Annu. Rev. Pharmacol. Toxicol. 1992. 32:89–107

CATALYTIC SITES OF HEMOPROTEIN PEROXIDASES

Paul R. Ortiz de Montellano

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143-0446

KEY WORDS: hemoprotein structure, cytochrome c peroxidase, horseradish peroxidase, chloroperoxidase, enzyme mechanisms

INTRODUCTION

Hemoprotein peroxidases catalyze the one-electron oxidation of micro- and macromolecular substrates at the expense of hydrogen peroxide or lipid hydroperoxides. Our understanding of the catalytic mechanisms of classical peroxidases (e. g. those with an imidazole as the fifth iron ligand) derives to a large extent from work with horseradish peroxidase (HRP) and cytochrome c peroxidase (CcP), the former because it is readily available and has been extensively studied by chemical and spectroscopic methods (1), and the latter because it is the only peroxidase for which a high resolution crystal structure is available (2, 3). These two enzymes, together with chloroperoxidase, a prototypical nonclassical peroxidase with a thiolate rather than imidazole iron ligand, provide a solid ground to explore the relationship between structure and function in the peroxidases. The relationships inferred from the analysis of these three enzymes appear to be broadly applicable, in principle if not always in detail, to other peroxidases, including the less accessible or tractable mammalian enzymes lactoperoxidase (4–6), thyroid peroxidase (7), myeloperoxidase (8), and eosinophil peroxidase (9).

0362-1642/92/0415-0089$02.00

COMPOUND I

Ferryl Complex

The minimal catalytic mechanism inferred from studies of HRP and CcP is initiated by the binding of a neutral molecule of hydrogen peroxide to the heme iron atom (Figure 1). As proposed by Poulos & Kraut from an analysis of the crystal structure of CcP, binding of the peroxide to the iron is facilitated by transfer of one of its hydrogens to the nitrogen of the active site imidazole (His-52 in CcP) (10). The hydrogen is then delivered by the imidazole to the terminal oxygen of the peroxide as the oxygen-oxygen bond is cleaved to give the ferryl ($Fe^{IV} = O$) species and a molecule of water. The oxygen-oxygen bond scission is promoted not only by the hydrogen transfer but also by an active site arginine that polarizes the dioxygen bond (10). Heterolytic cleavage of the dioxygen bond to give this compound I structure (sometimes called compound ES in CcP) requires two electrons, one of which comes from the iron atom. Extensive spectroscopic and kinetic evidence (1, 11), including Mössbauer (12–14), EXAFS (15), magnetic circular dichroism (16, 17), ENDOR (18), magnetic susceptibility (19), resonance Raman (20–23), and an actual crystal structure of CcP compound I (24), supports formulation of the iron as an $Fe^{IV} = O$ species in compound I of HRP and CcP. Extrapolation of this inference to other peroxidases is supported by spectroscopic evidence on those enzymes (4–9).

Source of the Second Electron

In HRP, the second electron required to cleave the peroxide bond comes from the porphyrin, producing a porphyrin radical cation (Figure 1; 25–28). The second electron may initially come from the porphyrin in CcP (29), but the porphyrin radical cation, if formed, is rapidly quenched by electron transfer from the protein to give a protein radical (3, 30). In the presence of reduced cytochrome c, however, electron transfer from cytochrome c may compete with electron transfer from the protein, possibly diminishing the role of the protein radical in normal catalytic turnover (30). The site of the protein radical in cytochrome c peroxidase has been elusive, but site specific mutagenesis studies (31–33) and EPR studies of compound I of CcP with deuterium-labeled tryptophan residues (34) indicate that the unpaired electron is centered on Trp-191. This tryptophan residue is adjacent to His-175, the fifth heme iron ligand, and is roughly parallel to it. Detailed analysis of the EPR spectra of several site-specific mutants suggests, in fact, that approximately 90% of the unpaired electron density is on Trp-191 and the other 10% on other (probably tyrosine) residues (33). This is consistent with the report that the tyrosine residues of the enzyme are cross-linked with time in the reaction of CcP with H_2O_2 (35, 36). The factors that determine whether the radical

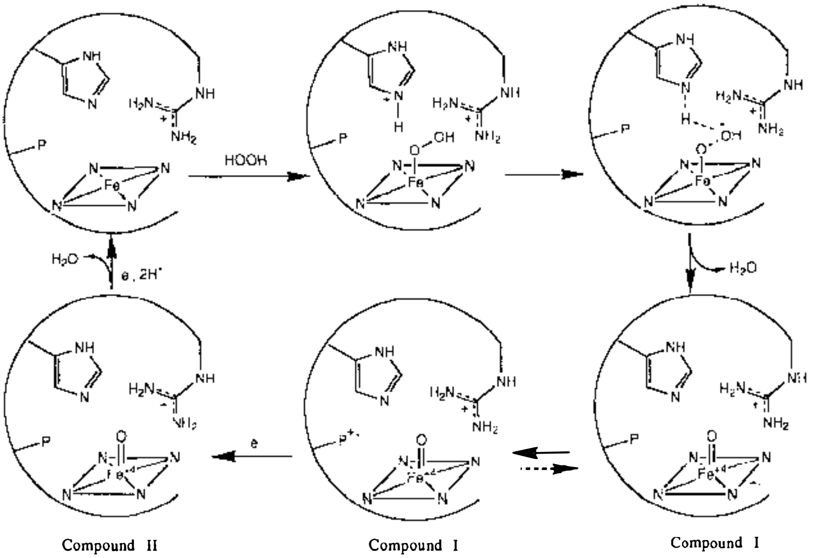

Figure 1 A general catalytic mechanism for classical peroxidases based on the mechanism proposed for cytochrome c peroxidase by Poulos & Kraut (10). The heme group is represented by the four nitrogen iron ligands. P stands for a protein residue. With some exceptions, only one of the two compound I structures shown in the scheme is detected for a given peroxidase.

density remains on the porphyrin, as in HRP, or is transferred to the protein, as in CcP, remain obscure, although it has been suggested that one factor may be the presence or absence of negatively charged groups near the porphyrin that stabilize the porphyrin radical cation (37).

Porphyrin-protein Radical Exchange

Lactoperoxidase and thyroid peroxidase react with H_2O_2 to give an $Fe^{IV}=O$/porphyrin radical cation analogous to that of HRP compound I (4–7). Recent work on the oxidation of iodine by these two peroxidases suggests, however, that the porphyrin radical oxidizes the protein at a relatively rapid rate to give a protein radical, much as in CcP (38–41). The key finding for lactoperoxidase is that the compound I structure with a porphyrin radical cation catalyzes both the iodination of tyrosine residues and the coupling of tyrosine residues to dimeric products, whereas only the latter reaction is catalyzed by an alternative two-electron oxidized compound I structure without the porphyrin radical cation spectrum (38). The existence of two two-electron oxidized states of lactoperoxidase was confirmed by titration with ferrocyanide at different pH values (39). The same research group demonstrated the formation of a transient thyroid peroxidase $Fe^{IV}=O$/porphyrin radical cation that catalyzes the iodination of tyrosine residues, and its decay to a second compound I species without the porphyrin radical cation that only promotes the coupling of tyrosine residues (40). The proposal that iodination requires the $Fe^{IV}=O$/porphyrin radical cation is supported by the finding that horseradish peroxidase catalyzes both the iodination and dimerization of tyrosine residues, whereas CcP only catalyzes tyrosine dimerization (41). The presence of two compound I species may be related to the observation that the oxidation of phenols by thyroid peroxidase switches from an apparent two-electron to a one-electron mechanism as the size of the 2,6-substituents of the phenol increases, whereas all the phenols are oxidized by HRP and lactoperoxidase via a one-electron mechanism (42). These results suggest that subtle factors determine whether the second oxidation equivalent is taken from the porphyrin or the protein, and indicate that the site of the unpaired electron has functional consequences.

COMPOUND II

Transfer of one electron to compound I of HRP, CcP, or other heme peroxidases gives compound II, in which the $Fe^{IV} = O$ species remains intact but the porphyrin or protein radical has been reduced (13, 15). The remaining oxidation equivalent is therefore located in the ferryl species in compound II of all the peroxidases (Figure 1). The catalytic activity of HRP compound II is linked to a group with a pKa of 8.6, probably His-42, which in the protonated state is hydrogen bonded to the ferryl oxygen (21, 23, 43). Compound II is inactive if this amino acid residue is not protonated (44). Compound II of CcP is generally not observed because it is rapidly reduced. Transfer of a second electron to the peroxidases in the final step of the catalytic cycle reduces compound II to the resting ferric state and results, after protonation of the oxygen, in release of the ferryl oxygen as a molecule of water.

PROPERTIES OF PEROXIDASE ACTIVE SITES

Iron Accessibility

A large body of indirect evidence suggests that an $Fe^{IV}=O$/porphyrin or protein radical is also the catalytic species of cytochrome P450 (45, 46). One of the challenges in the hemoprotein field has been to understand why cytochrome P450 enzymes, with occasional exceptions, transfer an oxygen atom to their substrates whereas the peroxidases generally remove an electron from theirs without transferring the oxygen atom. Recent work with HRP and other peroxidases provides some insight into this difference in the reactions catalyzed by compounds I and II of the peroxidases and the corresponding ferryl state of cytochrome P450. One key finding is that both HRP and cytochrome P450 are inactivated by phenylhydrazine, albeit by different mechanisms. Phenylhydrazine gives a relatively stable phenyl-iron complex with a characteristic long-wavelength absorption maximum with cytochrome P450 (47, 48) but does not give such a complex with HRP (49). Inactivation of HRP by phenylhydrazine is due to covalent binding to the protein as well as addition of the phenyl radical to the *δ-meso* position of the heme group (Figure 2; 49). In addition to forming δ-meso-phenylheme, the reaction also produces the 8-hydroxymethyl heme derivative due to competitive abstraction by the phenyl radical of a hydrogen from the 8-methyl group. Lignin peroxidase, manganese peroxidase, and *Coprinus macrorhizus* peroxidase also do not yield phenyl-iron complexes with phenylhydrazine (50–52). These results, given that all hemoproteins with accessible heme iron atoms so far examined give phenyl-iron complexes (53), suggest that the iron atom of the classical peroxidases is not readily accessible to substrates. Furthermore, exclusive modification of the *δ-meso* carbon and the vicinal 8-methyl group indicates that phenylhydrazine, and presumably other substrates, interact with this sector of the heme group.

δ-Meso Heme Edge

The inactivation of HRP by alkylhydrazines (54, 55), azide (56), cyclopropanone hydrate (57), and nitroalkanes (58) provides additional support for the hypothesis that the iron is not readily accessible and that substrates interact with the *δ-meso* heme edge (Figure 2). Unlike the reactions of the alkylhydrazines with cytochrome P450 (59), which result in N-alkylation of the prosthetic heme group, the reaction of HRP with several alkylhydrazines results in addition of the alkyl moieties exclusively to the *δ-meso* carbon (54). Cyclopropanone hydrate, nitromethane, and sodium azide similarly add to the *δ-meso* carbon after being oxidized by the enzyme to radical intermediates (56–58). The reaction with the alkylhydrazines is particularly instructive because it gives rise to a long-lived intermediate ($t_{1/2}$ of hours) with an absorption spectrum characteristic of an isoporphyrin (55). The substitution reaction therefore involves addition of the alkyl radical to the *δ-meso* carbon to give an isoporphyrin that slowly decays to the δ-meso-alkylheme by loss of the meso proton (Figure 2). The phenyl and azidyl radicals presumably add by the same mechanism, but the corresponding isoporphyrin intermediates apparently decay too rapidly to be observed. The same *δ-meso* regiospecificity is observed for the azide reaction with lignin, manganese, and *Coprinus macrorhizus* peroxidases (50–52).

As a result of these observations, it has been postulated that classical peroxidases suppress oxygen transfer reactions by allowing substrates to interact with the *δ-meso* heme edge while restricting their access to the ferryl oxygen, thereby forcing electron transfer to the heme edge (49, 53). Support for this proposal is provided by the finding that δ-meso-ethylheme-reconstituted HRP reacts normally with H_2O_2 to give a ferryl species but is catalytically inactive, whereas the enzyme reconstituted with hemin or δ-

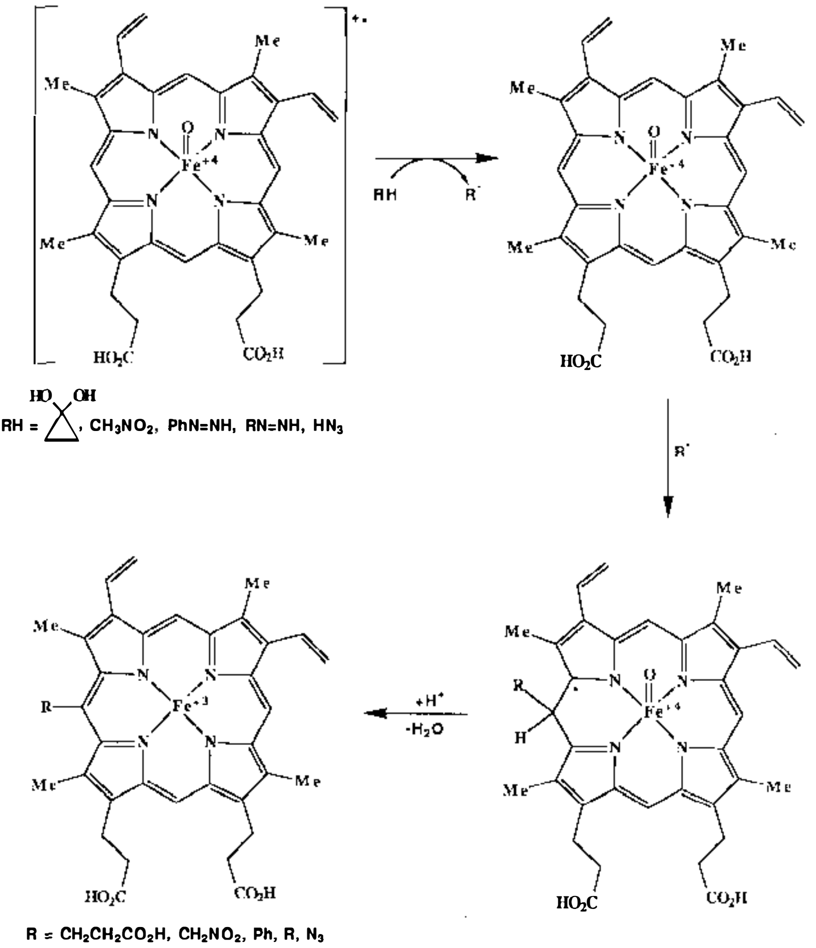

Figure 2 Meso-substitution of the prosthetic heme group of the peroxidases during the catalytic turnover of certain substrates. The radical cation of Compound I is shown outside the brackets to indicate that it could be located on the porphyrin or the protein.

meso-methylheme is active (54, 55). Retention of catalytic activity by the δ-meso-methyl but not -ethyl analogue demonstrates that the *δ-meso* substituent interferes with substrate oxidation by a steric mechanism rather than by critically altering the conformation or redox properties of the heme group. The argument that substrates interact with the *δ-meso* heme edge has received independent support from NMR nuclear Overhauser studies showing that resorcinol (60), 2-methoxy-4-methylphenol (60), and thiocyanate (61) bind to the ferric enzyme in the vicinity of the heme 8-methyl group. The estimated distances of the aromatic protons of the first two substrates from the iron are 8.4–11.0 Å, and of the thiocyanate nitrogen 6.8 Å. A schematic model for the active site of horseradish peroxidase which only exposes the δ-*meso* heme edge and the 8-methyl group is suggested by these results (Figure 3).

Monooxygenase Activity

The observation that catalytic turnover of HRP occasionally results in substrate oxygenation suggests that HRP functions, under certain circumstances, as a monooxygenase. In most of these cases, however, the oxygen is introduced by reaction of a normal peroxidative product with oxygen or water rather than by transfer of the ferryl oxygen to the substrate. Thus, the HRP-catalyzed oxidation of 2,4,6-trimethylphenol produces 2,6-dimethyl-4-hydroxymethylphenol, but ^{18}O-studies show that the oxygen does not come from H_2O_2 and therefore is not introduced by a P450-like monooxygenation reaction (62). The O-demethylation of 9-methoxyellipticine by HRP proceeds with replacement of the methoxy oxygen by an oxygen from the medium, so that the reaction involves addition of water to a peroxidatively generated quinone-imine intermediate rather than a P450-like hydroxylation of the methyl group (63, 64). A similar mechanism accounts for the O-demethylation observed in the oxidation of 1,2,4,5-tetramethoxybenzene (65). N-methylcarbazole has been used to probe the mechanisms of nitrogen demethylation reactions because the N-hydroxymethyl intermediate is sufficiently stable to be isolated. The hydroxymethyl intermediate isolated in the reaction catalyzed by HRP has been shown to incorporate oxygen from the medium (66). Furthermore, the formation of this species and its more highly oxidized N-formyl derivative has been shown to be oxygen dependent. The reaction thus involves oxidation of the nitrogen to the radical cation, deprotonation to give the vicinal carbon radical, and either reaction of the

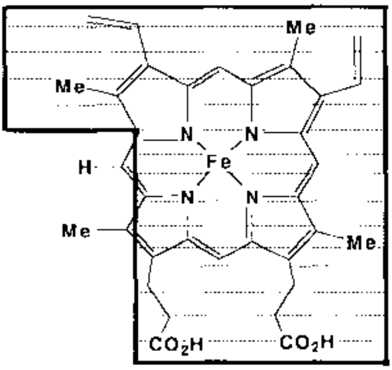

Figure 3 Hypothetical model of the active site of horseradish peroxidase based on the regiochemistry of the reactions of the prosthetic heme with catalytically generated substrate radicals.

carbon radical with molecular oxygen or further peroxidation to give the imminium cation that reacts with water (66). In agreement with its ineffectiveness as a ferryl oxygen transfer catalyst, HRP does not mediate the epoxidation of either styrene (62) or butadiene (67). The exception to the otherwise general observation that HRP does not catalyze oxygen transfer reactions is the oxidation of aryl thioethers to the corresponding sulfoxides, which proceeds with partial incorporation of oxygen from the peroxide (68). Thus, 66% and 85%, respectively, of the oxygen incorporated into the sulfoxides obtained from *p*-methoxy- and *p*-methylthioanisoles derives from the peroxide. In the case of the *p*-methoxy analogue, 10% of the sulfoxide oxygen derives from water. Despite the incorporation of peroxide oxygen, the sulfoxide produced from *p*-methylthioanisole is achiral (69). Kinetic and spectroscopic evidence suggests that the sulfur radical cation is an intermediate in this ferryl oxygen transfer reaction. The sulfoxidation rates increase with the electron-releasing ability of the aromatic substituent (69) and correlate with the voltammetric peak oxidation potentials for the substrates (70), as expected for a radical cation mechanism. Pseudo-first order and single turnover studies of the sulfoxidation reaction provide kinetic evidence for the formation of an intermediate, presumably the sulfur radical cation, in the reduction of compound I by thioanisoles, and for the reaction of compound II with both the radical cation intermediate and the parent thioanisole (71, 72). The kinetic evidence suggests, in fact, that decay of both compounds I and II at high thioether concentrations involves binding of a second sulfide molecule to the enzyme-thioanisole radical cation complex formed by oxidation of the first substrate molecule (72). Thioethers and related sulfur compounds are clearly oxidized by HRP in an exceptional manner that culminates in ferryl oxygen transfer. This finding needs to be reconciled with evidence that the ferryl species is shielded in peroxidases, leading to interaction of substrates with the heme edge. One possibility is that the charged sulfur radical cation perturbs the residues shielding the ferryl moiety in such a way that ferryl oxygen transfer becomes possible. The proposed involvement of enzyme-radical cation complexes in the reaction is consistent with strong interaction of the one-electron oxidation product with the protein (72). Kinetic evidence for a similar kinetically competent radical cation-enzyme complex has been reported for the HRP-catalyzed dimerization of dimethylaniline, a reaction thought to proceed via the dimethylanilinium radical cation (73).

ELECTRON TRANSFER: THE CASE OF CYTOCHROME C PEROXIDASE

CcP, as already noted, is the only peroxidase for which a crystal structure is available (2). It is anomalous, however, in that its substrate is cytochrome c, a hemoprotein, rather than a small organic or inorganic molecule (3). Because of this anomaly, it is less critical for proper peroxidative functioning of the enzyme that access to the ferryl oxygen be strictly limited. In addition to the oxidation of cytochrome c, CcP catalyzes the in vitro peroxidation of guaiacol and other small molecules and, more interestingly, the epoxidation of styrene and substituted styrenes by what appears to be a conventional ferryl oxygen transfer mechanism (G. D. DePillis, G. Mauk, P. R. Ortiz de Montellano, unpublished data). Furthermore, unlike HRP and other classical peroxidases, CcP reacts with phenylhydrazine to give the phenyl-iron complex characteristic of an open active site. Both the peroxidation of guaiacol and the epoxidation of styrene, but not the one-electron oxidation of ferrocytochrome c, are suppressed when the enzyme is reconstituted with δ-meso-ethylheme. The *δ-meso* substituent clearly interferes with small substrate oxidation but not with formation of the ferryl/protein radical complex or oxidation of cytochrome c, the normal redox partner. Differential processing of small substrates and cytochrome c is confirmed by the finding that reconstitution of the enzyme with *γ-meso*-ethylheme yields an enzyme that oxidizes small molecules normally but is defective in its ability to oxidize cytochrome c (74). The crystal structure of CcP readily rationalizes these results. Poulos & Kraut proposed, on the basis of modeling studies, that electron transfer from cytochrome c occurs when it is bound to the surface of CcP at a site facing the *γ-meso* edge (75). Brownian motion calculations suggest that the binding site may not be as rigidly defined as originally proposed by Poulos & Kraut, but nevertheless concur with the assessment that electron transfer is most favored when cytochrome c is bound to the surface facing the γ-edge of the heme group (76). Support for some degree of conformational searching in the binding of cytochrome c to cytochrome c peroxidase is provided by the fact that the rate of intramolecular electron transfer decreases under conditions of very low ionic strength that favor very tight complex formation (77). In contrast, H_2O_2 is thought to reach the enzyme through a 6Å-wide (11 Å-long) channel that leads from the outside of the protein into the heme crevice directly above the *δ-meso* position. Substrates migrating up the channel therefore interact first with the *δ-meso* edge, as in other classical peroxidases, but are able to migrate into the actual heme crevice and to react with the ferryl oxygen (Figure 4). δ-Meso-alkyl substituents sterically obstruct the access channel and interfere with the oxidation of small substrates but do not block the reaction with H_2O_2 or interfere with oxidation of cytochrome c at the protein surface. The results demonstrate that the ferryl oxygen of a peroxidase can be transferred to an olefin even when it interacts with proximal histidine and arginine residues and is paired with a protein rather than porphyrin radical. The fact that CcP behaves as a hemoprotein with a relatively open active site, in agreement with its crystal structure, reinforces the hypothesis that HRP and other classical peroxidases do not have such open sites.

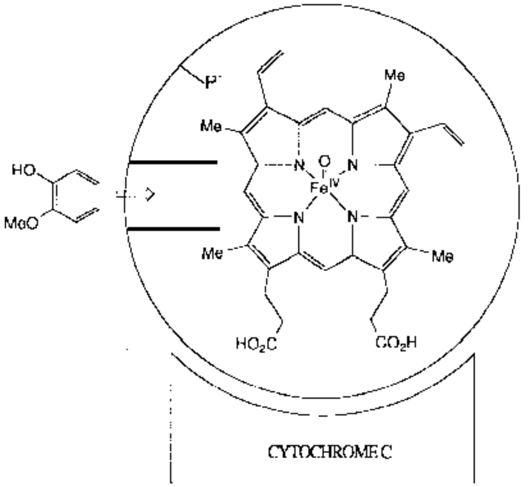

Figure 4 Schematic model for cytochrome c peroxidase illustrating the separate reaction sites for the oxidation of small substrates and cytochrome c.

LACTOPEROXIDASE, A MAMMALIAN PEROXIDASE

Prosthetic Group

The prosthetic group of lactoperoxidase can only be extracted under harsh acidic conditions but is readily extracted with acidic butanone if the protein is first digested by pronase (78). Absorption spectroscopy and thin layer chromatography suggest that these procedures yield iron protoporphyrin IX, although they cause appreciable degradation of the prosthetic group. These results led to the conclusion that the prosthetic group is iron protoporphyrin IX and that it is not, contrary to earlier claims, covalently bound to the protein by an ester or amide linkage (78). The prosthetic group has recently been re-isolated by mild treatment with mercaptoethanol in 8 M urea, however, and has been shown by NMR and mass spectrometric methods to be a heme with a thiol group on either the 5- or 8-methyl group (Figure 5; 79). Nichol et al have therefore proposed that the prosthetic group is bound in the active site of the enzyme via a disulfide bond between a cysteine residue and the sulfhydryl group of the modified heme. The porphyrin structural assignment appears to be on solid ground but the nature of the heme-protein link in the intact enzyme is not yet clear, although a hyperfine-shifted pair of methylene protons has been tentatively assigned to the -CH_2-S-moiety by NMR studies (80). Resonance Raman studies suggest that the vinyl groups of the heme are intact in the native enzyme and are not involved in covalent bonds (81). The probability that the thiol moiety is on the 8-methyl is of some interest because the

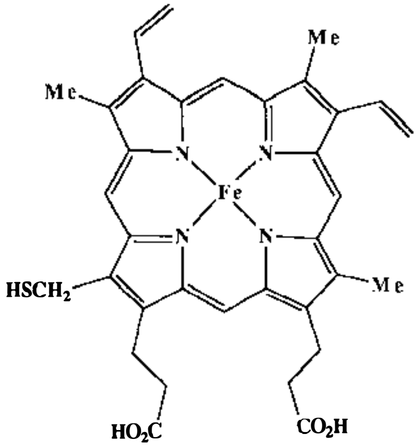

Figure 5 Structure of the prosthetic group isolated from lactoperoxidase treated with mercaptoethanol and 8 M urea. The structural data indicate that the thiol group is located either on the 8-methyl, as shown, or the 5-methyl.

same methyl group is converted to a hydroxymethyl group in the reaction of HRP with phenylhydrazine by a reaction that involves hydrogen atom abstraction from the methyl group by the phenyl radical (49). The intriguing possibility thus exists that the prosthetic group is covalently bound during turnover of a pro-enzyme form due to catalytic generation of a protein radical that abstracts a hydrogen from the 8-methyl group of the initially noncovalently bound heme.

Catalytic Site

The active site of lactoperoxidase has the general characteristics of a classical peroxidase. The bovine cDNA sequence encodes a protein of 78,500 kd exhibiting strong sequence identity with human myelo-, thyro-, and eosinophil peroxidases (82). Five imidazole residues are conserved in all four proteins, one of which is presumably the heme fifth ligand and another is the catalytic histidine. A histidine is implicated as the fifth heme ligand by EPR (83), magnetic circular dichroism (84), resonance Raman (85), and NMR data (80, 86, 87). The suggestion from magnetic circular dichroism studies that the sixth ligand is a carboxylate anion (84) has given way to the proposal, based on NMR data, that the sixth coordination site is vacant or is only loosely coordinated to a water molecule (87). Protons with dipolar connectivities similar to those of the distal histidine and arginine in HRP are observed by NMR and are consistent with the presence of these residues in lactoperoxidase (80). No other catalytic residues have been clearly identified although inactivation of the enzyme correlates well with derivatization of a tyrosine residue by diazotized sulfanilate (88). Chemical derivatization studies suggest that carboxyl-containing (89) but not tryptophan or arginine residues are also important (90), but such studies do not distinguish between functionally and structurally important residues.

The reaction of lactoperoxidase, as already discussed, yields an HRP-like ferryl/porphyrin radical cation that decays to a CcP-like ferryl/protein radical (40, 41). The mechanism of the interaction of substrates with lactoperoxidase is not yet clear, but the available evidence suggests that access to the heme iron is perhaps even more restricted than in HRP (91–93). The difference spectra for lactoperoxidase in the presence of phenols, hydroquinones, aryl amines, and hydroxamic acids, all of which slightly perturb the lactoperoxidase spectrum, are very similar, suggesting that the substrates do not interact with the iron atom (91). In contrast, the spectra are somewhat different for the corresponding HRP complexes (92). NMR proton relaxation studies indicate that several aromatic substrates bind to the enzyme at a site that places their protons 9.4–11.1 Å from the iron atom, approximately 2–3 Å further than the proton-iron distances estimated for HRP (93). Competitive studies show that iodide and thiocyanate compete for binding at the same site, whereas coordination of cyanide to the iron does not effect substrate binding (93–95). The observation that protonation of a residue with a pK_a of ~6 facilitates substrate binding has led to the proposal that substrates bind near, and possibly hydrogen bond with, the proposed catalytic histidine residue (93, 95). Further support for a closed active site is provided by the fact that reaction of lactoperoxidase with phenylhydrazine inactivates the enzyme (91, 96) concomitant with a spectroscopic alteration similar to that observed with HRP (91). This suggests that phenylhydrazine, as with HRP (49), reacts with the heme edge rather than the heme iron. Lactoperoxidase oxidizes benzylmethylsulfide and thioanisole slowly, and thiobenzamide more rapidly, to the corresponding sulfoxides (70). It is not yet known whether these oxidations, like those catalyzed by HRP (68, 69), proceed with incorporation of oxygen from the peroxide. Sulfur oxidation by lactoperoxidase probably also involves ferryl oxygen transfer and therefore must be reconciled, as discussed for HRP, with evidence that the heme iron is not readily accessible to most substrates. Lactoperoxidase is inactivated by hydrogen sulfide and the thiocarbamides by mechanisms that involve sulfur oxidation, covalent binding of the agents to the enzyme, and heme modification, but the nature of the heme reaction is unclear (97–99).

CHLOROPEROXIDASE, A PROTOTYPICAL NONCLASSICAL PEROXIDASE

Chloroperoxidase, a nonclassical peroxidase from *Caldariomyces fumago* with a cysteine (Cys-29) thiolate as the fifth heme ligand (100–103), is much poorer as a peroxidase than HRP (104, 105). The electron-rich thiolate ligand may help cleave the peroxide oxygen-oxygen bond in the absence of some of the assistance provided by polar active site residues in HRP, although there is evidence for the involvement of a group of pK_a 5.5, probably a histidine, in chloroperoxidase catalysis (100, 106, 107). This group may play the same role in chloroperoxidase as it does in horseradish and cytochrome c peroxidase. Inactivation of the enzyme by diethylpyrocarbonate, a histidine-specific agent, and sequence comparison with CcP, suggest that His-38 may be the residue in question (108). The catalytic intermediates involved in the peroxidatic reactions appear to be an HRP-like ferryl/porphyrin radical cation compound I and the usual ferryl compound II (100, 109–112). Like myeloperoxidase, chloroperoxidase catalyzes the H_2O_2-dependent activation of chloride ion to a chlorinating species. It is controversial whether the chlorinating agent is an enzyme-bound (e. g. Fe-O-Cl) species or the catalytically generated hypochlorous acid (100, 101, 112). In the absence of chloride ion, chloroperoxidase catalyzes monooxygenation reactions. These include olefin epoxidation (62, 67, 113, 114) and thioether sulfoxidation (68–70, 115, 116). The oxygen incorporated into the product in the epoxidation of styrene (62) and the sulfoxidation of two thioanisoles (68) derives primarily or exclusively from the peroxide. As expected for a P450-like ferryl oxygen transfer, the epoxidation of trans-[1-^{2}H]styrene proceeds with complete retention of the olefin stereochemistry (62). Furthermore, the sulfoxidations catalyzed by chloroperoxidase are enantioselective (69, 116) even though the chlorination reactions catalyzed by the same enzyme yield racemic products (107, 117–119). Further support for a P450-like rather than HRP-like active site is provided by the fact that its reaction with phenylhydrazine yields a phenyl-iron complex but not a δ-mesophenyl heme adduct (V. M. Samokyszyn, P. R. Ortiz de Montellano, submitted). Chloroperoxidase, like CcP, is therefore an anomalous peroxidase in that it catalyzes P450-like monooxygenation reactions.

SUMMARY

The structures of the active sites of horseradish and cytochrome c peroxidase, prototypical peroxidases with an imidazole heme ligand, suggest that small substrates are generally oxidized by peroxidases at the δ-*meso* edge of the heme group. This inference is supported by experimental results on the *Coprinus macrorhizus* peroxidase (52), manganese peroxidase (51), lignin peroxidase (50) and, less definitively, lactoperoxidase (90). Macromolecular substrates, exemplified by the cytochrome c peroxidase-cytochrome c interaction, are likely to be oxidized at peroxidase surface sites bearing no specific relationship to the δ-*meso* heme edge. The second oxidation equivalent in the two-electron Compound I states of the peroxidases is stored either as a porphyrin radical or as a protein radical, although some peroxidases have both types of compound I. The factors that control the location of the second oxidation equivalent remain unclear. Classical peroxidases do not generally catalyze olefin epoxidation and other monooxygenations but do catalyze sulfoxidation reactions. This is best rationalized by physical separation of the substrate from the ferryl oxygen, possibly by a protein barrier, because results with cytochrome c peroxidase show that there is no inherent mechanistic reason for the inability of peroxidases to epoxidize olefins. It is not yet clear why the barrier to oxygen transfer reactions is circumvented during sulfur oxidation reactions, although one possibility is that the relatively stable sulfur cation radical that is initially formed disrupts the barrier. Chloroperoxidase, the principal nonclassical hemoprotein peroxidase so far examined, has an open active site that readily catalyzes P450-like monooxygenation reactions. The active site of chloroperoxidase is a potentially useful model for that of myeloperoxidase, but caution must be used in extrapolating from one to the other because myeloperoxidase has a histidine rather than thiolate fifth heme ligand and therefore is a classical rather than nonclassical peroxidase.

Literature Cited

1. Dunford, H. B. 1991. Horseradish peroxidase: structure and kinetic properties. In *Peroxidases in Chemistry and Biology*, ed. J. Everse, K. E. Everse, M. B. Grisham, 2:1–24. Boca Raton: CRC. 264 pp.
2. Finzel, B. C., Poulos, T. L., Kraut, J. 1984. Crystal structure of yeast cytochrome c peroxidase refined at 1.7-Å resolution. *J. Biol. Chem.* 259: 13027–36
3. Bosshard, H. R., Anni, H., Yonetani, T. Yeast cytochrome c peroxidase. See Ref. 1, pp. 51–84
4. Thomas, E. L., Bozeman, P. M., Learn, D. B. Lactoperoxidase: structure and catalytic properties. In *Peroxidases in Chemistry and Biology*, ed. J. Everse, K. E. Everse, M. B. Grisham, 1: 123–42. Boca Raton: CRC. 355 pp.
5. Paul, K-G., Ohlsson, P. 1985. The chemical structure of lactoperoxidase. In *The Lactoperoxidase System. Chemistry and Biological Significance*, ed. K. M. Pruitt, J. O. Tenovuo, pp. 15–29. New York: Marcel Dekker, 257 pp.
6. Kohler, H., Jenzer, H. 1989. Interaction of lactoperoxidase with hydrogen peroxide. Formation of enzyme intermediates and generation of free radicals. *Free Rad. Biol. Med.* 6:323–39
7. Magnussen, R. P. Thyroid peroxidase. See Ref. 4, pp. 199–219
8. Hurst, J. K. Myeloperoxidase: Active site structure and catalytic mechanisms. See Ref. 4, pp. 37–62
9. Henderson, W. R. Eosinophil peroxidase: occurrence and biological function. See Ref. 4, pp. 105–21
10. Poulos, T. L., Kraut, J. 1980. The stereochemistry of peroxidase catalysis. *J. Biol. Chem.* 255:8199–205
11. Adediran, S. A., Dunford, H. B. 1983. Structure of horseradish peroxidase compound I. Kinetic evidence for the incorporation of one oxygen atom from the oxidizing substrate into the enzyme. *Eur. J. Biochem.* 132:147–50
12. Moss, T. H., Ehrenberg, A., Bearden, A. J. 1969. Mössbauer spectroscopic evidence for the electronic configuration of iron in horseradish peroxidase and its peroxide derivatives. *Biochemistry* 8: 4159–62
13. Schultz, C. E., Rutter, R., Sage, J. T., Debrunner, P. G., Hager, L. P. 1984. Mössbauer and electron paramagnetic resonance studies of horseradish peroxidase and its catalytic intermediates. *Biochemistry* 23:4743–54
14. Lang, G., Spartalian, K., Yonetani, T. 1976. Mössbauer spectroscopic study of compound ES of cytochrome c peroxidase. *Biochim. Biophys. Acta* 451: 250–58
15. Penner-Hahn, J. E., Eble, K. S.,

McMurry, T. J., Renner, M., Balch, A. L., et al. 1986. Structural characterization of horseradish peroxidase using EXAFS spectroscopy. Evidence for Fe = O ligation in compounds I and II. *J. Am. Chem. Soc.* 108:7819–25

16. Browett, W. R., Gasyna, Z., Stillman, M. J. 1988. Temperature dependence and electronic transition energies in the magnetic circular dichroism spectrum of horseradish peroxidase compound I. *J. Am. Chem. Soc.* 110:3633–40
17. Myers, D., Palmer, G. 1985. Magnetic circular dichroism studies on the heme and tryptophan components of cytochrome c peroxidase. *J. Biol. Chem.* 260:3887–90
18. Roberts, J. E, Hoffman, B. M., Rutter, R., Hager, L. P. 1981. ^{17}O ENDOR of horseradish peroxidase compound I. *J. Am. Chem. Soc.* 103:7654–56
19. Iizuka, T., Kotani, M., Yonetani, T. 1968. A thermal equilibrium between high- and low-spin states in ferric cytochrome c peroxidase and some discussion on the enzyme-substrate complex. *Biochim. Biophys. Acta* 167:257–67
20. Hashimoto, S., Teraoka, J., Inubushi, T., Yonetani, T., Kitagawa, T. 1986. Resonance Raman study on cytochrome c peroxidase and its intermediate. Presence of the Fe(IV)=O bond in compound ES and heme-linked ionization. *J. Biol. Chem.* 261:11110–18
21. Hashimoto, S., Tatsuno, Y., Kitagawa, T. 1986. Resonance Raman evidence for oxygen exchange between thc Fe^{IV} = O heme and bulk water during enzymic catalysis of horseradish peroxidase and its relation with the heme-linked ionization. *Proc. Natl. Acad. Sci. USA* 83:2417–21
22. Oertling, W. A., Babcock, G. T. 1985. Resonance Raman scattering from horseradish peroxidase compound I. *J. Am. Chem. Soc.* 107:6406–7
23. Sitter, A. J., Reczek, C. M., Terner, J. 1985. Heme-linked ionization of horseradish peroxidase compound II monitored by the resonance Raman Fe(IV) = O stretching vibration. *J. Biol. Chem.* 260:7515–22
24. Edwards, S. L., Xuong, H. X., Hamlin, R. C., Kraut, J. 1987. Crystal structure of cytochrome c peroxidase compound I. *Biochemistry* 26:1503–11
25. La Mar, G. N., de Ropp, J. S., Smith, K. M., Langry, K. C. 1981. Proton nuclear magnetic resonance investigation of the electronic structure of compound I of horseradish peroxidase. *J. Biol. Chem.* 256:237–43
26. Dolphin, D., Forman, A., Borg, D. C., Fajer, J., Felton, R. H. 1971. Compounds I of catalase and horse radish peroxidase: π-cation radicals. *Proc. Natl. Acad. Sci. USA* 68:614–18
27. Roberts, J. E, Hoffman, B. M., Rutter, R., Hager, L. P. 1981. Electron-nuclear double resonance of horseradish peroxidase compound I. Detection of the porphyrin π-cation radical. *J. Biol. Chem.* 256:2118–21
28. Palaniappan, V., Terner, J. 1989. Resonance Raman spectroscopy of horseradish peroxidase derivatives and intermediates with excitation in the near ultraviolet. *J. Biol. Chem.* 264:16046–53
29. Summers, F. E., Erman, J. E. 1988. Reduction of cytochrome c peroxidase compounds I and II by ferrocytochrome c. A stopped-flow kinetic investigation. *J. Biol. Chem.* 263:14267–75
30. Yonetani, T., Schleyer, H., Ehrenberg, A. 1966. Studies on cytochrome c peroxidase. VII. Electron paramagnetic resonance absorption of the enzyme and complex ES in dissolved and crystalline forms. *J. Biol. Chem.* 241:3240–43
31. Mauro, J. M., Fishel, L. A., Hazzard, J. T., Meyer, T. E., Tollin, G., et al. 1988. Tryptophan-191 → phenylalanine, a proximal-side mutation in yeast cytochrome c peroxidase that strongly affects the kinetics of ferrocytochrome oxidation. *Biochemistry* 27:6243–56
32. Erman, J. E., Vitello, L. B., Mauro, J. M., Kraut, J. 1989. Detection of an oxyferryl porphyrin π-cation-radical intermediate in the reaction between hydrogen peroxide and a mutant yeast cytochrome c peroxidase. Evidence for tryptophan-191 involvement in the radical site of compound I. *Biochemistry* 28:1992–95
33. Fishel, L. A., Farnum, M. F., Mauro, J. M., Miller, M. A., Kraut, J., et al. 1991. Compound I radical in site-directed mutants of cytochrome c peroxidase as probed by electron paramagnetic resonance and electron-nuclear double resonance. *Biochemistry* 30:1986–96
34. Sivaraja, M., Goodin, D. B., Smith, M., Hoffman, B. M. 1989. Identification by ENDOR of Trp^{191} as the free-radical site in cytochrome c peroxidase compound ES. *Science* 245:738–40
35. Coulson, A. F. W., Yonetani, T. 1972. Oxidation of cytochrome c peroxidase with hydrogen peroxide: Identification of the 'endogenous donor'. *Biochem. Biophys. Res. Commun.* 49:391–97
36. Spangler, B. D., Erman, J. E. 1986.

Cytochrome c peroxidase compound I: formation of covalent protein cross-links during the endogenous reduction of the active site. *Biochim. Biophys. Acta* 872:155–57

37. Ator, M. A., David, S. K., Ortiz de Montellano, P. R. 1989. Stabilized isoporphyrin intermediates in the inactivation of horseradish peroxidase by alkylhydrazines. *J. Biol. Chem.* 264: 9250–57
38. Courtin, F., Deme, D., Virion, A., Michot, J. L., Pommier, J., Nunez, J. 1982. The role of lactoperoxidase-H_2O_2 compounds in the catalysis of thyroglobulin iodination and thyroid hormone synthesis. *Eur. J. Biochem.* 124:603–9
39. Courtin, F., Michot, J-L., Virion, A., Pommier, J., Deme, D. 1984. Reduction of lactoperoxidase-H_2O_2 compounds by ferrocyanide: indirect evidence of an apoprotein site for one of the two oxidizing equivalents. *Biochem. Biophys. Res. Commun.* 121:463–70
40. Virion, A., Courtin, F., Deme, D., Michot, J. L., Kaniewski, J., Pommier, J. 1985. Spectral characteristics and catalytic properties of thyroid peroxidase-H_2O_2 compounds in the iodination and coupling reactions. *Arch. Biochem. Biophys.* 242:41–48
41. Deme, D., Virion, A., Michot, J. L., Pommier, J. 1985. Thyroid hormone synthesis and thyroglobulin iodination related to the peroxidase localization of oxidizing equivalents: studies with cytochrome c peroxidase and horseradish peroxidase. *Arch. Biochem. Biophys.* 236:559–66
42. Nakamura, M., Yamazaki, I., Kotani, T., Ohtaki, S. 1985. Thyroid peroxidase selects the mechanism of either 1- or 2-electron oxidation of phenols, depending on their substituents. *J. Biol. Chem.* 260:13546–52
43. Makino, R., Uno, T., Nishimura, Y., Iizuka, T., Tsuboi, M., Ishimura, Y. 1986. Coordination structures and reactivities of compound II in iron and manganese horseradish peroxidases. A resonance Raman study. *J. Biol. Chem.* 261:8376–82
44. Dunford, H. B., Stillman, J. S. 1976. On the function and mechanism of action of peroxidases. *Coord. Chem. Rev.* 19:187–251
45. Ortiz de Montellano, P. R. Oxygen activation and transfer. In *Cytochrome P-450: Structure, Mechanism, and Biochemistry*, ed. P. R. Ortiz de Montellano, pp. 217–71. New York: Plenum. 556 pp.
46. Dawson, J. H. 1988. Probing structure-function relationships in heme-containing oxygenases and peroxidases. *Science* 240:433–39
47. Raag, R., Swanson, B. A., Poulos, T. L., Ortiz de Montellano, P. R. 1990. Formation, crystal structure, and rearrangement of a cytochrome P-450_{cam} iron-phenyl complex. *Biochemistry* 29: 8119–26
48. Swanson, B. A., Dutton, D. R., Lunetta, J. M., Yang, C. S., Ortiz de Montellano, P. R. 1991. The active sites of cytochromes P450 IA1, IIB1, IIB2, and IIE1. Topological analysis by in situ rearrangement of phenyl-iron complexes. *J. Biol. Chem.* 266:19258–64
49. Ator, M. A., Ortiz de Montellano, P. R. 1987. Protein control of prosthetic heme reactivity. Reaction of substrates with the heme edge of horseradish peroxidase. *J. Biol. Chem.* 262:1542–51
50. DePillis, G. D., Wariishi, H., Gold, M. H., Ortiz de Montellano, P. R. 1990. Inactivation of lignin peroxidases by phenylhydrazine and sodium azide. *Arch. Biochem. Biophys.* 280:217–23
51. Harris, R. Z., Wariishi, H., Gold, M. H., Ortiz de Montellano, P. R. 1991. The catalytic site of manganese peroxidase. Regiospecific addition of sodium azide and alkylhydrazines to the heme group. *J. Biol. Chem.* 266:8751–58
52. DePillis, G. D., Ortiz de Montellano, P. R. 1989. Substrate oxidation by the heme edge of fungal peroxidases. Reaction of *Coprinus macrorhizus* peroxidase with hydrazines and sodium azide. *Biochemistry* 28:7947–52
53. Ortiz de Montellano, P. R. Control of the catalytic activity of prosthetic heme by the structure of hemoproteins. *Acc. Chem. Res.* 20:289–94
54. Ator, M. A., David, S. K., Ortiz de Montellano, P. R. 1987. Structure and catalytic mechanism of horseradish peroxidase. Regiospecific meso alkylation of the prosthetic heme group by alkylhydrazines. *J. Biol. Chem.* 262: 14954–60
55. Ator, M. A., David, S. K., Ortiz de Montellano, P. R. 1989. Stabilized isoporphyrin intermediates in the inactivation of horseradish peroxidase by alkylhydrazines. *J. Biol. Chem.* 264: 9250–57
56. Ortiz de Montellano, P. R., David, S. K., Ator, M. A., Tew, D. 1988. Mechanism-based inactivation of horseradish peroxidase by sodium azide. Formation of meso-azidoprotoporphyrin IX. *Biochemistry* 27:5470–76
57. Wiseman, J. S., Nichols, J. S., Kolpak,

M. X. 1982. Mechanism of inhibition of horseradish peroxidase by cyclopropanone hydrate. *J. Biol. Chem.* 257: 6328–32

58. Porter, D. J. T., Bright, H. J. 1983. The mechanism of oxidation of nitroalkanes by horseradish peroxidase. *J. Biol. Chem.* 258:9913–24
59. Ortiz de Montellano, P. R., Augusto, O., Viola, F., Kunze, K. L. 1983. Carbon radicals in the metabolism of alkyl hydrazines. *J. Biol. Chem.* 258:8623–29
60. Sakurada, J., Takahashi, S., Hosoya, T. 1986. Nuclear magnetic resonance studies on the spatial relationship of aromatic donor molecules to the heme iron of horseradish peroxidase. *J. Biol. Chem.* 261:9657–62
61. Modi, S., Behere, D. V., Mitra, S. 1989. Interaction of thiocyanate with horseradish peroxidase. ^{1}H and ^{15}N nuclear magnetic resonance studies. *J. Biol. Chem.* 264:19677–84
62. Ortiz de Montellano, P. R., Choe, Y. S., DePillis, G., Catalano, C. E. 1987. Structure-mechanism relationships in hemoproteins. Oxygenations catalyzed by chloroperoxidase and horseradish peroxidase. *J. Biol. Chem.* 262:11641–46
63. Meunier, G., Meunier, B. 1985. Peroxidase-catalyzed O-demethylation reactions. Quinone-imine formation from 9-methoxyellipticine derivatives. *J. Biol. Chem.* 260:10576–82
64. Meunier, B. 1991. N- and O-demethylations catalyzed by peroxidases. See Ref. 1, pp. 201–17
65. Kersten, P. J., Kalyanaraman, B., Hammel, K. E., Reinhammar, B., Kirk, T. K. 1990. Comparison of lignin peroxidase, horseradish peroxidase and laccase in the oxidation of methoxybenzenes. *Biochem. J.* 268:475–80
66. Kedderis, G. L., Rickert, D. E., Pandey, R. N., Hollenberg, P. F. 1986. ^{18}O Studies of the peroxidase-catalyzed oxidation of N-methylcarbazole. Mechanisms of carbinolamine and carboxaldehyde formation. *J. Biol. Chem.* 261: 15910–14
67. Elfarra, A. A., Duescher, R. J., Pasch, C. M. 1991. Mechanisms of 1,3-butadiene oxidations to butadiene monoxide and crotonaldehyde by mouse liver microsomes and chloroperoxidase. *Arch. Biochem. Biophys.* 286:244–51
68. Kobayashi, S., Nakano, M., Goto, T., Kimura, T., Schaap, A. P. 1986. Evidence for the peroxidase-dependent oxygen transfer from hydrogen peroxide to sulfides. *Biochem. Biophys. Res. Commun.* 135:166–71
69. Kobayashi, S., Nakano, M., Kimura, T., Schaap, A. P. 1987. On the mechanism of the peroxidase-catalyzed oxygen-transfer reaction. *Biochemistry* 26:5019–22
70. Doerge, D. R. 1986. Oxygenation of organosulfur compounds by peroxidases: evidence of an electron transfer mechanism for lactoperoxidase. *Arch. Biochem. Biophys.* 244:678–85
71. Pérez, U., Dunford, H. B. 1990. Spectral studies on the oxidation of organic sulfides (thioanisoles) by horseradish peroxidase compounds I and II. *Biochim. Biophys. Acta* 98–104
72. Pérez, U., Dunford, H. B. 1990. Transient-state kinetics of the reactions of 1-methoxy-4-(methylthio)benzene with horseradish peroxidase compounds I and II. *Biochemistry* 29:2757–63
73. Slaughter, D., O'Brien, P. J. 1986. A kinetic analysis of peroxidase-catalyzed N,N-dimethylaniline dimerization. *Biochim. Biophys. Acta* 883:225–32
74. DePillis, G. D., Sishta, B. P., Mauk, A. G., Ortiz de Montellano, P. R. 1991. Small substrates and cytochrome c are oxidized at different sites of cytochrome c peroxidase. *J. Biol. Chem.* 266: 19334–41
75. Poulos, T. L., Kraut, J. 1980. A hypothetical model of the cytochrome c peroxidase-cytochrome c electron transfer complex. *J. Biol. Chem.* 255:10322–30
76. Northrup, S. H., Boles, J. O., Reynolds, J. C. L. 1988. Brownian dynamics of cytochrome c and cytochrome c peroxidase association. *Science* 241:67–70
77. Hazzard, J. T., McLendon, G., Cusanovich, M. A., Tollin, G. 1988. Formation of electrostatically-stabilized complex at low ionic strength *inhibits* interprotein electron transfer between yeast cytochrome c and cytochrome c peroxidase. *Biochem. Biophys. Res. Commun.* 151:429–34
78. Sievers, G., 1979. The prosthetic group of milk lactoperoxidase is protoheme IX. *Biochim. Biophys. Acta* 579:181–90
79. Nichol, A. W., Angel, L. A., Moon, T., Clezy, P. S. 1987. Lactoperoxidase haem, an iron-porphyrin thiol. *Biochem. J.* 247:147–50
80. Thanabal, V., La Mar, G. N. 1989. A nuclear Overhauser effect investigation of the molecular and electronic structure of the heme crevice in lactoperoxidase. *Biochemistry* 28:7038–44
81. Manthey, J. A., Boldt, N. J., Bocian, D. F., Chan, S. I. 1986. Resonance Raman studies of lactoperoxidase. *J. Biol. Chem.* 261:6734–41

82. Dull, T. J., Uyeda, C., Strosberg, A. D., Nedwin, G., Seilhamer, J. J. 1990. Molecular cloning of cDNAs encoding bovine and human lactoperoxidase. *DNA Cell Biol.* 9:499–509

83. Lukat, G. S., Rodgers, K. R., Goff, H. M. 1987. Electron paramagnetic resonance spectroscopy of lactoperoxidase complexes: clarification of hyperfine splitting for the NO adduct of lactoperoxidase. *Biochemistry* 26:6927–32

84. Sievers, G., Gadsby, P. M. A., Peterson, J., Thomson, A. J. 1983. Assignment of the axial ligands of the haem in milk lactoperoxidase using magnetic circular dichroism spectroscopy. *Biochim. Biophys. Acta* 742:659–68

85. Kitagawa, T,, Hashimoto, S., Teraoka, J., Nakamura, S., Yajima, H., Hosoya, T. 1983. Distinct heme-substrate interactions of lactoperoxidase probed by resonance Raman spectroscopy: difference between animal and plant peroxidases. *Biochemistry* 22:2788–92

86. Behere, D. V., Gonzalez-Vergara, E., Goff, H. M. 1985. Comparison of heme environments and proximal ligands in peroxidases and other hemoproteins through carbon-13 nuclear magnetic resonance spectroscopy of carbon monoxide complexes. *Biochim. Biophys. Acta* 131:607–13

87. Shiro, Y., Morishima, I. 1986. Structural characterization of lactoperoxidase in the heme environment by proton NMR spectroscopy. *Biochemistry* 25:5844–49

88. Mäkinen, K. K., Mäkinen, P-L. 1982. Preparation of azolactoperoxidase. Evidence for the dependence of lactoperoxidase activity on tyrosyl residues. *Biochem. Biophys. Res. Commun.* 105:1402–07

89. Mäkinen, K. K. 1982. Evidence for an active carboxyl group in bovine milk lactoperoxidase. *Biochem. Int.* 5:375–80

90. Mäkinen, K. K., Mäkinen, P-L. 1982. Photochemical inactivation of lactoperoxidase sensitized by carbonyl compounds. *Eur. J. Biochem.* 123:171–78

91. Hosoya, T., Sakurada, J., Kurokawa, C., Toyoda, R., Nakamura, S. 1989. Interaction of aromatic donor molecules with lactoperoxidase probed by optical difference spectra. *Biochemistry* 28:2639–44

92. Paul, K-G., Ohlsson, P-I. 1978. Equilibria between horseradish peroxidase and aromatic donors. *Acta Chem. Scand.* B32:395–404

93. Modi, S., Behere, D. V., Mitra, S. 1989. Binding of aromatic donor molecules to lactoperoxidase: proton NMR and optical difference spectroscopic studies. *Biochim. Biophys. Acta* 996: 214–25

94. Modi, S, Behere, D. V., Mitra, S. 1989. Binding of thiocyanate to lactoperoxidase: ^{1}H and ^{15}N nuclear magnetic resonance studies. *Biochemistry* 28:4689–94

95. Sakurada, J., Takahashi, S., Shimizu, T., Hatano, M., Nakamura, S., Hosoya, T. 1987. Proton and iodine-127 nuclear magnetic resonance studies on the binding of iodide by lactoperoxidase. *Biochemistry* 26:6478–83

96. Allison, W. S., Swain, L. C. S., Tracy, M., Benitez, L. V. 1973. The inactivation of lactoperoxidase and the acyl phosphatase activity of oxidized glyceraldehyde-3-phosphate dehydrogenase by phenylhydrazine and phenyldiimide. *Arch. Biochem. Biophys.* 155:400–04

97. Nakamura, S, Nakamura, M., Yamazaki, I., Morrison, M. 1984. Reactions of ferryl lactoperoxidase (compound II) with sulfide and sulfhydril compounds. *J. Biol. Chem.* 259:7080–85

98. Doerge, D. R. 1986. Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. *Biochemistry* 25: 4724–28

99. Doerge, D. R. 1988. Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. Identification of turnover and inactivation pathways. *Biochemistry* 27:3697–70

100. Dawson, J. H., Sono, M. 1987. Cytochrome P-450 and chloroperoxidase: thiolate-ligated heme enzymes. Spectroscopic determination of their active site structures and mechanistic implications of thiolate ligation. *Chem. Rev.* 87:1255–76

101. Griffin, B. W. 1991. Chloroperoxidase: a review. See Ref. 1, pp. 85–137

102. Blanke, S. R., Hager, L. P. 1988. Identification of the fifth axial heme ligand of chloroperoxidase. *J. Biol. Chem.* 263:18739–43

103. Lukat, G. S., Goff, H. M. 1990. A nuclear magnetic resonance study of axial ligation for the reduced states of chloroperoxidase, cytochrome P-450$_{cam}$, and porphinatoiron(II) thiolate complexes. *Biochim. Biophys. Acta* 1037: 351–59

104. Thomas, J. A., Morris, D. R., Hager, L. P. 1970. Chloroperoxidase. VII. Classical peroxidatic, catalatic, and halogenating forms of the enzyme. *J. Biol. Chem.* 245:3129–34

105. Lambeir, A-M., Dunford, H. B., Pickard, M. A. 1987. Kinetics of the oxidation of ascorbic acid, ferrocyanide and

p-phenolsulfonic acid by chloroperoxidase compounds I and II. *Eur. J. Biochem.* 163:123- 27

106. Sono, M., Dawson, J. H., Hall, K., Hager, L. P. 1986. Ligand and halide binding properties: peroxidase-type active site heme environment with cytochrome P-450 type endogenous axial ligand and spectroscopic properties. *Biochemistry* 25:347–56
107. Campbell, B. A., Araiso, T., Reinisch, L., Yue, K. T., Hager, L. P. 1982. A kinetic study of the binding of carbon monoxide to ferrous chloroperoxidase. *Biochemistry* 21:4343–49
108. Blanke, S. R., Hager, L. P. 1990. Chemical modification of chloroperoxidase with diethylpyrocarbonate. Evidence for the presence of an essential histidine residue. *J. Biol. Chem.* 265:12454–61
109. Palcic, M. M., Rutter, R., Araiso, T. Hager, L. P., Dunford, H. B. 1980. Spectrum of chloroperoxidase compound I. *Biochem. Biophys. Res. Commun.* 94:1123–27
110. Rutter, R., Hager, L. P. 1982. The detection of two electron paramagnetic resonance radical signals associated with chloroperoxidase compound I. *J. Biol. Chem.* 257:7958–61
111. Rutter, R., Hager, L. P., Dhonau, H., Hendrich, M., Valentine, M., Debrunner, P. 1984. Chloroperoxidase compound I: electron paramagnetic resonance and Mössbauer studies. *Biochemistry* 23:6809–16
112. Neidleman, S. L., Geigert, J. 1986. *Biohalogenation: Principles, Basic Roles and Applications,* pp. 105–12. New York: Ellis Horwood. 203 pp.
113. Geigert, J., Lee, T. D., Dalietos, D. J., Hirano, D. S., Neidleman, S. L. 1986. Epoxidation of alkenes by chloroperoxidase catalysis. *Biochem. Biophys. Res. Commun.* 136:778–82
114. McCarthy, M-B., White, R. E. 1983. Functional differences between peroxidase compound I and the cytochrome P450 reactive oxygen intermediate. *J. Biol. Chem.* 258:9153–58
115. Geigert, J., De Witt, S. K., Neidleman, S. L., Lee, G., Dalietos, D. J., Moreland, M. 1983. DMSO is a substrate for chloroperoxidase. *Biochem. Biophys. Res. Commun.* 116:82–85
116. Colonna, S., Gaggero, N., Manfredi, A., Casella, L., Gullotti, M., et al. 1990. Enantioselective oxidations of sulfides catalyzed by chloroperoxidase. *Biochemistry* 29:10465–68
117. Ramakrishnan, K., Oppenhuizen, M. E., Saunders, S., Fisher, J. 1983. Stereoselectivity of chloroperoxidase-dependent halogenation. *Biochemistry* 22:3271–77
118. Yamada, H., Itoh, N., Izumi, Y. 1985. Chloroperoxidase-catalyzed halogenation of *trans*-cinnamic acid and its derivatives. *J. Biol. Chem.* 260:11962–69
119. Franssen, M. C. R., van der Plas, H. C. 1987. The chlorination of barbituric acid and some of its derivatives by chloroperoxidase. *Bioorg. Chem.* 15:59–70