions, whereas others result in modification of the amine group (154–158, 300, 301, 304, 310, 311, 313, 321, 339, 340, 342). One of the latter pathways is hydrolysis, which yields aldehydes, probably *via* imine intermediates (Fig. 2; reactions 1 and 2) (15, 160, 166, 370). Aldehyde formation from bromamines occurs more readily than from the corresponding chloramines (156, 158, 370). The resulting carbonyl species can react with protein or lipid amine groups to generate Schiff base imines (see Fig. 2; reaction 3), which can ultimately yield advanced glycation end products (AGEs) (36, 306); the latter have been linked to vascular disease [reviewed in (36, 306)]. Hydrolysis of His side-chain chloramines is a potential pathway for the formation of 2-oxo-His (160).

Halamines and halamides can decompose *via* radical pathways, with these reactions promoted by low-valent redox-active metal ions (Fe^{2+}, Cu^+) and $O_2^{-\bullet}$. These processes yield

$$RCH(CO_2H)NHCl \rightarrow RCH{=}NH + CO_2 + HCl \quad (1)$$

$$RCH{=}NH + H_2O \rightarrow RCHO + NH_3 \quad (2)$$

$$RCHO + R'NH_2 \rightarrow RCH{=}NR' + H_2O \quad (3)$$

FIG. 2. Decomposition reactions of chloramines.

nitrogen-centered radicals ($RNH^{\bullet}$) (154, 155, 157, 159, 339, 340, 342) that can undergo a range of intra- and intermolecular reactions, resulting in further oxidative damage. A number of these reactions involving amino acids and proteins have been reviewed (160).

As halamines and halamides retain the oxidizing capacity of the hypohalous acid from which they were generated, they are potentially capable of mediating cellular damage. Thus, evidence has been presented for halamines, rather than HOCl/HOBr, being major mediators of toxicity, as they are long-lived species that can diffuse through cellular membranes and hence mediate oxidative damage at remote locations away from their site of generation (111, 311, 403). Halamines are capable of oxidizing thiols and thioethers, such as Cys and Met, as described earlier for the parent hypohalous acids (310, 311), although halamines do not appear to produce the diversity of products observed with HOCl and HOBr. Thus, only sulfonic acids and disulfides have been detected on oxidation of thiols by halamines, with no evidence for the formation of glutathione sulfonamide (153). Halamines and halamides display a far greater selectivity in their reactions than do the hypohalous acids, with low-pK_a thiols (*e.g.*, those present in some enzymes) particularly susceptible to oxidation by chloramines (310–312). Halamines have been shown to modulate apoptotic pathways (431), inactivate intracellular enzymes (311, 312, 403), and induce cell death (311, 403).

In addition to reactions with nucleophiles, HOCl and HOBr also react with aromatic rings and double bonds, including some amino acids (Tyr and Trp), nucleobases, and fatty acid side chains [reviewed in (160, 301)]. Similar reactions also appear to occur with chloramines and bromamines, although at slower rates and with lower efficiency. Reactions with the phenolic side chain of Tyr are of particular importance, as these reactions result in the formation of 3-chlorotyrosine (3-chloro-Tyr) and 3-bromotyrosine (3-bromo-Tyr). With large excesses of oxidant, these products are further halogenated to form 3,5-dichorotyrosine (3,5-dichloro-Tyr) and 3,5-dibromotyrosine (3,5-dibromo-Tyr). These compounds constitute the only known specific biomarkers for HOCl-, HOBr-, or halamine-mediated damage to proteins (8, 103, 160, 165, 448). A number of studies have demonstrated that halamines are important intermediates in the formation of these materials on isolated proteins (40, 103, 156, 158, 370), although they are also formed *via* direct reactions with HOCl and HOBr, respectively (370). Oxidation of Tyr residues by HOCl and HOBr can also generate Tyr dimers (*o-o′* dityrosine; often known as di-Tyr) in low yield (156).

Oxidation of the Trp side chain by HOCl yields the 2-oxindole, possibly *via* an initial 3-chloroindole adduct, and subsequent hydrolysis (124, 160, 355); this product may also be generated by HOBr and halamines (304). Recent studies have shown that the products formed on oxidation of Trp in peptides or proteins depend on the local sequence, with a cyclized product detected when the neighboring residue is a Gly or Ala residue (124); with other side chains, mono- or dioxygenated derivatives of Trp are formed. Whether analogous products are formed with HOBr remains to be established.

Reaction of HOCl and HOBr with nucleobases generates stable halogenated products in addition to unstable halamines (see earlier). These products all feature new carbon-halogen bonds on the aromatic ring, and include 5-chlorocytosine, 5-chloro(2′-deoxy)cytidine, 5-chlorouracil, 8-chloroadenine, 8-chloro(2′-deoxy)adenosine, and 8-chloro(2′-deoxy)guanosine, and the corresponding brominated derivatives (81, 141, 176–179, 211, 257, 373, 443, 444). Of these products, 5-chloro- and 5-bromouracil have been detected in samples of human inflammatory tissue (177) and atherosclerotic lesions (393). HOCl and HOBr also generate a series of hydroxylated and ring-opened nucleobase-derived products (257, 443).

HOCl/HOBr and bromamines, but not chloramines, react with double bonds in unsaturated fatty acid side chains and cholesterol to give halohydrins ($RCH{=}CHR' + HOX \rightarrow RCH(X)\text{-}CH(OH)R'$); these can undergo further reactions to yield epoxides (18, 71, 72, 175, 199, 294, 381, 424). Evidence suggests that phospholipid halohydrins disrupt membrane structure, causing cell lysis (74, 429, 430), and are generated in HOX-treated lipoproteins (74, 75, 175, 448). Plasmalogens, which contain a vinyl ether linkage, rather than the normal ester linkage present in phospholipids, undergo rapid reaction with HOCl/HOBr, with this resulting in facile cleavage of the ether linkage, to give an α-halogenated aldehyde and a lysophospholipid (6, 409). Elevated levels of such chlorinated aldehydes have been detected in human atherosclerotic lesions (408).

B. Hypothiocyanous acid

MPO, EPO, and LPO are all capable of converting SCN^- into the corresponding (pseudo)hypohalous acid, hypothiocyanous acid (HOSCN) (13, 317, 319, 378, 401, 420, 422, 435). This species can also be generated by direct reaction of HOCl or HOBr with SCN^- (20, 279), and it has been suggested that the majority of HOBr generated under physiologically relevant conditions is converted to HOSCN (279). The pK_a of HOSCN is 5.3 (325, 399); thus, at pH 7.4, hypothiocyanous acid is present predominantly in the form of its conjugate base, $OSCN^-$. This anion exhibits a moderately intense absorption band in the UV region of the spectrum (λ_{max}, 235 nm), but some variation exists in the reported extinction coefficients [ε_{235} 1,290/*M*/cm (317), 1,480/*M*/cm (280)]. This peak is often obscured by the strong absorbance (<260 nm) of excess SCN^- in experimental studies, but a weak, characteristic absorbance at 376 nm (E_{376} 26.5/M/cm) has been attributed to $OSCN^-$ at high pH (280, 281).

Debate exists as to nature of the oxidizing species generated from SCN^- by peroxidases (13, 112, 116, 245, 280, 281, 317, 400, 422). As well as evidence for the formation of HOSCN, some data support the production of thiocyanogen $(SCN)_2$ (116, 281), cyanosulfurous acid (HO_2SCN), cyanosulfuric acid (HO_3SCN), and cyanide (CN^-) (400). It has also been demonstrated that $(SCN)_2$ can react further, for example, with excess SCN^-, to yield trithiocyanate $(SCN)_3^-$ (34). Furthermore, nonenzymatic reactions of HOSCN with excess H_2O_2 generate $(SCN)_2$ and HO_2SCN (116, 317). HO_2SCN and HO_3SCN decompose to cyanate (OCN^-), with the latter believed to be a major product of the EPO/H_2O_2/SCN^- system, together with $OSCN^-$ (13). In addition to species described earlier, it has been suggested that radicals including $SCN^{\bullet}$, $OSCN^{-\bullet}$, and/or $(SCN)_2^{-\bullet}$ may also be formed (112, 245, 420). For ease of reading, the oxidant species formed from SCN^- is designated later

as HOSCN, although it should be remembered that this may not be a true reflection of the exact structure of this oxidant.

Product studies indicate that HOSCN is much more selective than HOCl and HOBr, with strong evidence that the major targets for HOSCN are thiols (either on proteins, or low-molecular-mass species such as GSH) (13, 25, 146). The primary products of reaction of HOSCN with thiols are RS-SCN species and disulfides (13, 25). These adducts can react further (*e.g.*, to sulfenic acids) or be repaired by reductants (13, 25).

Limited evidence exists for damage to other targets such as aromatic residues, with modification of Tyr, His, and Trp detected after exposure of proteins and polypeptides (particularly those without Cys residues) to SCN^--derived oxidants (25). It has been postulated that these reactions occur *via* addition of ^+SCN from $(SCN)_2$ to the aromatic ring, rather than *via* reaction of HOSCN (25). It has also been suggested that RN-SCN species are generated with imidazole groups such as His (399), and recent studies have confirmed that HOSCN can modify His and Trp residues (Hawkins *et al.*, unpublished results).

Exposure of proteins to SCN^--derived oxidants has been shown to result in the loss of Lys residues and the formation of carbamylated Lys derivatives [$RN\text{-}C(O)NH_2$] (13). These products are formed *via* the irreversible addition of the HOSCN decay product, OCN^-, to the Lys amine group (13). Reagent OCN^-, and that formed *via* the decomposition of urea, react similarly and have been shown to target other (nucleophilic) moieties such as the α-amino groups of amino acids, peptides, and proteins, and (reversibly) thiol groups (382). Limited evidence exists for damage to biomolecules other than amino acids and proteins by HOSCN, although it might be expected that the decay product OCN^-, which reacts with amine groups, might yield carbamylated products with the amine functions present on amino sugars, phospholipid head groups, and nucleobases. Carbamylation of phospholipids and nucleobases occurs with reagent cyanate and isocyanates (239, 413), but has not been demonstrated with a peroxidase-mediated system. A couple of short reports suggest that $(SCN)_2$ can undergo addition across double bonds (48, 149), resulting in products that are analogous to chloro- and bromohydrins, but again, this has not been verified with phospholipids in a peroxidase-mediated system.

Although HOSCN is a much less powerful oxidant than HOCl and HOBr (13), considerable evidence indicates that this species can exert considerable biologic damage as a result of its greater specificity, particularly for thiols. Thus, SCN^--derived oxidants can inactivate a range of thiol-containing enzymes [*e.g.*, glyceraldehyde-3-phosphate dehydrogenase, glutathione *S*-transferases, and membrane ATPases (13, 146, 378)] and deplete intracellular GSH. HOSCN inactivates membrane ATPases 10 to 1,000 times more effectively than HOCl and HOBr because of this enhanced selectivity for critical thiol sites (13). Exposure of endothelial cells to HOSCN also induces tissue factor activity (437) and promotes the expression of cell-adhesion molecules (436), with these effects postulated to be due to specific oxidation of redox-sensitive thiols and resulting activation of NF-κB. If this is the mechanism, it would be expected that a large number of genes may be upregulated after exposure of cells to HOSCN (436).

C. Reactive nitrogen species

Nitrogen dioxide ($NO_2^\bullet$) has been reported to be formed as a result of the oxidation of NO_2^- by MPO, EPO, and LPO in the presence of H_2O_2 (60, 108, 135, 357, 421, 423, 435). $NO_2^\bullet$ is believed to be a key intermediate in the peroxidase-mediated nitration of Tyr residues, and it has been suggested that this could occur *via* peroxidase-mediated tyrosyl radical formation, followed by radical–radical termination with $NO_2^\bullet$ (421, 423). Formation of protein-bound tyrosyl radicals is postulated to occur *via* hydrogen atom abstraction from the protein-bound Tyr residue by small phenoxyl radicals (*e.g.*, on free Tyr) or by $NO_2^\bullet$ generated by the peroxidases (421, 423). $NO_2^\bullet$ has also been implicated as a key intermediate in peroxidase-mediated lipid peroxidation in the presence of NO_2^-, both *in vitro* (62) and in animal models of inflammation (467).

Recent studies have suggested that the reaction of $NO_2^\bullet$ with $O_2^{\bullet-}$ to generate peroxynitrate (O_2NOO^-) and the corresponding acid, peroxynitric acid (O_2NOOH) may be physiologically relevant at sites of inflammation (421). Peroxynitrate would be the dominant species at physiologic pH, as it has a pK_a of 5.85, but the role of this species in biologic damage may be limited, as it does not readily nitrate Tyr residues (unlike peroxynitrite, $ONOO^-$/ONOOH), and readily decomposes to NO_2^- and O_2 (224). At the mildly acidic pH values found at sites of inflammation (229), formation of peroxynitric acid would be favored; this is a stable and strong oxidant (224). Thus, peroxynitric acid has been shown to oxidize aromatic compounds such as Tyr, as well as NADH and Met [reviewed in (224)] and could contribute to peroxidase-mediated damage by NO_2^-.

In addition to these reactive nitrogen species, nitryl chloride (NO_2Cl) has been postulated to be formed by activated leukocytes (108). Formation of this product may be indirectly mediated by peroxidases, as it is generated *via* the reaction of peroxidase-derived HOCl with NO_2^- (107). This reaction occurs relatively slowly [$k \sim 10^4/M$/sec at pH 7.2, 22°C (293)] when compared with other reactions of HOCl that can occur *in vivo*; thus, the physiologic importance of this species may be limited (73, 135, 445). *In vitro* studies of the reactions of NO_2Cl have shown that it can both nitrate and chlorinate phenolic compounds such as Tyr residues (108) and DNA bases (82), and can also induce Tyr dimerization (108). A role for NO_2Cl in mediating lipid peroxidation has been discounted (62), with the observed oxidation believed to be mediated by $NO_2^\bullet$.

D. Singlet oxygen

Singlet oxygen is the first excited singlet state ($^1\Delta_g$, $^1O_2$) of molecular oxygen and has a relatively long lifetime of a few microseconds [reviewed in (98)]. It is one of the most important excited-state species generated in biologic systems and is highly reactive (98). It has been suggested for many years that $^1O_2$ is generated by activated leukocytes, although much debate has occurred on the validity of these assertions, as the specificity of the probe compounds that were initially used to detect $^1O_2$ has been questioned [reviewed in (229, 379)]. However, now considerable evidence indicates that $^1O_2$ can be generated by leukocytes, and that these reactions are mediated by products of peroxidase enzymes (12, 205, 207, 208, 225, 383, 384, 395). Kanofsky *et al.* (205, 208) demonstrated that LPO and

EPO generate $^1O_2$ in the presence of Br^- ions by monitoring photon emission at 1,268 nm, which is characteristic of the production of $^1O_2$; however, the concentrations of Br^- required to detect this species are physiologically relevant only for the EPO system. Initial evidence suggested that MPO could generate $^1O_2$ only at supraphysiologic levels of Br^- and low pH (<5) (207); however, more recent studies detected $^1O_2$ luminescence from reactions of MPO at physiologic pseudo(halide) concentrations at neutral pH (225). Formation of $^1O_2$ by neutrophils undergoing phagocytosis, and activated macrophages, has been demonstrated and quantified by using compounds that specifically trap $^1O_2$ (12, 383, 384).

It has been concluded that $^1O_2$ production arises primarily *via* reaction of HOBr with H_2O_2, as the yields of $^1O_2$ produced are maximal with elevated Br^- concentrations, or with EPO, which favors oxidation of Br^-. An equivalent reaction is known to occur with HOCl and H_2O_2 in isolated systems, but it appears that this is kinetically unable to compete with other reactions of HOCl at physiologic concentrations [reviewed in (395)], thus, $^1O_2$ is unlikely to be formed in high yields in the neutrophil phagosome (449). However, experimental data suggest that $^1O_2$ can account for up to 20% of the O_2 consumed by leukocytes under the conditions investigated (12, 208, 225, 383). The physiologic and pathologic relevance of these studies remains to be verified, as many of the systems studied have not contained nitrogenous substrates, at physiologic concentrations, which would be expected to convert a significant proportion of any HOCl or HOBr generated to chloramines and bromamines (160, 302, 448). The reaction of bromamines or dibromamines with H_2O_2 also generates $^1O_2$, but this is not the case with chloramines (206). Furthermore, studies that have included physiologic concentrations of SCN^- suggest that little, or no, $^1O_2$ production occurs (208, 225), probably as a result of the preferential oxidation of this anion to HOSCN (13, 279, 378, 420, 422), which does not react with H_2O_2 to form $^1O_2$.

It has been demonstrated that HOCl can also react with lipid hydroperoxides to yield $^1O_2$ *via* the intermediate formation of peroxyl radicals (264), although $^1O_2$ production *via* this mechanism has previously been reported not to be important (292). It is also possible that $^1O_2$ is generated by activated leukocytes *via* the spontaneous dismutation of $O_2^{\bullet -}$, in a mechanism independent of peroxidase enzymes (384, 395).

Whether or not $^1O_2$ is generated in appreciable yields by leukocytes, it would be expected that proteins would be the main target for further reactions, given their biologic abundance. Of the common amino acids present in proteins, Trp, His, Tyr, Met, and Cys are the primary targets of $^1O_2$ at physiologic pH values (98). Reactions of $^1O_2$ with Trp, His, and Tyr generate unstable endoperoxide or dioxetane intermediates, which can ring open to give hydroperoxides [reviewed in (98)]. Subsequent decomposition of these species results in the formation of mono- or dihydroxylated species and, in some cases, cyclized and ring-opened (for Trp and His) products (5, 282, 354, 452, 453). 3,4-Dihydroxyphenylalanine (DOPA) and di-Tyr may also be generated from Tyr oxidation in proteins (296), although this is disputed (29, 30). Many of the products generated by $^1O_2$ can undergo further reactions (371, 372), including the formation of crosslinks (*e.g.*, *via* the reaction of nucleophilic nitrogens, for example on His or Lys side chains, with the keto group of the oxidized His (5, 98, 412). Reaction of $^1O_2$ with Met and Cys generates Met sulfoxide and disulfides and (probably) cysteic acid, respectively, *via* $^1O_2$ attack at the sulfur center to form a zwitterionic intermediate (R_2S^+-OO^-) [reviewed in (98)].

$^1O_2$ also induces damage to other biomolecules with oxidation of DNA by $^1O_2$ resulting in the specific modification of guanine (63). A major product of such reaction is the common DNA oxidation product 8-oxo-guanine, with this arising *via* an endoperoxide generated by cycloaddition of $^1O_2$ onto the imidazole ring of guanine (63, 374). Oxidation of phospholipids and cholesterol also occurs *via* stereospecific addition of $^1O_2$ to the unsaturated double bonds, resulting in peroxide formation, and ultimately epoxide and hydroxylated derivatives. As the isomer distribution generated by $^1O_2$ is different from that induced by radical-mediated oxidation, the product distribution detected can yield definitive information on the generation of this oxidant (136, 139).

The peroxides generated on reaction of $^1O_2$ with biologic targets are themselves potentially damaging species. They can decompose to form free radicals and also are capable of oxidizing further susceptible species such as protein thiols. Thus, peptide and protein peroxides have been shown to inactivate enzymes (269), deplete low-molecular-mass antioxidants (270), and can mediate DNA damage such as strand breaks and DNA-protein adducts (247). They could also be involved in mediating further lipid damage (292).

E. Ozone

The generation of ozone (O_3) by activated leukocytes and peroxidase enzymes has been the subject of considerable controversy (28, 220, 229, 286, 328, 375, 379, 442). Data suggest that secondary reactions of $^1O_2$ can generate O_3, as well as H_2O_2 , and that these reactions occur in activated neutrophils (28, 286, 442). However, the dyes used to detect O_3 (*e.g.*, indigo carmine) are bleached by multiple oxidants including HOCl, $^1O_2$, and $O_2^{\bullet -}$ (28, 220, 286, 442). Data obtained with additional O_3 traps (*e.g.*, 3- and 4-vinylbenzoic acid) are also consistent with O_3 formation (28, 286). However, the proposed mechanism of O_3 formation requires the generation of significant yields of $^1O_2$, which, as outlined earlier, is far from certain, with a number of studies suggesting that this is physiologically improbable (395, 449). Kinetic considerations would suggest that, at the levels of HOCl, H_2O_2, and $^1O_2$ present in neutrophils (449), reaction to form O_3 would be highly unfavored.

F. Phenoxyl radicals

It is well established that MPO can mediate the formation of phenoxyl radicals from phenolic substrates in the presence of H_2O_2, in *in vitro* model systems, cells, and animal models (49, 170, 360, 416). Thus, free Tyr can be converted to di-Tyr, *via* radical–radical termination of two tyrosyl radicals (173, 410). Free tyrosyl radicals can also oxidize protein-bound Tyr residues, thereby creating di-Tyr crosslinks in proteins (174). Peptide and protein Tyr residues do not appear to be directly oxidized to tyrosyl radicals by MPO, presumably because of steric interactions with the enzyme (410). In addition to forming crosslinks, tyrosyl radicals can abstract hydrogen atoms

from suitable targets, including ascorbate and unsaturated phospholipids (170). It has been suggested that MPO-derived tyrosyl radicals are mediators of lipid peroxidation of LDL in *in vitro* studies (360), although the relevance of this process *in vivo* has been questioned (467).

A wide range of other phenolic compounds are also substrates for MPO-H_2O_2 systems, with this resulting in the formation of the corresponding phenoxyl radicals. Thus, paracetamol-derived radicals can mediate lipid peroxidation in LDL when generated *via* MPO or neutrophil systems (210). Similarly, it has been demonstrated that phenol is a good substrate for MPO, although it has been suggested that, in the HL60 cell line, the observed lipid peroxidation is mediated not by phenoxyl radicals directly, but instead *via* glutathionyl radicals ($GS^{\bullet}$) formed *via* hydrogen-abstraction from GSH by the phenoxyl radicals (49). It has also been shown that phenoxyl radicals from phenol can oxidize carotenoids and ascorbate (416).

G. Hydroxyl radicals

The formation of hydroxyl radicals ($HO^{\bullet}$) by activated leukocytes has been postulated since the 1970s, when it was first discovered that $O_2^{\bullet-}$ and H_2O_2 are generated by these cells (27). Although numerous studies have provided indirect evidence for the formation of $HO^{\bullet}$ by neutrophils and other activated phagocytes, little convincing evidence exists for direct $HO^{\bullet}$ formation by peroxidase enzymes (85, 151, 351), and $HO^{\bullet}$ generation by activated leukocytes is unlikely to occur in sufficiently high yields to be physiologically or pathologically important (229, 449). Thus, it is unlikely that significant concentrations of redox-active transition metal ions exist in activated phagocytes to catalyze Fenton chemistry (85, 151, 229), and the high concentrations of peroxidase enzymes would be expected to consume H_2O_2 efficiently (449). Peroxidase enzymes may also modulate a further potential source of $HO^{\bullet}$—the breakdown of peroxynitrite ($ONOO^-$) [reviewed in (276, 327, 331)]—as a result of the potential consumption of $O_2^{\bullet-}$, required for the formation of peroxynitrite, by intermediates in the enzymatic cycle of the peroxidase enzymes (see earlier). $HO^{\bullet}$ may also be generated indirectly *via* reaction of $O_2^{\bullet-}$ with HOCl (65); however, the physiologic relevance of this process is again questionable, as HOCl is extremely reactive with a wide range of biologic targets, and its reaction with $O_2^{\bullet-}$ may not compete significantly with other reactions of HOCl (449). The corresponding reaction of HOBr with $O_2^{\bullet-}$ is not believed to be a significant source of $HO^{\bullet}$, as one-electron reduction of HOBr is reported to yield HO^- and bromine atoms ($Br^{\bullet}$) instead of $HO^{\bullet}$ and Br^- (390).

V. INHIBITION OF OXIDANT GENERATION BY MYELOPEROXIDASE, EOSINOPHIL PEROXIDASE, AND LACTOPEROXIDASE

A. Limiting the availability of substrates for oxidant production

The availability of H_2O_2 for peroxidase-mediated oxidant generation is a key factor in determining the extent of oxidant production. The levels of H_2O_2 are determined to a major extent within cells, and at sites of inflammation, by the large number of enzymes that can efficiently remove this substrate for the heme peroxidases. Thus, the levels of catalase, glutathione peroxidases, and other enzymes that consume H_2O_2 in competition with MPO, EPO, and LPO can have an important bearing on the yield of oxidants generates by these heme peroxidases. The availability of H_2O_2 can also be limited by inhibition of the activity of the membrane-bound NADPH oxidase complexes present in phagocytic cells that generate $O_2^{\bullet-}$ and hence H_2O_2 *via* dismutation (26). Related NADPH oxidase complexes present in other cell types, including vascular cells (463, 466), may also be important targets for inhibition. A range of compounds has been identified that suppress NADPH oxidase activity *in vitro* and *in vivo* (104), and some can selectively inhibit vascular NADPH oxidase activity (105). The most commonly used agent to inhibit such activity is diphenylene iodonium chloride (DPI) (110, 150), although it should be noted that this agent also affects other sources of $O_2^{\bullet-}$ and hence H_2O_2, including mitochondrial sources (244).

$NO^{\bullet}$ can suppress NADPH oxidase activity in endothelial cells *via S*-nitrosylation of a key subunit of the enzyme complex, $p47^{phox}$ (367) and thus may limit peroxidase-mediated oxidation by decreasing vascular H_2O_2 production. However, inhibition of NOS *in vivo* has consistently been shown to inhibit protein nitration (330), highlighting the importance of preventing excessive production of $NO^{\bullet}$ and its downstream products, which may participate in peroxidase-mediated nitration reactions. Oxyhemoglobin oxidizes $NO^{\bullet}$ and NO_2^- to the redox-inactive product NO_3^- and may be an important intravascular inhibitor of peroxidase-dependent and -independent nitration reactions (330).

B. Inhibition of MPO, EPO, and LPO activity

As yet, no reported specific and potent inhibitors of MPO are known, although a large number of known agents act in a nonspecific manner, including general heme poisons such as azide and cyanide.

The copper-containing plasma protein ceruloplasmin avidly binds MPO (366) and inhibits its peroxidase and halogenation activities (295, 366) and therefore could participate in the clearance and inactivation of MPO *in vivo*; anti-MPO antibodies have been shown to reverse inhibition of the peroxidase activity of MPO by ceruloplasmin (145). The polyanionic glycosaminoglycan heparin, which is a widely used anticoagulant, binds the cationic MPO protein electrostatically (95) and can liberate vessel-associated MPO (33). Limitation of MPO activity within the vessel wall in this fashion could be an important, additional function of heparin. EPO has a pI similar to that of MPO, and heparin may also alter its tissue distribution.

Given the evidence for the role of hypohalous acid–mediated damage in inflammation-induced tissue injury, a great deal of interest exists in the therapeutic potential of peroxidase substrates that divert the catalytic activity of the enzymes from their halogenation cycles or irreversibly inhibit them. One approach to achieve the former objective involves the use of substrates that react readily with compound I and compound II, and thereby competitively inhibit oxidation of halides by compound I. The potential of competitive inhibitors is limited by the rel-

atively high concentrations required to compete with halide oxidation. It has been shown that physiologically relevant concentrations of paracetanol (<130 μM) (42) can effectively inhibit HOCl, HOBr, and HOSCN production by MPO-H_2O_2-halide systems, as assessed by measurement of total oxidant production (Rees *et al*., unpublished data). It should, however, be noted that paracetanol has previously been reported to enhance HOCl production by MPO by recycling compound II and compound III (254); however, in these studies, the initial rate of HOCl production, not the overall yield, was measured.

Poor peroxidase substrates can inhibit oxidant production by MPO at low concentrations by promoting compound II formation (196, 218). These substrates are relatively inefficient inhibitors of neutrophil HOCl production in the presence of a source of $O_2^{\bullet -}$, which efficiently recycles compound II (216, 218). On the basis of these data, it would be expected that $O_2^{\bullet -}$ will antagonize MPO inhibition by poor peroxidase substrates *in vivo*. Superoxide dismutase (SOD) may limit this process extracellularly (216, 218) but is unlikely to do so within the phagosome, where its concentration and access are limited. Differences in the redox properties between the peroxidase enzyme family may enable the development of specific, poor peroxidase inhibitors for MPO (196). Such compounds are likely to be those whose oxidation potentials lie between those for the compound I/compound II couple of MPO (1.35 V) and the corresponding couples for the other enzymes (*ca*. 1.1 V). Some substrates, such as hydroquinone (213) and amsacrine (222), can divert MPO from HOCl production in an analogous manner by promoting formation of compound III; again, $O_2^{\bullet -}$ would be expected to antagonize this inhibition by recycling compound III to the native enzyme.

The most effective inhibitors of the peroxidase family are those that mediate irreversible enzyme inhibition ("suicide" substrates). Hydrazines ($RNHNH_2$) and hydrazides ($RCONHNH_2$) are general suicide substrates for MPO (215), LPO (9), and other peroxidase enzymes such as horseradish peroxidase (HRP) (23). All of these inactivation reactions involve irreversible heme destruction. In the case of HRP, this is believed to involve direct, covalent modification of the heme group by substrate-derived radicals (23). In contrast, inhibition of MPO by benzoic acid hydrazides, which include the most potent MPO inhibitor identified to date, 4-aminobenzoic acid hydrazide (ABAH) (219), has been proposed to involve heme destruction *via* generation of ferrous MPO and subsequent reduction of this species (59); however, the precise mechanism is uncertain. Irreversible inhibition of MPO by the hydrazide derivative isoniazid, an antituberculosis drug, has been proposed to involve heme modification *via* a mechanism initiated by compound III formation (427).

Important limitations exist on the development of physiologic inhibitors of MPO, EPO, and LPO. In many cases, the concentrations of the inhibitor required to modulate oxidant production effectively may be toxic or be above the levels achievable by oral administration. Inhibition may be confounded by physiologic ligands of the enzymes, which restrict access to the active site; this effect has been observed in the inhibition of MPO-mediated LDL oxidation by thiol derivatives, in which bulky thiols such glutathione exhibited decreased ability to inhibit MPO in the presence of LDL (419). The effectiveness of inhibitors will also be reduced in an acidic milieu, because of the predominance of the halogenation activity of the enzymes at these pH values. Small suicide substrates, which may inhibit at very low concentrations, are an attractive class of compounds for development. Underlining the potential for this mode of inhibition, the suicide substrate ABAH has been shown to prevent experimental arterial injury by a MPO-H_2O_2-halide system in rats (457). Conversely, inhibitors that have high oral bioavailability and low toxicity have considerable therapeutic potential, even if their inhibition characteristics are comparatively modest.

VI. KINETICS AND SELECTIVITY OF DAMAGE INDUCED BY HEME PEROXIDASE–DERIVED OXIDANTS

A. Halogenating oxidants

Reported second-order rate constants for the reactions of HOCl with biologic substrates span >10 orders of magnitude (*k ca*. 10^7–10^{-3}/*M*/sec); the upper limit of this scale corresponds to reactions that are complete in a few milliseconds, suggesting that the lifetime of HOCl *in vivo* is of this magnitude (14, 15, 118, 272, 278, 298, 302, 303, 320, 325). The corresponding rate constants for HOBr typically display less variation than those for HOCl, but still span several orders of magnitude (279, 299, 302, 322, 325, 434). Rate constants for some chloramine-mediated reactions have also been determined, and these are generally a few orders of magnitude (up to 10^4 times) slower than for HOCl, but display a similar pattern of selectivity (298, 300–302, 304, 310, 311, 313, 321–323). The rate constants for bromamine reactions have not been studied as extensively [reviewed in (302)]. These data were reviewed recently (302) and are summarized briefly later.

In considering reported rate constants for reactions of HOCl and HOBr, it should be noted that these rate constants are markedly pH dependent, as the reactivities of the acids (HOCl and HOBr) differ dramatically from their conjugate bases ($^-$OCl and $^-$OBr) [reviewed in (15)], and this is also likely to be the case for the halamines. The pK_a values of HOCl and HOBr are close to physiologic pH values, and hence small pH variations may dramatically alter the rates of reaction (14, 15, 118, 298, 302). Furthermore, many target molecules (*e.g*., amines, amides, thiols) are readily protonated or ionized at pH values near physiologic, thereby creating further complications when considering the pH dependence of the reactions (10, 14, 15, 118, 298, 302). Thus, the reported rate data must be used with care at alternative pH values and temperatures; most of the kinetic data reported were obtained at physiologic pH (pH 7.0 to 7.5) and room temperature (*ca*. 20°C to 25°C).

1. Hypochlorous acid. Reactions with sulfur-containing compounds such as thiols and thioethers (*e.g*., Cys and Met side chains and GSH) are the fastest known, biologically relevant, reactions of HOCl (16, 298, 302, 447). These reactions have second-order rate constants *ca*. 3×10^7/*M*/sec at physiologic pH and 22°C (Fig. 3) (298); reaction with GSH is similarly rapid (118, 310, 447). The acidity of the sulfhydryl group in a range of thiols has little effect on the observed second-or-

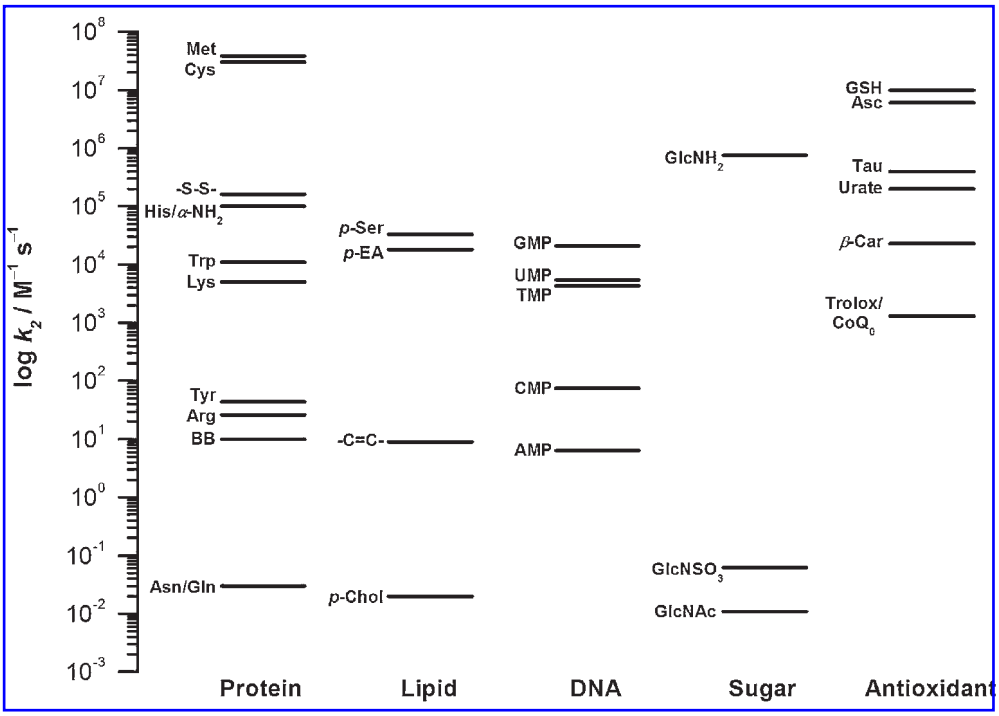

FIG. 3. Plot summarizing the second-order rate constants (on a log scale) for the reactions of HOCl with model compounds of protein, lipid, and carbohydrate components, nucleobases and antioxidants [reviewed in (302)]. The majority of the rate constants were acquired in phosphate-buffered solutions at pH 6.8 to 7.5 and 20°C to 25°C (118, 298, 303, 320, 321, 341, 447); however, that for β-carotene was determined in a detergent solution at pH 4.5 (7). -S-S-, disulfide bond; α-NH_2, α-amino group; BB, backbone amides; *p*-Ser, phosphoryl-Ser; *p*-EA, phosphoryl-ethanolamine; —C=C—, double bond; *p*-Chol, phosphoryl-choline; $GlcNH_2$, glucosamine; $GlcNSO_3$, *N*-sulfated glucosamine; GlcNAc, *N*-acetylated glucosamine; Asc, ascorbate; β-Car, β-carotene; CoQ_0, ubiquinol-0.

der rate constant (310). HOCl also reacts readily with disulfide groups, but with considerably lower rate constants (*k ca.* 2×10^5/*M*/sec) (see Fig. 3) (298, 302).

The next most reactive class of compounds–amines–are also nucleophiles. The second-order rate constants for these species cover a wide range of values (*k ca.* 10^6–10/*M*/sec), as the reactivity of these species is markedly structure dependent [reviewed in (302)]. The highest second-order rate constants (*k* 10^4–10^5/*M*/sec at pH 7.4 and 22°C) are generally for α-amino groups present in amino acids, peptides, and phospholipid models (*e.g.*,. phosphoryl-Ser) (10, 14, 15, 272, 278, 298, 302, 303), although reaction of HOCl with the primary amine group of the amino sugar, glucosamine has *k ca.* 8×10^5/*M*/sec (341), whereas that for the β-sulfonated amino acid, taurine, has *k ca.* 5×10^5/*M*/sec at pH 7.0 (118). Secondary amines (*e.g.*, those in cyclic structures, such as in nucleobases (320, 321) or the His side chain (298, 301)), react with similar or slightly slower rate constants. Nonactivated primary amines (*e.g.*, those on Lys side chains or phosphoryl-ethanolamine) have *k ca.* 5×10^3/*M*/sec) (298, 303). Surprisingly, the rate constants for reaction of HOCl with the exocyclic amines of nucleobases are much lower [1–100/*M*/sec; (320, 321)]. These rate constants are of a magnitude similar to those for other nitrogenous targets such as Arg side chains (298) and amide groups (298, 322). A wide range of values have been reported for amide groups, with those in cyclic dipeptides the most reactive (*k ca.* 10–100/*M*/sec); those for *N*-acetylated amino acids are much lower (*ca.* 10^{-3}/*M*/sec) (298, 322). The presence of nearby charge has a profound effect on these apparent rate constants (298). Rate constants for secondary chlorination of chloramines (to form dichloramines) have not been extensively studied; that for reaction of HOCl with monochlorinated glucosamine is *ca.* 14/*M*/sec (341).

Reaction of HOCl with aromatic and unsaturated compounds is much slower than with nucleophilic substrates, with chlorination of the Tyr ring occurring with *k ca.* 50/*M*/sec (298) and addition across double bonds (to give chlorohydrins) having *k* of 1–10/*M*/sec (303). The low rate constants for these reactions, which generate products that are used as biomarkers of HOCl damage, imply that if these products are observed *in vivo*, extensive damage has occurred. Trp residues are readily oxidized by HOCl with *k ca.* 1×10^4/*M*/sec; thus, in contrast to Tyr chlorination, Trp oxidation is likely to occur in tandem with chloramine formation and disulfide oxidation on proteins (298).

The water-soluble antioxidants ascorbate and urate react with HOCl with second-order rate constants of *ca.* 6×10^6/*M*/sec and 2×10^5/*M*/sec, respectively (see Fig. 3) (118, 447). In contrast, model compounds of lipid-soluble antioxidants react much less rapidly with HOCl (*k ca.* 10^3/*M*/sec) (Fig. 3) (303). These numbers imply that the scavenging effect of these antioxidants against HOCl generated *in vivo* is likely to be low, consistent with experimental data (162).

HOCl-mediated oxidation of heme groups (*e.g.*, iron protoporphyrin IX) is of potential significance, given the importance

of this prosthetic group in multiple enzymes and oxygen-storage and transport proteins. The rate constant for such reactions is, however, unresolved, with k for reaction of HOCl with isolated ferriprotoporphyrin IX under acidic conditions (pH 4.5) reported as being $2 \times 10^3/M$/sec (7), whereas reaction of HOCl with the heme moiety of MPO has been reported to be rapid ($k > 10^7/M$/sec) at neutral pH (126). It is, therefore, unclear whether direct heme modification by HOCl is physiologically/pathologically relevant.

2. Hypobromous acid. The known rate constants for the reactions of HOBr are much fewer than those for HOCl and are summarized in Fig. 4. This is likely to be due, at least in part, to the increased reactivity of HOBr that makes these rate constants difficult to measure. Much of the data obtained indicate that the HOBr rate constants are typically at least 10-fold larger than those for HOCl (299, 302).

As with HOCl, a large variation exists in the reported rate constants. Whereas those for amines and amides in amino acids, peptides, and proteins are 5 to 100 times higher than those for HOCl (*e.g.*, HOBr with the Lys side chain has k $3.6 \times 10^5/M$/sec) (298, 299, 302, 434), the values for reactions with Cys and Met residues (k $4 \times 10^6/M$/sec for Met, and k $1.2 \times 10^7/M$/sec for Cys) are slightly slower than with HOCl (298, 299, 302, 325). The values for disulfide bonds are similar for both oxidants (298, 299, 302, 325). The rate constants for reactions of HOBr with aromatic and unsaturated substrates display a much greater increase relative to HOCl than do the nucleophilic substrates. Thus, bromination of the Tyr side chain occurs with k *ca.* $2.6 \times 10^5/M$/sec, almost 5,000 times greater than that for chlorination by HOCl (299, 302). Similarly, Trp oxidation occurs ~ 450 times faster with HOBr (k $3.7 \times 10^6/M$/sec) than HOCl (299, 302). Reactions of HOBr with 3-pentenoic acid and sorbate, models of the double bonds of unsaturated fatty acid side chains, also occur much more rapidly (up to 1,000-fold greater) than with HOCl (377).

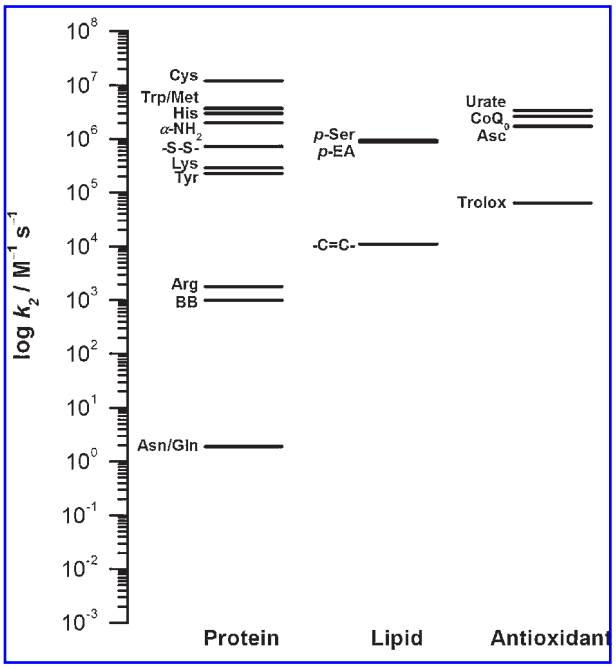

FIG. 4. Plot summarizing the known second-order rate constants (on a log scale) for the reactions of HOBr with model compounds of protein and lipid components and antioxidants [reviewed in (302)]. The majority of the rate constants were acquired in phosphate-buffered solutions at pH 7.2 to 7.5 and 22°C (299, 377). —S-S—, disulfide bond; α-NH_2, α-amino group; BB, backbone amides; *p*-Ser, phosphoryl-Ser; *p*-EA, phosphoryl-ethanolamine; —C=C—, double bond; Asc, ascorbate; CoQ_0, ubiquinol-0.

The greater rates of reaction of HOBr with unsaturated and aromatic targets, compared with HOCl, have important implications for the use of halogenated Tyr residues and phospholipid halohydrins as biomarkers in disease. The high rate constants for HOBr indicate that higher background levels of 3-bromo-Tyr and bromohydrins are likely to be present in control samples than the corresponding chlorinated derivatives. Second, whereas detection of increased levels of these brominated species in samples *vs.* controls demonstrates that HOBr-mediated processes have occurred, comparison of the extent of increase with the levels of the chlorinated materials is unwise.

HOBr reacts rapidly with SCN^- to yield HOSCN, with the rate constant for this process measured at pH >12, as the reaction was too fast to determine accurately at physiologic pH; these experiments yield values of $k(HOBr + SCN^-)$ of $2.3 \times 10^9/M$/sec and $k(^-OBr + SCN^-)$, of $3.8 \times 10^4/M$/sec) (279). These values indicate that SCN^- is likely to compete with organic targets for HOBr in plasma (279), even though it is present only at relatively low concentrations (*ca.* 50 to 200 μM). It has been proposed that SCN^- is a highly efficient scavenger of HOBr, which limits the ability of HOBr to cause biologic damage (279), although whether this is the case remains to be determined, as the HOSCN formed may be as damaging as, or more damaging than HOBr (see earlier). The corresponding reactions of HOCl/$^-$OCl with SCN^- are >2 orders of magnitude slower than for HOBr/$^-$OBr (20, 279), and hence are less likely to play a significant role.

3. Chloramines. These data indicate that chloramines are likely to be major intermediates in HOCl-mediated damage and can initiate further oxidation. The kinetics of chlorine-transfer reactions from chloramines formed on amino acids, small peptides, and nucleobases to other substrates have been studied in some detail (300, 301, 304, 310, 311, 313, 321–323); limited kinetic data also are available for the inorganic chloramine, NH_2Cl (191, 380). These data are summarized in Fig. 5 and elsewhere (302).

The available kinetic data for chloramine reactions indicate that the initiation of further damage is critically dependent on the structure of the chloramine. Thus, chloramines generated on endocyclic amines, such as those formed on the imidazole group of His (300, 301, 304), or certain nucleobases [thymidine, TMP; guanosine, GMP; and inosine (321, 322)], are much more reactive than chloramines formed on primary amines (300, 301, 304, 310, 311, 313, 321, 322). Thus, imidazole chloramines oxidize and chlorinate protein targets with rate constants that are typically only 5 to 25 times lower than those for HOCl (300, 301). Similarly, the ring chloramine of TMP reacts rapidly (k $4.3 \times 10^6/M$/sec at pH 6.9) with GSH (321), and chlorine transfer to peptide amine or amide groups occurs readily with all of the endocyclic nucleobase chloramines (321, 322).

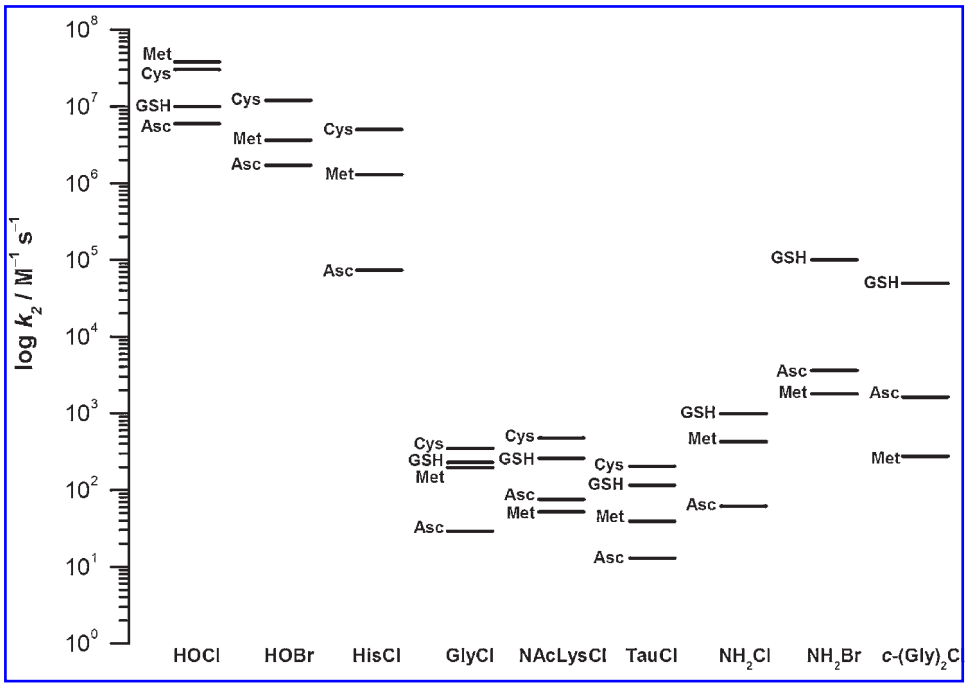

FIG. 5. Plot summarizing the known second-order rate constants (on a log scale) for the reactions of HOCl, HOBr, and various halamines with model compounds of the Met and Cys side chains, GSH, and ascorbate [reviewed in (302)]. The majority of the rate constants were acquired in phosphate-buffered solutions at pH 7.2 to 7.5 and 22°C (Pattison, Hawkins, and Davies, unpublished data) (118, 298–300, 310, 322, 326, 377). Asc, ascorbate; HisCl, chloramine of the His side chain; GlyCl, chloramine of the α-amino group of Gly; NAcLysCl, chloramine of the side-chain (ε) amino group of *N*-α-acetyl-Lys; TauCl, chloramine of the amine group of taurine; *c*-$(Gly)_2Cl$; monochloramide of the cyclic dipeptide, cyclo-$(Gly)_2$.

In contrast, primary chloramines formed on the α-amino group of Gly, the side chain of Lys, or on taurine react with substrates such as Met, Cys, GSH, disulfides, and Trp residues with second-order rate constants that are >5 orders of magnitude lower than the corresponding HOCl reactions (304, 310).

One notable consistent factor for such chloramine reactions, irrespective of their structure, is that the rate constants for reaction with Met are significantly lower than those for Cys, typically by 1 order of magnitude [reviewed in (302)]. Thus, unlike HOCl, chloramines appear to oxidize thiols selectively over Met residues. This selectivity is further enhanced in some cases by the structure dependence of these reactions, as the second-order rate constants for reaction of a range of thiols with taurine, Gly, and Lys side chain chloramines vary by a factor of 20, with a strong inverse correlation observed with the pK_a of the thiol target (310); this dependence is not observed with HOCl (310). These data imply that some thiols, particularly those with low pK_a values, may be especially susceptible to oxidation by chloramines; indeed, taurine chloramine has been shown to be more effective than HOCl at inhibiting the thiol-dependent enzymes, creatine kinase and GAPDH (312).

In addition to oxidation of sulfur-containing residues, chloramines of primary amines can undergo transchlorination reactions with other amines; thus, slow equilibration of chloramines has been observed between taurine, Gly, and histamine (313).

Chloramides also retain the oxidative potential of HOCl and can oxidize Cys, Met, and GSH, but at greatly diminished rate constants compared with HOCl (322). The reactions of cyclo-$(Gly)_2$ chloramide display an even greater selectivity for thiol (Cys) oxidation over Met oxidation, than those observed with chloramines [reviewed in (302)]. Interestingly, the rate constants for oxidation of ascorbate by chloramines and chloramides are typically reduced by a similar factor to that in thiols (118, 310, 311, 322); thus, ascorbate may be a more effective antioxidant toward chloramines and chloramides than HOCl [reviewed in (302)].

Several studies have shown that chloramines can mediate Tyr chlorination (40, 103, 156, 370); these reactions are, however, too slow to obtain accurate kinetic data. Product studies have shown that Tyr chlorination occurs with a range of model chloramines with varying rates and efficiency; imidazole chloramines chlorinate *N*-acetyl-Tyr rapidly, whereas primary chloramines on Gly or the Lys side chain react slowly (300, 301). The efficiency of chlorination by the less-reactive primary chloramines is decreased as a result of competing decomposition pathways that do not result in Tyr chlorination (300, 301).

4. Bromamines. Bromamines, as with chloramines, are likely to be major products of HOBr-mediated damage to a range of biologic materials, but to date, few studies exist on the kinetics of these reactions. One exception is NH_2Br, for which the second-order rate constants for the reactions with Met, GSH, and ascorbate have been determined (see Fig. 5) (326). From these data, it appears that the selectivity for oxidation of thiols and ascorbate, over Met, by bromamines may be even more pronounced than that observed for HOBr and chloramines (see earlier) (302).

B. Nitrogen dioxide

In contrast to the extensive kinetic data available for the halogenating oxidants described earlier, relatively few data are available for $NO_2^{\bullet}$. The data available have been reviewed quite recently (24, 224). It has been shown that protein Tyr and Cys residues are likely to be the major kinetic targets for $NO_2^{\bullet}$ (324). Although a rate constant for reaction with Tyr (in the dipeptide Gly-Tyr) has been determined at pH 7.5 [*k ca.* 3×10^5/*M*/sec (324)], the available data for Cys were determined at pH 9.2 [*k* 2.4×10^8/*M*/sec (324)], where the thiolate form predominates. Thus, the importance of the -SH group of Cys at physiologic pH is unclear. A rate constant for reaction with the Trp residue of Gly-Trp, at pH 6.5, has been estimated as *k ca.* 10^6/*M*/sec, although the expected indolyl intermediate could not be detected (324). No reaction was detected with the Met side chain or the disulfide bond of cystine (324). Linoleate and arachidonate react with $NO_2^{\bullet}$ with *k ca.* 10^5–10^6/*M*/sec at pH 9.2 (324), but later studies at pH 7.5 suggested that *k* is $<5 \times 10^4$/*M*/sec (120). $NO_2^{\bullet}$ also reacts with the isolated nucleotides of DNA and RNA with moderate rate constants at pH 8.5 [*k*, 10^6/*M*/sec (50)], but no reaction of $NO_2^{\bullet}$ was observed with duplex DNA at pH 6 to 7 (324). Competitive studies using oxidation of ABTS as a reference reaction have shown that $NO_2^{\bullet}$ oxidizes ascorbate at pH 6.5 with $k \sim 2 \times 10^7$/*M*/sec (120).

C. Phenoxyl radicals

Few reported rate constants are known for reaction of phenoxyl radicals in solution, with most of the available values limited to reactions in organic solvents (121). As the reactions of these species are likely to be solvent dependent, these data are not discussed further here.

VII. BENEFICIAL EFFECTS OF HEME PEROXIDASES IN DISEASE PREVENTION

A. Overview

The oxidants generated by peroxidases play a key role in defending the body against disease. Neutrophil-derived MPO is particularly important in mediating bacterial cell killing [reviewed in (151)], whereas EPO from eosinophils is largely responsible for destroying invading parasites [reviewed in (260)]. LPO is a potent antibacterial agent in milk, saliva, and tears [reviewed in (100, 345)] and has recently been demonstrated to play a role in the bacterial clearance of airways [reviewed in (89)]. As this area has been extensively reviewed elsewhere (see earlier and later), this topic is discussed only briefly for reasons of space.

B. Bacterial cell killing

The role of MPO in bacterial cell killing has been reviewed extensively [*e.g.*, (151, 229, 365)], so this topic is discussed in brief. *In vitro* studies have demonstrated that purified MPO, in the presence of H_2O_2 and halide ions, can effectively kill bacteria [*e.g.*, (227)]. It is generally believed that MPO-derived oxidants are the agents responsible for neutrophil-mediated bacterial cell killing (151), although this has been disputed recently (365). The importance of MPO in neutrophil-mediated killing is supported by studies using peroxidase inhibitors (azide and cyanide), where decreased killing ability was observed (228); it should, however, be noted that these species are general heme poisons and are not specific for MPO (see earlier). More recently, MPO-knockout mice have been shown to be more susceptible to bacterial infections, compared with wild-type, control animals, further supporting MPO as a key player in neutrophil-mediated defense (11, 182). Considerable evidence supports the generation of HOCl in the neutrophil phagosome, and subsequent reaction of this oxidant with bacterial proteins (79, 352). Other investigators have proposed that $^1O_2$ (12), $HO^{\bullet}$ (55), and O_3 (28) play important roles in bactericidal action, although the formation of O_3 has been disputed (220). Less-compelling direct evidence exists for a role for these species, because of the absence of specific reaction products generated by these species (see also earlier).

Despite this body of data supporting a role for MPO, the observation that the majority of MPO-deficient patients are not markedly more susceptible to severe or persistent infections, has resulted in questions as to the significance of MPO-mediated oxidant generation (407). Similarly, it has been shown that it is mainly the neutrophil-derived proteins, rather than the bacterial proteins, that are subject to chlorination in the phagosome in *in vitro* experiments (79). Neutrophil-mediated killing may involve the movement of potassium ions into the phagosome, which increases ionic strength and aids the solubilization and enzymatic action of neutrophil granule proteins, resulting in an environment incompatible with bacterial survival [reviewed in (365)]. However, this proposal has been disputed [*e.g.*, (114)]. Kinetic-modeling studies of the neutrophil phagosome are consistent with the formation of high fluxes of HOCl (449), and it has been predicted that the majority of HOCl will react with released neutrophil granule products, in accord with the observed experimental data (79). These data have led to the suggestion that chloramines and their subsequent reactions may be responsible for antimicrobial effects (449).

Similarly, it has been shown that purified EPO is bactericidal in the presence of H_2O_2 and either I^-, Br^-, Cl^-, or SCN^-, suggesting a role for hypohalous acid production by eosinophils in bacterial cell killing (201). Indeed, recent studies have shown that *Escherichia coli* organisms are rapidly and effectively killed by eosinophils by an O_2-dependent pathway, with the NADPH oxidase system acting in conjunction with EPO, suggesting that EPO-derived oxidants are the bactericidal agents (309). However, eosinophils are less efficient at killing bacteria than are neutrophils (459). This reduced bactericidal effect is generally attributed to a lower extent of phagocytosis and less efficient perforation of the bacterial envelope by eosinophils, rather than differences between MPO and EPO (459). However, EPO has also been reported to be less efficient at inactivating bacterial enzymes, which may contribute to cell death, compared with MPO (102, 459).

C. Destruction of parasites and other invading pathogens

The primary function of eosinophils is widely believed to be the destruction of invading parasites [reviewed in (260)]. Early

in vivo studies demonstrated a close association between dead or damaged parasites and eosinophils, and a correlation between resistance to parasites and the ability to induce eosinophilia after infection (260). These results have been confirmed in *in vitro* studies, in which it has been shown that eosinophils must be activated to achieve optimal killing efficiency, suggesting that EPO plays an important role in parasite killing (333). Indeed, isolated EPO, in combination with H_2O_2 and halide ions, is capable of killing schistosomula of the *Schistosoma mansoni* parasite (202). In addition to mediating the production of destructive oxidants, EPO has been shown to bind to the surface of schistosomula, which results in a significant improvement in the ability of neutrophils to destroy these parasites (202).

Eosinophils are also important in combating viral infections [reviewed in (140)]. Both activated eosinophils and isolated EPO in the presence of H_2O_2 and halide ions are virucidal to human immunodeficiency virus type 1 (HIV-1) (230). The destructive ability of activated eosinophils was compromised in the presence of the (nonspecific) peroxidase inhibitor azide, and H_2O_2-removing enzyme catalase, providing further evidence for the importance of EPO and its associated oxidants (230).

D. Oral hygiene

Salivary peroxidase (SPO), which is closely related to or identical to LPO, is the major peroxidase present in human saliva (319). Evidence also exists for the presence of MPO in saliva, due to release from oral leukocytes, in amounts proportional to the degree of gingival inflammation (67). The agent responsible for the antimicrobial properties of saliva is reported to be $^{-}$OSCN/HOSCN, produced by SPO- and MPO-catalyzed oxidation of SCN^{-} [*e.g.*, (68, 401)]. A number of *in vitro* studies have investigated the antibacterial effects of purified LPO on oral bacteria [reviewed in (318)]. It has been established that LPO-catalyzed oxidants readily inhibit glucose-stimulated metabolic events of *Streptococcus mutans* [*e.g.*, (69, 404)]. Similar inhibition of the growth of oral streptococci has been observed with purified SPO (252) and MPO (398) systems, and also with whole human saliva (246). Other strains of oral bacteria, including *Porphyromonas gingivalis*, which is known to be associated with periodontal diseases, are highly susceptible to $^{-}$OSCN/HOSCN–mediated damage (113). In addition, SCN^{-}-derived oxidants produced by peroxidases in saliva have antifungal effects, with oral fungi, including *Candida albicans*, displaying a loss in viability (243). Finally, it has been suggested that peroxidases may protect oral bacteria from H_2O_2-mediated toxicity, because of the conversion of H_2O_2 to a less-toxic oxidizing agent [*e.g.*, (4)]. However, the combination of LPO, H_2O_2, and SCN^{-} is much more effective than H_2O_2 alone, as an inhibitor of bacterial metabolism and growth under physiologic conditions, but if high levels of H_2O_2 are sustained for a long period, then H_2O_2 is an effective bactericidal agent, and LPO exerts a protective effect (405).

E. Airway mucosa

The body is protected against inhaled toxins and particles, including infectious agents, *via* a complex defense system involving the airway mucosa. The secreted mucus, in addition to providing a physical barrier against infection, contains a number of bactericidal agents, including the defensins (antimicrobial peptides) and lysozyme (antibacterial enzyme) (338). Cytochemical studies of airway mucosa provided evidence for the presence of active peroxidases (84). Studies in sheep have revealed that this peroxidase activity is most likely due to LPO (89, 137). It has been demonstrated that airway secretions contain sufficient SCN^{-} and H_2O_2 to support LPO-catalyzed production of HOSCN [reviewed in (89)]. Inhibition of LPO resulted in a significant decrease in the extent of bacterial clearance from the airway, suggesting that LPO plays a key role in airway defense (137). These studies have been extended recently, with evidence obtained for the presence of catalytically active LPO in human airways (446). The importance of LPO in airway defense has been highlighted by two recent studies that have shown that this protective mechanism is defective in patients with cystic fibrosis, due to insufficient SCN^{-} secretion (90, 275).

VIII. INVOLVEMENT OF HEME PEROXIDASES IN THE INITIATION AND PROGRESSION OF DISEASE

A. Overview

Although the oxidants generated by peroxidases play a key role in microbial killing and viral inactivation, excessive or misplaced generation of these reactive species has been linked to tissue damage. Increasing evidence indicates that the initiation and accumulation of oxidative damage mediated by peroxidases plays an important role in the progression of multiple diseases and particularly those with a major inflammatory component. The presence of active peroxidases, and the specific biomarkers for HOCl- and HOBr-mediated damage, 3-chloro-Tyr and 3-bromo-Tyr, respectively, in diseased tissue is consistent with a role for MPO and EPO in multiple pathologies. LPO may also play an important role in disease, particularly in the development of some cancers (78, 203).

It is becoming increasingly recognized that genetic polymorphisms of peroxidases, particularly MPO, may play an important role in disease development. The MPO G-463A polymorphism, in particular, has been studied in detail. This G/A transition is within an Alu sequence promoter region containing a hormone-response element (315). The presence of the G-allele results in high levels of MPO gene expression, because of increased binding at the SP1 transcription factor site, which has been postulated to have a detrimental effect, leading to an increased incidence of disease development (21, 277, 346, 347, 349). The GG genotype is present in ~60–65% of the population in the United States (346). In contrast, the A-allele (GA/AA) is associated with decreased SP1 binding, and hence lower MPO gene expression, which has been associated with a decrease in disease incidence (361). It has also been demonstrated that an EPO polymorphism (Pro-358-Leu) is strongly involved in the development of a common allergic disorder, pollinosis (hayfever) (283).

B. Atherosclerosis and cardiovascular disease

The evidence for an involvement of MPO in cardiovascular disease is particularly compelling [reviewed in (171, 285)]. En-

zymatically active MPO protein is present in all grades of human atherosclerotic lesions (97). Immunostaining of arterial samples with anti-human MPO antibodies has shown that MPO is present throughout the intima of lesions both intra- and extracellularly, with particularly high levels in the shoulder regions where rupture often occurs (97). Similarly, the intensity of staining arising from a monoclonal antibody (HOP-1) specific for HOCl- (and probably HOBr-) damaged proteins correlates with intimal thickening in human lesions of differing severity (164). Marked increases in the levels of 3-chloro-Tyr have also been detected in human atherosclerotic lesions compared with healthy tissue (165). Similarly, other markers of MPO-mediated damage, including *p*-hydroxyphenylacetaldehyde, α-chloro fatty aldehydes, unsaturated lysophosphatidylcholine, and 5-chloro-uracil, are elevated in lesions (168, 393, 408), supporting the presence of enzymatically active MPO in lesions and generation of chlorinating oxidants during the development of atherosclerosis.

Both an active MPO/H_2O_2/Cl^- system and reagent HOCl promote the oxidation of low-density lipoprotein (LDL), which may contribute to the progression of atherosclerosis (161, 170, 171). Reaction of HOCl with LDL results in the transformation of the protein into a form that is taken up readily by macrophages, leading to the formation of lipid-laden foam cells (161), an early and defining feature of atherosclerotic lesions. MPO-derived, chlorinating oxidants are believed to play an important role in LDL oxidation in the artery wall, as evidenced by the presence of 3-chloro-Tyr in LDL isolated from lesions at significantly higher levels (*ca*. 30-fold) than in circulating LDL (165). The role of MPO and reagent HOCl in the modification of LDL was reviewed recently (251). MPO has also been postulated to damage LDL in the artery wall *via* the generation of tyrosyl radicals (170, 240) and reactive nitrogen species (169) (see also earlier).

MPO has been reported to reverse the cardioprotective effects of high-density lipoproteins (HDL) *via* the selective modification of apolipoprotein A-1 (apoA-1), the primary protein component of HDL, with this postulated to arise from the generation of chlorinating oxidants and reactive nitrogen species (40, 41, 469, 470). This damage is believed to be of importance in cardiovascular disease (40, 307, 469, 470). Thus, the apoA-1 from HDL extracted from human atherosclerotic lesions is enriched in 3-chloro-Tyr and 3-nitro-Tyr (40, 307, 470). In addition, patients with cardiovascular disease have HDL that contains higher levels of both chlorinated and nitrated apoA-1 compared with healthy control subjects (470). *In vitro*, HDL appears to lose its antiatherogenic properties on treatment with HOCl, as evidenced by an impairment in the capacity of HDL to efflux cholesterol, and the conversion of this lipoprotein to a form readily taken up by macrophages (39). Similarly, analysis of circulating HDL, and HDL associated with the artery wall in patients with cardiovascular disease, revealed that MPO-mediated modifications to apoA-1 correlated with an impairment of the ABCA1-dependent reverse cholesterol-transport capacity of the lipoprotein (40, 469, 470). Immunoprecipitation studies have revealed that MPO binds to apoA-1 in plasma, which may facilitate the oxidation of HDL observed *in vivo* (470).

MPO has been postulated to contribute to cardiovascular disease by promoting endothelial dysfunction. Studies with a rodent model of traumatic shock revealed that endothelial dysfunction was accompanied by markedly elevated levels of MPO (64). It was demonstrated that the dilatation of isolated perfused guinea pig hearts, induced by acetylcholine and other vasodilators, was prevented on treatment with HOCl (242), suggesting a role for MPO-derived oxidants in endothelial dysfunction. Similarly, exposure of arterial rings to HOCl resulted in an impairment of endothelium-dependent relaxation mediated by acetylcholine, which stimulates $NO^\bullet$ production by endothelial cells (385, 464). In one study, this effect was reversed by addition of L-Arg (464), and it was found that chlorinated L-Arg derivatives could act as effective nitric oxide synthase inhibitors (465). Other studies have attributed the impaired endothelium-dependent relaxation to a reduction in endothelial nitric oxide synthase dimer stability (385). It has also been suggested that the altered vascular responsiveness of precontracted rings to acetylcholine is due to the catalytic consumption of $NO^\bullet$ by substrate radicals generated by MPO, rather than reactions induced by HOCl (109). Recently, it was shown that serum MPO levels independently predict endothelial dysfunction in humans, highlighting the potential importance of these reactions (432).

Finally, recent evidence supports a role for MPO in plaque rupture. Thus, macrophage MPO expression and HOCl-modified proteins colocalize in lesions from patients that experienced sudden cardiac death (388). Formation of HOCl by MPO in the subendothelium is postulated to be involved in plaque erosion and thrombogenesis, as treatment of endothelial cells with MPO-generated HOCl induces apoptosis and tissue factor activity at low, sublethal doses of oxidant (389). It has been suggested that degranulation of phagocytes and subsequent release of MPO may also promote plaque rupture *via* the activation of matrix metalloproteinases (188). Recently, it was shown that the level of MPO in the circulation of patients with acute coronary syndromes can independently predict an early risk of myocardial infarction (31, 53), which suggests that the ability of MPO to destabilize plaques is of particular importance in humans with cardiovascular disease.

Although convincing evidence is found for MPO-induced damage in human atherosclerotic lesions, the situation in mice is less clear. MPO does not appear to play a significant role in lesion development in LDL receptor–deficient mice, with MPO-knockout mice actually displaying enhanced lesion development (54). Physiologic differences between species may explain these discrepancies (54), as it has been shown that (a) MPO levels are markedly higher in humans than in mice; (b) MPO regulation and induction vary between species; (c) MPO is not present in lesions in the parent mouse strain of the knockout mice, whereas it is readily detectable in human lesions; and (d) 3-chloro-Tyr, a marker of MPO-derived oxidants, is undetectable in mouse lesions, whereas it is highly elevated even in early human lesions. Thus, MPO appears to play a much greater role in humans than in mice.

It was suggested recently that human MPO transgenes may enhance the utility of mouse models for diseases such as atherosclerosis, as the mouse MPO gene lacks the primate-specific Alu-receptor response element (236). Thus, mice made transgenic with the human MPO gene show enhanced atherosclerosis (259). Similarly, transgenic mice expressing the different human MPO alleles with either G or A at position 463, crossed with LDL receptor–deficient mice, exhibited significantly

larger aortic lesions compared with control LDL receptor–deficient mice (77). In addition, mice expressing the MPO G allele developed significantly higher serum cholesterol, triglycerides, and glucose levels, which correlated with increased weight gain and obesity, supporting a causative role of the higher-expressing genotype in cardiovascular disease (77).

Epidemiologic studies on the role of MPO genetic polymorphisms and incidence of cardiovascular disease in humans are conflicting. It has been reported that the A allele is associated with a decreased risk of developing coronary artery disease (287). This conclusion is supported by a study that showed an increased risk of cardiovascular events in patients with the higher-expressing G allele compared with the A allele (21). Similarly, patients with chronic kidney disease were more likely to develop cardiovascular disease if they had the G allele of the MPO gene (142). However, the presence of the A allele is associated with increased lipid levels in the healthy population, a known risk factor for cardiovascular disease (184). In addition, the low-expression MPO genotypes (containing the A allele) have been associated with larger fibrotic and calcified lesions in the thoracic and abdominal aorta, suggesting a protective role of the G allele in atherosclerosis development (249). Thus, the precise role of the MPO gene polymorphism in cardiovascular disease remains to be fully established.

C. Carcinogenesis

It is well established that chronic inflammation caused by infectious agents (*e.g.*, hepatitis B and C, *Helicobacter pylori*, schistosomiasis) and noninfectious particulate irritants (*e.g.*, asbestos) or allergens in asthmatics is associated with a greater incidence of cancer [reviewed in (440)]. The chronic cell killing that is observed at sites of inflammation is believed to result in a compensatory increase in cell division of surviving cells. If the genomic DNA in these cells is damaged by the oxidants produced by MPO and EPO *via* the immune response, this may lead to mutagenesis and the development of cancer (441). Thus, stimulation of neutrophils has been shown to induce genetic damage in cocultured cells [*e.g.*, (438)]. This genotoxicity was not observed when neutrophils that lack the ability to generate oxidants (isolated from humans with chronic granulomatous disease) were used (439). The addition of SOD, catalase, and low-molecular-mass antioxidants inhibited these deleterious effects, supporting a role of MPO-derived oxidants in mediating cellular DNA damage (438). Recent *in vivo* studies provided evidence for elevated levels of MPO and markers of oxidative stress in colonic tumors, compared with normal tissue, supporting the hypothesis that MPO-derived oxidants produced by activated neutrophils play a role in carcinogenesis (334).

Evidence has been presented for the presence of both chlorinated and brominated DNA bases in human inflammatory tissue, suggesting that these reactions are pathologically relevant (177). Halogenated DNA bases have been shown to induce downstream cellular effects, with chlorinated pyrimidine bases, on conversion to deoxyribonucleotides, being cytotoxic, effective mutagens, clastogens, and inducers of sister-chromatid exchange [reviewed (273)]. Thus, 5-chloro- (and 5-bromo-) cytosine can mimic 5-methylcytosine, resulting in enhanced sequence-specific DNA–protein interactions; this could result in unintended, potentially inheritable, gene silencing that has been postulated as a mechanism in cancer development (418).

MPO has been detected in human lung bronchoalveolar fluid in healthy subjects, consistent with the presence of activated neutrophils at such sites (363). The levels of MPO detected, arising from neutrophil infiltration, are increased after exposure to cigarette smoke and particulate irritants including asbestos (187). This led to the hypothesis that MPO present in the lung can contribute to the development of lung cancer *via* the activation of specific procarcinogens contained in cigarette smoke, including benzo[*a*]pyrene intermediates, 4-aminobiphenyl, and arylamines (93, 314, 415). Similarly, peroxidase-mediated activation of arylamines, postulated to be mediated by LPO present in breast milk, has been proposed to play an important role in the development of breast cancer (203). LPO has also been proposed to be involved in breast carcinogenesis because of oxidation *via* one-electron reduction, of estrogenic hormones (78). The resulting estrogenic quinones have been shown to interact with DNA and form mutagenic adducts that can initiate cancer (78).

Epidemiologic studies have supported the hypothesis that MPO plays a direct role in carcinogenesis *in vivo*, *via* correlation of disease incidence and the G-to-A substitution (-463G/A) polymorphism in the promoter region of the MPO gene. The variant 463A allele of MPO, which is responsible for decreased expression of MPO, has been associated with a reduced risk of lung cancer in several independent studies [reviewed in (226, 361)]. In contrast, the 463G allele, which is known to activate transcription of MPO, is overrepresented in acute promyelotic leukemia, suggesting that higher levels of MPO are associated with an increased risk of developing this type of leukemia (346).

D. Lung disease, respiratory damage, and allergens

1. Cystic fibrosis. It has been proposed that the progressive lung dysfunction and chronic pulmonary inflammation associated with cystic fibrosis is related to the formation of MPO-derived oxidants produced by neutrophils [*e.g.*, (57, 450]. Sputum samples collected from patients (young children and adults) with cystic fibrosis contain large amounts of active MPO (223, 425). It has been shown that the peroxidase activity in sputum from patients with cystic fibrosis correlates with the severity of the disease, supporting the hypothesis that peroxidase-derived oxidants contribute to lung dysfunction (343). More recently, it was demonstrated that the MPO activity of circulating neutrophils correlates with airway obstruction and sputum production in cystic fibrosis patients, suggesting that circulating neutrophils may deliver active MPO to the airway, thus contributing to airway injury (134).

MPO-derived HOCl is believed to be particularly important in mediating lung damage in cystic fibrosis patients, as evidenced by the detection of high levels of proteins containing the biomarker 3-chloro-Tyr in sputum and bronchoalveolar lavage fluid (223, 425); the detection of elevated levels of this biomarker, even in young children with cystic fibrosis, implies a role for HOCl in early lung damage (223). Oxidants produced by MPO also increase airway epithelial permeability in a rat trachea model (344); such data may explain the leakage of plasma proteins into the airway lumen of humans with cystic fibrosis (343, 344).

It has been reported that α_1-antitrypsin isolated from cystic fibrosis patients was partially oxidized, as it was truncated by porcine pancreatic elastase (66). Thus, MPO may also be responsible for indirect injury to the lung in cystic fibrosis by mediating the inactivation of this protease inhibitor, with resulting increased enzyme-induced damage (43). Similarly, MPO activates latent collagenase and the metalloproteinase gelatinase (308). In combination, these effects may enhance protease-mediated damage to the airway epithelium in cystic fibrosis patients (43).

Eosinophils and EPO also play a role in the lung dysfunction observed in cystic fibrosis. Evidence has been presented for the presence of elevated levels of EPO and other eosinophil proteins in patients with cystic fibrosis (231, 232). However, no significant elevation was found in the eosinophil cell numbers observed in these patients compared with control subjects, suggesting that these cells were activated in the patients but not in the controls (231, 232). The levels of EPO and other eosinophil proteins correlated strongly with lung dysfunction in the cystic fibrosis patients, suggesting that eosinophil activation and the formation of oxidants may play a role in pulmonary damage (232). This hypothesis is supported by the detection (by NMR spectroscopy) of elevated levels of both chlorinated and brominated Tyr derivatives in the sputum of patients with cystic fibrosis (359). Thus, it appears that both HOCl, produced by MPO, and HOBr, produced by EPO, may play a role in mediating lung damage in cystic fibrosis patients.

Finally, the proposed beneficial role of LPO in maintaining airway defenses by catalyzing the formation of HOSCN, a potent bactericidal agent (see earlier), is not applicable in cystic fibrosis patients (90, 275). Thus, the levels of SCN^- in the cystic fibrosis epithelia were significantly reduced compared with normal epithelia, because of a lower SCN^--transport rate, resulting in reduced SCN^- accumulation (90). The lack of SCN^- was found to render the LPO antimicrobial system inactive (275), even in the presence of elevated levels of LPO or H_2O_2 (90).

2. Asthma. The association of eosinophils with asthma and allergic diseases was established in the 1880s [reviewed in (263)]. Airway function in children with asthma correlates strongly with serum markers of eosinophil activation (337). Similarly, the level of EPO in asthmatics was found to be dependent on the severity of the disease, with significantly higher levels of EPO detected in people with severe asthma, compared with those with mild or moderate disease (297, 358). These studies provided indirect evidence that EPO and EPO-derived oxidants may play a role in lung damage.

The recent detection of 3-bromo-Tyr has provided the first direct evidence for the involvement of EPO and brominating oxidants in the tissue damage associated with asthma [reviewed in (172)]. Thus, elevated levels of the HOBr biomarker 3-bromo-Tyr have been detected on bronchoalveolar lavage proteins from people with asthma compared with controls (455). Similarly, significantly higher levels of EPO and 3-bromo-Tyr, but not MPO and 3-chloro-Tyr, were observed in sputum samples from people with asthma compared with healthy controls, further supporting a role for HOBr (8). MPO appears to play a role in the disease process only in the case of very severe asthma (197) or in the presence of a bacterial infection (396).

Eosinophils have also been proposed to be a major source of reactive nitrogen species in asthma (248, 454). Thus, EPO can use NO_2^- to generate species capable of nitrating protein Tyr residues (454), with bronchoalveolar lavage fluid from patients with severe asthma found to contain 10-fold higher levels of 3-nitro-Tyr compared with control subjects (248).

E. Neurodegenerative diseases

Evidence has been obtained for an increased expression of MPO in the brains of patients with Alzheimer's disease (143, 347), Parkinson's disease (83), and multiple sclerosis (277). These data, together with the detection of increased levels of 3-chloro-Tyr, suggest that MPO and chlorinating oxidants may play an important role in the progression of neurodegenerative diseases [reviewed in (458)]. Epidemiologic studies have also reported a correlation between the incidence of both Alzheimer's disease (347) and multiple sclerosis (277, 460) with the polymorphism in the promoter region of the MPO gene, associated with increased MPO expression.

Alzheimer's disease is characterized by the presence of insoluble plaques containing high levels of β-amyloid peptide (368). An increase in MPO expression has been suggested to contribute to the pathology of Alzheimer's disease by promoting the aggregation of β-amyloid peptide, as MPO has been shown to colocalize with β-amyloid peptide in senile plaques in cerebral cortex sections of brain tissue from Alzheimer's patients (347). A later study confirmed these findings and revealed that the MPO in brain tissue with Alzheimer's pathology was enzymatically active, and that the MPO-specific oxidation product, 3-chloro-Tyr, was also present at elevated levels, consistent with a role of HOCl in mediating damage to brain tissue (143). Alzheimer's disease is also associated with defects in cerebral energy metabolism, which may be due to a reduction in the activity of α-ketoglutarate dehydrogenase complex (138). MPO-derived, chlorinating oxidants can inhibit both purified and cellular α-ketoglutarate dehydrogenase (198), thereby providing a potential pathway by which MPO can contribute to neurodegenerative disease.

Epidemiologic studies on the incidence of Alzheimer's disease and the MPO −463G/A polymorphism have reported that overrepresentation of the G allele, leading to higher expression of MPO, is important in disease development in women (241, 347). It was later shown that the presence of the higher-expressing MPO G allele was associated with Alzheimer's disease in a white, but not in a Hispanic, population, although in this case, no relation was found with gender (92). In contrast, risk of Alzheimer's disease in Finnish men (not women) was found to increase if the lower-expressing MPO A allele and APOE ϵ4 allele were both present (348). Two other studies have reported negative data for an association between the MPO gene polymorphism and incidence of Alzheimer's disease (88, 386).

MPO has been implicated in Parkinson's disease indirectly, by a study that has shown that inflammation increases the risk of developing this neurologic disorder (80). This disease is characterized by the loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta region of the brain (96). It was shown recently that the level of MPO expression is increased markedly in the substantia nigra pars compacta from patients with Parkinson's disease, and mice treated with the

neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to induce loss of dopaminergic neurons (83). Elevated levels of 3-chloro-Tyr and HOCl-modified proteins were observed in the brains of mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, suggesting that oxidants produced by MPO may mediate tissue damage in this model of Parkinson's disease (83).

MPO has also been suggested to play a role in the progression of the inflammatory disease of the central nervous system, multiple sclerosis. This degenerative disease results in the gradual demyelination and transection of neuron axons, which causes lesions (scleroses) throughout the brain and spinal cord. MPO is present in macrophages/microglia in lesions from patients with multiple sclerosis (277). It was suggested that the increased expression of MPO by the macrophages/microglia may accelerate damage to the myelin sheath *via* the production of oxidants, although no evidence for the presence of 3-chloro-Tyr or HOCl-modified proteins was obtained (277). Multiple sclerosis has been reported to be associated with the presence of a genetic polymorphism that results in increased MPO expression, particularly in early-onset disease in females (277), and severe tissue damage (460). These findings are supported by a study that found MPO levels in opticospinal multiple sclerosis patients in remission were related to severe tissue destruction (262). However, these findings have been disputed. Thus, it has been demonstrated that no association exists between genetic variants of the MPO promoter gene and disease onset, susceptibility to, or the course and severity of multiple sclerosis (209). Similarly, patients with multiple sclerosis have been reported to have lower leukocyte MPO activity compared to healthy control subjects (335).

F. Kidney disease

MPO has been implicated in the pathogenesis of renal disease [reviewed in (229, 250)]. Early studies demonstrated that MPO may be important in neutrophil-mediated glomerulonephritis, by infusing MPO into the renal artery of rats, followed by nontoxic amounts of H_2O_2 and Cl^-; this treatment resulted in glomerular injury and proteinuria (200). The authors postulated that oxidants, particularly HOCl, were likely to be the mediators of the damage observed (200). The detection of elevated levels of neutrophils and MPO in renal biopsies of patients with Wegener granulomatosis (56), and a rat model of renal dysfunction (180), support a role for MPO in these pathologies. It was further demonstrated that inflamed kidney tissue contains significantly elevated MPO activity compared with noninflamed, control kidneys in a rat renal disease model (180). Similarly, MPO activity was increased in the kidneys of mice during glomerulonephritis (291). Patients with glomerulonephritis have been shown to have increased levels of MPO in their glomeruli, together with an associated increase in MPO-specific, anti-neutrophil cytoplasmic antibodies (MPO-ANCA) (353). The infusion of anti-MPO antibodies into immunodeficient mice resulted in the development of vasculitis and glomerulonephritis, suggesting that in humans, MPO-ANCA may play a causal role in kidney disease (94). Direct evidence for the presence of MPO-derived oxidants, particularly HOCl, has been obtained by the immunohistochemical detection of HOCl-modified proteins, which were often found to be colocalized with MPO, in renal tissue from patients with kidney disease (147). Elevated MPO activity in chronic hemodialysis patients also has been reported, as evidenced by the detection of increased amounts of chlorinated plasma proteins in these patients (181).

G. Rheumatoid arthritis

It has been proposed that the joint damage observed in rheumatoid arthritis may, at least in part, be attributed to the production of oxidants by activated neutrophils, as large numbers of these cells are found in the synovial fluid of patients with this condition [reviewed in (362)]. A considerable number of *in vitro* studies demonstrated that MPO-derived oxidants, including HOCl, can effectively degrade cartilage, hyaluronic acid, and other joint components [reviewed in (160, 362)]. *In vivo* evidence that neutrophils participate in the cartilage erosion characteristic of this disease has been obtained from studies in which it was shown that high numbers of neutrophils were present in the immediate vicinity of damaged cartilage (267). It was later shown that degranulation of these neutrophils results in the detection of MPO in synovial fluid (106), and that the MPO present in synovial fluid from arthritis patients is catalytically active (289). Similarly, levels of serum MPO are significantly elevated in rheumatoid arthritis patients and found to correlate with markers of oxidation (35).

H. Inflammatory bowel disease and related disorders

Significant neutrophil infiltration in injured mucosa is a characteristic feature of ulcerative colitis (350). Immunohistochemical analysis of the epithelium and lamina propria of patients with inflammatory bowel disease revealed increased numbers of MPO-positive monocytes, macrophages, and neutrophils (234). Similarly, in both ulcerative colitis and Crohn disease patients, intestinal inflammation is associated with increased activity of MPO (234). The importance of MPO-derived oxidants was demonstrated in a related study, which showed an association between MPO levels and markers of both protein and lipid oxidation (233). Eosinophils have also been identified in elevated levels in patients with inflammatory bowel disease and related disorders [*e.g.*, (44, 91, 288)]. Several studies have shown that the eosinophils present in colonic biopsies of patients with a number inflammatory bowel disorders, including ulcerative colitis, are activated, resulting in the detection of increased amounts of EPO [*e.g.*, (288)]. The activation of these cells is known to correlate with disease severity and gastrointestinal dysfunction (44). Similarly, it has been demonstrated in an experimental mouse model that degranulation of eosinophils and release of EPO can induce a progressive colitis, which is suppressed on genetic manipulation or drug treatment specifically to inhibit EPO activity, suggesting that EPO-derived oxidants play a key role in the development of these inflammatory bowel disorders (119).

IX. HEME PEROXIDASES AS MARKERS AND PROGNOSTIC AGENTS OF DISEASE

A. Cardiovascular disease

The role of MPO in the diagnosis and prognosis of cardiovascular disease was reviewed recently (414). In brief, MPO levels

have been recognized as a major risk factor for coronary artery disease (CAD), with both blood and leukocyte MPO levels significantly elevated in people with CAD (468). In multivariable models, adjusted for other factors, MPO levels were significantly associated with CAD [odds ratio, 11.9 for highest, *vs*. lowest, quartiles of leukocyte-MPO; 20.4 for the highest *vs*. lowest quartiles of blood-MPO (468)]. These values make leukocyte-MPO levels the strongest known independent predictor of CAD (468) (cf. an odds ratio of 4.2 for the highest *vs*. lowest quintiles of cholesterol, when measured as a ratio of total cholesterol to HDL cholesterol). Plasma MPO levels are also strongly associated with the prevalence of heart failure, with the highest levels of MPO found in the patients with severe chronic disease (394). Similarly, serum MPO levels appear independently to predict endothelial dysfunction, another key feature of cardiovascular disease (432). MPO levels have also been reported to be a powerful predictor of health outcomes in people with chest pain (53), in patients with acute coronary syndromes (31), and in patients that have had a myocardial infarction (266).

B. Other diseases

The presence of elevated levels of MPO has been used as a routine cytochemical test for the diagnosis of acute leukemia for many years (38). The MPO reaction (Sudan Black B staining) is positive for all acute leukemias, but is especially useful in making a distinction between the myeloblastic and lymphoblastic forms of the disease (38). It has been reported that serum markers of eosinophil activation, including EPO, may be of value in assessing the severity of childhood asthma (337). Indeed, serum EPO levels have been shown to be a more sensitive marker for bronchial hyperresponsiveness in asthma, compared with other markers of eosinophil activation (45). Similarly, serum EPO levels have been used for the clinical monitoring of cystic fibrosis (232). Lastly, MPO-ANCA are associated with various forms of systemic vasculitis, particularly glomerulonephritis and related kidney disorders [reviewed in (204)] and have been reported to be a useful diagnostic tool in primary systemic vasculitides, with current data showing that these markers correlate well with disease activity (204).

X. CONCLUDING REMARKS

The data reviewed indicate that peroxidase enzymes may play a key role in a number of human diseases, and that the activity of these species can be both beneficial and detrimental. Recent years have seen a dramatic increase in our understanding of the enzymology, biochemistry, and biologic roles of these species, although much remains to be discovered with regard to the nature of the major damaging species generated by these enzymes, the critical targets of these oxidants, and their role in both beneficial reactions and tissue pathology.

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ABBREVIATIONS

ABC-A1, ATP-binding cassette transporter A1; AGE, advanced glycation end product; ApoA-1, apolipoprotein A-1; Br^-, bromide ion; CAD, coronary artery disease; Cl^-, chloride ion; 3-bromo-Tyr, 3-bromotyrosine; 3-chloro-Tyr, 3-chlorotyrosine; 3,5-dibromo-Tyr, 3,5-dibromotyrosine; 3,5-dichloro-Tyr, 3,5-dichorotyrosine; di-Tyr, *o-o′* di-tyrosine; EPO, eosinophil peroxidase; $Fe^{IV}=O$, oxy-ferryl species; GSH, reduced glutathione; HDL, high-density lipoproteins; $HO^{\bullet}$, hydroxyl radical; H_2O_2, hydrogen peroxide; HOBr, the physiologic mixture of hypobromous acid and its anion; HOCl, the physiologic mixture of hypochlorous acid and its anion, HOSCN, the physiologic mixture of hypothiocyanous acid and its anion; LDL, low-density lipoproteins; LPO, lactoperoxidase; MMP-7, matrix metalloproteinase 7; MPO, myeloperoxidase; $NO^{\bullet}$, nitric oxide radical; NO^+, nitrosonium ion; $NO_2^{\bullet}$, nitrogen dioxide radical; NO_2^-, nitrite ion; NO_3^-, nitrate ion; $^1O_2$, molecular oxygen in its $^1\Delta_g$ excited singlet state; $O_2^{\bullet -}$, superoxide radical anion; O_3, ozone; OCN^-, cyanate ion; $ONOO^-$, peroxynitrite anion; ONOOH, peroxynitrous acid; SCN^-, thiocyanate ion.

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