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MINI-REVIEW

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Heme-thiolate haloperoxidases: versatile biocatalysts with biotechnological and environmental significance

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Abstract Heme-thiolate haloperoxidases are undoubtedly the most versatile biocatalysts of the hemeprotein family and share catalytic properties with at least three further classes of heme-containing oxidoreductases, namely, classic plant and fungal peroxidases, cytochrome P450 monooxygenases, and catalases. For a long time, only one enzyme of this type—the chloroperoxidase (CPO) of the ascomycete *Caldariomyces fumago*—has been known. The enzyme is commercially available as a fine chemical and catalyzes the unspecific chlorination, bromination, and iodation (but no fluorination) of a variety of electrophilic organic substrates via hypohalous acid as actual halogenating agent. In the absence of halide, CPO resembles cytochrome P450s and epoxidizes and hydroxylates activated substrates such as organic sulfides and olefins; aromatic rings, however, are not susceptible to CPO-catalyzed oxygen-transfer. Recently, a second fungal haloperoxidase of the heme-thiolate type has been discovered in the agaric mushroom *Agrocybe aegerita*. The UV–Vis adsorption spectrum of the isolated enzyme shows little similarity to that of CPO but is almost identical to a resting-state P450. The *Agrocybe aegerita* peroxidase (AaP) has strong brominating as well as weak chlorinating and iodating activities, and catalyzes both benzylic and aromatic hydroxylations (e.g., of toluene and naphthalene). AaP and related fungal peroxidases could become promising biocatalysts in biotechnological applications because they seemingly fill the gap between CPO and P450 enzymes and act as "self-sufficient" peroxygenases. From the environmental point of view, the existence of a halogenating mushroom enzyme is interesting because it could be linked to the multitude of halogenated compounds known from these organisms.

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Introduction

The advantages of enzymes as catalysts in chemical syntheses are increasingly recognized. They make chemical reactions possible under mild, environmentally friendly conditions in aqueous reaction mixtures and mostly, enzymes show specificity and selectivity that cannot be achieved by traditional chemical methods. Thus, certain enzymes catalyze regio-and stereoselective transformations leading to products that are useful as fine chemicals or pharmaceuticals. Despite several biocatalytic processes which have successfully put on the market—for example, the production and the manufacture of the sweetener aspartame (Schmid et al. 2001, 2002)—much research remains to be done before enzymes can be used routinely throughout the chemical industry (Schoemaker et al. 2003; Poliakoff et al. 2002).

In this context, selective oxygenations and halogenations of organic substrates are among the most desired reactions (van Beilen et al. 2003). Both transformations usually require aggressive chemicals and can lead to the formation of undesired and toxic by-products. There is a huge number of oxygenases in all kinds of organisms (bacteria, fungi, plants, animals) introducing one or two oxygen atoms specifically into substrate molecules (more than 50 dioxygenases and 150 monoxygenases; EC 1.13 and EC 1.14; Enzyme Nomenclature 1992). The number of halogenating enzymes is considerably smaller but there are a few types of biocatalysts catalyzing the incorporation of chlorine, bromine or iodine into organic molecules. Biological fluorination (i.e., C–F bond formation) is extremely rare and seemingly catalyzed by a specific fluorinase that mediates the reaction between the fluoride ion and *S*-adenosyl-L-methionine (Cadicamo et al. 2004; Deng et al. 2005).

Halogenating enzymes belong to three main groups (Murphy 2003): unselective haloperoxidases and perhydrolases (originally regarded as metal-free haloperoxidases; Picard et al. 1997), methyl transferases, which transfer a methyl group from *S*-adenosylmethionine to a halide (Wuosmaa and Hager 1990) as well as selective flavin-dependent halogenases. The latter require the support of a flavin reductase and produce hypohalous acid "trapped" and

guided inside the active site's tunnel, which leads to the regioselective halogenation of substrates. van Peé and Patallo (2006) have recently reviewed the present knowledge in the field of flavin-dependent halogenases and discussed their potential for (bio)chemical syntheses. Perhydrolases take effect via primarily formed peracids, which in turn oxidize halide ions (X^-) into hypohalous acid (XOH) acting as a free-diffusible halogenating agent (van Peé 2001).

This review deals with recent findings on haloperoxidases with focus on heme-thiolate chloroperoxidase (CPO) from the mold *Caldariomyces fumago*, that has been studied for almost 50 years, and a second enzyme of this type that has recently been discovered in a basidiomycetous fungus.

Haloperoxidases

Haloperoxidases contain either vanadium (V) or ferric heme (ferri-protoporphyrin IX) in the active site and generate, as perhydrolases, hypohalous acid but by the direct H_2O_2-dependent oxidation of halides (Murphy 2003). A vanadium bromoperoxidase was first isolated from the brown macroalga *Ascophyllum nodosum* (Vilter 1983). Later, similar enzymes with brominating or chlorinating activities, which show structural and functional similarities with acid phosphatases (Littlechild et al. 2002), were also found in other brown algae (e.g., *Laminaria* spp., Almeida et al. 2001), red algae (e.g., *Corallina* spp., Sheffield et al. 1993), gram-negative bacteria (e.g., *Pseudomonas aureofaciens*, van Peé and Lingens 1985), streptomycetes (e.g., *Streptomyces aureofaciens*, van Peé et al. 1987), as well as in the marine snail *Murex trunculus* (Jannun and Coe 1987), the lichen *Xanthoria parietina* (Plat et al. 1987), and the ascomycetous fungus *Curvularia inaequalis* (Simons et al. 1995; ten Brink et al. 2000). Comprehensive reviews on vanadium haloperoxidases focusing on their function in marine organisms were published by Butler and coworkers (Butler and Walker 1993; Butler 1999; Butler et al. 2001). An interesting recent approach tries to use vanadium haloperoxidases to disinfect surfaces on the basis of the formation of free hypochlorous acid (Hansen et al. 2004).

There are several animal and human heme peroxidases which can oxidize halides, for example, the flavin-heme chloroperoxidase from the marine polychaete *Notomastus lobatus*, myelo- and eosinophil peroxidases from human leukocytes as well as bovine lacto- and human thyroid peroxidases. Also bacterial, fungal, and plant heme peroxidases possess halogenating activities; thus, certain *Streptomyces* peroxidases oxidize chloride and bromide; lignin, manganese, soybean, and several algae peroxidases show brominating and horseradish peroxidase iodating activities. An overview of heme peroxidases with halogenating activity including respective references is given in Table 1. Haloperoxidases may oxidize chloride (Cl^-), bromide (Br^-), and/or iodide (I^-) but no fluoride (F^-). Iodine has the lowest redox potential and iodation is the most favored reaction, with bromination and chlorination requiring increasing levels of activation of the active site for halogenation (Robinson et al. 2000). Excellent reviews on biological halogenation and the involvement of halogenating enzymes including haloperoxidases were published by Littlechild

Table 1 Occurrence and properties of heme peroxidases with halogenating activity

Organism	Taxonomic Group	Enzyme	Halide	Reference
Caldariomyces fumago	Ascomycetous fungus	Chloroperoxidase (CPO)	Cl^-, Br^-, I^-	Morris and Hager 1966; Dunford et al. 1987
Agrocybe aegerita	Basidiomycetous fungus	*A. aegerita* peroxidase (AaP)	(Cl^-), Br^-, (I^-)	Ullrich et al. 2004; Ullrich and Hofrichter 2005
Phanerochaete chrysosporium	Basidiomycetous fungus	Lignin peroxidase (LiP)	Br^-, I^-	Farhangrazi et al. 1992
		Manganese peroxidase (MnP)	Br^-, I^-	Sheng and Gold 1997
Glycine max	Spermatophyta	Soybean peroxidase	Br^-, I^-	Munir and Dordick 2000
Armoracia rusticana	Spermatophyta	Horseradish peroxidase (HRP)	I^-	Adak et al. 2001
Penicillus capitatus	Chlorophyta	Bromoperoxidase	Br^-, I^-	Baden and Corbett 1980; Manthey and Hager 1981
Cystoclonium purpureum	Rhodophyta	Bromoperoxidase	Br^-, I^-	Pedersén 1976
Porphyridium purpureum	Rhodophyta	Iodoperoxidase	I^-	Murphy et al. 2000
Notomastus lobatus	Polychaete	Chloroperoxidase	Cl^-, Br^-, I^-	Chen et al. 1991
Homo sapiens	Mammalia	Myeloperoxidase	Cl^-, Br^-, I^-	Harrison and Schultz 1976; Thomas et al. 1995
		Eosinophil peroxidase	Cl^-, Br^-, I^-	Thomas and Fishman 1986
		Thyroid peroxidase	I^-	Rawitch et al. 1979
Bos taurus	Mammalia	Lactoperoxidase	Br^-, I^-	Taurog and Dorris 1991
Streptomyces phaeochromogenes	Gram-positive bacterium	Bromoperoxidase	Br^-	van Peé and Lingens 1984, 1985
Streptomyces toyocaensis	Gram-positive bacterium	Chloroperoxidase Bromoperoxidase	Cl^-, Br^- Br^-	Marshall and Wright 1996

(1999), Murphy (2003), Neilson (2003), and van Peé and Zehner (2003).

Among the halogenating heme peroxidases, *Caldariomyces fumago* CPO is particularly interesting because it bears a cysteine residue ligated to the proximal side of heme iron (heme-thiolate protein; Sundaramoorthy et al. 1995). In contrast, most other heme peroxidases have a histidine at this position (Dunford 1999). In consequence, CPO does not only halogenate organic substrates susceptible to electrophilic attack but possesses oxygenase properties as well. In this way, it resembles cytochrome P450-dependent monooxygenases belonging also to the heme-thiolate proteins (Sono et al. 1996) and catalyzing a multitude of biotechnologically important oxygen transfer reactions (Urlacher et al. 2004).

Caldariomyces fumago chloroperoxidase (CPO)

Chloroperoxidase (CPO; EC 1.11.1.10) was discovered by Hager and coworkers (Shaw and Hager 1959; Morris and Hager 1966) in the ascomycetous fungus *C. fumago* that belongs to the so-called sooty molds. These fungi colonize living-plant surface habitats and are characterized by darkly pigmented cell walls (Reynolds 1999). Already in the 1940s, *C. fumago* was found to produce chlorinated metabolites (Clutterbuck et al. 1940), among which caldariomycin (2,2-dichloro-1,3-cyclopentanediol) is the major product. Later, tracer experiments and enzymatic studies tried to substantiate the role of CPO in the biosynthesis of caldariomycin but despite the fact that CPO can chlorinate caldariomycin precursors in vitro, there is no nonambiguous proof for that in vivo (Beckwith and Hager 1963, van Pée 2001).

CPO is the most extensively studied halogenating enzyme and commercially available as a fine chemical (e.g., from Sigma-Aldrich and Jülich Fine Chemicals GmbH). It is a heavily glycosylated protein (25–30 % carbohydrates, two high-mannose *N*-glycosylation sites) with a molecular mass of 42 kDa and an isoelectric point (pI) of 4.0 (Morris and Hager 1966; Hollenberg and Hager 1973; Hashimoto and Pickard 1984; Kenigsberg et al. 1987). Two CPO isoenzymes (A and B) were reported (Sae and Cunningham 1979) but later work showed that there are multiple forms, differing only in the carbohydrate content (Hashimoto and Pickard 1984). CPO can be produced at concentrations up to 280 and 600 mg l^{-1} in batch airlift and semicontinuous flow bioreactors, respectively (Carmichael and Pickard 1989; Blanke et al. 1989). The CPO gene was isolated and cloned, the DNA sequence determined (Fang et al. 1986; Nuell et al. 1988), and the enzyme can be expressed heterologously in *Aspergillus niger* (Conesa et al. 2001). Directed evolution of CPO was successful and led to mutant proteins with improved catalytic properties (Rai et al. 2001). It is interesting to note that putative chloroperoxidase sequences showing about 30 % identity to that of CPO have been found in the ascomycete *Emericella nidulans* and the common basidiomycete *Agaricus bisporus* (white button mushroom) in protein database searches (van Peé 2001).

Catalytic cycle of CPO

The basic mechanism of CPO-catalyzed halogenations can be summarized as follows (Fig. 1; Robinson et al. 2000; van Peé 2001): the native ferric enzyme (resting state) contains ferric protoporphyrin IX in the active site (heme Fe^{3+}), and the catalytic cycle is initiated by the heterolytic cleavage of a loosely bound H_2O_2 molecule, which requires a two-electron transfer from the heme and leads to the formation of one molecule of water and CPO compound I (H_2O_2 can be generated by extracellular oxidases and peroxidases usually need levels between 50 and 2,000 μM; Hattaka 2001). Compound I is a typical intermediate of heme peroxidases (Dunford 1999), and chemically an oxo-ferryl porphyrin cation-radical complex [heme $(Fe^{4+}=O)^{\bullet+}$]. Compound I is unstable and proposed to react with the halide (X^-) to form a hypothetical ferric hypohalite adduct termed compound X (heme Fe^{3+}–O–X). Compound X is also very unstable and decomposes to yield the resting enzyme and a halonium ion (X^+) that has—in aqueous solution—hydroxyl (OH^-) as the counterpart and, hence, is a hypohalous acid (HOX) (Griffin 1983).

If the hypohalous acid would be bound to the active site, it may catalyze selective halogenations but if it diffuses into free solution it will show the same reactivity and low

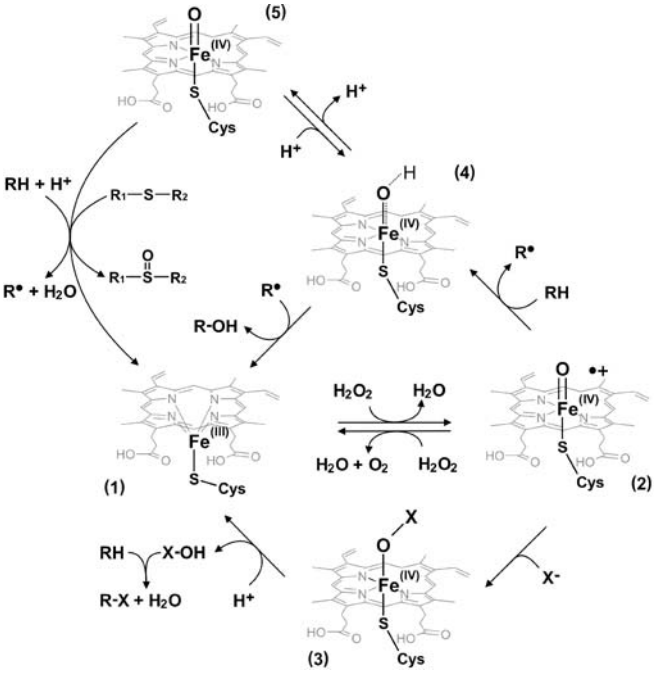

Fig. 1 Catalytic cycle of a heme-thiolate haloperoxidase (modified according to Anderson and Dawson 1991; Hollenberg 1992; Green et al. 2004; Ortiz de Montellano and de Voss 2005). (*1*) Resting ferric enzyme, (*2*) compound I, (*3*) compound X that releases hypohalous acid (HOX), (*4*) protonated compound II, (*5*) compound II. Classic peroxidase reaction (e.g., phenol oxidation): (*2*) ⇒ [(*4*)] ⇒ (*5*) ⇒ (*1*) (without oxygen transfer to the substrate); halogenation: (*3*) ⇒ (*1*); hydroxylation (e.g. OH-transfer to benzylic carbon, see also Fig. 3: (4) ⇒ (1); oxygenation (e.g. sulfoxidation): (*5*) ⇒ (*1*) (oxygen transfer to the substrate)

selectivity as chemically produced hypohalous acid. Both possibilities have been subject of a long controversial discussion in the literature (Wagenknecht and Woggon 1997; Sundaramoorthy et al. 1998; Littlechild 1999; Murphy 2003), but actual findings based upon the chlorination of 35 different substrates of different reactivity and size indicate that the chlorine transfer to the final substrate occurs most probably outside the active site and is mediated by diffusible electrophilic species (Manoj and Hager 2005).

Halogenation by CPO requires a low pH (<3) and leads rapidly to the inactivation of the enzyme due to reaction with hypohalous acid (Wagenknecht and Woggon 1997). Substrates susceptible to halogenation include a variety of organic compounds and structures, for example, alicyclic ketones (e.g., monochlorodimedone, the mostly used substrate to assay haloperoxidases; Hager et al. 1966; Hallenberg and Hager 1978), phenols (Wannstedt et al. 1990), flavonoids (Yaipakdee and Robertson 2001), aromatic acids (Yamada et al. 1985), polycyclic aromatic hydrocarbons (Niu and Yu 2004; Vazquez-Duhalt et al. 2001), biphenyls (Speicher 2000), antipyrine (Ashley and Griffin 1981), lignin and lignin model compounds (Ortiz-Bermúdez et al. 2003), fulvic and humic acids (Niedan et al. 2000; Matucha et al. 2003), steroids (Neidleman and Levine 1968), acetic and other aliphatic short-chain carboxylic acids (Hoekstra et al. 1999; Laturnus et al. 2005), as well as alkenes and alkyns (Geigert et al. 1983a–c). Some examples of CPO-catalyzed halogenation reactions are given in Fig. 2. Similar patterns of halogenation were reported for hypohalous acid in free solution (Albrich et al. 1981; Folkes et al. 1995).

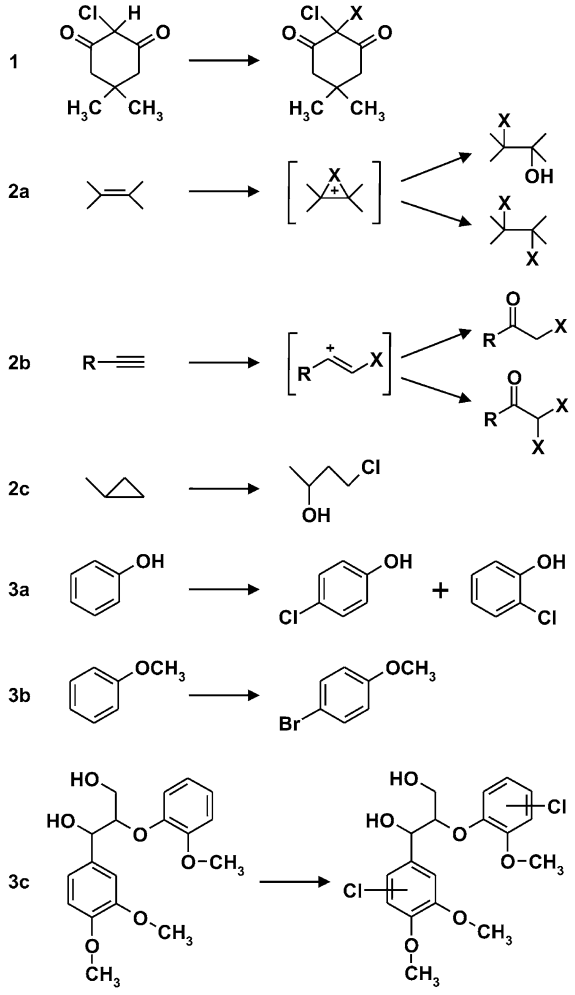

Fig. 2 Some halogenations catalyzed by chloroperoxidase from *Caldariomyces fumago*. *1* Chlorination and bromination of β-diketones such as monochlorodimedone (Morris and Hager 1966); *2a* halogenation of alkenes (Geigert et al. 1983b,c); *2b* halogenation of alkynes (Geigert et al. 1983a,c); *2c* chlorination and bromination of cycloalkanes, e.g., of methylcyclopropane (Geigert et al. 1983a); *3a* chlorination of phenol (Wannstedt et al. 1990), *3b* bromination of anisole (Pickard et al. 1991); *3c* chlorination of a non-phenolic lignin model compound (Ortiz-Bermudéz et al. 2003)

Non-halogenating reactions of CPO

Besides halogenation, CPO is able to catalyze a number of other oxidations independent of halide ions including *N*-dealkylation (Kedderis et al. 1980; Okazaki and Guengerich 1993), oxidation of amino into nitroso groups (Corbett et al. 1978), epoxidation of olefins (Lakner and Hager 1996), benzylic and propargylic hydroxylations (Miller et al. 1995; Hu and Hager 1998, 1999), sulfoxidations (Colonna et al. 1990), oxidation of alcohols and aldehydes (Baciocchi et al. 1999), as well as classic peroxidase reactions (phenol oxidation; La Rotta and Bon 2002) (Fig. 3). In the absence of halide and organic substrate, the prime reaction of catalases is observed, the disproportionation of H_2O_2 to dioxygen and water (Sun et al. 1994).

Some of these reactions have a promising biotechnological potential because they lead to the regio- and enatioselective oxygenation of substrate molecules and, hence, to prochiral or chiral products (Littlechild 1999). The mechanism of oxygen transfer is peroxygenation (Figs. 1 and 3), i.e., the oxygen comes from H_2O_2 (Manoj and Hager 2001), and it has been proposed to resemble the so-called "peroxide-shunt" in the catalytic cycle of cytochrome P450 enzymes (Nordblom et al. 1976; Doerge and Corbett 1991; Sundaramoorthy et al. 1995; Matsunaga et al. 2002; Makris et al. 2005; see also Fig. 7). Recent studies with the use of X-ray spectroscopy indicate that CPO catalysis involves a unique compound II that is a protonated oxo-ferryl porphyrin (heme Fe^{4+}–O–H) and a good model for the hypothetical rebound intermediate in the P450 oxygenation cycle (Green et al. 2004) (Figs. 1 and 3).

CPO hydroxylates the cyclo-olefinic ring in indene via an epoxide intermediate (Manoj et al. 2000) and oxidizes one methyl group in *p*-xylol to alcohol, aldehyde, and carboxyl functions (Morgan et al. 2002) but it seemingly cannot catalyze aromatic hydroxylations as P450s can. The reason for this has been attributed to significant differences at the active sites of both enzymes (McCarthy and White 1983; Lukat and Goff 1990; Sundaramoorthy et al. 1998).

Several attempts have been made to make use of the biocatalytic potential of CPO, and promising catalytic

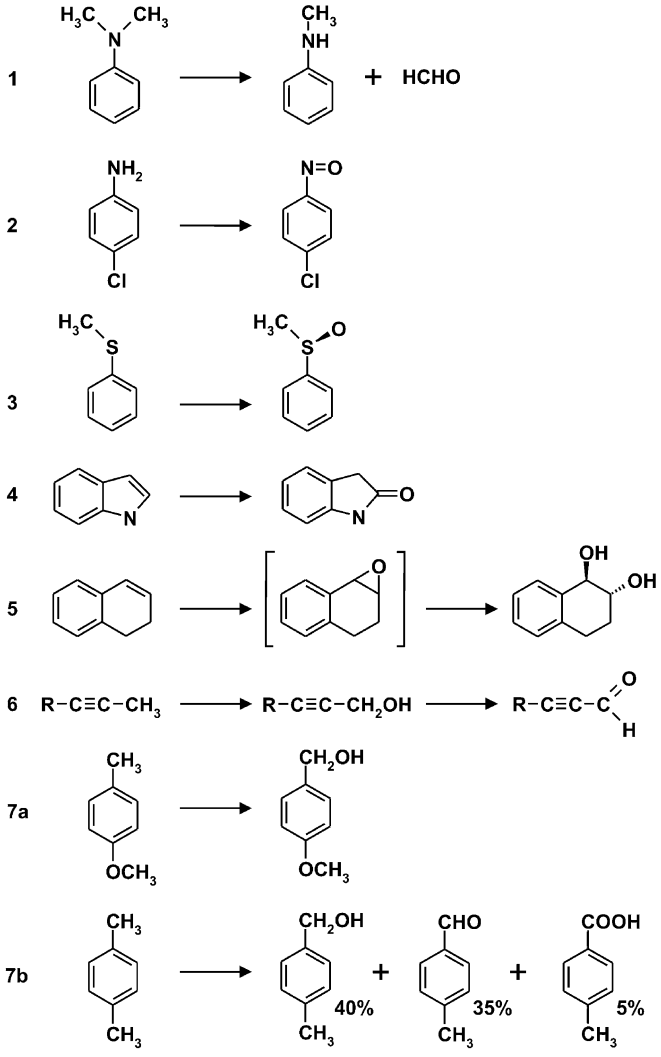

Fig. 3 Selected nonhalogenating oxidations catalyzed by CPO. *1N*-dealkylation, e.g., demethylation of *N*,*N*-dimethylaniline (Kedderis et al. 1980); *2* oxidation of the amino group of chloroaniline into the corresponding nitroso compound (Corbett et al. 1978, Doerge and Corbett 1991); *3* enantioselective sulfoxidation of thioanisole into the corresponding (*R*)-sulfoxide (van de Velde et al. 2001); *4* indole oxidation into oxindole (Seelbach and Kragl 1997); *5* epoxidation/hydroxylation of 1,2-dihydronaphthalene (Sanfilippo et al. 2004); *6* propargylic hydroxylation (Hu and Hager 1998); *7a* benzylic hydroxylation of *p*-methylanisole (Miller et al. 1995); *7b* selective hydroxylation and subsequent oxidation of one methyl group in *p*-xylene (Morgan et al. 2002)

transformations in different reaction systems have been described, even though still no industrial application has been established (Franssen and van der Plas 1992; Franssen 1994; Holland and Weber 2000; Murphy 2003; Dembitsky 2003). Table 2 gives an overview on recent developments in this field with focus on selective oxidations and the improvement of enzyme stability.

A novel heme-thiolate haloperoxidase from the mushroom *Agrocybe aegerita*

Recently, we have discovered—or rather recognized as such—a second heme-thiolate haloperoxidase in the fungus *Agrocybe aegerita* strain A1 (Ullrich et al. 2004). This agaric basidiomycete is a popular edible mushroom in Mediterranean countries and grows preferably on poplar stumps, bark, and mulch, often in tight contact with soil (Stamets 2000; Zadrazil 2000). The enzyme was first described as an "alkaline lignin peroxidase" (Upadhyay 1995) because it oxidizes veratryl alcohol into veratryl aldehyde at pH 7 [true lignin peroxidase (LiP) catalyzes this reaction only under acidic conditions between pH 2.5 and 5; Kirk and Farrell 1987]. However later, it turned out that the *Agrocybe aegerita* peroxidase (AaP) does not oxidize nonphenolic lignin moieties (β-O-4 structures) that way as LiP does, and the enzyme was referred to as an "unusual peroxidase". Unfortunately, AaP was difficult to produce during this time and not enough protein was available for its purification and detailed characterization. This changed only in 2003, when Ullrich et al. (2004) found out that *A. aegerita* produces relatively high and stable peroxidase levels in complex liquid media based on soybeans.

AaP can now be produced in stirred-tank bioreactors, and it was purified to homogeneity and characterized with respect to its physical and catalytic properties (Ullrich et al. 2004). Two AaP fractions (AaP I and II) with the same molecular mass of 46 kDa were separated by ion exchange chromatography, and their further analysis by 2-D gel electrophoresis revealed the presence of six isoforms with different isoelectric points (4.6–5.4). Current studies indicate that these isoforms are simply differently glycosylated proteins (~10–20 % carbohydrates; Ullrich 2005, unpublished result).

Figure 4 shows the alignment of N-terminal amino acids of AaP and several heme peroxidases as well as of a fungal cytochrome P450 ($P450_{nor}$). While AaP shows very little sequence identity to most heme peroxidases, five out of 14 determined amino acids at the N-terminus of AaP are identical to those of CPO; nitric oxide oxidase ($P450_{nor}$) from the ascomycete *Cylindrocarpon tonkinense* shares three amino acids with AaP (Fig. 4).

Spectral properties of AaP

The spectroscopic and catalytic properties of AaP confirm its possible relationship both to CPO and P450s. The Soret absorption band (A_{max}) of the reduced carbon monoxide complex [AaP–Fe^{2+}=CO; carbomonoxy-ferrous heme] has its maximum at 445 nm, which fits well to that of CPO (443–445 nm; Fig. 5c; Hollenberg and Hager 1973) and is a clear indication for a cysteine-ligated heme in the active site (Lewis 2001). The Soret maxima of cytochrome P450 CO-complexes range between 446 and 452 nm, and this characteristic absorption behavior was deciding for their nomenclature (Lewis 2001; Correia 2005). It is interesting

Table 2 Recent developments in *Caldariomyces fumago* chloroperoxidase (CPO) catalysis

Reactions	Comments	References
Olefine oxidation	Epoxidations of styrene in nonaqueous media	Santhanam and Dordick 2002
Olefine oxidation	Oxidation of cyclic conjugated dienes	Sanfilippo and Nicolosi 2002
Olefine oxidation	CPO conjugated with polystyrene for interfacial styrene epoxidations	Zhu and Wang 2005
Sulfoxidation	Oxidation of sulfides in diesel fuel	Ayala et al. 2000
Sulfoxidation, indole oxidation	Enatioselective oxidations in hydrophobic media	van de Velde et al. 2001
Sulfoxidation	Stereoselective oxidation of methionine derivatives	Holland et al. 2002
Sulfoxidation	Microencapsulated CPO for the enatioselective oxidation of sulfides	Trevisan et al. 2004
Sulfoxidation	Oxidation 4,6-dimethyldibenzothiophene	Torres and Aburto 2005
Sulfoxidation	Model sulphur compounds as mechanistic probes for CPO catalyzed sulfoxidations	Peñéñory et al. 2004
Hydroxylation	Selective hydroxylation of the furan ring	Alvarez et al. 2001
Hydroxylation	Catalysis in ionic liquids, dihydronaphthalene hydroxylation	Sanfilippo et al. 2004
Alcohol oxidation	Prochiral selectivity in the oxidation of arylalkanols	Baciocchi et al. 2001
Alcohol oxidation	Enatioselective oxidation of *cis*-cyclopropylmethanols to corresponding aldehydes	Hu and Dordick 2002
Chlorination	Chloride analysis using CPO and a benzidine	Keener and Watwood 2004
Enzyme assay	Spectrofluorometrical method to measure haloperoxidase activities	Jacks (2005)
General	Stabilization of CPO by polyethylene glycol	Spreti et al. 2004
General	Enzyme stabilization by mesoporous sol-gel glass complexes	Borole et al. 2004
General	Immobilization of CPO in nanophase-separated amphiphilic networks	Bruns and Tiller 2005
General	Enzyme stabilization by reductive alkylation, amidation and cross-linking	La Rotta Hernandez et al. 2005
Enzyme assay	A colorimetric method for detection and quantification of chlorinating activity of hemeperoxidases	Manoj and Hager 2006

to note that all absorption characteristics of the native AaP (enzyme as isolated; A_{max}=420 nm) are almost identical to resting state P450s (A_{max}=416–420 nm; Lewis 2001) and differ noticeably from native CPO (A_{max} 401 nm; Table 3; Fig. 5a). Then again, the Soret bands of the dithionite-reduced forms of AaP, CPO, and P450s absorb in the same range (Fig. 5b). Also the spectral properties of the native flavin-chloroperoxidase from the polychaete *Notomastus lobatus* (*Nl*CPO) are quite similar to AaP and P450s (Table 3) but the dithionite-reduced *Nl*CPO and its CO-complex absorb in the shorter wavelength range (<430 nm) because the iron is seemingly ligated to a histidine instead to a cystein residue (Roach et al. 1997). In summary, the spectral properties of AaP, in all states studied so far, bear great resemblance to cytochrome P450s. Further selective inhibition tests (e.g., with azide, cyanide, fluoride) and spectral studies will have to substantiate this fact, and the question will have to be answered whether the resting state AaP contains low-spin aquo-ferric iron (Fe^{3+}–H_2O) as most P450s (Makris et al. 2005) or oxy-ferrous iron (Fe^{2+}=O_2) as presumed for *Nl*CPO (Roach et al. 1997).

Enzyme	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AaP	E	P	G	K	P	P	G	P	P	(E)	E	S	S	A
CPO	E	P	G	S	G	I	G	Y	P	Y	D	N	N	T
$P450_{nor}$	E	P	P	S	F	P	F	Q	R	A	S	G	M	E
CiP	Q	G	P	G	G	G	G	S	V	T	C	P	G	G
*Pc*LiP	A	T	C	S	N	G	K	T	V	G	D	A	S	S
*Ab*MnP	A	Q	C	A	D	G	T	-	T	V	S	N	E	A
VP	A	T	D	A	D	G	R	T	T	A	-	N	A	A
MP	C	P	E	Q	D	K	X	R	T	I	T	G	M	C
CcP	M	Q	S	S	Q	L	L	P	L	G	S	L	L	L
HRP	E	L	T	P	T	F	Y	D	N	S	C	P	N	V

Fig. 4 N-terminal amino acid sequences of *Agrocybe aegerita* peroxidase (AaP major 2-D spot of fraction II) and different fungal, animal and plant heme peroxidases as well as of a fungal cytochrome P450 (modified according to Ullrich et al. 2004, Dunford 1999). CPO *Caldariomyces fumago* chloroperoxidase; $P450_{nor}$ nitric oxide reductase (CYP55A2) from the ascomycete *Cylindrocarpon tonkinense* (Kudo et al. 1996; Stündl et al. 2000); CiP *Coprinus cinereus* peroxidase; *Pc*LiP *Phanerochaete chrysosporium* lignin peroxidase 8; *Ab*MnP manganese peroxidase from *Agaricus bisporus* (Lankinen et al. 2001); VP versatile peroxidase of *Pleurotus eryngii* (Martínez et al. 1996); MP human myeloperoxidase; CcP Cytochrome c peroxidase of *Pseudomonas aeruginosa*; HRP horseradish peroxidase

Catalytic activities of AaP

AaP has strong brominating and weak chlorinating activities and converts phenol in the presence of KBr into 4- and 2-bromophenol at the ratio of 4:1. Substantial amounts of polybrominated phenols as in case of CPO (Wannstedt et al. 1990) and *Nl*CPO (Yoon et al. 1994) were not observed (Ullrich et al. 2004). With Cl^- as halide, only traces of 2-chlorophenol were formed and the chlorinating activity is roughly 10,000-fold lower than the brominating one. Also the iodide-oxidating activity of AaP is little pronounced and more than 200-fold lower than those of CPO, HRP, or Mn peroxidase (Ullrich 2005). The reason for such different reactivity may be rather connected to the halide-binding site of AaP than to its redox potential or the reactivity of halides.

AaP possesses also different oxidative activities independent of halide and oxidizes among others aromatic alcohols (e.g., benzyl, anisyl, vanillyl or 4-ethoxy-3-methoxybenzyl alcohols) into the corresponding aldehydes and further into benzoic acids (Ullrich et al. 2004). Similar

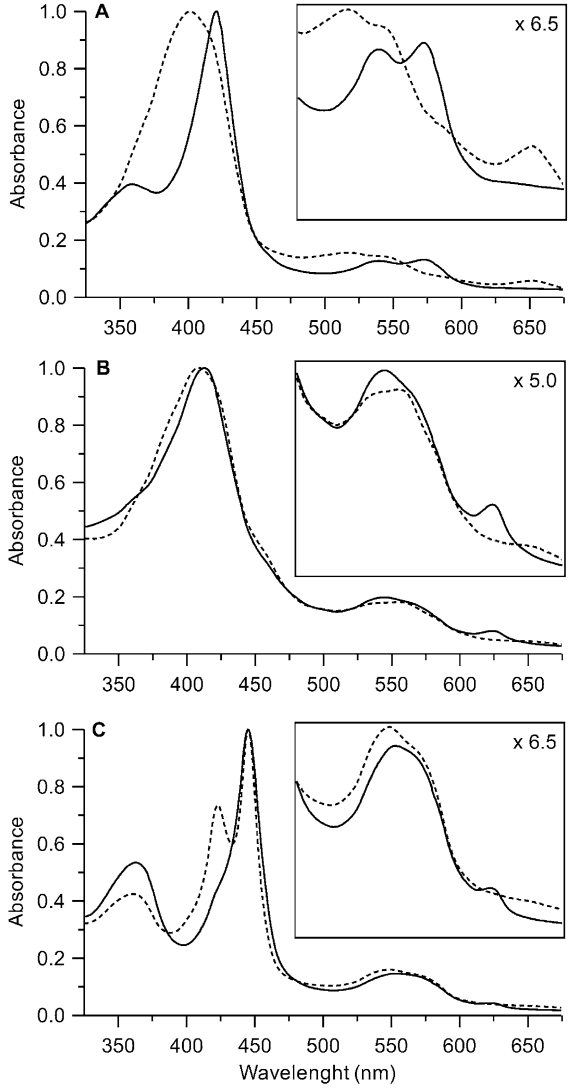

Fig. 5 UV–Vis absorption spectra of native *Agrocybe aegerita* peroxidase (AaP; *solid line*) and native *Caldariomyces fumago* CPO (*dashed line*) (**a**) as well as of the respective dithionite-reduced forms (**b**) and the reduced CO-complexes (**c**). Spectra were recorded in 20 mM phosphate buffer (pH 7) (modified according to Ullrich et al. 2004, Ullrich 2005, Ullrich and Hofrichter 2005). *Insets* represent the fivefold or 6.5-fold magnified spectra in the region from 475 to 625 nm

reactions have been reported for CPO though the final oxidation of benzaldehyde to benzoic acids is less pronounced (Baciocchi et al. 1999). The CPO reaction has been proposed to occur via an attack on the benzylic carbon, resulting in the formation of the benzylic radical and cation (Baciocchi et al. 2001), which may also apply to AaP (Ullrich et al. 2004). Later, it turned out that AaP does not only oxidize the benzylic carbon of aryl alcohols but also the benzylic carbon of toluene (Ullrich and Hofrichter 2005). This reaction requires the transfer of oxygen and is only ineffectively catalyzed by CPO (Miller et al. 1995). The most remarkable activity concerning AaP catalysis, however, is the hydroxylation of the aromatic ring. Thus, toluene oxidation by AaP yields also *p*- and *o*-cresol as well as methyl-*p*-benzoquinone. Naphthalene hydroxylation proceeds regioselectively and leads to the formation of 1-naphthol and traces of 2-naphthol (Fig. 6). It is interesting to note that the ratio of 1-naphthol vs 2-naphthol (36:1) is almost the same as that reported for the oxidation of naphthalene by cytochrome $P450_{cam}$ (32:1; England et al. 1998). As in case of CPO, a peroxygenation mechanism has been proposed for AaP (Ullrich and Hofrichter 2005). The hydroxylation of other aromatic compounds by AaP is currently under investigation.

Due to the properties of AaP and its ability to catalyze both the bromination and hydroxylation of aromatic substrates, we propose that it represents a "missing link" between CPO and cytochrome P450s. Its catalytic cycle may comprise similar intermediates and reactions as shown in Fig. 2 for CPO as well as in Fig. 7, for the peroxide-shunt of cytochrome P450s. The latter pathway is a peroxidase-like side-reaction of some P450 monooxygenases and proceeds directly from the ferric enzyme-substrate complex (Fe^{3+}–R–H) via a protonated ferric peroxo-intermediate (Fe^{3+}–O–OH, compound 0, Shaik and de Visser 2005) to a putative ferryl-oxo species (Fe^{4+}=O; compound I) that is thought to be the actual hydroxylating agent in most P450

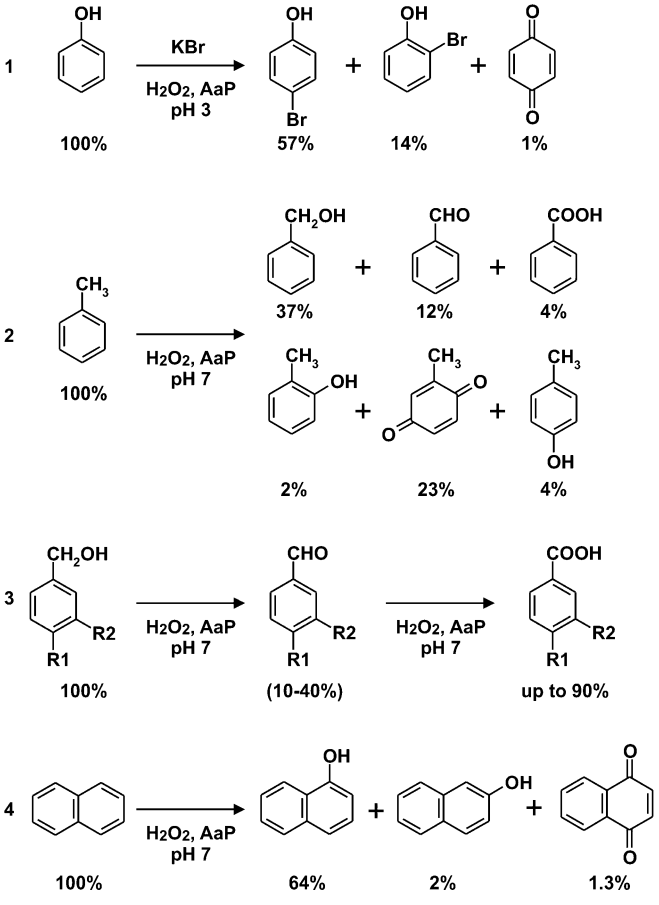

Fig. 6 Oxidation reactions catalyzed by AaP (according to Ullrich et al. 2004; Ullrich 2005; Ullrich and Hofrichter 2005). *1* Bromination of phenol; *2* benzylic and aromatic hydroxylation of toluene; *3* stepwise oxidation of substituted benzyl alcohols (e.g., anisyl, vanillyl, or veratryl alcohols) via the corresponding transient aldehydes into benzoic acids; *4* hydroxylation of naphthalene

Table 3 Spectral characteristics of selected heme peroxidases with halogenating activity and cytochrome P450 enzymes (according to Dunford 1999; Lewis 2001; Ullrich 2005). α, β, δ-absorption bands for UV and visible light; CT1, CT2–charge transfer bands

		Soret maxima (nm)			Further maxima (nm) of native enzymes				
Organism	Enzyme	Resting	Reduced	CO adduct	CT1	α	β	CT2	δ
Caldariomyces fumago[1]	Chloroperoxidase	401	409	443	652	–	(545)	517	–
Penicillus capitatus[2]	Bromoperoxidase	413	447	426	635	–	545	495	–
Bjerkandera adusta[3]	Mn peroxidase	408	439	424	640	–	(541)	504	–
Armoracia rusticana[4]	Horseradish peroxidase	402.5	439	423	643	–	(530)	498	–
Oryctolagus cuniculus[4]	Myeloperoxidase[5]	430	475	462	620	571	–	496	–
Notomastus lobatus[6]	Flavin/Chloroperoxidase	417	426	422	–	575	540	–	355
Agrocybe aegerita[7]	Haloperoxidase	420	413	445	–	572	540	–	359
Fusarium oxysporum[8]	$P450_{nor}$	414	406	447	–	565	533	–	–
Rattus norvegicus[9]	CYP1A1	418	413	447	–	568	535	–	357
Mycobacterium tuberculosis[10]	CYP121	416.5	N.D.	448	–	565	538	–	–

[1]Morris and Hager (1966), Hollenberg and Hager (1973)
[2]Manthey and Hager (1981)
[3]Ullrich (2005)
[4]Dunford (1999)
[5]Morel and Vignais (1984), Kooter et al. (1999)
[6]Chen et al. (1991), Roach et al. (1997)
[7]Ullrich et al. (2004), Ullrich and Hofrichter (2005)
[8]Shiro et al. (1995)
[9]Lewis (2001)
[10]McLean et al. (2002)

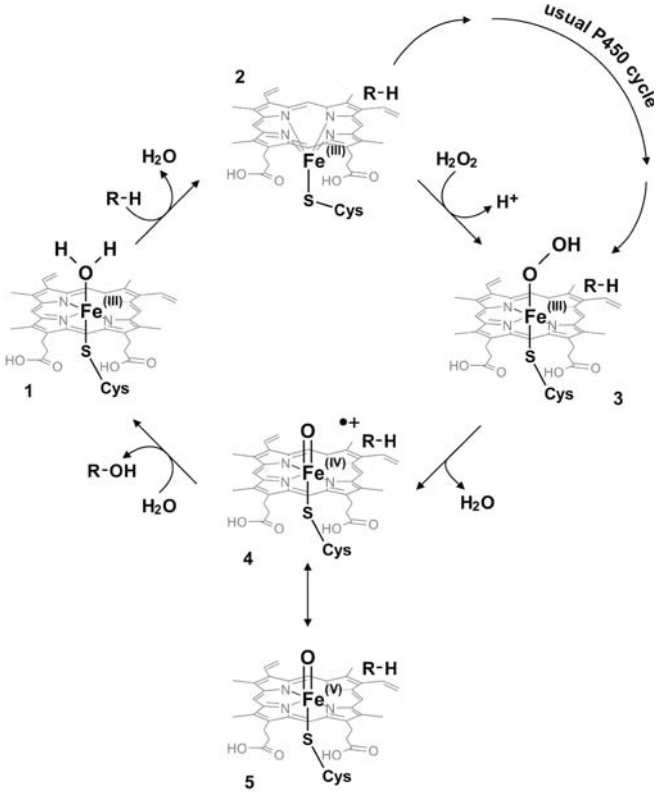

Fig. 7 "Peroxide shunt" pathway of cytochrome P450 enzymes (modified according to Ortiz de Montellano and de Voss 2005; Makris et al. 2005; Ullrich 2005). *1* native hydro-ferric enzyme (Fe^{3+}–H_2O), *2* ferric enzyme-substrate complex (Fe^{3+}–R–H), *3* ferric hydro-peroxo complex (Fe^{3+}–O–OH; compound 0), *4* oxo-ferryl radical complex [$(Fe^{4+}=O)^{\bullet+}$, compound I], *5* oxo-iron(V) complex (Fe^{5+}=O)

reactions but has not unambiguously been identified so far (Fig. 6; Hlavica 2004; Poulos and Johnson 2005).

It is still too early to make a statement on the actual function of AaP in *A. aegerita*. There are several possibilities, for example, the involvement of AaP in lignin and humus transformation or in the synthesis of hydroxylated and/or halogenated metabolites (e.g., antibiotics). But it is possible just as well that it has a completely different function.

AaP-like enzymes of other organisms?

A few reports in the literature and own recent findings indicate that the haloperoxidase of *A. aegerita* A1 is not a "freak of nature" (i.e., a very rare enzyme) and that other mushrooms can produce similar enzymes. Thus, we found AaP-like activities in at least four other strains of *A. aegerita* (Ullrich et al. 2004), and recently, we have detected hydroxylating peroxidase activities in two strains of the closely related species *A. chaxingu* as well as in two *Coprinus* spp. (Ullrich and Hofrichter, unpublished results). The latter finding is surprising because the genus *Coprinus* belongs to family Coprinaceae and not to the Bolbitiaceae as *Agrocybe* does. An unusual peroxidase, that oxidized the methyl group of toluene and a number of its derivatives via benzyl alcohols into the corresponding aldehydes, were reported for a not further characterized *Coprinus* sp. strain (Russ et al. 2002). As AaP, the enzyme was produced in a liquid soybean medium (Hauer et al. 2003). Whether this *Coprinus* peroxidase catalyzes halogenations or aromatic hydroxylations or bears a

heme-thiolate at the active site is unclear because its purification and characterization is still pending.

The existence of hydroxylating peroxidases is seemingly not confined to fungi. The aromatic hydroxylation of 4-hydroxybenzyl alcohol by a bromoperoxidase of the red alga *Cystoclonium purpureum* was already described in the 1970s (Pedersén 1976). The reaction required a pH above 5, H_2O_2, and the presence of 2,5-dihydroxyphenyl acetic acid and led to the formation of 4,5-dihydroxybenzyl alcohol. Hydroxylation competed with bromination that was favored at more acidic pH (<5). Unfortunately, detailed information on the enzyme is lacking because it has not been purified and characterized yet.

More information on AaP and in particular on its relation to P450s and CPO will be available when the AaP gene will have been isolated, cloned, and sequenced as well as when AaP-like enzymes will have been isolated from other fungal species (e.g., *Coprinus* spp.). Respective studies are currently under investigation.

Conclusions and outlook

The existence of extracellular halogenating and hydroxylating enzymes in mushrooms may be of ecological and environmental as well as biotechnological relevance. The natural occurrence of organohalogens (AOX), mostly chlorinated substances in terrestrial and brominated compounds in marine environments, is a well-known phenomenon which has gained more and more attention during the last decade (Gribble 1996; Öberg 2002; Myneni 2002; Neilson 2003). Basidiomycetous fungi and in particular agaric mushrooms as *Hypholoma* spp. or *Mycena* spp. have been found to produce high amounts of chlorinated metabolites in forest litter and the laboratory (Verhagen et al. 1996, 1999). The responsible enzymatic reactions, however, remained unclear though the involvement of haloperoxidases was repeatedly discussed and chloroperoxidase activities were detected in soil (Asplund et al. 1993; de Jong and Field 1997; Neilson 2003). With the haloperoxidase of *A. aegerita*, the first enzyme of a basidiomycetous fungus with a low but detectable chlorinating and a strong brominating activity has been found. The specific search for similar enzymes among the huge number of basidiomycetous fungi colonizing litter or lignicelluloses will surely result in the discovery of further haloperoxidases and may help to understand better the biological formation of AOX in terrestrial ecosystems.

The great biotechnological potential of peroxygenases has generally been recognized and there are several attempts to "create" improved biocatalysts of this type. One attempt is the laboratory evolution of peroxide-mediated cytochrome P450s hydroxylation (i.e., the improvement of the peroxide-shunt pathway), which led already to mutants of $P450_{cam}$ with 20-fold higher peroxygenase activity towards naphthalene than the native enzyme has (Joo et al. 1999). Other approaches make use of engineered peroxidases (e.g., horseradish and *Coprinus cinereus* peroxidases) and try to improve their oxygen transfer potential (van Rantwijk and Sheldon 2000). Microperoxidase 8 is another interesting example of a hydroxylating biocatalyst. It is a minienzyme that consists of a heme-containing octapeptide obtained by the proteolytic digestion of horse-heart cytochrome c and hydroxylates phenols, anilines, and anthracene by means of H_2O_2 (Osman et al. 1996; Dorovska-Taran et al. 1998). Moreover, a myoglobin mutant catalyzing the aromatic monooxygenation of tryptophan has been recently described (Pfister et al. 2005). Complementary to these developments, our results demonstrate that natural peroxygenases do also exist, which could become versatile biotechnological tools.

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References

Adak S, Bandyopadhyay D, Bandyopadhyay U, Banerjee RK (2001) An essential role of active site arginine residue in iodide binding and histidine residue in electron transfer for iodide oxidation by horseradish peroxidase. Mol Cell Biochem 218:1–11

Albrich JM, McCarthy CA, Hurst JK (1981) Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leucocyte myeloperoxidase. Proc Natl Acad Sci USA 78:210–214

Almeida M, Filipe S, Humanes M, Maia MF, Melo R, Severino N, da Silva JA, Frausto da Silva JJ, Wever R (2001) Vanadium haloperoxidases from brown algae of the Laminariaceae family. Phytochemistry 57:633–642

Alvarez RG, Hunter IS, Suckling CJ, Thomas M, Vitinius U (2001) A novel biotransformation of benzofurans and related compounds catalysed by a chloroperoxidase. Tetrahedron Lett 57:8581–8587

Andersson LA, Dawson JH (1991) EXAFS spectroscopy of hemecontaining oxygenases and peroxidases. Struct Bond 64:1–40

Ashley PL, Griffin BW (1981) Chloroperoxidase-catalyzed halogenation of antipyrine, a drug substrate of liver microsomal cytochrome P-450. Arch Biochem Biophys 210:167–178

Asplund G, Christiansen JV, och Grimvall A (1993) A chloroperoxidase-like catalyst in soil: detection and characterization of some properties. Soil Biol Biochem 25:41–46

Ayala M, Robeldo N, Lopez-Munguia A, Vazquez-Duhalt R (2000) Substrate specificity and ionization potential in chloroperoxidase-catalyzed oxidation of diesel fuel. Environ Sci Technol 34:2804–2809

Baciocchi E, Lanzalunga O, Manduchi L (1999) Prochiral selectivity and deuterium kinetic isotope effect in the oxidation of benzyl alcohol catalyzed by chloroperoxidase. Chem Commun (Camb) 17:1715–1716

Baciocchi E, Fabbrini M, Lanzalunga O, Manduchi L, Pochetti G (2001) Pro-chiral selectivity in H_2O_2-promoted oxidation of arylalkanoles catalysed by chloroperoxidase. Eur J Biochem 268:665–672

Baden DG, Corbett MD (1980) Bromoperoxidases from *Penicillus capitatus*, *Penicillus lamourouxii* and *Rhipocephalus phoenix*. Biochem J 187:205–211

Beckwith JR, Hager LP (1963) Biological chlorination. VIII. Late intermediates in the biosynthesis of caldariomycin. J Biol Chem 238:3091–3094

Blanke SR, Yi S, Hager LP (1989) Development of semi-continuous and continuous flow bioreactors for the high level production of chloroperoxidase. Biotechnol Lett 11:769–774

Borole A, Dai S, Cheng CL, Rodriguez M, Davison BH (2004) Performance of chloroperoxidase stabilization in mesoporous sol-gel glass using in situ glucose oxidase peroxide generation. Appl Biochem Biotechnol 113:273–286

Bruns N, Tiller JC (2005) Amphiphilic network as nanoreactor for enzymes in organic solvents. Nano Lett 5:45–48

Butler A (1999) Mechanistic considerations of the vanadium haloperoxidases. Coord Chem Rev 187:17–35

Butler A, Walker JV (1993) Marine haloperoxidases. Chem Rev 93:1937–1944

Butler A, Carter JN, Simpson MT (2001) Vanadium in proteins and enzymes. In: Bertini I, Sigel A, Sigel H (eds) Handbook on metalloproteins. Marcel Dekker, New York, pp 153–179

Cadicamo CD, Courtieu J, Deng H, Meddour A, O'Hagan D (2004) Enzymatic fluorination in *Streptomyces cattleya* takes place with an inversion of configuration consistent with an SN2 reaction mechanism. Chembiochem 5:685–690

Carmichael RD, Pickard MA (1989) Continous and batch produktion of chloroperoxidase by mycelial pellets of *Caldariomyces fumago* in an airlift fermenter. Appl Environ Microbiol 55:17–20

Chen YP, Lincoln DE, Woodin SA, Lovell CR (1991) Purification and properties of a unique flavin-containing chloroperoxidase from the capitellid polychaete *Notomastus lobatus*. J Biol Chem 266:23909–23915

Clutterbuck PW, Mukhopadhaya SL, Oxford AE, Raistrick H (1940) Studies in the biochemistry of micro-organisms. 65 A) a survey of chlorine metabolism by moulds B) caldariomycin, C5H8O2Cl2, a metabolic product of *Caldariomyces fumago*. Biochem J 34:664–667

Colonna S, Gaggero N, Manfredi A, Casella L, Gulotti M, Carrea G, Pasta P (1990) Enantioselective oxidations of sulfides catalyzed by chloroperoxidase. Biochemistry 29:10465–10468

Conesa A, van de Velde F, van Rantwijk F, Sheldon RA, van den Hondel CAMJJ, Punt PJ (2001) Expression of the *Caldariomyces fumago* chloroperoxidase in *Aspergillus niger* and characterization of the recombinant enzyme. J Biol Chem 276:17635–17640

Corbett MD, Chipko BR, Baden DG (1978) Chloroperoxidase-catalysed oxidation of 4-chloroaniline to 4-chloronitrosobenze. Biochem J 175:353–360

Correia MA (2005) Human and rat liver cytochromes P450: functional markers, diagnostic inhibitor probes, and parameters frequently used in P450 studies. In: Ortiz de Montellano PR (ed) Cytochrome P450 - structure, mechanism and biochemistry, 3rd edn. Plenum, New York, pp 619–657

de Jong E, Field JA (1997) Sulfur tuft and turkey tail. Annu Rev Microbiol 51:375–414

Dembitsky VM (2003) Oxidation, epoxidation and sulfoxidation reactions catalysed by haloperoxidases. Tetrahedron Lett 59:4701–4720

Deng H, O'Hagan D, Schaffrath C (2005) Fluorometabolite biosynthesis and the fluorinase from *Streptomyces cattleya*. ChemInform. DOI 10.1002/chin.200514282

Doerge DR, Corbett MD (1991) Peroxygenation mechanism for chloroperoxidase-catalyzed N-oxidation of arylamines. Chem Res Toxicol 4:556–560

Dorovska-Taran V, Posthumus MA, Boeren S, Boersma MG, Teunis CJ, Rietjens IM, Veeger C (1998) Oxygen exchange with water in heme-oxo intermediates during H_2O_2-driven oxygen incorporation in aromatic hydrocarbons catalyzed by microperoxidase-8. Eur J Biochem 253:568–659

Dunford HB (1999) Heme peroxidases. Wiley-VCH, New York

Dunford HB, Lambeir AM, Kashem MA, Pickard M (1987) On the mechanism of chlorination by chloroperoxidase. Arch Biochem Biophys 252:292–302

England PA, Harford-Cross, Stevenson JA, Rouch DA, Wong LL (1998) The oxidation of naphthalene and pyrene by cytochrome P450cam. FEBS Lett 424:271–274

Fang GH, Kenigsberg P, Axley MJ, Nuell M, Hager LP (1986) Cloning and sequencing of chloroperoxidase cDNA. Nucleic Acids Res 14:8061–8071

Farhangrazi ZS, Sinclair R, Yamazaki I, Powers LS (1992) Haloperoxidase activity of *Phanerochaete chrysosporium* lignin peroxidases H2 and H8. Biochemistry 31:10763–10768

Folkes LK, Candeias LP, Wardman P (1995) Kinetics and mechanisms of hypochlorous acid reactions. Arch Biochem Biophys 323:120–126

Franssen MCR (1994) Haloperoxidases: useful catalysts for halogenation and oxidation reactions. Catal Today 22:441–457

Franssen MCR, van der Plas HC (1992) Haloperoxidases: their properties and their use in organic synthesis. Adv Appl Microbiol 37:41–99

Geigert J, Neidleman SL, Dalietos DJ (1983a) Novel haloperoxidase substrates. Alkynes and cyclopropanes. J Biol Chem 258:2273–2277

Geigert J, Neidleman SL, Dalietos DJ, de Witt SK (1983b) Haloperoxidases: enzymatic synthesis of α,β-halohydrins from gaseous alkenes. Appl Environ Microbiol 45:366–374

Geigert J, Neidleman SL, Dalietos DJ, DeWitt SK (1983c) Novel haloperoxidase reaction: synthesis of dihalogenated products. Appl Environ Microbiol 45:1575–1581

Green MT, Dawson JH, Gray HB (2004) Oxoiron(IV) in chloroperoxidase compound II is basic: implications for P450 chemistry. Science 304:1653–1656

Gribble GW (1996) Naturally occuring organohalogen compounds - a comprehencive survey. In: Herz G, Kirby GW, Moore RE, Steglich W, Tamm C (eds) Progress in the chemistry of organic natural products. Springer, Berlin Heidelberg New York, pp 1–498

Griffin BW (1983) Mechanism of halide-stimulated activity of chloroperoxidase evidence for enzymatic formation of free hypohalous acid. Biochem Biophys Res Commun 116:873–879

Hager LP, Morris DR, Brown FS, Eberwein H (1966) Chloropeoxidase. II. Utilization of halogen anions. J Biol Chem 241:1769–1777

Hallenberg PF, Hager LP (1978) Enzymatic assay of chloroperoxidase (EC 1.11.1.10). Methods Enzymol 52:521–529

Hansen EH, Schembri MA, Klemm P, Schäfer T, Molin S, Gram L (2004) Elucidation of the antibacterial mechanism of the *Curvularia* haloperoxidase system by DNA microarray profiling. Appl Environ Microbiol 70:1749–1757

Harrison JE, Schultz J (1976) Studies on the chlorinating activity of myeloperoxidase. J Biol Chem 251:1371–1374

Hashimoto A, Pickard PA (1984) Chloroperoxidases from *Caldariomyces* (*Leptoxyphium*) cultures: glycoproteins with variable carbohydrate content and isoenzymic forms. J Gen Microbiol 130:2051–2058

Hattaka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbüchel A (eds) Biopolymers: lignin, humic substances and coal. Wiley-VCH, Weinheim, pp 129–180

Hauer B, Zelinski T, Habicher T, Breuer M, Anke T, Russ R (2003) Verfahren zur selektiven oxidation von substituierten Toluolen durch mikrobielle Peroxidasen aus *coprinus*-procedure for the selective oxidation of substituted toluens with microbial peroxidases from *coprinus*. German Patent Office München No DE 10149266A1

Hlavica P (2004) Models and mechanisms of O–O bond activation by cytochrome P450. A critical assessment of the potential role of multiple active intermediates in oxidative catalysis. Eur J Biochem 271:4335–4360

Hoekstra EJ, de Leer EWB, Brinkman UAT (1999) Findings supporting the natural formation of trichloroacetic acid in soil. Chemosphere 38:2875–2883

Holland HL, Weber HK (2000) Enzymatic hydroxylation reactions. Curr Opin Biotechnol 11:547–553

Holland HL, Brown FM, Lozada D, Mayne BW, Szerminski R, van Vliet AJ (2002) Chloroperoxidase-catalyzed oxidation of methionine derivatives. Can J Chem 80:633–639

Hollenberg PF (1992) Mechanisms of cytochrome P450 and peroxidase-catalyzed xenobiotic metabolism. FASEB J 6:686–694

Hollenberg PF, Hager LP (1973) The P-450 nature of the carbon monoxide complex of ferrous chloroperoxidase. J Biol Chem 248:2630–2633

Hu S, Dordick JS (2002) Highly enantioselective oxidation of cis-cyclopropylmethanols to corresponding aldehydes catalyzed by chloroperoxidase. J Org Chem 67:314–317

Hu S, Hager LP (1998) Unusual propargylic oxidations catalyzed by chloroperoxidase. Biochem Biophys Res Commun 253:544–546

Hu S, Hager LP (1999) Highly enantioselective propargylic hydroxylations catalyzed by chloroperoxidase. J Am Chem Soc 121:872–873

Jacks TJ (2005) Evaluation of kojic acid for determining heme and nonheme haloperoxidase activities spectrofluorometrically. Anal Lett 38:921–927

Jannun R, Coe EL (1987) Bromoperoxidase from the marine snail *Murex trunculis*. Comp Biochem Physiol 88:917–922

Joo H, Arisawa A, Lin Z, Arnold FH (1999) A high-throughput digital imaging screen for the discovery and directed evolution of oxygenases. Chem Biol 6:699–706

Kedderis GL, Koop DR, Hollenberg PF (1980) *N*-Demethylation reactions catalyzed by chloroperoxidase. J Biol Chem 255:10174–10182

Keener WK, Watwood ME (2004) Chloride analysis using 3,3′,5,5′-tetramethyl-benzidine and chloroperoxidase. Anal Biochem 334:406–408

Kenigsberg P, Fang GH, Hager LP (1987) Post-translational modifications of chloroperoxidase from *Caldariomyces fumago*. Arch Biochem Biophys 254:409–415

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Kooter IM, Koehler BP, Moguilevsky N, Bollen A, Wever R, Johnson MK (1999) The Met243 sulfonium ion linkage is responsible for the anomalous magnetic circular dichroism and optical spectral properties of myeloperoxidase. J Biol Inorg Chem 4:684–691

Kudo T, Tomura D, Liu DL, Dai XQ, Shoun H (1996) Two isozymes of P-450nor of *Cylindrocarpon tonkinense*. Biochimie 78:792–799

La Rotta CE, Bon EP (2002) 4-chlorophenol degradation by chloroperoxidase from *Caldariomyces fumago*: formation of insoluble products. Appl Biochem Biotechnol 98:191–203

La Rotta Hernandez CE, Lütz S, Liese A, Bon EPS (2005) Activity and stability of *Caldariomyces fumago* chloroperoxidase modified by reductive alkylation, amidation and cross-linking. Enzyme Microb Technol 37:582–588

Lakner FJ, Hager LP (1996) Chloroperoxidase as enantioselective epoxidation catalyst: an efficient synthesis of (*R*)-(−)-mevalonolactone. J Org Chem 61:3923–3925

Lankinen VP, Bonnen AM, Anton LH, Wood DA, Kalkkinen N, Hatakka A, Thurston (2001) Characteristics and N-terminal amino acid sequence of manganese peroxidase from solid substrate cultures of *Agaricus bisporus*. Appl Microbiol Biotechnol 55:170–176

Laturnus F, Fahimi I, Gryndler M, Hartmann A, Heal MR, Matucha M, Schöler HF, Schroll R, Svensson T (2005) Natural formation and degradation of chloroacetic acids and volatile organochlorines in forest soil. Environ Sci Pollut Res Int 12:233–244

Lewis DFV (2001) Guide to cytochromes P450: structure & function. CRC Press, Boca Raton, FL

Littlechild J (1999) Haloperoxidases and their role in biotransformation reactions. Curr Opin Chem Biol 3:28–34

Littlechild J, Garcia-Rodriguez E, Dalby A, Isupov M (2002) Structural and functional comparisons between vanadium haloperoxidase and acid phosphatase enzymes. J Mol Recognit 15:291–296

Lukat GS, Goff HM (1990) A nuclear magnetic resonance study of axial ligation for the reduced states of chloroperoxidase, cytochrome P-450cam, and porphinatoiron(II) thiolate complexes. Biochim Biophys Acta 1037:351–359

Makris TM, Denisov I, Schlichting I, Sligar SG (2005) Activation of molecular oxygen by cytochrome P450. In: Ortiz de Montellano PR (ed) Cytochrome P450—structure, mechanism and biochemistry. Plenum, New York, pp 149–182

Manoj KM, Hager LP (2001) Utilization of peroxide and its relevance in oxygen insertion reactions catalyzed by chloroperoxidase. Biochim Biophys Acta 1547:408–417

Manoj KM, Hager LP (2005) New insight into chloroperoxidase catalyzed reactions. Proceedings of the 14th International Conference on Cytochrome P450, Dallas (Texas), May 31-June 5, 2005, p 139

Manoj KM, Hager LP (2006) A colorimetric method for detection and quantification of chlorinating activity of hemeperoxidases. Anal Biochem 348:84–86

Manoj KM, Lakner FJ, Hager LP (2000) Epoxidation of indene by chloroperoxidase. J Mol Catal, B Enzym 9:107–111

Manthey JA, Hager LP (1981) Purification and properties of bromoperoxidase from *Penicillus capitatus*. J Biol Chem 256:11232–11238

Marshall GC, Wright GD (1996) Purification and characterization of two haloperoxidases from the glycopeptide antibiotic producer *Streptomyces toyocaensis* NRRL 15009. Biochem Biophys Res Commun 219:580–583

Martínez MJ, Ruiz-Duenas FJ, Guillen F, Martinez AT (1996) Purification and catalytic properties of two manganese peroxidase isoenzymes from *Pleurotus eryngii*. Eur J Biochem 237:424–432

Matsunaga I, Sumimoto T, Ayata M, Ogura H (2002) Functional modulation of a peroxygenase cytochrome P450: novel insight into the mechanisms of peroxygenase and peroxidase enzymes. FEBS Lett 528:90–94

Matucha M, Forczek ST, Gryndler M, Uhlirova H, Fuksova K, Schroder P (2003) Trichloroacetic acid in Norway spruce/soil-system. I. Biodegradation in soil. Chemosphere 50:303–309

McCarthy MB, White RE (1983) Functional differences between peroxidase compound I and the cytochrome P-450 reactive oxygen intermediate. J Biol Chem 158:9153–9158

McLean KJ, Cheesman MR, Rivers SL, Richmond A, Leys D, Chapman SK, Reid GA, Price NC, Kelly SM, Clarkson J, Smith WE, Munro AW (2002) Expression, purification and spectroscopic characterization of the cytochrome P450 CYP121 from *Mycobacterium tuberculosis*. J Inorg Biochem 91:527–541

Miller VP, Tschirret-Guth RA, Ortiz de Montellano PR (1995) Chloroperoxidase-catalyzed benzylic hydroxylation. Arch Biochem Biophys 319:333–340

Morel F, Vignais PV (1984) Examination of the oxidase function of the b-type cytochrome in human polymorphonuclear leucocytes. Biochim Biophys Acta 764:213–225

Morgan JA, Lu Z, Clark DS (2002) Toward the development of a biocatalytic system for oxidation of p-xylene to tetraphtalic acid: oxidation of 1,4-benzenedimethanol. J Mol Catal, B Enzym 18:147–154

Morris DR, Hager LP (1966) Chloroperoxidase. I. Isolation and properties of the crystalline glycoprotein. J Biol Chem 241:1763–1768

Munir IZ, Dordick JS (2000) Soybean peroxidase as an effective bromination catalyst. Enzyme Microb Technol 26:337–341

Murphy CD (2003) New frontiers in biological halogenation. J Appl Microbiol 94:539–548

Murphy CD, Moore RM, White RL (2000) Peroxidases from marine microalgae. J Appl Phycol 12:507–513

Myneni SCB (2002) Formation of stable chlorinated hydrocarbons in weathering plant material. Science 295:1039–1041

Neidleman SL, Levine SD (1968) Enzymatic bromohydrin formation. Tetrahedron Lett 37:4057–4059

Neilson AH (2003) Organic bromine and iodine compounds: biological effects and biosynthesis of brominated metabolites. In: Neilson AH (ed) Handbook of environmental chemistry. Springer, Berlin Heidelberg New York, pp 75–204

Niedan V, Pavasars I, Oberg G (2000) Chloroperoxidase-mediated chlorination of aromatic groups in fulvic acid. Chemosphere 41:779–785

Niu J, Yu G (2004) Molecular structural characteristics governingbiocatalytic chlorination of PAHs by chloroperoxidase from *Caldariomyces fumago*. Environ Res 15:159–167

Nordblom GD, White RE, Coon MJ (1976) Studies on hydroperoxide-dependent substrate hydroxylation by purified liver microsomal cytochrome P450. Arch Biochem Biophys 175:524–533

Nuell MJ, Fang GH, Axley MJ, Kenigsberg P, Hager LP (1988) Isolation and nucleotide sequence of the chloroperoxidase gene from *Caldariomyces fumago*. J Bacteriol 170:1007–1011

Öberg G (2002) The natural chlorine cycle—fitting the scattered pieces. Appl Microbiol Biotechnol 58:565–581

Okazaki O, Guengerich FP (1993) Evidence for specific base catalysis in *N*-dealkylation reactions catalyzed by cytochrome P450 and chloroperoxidase. J Biol Chem 268:1546–1552

Ortiz de Montellano PR, de Voss JJ (2005) Substrate oxidation by cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) Cytochrome P450—structure, mechanism and biochemistry, 3rd edn. Plenum, New York, pp 183–245

Ortiz-Bermudez P, Srebotnik E, Hammel KE (2003) Chlorination and cleavage of lignin structures by fungal chloroperoxidase. Appl Environ Microbiol 69:5015–5018

Osman AM, Koerts J, Boersma MG, Boeren S, Veeger C, Rietjens IM (1996) Microperoxidase/H_2O_2-catalyzed aromatic hydroxylation proceeds by a cytochrome-P-450-type oxygen-transfer reaction mechanism. Eur J Biochem 240:232–238

Pedersén M (1976) A brominating hydroxylating peroxidase from the red alga *Cystoclonium purpureum*. Plant Physiol 37:6–11

Peñéñory AB, Argüello JE, Puiatti M (2004) Novel model sulfur compounds as mechanistic probes for enzymatic and biomimetic oxidations. Eur J Org Chem 2005:114–122

Pfister TD, Ohki T, Ueno T, Hara I, Adachi S, Makino Y, Ueyama N, Lu Y, Watanabe Y (2005) Monooxygenation of an aromatic ring by F43W/H64D/V68I myoglobin mutant and hydrogen peroxide myoglobin mutants as a model for P450 hydroxylation chemistry. J Biol Chem 280:12858–12866

Picard M, Gross J, Lübbert E, Tölzer S, Krauss S, van Pee K-H, Berkessel A (1997) Metal-free bacterial haloperoxidases as unusual hydrolases: activation of H_2O_2 by the formation of peracetic acid. Angew Chem Int Ed Engl 36:1196–1199

Pickard MAK, Kadima TA, Carmichael RD (1991) Chloroperoxidase, a peroxidase with potential. J Ind Microbiol Biotechnol 7:235–241

Plat H, Kren BE, Wever R (1987) The bromoperoxidase from the lichen *Xanthoria parietina* is a novel vanadium enzyme. Biochem J 248:277–279

Poliakoff M, Fitzpatrick JM, Farren TR, Anastas PT (2002) Green chemistry: science and politics of change. Science 297:807–810

Poulos TL, Johnson EF (2005) Structures of cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) Cytochrome P450—structure, mechanism, and biochemistry. Plenum, New York, pp 87–114

Rai GP, Sakai S, Florez AM, Mogollon L, Hager LP (2001) Directed evolution of chloroperoxidase for improved epoxidation and chlorination catalysis. Adv Synth Catal 343:638–645

Rawitch AB, Taurog A, Chernoff SB, Dorris ML (1979) Hog thyroid peroxidase: physical, chemical, and catalytic properties of the highly purified enzyme. Arch Biochem Biophys 194:244–257

Reynolds DR (1999) *Capnodium citri*: the sooty mold fungi comprising the taxon concept. Mycopathologia 148:141–147

Roach MP, Chen YP, Woodin SA, Lincoln DE, Lovell CR, Dawson JH (1997) *Notomastus lobatus* chloroperoxidase and *Amphitrite ornata* dehaloperoxidase both contain histidine as their proximal heme iron ligand. Biochemistry 36:2197–2202

Robinson GK, Jackman SA, Stratford J (2000) Halocompounds. In: Rehm H-J, Reed G, Pühler A, Stadler P (eds) Biotechnology, 2nd edn. Wiley-VCH, New York, pp 173–220

Russ R, Zelinski T, Anke T (2002) Benzylic biooxidation of various toluenes to aldehydes by peroxidase. Tetrahedron Lett 43:791–793

Sae ASW, Cunningham BA (1979) Isolation and properties of chloroperoxidase isoenzymes. Phytochemistry 18:1785–1787

Sanfilippo C, Nicolosi G (2002) Catalytic behavior of chloroperoxidase from *Caldariomyces fumago* in the oxidation of cyclic conjugated dienes. Tetrahedron Asymmetry 13:1889–1892

Sanfilippo C, D'Antona N, Nicolosi G (2004) Chloroperoxidase from *Caldariomyces fumago* is active in the presence of an ionic liquid as co-solvent. Biotechnol Lett 26:1815–1819

Santhanam L, Dordick JS (2002) Chloroperoxidase-catalysed epoxidation of styrene in aqueous and nonaqueous media. Biocatal Biotransform 20:265–274

Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M, Witholt B (2001) Industrial biocatalysis today and tomorrow. Nature 409:258–268

Schmid A, Hollmann F, Park JB, Bühler B (2002) The use of enzymes in the chemical industry in Europa. Curr Opin Biotechnol 13:359–366

Schoemaker HE, Mink D, Wubbolts MG (2003) Dispelling the myths—biocatalysis in industrial synthesis. Science 299:1694–1697

Seelbach K, Kragl U (1997) Nanofiltration membranes for cofactor retention in continuous enzymatic synthesis. Enzyme Microb Technol 20:389–392

Shaik S, de Visser SP (2005) Computational approaches to cytochrome P450 function. In: Ortiz de Montellano PR (ed) Cytochrome P450—structure, mechanism and biochemistry, 3 edn. Plenum, New York, pp 45–85

Shaw PD, Hager LP (1959) Biological chlorination. III. β-Ketoadipate chlorinase: a soluble enzyme system. J Biol Chem 234:2565–2569

Sheffield DJ, Smith AJ, Harry TR, Rogers LJ (1993) Thermostability of the vanadium bromoperoxidase from *Corallina officinalis*. Biochem Soc Trans 21:445

Sheng D, Gold MH (1997) Haloperoxidase activity of manganese peroxidase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 345:126–134

Shiro Y, Fujii M, Iizuka T, Adachi S, Tsukamoto K, Nakahara K, Shoun H (1995) Spectroscopic and kinetic studies on reaction of cytochrome P450nor with nitric oxide implication for its nitric oxide reduction mechanism. J Biol Chem 270:1617–1623

Simons BH, Barnett P, Vollenbroek EGM, Dekker HL, Muijsers AO, Messerschmidt A, Wever R (1995) Primary structure and characterization of the vanadium chloroperoxidase from the fungus *Curfularia inaequalis*. Eur J Biochem 229:566–574

Sono M, Roach MP, Coulter ED, Dawson JH (1996) Heme-containing oxygenases. Chem Rev 96:2841–2887

Speicher A (2000) Chemical and in vitro syntheses of brominated and chlorinated 3,4′-dihydroxybibenzyles (halogenated lunularins). J Prakt Chem 342:162–168

Spreti N, Germani R, Incani A, Savelli G (2004) Stabilization of chloroperoxidase by polyethylene glycols in aqueous media: kinetic studies and synthetic applications. Biotechnol Prog 20:96–101

Stamets P (2000) Growing gourmet and medicinal mushrooms, 3 edn. Ten Speed Press, Berkeley

Stündl UM, Patzak D, Schauer F (2000) Purification of a soluble cytochrome P450 from *Trichosporon montevideense*. J Basic Microbiol 40:289–292

Sun W, Kadima TA, Pickard MA, Dunford HB (1994) Catalase activity fo chloroperoxidase and its interaction with peroxidase activity. Biochem Cell Biol 72:321–331

Sundaramoorthy M, Terner J, Poulos TL (1995) The crystal structure of chloroperoxidase: a heme peroxidase-cytochrome P450 functional hybrid. Structure (Camb) 3:1367–1377

Sundaramoorthy M, Terner J, Poulos TL (1998) Stereochemistry of the chloroperoxidase active site: crystallographic and molecular-modeling studies. Chem Biol 5:461–473

Taurog A, Dorris ML (1991) Peroxidase-catalyzed bromination of tyrosine, thyroglobulin, and bovine serum albumin: comparison of thyroid peroxidase and lactoperoxidase. Arch Biochem Biophys 287:288–296

ten Brink HB, Dekker HL, Schoemaker HE, Wever R (2000) Oxidation reactions catalyzed by vanadium chloroperoxidase from *Curvularia inaequalis*. J Inorg Biochem 80:91–98

Thomas EL, Fishman M (1986) Oxidation of chloride and thiocyanate by isolated leucocytes. J Biol Chem 261:9694–9702

Thomas EL, Bozeman PM, Jefferson MM, King CC (1995) Oxidation of bromide by the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. J Biol Chem 270:2906–2913

Torres E, Aburto J (2005) Chloroperoxidase-catalyzed oxidation of 4,6-dimethyldibenzothiophene as dimer complexes: evidence for kinetic cooperativity. Arch Biochem Biophys 437:224–232

Trevisan V, Signoretto M, Colonna S, Pironti V, Strukul G (2004) Microencapsulated chloroperoxidase as a recyclable catalyst for the enantioselective oxidation of sulfides with hydrogen peroxide. Angew Chem Int Ed Engl 116:4189–4191

Ullrich R (2005) The haloperoxidase of the fungus *Agrocybe aegerita*: an extracellular P450 enzyme (in German). Ph.D. thesis, International Graduate School of Zittau (Germany)

Ullrich R, Hofrichter M (2005) The haloperoxidase of the agaric fungus Agrocybe aegerita hydroxylates toluene and naphthalene. FEBS Lett 579:6247–6250

Ullrich R, Nüske J, Scheibner K, Spantzel J, Hofrichter M (2004) Novel haloperoxidase from the agaric basidiomycete *Agrocybe aegerita* oxidizes aryl alcohols and aldehydes. Appl Environ Microbiol 70:4575–4581

Upadhyay RC (1995) Metabolism of phenolic substances by *Pleurotus flabellatus* (Berk and Br.) and *Agrocybe aegerita* (Brig.) Sing. Ph.D. thesis, Faculty of Biology and Pharmacy, Friedrich Schiller University of Jena (Germany)

Urlacher VB, Lutz-Wahl S, Schmid RD (2004) Microbial P450 enzymes in biotechnology. Appl Microbiol Biotechnol 64:317–325

van Beilen JB, Duetz WA, Schmid A, Witholt B (2003) Practical issues in the application of oxygenases. Trends Biotechnol 21:170–177

van de Velde F, Bakker M, van Rantwijk F, Sheldon RA (2001) Chloroperoxidase-catalyzed enantioselective oxidations in hydrophobic organic media. Biotechnol Bioeng 72:523–529

van Peé K-H (2001) Microbial biosynthesis of halometabolites. Arch Microbiol 175:250–258

van Peé K-H, Lingens F (1984) Detection of a bromoperoxidase in *Streptomyces phaeochromogenes*. FEBS Lett 173:5–8

van Peé K-H, Lingens F (1985) Purification and molecular and catalytic properties of bromoperoxidase from *Streptomyces phaeochromogenes*. J Gen Microbiol 131:1911–1916

van Peé K-H, Zehner S (2003) Natural production of organohalogen compounds: enzymology and molecular genetics of biological halogenation. In: Gribble GW (ed) The handbook of environmental chemistry. Springer, Berlin Heidelberg New York, pp 171–199

van Peé K-H, Patallo EP (2006) Flavine-dependent halogenases involved in secondary metabolism in bacteria. Appl Microbiol Biotechnol, (in press)

van Peé K-H, Sury G, Lingens F (1987) Purification and properties of a non-heme bromoperoxidase from *Streptomyces aureofaciens*. Biol Chem Hoppe-Seyler 368:1225–1232

van Rantwijk F, Sheldon RA (2000) Selective oxygen transfer catalysed by heme peroxidases: synthetic and mechanistic aspects. Curr Opin Biotechnol 6:554–564

Vazquez-Duhalt R, Ayala M, Marquez-Rocha FJ (2001) Biocatalytic chlorination of aromatic hydrocarbons by chloroperoxidase of *Caldariomyces fumago*. Phytochemistry 58:929–933

Verhagen FJM, Kuyper TW, Wijnberg JBPA, Field JA (1996) The ubiquity of natural adsorbable organic halogen production among basidiomycetes. Appl Microbiol Biotechnol 45:710–718

Verhagen FJM, van Assema FBJ, Boekema BKHL, Swarts HJ, Wijnberg JBPA, Field JA (1999) Dynamics of organohalogen production by the ecologically important fungus *Hypholoma fasciculare*. FEMS Microbiol Lett 158:167–178

Vilter H (1983) Peroxidases from phaeophyceae. 3. Catalysis of halogenation by peroxidases from Ascophyllum-nodosum. Le-Jol Bot Mar 26:429–435

Wagenknecht HA, Woggon WD (1997) Identification of intermediates in the catalytic cycle of chloroperoxidase. Chem Biol 4:367–372

Wannstedt C, Rotella D, Siuda JF (1990) Chloroperoxidase mediated halogenation of phenols. Bull Environ Contam Toxicol 44:282–287

Wuosmaa AM, Hager LP (1990) Methyl chloride transferase: a carbocation route for biosynthesis of halometabolites. Science 249:160–162

Yaipakdee P, Robertson LW (2001) Enzymatic halogenation of flavanones and flavones. Phytochemistry 57:341–347

Yamada H, Itoh N, Izumi Y (1985) Chloroperoxidase-catalyzed halogenation of trans-cinnamic acid and its derivatives. J Biol Chem 260(22):11962–11969

Yoon KS, Chen YP, Lovell CR, Lincoln DE, Knapp LW, Woodin SA (1994) Localization of the chloroperoxidase of the capitellid polychaete *Notomastus lobatus*. Biol Bull 187:215–222

Zadrazil F (2000) Industrial production of *Agrocybe aegerita* (Brig.) Sing.: will *Agrocybe aegerita* become a new culture mushroom. (In German). Champignon 412:296–301

Zhu G, Wang P (2005) Novel interface-binding chloroperoxidase for interfacial epoxidation of styrene. J Biotechnol 117:195–202